# 2004 International *Dictyostelium* Conference

## Sainte-Adèle, Québec, Canada


# 2004 International *Dictyostelium* Conference

Sainte-Adèle, Québec, Canada
August 15th to August 20th, 2004

Local Organizing Committee:

David Cotter
Barrie Coukell
Samantha LaRue
Adrian Tsang
Gerald Weeks

# SPONSORS

The organizing committee wishes to thank the following sponsors for contributing to and supporting the 2004 ***Dictyostelium*** meeting:

Faculty of Arts and Science, Concordia University

Office of the Provost and Vice-Provost Research, Concordia University

Centre for Structural and Functional Genomics, Concordia University

Invitrogen

Fisher Scientific

The University of Windsor

Concordia
UNIVERSITY

Fisher Scientific

## Sunday, August 15, 2004

15:30 - 19:00 **Registration**

19:00 - 23:00 **Welcome Reception**

## Monday, August 16, 2004

7:00 - 8:30 Breakfast

**Session I - Growth and the Growth/Development Transition**
**Chair: Richard Firtel**



10:10 - 10:30 **Break**

**Session II - Early Development**
**Chair: Yasuo Maeda**



**Session III - cAMP Signalling I**
**Chair: Robert Dottin**

**Session IV - The Dicty Genome**
**Chair: Rex Chisholm**

19:00 - 20:30 **Dinner**

20:30 - 22:00 **Poster Session I**

## Tuesday August 17, 2004

7:00 - 8:30 **Breakfast**

**Session V- cAMP Signalling II**
**Chair: William Loomis**



10:10-10:30 **Break**

**Session VI - cAMP Signalling III**
**Chair: Janet Smith**



**Session VII – Evolution**
**Chair: Edward Cox**

16:30 - 16:50 **Cheaters that don't prosper: pleiotropy as a mechanism to stabilise cooperation.** **39**
Kevin Foster, Gad Shaulsky, Joan Strassmann, David Queller, and Chris Thompson

16:50 - 17:10 **Break**

**Session VIII - Late Development I**
**Chair: Catherine Pears**

17:10 - 17:30 **Filamin regulated f-actin assembly controls phototaxis in *Dictyostelium*.** **40**
Nandkumar Khaire and Angelika A. Noegel

17:30 - 17:50 **A retinoblastoma-like gene in *Dictyostelium*.** **41**
A. Ceccarelli, G. Dollman, K. Doquang, R. Pedrola, A. Tsang, and H.K. MacWilliams

17:50 - 18:10 **Analysis of actin cytoskeleton dynamics during cell movement in slugs.** **42**
Dirk Dormann and Kees Weijer

18:10 - 18:30 **The Disintegrin domain protein, ampa, has effects on cell-cell adhesion, cytoskeletal organization and motility.** **43**
C. Petty, H.N. Ho, T. R. Varney, and D.D. Blumberg

19:00 - 20:30 **Dinner**

## Wednesday, August 18, 2004

7:00 - 8:30 **Breakfast**

**Session IX - Late Development II**
**Chair: Charles Saxe**

8:30 - 8:50 **The secreted peptide SDF-2 modulates phosphorelayation from DhkA to control terminal differentiation.** **44**
Christophe Anjard and William F. Loomis

**Session X - Cytoskeleton and Cell Movement**
**Chair: Chi-Hung Siu**

## Thursday August 19, 2004



**Session XI - Intracellular Pathways**
**Chair: David Ratner**

10:10 - 10:30 **Break**

**Session XII - Models for Human Disease**
**Chair: Joan Strassman**



12:10 - 15:00 **Lunch**

**Session XIII - New Approaches I**
**Chair: Robert Kay**

## Friday August 20, 2004

| | |
|---|---|
| 8:30 - 10:30 | **Breakfast** |
| 10:30 | **Departure** |

## Poster Sessions

**POSTER SESSION I**
**MONDAY AUGUST 16TH, 2004**

**P1** A pharmacogenetics approach to defining the molecular mechanism of valproic acid in bipolar affective disorder treatment.
K. Adley and R.S.B. Williams

**P2** Establishment of zyg1 overexpression under the control of V18 promotor in *Dictyostelium* mucoroides.
A.Amagai and H. K. MacWilliams

**P3** A functional proteomics approach to dissecting the DIF-1 signalling pathway: DIF-1 rapidly modulates the tyrosine phosphorylation of several cellular proteins.
Tsuyoshi Araki and Jeffrey Williams

**P4** Investigating transcriptional responses to DIFs -1, -2 and -3.
G. Bloomfield, J. Skelton, T. Saito, N. Nikolaidou-Katsaridou, B. Barrell, R. Kay, and A. Ivens

**P5** Cloning putative palmitoyltransferases in *Dictyostelium discoideum.* Bethany Bodwell and Robert E. Gundersen

**P6** Altered stalk length and loss of tip dominance in *Dictyostelium* cell lines with RNAi-mediated reduction of calcineurin B expression.
Katrina Boeckeler, Barbara Weissenmayer, and Rupert Mutzel

**P7** Assigning function to genes using microarray profiling of mutant strains.
Ezgi O. Booth and Gad Shaulsky

**P8** Differential effects of Nramp1 expression on phagocytosis and resistance to infection by pathogenic bacteria.
A. Balest, B. Peracino, C. Skriwan, M. Steinert, and S. Bozzaro

**P9** Looking for PI3K independent pathways regulating actin polymerization in chemotaxis.
L. Chen, M. Iijima, Y. Huang, C. Janetopoulos, and P.N. Devreotes

**P10** A phospholipase D negatively regulates quorum sensing in *Dictyostelium discoideum* development.
Yi Chen, Vanessa Rodrick, Yi Yan, and Derrick Brazill

**P11** Visualisation of the *Dictyostelium* exocyst complex in moving amoebae using fluorescently labelled proteins.
Margaret Clotworthy and David Traynor

**P12** Study of *Dictyostelium discoideum* Nramp homologs.
L. Cortes, L. Wilson, K. Doquang, A. Tsang, and M. Cellier

**P13** Disruption of the NCS-1/Frequenin-related gene (ncsA) in *Dictyostelium discoideum* accelerates development.
B. Coukell, A. Cameron, S. Perusini

**P14** Copines in *Dictyostelium.*
Damer, C.K., Bayeva, M., Hahn, E.S., Naliboff, L., Rivera, J., and Socec, C.I.

**P15** *Dictyostelium discoideum* paxB- Show an Aberrant Phenotype in Fruiting Body Formation.
M. Berenice Duran and Derrick Brazill

**P16** A model of cell localizaton in the migrating slug of *Dictyostelium discoideum*: The roles of differential sensitivity to cAMP chemotaxis and of differential sensitivity to suppression of such chemotaxis by ammonia.
I.N. Feit, J.S. Pawlikowski and B.S. Davis

**P17** A novel disintegrin domain protein functions extracellularly to regulate early cell type specification in *Dictyostelium discoideum.*
H.N.Ho, B.L. Ford, and D.D. Blumberg

**P18** Up-regulation of rnrB expression in response to DNA damage is important for survival.
Pascale Gaudet, Harry MacWilliams, and Adrian Tsang

**P19** A mathematical model of cAMP-induced phosphatidylinositol metabolism during chemotaxis.
J.S. Gruver, P.S. Crooke, and C.Y. Chung

**P20** The role of myosin II phosphorylation in motility and chemotaxis.
P.J. Heid, K. J. Daniels, D. Wessels, D. P. Gibson, H. Zhang, E. Voss, T. T. Egelhoff and D. R. Soll

**P21** Analysis of *Legionella pneumophila* pathogenesis in *Dictyostelium discoideum.*
D.W. Hilbert, R.J. Fitzhenry, Otto G.P, Wu M.Y., H.A. Shuman, and R.H. Kessin

**P22** Nuclear fusion and microtubule organization in the early stages of sexual development in *Dictyostelium discoideum.*
K.Ishida and H.Urushihara

**P23** Genetic analysis of differentiation mutants in *Dictyostelium discoideum.*
Mariko Katoh, Adam Kuspa, and Gad Shaulsky

**P24** Determination of the elastic properties of *Dictyostelium discoideum* cells using the atomic force microscope.
B. J. Haupt, R. Spanhoff, M. Osbourn, S. de Keijzer, E. Snaar-Jagalska, T.Schmidt

**P25** Novel mode of cell growth regulation by Tuberous sclerosis protein 2 (TSC2); the role of *Dictyostelium* TSC2 and Rheb in phagocytosis.
D. Rosel and A.R. Kimmel

**P26** A Rab21/LIM-only complex regulates phagocytosis by repressing the action of an associated CH-LIM protein.
T. Khurana and A. R. Kimmel

**P27** Analysis of the spore matrix of the social amoebae, *Dictyostelium discoideum.*
K.Z.Kirmani, S.Kiev, V.Chow, M.Askew, E.K.M Squires, L.Sovran, M.Sameshima, and
D.A.Cotter.

**P28** An analysis of upstream sequences of *Dictyostelium discoideum* using a distributed computer system.
N.Kobayashi, M.Marin, T.Morio, Y.Tanaka, and H.Urushihara

**P29** Vesicle trafficking is important for the proper cellular distribution and efficient activation of adenylyl cyclase in chemotaxing cells.
P.W. Kriebel, V.A. Barr, and C.A. Parent

**P30** Exploiting new terrain: an advantage to sociality in the slime mould *Dictyostelium discoideum.*
J.J.Kuzdzal, K.R.Foster, D.C.Queller, and J.E.Strassmann

**POSTER SESSION II**
**WEDNESDAY, AUGUST 18TH, 2004**

**P31** Using *Dictyostelium discoideum* to understand how different functions of the Adenomatous Polyposis tumour suppressor protein are co-ordinated.
H.-H. S. Lin, I. P. Newton, and I. S. Näthke

**P32** Fascinated behaviors of *Dictyostelium* homologues of TRAP1 and GRP94 during development, revealed by immuno-electron microscopy.
Hitomi Yamaguchi, Tsuyoshi Morita, Aiko Amagai, and Yasuo Maeda

**P33** A new late step in spore coat biogenesis in *Dictyostelium.*
Talibah Metcalf, Hanke van der Wel, Yunyan Zhang and Christopher M. West

**P34** Reverse genetic analyses of gamete-enriched genes revealed a novel regulator of cAMP signaling pathway in *Dictyostelium discoideum.*
T. Muramoto and H. Urushihara

**P35** Role of the WH1 domain of WASP in the regulation of F-actin polymerization and chemotaxis.
S.A. Myers, J. Moon, and C.Y. Chung

**P36** FbiA, a potential target of ubiquitin-mediated degradation, regulates cell-type proportioning in *Dictyostelium discoideum*.
C. J. Mason, E. E. Staley, J. T. Maxwell, M. J. Niederst, B. L. Lubawy, D. S. McGill, K. A. McFeaters, C. Moré, J. A. Christman, T. Abe, and M. K. Nelson

**P37** Cloning and Characterization of rnoA, a Gene Coding for a Putative Guanine Nucleotide Exchange Factor in *Dictyostelium.*
Liem Nguyen and Derrick Brazill

**P38** A PKB/AKT related kinase is essential for both slug migration and ecmB expression in core region of slug.
H. Ochiai, S. Watanabe, K. Naito, T. Saito, K. Takeda, A. Kato

**P39** *Dictyostelium discoideum* disrupted in sepiapterin reductase provides a model system to investigate cellular functions of tetrahydrobiopterin.
Yong Kee Choi, Jeong Soon Park, Jin Seon Kong, and Young Shik Park

**P40** Aark: a novel kinase required for PsA expression.
W.J. Ryves, J. Reynolds, and A. J. Harwood

**P41** Looking for social genes in the social mold *Dictyostelium discoideum.*
L. Santorelli, D. Queller, J. Strassmann, C. Thompson, and G. Shaulsky

**P42** Analyses of srfA-dependent gene promoters.
Patricia Torija, Carmen Valenzuela, Juan-Jesús Vicente, Ricardo Escalante, and Leandro Sastre

**P43** RasG affects early developmental adhesion in *Dictyostelium* through the phosphorylation of DdCAD-1.
D. M. Secko, C. H. Siu, G. B. Spiegelman, and G. Weeks

**P44** Progress of the *Dictyostelium* DNA microarray resource.
J. Skelton, G. Bloomfield, N. Nikolaidou-Katsaridou, B. Barrell, R. Kay, and A. Ivens.

**P45** Importance of myosins, actin dynamics and membrane trafficking in phagocytosis and cell motility.
Y. von Heyden, M. Saha, K. Yoshida, and T. Soldati

**P46** Proteomic analysis of the GSKA-ZAK1 signalling pathway.
L. Strmecki, T. Araki, C. Schilde, D.M. Greene, A.J. Harwood, and J.G. Williams and C. Pears

**P47** Collosin: a new giant protein.
G. Chen, J. Song, R. Sucgang, and A. Kuspa

**P48** Aphidicolin addition just before electroporation increases the transformation efficiency in *Dictyostelium discoideum.*
K.S.K. Uchida, S. Kuhara, and Y. Tanaka

**P49** The two RasGEF containing proteins GbpC and GbpD have opposite effects on cell polarity and chemotaxis in *Dictyostelium discoideum.*
Leonard Bosgraaf, Arjen Waijer, Ruchira, Ton Visser, Deborah Wessels, David Soll, and Peter J.M. van Haastert

**P50** The Phosducin-like protein Phlp1 is essential for Gbeta-gamma dimer formation in *Dictyostelium.*
Jaco C. Knol, Ruchira Engel, Mieke Blaauw, Antonie J.W.G. Visser, and Peter J.M. van Haastert

**P51** Dock180 homologues and their roles in regulation of *Dictyostelium* behaviour.
Jun Wang and Kees Weijer

**P52** How TRE5-A retrotransposons select tRNA genes as integration targets - an update.
Oliver Siol, Theodor Dingermann, and Thomas Winckler

**P53** Phylogenetic analysis of the *Dictyostelium* Ras subfamily.
Michelle Wyse, Matthew Rogers, Patrick Keeling, George Spiegelman, and Gerry Weeks

**P54** Transcriptional transitions in *Dictyostelium* spore germination.
Q.Xu, M.Ibarra, D.Mahadeo, C.Shaw, E.Huang, A.Kuspa, D.Cotter, and G.Shaulsky

**P55** GOAT: A Gene Ontology Analysis Tool Package.
Q.Xu, C.Shaw, N.Whitehouse, and G.Shaulsky

**P56** Identify novel functions of DNA Mismatch Repair (MMR) protein using *D. discoideum* as a model system.
X. S. Xu, Y. Zhang, and G. Wang

**P57** Dynamics of chemotactic gradient sensing in *Dictyostelium* revealed by quantitative live cell imaging.
Xuehua Xu, Martin Meier-Schellersheim, Lauren E. Nelson, and Tian Jin

# Oral Presentations

Abstract 1

# Interaction between GSK-3 and the *Dictyostelium* mitotic spindle.

A. J. Harwood [1], H. Williams [1], and A. Mueller-Taubenberger [2]

[1]MRC-Laboratory for Molecular Cell Biology & Dept of Biology, University College London London, WC1E 6BT, UK; [2] Max-Planck-Institute for Biochemistry, Martinsried, Germany

The protein kinase GSK-3 is a well-characterized component of a number of important signaling pathways, including those mediated by insulin and Wnt in animals. It is also directly involved in a number of important cellular regulatory events, including interactions with the cytoskeleton [1]. In cultured mammalian cells, GSK-3 associates with the mitotic spindle and is required for a high fidelity of chromosomal inheritance [2].

We show by use of GFP fusions and live cell imaging that GskA, the Dictyostelium homologue of GSK-3, also associates with centrosomes and the spindle during mitosis. Cells lacking GskA divide more slowly, and have an increased frequency of multinucleate cells when grown in shaking suspension. The mitotic spindles of dividing gskA null cells however have a similar appearance to those of wild type cells. In contrast, expression of a mutant GskA
protein, which possesses a restricted range of substrates, shows a more extreme phenotype in shaking suspension. In this mutant the mitotic spindle fails to disassemble during cytokinesis preventing cell division. These results suggest the GskA plays multiple regulatory roles during cell division to control the behaviour of the mitotic spindle.

1. A.J. Harwood & Braga, V.M.M. (2003) Cdc42 & GSK-3: signals at the crossroads. Nat. Cell Biol. 5, 275-277.

2. Wakefield, J. G., Stephens, D. J. & Tavare, J. M. (2003) A role for glycogen synthase kinase-3 in mitotic spindle
dynamics and chromosome alignment J. Cell Sci. 116, 637-646.

Abstract 2

## Motility patterns of *Dictyostelium* vegetative amoebae: vectorial analysis reveals a chemokinetic component of folate attraction.

Jared L. Rifkin and Robert R. Goldberg

Queens College of CUNY, Flushing, NY 11367-1597
USAMotility patterns of Dictyostelium vegetative amoebae: a vectorial analysis.

Dictyostelium discoideum vegetative amoebae respond chemotactically to folate and pterin but the response appears to be greater towards folate. Both behavioral and kinetic studies indicate that folate and pterin have separate receptors and our numerous observations suggest that the motility reponses to pterin and folate are different in kind. Because chemotactic data are usually reported as velocities, the vector sums of speed and orientation angle, we undertook a vectorial analysis of such movements. NC-4H Dd amoebae were allowed to migrate on agar in both static fields and dynamic gradients of folate and pterin with standard saline as control. Time lapse video images, @10 sec intervals for 1.5 hr, were taken of amoebae moving in steady state conditions and digitized. These records were thresholded, each amoeba was assigned a single-pixel position in each frame, and both distance and angle of displacement were determined for each amoeba in successive frames. Compared with control, the vectorial analyses reveal that pterin stimulates only a contraction in orientation angle but folate stimulates both this angular effect and an increase in speed. Hence, pterin has only a chemotactic effect but folate stimulates both chemotaxis and chemokinesis. This explains the greater velocity of amoebae migrating toward folate vs pterin. Whether the different receptors are tied to separate signal transduction pathways and what other factors may be involved in the different behavioral outcomes is a matter for future studies.

Abstract 3

## A histone methyltransferase homolog required for the normal timing of the growth-differentiation transition

Jonathan R. Chubb, Wendy A. Bickmore, and Robert H. Singer

Department of Anatomy and Structural Biology, Albert Einstein College of Medicine
1300 Morris Park Avenue, Bronx, NY10461

Histone modifying enzymes have enormous potential as regulators of the large-scale changes in gene expression occurring during differentiation, as they act over much of the genome. It is presently unclear how different combinations of histone modification coordinate regimes of transcription during differentiation. Dictyostelium in an ideal organism for studying the impact of different histone modifications on programmes of gene expression, by virtue of its relative developmental simplicity, genetic amenability and the full complement of higher eukaryotic chromatin proteins it appears to encode. We have studied the function of SET1, a histone methyltransferase homolog in *Dictyostelium*. SET1 mutant cells lack mono, di and tri-methylation of the lysine 4 residue of histone H3. The different methylation states mark different developmental phases of the organism. Depletion of SET1 gives rise to cells displaying unusually rapid development, characterised by precocious aggregration of amoebae into multicellular aggregates after nutrient removal. This phenotype is paralleled by precocious expression of aggregation stage genes and an early decline in expression of growth genes. Early starvation markers are abundantly expressed during growth, and the cell signaling events underlying this timing defect are being determined.

Abstract 4

## A novel Frizzled -like protein in *Dictyostelium discoideum.*

Deenadayalan Bakthavatsalam, Francisco Rivero, and Angelika A. Noegel

Institute of Biochemistry I, Medical Faculty, University of Cologne, Joseph-Stelzmann-Str.52, 50931 Cologne, Germany

Frizzled, a membrane protein with an N-terminal cysteine rich domain (FRI) and a C-terminal transmembrane domain (Frizzled), belongs to the G-protein coupled receptor (GPCR) superfamily. Members of a large family of putative transmembrane receptors homologous to the Frizzled protein in *Drosophila* have been identified from invertebrates to vertebrates and have been shown to regulate symmetry of structure and patterning. Frizzled not only contributes to symmetry, but also to tissue polarity, a process for cells to make the correct choice of which direction to turn (cell polarity cascade), and is also involved in the positional fate specification of individual cells (wingless cascade).
In search for cell polarity specific genes in *Dictyostelium* we identified a Frizzled like protein (DdFrzA) that lacks a FRI domain but has an N-terminal transmembrane domain (Frizzled) and a C-terminal domain homologous to phosphatidylinositol-4-phosphate 5-kinase (PIP5K). The Frizzled transmembane region is known to bind G proteins to induce a Wnt-$Ca^{2+}$ singal transduction pathway and the PIP5K is well understood in catalysing the formation of phosphoinositol-4,5-bisphosphate via the phosphorylation of phosphatidylinositol-4-phosphate, a precursor in the phosphoinositide signaling pathway that has a role in cellular processes such as cell proliferation, death, motility, cytoskeletal regulation, intracellular vesicle trafficking and cell metabolism.

The 95kDa *Dictyostelium* Frizzled protein is unique in domain architecture and is found throughout all stages of the *Dictyostelium* life cycle. The knock out of this gene shows a defect in sensing starvation that prolongs the developmental cycle by 24 hrs. Furthermore, various early and late developmental genes are affected showing that the G protein independent and dependent signalling is altered. Here, we are trying to address the question, what role the DdFrzA has in the development of *Dictyostelium*?

Abstract 5

# The bi-directional fkbp2/dia1 promoter in *Dictyostelium* cells: The transcriptional control in regulation of growth/differentiation transition.

Taira Mayanagi[1], Shigenori Hirose[2], Catherine Pears[3], Aiko Amagai[1], and Yasuo Maeda[1]

[1]Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University; [2]Devision of Cell and Developmental Biology, Department of Biological Sciences, University of California, San Diego; [3]Department of Biochemistry, Oxford University

The development of *Dictyostelium* cells provides us an excellent experimental system for investigating the regulatory mechanisms from growth to differentiation. Besides, a critical checkpoint (PS-point) has been specified as a particular cell cycle position to enter the differentiation phase. We have identified *dia1* as one of genes specifically expressed in response to the initial differentiation from the PS point. The DIA1 protein is presumed to be a GPI-anchored protein, and negatively regulates the progression of early differentiation. The upstream of *dia1* genome shares a transcriptional regulatory region with *fkbp2* gene, which is encoded by near the reverse strand of the *dia1* gene. Although the function of FKBP2 has not been characterized thus far, the FKBP2 protein has a potential peptidiylprolyl cis-trans isomerase domain, which regulates protein folding. In contrast to the *dia1* specifically expressed coupling with differentiation, *fkbp2* dominantly expressed during the growth phase was rapidly decreased in response to starvation. Thus, the transcriptional activities and directions of the *fkbp2/dia1* promoter are switched in response to the growth/differentiation transition. We have analyzed the regulatory promoter region, which is composed of 653 bp with two conserved repeat sequences, referred to as repeat A and repeat B. Deletion analysis showed that 118 bp region just upstream of the *fkbp2* start ATG and repeat B region is involved in that the *fkbp2* expression during the vegetative growth phase. The expression of the *dia1* gene during differentiation phase also requires the *fkbp2* proximal region. Importantly, the repeat A region at the middle was found to repress the *dia1* expression during the growth phase. Electrophoretic mobility shift assay (EMSA) was used to identify factors that interact with the regulatory regions characterized by the deletion assay. As a result, it was found that several trans-factors interact with the regulatory regions, and that the binding states of the proteins are different between the growth phase and the differentiation phase. We are now trying to isolate and identify the *trans*-factors that bind with the regulatory *cis*-elements. The detailed analysis of the switching mechanism of the *fkbp2/dia1* promoter should shed new light on our understanding of transcriptional regulations in the growth/differentiation transition.

Abtract 6

## C-module-binding factor plays an essential role in the initiation of *Dictyostelium* development.

T.Winckler[1], N.Iranfar[2], P.Beck[1], I.Jennes[1], O.Siol[1], U.Baik[1], W.F.Loomis[2], and T.Dingermann[1]

[1] Institut für Pharmazeutische Biologie, Universität Frankfurt (Biozentrum), D-60439 Frankfurt am Main, Germany; [2] Cell and Developmental Biology, Division of Biology, University of California, San Diego, La Jolla, California, 92093, U.S.A.

We recently isolated a sequence-specific DNA-binding protein, CbfA, that interacts in vitro with a regulatory element present at the 3' end of the D. discoideum retrotransposon TRE5-A. CbfA cannot be inactivated by conventional gene disruption. We have previously generated a cbfA mutant strain by introducing an amber translation stop codon into the chromosomal copy of the cbfA gene that was partially supprerssed by an amber suppressor tRNA gene present in the same cell. Here we report on the developmental phenotype of a cbfA mutant that expresses CbfA at <5% of wild-type level. We found that aggregation of the mutant cells is highly compromised and that most cells stay as a monolayer almost indefinitely. CbfA-depleted cells responded properly to prestarvation conditions by expressing discoidin in a cell density-dependent manner. A genome- wide microarray-assisted expression analysis combined with Northern blot analyses revealed a failure of CbfA- depleted cells to properly induce the aggregation-specific adenylyl cyclase ACA and other genes required for cAMP signal relay that is necessary for aggregation and subsequent multicellular development. However, the cbfA mutant aggregated efficiently when mixed with as few as 5% wild-type cells. Moreover, pulsing cbfA mutant cells developing in suspension with nanomolar levels of cAMP resulted in induction of acaA and other early developmental genes. Although the response was less efficient and slower than in wild-type cells, it showed that cells depleted in CbfA are able to initiate development if given exogenous cAMP signals. Ectopic expression of the gene encoding the catalytic subunit of protein kinase A restored multicellular development of the mutant. We conclude that sensing of cell density and starvation are independent of CbfA, whereas CbfA is essential for the pattern of gene expression which establishes the genetic network leading to aggregation and multicellular development of D. discoideum.

Abstract 7

## Inositol phosphate regulated gene expression during *Dictyostelium* aggregation.

J. King, M. Keim, K. McQuillan, R. Williams*, and A. J. Harwood

MRC-LMCB, Department of Biology, UCL, London. UK.

Prolyl oligopeptidase (PO) has major effects on Inositol phosphate (InsP) based signalling during *Dictyostelium* aggregation. Loss of PO increases the basal level of inositol (1,4,5) trisphosphate ($InsP_3$), whereas PO over expression lowers $InsP_3$. Lithium and VPA reduce the intracellular concentration of myo-inositol and therefore lowers the InsP content. Lithium or VPA treatment of *Dictyostelium* cells dramatically reduces aggregation. This effect is reversed by loss or inhibition of PO, and cells become hypersensitive when PO is overexpressed. This argues for a role of InsP signalling during aggregation. We are investigating why lithium causes an aggregation defect and how PO interacts with InsP signalling to reverse this effect.

We have established that lithium reduces the persistence of cells during chemotaxis towards cAMP, making them turn at a higher frequency than wild type. It also suppresses expression of the cAMP pulse induced gene, csA (gp80). Loss of PO increases the persistence of cells, and induces premature expression of csA. We are using 2D-DIAS and microarray analysis to further investigating how PO affects cell behaviour.

We have also discovered that PO activity regulates expression of the inositol synthase (*ino1*) gene. This could explain how PO alters the cellular InsP content. We have found that alteration of PO activity leads to a marked change in the ratio of $InsP_6$ to $InsP_5$. Based on observations in yeast, this could lead to altered *ino1* expression through a InsP mediated modulation of Swi/Snf related chromatin remodelling factors. To test this hypothesis, we are manipulating the activity of two enzymes that control the conversion of $InsP_5$ to $InsP_6$. These are multiple inositol polyphosphate phosphatase (MIPP), which dephosphorylates higher order InsPs, and inositol polyphosphate multikinase (IMPK), which converts $InsP_4$ to $InsP_6$. We will report the effects of knockout or overexpression of these enzymes. Finally, we are investigating the effects of chromatin remodelling factors on cell behaviour, gene expression and lithium sensitivity.

Abstract 8

## Elucidating the *Dictyostelium* counting factor pathway.

R. Ammann[1], D. Brock[1], M. Coldiron[2], J. Dallon[3], B. Deery[1], K. Ehrenman[1], T. Gao[1], D. Hatton[1], W. Jang[1], D. Knecht[4], Y. Shamoo[2], Y. Tang[2], and R. Gomer[1]

[1]Howard Hughes Medical Institute; [2]Rice University, USA; [3]Department of Mathematics, Brigham Young University, USA; [4]Department of Molecular and Cellular Biology, University of Connecticut, USA

Despite impressive recent advances, much remains to be understood about how tissue size is regulated. *Dictyostelium* cells form relatively evenly sized groups during development, with our laboratory strains forming groups of about $2x10^4$ cells. A secreted 450 kDa protein complex called counting factor (CF) regulates group size by inducing stream breakup when there are too many cells in a stream. Stream breakup is induced by repressing cell-cell adhesion and myosin polymerization, and increasing actin polymerization and cell motility. Countin, CF45-1, and CF50 are components of CF, and recombinant versions of all three proteins induce the formation of small fruiting bodies. Under conditions where there are very low levels of other CF components, countin and CF50 bind to cells, whereas CF45 does not show detectable binding. Countin and CF50 require each other's presence for optimal activity, and cross-linking experiments suggest that they bind to different proteins on cell surfaces. Our working hypothesis is that cells have receptors for countin and CF50, and that CF45 does not bind directly to cells but instead potentiates the activity of some other CF component to affect group size.

Motility is regulated in part by Akt/PKB binding to PIP3 on membranes. We found that starving cells in the presence of PI3 kinase inhibitors increases group size, and that CF potentiates cAMP-stimulated PIP3 production, Akt/PKB translocation, and Akt/PKB activity. The CF-mediated increase in PIP3 could be due to either a potentiation of PI3 kinase or a repression of the PTEN phosphatase. CF increases PIP3 in PTEN knockout cells, suggesting that CF can regulate PIP3 levels in the absence of this PTEN. Recent computer simulations of stream formation and breakup suggested that directional changes in cells while they are moving in a stream could lead to local stresses and potentiate stream breakup. We observed that CF does slightly increase the turning angle of cells.

To identify other components of the CF signal transduction pathway, we have isolated second-site suppressors of *smlA*$^-$, a transformant that oversecretes CF and as a result forms small fruiting bodies. We have identified 13 transformants that form large fruiting bodies in a *smlA*$^-$ background and which are also insensitive to exogenous CF. Some of the genes that the REMI construct have integrated into in these transformants encode proteins with similarity to Frizzled/smoothened receptors, a cleft lip and palate-associated transmembrane protein, a heparan sulfate 6-O-transferase, a Toll-interacting protein, an adaptin, a Rab-like GTPase, two kinases, a protein methylase, a tenascin, an ankyrin-repeat-containing protein, and proteins of unknown function. We are currently disrupting the genes encoding these proteins in a wild-type background to begin elucidating their function.

Abstract 9

## NCF60: To be or not to be part of the counting factor complex.

D. Brock and R. Gomer

Howard Hughes Medical Institute and Rice University, USA

*Dictyostelium* cells use a secreted complex of proteins named counting factor (CF) to regulate group size. NCF60 is a secreted *Dictyostelium* protein with a putative ATP/GTP-binding site motif A (P-loop) and similarity to a Salmonella protein of undetermined function. Known CF components such as countin, CF50, and CF45-1 have been previously shown to be part of a ~450 kDa complex by using sieving gel chromatography of conditioned starvation medium. Although isolated from a semi-purified CF pool, NCF60 does not appear to be part of the CF complex as it elutes at ~150 kDa. NCF60 protein is present in vegetative cells and conditioned HL-5 medium, and in lower concentrations in conditioned starvation medium. Other CF components are most abundant in conditioned starvation medium with little present in vegetative cells or conditioned HL-5.

*ncf60*$^-$ cells growing on bacteria or in HL-5 display rapid proliferation compared to wild-type cells. The saturation density of *ncf60*$^-$ cells is double that of wild-type cells in growth curve experiments. Development on filter pads produced large aggregates similar to other CF component mutants, and growth and development on bacteria produced large, grossly aberrant structures with some moderate size fruiting bodies seen in the background.

Overexpression of NCF60 in the *ncf60*$^-$ background (*ncf60*$^-$*/actin15::ncf60*) rescues the *ncf60*$^-$ phenotype by increasing the aggregate number to wild type levels when starved on filter pads. The aberrant structures seen in *ncf60*$^-$ are replaced by fruiting bodies in *ncf60*$^-$*/actin15::ncf60* that are similar in size and number to wild type fruiting bodies. *ncf60*$^-$'s rapid proliferation is changed in *ncf60*$^-$*/actin15::ncf60* to less than half that of wild-type.

In many cell types, Erk2 has been shown to function in a protein kinase cascade that plays an important role in the regulation of cell growth and differentiation. The cAMP-stimulated activation of Erk2 is increased in ncf60- cells compared to wild-type cells and is conversely decreased in null cells overexpressing NCF60. Together our data suggest that NCF60 might function as part of a secreted signal that is not CF (ncf) to delay cell cycle progression through phosphorylation events in a signal transduction pathway.

Abstract 10

## Structure-function analysis of the Ca2+-dependent cell adhesion molecule DdCAD- 1 in *Dictyostelium discoideum.*

C.-H. Siu[1], Z. Lin[2], E.Huang[1], S.Sriskanthadevan[1], E.Wong[1], and D1. Yang[2]

[1] University of Toronto, Toronto, Canada; [2] National University of Singapore, Singapore

Expression of the $Ca^{2+}$-dependent cell adhesion molecule DdCAD-1 coincides with the onset of Dictyostelium development. DdCAD-1 mediates cell-cell adhesion by homophilic binding in a $Ca^{2+}$-dependent manner. DdCAD-1 is unique in that it is synthesized as a soluble cytoplasmic protein without a transmembrane domain. It is transported to the cell surface via the contractile vacuoles. Therefore, DdCAD-1 can function on the cell surface, extracellularly as a soluble protein, and intracellularly in the cytoplasm. Gene-knockout studies suggest that it plays a role in cell-type proportioning and cell sorting, in addition to cell-cell adhesion. Analysis of recombinant proteins containing partial deletions indicates that there are multiple $Ca^{2+}$-binding sites in DdCAD-1. Equilibrium dialysis shows that 4 moles of $Ca^{2+}$ are bound per mole of DdCAD-1. Sequences in the C-terminal two-thirds of the protein appear to bind three $Ca^{2+}$ and are involved in cell binding. To further investigate the mechanisms involved in the various functions of DdCAD-1, the structure of DdCAD-1 has been determined using multidimensional NMR spectroscopy. The NMR data reveal a structure containing two distinct domains. Both domains adopt the b-barrel fold linked by a bent hinge region. The whole protein contains potentially four $Ca^{2+}$-binding acidic pockets. The two halves of the molecule interact through a large loop from the N- terminal domain and constitute one of the four $Ca^{2+}$-binding sites, suggesting that this part of the molecule may have a regulatory function. The organization of the N- terminal domain shows close resemblance with the structure of protein S, which is a $Ca^{2+}$-binding protein associated with the spore coat of *Myxococcus xanthus*. It is, therefore, related to the b/g crystallin family of proteins. The C-terminal half of DdCAD-1 shows modest sequence identity with cadherin and its structure also appears to be related to those of the extracellular domains of classical cadherins.The putative functional domains revealed from the structural information are currently being evaluated by site-directed mutagenesis.

Abstract 11

# The intracellular role of adenylate cyclase in the suppression of lateral pseudopod formation during *Dictyostelium* chemotaxis.

V. Stepanovic [1], D. Wessels [1], W.F. Loomis [2], and D. R. Soll [1]

[1] W.M. Keck Dynamic Image Analysis Facility, Department of Biological Sciences, University of Iowa, Iowa City, IA 52242; [2] Division of Biological Science, University of California, San Diego, La Jolla, California 92039

The adenylate cyclase gene, acaA, is responsible for the production of the extracellular cAMP that constitutes chemotactic waves during aggregation (Pitt et al., 1992). A number of experiments performed with the acaA null mutant suggested that an intracellular cAMP signal did not play a significant role in the behavioral responses of cells to the chemotactic signal.

However, it was recently demonstrated that null mutants of the intracellular phosphodiesterase RegA and the cAMP-regulated protein kinase A regulatory subunit PKA-R were defective in chemotaxis (Wessels et al., 2000,2004). These results argued that intracellular cAMP, presumably synthesized by ACA, must play a fundamental role in chemotaxis. To investigate this directly, we used computer-assisted 2D and 3D methods (Soll, 1995; Soll and Voss, 1998) to analyze the basic motile behavior of acaA- mutants in the absence of a cAMP signal as well as their responses to the spatial, temporal and concentration components of the natural cAMP wave (Soll et al., 2003). Our results indicate that acaA plays a fundamental intracellular role in the suppression of lateral pseudopod formation in the front of a natural chemotactic wave, a behavior essential for normal chemotaxis in a natural wave of cAMP (Soll et al., 2003). Suppression of lateral pseudopod formation has previously been demonstrated to be under the regulation of RegA and PKA (Wessels et al., 2000, 2004). Interestingly, we were able to partially rescue some of the defects of acaA- mutants by increasing the concentration range of cAMP in simulated temporal waves, suggesting that ACA plays a role in intracellular amplification of cAMP.

We have now used computer-assisted motion analysis systems and the same set of experimental protocols to quantitate the basic motile behavior, and response to the spatial and temporal components of the wave, in the following Dictyostelium mutants: regA-, pkaR-, rasC-, mhc-, 3XALA, 3XASP, S13A, cAR1-, acaA-, acaA-/acrA-, myoA-, myoB-, myoA-/myoB-, myoF-, myoA-/myoF-, chc-, sglA. The data provide us with 40 behavioral parameters for each of seven experimental protocols per mutant, or 280 parameters per mutant for quantitative comparison. An emerging model of parallel pathways emanating from different phases of the wave and terminating in individual behaviors that, in sequence, represent the chemotactic response, will be discussed.

Abstract 12

## Cross-talk between two adenylyl cyclases is required for chemotaxis and aggregation in *Dictyostelium.*

B. Pergolizzi and S. Bozzaro

Department of Clinical and Biological Sciences, University of Turin, Ospedale San Luigi, 10043 ORBASSANO (Torino), Italy

Two adenylyl cyclases are expressed during development, ACA and ACB (the product of the *acrA* gene). Both adenylyl cyclases are expressed at pre-aggregative stage, though ACA reaches maximal activity during aggregation, while ACB activity increases thereafter. Null mutants for ACA or ACB fail to develop or form viable spores, respectively. This has led to the suggestion that ACA, but not ACB, play a role for aggregation, despite the fact that both proteins are expressed at pre-aggregative stage.

To study the relative function of both ACA and ACB in development, we have taken advantage of HSB1, a temperature-sensitive mutant for the G protein-dependent ACA regulator PIA (Pergolizzi et al., Dev. Biol. 251, 18-26 (2002)). At temperatures below 18°C, PIA, and thus ACA, are active, whereas at temperatures above 18°C, they are inactive. Consequently, cells aggregate and form fruiting bodies at permissive temperature, but fail to aggregate at 23°C.

We have generated a null mutant for acrA in the HSB1 background, to exploit the temperature-sensitivity of the HSB1 mutant. The rationale was that we could investigate the developmental role of ACA, in the absence of ACB, by shifting cells from the permissive to the non-permissive temperature, and vice-versa, at different developmental stages. Indirectly, the role of ACB during development would also be clarified.

Surprisingly, the new double mutant (HSB101) failed to aggregate also at permissive temperature, suggesting that ACB is somewhat required for aggregation. Constitutive overexpression of wild-type PIA partially rescued the mutant, which was able to form fruiting bodies, but no differentiated, viable spores, resembling the original acrA- mutant (Soderbom et al., Dev . Biol. 126, 5463-5471 (1999)).

Chemotaxis, cell adhesion and gene expression studies in HSB1, HSB101 and mutants expressing wild-type PIA lead us to propose a model whereby ACA and ACB are both required, but with distinct roles, during chemotaxis and aggregation.

Abstract 13

## Two Ras proteins play a role in the early development of *Dictyostelium discoideum*.

H. Kae, P. Bolourani, G.B. Spiegelman, and G. Weeks

Department of Microbiology and Immunology, University of British Columbia, 300-6174 University Boulevard, Vancouver, British Columbia, Canada, V6T 1Z3

*Dictyostelium discoideum* contains an extensive family of Ras proteins that participate in the signal transduction of events leading to the regulation of cell division, motility, the cytoskeleton, and development. Using the Ras Binding Domain of the *Schizosaccharomyces pombe* kinase Byr2 we were able to selectively bind the activated forms of RasC and RasG from cells grown under different conditions. We discovered that levels of both RasC and RasG showed a rapid and transient increase when aggregation competent cells were stimulated with the chemoattractant cAMP. While RasC has been explicitly linked to regulating events during aggregation, as *rasC* null cells are unable to aggregate and produce cAMP under certain conditions, the observation that cAMP stimulated RasG activation led us to investigate the role of RasG during early development by isolating new stable *rasG* null mutants in both Ax2 and JH10 backgrounds. Both of these *rasG* null strains were able to aggregate and develop normally, but the onset of aggregation is delayed by approximately 4 hours relative to their parental wild type cells. In addition, both the ACA activation and PKB phosphorylation that occurs in response to cAMP were reduced in the *rasG* null strains. These results indicate that while RasC and RasG clearly have distinct functions, their signalling pathways appear to converge at some point downstream of activation as both signalling pathways affect ACA activity and PKB phosphorylation. To investigate further the functions of RasC and RasG during early development, we have recently isolated *rasC/rasG* double null cells. The early developmental characteristics of these cells will be described.

Abstract 14

# Activation of soluble guanylyl cyclase at the leading edge during *Dictyostelium* chemotaxis

Douwe M. Veltman[1], Jeroen Roelofs[1], Ruchira Engel[2], Antonie J.W.G. Visser[2], and Peter J.M. Van Haastert[1]

[1]Department of Biochemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands; [2] MicroSpectroscopy Centre, Laboratory of Biochemistry, Wageningen University Dreijenlaan 3, 6703 HA Wageningen, The Netherlands

Dictyostelium contains two guanylyl cyclases, GCA, a twelve-transmembrane enzyme, and sGC, a homolog of mammalian soluble adenylyl cyclase. sGC provides nearly all chemoattractant-stimulated cGMP formation and is essential for efficient chemotaxis towards cAMP. We show that in resting cells the sGC-GFP fusion protein localizes to the cytosol (87%) and cell cortex (13%). With the artificial substrate Mn2+/GTP, sGC activity and protein exhibit a similar distribution between soluble and particulate fraction of cell lysates. However, with the physiological substrate Mg2+/GTP, sGC in the cytosol is nearly inactive, while the particulate enzyme shows similar activity as with Mn2+/GTP. Reconstitution experiments reveal that inactive cytosolic sGC acquires catalytic activity with Mg2+/GTP upon association to the membrane. Stimulation of cells with cAMP results in a 2-fold increase of membrane-localized sGC-GFP, which is accompanied by an increase of the membrane-associated guanylyl cyclase activity. In a cAMP gradient, sGC-GFP localizes to the anterior cell cortex, suggesting that in chemotacting cells, sGC is activated at the leading edge of the cell.

Abstract 15

## The Genome of *Dictyostelium discoideum.*

J. A. Pachebat[1,4*], G. Glöckner[2*], R. Sucgang[3*], L. Eichinger[4*], M.-A. Rajandream[5*], E. C. Cox[6], B. Tunggal[4], K. Szafranski[2], B. A. Konfortov[1], P. Farbrother[4], A. T. Bankier[1], R. Lehmann[2], J. Song[3], N. Bason[5], Q.Xu[12], M. Berriman[5], G.Chen[3], C. Churcher[5], E. Sodergren[12,13], J. Cooper[5], X. Nie[3], A. Cronin[5], L. Hemphill[3], R. Davies[5], P. Davis[5], B. Desany[3], I. Goodhead[5], N. Hall[5], N. van Driessche[12], N. Hamlin[5], D. Harper[5], D. Muzny[13], H. Hauser[5], D. Johnson[5], R. Lindsay[3], A. Kehornou[5], A. Knights[5], M. Quiles[13], C. Buchrieser[9], K. Mungall[5], C. Price[5], H. Loulseged[13], M. Quail[5], E. Rabbinowitsch[5], J. Ma[13], M. Sanders[5], J. Hernandez[13], D. Saunders[5], T. Morio[7], T. Saito[8], Y. Tanaka[7], D. Steffen[13], S. Sharp[5], G. Weinstock[12,13], M. Simmonds[5], G. Shaulsky[12], A. Tivey[5], W. F. Loomis[3], B. White[5], R. Gibbs[12,13], D. Walker[5], J. Woodward[5], T. Winckler[2], M. Schleicher[14], A. Rosenthal[2], F. Rivero[4], R. L. Chisholm[10], R. R. Kay[1], J. Williams[11], A. Kuspa[3,12‡], M. Platzer[2‡], A. A. Noegel[4‡], P. H. Dear[1‡], and B. Barrell[5‡]
[*]*These authors contributed equally to this work.* [‡]*co-senior authors*

[1]Laboratory of Molecular Biology, MRC Centre, Cambridge CB2 2QH, UK
[2]IMB Jena, Genome Analysis, Beutenbergstr. 11, D-07745 Jena, Germany; [3]Dept. of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030, USA; [4]Center for Biochemistry, University of Cologne, Joseph-Stelzmann-Str. 52, D-50931 Köln,Germany; [5]The Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire CB10 1SA,UK; [6]Department of Molecular Biology, Princeton University, Princeton, NJ08544-1003, USA; [7] Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan; [8] Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810 Japan; [9] Laboratoire de Genomique des Microorganismes Pathogenes, Institut Pasteur, 28 rue du Dr. Roux, 75724 Paris Cedex 15, France; [10] dictyBase, Center for Genetic Medicine, Northwestern University, 303 E Chicago Ave,Chicago, IL 60611, USA; [11] School of Biological Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK; [12]Dept. of Human and Molecular Genetics, Baylor College of Medicine, Houston, TX 77030, USA; [13]Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030, USA; [14]Adolf-Butenandt-Institute/Cell Biology, Ludwig-Maximilians-University, 80336 Munich, Germany

The 34 Mb *Dictyostelium discoideum* genome was assembled and finished to a high level of accuracy, and we will be presenting the notable features of this important model organism. With a composition of 77% A+T, the genome could not be cloned in BACs as large insert clones, and was assembled using a chromosome by chromosome strategy, anchored through the production of a high-resolution STS map by the *in vitro* HAPPY map technique, and through the use of tiled congruent YACs. The six chromosomes are gene-dense, with a gene estimated per 2.5 kb, comprising about 13,498 genes genome wide. Complex repetitive elements and retrotransposons make up about 10% of the genome; one type, DIRS, is found at one end of each chromosome in clusters that may mark the position of the centromeres. The ribosomal RNA genes are encoded on

extrachromosomal palindromic elements making up 20% of the nuclear DNA, but we also find copies embedded in the genome, indicating a more extensive interaction between these elements and the chromosomes. The genome sequence provides evidence that the chromosomes and the rRNA elements share a common telomere maintenance mechanism. A notable feature of the genome is a known 700 kb inverted duplication on Chromosome 2; we will discuss possible mechanisms by which this structure arose. The protein coding potential of the genome was evaluated through a combination of outputs from three different gene prediction programs and ongoing manual curation. At least 82% of the time, predicted gene models match curated genes perfectly; 89% of expressed sequence tag sequences map to gene models. We will discuss available online tools for accessing and taking advantage of the genome data.

Abstract 16

# Sequence analysis by the cDNA Project identified 60% of the gene repertoire and revealed interesting transcriptional properties in *Dictyostelium discoideum.*

H. Urushihara[1], T. Morio [1], H. Kuwayama [1], E. Koriki[1], N. Kobayashi[1], S. Obara[1], K. Uchida[1], M. Yoshida[1], M. Maeda[2], Y. Tanaka[1], and the *Dictyostelium* cDNA Project in Japan

[1]University of Tsukuba, Japan; [2]Osaka University, Japan

Since 1996, the cDNA Project in Japan continued efforts to determine the gene repertoire in *Dictyostelium discoideum* AX4. On closing the Project, we here summarize the analysis of sequences themselves. The cDNA libraries cover vegetative, aggregating, migrating and culminating stages, as well as gamete phase in the sexual development. They include conventional oligo-d(T) primed directional libraries with or without size-fractionation, full-length libraries generated by the oligo-capping method, and an SSH-mediated subtraction library. Nucleotide sequences were routinely determined from either end of the clones, but we also tried to fill the internal sequence gaps at the final stage of sequencing. Thus, our collection of sequence data is altogether 157,000 reads of 90,000 clones from fourteen cDNA libraries. The above sequences were assembled by the PHRAP program to yield 11,210 independent contigs, which were further clustered into 8,333 by considering read pairs. The number of independent genes corresponds to the sum of ordinary- and gapped-contigs and is 6,708, which is nearly 60% of the expected gene number in D. discoideum. The unidentified 40% genes are considered to represent extremely low-copy mRNAs or those expressed in unexamined cellular physiological status like under stressed conditions. There being no completion is one of the difficulties in the cDNA sequencing. Although we masked frequently appeared short repeat and apparently cohesive dutA sequences, some of the contigs showed abnormal properties (e.g. unacceptably large in size), and seemed to have been over-assembled. Therefore, qualities of the entire contigs were tested by size, clone number, homology, read alignment and so on, and we split and re-assembled them where necessary. During this process, interesting cases of anti-sense transcription and stage-dependent alternative initiation or termination were noted. Although careful examination of the analysis logs is necessary before concluding that those reflect physiological events, some more examples will be discovered. Besides the prominent molecular biological usage of individual or whole set of clones, combination of cDNA contigs and chromosome sequences will give us much information on genetic system in *D. discoideum.*

Abstract 17

## Analysis of the proteome of *Dictyostelium discoideum.*

L. Eichinger[1]*, M-A. Rajandream[2]*, G. Glöckner[3]*, R. Sucgang[4]*, M. Berriman[2], P. Fey[5], K. Pilcher[5], R. Olsen [6], K. Szafranski [3], P. Gaudet [5], S. Kummerfeld [7], M. Madera[7], N. Hall[2], C. Anjard[6], F. Rivero[1], R. Rost [8], S. Haydock[9], J. Pachebat [1,7], R. Lehmann[3], E. Just[5], M. Lu [4], J. Ma [4], B. Tunggal[1], M. Felder [3], H. Urushihara [10] Y. Kohara [11], S. Sugano [12] A. Rosenthal[3], M. Schleicher[8], G. Shaulsky [13], R. L. Chisholm[5], Weinstock[13,14], P. Dear[6], R. Gibbs[13,14], B. Barrell[2], M. Platzer[3], W. F. Loomis[6], R. R. Kay[7ý], J. Williams[15ý], A. Noegel[1ý], and A. Kuspa[4,13ý§]

[1]Center for Biochemistry University of Cologne, Joseph-Stelzmann-Str. 52, 50931 Köln, Germany; [2]The Sanger Centre, Hinxton, Cambridgeshire CB10 1SA, UK; [3]IMB Jena, Genome Analysis, Beutenbergstr. 11, 07745 Jena, Germany; [4]Dept. of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030, USA; [5]dictyBase, Center for Genetic Medicine, Northwestern University, 303 E Chicago Ave, Chicago, IL 60611, USA; [6]Section of Cell and Developmental Biology, Division of Biology, University of California, San Diego, La Jolla, CA 92093, USA; [7]Laboratory of Molecular Biology, MRC Centre, Cambridge CB2 2QH, UK; [8]Adolf-Butenandt-Institute/Cell Biology, Ludwig-Maximilian-University, 80336 Munich, Germany; [9]Biochemistry Department, University of Cambridge,Cambridge CB2 1QW, UK; [10]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan; [11]Centre for Genetic Resource Information, National Institute of Genetics, Mishima, Shizuoka 411-8540, Japan; [12]Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Minato, Tokyo 108-8639, Japan;[13]Dept. of Human and Molecular Genetics, Baylor College of Medicine, Houston, TX 77030, USA; [14]Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030, USA; [15]School of Biological Sciences, University of Dundee, Dow Street, Dundee DD1 5EH, UK.
*These authors contributed equally to this work. ý, co-senior authors.

We will report on our initial analysis of the predicted proteome of *Dictyostelium*, the first member of the Amoebozoa to be sequenced. As expected from Dictyostelium's reliance on cell motility and cell-cell communication, the actin cytoskeleton is complex, as is the array of G-protein coupled receptors and histidine kinase receptors. A large repertoire of protein kinases is present and although receptor tyrosine kinases appear to be absent, a diverse family of SH2-domain signaling proteins are clearly present. A number of other predicted cell signaling and adhesion proteins have structures similar to those of metazoa, but the paucity of recognizable transcription factors suggests that novel transcriptional regulators are yet to be identified. Enzymes for synthesizing, modifying and breaking down cellulose are present, as expected from cellulose's structural roles in development. About 3% of the genome is devoted to ABC transporters and polyketide synthases, suggesting the production and transport of dozens of small molecules for defense and signaling. We also observed genes that were likely acquired by horizontal transfer from bacteria, such as those encoding thymidylate synthase and polyphosphate kinase. A number of proteins show a high degree of similarity to confirmed human disease genes

that are absent from the yeasts. Thus, fundamental insights into cellular aspects of human diseases may come from studies in *Dictyostelium*. Comparison of the *Dictyostelium* proteome with representative plants, animals and fungi suggests that the last common ancestor of Eukaryotes had a greater repertoire of proteins than previously thought. The complete proteome of Dictyostelium provides a new perspective for studying its cell and developmental biology and should also lead to new insights into the basic features of eukaryotic cells.

Abstract 18

## Localized Ras signaling at the leading edge regulates PI3K, cell polarity, and directional cell movement.

Atsuo T. Sasaki, Cheryl Chun, Kosuke Takeda, and Richard A. Firtel

Section of Cell and Developmental Biology, Division of Biological Sciences, and Center for Molecular Genetics

During chemotaxis, the chemoattractant receptors and heterotrimeric G-protein subunits are distributed and activated almost uniformly along the cell membrane, whereas PI(3,4,5)P3, the product of PI3K, is accumulated locally at the leading edge. We have reported that Dictyostelium PI3K transiently translocates to the plasma membrane in response to chemoattractant stimulation and to the leading edge in chemotaxing cells, whereas PTEN, work also done in the Devreotes lab, exhibits a reciprocal pattern of localization. These regulatory events, at least in part, elicit the asymmetry in signaling that leads to directional sensing. However, the events of spatial regulators upstream from PI3K are unknown. Here we show that Ras is rapidly and transiently activated in response to chemoattractant stimulation and regulates PI3K activity. Ras activation occurs at the leading edge of chemotaxing cells. We show that directional both Ras activation and PIP3 accumulation occur in the absence of PTEN and local PI3K accumulation. These and other studies suggest that localization of PI3K and PTEN modulate the PIP3 gradient rather than create it. Furthermore, inhibition of Ras results in severe defects in directional movement and cell polarization. Our findings provide the evidence that locally PIP3 production is cooperatively regulated by locally Ras activation and PI3K localization and that Ras is an upstream component of the cell's compass.

Abstract 19

## Rac regulation of chemotaxis and morphogenesis in *Dictyostelium.*

Kyung Chan Park[1], Francisco Rivero[2], Ruedi Meili[1], Susan Lee[1], Fabio Apone[1], and Rick Firtel[1]

[1]Section of Cell and Developmental Biology,Division of Biological Sciences, Center for Molecular Genetics, University of California, San Diego, 9500 Gilman Drive La Jolla, CA 92093-0634; [2] Center for Biochemistry, Medical Faculty, University of Cologne, Joseph-Stelzmann-Str. 52n Cologne, D-50931, Germany

Movement up a chemoattractant gradient requires localized F-actin polymerization at the site of the plasma membrane closest to the chemoattractant source,. a process that is controlled by Rac/Cdc42 GTPases. We identify Dictyostelium RacB as an essential mediator of this process. RacB is activated in response to chemoattractant stimulation, exhibiting biphasic kinetics that parallel F-actin polymerization. racB null cells exhibit strong chemotaxis and morphogenesis defects and a severely reduced chemoattractant-mediated F-actin polymerization and p21-activated protein kinase PAKc activation. RacB activation is controlled, in part, by the PI3K pathway. pi3k1/2 null cells and wild-type cells treated with a PI3K inhibitor exhibit a significant reduction in the second peak of F-actin polymerization that is linked to pseudopod extension, whereas RacB activation in a PTEN hypomorph is elevated. We identify a RacGEF, RacGEF1, which exhibits a specificity for RacB in vitro. In racgef1 null cells, RacB activation is reduced and cells expressing mutant RacGEF1 proteins display chemotaxis and morphogenesis defects.

RacGEF1 localizes to sites of F-actin polymerization. Inhibition of this localization reduces RacB activation, suggesting a feedback loop from RacB via F-actin polymerization to RacGEF1. Our findings provide a critical linkage between chemoattractant stimulation, F-actin polymerization, and chemotaxis in Dictyostelium.

Abstract 20

## Regulation of WASP function during Dictyostelium chemotaxis.

Ji W. Han[1], Scott A. Myers[1], Francisco J. Rivero[2], and Chang Y. Chung[1]

[1]Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN, 37232-6600, USA; [2]Institut für Biochemie I, Medizinische Fakultät, Universität zu Köln, 50931 Köln, Germany.

The actin cytoskeleton controls the overall structure of cells and is highly polarized in chemotaxing cells, with F-actin assembled predominantly in the anterior leading edge and to a lesser degree in the cell's posterior. Wiscott-Aldrich syndrome protein (WASP) has emerged as a central player in controlling actin polymerization. We have investigated spatial-temporal regulation of WASP function in chemotaxing Dictyostelium cells and demonstrated the specific and essential role of WASP in organizing polarized F-actin assembly in chemotaxing cells. GFP-WASP preferentially localizes at the leading edge and uropod of chemotaxing cells and the B domain of WASP is required for the localization of WASP. We demonstrated that WASP localization can be differentially regulated by the interaction of the B domain with $PI(4,5)P_2$ and $PI(3,4,5)P_3$. Further, we provide evidence that WASP localization to the leading edge requires the basic domain, which preferentially interacts with $PI(3,4,5)P_3$ , the product of PI3 Kinase. In resting conditions, WASP family proteins exist in an auto-inhibitory conformation by interacting between VCA and GBD domain that prevents the binding of Arp2/3 complex. In activation of WASP, activated Cdc42 or Rac may bind to the GBD/CRIB motif, which allow Arp2/3 complex to bind to WASP. We initially found four Rac proteins (Rac1b, RacA, RacB, and RacC) are capable of binding the GBD domain of WASP by a yeast two-hybrid screening. We further characterized the interaction of these Rac proteins with the GBD domain of WASP via GST pull-dowm assay and FRET-based assay. Regulation of WASP function by Rac proteins during chemotaxis will be discussed.

Abstract 21

## Spatial-temporal dynamics of G-protein activation and PIP3 in a cell suddenly exposed to a steady cAMP gradient and models of gradient sensing

Xuehua Xu[1], Martin Meier-Schellersheim[2], Lauren E. Nelson[1], and Tian Jin[1]

[1]Laboratory of Immunogenetics; [2]Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, NIH

The central question in chemotaxis is how eukaryotic cells amplify a shallow gradient of extracellular chemoattractant exposure into a steep intracellular response that guides cell migration. Previous studies have identified many proteins that function during chemotaxis in the signal transduction pathway downstream of G-protein coupled receptors, and live cell localization studies of several of these components have also been conducted. Based on these studies, several models have been proposed to explain how cells achieve adaptation to uniform increases in chemoattractant concentration while displaying persistent, spatially polarized responses to gradients. Two key questions remain to be answered: at which step of the signal transduction pathway are slight differences in concentration of a chemattractant across the cell body amplified into sharply localized biochemical responses, and what is the dynamic process of gradient sensing?

We developed new imaging approaches to quantitatively measure temporal and spatial changes of receptor occupancy, G-protein activation by fluorescence resonance energy transfer (FRET) imaging, and PIP3 levels by monitoring the dynamics of PHCrac-GFP translocation in single living cells in response to different chemoattractant fields. Using a new experimental design, we also carried out the experiment of abruptly exposing a naïve cell (one which has not experienced chemoattractant) to a stable chemoattractant gradient and measured the temporal-spatial patterns of G-protein activation and PIP3 accumulation at different sides of the cell. Such an experiment has been previously proposed as a "thought experiment", but the experiment had never been done due to difficulty in precisely creating and monitoring the experimental conditions. By comparing the kinetics of three key signaling steps in different membrane regions of single cells, our results revealed the dynamics of the translation of differences in receptor occupancy between the front and back of a cell into G-protein activation and polarized PHCrac-GFP localization. Surprisingly, the dynamics of PHCrac-GFP accumulation in the front of the cell displayed a biphasic temporal pattern, which neither has been predicted nor can easily be explained by current models of gradient sensing. Our new findings lead to a revised model of gradient sensing that accounts for the observed temporal and spatial dynamics of PHCrac-GFP responses and provide the foundation for further quantitative modeling of chemotactic responses.

Abstract 22

## Sensitisation of *Dictyostelium* chemotaxis by PI3-kinase mediated self-organising signalling patches.

Harriët M. Loovers, Marten Postma, Christophe Erneux, Jeroen Roelofs, Joachim Goedhart, Antonie J.W.G. Visser, and Peter J.M. Van Haastert

Department of Biochemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, the Netherlands

The leading edge of *Dictyostelium* cells in chemoattractant gradients can be visualised using GFP tagged to the PH domain of CRAC, which presumable binds PI(3,4,5)P3. Uniform cAMP induces persistent translocation PHCrac-GFP from the cytosol to multiple patches, which are similar to the single patch of PHCrac-GFP at the leading edge in a cAMP gradient (Postma et al, 2003, MBC, 14: 5019-5027). The concentration of cAMP determines the probability of patch formation (half-maximal effect at 0.5 nM cAMP), but not size, lifetime or intensity of patches. A pseudopod is extended from the area of the cell with a PHCrac-GFP patch at about 10 s after patch formation. Cells treated with the F-actin inhibitor latrunculin A are round without pseudopodia; uniform cAMP still induces localised patches of PHCrac-GFP. These data indicate that PHCrac-GFP/PI(3,4,5)P3 patches are self-organising structures that regulate pseudopod formation (Postma et al, 2004, JCS, 117: 2925-2935). We investigated the importance of PI(3,4,5)P3 production in Dictyostelium using mutant cells with deletion of two PI3-kinase encoding genes and wild type cells treated with the PI3-kinase inhibitor LY294002, which blocks the PI(3,4,5)P3 response by more than 95%. Both starvation induced aggregation of cells and chemoattractant induced cAMP production strongly depend on PI3-kinase activity. However, cGMP production and actin polymerization are not affected at a wide range of chemoattractant concentrations. LY294002 inhibits PHCrac-GFP patches and inhibits chemotaxis towards nanomolar cAMP, but has no effect at higher cAMP concentrations. We conclude that very low cAMP concentrations induce self-organising PHCrac-GFP patches. These patches serve as a localizer, but not a strong activator, of actin polymerization.

Abstract 23

## The oscillator in periodic production of cAMP and directional motility.

William F. Loomis[1], Mineko Maeda[2], Gad Shaulsky[3], Adam Kuspa[3], Vesna Stepanovic[4], Deborah Wessels[4], and David Soll[4]

[1]Division of Biology, UCSD, La Jolla, CA 92093-0368, USA;[2]Department of Biology, Osaka University, Osaka 560-0043, Japan; [3]Molecular and Human Genetics, Baylor College of Medicine, Houston TX 77030, USA; [4]Department of Biological Sciences, University of Iowa, Iowa City, IA 52242, USA

A circuit connecting CAR1, ACA, ERK2, RegA and PKA has been proposed that can account for periodic production of cAMP as well as cyclic modifications to the cytoskeleton. Strains in which *erkB* is disrupted are unable to relay cAMP and so fail to aggregate. Suppressor studies have shown that ERK2 is not involved in activation of adenyly cyclase, as previously thought, but is involved in inhibiting RegA activity. In the absence of ERK2 the high level of RegA phosphodiesterase hydrolyzes cAMP as soon as it is synthesized. The oscillatory circuit has been modified in light of these results (Fig. 1). In this model ACA activity is either directly or indirectly controlled by PKA which may inhibit ligand bound CAR1 from activating ACA or lead to its inhibition. Support for this link is the observation by Mann et al.(1997) that the period of high ACA activity following stimulation with cAMP is prolonged in *pkaC*$^-$ null cells. This circuit accounts for the production of pulses of cAMP with a 7 minute periodicity. Spontaneous oscillations are predicted to occur when ACA activity accumulates to a threshold level during early development. Oscillations cease after 6 hours of development as the result of turnover of RegA by proteosome degradation following ubiquitinylation in a ERK2-dependent complex containing the cullin CulA and the F-box protein FbxA (Mohanty et al. 2001).

We have found that loss of any one of the components of the oscillator results in aberrant chemotactic motility in a natural wave of cAMP. Suppression of lateral pseudopods in a stable cAMP gradient or during the rising phase of a temporal wave fails to occur in *regA*$^-$, *pkaR*$^-$, and *acaA*$^-$ strains. Preliminary data indicate that it is also aberrant in *carA*$^-$ cells. These results suggest that the oscillator impinges on the cytoskeleton to generate periodic cortical tension. Coupling of the motile response to the production of the chemoattractant would ensure that cells move directionally only during the rising phase of the natural wave and will not back-track after the wave has passed over them and the direction of the gradient is reversed. Although the oscillator can account for the transient response to a pulse of cAMP, it cannot account for adaptation of the cells to steady state levels of cAMP which is likely to involve a CAR1 dependent inhibitory process.

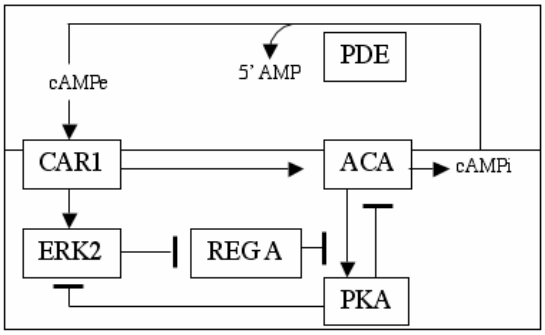



Figure 1. Proposed oscillatory circuit. Arrows indicate activation and bars indicate inhibition. The connections are not necessarily direct and may involve intermediates.

Abstract 24

## A genetic circuit optimizes the number of spiral wave cores.

Satoshi Sawai, Peter A. Thomason, and Edward C. Cox

Princeton University

We will introduce a time-delay embedding method that allows one to rigorously detect cores of spiral waves from dark-field optics movies. By applying this method to data obtained for regA, erkB and pkaR/Rm mutants, we demonstrate that their spatially disordered wave patterns result from appearance of unusually many spiral cores. Numerical simulations of a theoretical model that incorporates the cAMP pulse-induction of excitability successfully describe the observed wild-type and mutant phenotypes. The results suggest that PKA-mediated auto-regulation of cell excitability acts to optimize the number of signaling centers.

Abstract 25

## Functional linkages between CAR1 phosphorylation and Ga9 in control of cAMP oscillations and chemotaxis.

Joseph A. Brzostowski [1], Dale Hereld [2], Carole A. Parent [3], and Alan R. Kimmel [1]

[1] NIDDK/ National Institutes of Health, USA; [2] University of Texas Health Science Center, USA; [3] NCI/ National Institutes of Health, USA

The binding of the chemoattractant cAMP to its cognate G protein -coupled, 7 -TM receptor CAR1, releases the activating G βγ subunits to initiate the production of the second messages PI(3,4,5)P 3, cGMP, and cAMP. The activation of these second messenger pathways is transient (adaptive), but the mechanisms that control adaptation are largely unknown. Cycling between responsive and adaptive states ensures the outward propagation of an oscillating cAMP signal and the inward accretion of individual cells at centers of aggregation. We have recently defined a novel inhibitory pathway that is mediated by the G. protein subunit G α9 and that antagonizes G βγ function. Our data suggest that G α9 couples to CAR1 and functions to attenuate the production of second messenger pathways activated by G βγ. To explore functional linkages between CAR1 and G α9, we disrupted *Gα9* in cells expressing a v ariety of CAR1 mutants that are defective in ligand -mediated phosphorylation and analyzed production of cAMP and cGMP, chemotactic behavior, and actin polymerization. Disruption of the primary phosphorylation site did not alter adaptation events. Interesti ngly, major structural alterations of the carboxyl -terminal tail of CAR1 disrupted all phosphorylation and inhibited adaptation of adenylyl cyclase and proper chemotaxis. Cells expressing non - adapting receptors were also unable to initiate endogenous cAMP oscillations, indicating that periodic cAMP signaling can not be solely dependent on an inherent oscillatory circuit. Finally we show that defects in chemotaxis and cell polarity in CAR1 -mutant cells are suppressed by the disruption of *Gα9*, consistent with our previous studies that implicate Gα9 as negative regulator of chemotatic response.

Abstract 26

## Dominant-negative cAR1 mutants impair pulse-induced gene expression and cause constitutive adaptation of the cAR1-ERK2 pathway.

M. Goswami, M. Zhang, and D. Hereld

The University of Texas Medical School at Houston, U.S.A.

We have previously reported that cAR1 substitution mutants L100H and I104N have dominant-negative effects on the aggregation and subsequent development of *Dictyostelium*. The constitutive phosphorylation of these receptors, their dramatically elevated affinities for cAMP, and the location of the mutations at the cytoplasmic end of the third transmembrane domain suggest that they are constitutively activated. Systematic substitution of I104 revealed a strong correlation between activation and the polarity of the substituting amino acid, indicating an important role for a hydrophobic residue at this position in stabilizing the receptor's inactive conformation.

We hypothesized that these mutant receptors impair development by triggering persistent adaptation of cAR1 pathways. Consistent with this, cAR1-mediated responses known to be subject to adaptation (i.e., chemotaxis, cAMP and cGMP accumulation) were markedly impaired by the L100H and I104N mutants. In addition, the cAMP-pulse-dependent induction of genes including those encoding cAR1, Gα2, and ACA was assessed and was also found to be dramatically impaired, presumably due to adaptation. However, the failure to maximally express the products of these genes, particularly Gα2, complicates the interpretation of the aforementioned cAR1-mediated responses. For this reason, we evaluated the ability of the L100 and I104 substitution mutants to activate ERK2 because this response does not depend strongly on either Gα2 or development.

As is the case for cAR1-stimulated cAMP secretion, we found that adaptation of the cAR1-ERK2 pathway is cAMP-dose-specific and that subsequent challenge with a higher dose resulted in ERK2 reactivation to an extent proportional to the change in receptor occupancy. Similar results were obtained when we examined the folate-induced adaptation of the folate receptor-ERK2 pathway. Futhermore, adaptation to cAMP had little, if any, effect on folate's ability to activate ERK2, indicating that ERK2 adaptation is pathway-specific. Lastly, the collection of I104 substitution mutants were tested for their ability to activate ERK2 in car1/car3$^{-}$ cells. Those mutants which are wildtype-like in terms of their affinity and phosphorylation were also comparable to wild-type cAR1 in their ability to activate ERK2. The mutants exhibiting the characteristics of constitutive activation (i.e., high affinity, constitutive phosphorylation, dominant-negative active with respect to development) were greatly impaired in ERK2 activation (~0-50% of normal).

Taken together, these results suggest that polar substitutions of L100 and I104 result in constitutive activation of cAR1. The ability of these mutant receptors to dominantly block development apparently stems from triggering the adaptation of ERK2 activation, pulse-induced gene expression, and other vital cAR1-mediated responses.

Abstract 27

## Control of cell movement during later development.

Gerti Weijer, Gail Cargill, Andreas Schneider, Dirk Dormann, and Kees Weijer

Wellcome Trust Biocentre, University of Dundee, Dundee DD1 5EH UK

*Dictyostelium* morphogenesis is the result of rearranging differentiating cells. We have provided evidence that cAMP relay continues to control chemotactic cell movement during slug formation and culmination. The cAMP signal is transduced by a combination of 4 different cAMP receptors, but the role of each individual receptor in the control of cell polarisation and the dynamics of the actin cytoskeleton is still unresolved. We have made cAMP receptor mutants in cAR2, cAR3 and cAR4 various combinations to investigate their role in differentiation, signalling and regulation of the cytoskeleton in more detail and will update our findings so far.

We have started to investigate the physical basis underlying cell movement, via the measurement of movement and traction forces produced by migrating cells. We have adapted existing methods using elastic substrates for the measurement of movement and traction forces originally developed for the analysis of fibroblast movement to the analysis of chemotactic movement of individual *Dictyostelium* cells. We can with high spatial and temporal resolution measure the forces produced by chemotactically moving wildtype and mutant cells. We are now in the process of extending these methods to the analysis of slug movement, which will enable us to measure the spatial and temporal distribution forces produced by migrating slugs. This information should then enable us to determine which cells in slugs contribute when and where to its movement in wildtype and mutant slugs.

Abstract 28

## Comparative analyses of *gsk3* and *cAR* homolog between *Polysphondylium pallidum* and *Dictyostelium discoideum.*

Y.Kawabe, T.Morio, and Y.Tanaka

University of Tsukuba, Japan

The fruiting body of *Polysphondylium pallidum* is composed of whorls of branches along the axis of central stalk. Thus, in *P. pallidum*, structure is more complex than that of *Dictyostelium discoideum*. Comparison of molecular mechanism of fruiting body formation between two species may offer important clues to understand morphological diversity and evolution of multicellular organisms.

In *D. discoideum*, cAR3 and cAR4 regulatethe ratio of prespore and prestalk cells through regulation of the GSK3 activity. Previously, we characterized the *tasA* gene that is similar in sequence to *D. discoideum* cAMP receptor gene and functions at fruiting body formation. For further comparative analysis between two species, we isolated a *gsk3* gene and another cAR homolog gene. Firstly, we analyzed the *gsk3* of *P. pallidum*. Analysis of *gsk3* of *P. pallidum* showed that the phenotype of fruiting bodies of disruptant were normal. However, the *gsk3*-null cells showed tendency to differentiate into microcysts that are formed through single cell encystment. When the *gsk3*-null cells developed on a nutrient agar plate with bacteria, almost all cells that consumed bacteria formed microcysts. In shaking culture with bacto-milk as food, after consumption of the foods, about 90 % cells formed microcysts in the disruptant, but only 20 % in wild type cells. These results suggest that GSK3 of *P. pallidum* does not regulate fruiting body formation but regulate microcyst formation. The induction of microcyst formation of *gsk3*-null cells was observed in the conditioned medium of wild type cells as well as the mutant cells, but not in phosphate buffer. Therefore, in *P. pallidum*, the microsyst formation is induced by some small molecule (s) and one of the functions of GSK3 may reduce sensitivity to the molecule (s). Next, to examine existence other cAR homologs, we performed Southern blot analysis probing with *tasA* coding sequence at low stringency and found at least one more homolog. We isolated the gene by degenerate PCR and now further analyses are under process.

Abstract 29

## The evolution of cAMP signaling in the Dictyostelids.

Elisa Alvarez-Curto[1], Daniel E. Rozen[1], Alysson V. Ritchie[1],
Sandra L. Baldauf[2], and Pauline Schaap[1]

[1]School of Life Sciences, University of Dundee, UK; [2]Department of Biology, University of York, UK.

The life cycle of the Dictyostelids can be subdivided into an aggregation phase, where starving amebae collect into mounds, and a sporulation phase, where the mound transforms into a fruiting body, that consists of a stalk supporting a spore mass. Pulses of extracellular cAMP coordinate aggregation of the model amoeba *Dictyostelium discoideum*. cAMP is detected by the high affinity cAMP receptors, cAR1 and cAR3, while a cAMP-stimulated adenylyl cyclase, ACA, and an extracellular phosphodiesterase, PdsA, are essential components of the network that produces cAMP pulses. Typically, these components are expressed at high levels during aggregation and at reduced levels during sporulation.

In contrast to *D. discoideum*, most of the ~ 75 known Dictyostelid species do not use cAMP as chemoattractant. For example, the *Polysphondylium* species use a modified dipeptide, glorin, as attractant, while *D. minutum* and *D. fasciculatum* respectively use a folate analog and an unknown compound to aggregate. These species are very distant relatives of *D. discoideum*, and *D. fasciculatum*, in particular appears to be among the earliest branches off a long line of descent leading to *D. discoideum*.

To trace the evolution of cAMP signaling in the Dictyostelids, we identified cAMP receptor (cAR) genes in *D. minutum* and *D. fasciculatum*. The *D. minutum* cAR gene restored high affinity cAMP binding, pulsatile cAMP signaling and aggregation in a *D. discoideum* cAR null mutant. The *D. minutum* and *D. fasciculatum* cAR genes are predominantly expressed during sporulation and only this process, and not aggregation, was disrupted by inhibition of cAR function. Together, these data indicate that coordination of sporulation is the ancestral role for cAMP signaling in the Dictyostelids.

Abstract 30

## The amazing parasitism of *Dictyostelium caveatum*, revisited.

Clement Nizak[1], John Williams[2], Stanislas Leibler[1], and Richard H. Kessin[3]

[1] Rockefeller University, Center for Physics and Biology, Living Matter Laboratory, 1230 York Avenue New York NY, 10021, USA; [2] Kimmel Cancer Center & Dept. Microbiology and Immunology, Thomas Jefferson University,233 South 10th Street, BLSB; [3] Department of Anatomy and Cell Biology, Columbia University, 630 West 168th St. New York, New York

*Dictyostelium caveatum* is a species that is known to parasitize many other species of their clade. David Waddell and colleagues showed more than 20 years ago that when *D. caveatum* is allowed to develop in mixtures with other species, the fruiting bodies that emerge contain exclusively its spores. Using in vivo time-lapse microscopy, we confirm the prediction that *D. caveatum* cells phagocytose live cells of other species by a process previously described as "cell nibbling". We further show that *D. caveatum* possesses efficient and unusual capacities for rapid cell movement and aggressiveness toward other cell types, including mammalian cells. Details of these processes will be described. Moreover, we have elucidated part of the inhibitory mechanism that ensures total parasitism of other dictyostelids: *D. caveatum* secretes a small hydrophilic compound with a molecular mass of 583 Daltons, which diffuses rapidly in aggregates of developing prey amoebae and prevents their subsequent sporulation, even when *D. caveatum* cells are highly diluted in the aggregates. D. caveatum amoebae then feed on the "paralyzed" prey cells and develop when all cells of the other species are gone. Its spores are the only ones to emerge in fruiting bodies.

Abstract 31

## Cheaters that don't prosper: pleiotropy as a mechanism to stabilise cooperation

Kevin Foster[1], Gad Shaulsky[2], Joan Strassmann[1], David Queller[1] and Chris Thompson[2,3]

[1]Ecology and Evolution, Rice University, Houston, Texas, [2]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, [3]School of Biological Sciences, University of Manchester, Manchester, U.K.

Acts of cooperation like stalk formation in *D. discoideum* are a challenge for evolutionary biologists because of the potential for disruptive cheaters. Since genetically different clones of *D. discoideum* will aggregate together and form chimeric fruiting bodies, this suggests that a cheater that produces fewer stalk cells can gain a selfish advantage and raises the question of how stalk formation is maintained. Around half of prestalk cells in *D. discoideum*, known as the prestalkO cells, are induced to differentiate by the signalling molecule DIF-1. A viable cheating strategy, therefore, should be to ignore DIF-1 and overproduce spore cells in chimeras.

We used a knockout mutant to examine the effects of ignoring DIF-1. The recently discovered *dimA* gene encodes a central component of the DIF response pathway. A mutant with this gene disrupted (*dimA*$^-$) ignores DIF-1 and produces prespore cells in place of prestalkO cells. We found that *dimA*$^-$ cells co-aggregated normally with AX4 and were not lost from aggregations during the migratory slug stage. As predicted, *dimA*$^-$ behaved like a cheater by preferentially sorting to the prespore zone. Surprisingly, however, the cheating behaviour of *dimA*$^-$ in slugs did not result in a competitive advantage. When we examined the spores in the fruiting bodies that form after the slug stage, we found that *dimA*$^-$ cells ultimately lose out with almost half as many present as AX4 cells in the spores. The low number of *dimA*$^-$ spores is not simply because *dimA*$^-$ cells present in the spore head fail to make spores and remain as amoebae: there was no reduction in total spore number in the *dimA*$^-$/AX4 chimera compared to AX4 alone. These results suggested that in chimeras *dimA*$^-$ cells are replaced in the spore population by AX4 prestalk cells late in development. To test this we used a prestalk specific reporter *ecmAO-lacZ* as a marker of transdifferentiation. This revealed a dramatic increase in the number of *lacZ* positive (transdifferentiated) AX4 spores when developed in chimera with *dimA*$^-$ cells.

The *dimA* gene therefore has two contrasting effects. Cells that do not express *dimA* behave like a cheater in chimeric slugs by increasing their representation in the prespore population, presumably because they are able to ignore the signal of prestalk cell induction, DIF-1. However, they do not benefit from defection because of a second phenotype of *dimA*$^-$ cells that causes them to be competitively excluded from the spores late in development. This second pleiotropic effect of *dimA* makes ignoring the DIF-1 signal unprofitable and helps to limit cheating and ensure fair contribution to the stalk.

These results have great implications for studies of evolutionary and social biology. The spread of social genes will be promoted by mechanisms that make their loss costly. Our study provides such a mechanism. Although pleiotropy, is typically viewed as a major constraint on evolution because adaptive change in one trait may be compromised by others that are affected by the same genes; we have shown that pleiotropy can have the opposite effect and benefit one of the most enigmatic of adaptations – cooperation. Pleiotropy therefore provides one way to limit individual rebellion and allow stable cooperation to evolve. Where pleiotropic costs are strong, other means of control like high relatedness and policing may be less necessary.

Abstract 32

## Filamin Regulated F-Actin Assembly Controls Phototaxis in *Dictyostelium.*

Nandkumar Khaire and Angelika A. Noegel

*Center for Biochemistry, Medical Faculty, University of Cologne, 50931 Köln, Germany

Phototaxis plays a crucial role in *Dictyostelium* development. *Dictyostelium* strains lacking the F-actin crosslinking protein ddfilamin have a severe phototaxis defect at the multicellular slug stage. Slugs migrate in an angle towards the light and travel shorter distances. In experiments in which individual domains were tested for their rescue potential only filamin polypeptides that crosslink actin filaments re-establish correct phototaxis. Molecules that bind to but no longer crosslink F-actin rescue the migratory but not the phototactic defect. For correct phototaxis the presence of ddfilamin is only required at the tip of the slug as revealed by expressing the protein under the control of the cell type specific ecmA promoter and by mixing experiments in which chimeric slugs contain 10 percent of wild type cells which are primarily found at the tip. These results indicate that filamin is essential for the generation of a proper tip, which regulates phototactic migration and turning of *Dictyostelium* slugs. Data from microarray analyses of mutant and wild type slugs showed that the transcription of several genes involved in cAMP metabolism was upregulated. This may lead to altered chemotactic responses affecting the generation of cAMP waves necessary for the slug migration.

Abstract 33

## A retinoblastoma-like gene in *Dictyostelium.*

A. Ceccarelli[1], *G. Dollman[2], * K. Doquang[3], *R. Pedrola[1], A. Tsang[3], and H.K. MacWilliams[2]

[1] Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Ospedale S.Luigi, Orbassano, Torino, Italia; [2]Department Biologie II, Ludwig-Maximilians-Universität, München, Deutschland; [3]Biology Department, Concordia University, Montréal, Québec, Canada

*Dictyostelium rblA* shows homologies to the retinoblastoma gene, a well-known regulator of the cell cycle and differentiation in metazoans and higher plants, in the conserved RbA and RbB domains and the N-terminus. Although no ESTs have been described, we have detected a transcript by PCR in a developmental cDNA library and in Northern blots polyA-plus RNA prepared from developing cells; the Northern band is of the expected size and has been seen in late development. A reporter construct, in which sequences upstream of the *rblA* coding region are used to drive an unstable beta-galactosidase, also shows maximal activity in late development. Staining can be seen as early as the tipped aggregate stage; in this and in all subsequent stages the activity is restricted to cells of prespore/spore pathway. Other features also suggest arelationship with spore differentiation. The promoter shows vegetative activity (detectable with a chemoluminescent assay) that appears maximal in late G2, a time at which cells are known to show a preference for the spore pathway. Vegetative activity is decreased severalfold when glucose is omitted from the medium and is barely detectable in the stationary phase; under both of these conditions, cells are known to prefer stalk differentiation.

*rblA* was disrupted by replacing the RbA and RbB domains with a bsr cassette. Two independent KO clones were identified using PCR screening and verified by Southern blots.RblA-minus cells complete development and produce morphologically normal fruiting bodies. When mixed with wild-type cells, however, they show a strong preference for the prestalk/stalk pathway, suggesting a role for *rblA* in stabilizing the prespore state. *rblA*-minus cells also show accelerated early development, a characteristic associated in one study with stalk precursors. RblA-minus cells misregulate rnrB, a gene with a generally similar expression profile (primary expression in prespores; weak vegetative expression with a maximum in late G2). The *rblA* disruptant also misregulates the *rblA* reporter construct, with loss of temporal and cell-type specificity. Together these findings suggest that the retinoblastoma gene product is a transcriptional effector. We have been unable to obtain transformants that overexpress Rbl1, raising the possibility that overexpression leads to cell cycle arrest, as observed in other systems. We propose a model in which cell cycle arrest due to Rbl1 maintains cells in the portion of the cell cycle favoring the spore pathway. Since *rblA* is affected by both the cell cycle and nutritional state, this gene may act in a final common pathway regulating cell-type preference in *Dictyostelium*.

Abstract 34

## Analysis of actin cytoskeleton dynamics during cell movement in slugs.

Dirk Dormann and Kees Weijer

Wellcome Trust Biocentre, University of Dundee, Dundee, DD1 5EH, UK

*Dictyostelium* cells aggregate and form a migratory slug consisting of thousands of individual cells surrounded by an extracellular matrix, the slime sheath. The slug cells move in a co-ordinated manner in response to cAMP signals generated by the slug tip. Although slug movement has been studied in some detail, it remains unclear how cells move inside this multicellular 3-dimensional tissue. In order to address this question we studied the actin cytoskeleton in slug cells expressing the f-actin specific ABD-GFP. Our results show that the actin cytoskeleton around the central part of the cell body remains completely stationary with respect to the substratum while new actin polymerisation occurs at the leading edge and actin disassembly at the rear of the cell. Cells inside the slug and not in contact with the slime sheath can presumably move by gaining traction from the stationary cytoskeleton of their neighbour cells consistent with the observation that cells on the outside of the slug move at a similar speed compared to cells further inside the slug.

We also found novel f-actin containing structures at the leading edge of slug cells. These very dynamic structures appeared as lamellipodia like sheets of actin that were mainly orientated perpendicular to the slug surface. They were not detected during earlier development, during aggregation and in mounds, and in slugs they were mostly present in cells of the slug tip suggesting cell type specific differences. The possible function of these structures as well as the regulation of their formation will be discussed.

Abstract 35

## The Disintegrin Domain Protein, AmpA, has Effects on Cell-Cell Adhesion, Cytoskeletal Organization and Motility.

C. Petty, H.N. Ho, T. R. Varney, and D.D. Blumberg

Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, Md. U.S.A. 21250.

The *Dictyostelium* protein AmpA contains repeated domains homologous to two structurally distinct anticoagulants, the disintegrins found in snake venom and the ornatins found in leach saliva. The 28 KDa AmpA protein contains a hydrophobic leader sequence which iscleaved prior to secretion (2). During development expression of the *ampA* gene is restricted to a highly motile subset of cells, the anterior like cells, that represent less than 10% of the total cells in a mature fruiting body (1). Characterization of *ampA* null and overexpressing strains during development has indicated that the AmpA protein influences both cell-cell and cell-substrate adhesion as well as initial cell type specification (3). The anterior like cells require the AmpA protein in a cell autonomous (autocrine) manner to migrate to the upper cup while the prestalk cells require AmpA protein in a non-cell autonomous (paracrine) manner to migrate to the mound apex where they form the tip that later gives rise to the stalk (3). In the absence of AmpA this migration is delayed and cells remain stuck to the substratum (2&3). AmpA also acts in a paracrine manner to prevent cells at the mound periphery from entering the prespore pathway (3).

Here we show that in growing cells the AmpA protein accumulates as a function of cell density, is located at the cell periphery, and is necessary to prevent cells from clumping at high density in suspension culture. When cells are grown on a bacterial lawn, AmpA protein also influences the size of the plaques formed. We show that the plaque size alteration is not due to differences in phagocytosis of the bacteria, but, to alterations in cell adhesion and cell traction which influence whether cells can migrate out of the plaque into the bacterial lawn in search of food. Analysis of single cells migrating in 2 and 3 dimensional environments indicates that overexpression of AmpA results in a loss of traction that prevents the cells from moving against resistance in a 3D environment but they migrate efficiently in a 2D environment. The overexpressing cells extend more extensive pseudopods and display excessive actin polymerization relative to wild type. By contrast, a loss of AmpA results in defects in pseudopod extension and a reduction in the amount of actin polymerization. The degree of motility of the AmpA null cells depends on the amount of contact between the cell and its environment. The null cells migrate efficiently in a 3D environment but are defective in migrating in a 2D environment, just the opposite of the overexpressing cells. We propose that AmpA functions to signal a cytoskeletal reorganization that results in reduction of cell-cell and cell-substrate adhesion, preventing cell clumping at high density and promoting cell.

1) Casademunt, E., Varney, T. R., Dolman, J., Petty, C., and Blumberg, D. D. (2002). A gene encoding a novel anti-adhesive protein is expressed in growing cells and restricted to anterior-like cells during development of Dictyostelium. Differentiation 70, 23-35.

2) Varney, T. R., Casademunt, E., Ho, H., Petty, C., Dolman, J., and Blumberg, D. D. (2002a). A novel Dictyostelium gene encoding multiple repeats of adhesion inhibitor-like domains has effects on cell-cell and cell-substratum adhesion. Developmental Biology 243, 226-248.

3) Varney, T. R., Ho, H., Petty, C., and Blumberg, D. D. (2002b). A novel disintegrin domain protein affects early cell fate specification and pattern formation in *Dictyostelium*. Development 129, 2381-2389.

Abstract 36

## The secreted peptide SDF-2 modulates phosphorelay from DhkA to control terminal differentiation.

Christophe Anjard and William F. Loomis

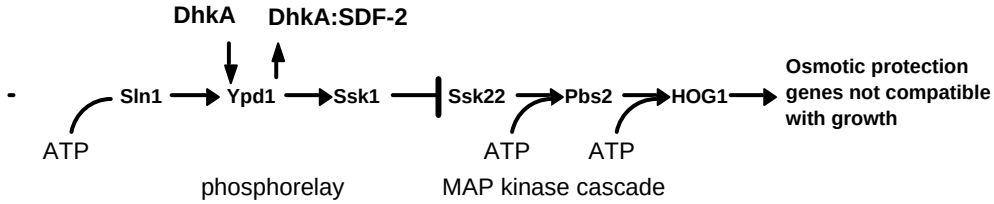
Division of Biology, UCSD, La Jolla, CA 92093-0368, USA.

Terminal differentiation is temporally coordinated among the cell types by the secreted peptide SDF-2 and its surface receptor DhkA which is a "two component" histidine kinase. Genetic and biochemical studies have indicated that ligand binding to DhkA results in reduced activity of the response regulator phosphodiesterase RegA such that cAMP can accumulate in the cells and lead to an increase in PKA activity that triggers encapsulation. The prespore gene, *acbA*, encodes the precursor of SDF-2 which is a small, well conserved, protein also found in yeast, plants and animals. AcbA has been shown to bind saturated and unsaturated $C_{14}$-$C_{22}$ acyl-CoA esters and shuttle them between membranes. We have found that bound palmitoyl-CoA protects recombinant Dictyostelium AbcA from processing by trypsin.

In mammals, various peptides are generated from the full length precursor and are used as intercellular signals both in the periphery and the central nervous system. Neuropeptides including a 36 amino acid form, TTN, are generated by glial cells and interact with the neuronal $GABA_A$ receptor/chloride channel complex where it inhibits binding of benzodiazapines such as Valium. Processing occurs by a trypsin-like cleavage following lysine residues. There is a tryptic peptide of Dictyostelium AcbA which shows 40% amino acid identity to human TTN. In fact, the human peptide is able to induce rapid encapsulation of Dictyostelium spores, but only if added to 100 nM. Chemically synthesized SDF-2 is maximally active at 0.1 pM. Full length recombinant AcbA is inactive in the bio-assay but trypsin treated AcbA is as active as the synthetic peptide. Using antibodies specific to AcbA we have found that it is present in growing cells, decreases >10 fold during aggregation, and accumulates in prespore cells during late development.

We have direct evidence that SDF-2 receptor DhkA can act as a constitutive histidine kinase in the absence of ligand and preliminary evidence that ligand binding shifts the equilibrium such that DhkA acts as a phosphatase. We expressed DhkA in a yeast strains and tested the effects of adding SDF-2. Under conditions where mutant cells lacking their single yeast histidine kinase, Sln1p, cannot grow, expression of DhkA rescued them. However, if 10 nM SDF-2 was added to the medium, the cells were no longer able to grow. These results indicate that ligand binding inhibits the kinase activity of DhkA. We studied a wild-type yeast strain expressing SLN1p as well as DhkA to demonstrate that SDF-2 converts DhkA from a kinase to a phosphatase. When SLN1 is active, the Hog1 MAP kinase cascade is inhibited and there is no measurable HOG1-phosphate. However, upon addition of SDF-2 to cells expressing both SLN1p and DhkA, HOG1-phosphate rapidly accumulates and can be recognized by anti-phosphotyrosine antibodies following electrophoretic separation of immunoprecipitated HOG1p. It appears that DhkA acts a

constitutive histidine kinase in absence of ligand and as a phosphatase upon addition of synthetic SDF-2. Such a "push-pull" mechanism could result in rapid changes in phosphorelay systems.



Abstract 37

## Evidence that TagA works with the SDF-2 precursor AcbA to modulate cell fate specification.

M. Cabral[1,2], G. Chen[1], C. Anjard[3], W. F. Loomis[3], and A. Kuspa[1,2]

[1]Departments of Biochemistry and Molecular Biology; [2]Human and Molecular Genetics, Baylor College of Medicine, Houston, TX 77030, USA; [3]Division of Biology, UCSD, La Jolla, CA 92093, USA.

The tagA, tagB and tagC genes are predicted to encode ABC transporters with a serine protease domain and have been hypothesized to mediate cell signaling by transporting peptides across membranes. TagA appears to play a role in cell fate determination at the onset of development (1). Wild type cells express tagA in all prespore cells and mature spores. However, only about 10 percent of tagA mutant cells activate the tagA promoter during development and these cells are unable to differentiate as spores, but instead form part of the outer basal disc and lower cup of the fruiting body. TagA mutant aggregates elaborate elongated or supernumerary prestalk tips. Cell counting experiments and the behavior of tagA- cells in chimerae indicate that tagA mutants have a cell-autonomous bias toward the prestalk cell fate. Thus, TagA is minimally required for the specification of an initial population of prespore cells. Additional studies suggest that TagA is more generally required for cell fate specification. Over-expression of the TagA protease domain causes a multiple-tip phenotype in wild-type cells. A delay in the normal transcriptional program (determined by cDNA microarrays) occurs in tagA mutants and this delay is coincident with the initial increase in tagA expression wild-type cells between 2 and 6 hours of development, suggesting that TagA is needed in most cells. In support of this, cell-type specific gene expression is perturbed in tagA mutants; the spore coat protein gene cotB is expressed within vacuolated stalk cells and the prestalk/stalk-specific ecmB gene is expressed in spores.

AcbA is a ubiquitous acyl-CoA carrier protein that can be processed into the SDF-2 peptides that coordinate spore encapsulation during culmination (2-4; see also the abstract by Anjard and Loomis). Genetic evidence suggested that the TagA homolog TagC is required for release of SDF-2 during culmination (2). We are exploring the hypothesis that the processing of AcbA by TagA mediates the cell fate regulation inferred from the tagA mutant studies. Both TagA and AcbA are expressed early in development and both become prespore enriched. SDF-2 is not released from tagA mutant cells, although another spore differentiation factor (SDF-1) is released normally, suggesting that AcbA is a substrate of TagA. In support of this idea, an acbA mutation suppresses the TagA protease over-expression phenotype.

Encouraged by the genetic interactions of acbA and tagA, we are carrying out biochemical and cell biological analyses of the relationship between AcbA and TagA. Indirect immuno-fluorescence studies show that anti-TagA and anti-AcbA antibodies stain puncta, presumably vesicles, that are distinct from the endoplasmic reticulum and Golgi apparatus. TagA is predominantly found on endosomal vesicles while AcbA is

predominantly found on endosomes but also occurs in other locations, consistent with the role of AcbA orthologs in lipid metabolism as determined in other organisms. Thus, it is possible that AcbA is accessible for processing by TagA within the cell. Interestingly, there is an inverse relationship between the levels of TagA and AcbA during development. We will describe our current attempts to explore the possible processing of AcbA by TagA.

Good, et al. (2003). Development 130: 2953-65.
Anjard, et al. (1998). Developmental Biology 193: 146-155.

Anjard, et al. (1998). Development 125: 4067-4075.
Wang, et al. (1999). Molecular Cellular Biology 19: 4750-4756.

Abstract 38

# A transcription factor gene *amvA* regulates morphogenetic cell movement during development in *Dictyostelium discoideum.*

T. Morio[1], K.S.K. Uchida[1], H. Kuwayama[1*], S. Obara[1], M. Katoh[1**], R. Yoshino[1], K. Tashiro[2], S. Kuhara[2], Y. Tanaka[1], and H. Urushihara[1]

[1]University of Tsukuba, Japan; [2]Kyushu University, Japan
Present addresses: [*]Osaka University, Japan, and [**]Baylor College of Medicine, U. S. A.

In order to elucidate the molecular network that regulates developmental gene expression, we are carrying out functional analyses of transcription factor genes identified through the cDNA and genome database search. One gene, *amvA* (aberrant morphogenetic cell movement) encodes a putative transcription factor containing a GATA-type zinc finger DNA binding motif. The *amvA* mRNA was developmentally regulated and accumulated during aggregation and thereafter. The *amvA* null cells can grow normally and form fruiting bodies with viable spores, however, some phenotypic aberrations were observed. First, the mutant slugs showed poor phototaxis. Second, there were fruiting bodies whose stalk tube wound their way through the spore mass. These phenotypes suggest that *amvA* is involved in the regulation of morphogenetic cell movement in cell aggregates. By comparison of gene expression profile during development using microarray technology, 90 differentially expressed genes were identified. Although about half of them could not be functionally annotated by similarity, some functionally related gene groups were found. One group includes genes involved in the regulation of cell movement such as PI3K homolog, ponticulin, and actin. These genes are expected to have a close relationship to the mutant phenotype. Another group consists of genes related to calcium signaling such as *cbpF*, *cbpG*, CaM-BP15 homologs and *nxnA*. Moreover, several known genes such as discoidin-1 alpha, ABC transporter *abcG3*, and transcriptional repressor *tupA* were also differentially expressed in *amvA* null cells. Taken together, *amvA* would take a part in the regulatory mechanism of morphogenetic cell movement by the transcriptional control of genes involved in cell movement and some related regulatory activities.

Abstract 39

## A MYB transcription factor, of a class previously found only in plants, mediates the activation of *Dictyostelium* gene expression by DIF-1.

M. Fukuzawa, M. Tsujioka, and J. G. Williams

University of Dundee, MSI/WTB Complex, Dow Street, Dundee DD1 5EH UK

DIF-1 is a low molecular weight signalling molecule that induces pstO cell differentiation. We show that a 22nucleotide region from the promoter of the ecmA, prestalk-specific gene is both necessary and sufficient for DIF-inducible gene expression. The 22-mer was used to purify a novel MYB protein. This protein, DdMyb5, contains a single MYB DNA binding domain of a type thus far found only in one very large family of plant MYB proteins: the"SHAQKY" family. Expression directed by the 22-mer is completely non DIF-inducible in a mybE null strain. Furthermore, expression profiling shows that the majority of DIF-1 induced genes are either partially or wholly dependent upon DdMyb5 activity for their induction. The situation is, however, surprisingly complex and we also present evidence for redundancy in the DIF-1 signalling pathway and for signalling events that are dependent upon the acquisition of multicellularity. The differentiation of the other major prestalk cell sub-type, the pstA cells, is known to be subject to negative regulation that is by mediated by a DIF-1 activated STAT. Thus two DIF-regulated signalling pathways, one employing a STAT protein the other employing a MYB protein, control different aspects of prestalk cell differentiation.

Abstract 40

## Ammonia transporter C is required for tip cell regulation of the transition from slug to culminant in *Dictyostelium discoidium*.

J.H. Kirsten, Y. Xiong, M. Rai, and C.K. Singleton

Department of Biological Sciences, Vanderbilt University, Nashville TN USA

Ammonia is an important signaling molecule involved in the regulation of development in *Dictyostelium*. During aggregation, ammonia gradients are established and the ammonia concentration in the immediate environment or within a particular cell throughout development may vary. This is due to the rate of cellular ammonia production, to its rate of loss by evaporation to the atmosphere or by diffusion into the substratum and to cellular transport by ammonia transporters (AMTs). One ammonia transporter is AmtC, which is expressed in the prespore region and at the tip of the prestalk region during the first finger and slug stages in wildtype (Ax4) cells (1). The *amtC* null strain results in a slugger phenotype and the misregulation of several tip specific genes. The prestalk specific promoters ecmA, ecmB and ecmO are grossly under-expressed at all developmental stages and confined to the anterior-like cells. RT-PCR reveals that the tip specific gene *cudA*, and the cDNAs SLF308 and SLB233 (2) are under-expressed or absent. Preliminary data suggests that adenylyl cyclase A and StatA nuclear localization are mis-regulated at the tip.

The slugger phenotype of the *amtC* null strain is rescued when cudA is expressed with the Actin15 promoter or when the *amtC* null strain is mixed with as few as 10% wildtype Ax4 cells. In mixing experiments, the wildtype cells tend to transiently localize to the tip during early first finger formation. When *amtC* is expressed with the ecmA or pspA promoters, no rescue has been observed. Because the ecmA promoter does not function in the tip cells in *amtC-*, these finding suggest the slugger phenotype is due to a lack of AmtC in tip cells. All of the above mentioned evidence indicates that ammonia transporter C is active in the pathways regulated by adenlyl cyclase A activity in the tip. In addition, we are investigating the possibility of a role for *amtC* in the *dhkC* phospho-relay system. Knocking out *amtC* in a *regA* null background results in the *regA*- phenotype of precocious sporulation rather than the slugger phenotype seen in the wildtype background, suggesting that it does indeed play a role.

1. Follstaedt, S, Kirsten, J and Singleton, C. (2003) *Differentiation,* 71, p. 557-566.

2. Maeda, M, Sakamoto, H, Iranfar, N, Fuller, D, Maruo, T, Ogihara, S, Morio, T., Urushihara, H, Tanaka, Y and Loomis, W. (2003) *Eukarotic Cell,* 2(3), p. 627-637.

Abstract 41

## Motile microtubules in *Dictyostelium*.

D. Brito, A. Khodjakov, I. Tikhonenko, and M. Koonce

Division of Molecular Medicine, Wadsworth Center, Albany, NY 12201-0509

The interphase microtubule cytoskeleton in Dictyostelium is firmly anchored at one end to the centrosome and extends to form a radial cytoplasmic array that is common in most eukaryotic cells. We have examined microtubule motions in Dictyostelium using a combination of GFP-tubulin, a dominant negative dynein mutant, and a laser microbeam. Unlike many mammalian cells, individual microtubules in Dictyostelium undergo rapid lateral bending and sweeping motions. At least part of this motion is driven by a robust amount of organelle movement; in addition there are clear force-producing activities at the cell cortex. Surprisingly, these motions are visible in real time and make quantitation of microtubule end dynamics difficult. Laser ablation of the centrosome results in full-scale depolymerization of the microtubule array, from the newly-exposed minus ends. In dynein mutant cells, the centrosome is motile, and moves through the cytoplasm at rates up to 2.5 um/second (Koonce et al 1999. EMBO J. 18:6786-6792). Irradiation of the trailing, comet-like tail of microtubules suggests that a pushing force contributes significantly to this movement. Our data support the existence of two cortical forces acting on the interphase microtubules: a dynein dependent pulling force and a kinesin-like pushing action. Together, these activities may act to balance each other to support the radial character of the interphase array. We feel that this mechanism is particularly important in highly motile cells such as Dictyostelium, in maintaining the microtubule array as cells change shape and direction.

Abstract 42

## The role of the plasma membrane in the mechanics of cell motility.

D. Traynor, S. Reichelt, and R.R. Kay

MRC Laboratory of Molecular Biology, Cambridge, England

Early observations, tracking the rearward movement of particles attached to the surface of moving cells, led to the suggestion by Abercrombie that new membrane is inserted into the front of a moving cell and flows backwards from it. Later emphasis on the cytoskeleton caused this idea to be neglected, and it came to be implicitly assumed that the plasma membrane has little direct role in the dynamics of cell movement and just passively follows the shape dictated by the cytoskeleton. Here we provide evidence for a much more central role of the plasma membrane. We devised a method for measuring the surface area of individual moving cells, using 4-D confocal microscopy and computer reconstruction of the surface.When a rounded cell is induced to throw out a pseudopod, its surface area increases by around 30% within a few minutes. Randomly moving and chemotacting cells show fluctuations in surface area of similar magnitude (both increases and decreases of 30 % in total surface area per minute are common), and these are especially noticeable when a cell stops or changes direction. These observations indicate that extensive exocytosis and endocytosis are probably coupled to cell movement. If the surface of a cell is pre-labelled with fluorescent ConA, and it is then induced to form a pseudopod, this pseudopod is essentially unlabelled. This strongly suggests that the pseudopod membrane derives from internal (unlabelled) stores by local exocytosis. We have also shown, using a temperature-sensitive NSF mutant in which endocytosis and exocytosis are strongly inhibited at the restrictive temperature, that membrane trafficking is essential for a pseudopod to expand. Combined, these results indicate that cell movement is accompanied by constant changes in the cell's surface area, to accommodate its changing shape, that a pseudopod's membrane expands by localized exocytosis of internal membrane and that this process is essential for a pseudopod to expand.

Abstract 43

# Structure and mechanical unfolding of the F-actin crosslinking protein Ddfilamin.

Michael Schleicher[1], Angelika Noegel[2], Tad Holak[3], and Matthias Rief[4]

[1] Institut für Zellbiologie der LMU, Schillerstr. 42, 80336 München, Germany; [2] Institut für Biochemie I, Med. Fak. der Univ. zu Köln, Joseph-Stelzmann-Strasse 52, 50931 Köln, Germany; [3] Max-Planck-Institut für Biochemie, 82152 Martinsried, Germany; [4] Lehrstuhl für Angewandte Physik, LMU München, Amalienstr. 54, 80799 München, Germany

In a moving cell the cytoskeleton undergoes constant reorganization, and the protein components in the cytoskeletal meshwork are therefore subject to mechanical deformation. Despite its obvious importance, we know little about the mechanical response of the individual cytoskeletal components to a force. Many F-actin crosslinking proteins share a common building scheme: two actin-binding domains are connected by a rod domain. The length and structure of the connecting rod varies considerably among actin crosslinkers. Depending on the specific filamin isoform the number of rod domains can vary from 6 to 24. *Dictyostelium discoideum* filamin (gelation factor, ABP120) is a two-chain F-actin crosslinking protein with an N-terminal actin-binding domain and a rod domain constructed from six tandem repeats of a 100-residue motif that has an immunoglobulin fold. The two chains are arranged in an antiparallel fashion and form an elongated element. We have used single molecule mechanical experiments in combination with protein mutation to characterize the mechanical response to a stretching force of the rod domains of the Ddfilamin. We find that one of the 6 Ig-domains unfolds at lower forces than all other domains and exhibits a stable mechanical unfolding intermediate on its mechanical unfolding pathway. Inserts of 5 amino acid residues into various loops of this domain lead to length changes in the single molecule unfolding pattern which allowed us to map the stable core of ~60 amino acids that constitute the unfolding intermediate. Fast refolding in combination with low unfolding forces suggest a potential in-vivo role for this domain as a mechanically extensible element within the Ddfilamin rod. With its low unfolding forces and fast refolding kinetics domain 4 may act as an extensible element within the rod which would allow the rod to double in length compared to its folded length.

McCoy, A.J. et al. (1999). Structural basis for dimerization of the Dictyostelium gelation factor (ABP120) rod. **Nature Struct. Biol.** *6*, 836-841.

Stossel, T.P., et al. (2001). Filamins as integrators of cell mechanics and signalling. **Nature Reviews Molecular Cell Biology** *2*, 138-145.

Schwaiger, I., et al. (2004). A mechanical unfolding intermediate in an actin-crosslinking protein. **Nature Struct. Mol. Biol.** *11*, 81-85.

Popowicz, G.M., et al. (2004). Molecular structure of the rod domain of *Dictyostelium* filamin, submitted

Abstract 44

## The Diaphanous-related formin dDia2 is required for the formation and maintenance of filopodia.

Antje Schirenbeck [1], Till Bretschneider [2], Rajesh Arasada [1], Michael Schleicher [1], and Jan Faix [1]

[1]A. Butenandt-Institut / Zellbiologie, Ludwig-Maximilians-Universität, Schillerstr. 42, 80336 München, Germany; [2] AG Zelldynamik, Max-Planck-Institut für Biochemie, Am Klopferspitz 18a, 82152 Martinsried, Germany.

Complex cellular functions such as the establishment of cell morphology and cell movement are mediated by the dynamic reorganization of the actin cytoskeleton. The polymerization of actin and assembly of F-actin with accessory cytoskeletal components to ordered structures is precisely controlled in a temporal and spatial manner. Recent findings suggest that besides the Arp2/3 complex, formins are key players regulating the dynamic rearrangement of the microfilament system. Formins constitute a family of proteins that contain two highly conserved formin-homology domains, FH1 and FH2. Diaphanous-related formins (Drfs) are a subfamily specified by an additional FH3 domain required for subcellular localization and two regulatory regions. The Drfs are activated by Rho GTP-binding proteins and induce the polymerization of unbranched actin filaments. The *Dictyostelium* genome indicates that this microorganism contains at least 10 different *formin* genes. In an attempt to characterize the *Dictyostelium* formins we have generated a large collection of single and multiple formin-null mutants employing the cre/loxP system and as yet, we have analyzed the Diaphanous-related formin dDia2 in detail. dDia2 is an effector of small Rho GTPase Rac1A and interacts with profilin II. A recombinant FH1FH2 fragment of dDia2 promoted actin assembly, removed capping protein from capped filament ends and prevented de-polymerization of F-actin. Thus, dDia2 appears to act as a processive cap that walks with the barbed end as it elongates. Elimination and overexpression studies demonstrated that, besides its function in cell migration, dDia2 plays a pivotal role in formation, elongation and maintenance of filopodia. We have gathered strong evidence that dDia2 specifically controls filopodial dynamics by regulating the actin turnover at the barbed ends of actin filaments.

Abstract 45

## Phg2, a novel kinase regulating focal site modeling and adhesion in *Dictyostelium discoideum.*

Leigh Gebbie[1], Mohammed Benghezal[1], Sophie Cornillon[1], Romain Froquet[1], Nathalie Cherix[1], Marilyne Malbouyres[2], Yaya Lefkir[2], Christophe Grangeasse[2], Sébastien Fache[3], Jérémie Dalous[3], Steve J Charette[1], Franz Brückert[3], François Letourneur[2], and Pierre Cosson[1]

[1] Université de Genève, Centre Médical Universitaire, Département de Morphologie, 1 rue Michel Servet, CH-1211 Genève 4, Switzerland; [2] Institut de Biologie et de Chimie des Protéines, UMR 5086 CNRS, Université Lyon I, 7 passage du Vercors, 69367 Lyon Cedex 07, France; [3] Laboratoire de Biochimie et Biophysique des Systèmes Intégrés, UMR5092, CNRS, CEA, Grenoble, France

A new adhesion-defective mutant named phg2 was isolated in the amoeba *Dictyostelium discoideum* model. The product encoded by the mutated gene bears both a serine/threonine kinase and a ras-binding domain. We compared the phenotype of phg2 null cells to other previously isolated adhesion mutants to evaluate the specific role of each gene product. Phg1, Phg2, myosin VII and talin all play similar roles in cellular adhesion. Like myosin VII and talin, Phg2 is also involved in the organization of the actin cytoskeleton. In addition phg2 mutant cells have defects in the organization of the actin cytoskeleton at the cell-substrate interface, and in cell motility. Since these last two defects are not seen in phg1, myoVII or talin mutants, this suggests a specific role for Phg2 in the control of local actin polymerization/depolymerization. This study establishes a functional hierarchy in the roles of Phg1, Phg2, myosinVII and talin in cellular adhesion, actin cytoskeleton organization and motility.

Abstract 46

## Mitochondrial protein import in *Dictyostelium discoideum.*

A. U. Ahmed [1], P. R. Gilson [2], P. L. Beech [2], and P. R. Fisher [1]

[1] Department of Microbiology, La Trobe University, Bundoora, VIC, Australia 3086;
[2] Centre for Cellular and Molecular Biology, School of Biological and Chemical Sciences, Deakin University, VIC, Australia 3125

To study mitochondrial protein import in *Dictyostelium discoideum*, we utilized two different reporter proteins - green fluorescent protein (GFP) and aequorin (a $Ca^{2+}$-sensitive luminescent protein). Both GFP and aequorin were fused either to variable N-terminal regions of chaperonin 60 (the first 23, 40 and 150 amino acids) or to the mitochondrial targeting sequence of DNA topoisomerase II and expressed in AX2 cells under the actin-15 promoter. Fluorescence images of GFP transformants confirmed that Dictyostelium chaperonin 60 is a mitochondrial protein. Expression levels were assessed by Northern and Western blots and (in the case of GFP) by fluorescence microscopy or (in the case of aequorin) by bioluminescence. In the latter case, transformants were lysed in a buffer containing excess $Ca^{2+}$ in order to discharge total aequorin after its *in vivo* reconstitution with the cofactor coelenterazine h and the total emitted light was used as a measure of aequorin activity.

The activities of both the mitochondrially targeted GFP and aequorin fusion proteins were unexpectedly much lower than the non-targeted (cytoplasmic) forms. The distinction between targeted and non-targeted protein activities was investigated at both transcriptional and translational levels in vivo. We found that targeting GFP to the mitochondria results in reduced levels of the fusion protein even though transcription of the fusion gene and the stability of the protein are unaffected. The results indicate a novel phenomenon for GFP fusion proteins – import-mediated translational inhibition whereby protein import into the mitochondria limits the rate of translation. The simplest explanation for this is that the GFP fusion protein import occurs cotranslationally i.e. protein synthesis and import into mitochondria are coupled events. Our results also demonstrate that the leader peptide of GFP fusions is susceptible to degradation in the cytoplasm but not in the mitochondria, whereas GFP itself is quite stable in both compartments. We suggest that the cotranslational import of GFP fusion proteins results in their avoidance of cytosolic degradation of the leader peptide which would result in the formation of a stable, import-incompetent structure of GFP. By contrast, the results with aequorin transformants have shown that aequorin expression is quite comparable at both the transcriptional and translational levels regardless of whether or not it has been successfully targeted to the mitochondria. The reduced activity of aequorin in mitochondrially targeted fusion proteins presumably results from interference by the additional N-terminal amino acids either with their proper folding or with some aspect of their catalytic activity.

Abstract 47

## Cytoplasmic glycosylation of protein-hydroxyproline in *Dictyostelium* Skp1 and its relationship to other glycosylation pathways.

Christopher M. West[1], Hanke van der Wel[1], Altan Ercan[1], Slim Sassi[2], and Eric A. Gaucher[3]

[1] Dept. of Biochemistry & Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, 73104 USA;[2] Dept. of Anatomy & Cell Biology, University of Florida, Gainesville, FL, 32610 USA; and[3] Foundation for Applied Molecular Evolution, Gainesville, FL, 32601 USA

The Skp1 protein, best-known as a subunit of $E3^{SCF}$-ubiquitin ligases, is subject to complex glycosylation in the cytoplasm of the cellular slime mold *Dictyostelium*. Pro143 of this protein is sequentially modified by a prolyl hydroxylase and five soluble glycosyltransferases, to yield the structure Galα1,Galα1,3Fucα1,2Galβ1,3GlcNAcα1-HyPro143. These enzymes are unusual in that they are expressed in the cytoplasmic compartment of the cell, rather than the secretory pathway where hydroxylation and complex glycosylation of proteins usually occurs. The first enzyme in the pathway appears to be related to the soluble animal prolyl 4-hydroxylases that modify the transcriptional factor subunit HIF-1α in the cytoplasm, and more distantly to the prolyl 4-hydroxylases that modify collagen and other proteins in the rER, based on biochemical analysis of a recombinantly expressed sequence predicted using an informatics approach. The soluble αGlcNAc-transferase acting on Skp1 is distantly related to the mucin-type polypeptide αGalNAc-transferases in the Golgi of animals. Its characterization has led to the discovery of a family of related polypeptide αGlcNAc-transferases in the Golgi of selected lower eukaryotes; the *Dictyostelium* enzyme shares cross-sensitivity to inhibitors of the mammalian enzymes. The Skp1 GlcNAc is extended by a bifunctional diglycosyltransferase that sequentially and apparently processively and constitutively adds β1,3Gal and α1,2Fuc. Though this structure is also formed in the animal secretory pathway, the glycosyltransferases involved are dissimilar. Preliminary studies suggest that the α3-linked Gal residue is conditionally attached by an enzyme distantly related to glycogenin and glycogen synthase, which also function in the cytoplasm. Characteristics of these enzymes suggest that glycosylation contributes to quality control of Skp1 folding and in turn influences Skp1 nuclear compartmentalization. Conceptual translation of available genomes suggests that this kind of complex cytoplasmic glycosylation also occurs in other eukaryotic microorganisms, including diatoms, oomycetes, and possibly *Chlamydomonas* and *Toxoplasma*, and an evolutionary precursor of this pathway may also occur in prokaryotes.

Abstract 48

## Massive intracellular digestion by autophagy is essential for development of *D. discoideum.*

Turgay Tekinay, Mary Y. Wu, Grant P. Otto, O. Roger Anderson[1], and Richard H. Kessin

Department of Anatomy and Cell Biology and [1]Lamont-Doherty Earth Observatory, Columbia University, New York, New York, 10032, USA

We have defined a number of *Dictyostelium* genes that are essential for turnover of protein and other material during development. The genes participate in an autophagic mechanism whereby a membrane surrounds cellular material, including organelles, and encapsulates it in an autophagic vacuole. This vacuole is then ferried to the lysosomal system where the materials are degraded. Mutations in atg1, atg5, atg6, atg7, atg8 and atg16 (tipD) are essential to autophagy in budding yeast. Mutations in these genes also block *Dictyostelium* development. These mutants have characteristic phenotypes – they die rapidly in nitrogen free medium (which a parental strain does not), they do not aggregate well on lawns of bacteria (but do better on Millipore filters), and they do not turn over cellular organelles or decrease their protein contents. They also have altered density on Percoll gradients after they have starved for a day or more. Autophagy mutants retain the density of vegetative cells when starved. Parental cells degrade their organelles and have altered density. An inability to do this is lethal.

We have used the phenotypes of autophagy mutants to ask whether in mutant collections, there are any other genes involved in autophagy that were not previously known from work in yeast. We have isolated one such mutant, temporarily called HRM13, which affects an early step in the induction of autophagy. The mutants of HRM13 have all of the phenotypes of autophagy mutants, but the gene, though found in *C. elegans*, *D. melanogaster*, and humans, is not found in yeast. Studies of autophagy in *Dictyostelium*, which previously derived from studies of *Saccharomyces cerevisiae*, have now become independent and have yielded a new autophagy gene. In addition, we have learned that regulation of autophagy by ATG1 differs in *Dictyostelium* and *Saccharomyces*.

Abstract 49

## Molecular pathways of *Dictyostelium* cell death.

Pierre Golstein, Artemis Kosta, Marie-Françoise Luciani, Céline Roisin-Bouffay, and Catherine Laporte

Centre d'Immunologie de Marseille-Luminy, Campus de Luminy, Case 906, 13288 Marseille Cedex 9, France

Few model organisms exist to study caspase-independent cell death mechanisms. *Dictyostelium discoideum*, which is haploid and genetically tractable, shows programmed cell death (PCD). In *Dictyostelium*, development upon starvation leads to a fruiting body with a mass of spores at the tip of a stalk that is made of cells having undergone PCD. Methods exist to trigger differentiation in vitro without morphogenesis: these cells dying as a monolayer are easier to study than cells in a stalk. This developmentally regulated PCD was shown to be independent of conventional caspases, and, recently, of paracaspase. More precisely, cell death in *Dictyostelium* can be used as a model for caspase-independent autophagic vacuolar PCD.

Using a combination of cell biology and molecular biology approaches, we showed that in *Dictyostelium* cell death cell polarization, immobilization and rounding, with concommitant actin segregation then depolymerization, were early signs on the death pathway, corresponding to committment to cell death as shown using a clonogenic assay, and occurring long before vacuolization. Inactivation of the cellulose synthase gene showed that cellulose encasing was not required for cell death. Late events included massive vacuolization then membrane lesions without DNA fragmentation. These and other results allowed a provisional definition of a cascade of events in *Dictyostelium* PCD. Current studies take advantage of the double triggering of this cell death (through starvation and DIF) and of gene knock-out technology to try to analyse the role of autophagy in this autophagic vacuolar PCD model.

Abstract 50

## Rho-dependent signaling in *Dictyostelium discoideum*: role of RacH.

Baggavalli P. Somesh, Carola Neffgen, and Francisco Rivero

Center for Biochemistry, Medical Faculty, University of Cologne, Joseph-Stelzmann-Str. 52, 50931 Köln, Germany

The small GTPases of the Rho family act as molecular switches, cycling between an active GTP-bound state and an inactive GDP-bound state. In its active state Rho GTPases interact with a multitude of effectors that relay upstream signals to downstream targets. Although initially described as major regulators of cytoskeletal remodeling, Rho GTPases have been shown to be also involved in the regulation of cellular processes as diverse as endocytosis, vesicle trafficking, morphogenesis, cytokinesis, transcriptional activation and cell cycle progression. *Dictyostelium discoideum* is an attractive model to investigate structural and regulatory aspects of the actin cytoskeleton. In addition to 15 Rho GTPases, other components of Rho-regulated signalling pathways are also present in this organism, like RhoGDI, the Arp2/3 complex, PAK, Scar, WASP, formins and numerous RhoGEFs and RhoGAPs.

We report on our studies aimed to uncover the role of RacH, which is constitutively expressed throughout the complete developmental cycle of *Dictyostelium*. We studied RacH function with the help of strains that overexpress the wild-type, constitutively active or dominant negative protein. RacH appears to be primarily involved in regulation of endocytosis and cytokinesis, and has no impact on cell motility or chemotaxis. In a cell- free system RacH stimulated actin polymerization, suggesting that it might be involved in actin-based trafficking of vesicular compartments. In fact, *Dictyostelium* RacH was found to be targeted to intracellular membranes of the nuclear envelope, ER and Golgi apparatus. We have addressed the requirements for subcellular localization of RacH by means of chimeric constructs and alanin exchange mutants. In search for effectors of RacH we are examining interaction with a number of candidates by means of yeast two-hybrid experiments. Generation of a RacH knockout strain is underway.

Abstract 51

## Expression of *Yersinia* Yop proteins in *Dictyostelium*.

Bettina Wagner, Oxana Schmidt, Rupert Mutzel, and Barbara Weissenmayer

Fachbereich Biologie, Chemie, Pharmazie, Institut für Biologie - Mikrobiologie, Freie Universität Berlin, Königin-Luise-Strasse 12-16, D-14195 Berlin, Germany

Three pathogenic *Yersinia* species including *Yersinia pestis*, the causer of bubonic plague, are known. They all share a virulence system which involves the delivery of bacterial proteins inside eukaryotic cells by extracellular bacteria. Depending on proteins being translocated *Yersinia* can escape the immune system of the host by inhibiting macrophage phagocytosis and cytokine production.

By focusing on relatively well understood *Yersinia* outer proteins (Yop) we investigated whether *Dictyostelium discoideum* can be utilized as model organism for *Yersinia* infection. For this purpose the effects of *Yersinia* cytotoxins on vegetative growth, cell differentiation and morphogenesis of *Dictyostelium* were studied by expressing *yopE* and *yopH* of *Yersinia pseudotuberculosis* in the MB38 tetracycline-controlled vector system (Blaauw et al 2000). In mammalian cells YopE was recently shown to have RhoGAP activity which leads to disruption of actin microfilament structure. As a consequence cell cycle progression is blocked and phagocytosis is inhibited. Expression of YopE in *Dictyostelium* severely inhibited growth in axenic medium. YopE producing cells were small and rounded. Growth on a bacterial lawn was not affected. Spontaneous reversion of this phenotype was observed.

In contrast to YopE, which proved to be cytotoxic for *Dictyostelium*, expression of YopH in *Dictyostelium* had no effect on growth. BothYopE or YopH producing cells developed as the vector-control cell line.

Abstract 52

## Endocytic trafficking is altered at multiple steps in *Dictyostelium* amoebae infected with *Legionella pneumophila*.

Margaret Clarke and Hao Lu

Oklahoma Medical Research Foundation, 825 N.E. 13th St., Oklahoma City, OK, 73104

We are tracking early steps in the infection of *Dictyostelium* cells by *Legionella pneumophila*, the bacterial pathogen that causes Legionnaire's disease. Our approach is confocal microscopy of living cells expressing fluorescent markers (DsRed-Express for *Legionella*, and GFP for *Dictyostelium* organelles and proteins that have been implicated in the infection process). The latter proteins include VatM (a subunit of the V-ATPase), the actin-binding protein coronin/TACO, the ER proteins calnexin and calreticulin, alpha-tubulin, and the pleckstrin homology domain of CRAC, which binds to phosphatidyl-inositol-(3,4,5)-trisphosphate (PIP3). Our marker for phagosome-lysosome fusion is delivery of the V-ATPase to the phagosome membrane, a process that begins for food particles about 90 seconds after a new phagosome is internalized. Coronin-GFP shows that newly-formed *Legionella*-containing phagosomes are surrounded by an actin coat that is shed shortly after uptake (and thus cannot be responsible for blocking phagosome-lysosome fusion). The phagosome then undergoes rapid microtubule-based transport about the cell. In these respects, the behavior of a *Legionella*-containing phagosome resembles that of a phagosome containing a food particle. However, there is no delivery of the V-ATPase. Furthermore, the transient enrichment of PIP3 that is observed in the membrane of phagosomes containing food particles is substantially prolonged for phagosomes containing *Legionella*. This is true not only for wild-type *Legionella* (strain JR32) but also for an *icmT* mutant, which cannot replicate in macrophages or *Dictyostelium* cells. These results suggest that the membrane composition of a *Legionella*-containing phagosome is altered at the time of uptake or immediately thereafter. ER markers begin to associate with phagosomes containing wild-type *Legionella* within ~30 minutes after uptake, correlating with a cessation of rapid phagosome movement. The accumulation of GFP bearing HDEL, an ER localization signal, can be detected somewhat earlier than that of calnexin and calreticulin, resident ER proteins. ER markers accumulate to high levels over the next several hours. At about the same time that ER markers begin to associate with phagosomes containing wild-type *Legionella*, phagosomes containing *icmT* bacteria become surrounded by VatM-GFP. We infer that endocytic trafficking is altered by the pathogen in multiple steps, since the PIP3 probe revealed that phagosomes formed to ingest either wild-type and *icmT Legionella* differ in composition from phagosomes surrounding food particles, while the phagosomes containing these two types of *Legionella* first diverge in trafficking behavior about 30 minutes after uptake.

Abstract 53

## Investigation of the transcriptional changes in *Dictyostelium* upon infection with Legionella using DNA microarrays.

Patrick Farbrother [1], Michael Steinert [2], Carina Wagner [2], Takahiro Morio [3], Hideko Urushihara[3], Yoshimasa Tanaka[3], Michael Schleicher[4], and Ludwig Eichinger[1]

[1] Institut für Biochemie I, Medizinische Fakultät, Universität zu Köln, Joseph-Stelzmann-Str. 52, D-50931 Köln; [2] Institut für Molekulare Infektionsbiologie, Universität Würzburg, Röntgenring 11, D-97070 Würzburg; [3] Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan; [4] Institut für Zellbiologie, Medizinische Fakultät, Ludwig-Maximilians-Universität München, D-80336 München

Freshwater amoebae are the natural host of *Legionella pneumophila*, the causative agent of Legionnaires' disease. The social amoeba *Dictyostelium discoideum* is a well-established model organism for the study of basic aspects of differentiation, signal transduction, phagocytosis, cytokinesis and cell motility. In its natural habitat, the forest soil, it lives on bacteria that are taken up by phagocytosis. Furthermore it was shown that the medically relevant infection of host cells by pathogenic microorganisms could be investigated with *D. discoideum* as host and the facultative intracellular parasite L. pneumophila. Differential gene expression of the host after infection with L. pneumophila was investigated using DNA-microarrays, that carry more than 5,000 non-redundant EST clones, 450 probes derived from published genes as well as appropriate positive and negative controls.

The differential gene expression of *Dictyostelium* infected with L. pneumophila was investigated during a 48-hour time course of infection. The results showed, that most of the transcriptional changes occur three and 24 hours after infection. For the 24-hour time point post-infection experiments were performed in comparison to three controls, uninfected cells and co-incubation with L. hackeliae as well as *L. pneumophila* ΔdotA. L. hackeliae and L. pneumophila ΔdotA are Legionella strains with reduced pathogenicity and a deleted pathogenicity gene, respectively. 140 differentially expressed *D. discoideum* genes were identified as common to all three experiments. Based on the comparisons, these genes are thought to be involved in the pathogenic response. A few of these genes code for known D. discoideum proteins. Among these are RtoA, Discoidin I, CotB and the lysosomal α-mannosidase. Functions could be assigned to others by homology searches. The molecular chaperone ClpB, β'-COP and three small calcium binding proteins are some of these. With the aid of a categorization of probes by cellular processes, the alterations in gene expression were analyzed on a functional level. It could be shown, that among the genes that are differentially regulated, those are over-represented, whose products are involved in nucleotide metabolism or that are ribosomal proteins. The differential expression of ten genes identified with DNA microarrays was confirmed by quantitative PCR and northern blots. The results provide the basis for a better understanding of the complex host-pathogen interactions and for further studies on the *Dictyostelium* response to Legionella infection.

Abstract 54

# Sphingolipid control of growth, differentiation and drug resistance in *Dictyostelium discoideum* and human cells.

Junxia Min[1], David Traynor[2], Andrew Stegner[1], Hannah Alexander[1], and Stephen Alexander[1]

[1] Division of Biological Sciences, University of Missouri, Columbia, MO 65211-7400, USA; [2] MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Cancer is frequently treated with chemotherapy, but efficacy is often limited by drug resistance. In the case of the widely used platinum based drug cisplatin, the basis for the resistance of tumor cells is not well understood. We have used *D. discoideum* as a model with which to understand the basis of resistance to cisplatin and identify new drug targets. A cisplatin resistant mutant with an inactivated sphingosine-1-phosphate (S-1-P) lyase (*sglA*) gene was recovered from a REMI based screen. This result suggested that increased S-1-P was an underlying cause for drug resistance. We have now produced mutants that overexpress the S-1-P lyase, and as predicted they are more sensitive to cisplatin. In addition, we have overexpressed and disrupted the sphingosine kinase A and B (*sgkA* and *sgkB*) genes. Again as predicted, overexpression increases resistance to cisplatin while disruption increases sensitivity. Importantly, the changes in cisplatin sensitivity are also observed with carboplatin, another platinum based drug, but not other DNA damaging drugs which is important for treating tumors with multiple drugs. DMS, an inhibitor of sphingosine kinase, at subtoxic concentrations also increases sensitivity to cisplatin. We have now used REMI to isolate mutants that are resistant to DMS. We have validated these results in human cells by overexpressing the human S-1-P lyase and kinase in human embryonic kidney cells and shown that this alters sensitivity to cisplatin as predicted. In addition, we have examined the roles of ERK and p38 in modulating the effect in human cells. In *D. discoideum*, altering either the S-1-P lyase or sphingosine kinase levels alters growth rate in both axenic medium and on agar in association with bacteria. S-1-P lyase mutants have a number of significant developmental defects including cell shape, gene expression, slug migration and spore maturation. Overall, using *D. discoideum* we have identified two new targets for improving the effect of cisplatin. We are continuing to use this system to identify mutant that are resistant to other chemotherapy drugs.

Abstract 55

## Rac signalling and VPA targets.

K. Adley[1], A. Mueller-Taubenberger[2], X. Xu[3], T. Jin[3], and R.S.B. Williams[1]

[1]Department of Biology, UCL, Gower St, WC1 E6BT, UK; [2]Max-Planck-Institute for Biochemistry, Martinsried, Germany; [3] National Institute of Allergy and Infectious Diseases, NIH, Rockville, Maryland USA.

Bipolar disorder or manic depression is a major problem worldwide, causing a substantial reduction in quality of life, with current treatments (eg. lithium and valproic acid - VPA) still giving a poor prognosis for recovery. The way these treatments work to combat the disorder remains unknown. We have previously used *Dictyostelium* to identify genes involved in the response to lithium. This has provided insight into how specific enzymes found to be elevated in patients may be involved in this disorder (1, 2). We are now also using *Dictyostelium* to define the therapeutic mechanism of the first-choice treatment for bipolar disorder in the US, VPA. VPA has both acute and long term effects on *Dictyostelium.* At concentrations seen in human blood plasma (1 mM), VPA slows *Dictyostelium* growth on bacterial lawns as well as development by about 5 hours. Acute effects are seen using aggregating cells, where 1 mM VPA blocks chemotaxis to cAMP, but this effect occurs whilst cells can still sense direction. VPA also causes a rapid increase in the number of filopodia, a phenotype closely resembling that of cells containing inactivated Rac1A protein. To look for effects of VPA on Rac1A activity, we treated wild-type cells containing constitutively active Rac1A (showing numerous lamellipodia) with VPA. This blocked the formation of lamellipodia in these cells within 10 minutes, suggesting VPA may function through inactivation of Rac. One possible link between Rac signalling and the action of VPA is through the reduction of phosphatidylinositol-bis-phosphate (PIP2) caused by inositol depletion, as this may attenuate the production of PIP3 by PI3 kinase. To analyse this, we have used a PIP3-GFP binding construct to measure changes in PIP3 following VPA treatment. We found no reduction in total PIP3 levels, although VPA reduced the period required for maximal PIP3 production, suggesting that VPA may inhibit this signalling pathway. As a reduction in PIP3 levels can also be caused by the inhibition of PI3 kinase, we exposed cells containing dominant active Rac1A to a PI3 kinase inhibitor and found this inhibitor also reversed the activated Rac phenotype. These results therefore suggest that either PI3 kinase is downstream from Rac signalling or that both these activities are affected by VPA.

1. Breen, G., Harwood, A.J., Gregory, K., Sinclair, M., Collier, D., St. Clair, D. and Williams, R.S.B. (2004) Prolyl oligopeptidase and Z-prolinal-insensitive peptidase activity decrease in bipolar disorder not schizophrenia. *Bipolar Disorders*, **6**, 156-161.

2. Williams, R.S.B., Cheng, L., Mudge A.W. and Harwood, A.J. (2002) A common mechanism of action for three mood-stabilizing drugs. *Nature,* **417**, 292-295.

Abstract 56

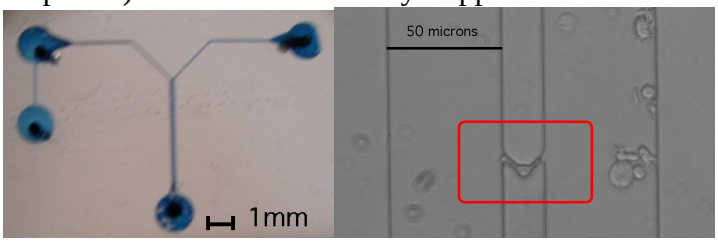
# Microfluidic technology introduces new possibilities for studies of internal protein dynamics.

Danica Wyatt[1], Eberhard Bodenschatz[1], and William Loomis[2]

[1] Cornell University, USA; [2] University of California, San Diego, USA

Microfluidic technology reduces the dimension of an experimental environment to the cellular scale, thus increasing the precision and repeatability of an applied cAMP stimulus. We bring these advantages to new experiments involving PH-domain protein response to external cAMP stimulus.

We have designed a device where cells are confined in a hole between two parallel channels while static or dynamic patterns of cAMP are introduced from external fluid reservoirs. The image below shows a macroscopic view of a typical device on the left with a magnified view of the central channel on the right in which a cell (boxed in red for emphasis) has been successfully trapped.




The fluid streams that flow past the cells are controlled by a custom built syringe pump system driven by high-resolution linear motors. Replicating well-established results from previous experiments has tested the validity of observations made in this environment. For example, cells confined in the channel show the expected uniform translocation of PH-domain containing proteins in response to global stimulus with cAMP.

This system delivers a well-quantified concentration of cAMP on either side of the cell that can be kept constant indefinitely, or changed on a time scale shorter than one second, allowing precise triggering and observation of early directional responses. We will present the technical capabilities of this microfluidic approach as well as results from *in vivo* studies of PH-domain protein dynamics.

Abstract 57

# The study of *Dictyostelium* chemotaxis using microfluidic devices and imaging microscopy.

L. Song[1], S. M. Nadkarni[1], W. F. Loomis[2], H. Levine[3], and E. Bodenschatz [1]

[1]Department of Physics, Cornell University, Ithaca, NY; [2]Division of Biological Sciences; [3]Department of Physics, University of California at San Diego, La Jolla, CA.

Chemotaxis plays an important role in a wide range of physiological processes such as embryonic development, inflammation, wound healing, and cancer metastasis. In the past, chemotaxis has been widely investigated using traditional experimental techniques such as the Zigmond or Dunn chambers and micropipette assays. We have made microfluidic networks similar to those of G. Whitesides and colleagues (Jeon et al.) A pyramidal network is used to create a spatially linear, temporally constant gradient. Dictyostelium discoideum cells with green fluorescent protein-tagged cytosolic regulator of adenylyl cyclase (gfp::CRAC) were developed for 4 hours under starvation conditions inside the microfluidic chamber, and were then exposed to a stable linear cAMP gradient. Our experiments so far have demonstrated that stable linear cAMP gradients generated in such microfluidic devices can indeed elicit a chemotactic response from the cells. During the first 90 minutes of a linear gradient, cells moved from a random distribution throughout the width of the chamber (525 micrometers) to the side of the higher cAMP concentration. Reversal of the direction of the cAMP gradient leads to reversal of the migration direction of the cells. Thus far, our microfluidic device has demonstrated its ability to provide well-controlled, well-defined, temporally and spatially stable and quantifiable chemical environments. In these flow-through systems, chemicals of interest can be added to and removed from the environment at will. The latter is important, because it provides the opportunity of generating temporally or chemically different environments to the same cell population. Our ongoing experiments aim to arrive at a better understanding of the threshold sensitivity for *Dictyostelium* cAMP chemotaxis.

Acknowledgment: Nanobiotechnology Center (NBTC) grant No. ECS-9876771 and National Science Foundation Bio-Complexity grant No. 0083704. NBTC and Cornell Nanofabrication Facility (CNF).

Abstract 58

## Dynamics of actin patterns as revealed by TIRF-microscopy.

T. Bretschneider[1], A. Müller-Taubenberger[1], S. Diez[2], K. Anderson[2], and G. Gerisch[1]

[1] Max-Planck-Institute of Biochemistry, Germany; [2] Max-Planck-Institute of Molecular Cell Biology and Genetics, Germany

We recently made significant progress in visualising cortical actin dynamics in live *Dictyostelium* cells based on the combination of: 1) using a fluorescent probe which specifically binds to polymerised F-actin, Lim-delta-coil and 2) applying total internal reflection fluorescence microscopy (TIRF), which selectively excites fluorescence in the substrate attached cell cortex and eliminates background signal from upper levels of the cell. Recordings at intervals of down to 50 milliseconds reveal a highly dynamic network which undergoes complete reorganisation on the timescale of approximately two seconds. Polymerisation of new filament bundles proceeds with an average speed of 3µm/sec. Anchored within this network are stationary Arp2/3 containing patches with a lifetime of around 10 seconds. Eventually these patches can be organised into patterns of propagating wave fronts which can be efficiently induced by sequestering G-Actin at moderate levels of Latrunculin A. We are currently working on double-labeling experiments (GFP, mRFP) to characterise the temporal and spatial organisation of proteins within the observed supramolecular actin complexes (see abstract by A. Müller-Taubenberger).

Abstract 59

## New fluorescent probes for visualizing rapid reorganization of cytoskeletal structures.

A.Müller-Taubenberger[1], K.I.Anderson[2], T.Bretschneider[1], S.Diez[2], and G.Gerisch[1]

[1] Max-Planck-Institute of Biochemistry, Martinsried, Germany; [2] Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Combining the expression of fluorescent fusion proteins as specific probes for the cytoskeleton with total internal reflection (TIRF) microscopy revealed new insights into actin dynamics in live cells (Bretschneider *et al*., Curr. Biol. 14, 1-10, 2004). We have analyzed actin-myosin dynamics at the substrate-attached surface of migrating *Dictyostelium* cells. Both actin and myosin II form networks of motile filaments immediately beneath the cell surface. Two patterns of actin assembly can be distinguished: highly dynamic, loose meshworks and dense filament assemblies. Actin meshworks are present allover the substrate-attached surface and are characterized by fast reorganization. To visualize actin filament dynamics in combination with TIRF microscopy we also tested a photoactivatable GFP probe.

The dense actin assemblies are sites of Arp2/3 complex recruitment, prominent at leading edges, but also transiently found at distal foci and in propagating actin waves. In order to further dissect the mechanism of Arp2/3 complex induced actin filament assembly we have employed *in vivo* double labeling using either GFP or YFP tagged and monomeric RFP (mRFP) tagged proteins. For this purpose we developed a new version of mRFPmars that is brighter and more stable than the previous mRFP1, and is adapted to the *Dictyostelium* codon usage. We employed *in vivo* double labeling to visualize components interacting with the Arp2/3 complex.

Our studies suggest that the rapidly restructured network of single or bundled actin filaments provides a scaffold for the assembly of differentiated actin complexes. A combination of different fluorescently labeled proteins involved in this regulated process enabled us to study their assembly during wave and lamellipodia formation *in vivo*.

Abstract 60

## Time-resolved molecular fingerprinting of phagosome maturation.

V. Blancheteau[1], D. Gotthardt[2], and T. Soldati[1,3]

[1]Department of Biological Sciences, Imperial College, London, UK; [2]MPI for medical Research, Heidelberg, Germany; [3]Department of Biochemistry, University of Geneva, Switzerland

Investigations of molecular mechanisms of endocytosis in *Dictyostelium*, a genetically and biochemically tractable professional phagocyte, reveal striking similarities to higher eukaryotic cells. Using an improved phagosome purification method, pulse-chase feeding experiments had allowed us to begin the completion of a precise time-dependent profiling of phagosomal markers. The resulting complex molecular fingerprint of phagosome biogenesis revealed distinct phases. First, a signaling phase, when trimeric G proteins, proteins involved in the nucleation of actin filaments (Abp1, Arp2/3 complex), and other actin associated proteins (coronin and MyoK) are concentrated. Second, at least two phases of delivery of lysosomal hydrolases (CatD, CP34) accompanied by recycling of plasma membrane components (PM4C4, biotinylated surface proteins, p25). Third, a phase of late maturation before final exocytosis of undigested material which includes quantitative recycling of the V-ATPase, hydrolases and association with specific markers, such as vacuolin. Lysosomal glycoproteins of the same family, LmpA, LmpB and LmpC showed distinctive trafficking kinetics. This heavy membrane traffic is accompanied by the appearance and disappearance of adaptor complexes, clathrin heavy chain, Rab GTPases and endosomal v-SNAREs. The delivery and recycling of CatD was also visualised by confocal microscopy.

We have carried out a comprehensive proteomic cartography of phagosome maturation. Over 400 spots were analysed by MALDI-TOF, leading to the identification of about 300 phagosomal proteins. Among these, about two dozens have also been uncovered by proteomic analysis of the constituents of the mouse phagosome. The majority can be assigned to the functional classes of signalling, membrane trafficking, cytoskeleton associated, chaperones and metabolic enzymes. About 50 spots correspond to novel proteins, most of which being conserved in other higher eukaryotes. The Phoretix-Evolution software allowed us to match the spots from a series of 2D gels acquired from samples at different stages of maturation, generating a time resolved database of phagosomal components. Cluster analysis is being used to identify potential functional groups of phagosomal proteins.

We also started a comprehensive comparative proteomics project that uses fluorescence two-dimensional difference gel electrophoresis (DIGE) technology to map differences in phagosomal protein composition between wild type and a range of mutant strains deficient in MyoB, MyoK, Abp1 and CARMIL.

Abstract 61

## Epistasis analysis with transcriptional microarray phenotypes.

N. Van Driessche[1,2], E. Okyay[3,1], A. Kuspa[1,2,4], and G. Shaulsky[1,2,3]

[1] Department of Molecular and Human Genetics; [2] Developmental Biology Program; [3] Structural and Computational Biology and Molecular Biophysics Program; [4] Department of Biochemistry and Molecular Biology Baylor College of Medicine, Houston, TX 77030

Genome-scale profiles of gene expression can serve as surrogate measures of cell physiology. Expression array data have been used as diagnostics in cancer, as means of associating mutant strains in yeast, etc. We hypothesized that transcriptional profiles could be used to determine epistatic relations in genetic analysis. In epistasis, gene A is said to precede gene B in a linear pathway if mutations in either gene have distinct phenotypes and if mutating both genes in one organism gives the phenotype of mutating gene B alone. We tested our hypothesis on a 6-gene pathway that regulates development in Dictyostelium by controlling the activity of PKA (cAMP-dependent protein kinase). Comparing the developmental transcriptional profiles of single- and double-gene mutant strains we confirmed all of the known epistatic relations in the pathway. Moreover, we found new genetic interactions that were either unknown or known only from biochemical assays. The microarray profile analysis also revealed that the common concept of epistasis is limited. In most cases we found evidence for additional pathways where the genes act independently of each other or in a reversed manner relative to the widely accepted interactions. In summary, we have shown that microarray profiles can be used as phenotypes in complicated genetic analyses. This finding provides an enormous advantage over common methods because microarray measurements are independent of prior knowledge of gene function in the pathway. Classical genetics can only analyze pathways where the outcome is measurable, meaning that the investigator must know some of the gene functions before the experiment. Also, evaluating the outcome in classical genetics is sometimes subjective or requires a variety of expertise. Microarrays provide an unbiased and quantitative means of evaluating mutant phenotypes that is independent of prior knowledge and requires only expertise in microarray data analysis.

Abstract 62

## Development of RNAi as a silencing tool in *Dictyostelium discoideum*.

Sebastian Mana-Capelli and Denis A. Larochelle

Department of Biology,Clark University, 950 Main St., Worcester, Massachusetts 01610

To further explore the utility of dsRNA-mediated gene silencing in *Dictyostelium discoideum* we have tested a series of different constructs for their ability to induce gene silencing. RNA-mediated interference, or RNAi, is a conserved cellular mechanism for post-transcriptional gene silencing. RNAi has been successfully used in *D. discoideum* to silence both endogenous and exogenous genes. However, long fold-back dsRNA molecules were used. These constructs have been shown to also effect the expression of genes other than the target gene. We therefore decided to generate a series of constructs (Figure 1.) that produce dsRNA molecules of different lengths and conformations and test these for their ability to induce gene silencing. As a target gene for these experiments we chose *racE*. RacE is required for cytokinesis A to proceed and mutations at this gene locus result in large and multinucleated cells when grown in suspension culture. Such cells are either mono or bi-nucleated when grown as attached cultures. Therefore, we could easily screen cells for gene silencing by comparing their growth in suspension culture to that in stationary culture. Palindromic sequences of varying lengths (as described in Figure 1) were amplified by PCR and cloned behind the A15 promoter into a suitable plasmid for constitutive expression in vegetative *Dictyostelium* cells. Following transfection into DH1 cells, clonal cell lines were selected and screened for their ability to divide. Growth curves were generated for stationary and shaking cultures and the number of nuclei per cell (visualized by DAPI staining) was determined. As expected, all cell lines divided normally when grown as an attached culture. The long-sense and tandem constructs produced the strongest phenotypes (14.69 ± 17.00 and 9.07 ± 11.55 nuclei per cell, respectively, after 3 days in suspension culture) (Fig 2). This is in agreement with racE null cells (21.89 ± 14.24). Although we have not examined this, the large variability in the number of nuclei per cell may reflect varying levels of expression. Interestingly, the siRNA constructs (generated by annealing complementary, gene-specific oligonucleotides) produced less variability in the number of nuclei per cell, although the total number of nuclei per cell was not as great (6.46 ± 5.38). All constructs tested produced some degree of gene silencing, however, the short 21-nt dsRNA generated the most consistent phenotypes (in terms of number of nuclei per cell). This should enable *Dictyostelium* researchers to take advantage of the ease of synthesis and the specificity of the 21-nt dsRNA constructs to rapidly target genes of interest.

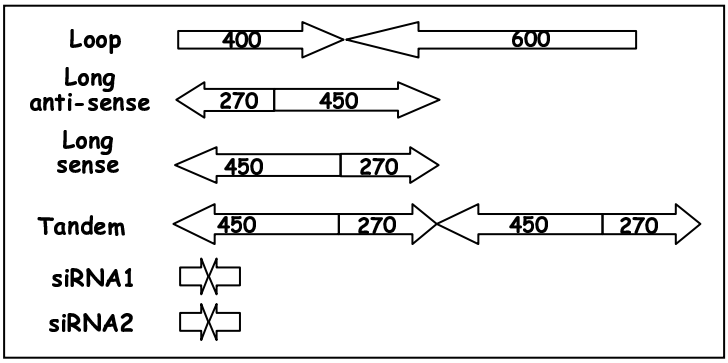



**Fig. 1:** scheme of the different RNAi constructs.

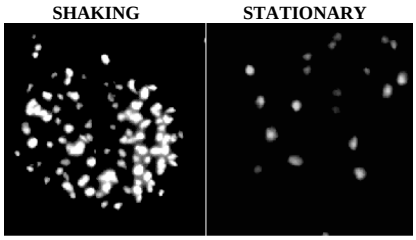



**Fig 2:** DAPI staining showing a representative phenotype of DH1 cells after silencing with the Long anti-sense construct.

Abstract 63

# Imaging of individual G-protein coupled cAMP receptors on the plasma membrane of *Dictyostelium discoideum in vivo.*

S. de Keijzer[1,2*], A. Sergé[2]*, A. P. Lommerse [1,2], F. van Hemert[1], H.P. Spaink[1],T. Schmidt[2], and B.E. Snaar-Jagalska[1]

[1]Molecular Cell Biology, IBL and 2, Physics of Life Processes, LION, Leiden University.

* These authors contributed equally to this work.

Single molecule microscopy is a new approach to monitor the behaviour of single molecules *in vivo* . The mobility of lipids and proteins in membranes is believed to play an important role in signal transduction processes. Using fluorescence labelled lipids and proteins, we are able to perform such experiments in membranes under physiologically conditions. Our experimental set-up consists of an inverted microscope, laser excitation between 450 and 640 nm, and image detection on an ultra-sensitive CCD-camera. This set-up allows images of individual fluorophores within a time-frame of a few milliseconds at a signal-to-background ratio of 30 with a positional accuracy of 40 nm. Since a few years, the technique, as being developed for artificial systems, is extended to the auto-fluorescent proteins. This will enable a multitude of cell biological systems to be analysed at the single molecule level.

Here we applied this technique to the chemotactic model of *Dictyostelium discoideum.* A construct of the cAMP receptor, cAR1, fused to the autofluorescent protein, YFP, was made. Our experimental system was optimised, concerning auto-fluorescence, photo-bleaching and mobility of the cells. The behaviour of single cAR1 receptors on the apical plasma membrane was followed in real time and the effect of cAMP stimulation on the mobility was investigated. This is investigated to find a possible role for receptor dynamics in the polarised activation and distribution of downstream signalling components at the plasma membrane during chemotactic movement. In unpolarized cells there were two populations of receptors found with different diffusion constants. A slow moving fraction (35%) with D = 0.04 $\mu m^2/s$ and a fast moving fraction (65%) with D = 0.2 $\mu m^2/s$. Biophysical properties of the receptors were different in polarized cells. Changes in the fractioning and the diffusion constants upon stimulation with cAMP and differences occurring at the anterior and posterior of a polarized cell, will be discussed. The effect of cAMP stimulation on the internalization of cAR1-YFP receptor was observed in real time and at the single molecule level.

Abstract 64

## Workshop: dictyBase and the Dicty Stock Center

**This workshop gives the research community the opportunity to browse dictyBase and the Dicty Stock Center and learn about the tools available to retrieve and analyze data. There will be a one hour introduction given by the curators, followed by a hands-on tutorial to help individuals browse the database themselves. We will provide work stations, but participants are encouraged to bring their laptop.**

**Introduction to dictyBase**

**Pascale Gaudet+, Karen E. Pilcher+, Petra Fey+, Eric M. Just, Sohel N. Merchant, Warren A. Kibbe, and Rex L. Chisholm**
**+authors making equal contributions**

**Northwestern University, USA**

DictyBase (http://dictybase.org) provides a single access point for information about *Dictyostelium discoideum*. It combines all information from the former 'Dictyostelium WWW server' with a comprehensive database that contains genomic and proteomic data. Components of the 'WWW server' such as 'Learn about Dictyostelium,' 'Read the dictyNews,' and 'Techniques,' are a part of dictyBase and are still being maintained and updated. In addition to these tools, dictyBase houses a powerful database that is constantly being improved. This includes the full *Dictyostelium* genome, GenBank genes, and ESTs, as well as genome and functional annotations, curated *Dictyostelium* literature, and tools for discovering homology and functional relationships; it also includes the *Dictyostelium* Stock Center and the Colleague database of *Dictyostelium* researchers. These pages are constantly updated, both manually and automatically.

The database is easily accessible through a search box located on every page, including the home page. Users can search any Gene name, Alias, Gene product, Gene Ontology (GO) term, Colleague, Author, GenBank Accession Number, or dictyBase ID. The search result lists the number of entities by topic. For example, the search for 'kinase' currently returns 106 gene products and 364 GO terms. Clicking on '106 gene products' displays a list of all gene products that contain the word 'kinase' and their associated genes. If there is only one result in the search, such as a search for a single gene name (e.g. mlkA), the user is automatically directed to the Locus Page for that gene. To broaden a search, use only part of a name and a wildcard (*) character.

In dictyBase, each gene has a Locus Page that can be accessed either through a search or through the Genome Browser. The Locus Page contains all available information about a gene such as names, sequences, literature, phenotypes, GO annotations, expression data, and colleagues. The amount of information on a Locus Page depends on the available information and whether a locus has been manually curated.
Manual curation is an ongoing process, and expert input by sending an Email to the curators (dictybase@northwestern.edu) is highly appreciated. In the near future, we are also planning to add pages for gene families such as kinases, transcription factors, and

cytoskeletal proteins. Through automatic updates, added features, and extensive manual curation, dictyBase is continually evolving.

**Sequence Information in dictyBase**

The *Dictyostelium* sequencing project is now essentially complete. The enormous effort of sequencing and assembly was accomplished through an international collaboration by the *Dictyostelium* Genome Consortium. Currently dictyBase stores 13,469 automated gene predictions that have been provided by the Sequencing Centers, all the *Dictyostelium* sequences published in GenBank (over 1,300 GenBank records), as well as the 155,032 ESTs generated by the *Dictyostelium* cDNA Project in Japan.

The genome sequence is analyzed by the Sequencing Centers using automated gene prediction software. At dictyBase, curators manually assess the quality of each gene prediction. Gene models provided by researchers (GenBank records, publications, or personal communications), mRNA, ESTs, and sequence similarities are all taken into account to determine the accuracy of the automated gene predictions. The Curated Model therefore represents the best gene model based on the available information. Roughly 1000 genes have been manually curated, including nuclear genes that have GenBank records. Each Curated Model has a Locus Note stating how the gene model was obtained (from the GenBank gene sequence, the automated gene prediction, or inferred by the curator) and its supporting evidence (mRNA, ESTs, and/or sequence similarity). In some cases a curated gene model cannot be created due to lack of supporting evidence; this is indicated in the Locus Note. Loci without Locus Notes have not yet been curated.

**Accessing the Sequences**

Sequences can be retrieved on the Locus Page, which can be accessed by typing a gene name or a dictyBase ID in the search box present on all dictyBase web pages or by clicking on a gene model in a Genome Browser window. Protein, coding, or genomic sequences can be downloaded in FASTA format. More details can be found in the help documentation.

The Genome Browser displays a graphical representation of all the sequence information in dictyBase, including manually curated gene models, sequencing center gene predictions, GenBank records, ESTs, and chromosomal contigs. The Genome Browser can be accessed by clicking on the minimap on the Locus Page, or directly through the link on the top menu on all the dictyBase pages. The window in the upper left corner of the Genome Browser can be used to search gene names or chromosomal coordinates, in the format Chr:start..stop. For example, 1:1..50000 displays the nucleotides 1 to 50,000 of chromosome 1. The Genome Browser can also be configured to show restriction sites. In addition, sequences of any length can be retrieved by using the 'dump sequence' function located below the image.

Sequences can also be accessed through the BLAST Server. The sequences are organized into seven different datasets: full chromosomes, primary features (one entry per predicted gene; the Curated Model where there is one, otherwise the Sequencing Center Gene Prediction), sequencing center gene predictions, HMM gene predictions, curated gene

models, GenBank records, and ESTs. The BLAST server can be accessed from the Locus Page or through the link on the top menu on all the dictyBase pages.

**Literature**

DictyBase contains approximately 8800 references from PubMed and the Franke Reference Library, including books, theses, and over 7000 journal articles. References from PubMed are reviewed by curators and linked with appropriate loci and literature topics. Literature related to a particular gene can be accessed through the gene's Locus Page. Every Locus Page has a Literature section with the option to view the Literature Guide, which consists of a list of all associated references and topics related to that gene. Other genes associated with the references are also listed here. From the Literature Guide, it is possible to link to the dictyBase reference information, the PubMed abstract, or the full-text article through the publisher's website for any given reference.

Literature may searched by author using the search box found on every page in dictyBase. Entering the last name of an author will retrieve all references that include that author's last name. Clicking on the 'dictyBase Paper' button displays the abstract and the loci associated with that reference.

**Gene Ontology (GO)**

The Gene Ontology (GO) is a standardized vocabulary developed and maintained by the GO Consortium (http://www.geneontology.org). The goal of GO is to provide consistent descriptions of gene products in different databases and facilitate electronic searching. Model organism databases, including dictyBase, use GO terms to annotate gene products with GO terms to describe molecular functions, biological processes, and cellular components. The use of GO terms creates connections between the gene products of different organisms and allows users to search for all gene products associated with a particular function or process.

GO terms both manually and automatically associated with a particular locus can be found on the Locus Page in the GO Annotations section. To view the supporting references for GO annotations, click on 'GO evidence and references.' Each annotation has an evidence code indicating the source of the annotation. All automated annotations have the evidence code IEA, an acronym for Inferred from Electronic Annotation. All other evidence codes represent annotations done manually by a curator, for example, IDA (Inferred from Direct Assay) and ISS (Inferred from Sequence or structural Similarity). Clicking on any GO term displays a list of all genes in dictyBase associated with that term. Alternatively, all GO terms may be searched using the search box, which retrieves a list of all GO terms and the loci to which they are annotated. Selecting any of the loci will direct the user to the Locus Page.

**Phenotypes**

Mutant phenotypes are assigned to genes by curators based on published literature and are displayed on the Locus Page. Mutant type, such as null or overexpression, is indicated in the left column. Similar to GO annotations, the references supporting each phenotypic

observation are available on the 'Phenotype details and references' page. Selecting any given phenotype will show a list of all *Dictyostelium* genes with that phenotype.

## Progress report of the Dicty Stock and Reference Center

Jakob Franke, Stephanie Marsh, and Richard H. Kessin

Columbia University, USA

The strain catalogue of the Dicty Stock Center has gone on line and contains more than 400 strains at this moment. We have stored the complete collections of Peter Newell, Rob Kay, and Peter Devreotes, and a number of smaller collections. Every week we scan the literature for new references, and if new strains and plasmids are published we request them immediately. If you have important collections, please discuss their preservation with Jakob Franke. So far, the response and cooperation have been excellent.

In addition we are storing a backup collection of more than 600 characterized REMI mutants from Baylor, and a catalogue for this collection will be available shortly. A plasmid collection is being assembled and will be brought on line in the coming year. In January we made a new update of 197 references available for the Franke literature database, which now contains 7756 references specific to the cellular slime molds. Another update is in the works and will be made available this summer.

At the moment the database is still maintained in Microsoft Access and periodically down-loaded, but we are approaching the point that the entire strain catalogue, including the inventory part, can be maintained on the dictyBase website. We are testing this now, and the goal is to achieve this capability by the end of July.

We get several requests a week at the moment, including requests for technical help, and believe we have been successful in promptly filling the requests for strains, plasmids, and general assistance. Please use the strain repository web page. Funding depends on increased use.

**Useful links**

BLAST server: http://dictybase.org/db/cgi-bin/blast.pl
BLAST database download:http://dictybase.org/db/cgi-bin/dictyBase/download/blast_databases.pl
Colleagues: http://dictybase.org/db/cgi-bin/dictyBase/colleague/colleagueSearch
Developmental Mutants: http://dictybase.org/DdDevelopmentMutants.htm
Gene Ontology (GO): http://dictybase.org/db/html/help/GO.html
GO evidence codes: http://dictybase.org/db/html/help/GOEvidence.html
Help files index: http://dictybase.org/FAQ/HelpFilesIndex.html
Sequence Retrieval: http://dictybase.org/db/html/help/sequence_retrieval.html
Stock Center: http://dictybase.org/StockCenter/StockCenter.html

# Poster Session I

Abstract P1

# A pharmacogenetics approach to defining the molecular mechanism of valproic acid in bipolar affective disorder treatment.

K. Adley and R.S.B. Williams

University College London, UK

Bipolar affective disorder (BAD), or manic depression, is a chronic psychiatric illness affecting approximately 2% of the world population. Lithium and valproic acid (VPA) are widely used treatments for BAD. VPA is a short chain fatty acid with both anti-manic and anti-epileptic functions, and unlike Li, has a low toxicity. However, VPA is teratogenic in 2% of cases causing neural tube defects if taken during the first trimester of pregnancy. The molecular mechanism of these functions is unknown. In order to define the mechanism of action of VPA in the treatment of BAD we have used a pharmacogenetics approach1. Growth and developmental screens of *Dictyostelium discoideum* mutants has identified 25 genes involved in VPA resistance, some isolated multiple times from both screens. Most of the mutant genes isolated can be classified by function into either: RNA transcription/modification, MAP kinase signalling, or lipids/small GTPase signalling.

Our research is currently focused on two genes:

VPR 3-26: a gene product which may modulate small GTPase activities

VPR 3-3: a gene product encoding a putative tyrosine kinase upstream of the MAP kinase signalling pathway.

Both these mutants have developmental resistance to lithium, and in the case of VPR 3-3, non-teratogenic VPA analogues. These resistant phenotypes suggest a possible role for these genes in the anti-manic, anti-epileptic or teratogenic function of VPA.

1 Williams, R. S. B. (2004) Pharmacogenetics in model systems: defining a common mechanism of action for mood stabilisers. Special Topic on Pharmacogenetics: Progress in Neuro-Psychopharmacology and Biological Psychiatry. IN PRESS.

Abstract P2

## Establishment of zyg1 overexpression under the control of V18 promotor in *Dictyostelium mucoroides.*

A. Amagai[1] and H. K. MacWilliams[2]

[1]Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Aoba, Sendai 980-8578, Japan; [2] Zoologisches Institut, Ludwig-Maximilians-Universitat, Luisenstrasse 14, 80333, Munich, Germany

A gene, zyg1, was isolated by differential screening from *Dictyostelium mucoroides*-7 (Dm7) cells, as one preferentially expressed during their sexual development. The zyg1 gene encodes a novel protein (ZYG1; a deduced molecular mass of 29.4 kDa) consisting of 268 amino acids. Although the ZYG1 protein has several predicted PKC phosphorylation sites, it has neither transmembrane domains nor specified signal sequences. When zyg1 was over-expressed using the actin15 or rnrB promotor, northern blots showed enhanced expression of zyg1 and a number of giant cells were formed, possibly by induction of sexual cell fusion. This indicates that the zyg1 expression is involved in zygote formation during sexual development. In order to demonstrate more clearly the function of zyg1 in zygote formation, we attempted to express zyg1 under the V18 promotor, which is known to be highly active in Dm 7 cells.

Here we obtained no significant zyg1 overexpression. In these experiments we used an expression vector in which a zyg1 cDNA was inserted at a Bgl2 site immediately after the ATG originally identified as the translation start. Recently, Dr. Daphne Blumberg's group has demonstrated that translation starts signficantly further upstream (Differentiation 65,73-88, 1999), so that the expressed protein would contain 35 extra amino acids added to the N-terminus. We have now constructed a corrected vector and introduced it into Dm7 cells. Since several positive clones have been selected by the use of 50 μg/ml G418, their phenotypic characterization is under investigation.

Abstract P3

# A functional proteomics approach to dissecting the DIF-1 signalling pathway: DIF-1 rapidly modulates the tyrosine phosphorylation of several cellular proteins.

Tsuyoshi Araki and Jeffrey Williams

Wellcome Trust Biocentre, School of Life Science, University of Dundee, Dow Street, Dundee DD1 5EH, United Kingdom

DIF-1 (Differentiation Inducing factor-1) is a chlorinated hexaphenone, produced by developing *Dictyostelium* cells, that induces pstO cell differentiation. DIF-1 signalling has, as one of its endpoints, a STAT (Signal Transducer and Activator of Transcription) but recent work has identified two additional transcriptional factors that are involved in DIF signalling; the bZIP/bRLZ transcription factor, DimA (Thompson *et al*., 2004) and the single MYB domain protein DdMyb5 (Fukuzawa, Tsujioka and Williams, these abstracts). The DIF-regulated STAT, Dd-STATc, is functionally and structurally similar to the STAT proteins that mediate cytokine function in mammalian cells. Upon stimulation with DIF-1, Dd-STATc is tyrosine phosphorylated, dimerizes and translocates to the nucleus. To dissect upstream steps in these DIF-1 signalling pathways we have performed functional proteomics. Within 3 min of DIF-1 addition to 4 hour starved cells in shaking culture Dd-STATc is tyrosine phosphorylated and our approach is based upon the fact that cytokines induce several essential tyrosine phosphorylation events prior to activating their target STAT. Profiles of tyrosine phosphorylation three minutes after DIF-1 stimulation were analyzed by 2D gel electrophoresis and anti-pTyr Western analysis. Tandem mass-spectrometry (MS/MS) was then used to identify spots of interest. Approximately fifteen species, localized in different cellular compartments, the membrane, cytosol and nucleus, change their level of tyrosine phosphorylation after DIF-1 induction. Thus far, we have putatively identified seven specific proteins that change their tyrosine phosphorylation state after DIF-1 treatment. These include a novel Rho-GAP and cyclase associated protein (CAP). However, because of technical limitations inherent in the method, these now need to be confirmed by analyzing null strains and over-expressors for the candidate proteins. Interestingly, we also find that the *Dictyostelium* GSK-3 homologue, GskA, undergoes a pI shift after stimulation by DIF-1. Thus DIF-1 rapidly modifies the tyrosine phosphorylation profile of the entire cell.

Fukuzawa, M., Araki, T., Adrian, I. and Williams, J. (2001) Tyrosine phosphorylation-independent nuclear translocation of a *Dictyostelium* STAT in response to DIF signalling.

Thompson, C., Fu, Q., Buhay, C., Kay, R. and Shaursky, G. (2004) A bZIP/bRLZ transcription factor required for DIF signaling in *Dictyostelium.*

Abstract P4

## Investigating transcriptional responses to DIFs -1, -2, and -3.

G. Bloomfield[1], J. Skelton[2], T. Saito[3], N. Nikolaidou-Katsaridou[2], B. Barrell[2], R. Kay[1], and A. Ivens[2]

[1]MRC Laboratory of Molecular Biology, UK; [2]Wellcome Trust Sanger Institute, UK; [3]Hokkaido University, Japan

Differentiation Inducing Factors (DIFs) -1, -2, and -3 are a related group of chlorinated alkyl phenones produced by developing *Dictyostelium* cells. They were originally isolated by virtue of their ability to induce stalk cell differentiation in low-density monolayers. DIF-1 has since been shown to be necessary to be essential for the induction for a subset of prestalk cells, however the functions of DIFs -2 and -3 within the developing organism remain unclear. We have used microarrays to catalogue the cells' different responses to these molecules as fully as possible: similarities and differences between the three will be presented and discussed.

Abstract P5

## Cloning putative palmitoyltransferases in *Dictyostelium discoideum.*

Bethany Bodwell and Robert E. Gundersen

Department of Biochemistry, Microbiology and Molecular Biology, University of Maine, Orono, Maine, USA

Heterotrimeric guanine nucleotide binding proteins (G-proteins) are crucial in a broad range of eukaryotic signaling pathways. They function by activating downstream effectors in response to receptor-ligand binding. Most G-proteins require a post-translational lipid modification of myristic and/or palmitic acid for correct localization and function. In the soil amoebae *Dictyostelium discoideum* the transition from unicellular to multicellular development during starvation is dependent on G-protein signaling pathways, in which the G-protein, Gα2, is palmitoylated. The mechanism by which the G-proteins are palmitoylated has only recently been defined. A Ras palmitoyltransferase gene, which contains a zinc finger (DHHC) cysteine rich domain was identified in *Saccharomyces cerevisiae*. A BLAST search of the palmitoyltransferase gene identified in yeast against the *Dictyostelium* genome identified 14 putative palmitoyltransferases, all containing a similar DHHC zinc finger. Experiments have been focusing on cloning two of these genes, termed PAZ4 and PAZ5.

To clone the potential PAZ4 and PAZ5 genes of *Dictyostelium*, a reverse transcriptase (RT) reaction with an oligo dT primer was preformed using total RNA isolated from growing cells. The cDNA from the RT reaction was then subjected to a polymerase chain reaction (PCR) using primers designed for PAZ4 and PAZ5 to isolate sufficient material for cloning. The cDNA from the PCR reactions was cloned into a vector (pCR4, Invitrogen) containing ampicillin resistance and transformed into competent *E. coli*. Plasmids containing insert (based on a restriction enzyme digestion) were sequenced for confirmation. To date, a complete PAZ5 gene has been obtained and a partial clone for PAZ4. The cloned PAZ5 cDNA has been expressed in an *in vitro* transcription/translation system using $^{35}$S-methionine. To determine if PAZ5 possesses palmitoyltransferase activity, $^{3}$H-palmitate labelling will be performed. In addition, PAZ5 will be over-expressed in *Dictyostelium* as well as generating gene knock-outs.

Abstract P6

## Altered stalk length and loss of tip dominance in *Dictyostelium* cell lines with RNAi-mediated reduction of calcineurin B expression.

Katrina Boeckeler, Barbara Weissenmayer, and Rupert Mutzel

Fachbereich Biologie, Chemie, Pharmazie, Institut für Biologie - Mikrobiologie, Freie Universität Berlin, Königin-Luise-Strasse 12-16, D-14195 Berlin, Germany

The *cnbA* gene in *Dictyostelium discoideum* codes for calcineurin B (CNB), the regulatory subunit of the $Ca^{2+}$/calmodulin-dependent protein phosphatase calcineurin. In order to analyze the role of CNB in *Dictyostelium*, the *cnbA* gene was silenced by expression of RNAi, a mechanism which was recently demonstrated to function in *Dictyostelium* (Martens *et al*., 2002).

We found a variety of silencing levels of *cnbA* mRNA induced by a construct expressing a stem-loop RNA directed against *cnbA*. We isolated recombinant cell lines expressing virtually wild type levels as well as clones with drastically reduced *cnbA* mRNAs. Reduction of the mRNA level in a given cell line correlated with a reduction of calcineurin B protein concentration. Cell lines with a significantly (>10-fold) reduced protein level completed development but formed approximately 50% shorter stalks than wild type fruiting bodies. Many of the culminating structures formed by these cells showed one or more additional, irregularly arranged tips protruding from the spore heads, suggesting that the protein phosphatase plays a crucial role in maintaining tip dominance and in the regulation of tissue size.

Abstract P7

## Assigning function to genes using microarray profiling of mutant strains.

Ezgi O. Booth and Gad Shaulsky

Baylor College of Medicine, W. M. Keck Center for Computational and Structural Biology

*Dictyostelium discoideum* provides a multicellular model for studying gene expression during development. Changes in gene expression represent physiological changes in the cell. Although most of the genome has been sequenced, many of the genes' functions are unknown. Our goal is to develop an efficient, high-throughput method for assigning function to unknown genes. Using microarray profiles as a phenotyping tool, we plan to propose gene function by analyzing several hundred mutants. Within the next couple of years, we expect to obtain 5000 *D. discoideum* mutants. Using our standard technique, it would be hard to analyze so many mutants. Thus it is necessary to develop methods that will enable us to obtain expression profiles of these mutants rapidly. In our previous studies, we found a considerable number of genes with identical expression patterns. We therefore looked for a smaller number of microarray targets that would provide the same information as the large array but would be easier to produce.

Through k-means clustering analysis, 57 unique expression groups were identified based on wild type and 17 *D. discoideum* mutant developmental time courses. Gene ontology analyses on these groups indicate that various gene expression groups belong to certain functional groups, enabling us to reduce the 7744 current microarray targets to a mini-chip that contains about 300 genes. The targets were chosen as representatives of each gene expression pattern identified in the wild type development and in our current mutant collection.

Targets for the mini-chip platform were selected from each group based on how well their expression profile represents the groups they belong to, their spot quality, reproducibility, and function. After selection, targets were tested computationally and experimentally to ensure consistent results with previous findings obtained using the 7744-target microarray.

Following the analysis of mutants, we will build an interactive database that will contain all the analyzed mutants allowing addition of more mutants at any time. In the database, the mutants will also be assigned to groups based on proposed function and similarity of their expression profiles, enabling researchers to propose specific tests for function of mutants. Our research aims will combine novel experimental and computational methods to provide a fast and powerful tool to assign function to genes.

Abstract P8

## Differential effects of Nramp1 expression on phagocytosis and resistance to infection by pathogenic bacteria.

A. Balest, B. Peracino, C. Skriwan*, M. Steinert*, and S. Bozzaro

Department of Clinical and Biological Sciences, University of Turin, Ospedale San Luigi, 10043 Orbassano, Italy; *Institut for Molecular Infection-biology, University of Wuerzburg, 97070 Wuerzburg, Germany

*Dictyostelium* amoebae share with mammalian phagocytes the lysosomal Nramp1 protein, a metal ion transporter that confers resistance to infection by invasive microorganisms, such as legionella, mycobacteria, salmonella, leishmania. *Dictyostelium* Nramp1 gene is transcribed during growth and down-regulated upon starvation. Disruption of the Nramp1 gene by homologous recombination or by an antisense expression results in cells with increased sensitivity to infection by *L. pneumophila* and *M. avium*. Constitutive overexpression of Nramp1 protects the wild-type AX2 cells from infection.

By using GFP fusion proteins with Nramp1 or the vatB subunit of the V-H+ATPase we have found that Nramp1 marks intermediate and late steps in the endocytic pathway. As expected, GFP-vatB is inserted in membranes of all acidic compartments, in addition to the contractile vacuole system. In contrast, GFP-Nramp1 decorates only a subset of acidic vesicles, the Golgi and post-lysosomal vesicles, which are positive for vacuolin and negative for vatB. Pulse-chase experiments with TRITC-dextran show that endosomes fuse first with vatB-positive and later on with Nramp1-positive vesicles.

To gain insights into the function of Nramp1, we have followed the in vivo dynamics of ingested pathogenic (*L. pneumophila* and *M. avium*) and non-pathogenic (*E. coli*) bacteria in pulse-chase experiments. The number of *E. coli* and *M. avium* fused with GFP-Nramp1 vesicles is comparable during the first minutes of chase, reaching a maximum of 70% at 30min, but later on diminishes for *E. coli* while remaining high for *M. avium*. In contrast, less than 30 % of *L. pneumophila* are found in GFP-Nramp1 vesicles. The data suggest that *L. pneumophila* avoids, while *M. avium* tends to stay in Nramp1-positive vesicles. Remarkably, during infection by *L. pneumophila*, in contrast to *M. avium*, and to incubation with *E. coli*, Nramp1 gene expression in AX2 is selectively repressed. A selective repression of Nramp1 gene expression is also found with iron, which together with zinc and manganese is transported by Nramp1. The results suggest that *L. pneumophila* and *M. avium* use different ways to neutralize Nramp1, probably linked to different requirements for the metal ions.

Abstract P9

## Looking for PI3K independent pathways regulating actin polymerization in chemotaxis.

L. Chen, M. Iijima, Y. Huang, C. Janetopoulos, and P.N. Devreotes

School of Medicine/Johns Hopkins University

The directional movement of cells in chemoattractant gradients requires regulation of actin cytoskeleton. Uniform exposure of D. discoideum to chemoattractant triggers two phases of actin polymerization. The fast phase of PI(3,4,5)P3 accumulation and actin polymerization is relatively resistant to PI3K inhibition. Cells under this condition can do inefficient but reasonably accurate chemotaxis. The slower phase of actin polymerization is sensitive to inhibition of PI3K. Based on these results, we believe that there is either further amplification steps downstream of PI3K or a parallel pathway independent of PI3K which also regulate actin polymerization. A genetic screen was carried out aimed to find possible components regulating the first phase of actin polymerization. Mutants which failed to aggregate in the presence of PI3K inhibitor were found. We are currently trying to identify and characterize these genes.

Abstract P10

## A phospholipase D negatively regulates quorum sensing in *Dictyostelium discoideum* development.

Yi Chen, Vanessa Rodrick, Yi Yan, and Derrick Brazill

Department of Biological Sciences, Hunter College, 695 Park Avenue, New York, NY 10021

Quorum Sensing, the ability to measure the local cell density, is required for animal cells to achieve proper cell growth, differentiation and development. *Dictyostelium discoideum* cells, when starved, are able to calculate the concentration of surrounding starving cells by simultaneously secreting and sensing a glycoprotein named conditioned medium factor (CMF). With a low density of starving cells, the corresponding low level of CMF obstructs the signaling through the chemoattractant cAMP receptor cAR1, thus inhibiting the aggregation. When the density of starving cells is high ($>10^5$ cells/$cm^2$), the high level of CMF allows the cAMP-cAR1 signal transduction to occur, followed by cell aggregation and development. Binding of cAMP to cAR1 activates a heterotrimeric G protein with a Gα2 alpha subunit. We have previously shown that CMF regulates cAMP signaling in part by decreasing the intrinsic GTPase activity of Gα2. CMF attains this by activating a signaling cascade that involves Gα1, phospholipase C and protein kinase C (Pkc).

Since Pkc stimulates several enzyme activities, including phospholipase D (Pld), in mammals, we have been examining the role of Pld in CMF signaling. The gene *pldB*, with high homology to mammalian Pld1, shows expression by 8 hours of development and peaks at 16 hours. Pld activity is three-fold higher by 24 hours of development compared to that of vegetative cells. *pldB* knockout cells are able to chemotax at the same speed as wild type cells and form similar fruiting bodies. But these *pldB*$^-$ cells develop at a faster pace than wild type cells, indicating that *pldB* is involved in the timing of development. To determine why *pldB*$^-$ cells develop early, we examined the timing of expression of cAR1, CRAC and ACA in *pldB*$^-$ cells. While CRAC and ACA showed expression kinetics similar to wild type cells, cAR1 showed earlier and higher levels of expression in the *pldB*$^-$ cells. Moreover, *pldB*$^-$ cells can aggregate at very low cell density, implying that *pldB* is a negative regulator of quorum sensing. This ability of *pldB*$^-$ cells is cell autonomous.

Abstract P11

## Visualisation of the *Dictyostelium* exocyst complex in moving amoebae using fluorescently labelled proteins.

Margaret Clotworthy and David Traynor.

MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, England

The exocyst is an octameric protein complex that is believed to be involved in tethering exocytic vesicles to specific regions of the plasmalemma undergoing membrane expansion, such as at the bud tip in budding yeast. The complex was first identified in yeast and has homologues in *Dictyostelium* and other genomes. The members of the complex are Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84.

In order to try to identify sites of active membrane addition in moving *Dictyostelium* amoebae, various components were tagged with fluorescent proteins and over expressed. Sec6, a component thought to reside on the target membrane for exocytic vesicular fusion, was observed to localise at the rear rim of moving amoebae. Sec4, a rab family GTPase thought to bind exocytic vesicles and regulate their interaction with the exocyst, also localised to vesicles at the rear edge of the uropod. A GTP-locked, and hence presumably constitutively active version of Sec 4 concentrated in the rear in a similar fashion.

Further characterisation of this complex may involve the generation of knock-ins and temperature-sensitive mutants to elucidate the possible role of the exocyst in cell motility.

Abstract P12

## Study of *Dictyostelium discoideum* Nramp homologs.

L. Cortes[1], L. Wilson[1], K. Doquang[2], A. Tsang[2], and M. Cellier[1]

[1]INRS-Institut Armand-Frappier, 531 Boul. des Prairies, Laval, Québec, Canada, H7V 1B7; [2]Centre for Structural and Functional Genomics, Concordia University, 7141 Sherbrooke West, Montreal, Québec, Canada, H4B 1R6

Members of the Natural Resistance-Associated Macrophage Protein (Nramp) family transport divalent cations (e.g. iron, $Fe^{2+}$, manganese, $Mn^{2+}$) and protons from outside of the cell (or the interior of an intracellular vacuole) towards the cytoplasm. The divalent cations $Fe^{2+}$ and $Mn^{2+}$ are vital nutrients and cofactors of many enzymes. Homologs of the Nramp family were characterized in eukaryotes (yeast, animals, plants) and in bacteria. Phylogenetic analyses suggest that this family emerged in procaryotes and that a bacterial Nramp gene was transferred to the eucaryotic nucleus after endosymbiosis of the mitochondria precursor; the resulting ancestral eukaryotic Nramp gene was then duplicated yielding a "prototype" and an "archetype" *Nramp* gene. One copy of each gene was found in *D. discoideum* (Dicty) while vertebrate Nramp, including Nramp1, key for host resistance to intracellular pathogens and Nramp2, required for iron homeostasis, were derived from the "archetype" *Nramp*. The present study aims at studying the physiological role of Dicty Nramps.

Sequences retrieved from dictyBase were analyzed using the softwares Clustal X, Mega 2 and Tree-puzzle. Genes were PCR amplified using purified genomic DNA, cloned in *E. coli* and sequenced. Disrupted alleles were created by cloning both halves of the orf (or exon 2) in 5' and 3' of a blasticidin resistance cassette in the vector pRHI. The constructs were sequenced and are used to generate *Nramp* knockout strains by electroporation, selection of blasticidin resistant clones and Southern blot analysis. A rabbit specific antiserum was raised against Dicty "prototype" Nramp N-terminus that was produced as a glutathione-S-transferase fusion polypeptide. The site of cellular expression of Dicty "prototype" Nramp protein will be investigated by in situ indirect immuno-fluorescence. A 1542 bp orf encoding Dicty "prototype" Nramp (514 a.a.) was cloned in *E. coli*. The deduced a.a. sequence contains 12 hydrophobic segments corresponding to predicted transmembrane domains. The predicted topology places both the N- and C-termini within the cytoplasm, a transmembrane organization that is most common among "archetype" Nramp. Phylogenetic analyses indicated that Dicty "prototype" has close homologs in the mosquito *A. gambiae* and in primitive plant *spp*., which are closer to bacterial Nramp homologs than to "archetype" Nramp. Expression of Dicty "prototype" Nramp in *E. coli* was not functional in the conditions tested and will be tested by transfection of Dicty.

Abstract P13

# Disruption of the NCS-1/Frequenin-related gene (ncsA) in *Dictyostelium discoideum* accelerates development.

B. Coukell, A. Cameron, and S. Perusini

York University, Toronto, Ontario, Canada

Neuronal calcium sensor (NCS) proteins are members of the calmodulin superfamily of $Ca^{2+}$-binding proteins. Proteins in this subgroup are N-myristoylated, about 190 amino acids in size, and possess four putative EF-hands (I-IV), although EF-hand I (and occasionally IV) are non-functional. NCS proteins have been implicated in the regulation of a variety of cellular processes including neurotransmitter release, cyclic nucleotide metabolism, polyphosphoinositide biosynthesis and gene expression. Although these proteins were originally discovered in neurons, they are now known to function in cells from yeast to mammals. To learn more about the role of intracellular $Ca^{2+}$ in *Dictyostelium* during growth and development, we searched the *Dictyostelium* cDNA and genomic databases for genes encoding NCS-related proteins. As a result, five new $Ca^{2+}$-binding proteins were identified represented by the cDNA clones SSL268, VSA595, SSD217, VSD748 and VSJ160. Based on amino acid sequence alignments and phylogenetic analyses, the protein encoded by SSL268 is most closely related to NCS proteins in other organisms. The gene was designated *ncsA* and its protein product NcsA. Northern blot analyses revealed that *ncsA* mRNA is expressed at very low levels during growth and early development but it is up-regulated substantially during late aggregation and the slug stage (9-18 h). Pure recombinant NcsA (expressed in *E. coli)* binds $^{45}Ca^{2+}$ in overlay experiments and exhibits a dramatic mobility shift on SDS-PAGs in the presence of $Ca^{2+}$. The single copy *ncsA* gene in strain AX2 was disrupted using a blasticidin S cassette. The *ncsA*-null cells were viable and grew normally in axenic medium. However, in lawns of *K. aerogenes*, the clones grew slowly and development began prematurely within the clones. In larger clones, the *ncsA*-null cells formed narrow growth zones (halos) with evenly spaced aggregates along the inner edge, and towards the middle, closely packed fruiting bodies with large spore caps. An analysis of intracellular cAMP production, development on PBS agar and stage-specific gene expression suggested that development of the *ncsA*-null cells is accelerated by about 3 h compared to control cells. Moreover, this increased rate of development seemed to be due primarily to a reduced sensitively of the cells to nutrients in the environment. In *S. cerevisiae* and in mammalian cells, the NCS protein NCS-1/Frequenin appears to function by binding to and activating phosphatidylinositol 4-kinase beta (PI4Kβ). In *Dictyostelium*, one PI4Kβ gene (*pikD*) has been identified (1). However, NcsA probably does not act through this enzyme because NcsA and PikD do not interact in pull-down assays and PikD appears to lack the 28 residue hydrophobic N-terminal motif essential for NCS-1/Frequenin binding (2). Interestingly, it has been reported recently that disruption of the NCS-1/Frequenin homolog in *S. pombe* (*ncs1*) results in nutrition-insensitive sexual development involving altered cAMP metabolism, possibly by altering the regulation of the glucose-sensing G protein-coupled receptor, Git3p (3).

Therefore, NcsA might function by regulating an analogous nutrient-sensing system in *Dictyostelium*. (Supported by the NSERC of Canada).

(1) Zhou K., Takegawa, K., Emr, S.D., Firtel, R.A. (1995). Mol. Cell. Biol.15: 5645-5656.
(2) Strahl, T., Grafelmann, B., Dannenberg, J., Thorner, J., Pongs, O. (2003). J. Biol. Chem. 278: 49589-49599.
(3) Hamasaki-Katagiri, N., Molchanova, T. Takeda, K., Ames, J.B. (2004). J. Biol. Chem. 279: 12744-12754.

Abstract P14

## Copines in *Dictyostelium*.

Damer, C.K., Bayeva, M., Hahn, E.S., Naliboff, L., Rivera, J., and Socec, C.I.

Vassar College

Copines are soluble, calcium-dependent membrane binding proteins found in a variety of eukaryotic organisms. This family of proteins is characterized by two C2 domains at the N-terminus and a region similar to the A domain found in integrins at the C-terminus. The *Dictyostelium discoideum* genome contains five copine genes, *cpnA*-*cpnE*, and a possibe sixth copine gene, *cpnF*. Our studies are focused on one of the copine genes, *cpnA*. RT-PCR and Western blot analyses indicate that *cpnA* is expressed throughout development. Membrane binding assays indicate that CpnA binds to native *Dicytostelium* membranes in a calcium-dependent manner *in vitro*. A GFP-tagged version of CpnA expressed in wildtype *Dictyostelium* also binds to native *Dictyostelium* membranes in a calcium-dependent manner. In flattened fixed cells, GFP-CpnA labels the plasma membrane and intracellular vacuoles. The size, shape, and number of these vacuoles suggest they are part of the contractile vacuole and/or endolysosomal systems. Cells were placed in hypo-osmotic conditions to increase the size of contractile vacuoles, and this resulted in a corresponding increase in the size of some of the GFP-CpnA labeled vacuoles. In addition, contractile vacuoles were labeled with an antibody to calmodulin, definitively identifying some of the GFP-CpnA labeled vacuoles as contractile vacuoles. Cells were treated with rhodomine-labeled dextran to label endolysosomal organelles. The rhodamine-labeled structures did not coincide with the GFP-CpnA labeled vacuoles. In intact live cells, GFP-CpnA fills the cytoplasm so that no membrane labeling is observed. However, when the integrity of the plasma membrane is compromised, labeling of the plasma membrane and vacuoles is observed. Fluorescence imaging of live broken cells in the presence and absence of calcium indicates that the binding of GFP-CpnA to membranes is calcium-dependent. We are currently working on a project to inhibit the function of CpnA *in vivo* by expressing a dominant negative mutant CpnA tagged with GFP in wildtype *Dictyostelium*.

Abstract P15

## *Dictyostelium discoideum* paxB- shows an aberrant phenotype in fruiting body formation.

M. Berenice Duran and Derrick Brazill

Department of Biological Science, Center for Study of Gene Structure and Function Hunter College, City University of New York Graduate Center, 695 Park Avenue, New York, NY 10021

The adhesion of cells to the surrounding extracellular matrix is a key element in the environmental cues necessary to control a cell's decision to survive, proliferate, differentiate or migrate. Cell adhesion is an important component of *Dictyostelium* morphogenesis and is required for multicellular development past the aggregation stage. As in animals, cell adhesion molecules have not only a mechanical function, but may also interact with the signal-transduction processes governing morphogenesis.
In mammals, the protein paxillin has been known to have functions as a focal adhesion molecule, mainly by serving as a docking site on the plasma membrane for signaling and structural proteins. Paxillin has been found to play a role in cell spreading and motility in mammalian cells. To gain a better understanding of the role and regulation of paxillin, we are studying the role of *Dictyostelium discoideum* paxillin homolog, PaxB. We have found *paxB* expression pattern occurs in all of the developmental stages, particularly higher levels of *paxB* expression are observed between eight and twelve hours of development. We have observed several phenotypes in transformed *paxB* knockouts, all of these involved additional fruiting bodies forming on top of spore mass. We are currently examining the protein production pattern and the effects of *paxB* overexpression in *Dictyostelium* development.

Abstract P16

## A model of cell localization in the migrating slug of *Dictyostelium discoideum*: the roles of differential sensitivity to cAMP chemotaxis and of differential sensitivity to suppression of such chemotaxis by ammonia.

I.N. Feit, J.S. Pawlikowski, and B.S. Davis

Franklin and Marshall College, Lancaster, PA 17603 USA

We present a model of localization of three cell types (pre-stalk, anterior-like and prespore cells) in the migrating slug of *Dictyostelium discoideum*.

Our model is centered on both the differential chemotactic response of the cell types to cAMP and the differential sensitivity of the cell types to suppression of such chemotaxis by ammonia.

Our data is based on a drop assay that we have reported previously [Feit, Medynski & Rothrock (2001) J Biosci. 26: 157-66].

Some of our data is preliminary and we plan to extend our studies to pre-stalk cell subtypes.

Abstract P17

# A novel disintegrin domain protein functions extracellularly to regulate early cell type specification in *Dictyostelium discoideum.*

H.N.Ho, B.L. Ford, and D.D. Blumberg

Department of Biological Sciences, University of Maryland, Baltimore County, Baltimore, Md. 21250

The ampA gene encodes a 28Kd secreted protein that shares homology with two structurally distinct anticoagulants, the disintegrins, a class of hemorrhagic peptides found in snake venom and the ornatins from leech saliva (1). In higher eukaryotes these anticoagulants function as competitive inhibitors of integrin receptors. We have previously demonstrated that AmpA modulates cell-cell and cell-substrate adhesions and plays a role in reorganizing the actin cytoskeleton to facilitate cell migration (1). AmpA additionally plays a role in initial cell type specification during development (2). Expression of the ampA gene is restricted to the population of anterior-like cells (ALCs) during development (3). The AmpA expressing ALCs are initially scattered throughout the aggregate but then sort to two locations; the majority are found at the mound periphery, while a small subset are located in the interior of the mound (3). Insertional inactivation of the ampA gene results in cells at the periphery of mounds, that will ultimately differentiate into prestalk cells, initially expressing a prespore reporter (2). Inclusion of only 10% wild type cells in chimeras with 90% AmpA null cells is sufficient to rescue the premature mis-expression of the prespore marker (2). Here we demonstrate that a supernatant source of AmpA protein added extracellularly can prevent this premature mis-expression of the prespore marker. We also demonstrate that a factor capable of inducing cells to activate prespore reporter expression accumulates extracellularly in the absence of AmpA protein. Synthetic oligopeptides and alanine substitution mutagenesis have been used to identify a 9 amino acid active site sequence that is important for preventing cells from premature prespore gene mis-expression. Alignment of the AmpA cysteine repeats with the cysteines of the disintegrin and ornatin peptides indicates that the active site of the AmpA peptide is in the same position as the integrin receptor binding site of the ornatin and disintegrin peptides. While *Dictyostelium* is not known to have integrin receptors, the structural and functional homology suggests that the binding motif is evolutionarily preserved. A model for AmpA acting through a lateral inhibition mechanism to prevent cells at the mound periphery from assuming a prespore fate is discussed.

1) Varney, T. R., Casademunt, E., Ho, H., Petty, C., Dolman, J., and Blumberg, D. D. (2002). A novel *Dictyostelium* gene encoding multiple repeats of adhesion inhibitor-like domains has effects on cell-cell and cell-substratum adhesion. Developmental Biology 243, 226-248.
2) Varney, T. R., Ho, H., Petty, C., and Blumberg, D. D. (2002). A novel disintegrin domain protein affects early cell fate specification and pattern formation in *Dictyostelium*. Development 129, 2381-2389.
3) Casademunt, E., Varney, T. R., Dolman, J., Petty, C., and Blumberg, D. D. (2002). A gene encoding a novel anti-adhesive protein is expressed in growing cells and restricted to anterior-like cells during development of *Dictyostelium*. Differentiation 70, 23-35.

Abstract P18

# Up-regulation of rnrB expression in response to DNA damage is important for survival.

Pascale Gaudet[1, 2], Harry MacWilliams[3], and Adrian Tsang[1]

[1]Center For Functional and Structural Genomics, Concordia University, Montreal, Canada; [2]Present address: dictyBase, Northwestern University, Chicago, IL, USA; [3]Ludwig-Maximilians-Universität, München, Germany

Ribonucleotide is the enzyme responsible for the conversion of ribonucleotides into deoxyribonucleotides. This enzymatic reaction is the first unique step for DNA synthesis. Therefore ribonucleotide reductase expression and activity are highly regulated. The enzyme has an $\alpha 2\beta 2$ structure, where the $\beta$ subunit is the catalytic subunit. In all organisms studied so far, expression of the small subunit varies according to the cell cycle and in response to DNA damaging agents (Reichard (1988) *Annu. Rev. Biochem.* 57:349, Engstrom *et al.* (1985) *J. Biol. Chem.* 260:9114, Elledge and Davis (1990) *Genes Dev.* 4:740). The gene appears to be essential, because a null mutant in inviable in yeast (Elledge and Davis (1997) *Mol Cell Biol.* 7:2783). The activity of ribonucleotide reductase is highly regulated allosterically. The tight regulation of gene expression provides an additional level of control of the ribonucleotide reductase activity in the cell. Thus far, the physiological importance of the transcriptional regulation of ribonucleotide reductase had not been investigated. In *Dictyostelium*, growth events are independent from developmental events. We took advantage of the unique properties of the *Dictyostelium discoideum* life cycle to address this question.

We have previously shown that the elements of the *rnrB* promoter located between -130 and -212 relative to the ATG are required for expression of this gene in vegetatively growing cells (Bonfils, Gaudet and Tsang (1999) *J Biol Chem* 274: 20384-90). To investigate the physiological importance of proper expression of *rnrB*, we have constructed a targeted disruption of the *rnrB* upstream regulatory elements (nucleotides -212 to -450). We show that the mutant still expresses *rnrB* during vegetative growth, but expression is not cell cycle-regulated. The mutant also does not express the *rnrB* gene during development. There is no up-regulation of the *rnrB* transcript after treatment with DNA damaging agents in the mutant. The mutant divides at a slower rate compared to the control.

We have investigated the physiological importance of up-regulation of the *rnrB* transcript in the presence of DNA damage. Our results indicate that the sensitivity of the mutant to DNA damaging agents is increased by more than two-fold. This is the first time that the up regulation of a DNA synthesis gene is shown to provide increased survival to exposure to DNA damaging agents

Abstract P19

## A mathematical model of cAMP-induced phosphatidylinositol metabolism during chemotaxis.

J.S. Gruver[1], P.S. Crooke[2], and C.Y. Chung[1]

[1]Department of Pharmacology, Vanderbilt University Medical Center, Nashville, TN 37232 USA; [2]Department of Mathematics, Vanderbilt University, Nashville, TN, USA

Chemotaxis requires a complex signal transduction pathway spanning from the cAMP receptor to the activation of many downstream effectors including adenylyl cyclase, the Ras and Rho-family GTPases, PI3 Kinase, and PTEN. Although genetic and cellular studies continue to expand the magnitude and the complexity of the cAMP sensing system we have identified cAMP-induced phosphatidylinositol metabolism for combined experimental and theoretical study. The metabolism of phosphatidylinositols displays interesting spatial and temporal dynamics in response to various chemoattractant stimuli and the spatially restricted production of the phosphatidylinositol PIP3 appears to serve as a directional cue during chemotaxis. A detailed ordinary differential equation model was developed with the aim of capturing the temporal response of the sensory system. The model is based upon experimentally established signaling pathways and includes receptor-ligand dynamics, G-protein activation, the reciprocal translocation of PI3K and PTEN and the transient membrane accumulation of the second messenger PIP3. The model will be experimentally validated using a combination of molecular and cellular techniques. The final iteration of this model will be extended to a partial differential equation based reaction-diffusion model to address the spatial and temporal control over phosphatidylinositol metabolism during chemotaxis.

Abstract P20

## The role of myosin II phosphorylation in motility and chemotaxis.

P.J. Heid[1], K. J. Daniels[1], D. Wessels[1], D. P. Gibson[1], H. Zhang[1], E. Voss[1], T. T. Egelhoff[2], and D. R. Soll[1]

[1]University of Iowa, USA; [2]Case Western Reserve University, USA

To assess the role of myosin II heavy chain (MHC) phosphorylation in basic motility and natural chemotaxis, the mhcA null mutant mhcA-, mutant cells with MHC that mimics the constitutively unphosphorylated state (3XALA) and mutant cells with MHC that mimics the constitutively phosphorylated state (3XASP), were analyzed in buffer and in response to the individual spatial, temporal and concentration components of the wave using computer-assisted methods. Each mutant strain exhibited unique defects in cell motility and chemotaxis. Although mhcA- cells crawled and chemotaxed in a spatial gradient of cAMP, they were very slow, more three-dimensional than control cells, underwent chemotaxis with greatly reduced efficiency, and were incapable of responding to temporal gradients of cAMP. 3XASP cells displayed defects similar to those of mhcA- cells, but were slightly faster and chemotactically more efficient. They failed to respond to temporal gradients of cAMP. 3XALA cells were fast, chemotaxed in spatial gradients of cAMP and were able to respond to temporal gradients of cAMP, but exhibited a 50% reduction in chemotactic efficiency, could not stream late in aggregation and could not enter the streams of control cells in mixed cultures. F-actin staining revealed that while the presence of unphosphorylated MHC was essential for the increase in F-actin in the cytoplasm in response to the increasing temporal gradients of cAMP, the actual dephosphorylation event was essential for the associated increase in cortical F-actin.The results of these studies indicate that MHC phosphorylation-dephosphorylation, like myosin II regulatory light chain phosphorylation-dephosphorylation, represents a potential downstream target of regulatory cascades emanating from different phases of the wave. Since the MHC and MRLC kinases would be the upstream phosphorylating enzymes, we have initiated studies of the null mutants of these enzymes.

Phosphorylation-dephosphorylation of MHC and MRLC, therefore, play fundamental roles in the behavior of *Dictyostelium* amoebae. Although the general distribution of myosin II has been analyzed by immunofluorescent staining, there are strong indications that its phosphorylation state dictates function and localization. To assess localization of the different phosphorylation states of myosin II, we have generated monoclonal antibodies that specifically identify: 1) the phosphorylated state of MHC; 2) the unphosphorylated state of MHC; 3) the phosphorylated state of MRLC; and 4) the unphosphorylated state of MRLC. Staining of *Dictyostelium* amoebae migrating in buffer reveals four staining patterns that depend upon the phosphorylated state of MHC and MRLC.

Abstract P21

## Analysis of *Legionella pneumophila* pathogenesis in *Dictyostelium discoideum*.

D.W. Hilbert, R.J. Fitzhenry, Otto G.P, Wu M.Y., H.A. Shuman, and R.H. Kessin

Columbia University, USA

*Legionella pneumophila* is an opportunistic pathogen that causes a severe pneumonia in humans. In the environment, *L. pneumophila* infects freshwater amoebae, such as *Acanthamoebae castellani*. When aerosolized and inhaled, the bacterium is internalized by alveolar macrophages. Unlike most internalized bacteria, the phagosome containing *L. pneumophila* does not fuse with lysosomes, but rather is remodeled into a replicative vacuole resembling the endoplasmic reticulum. In both protozoan and mammalian cells, *Legionella* alters the fate of its compartment by injecting a number of effector proteins into the host cell cytoplasm via a Type IVB translocation apparatus. *L. pneumophila* also infects the social amoeba *Dictyostelium discoideum*; morphogenesis of the replication vacuole and virulence factors required are similar to *A. castellani* and macrophages. We are pursuing a number of strategies using the unique genetic and cell biological properties of *D. discoideum* to learn more about the pathogenesis of *L. pneumophila*. Published reports suggested that the cellular recycling pathway of macroautophagy is involved in bacterial replication; however, a reverse genetic approach revealed that the process is dispensable. In order to identify host processes utilized by the pathogen, we have begun an exhaustive mutant screen using novel high-throughput technology. In addition, we are purifying the *L. pneumophila* phagosome at different stages of infection and using phage display and proteomic approaches to characterize this unique organelle in detail. Based on the similarity of infection process in multiple hosts, we believe that our findings will have broad applicability with regards to *L. pneumophila* infection and intracellular pathogenesis in general.

Abstract P22

## Nuclear fusion and microtubule organization in the early stages of sexual development in *Dictyostelium discoideum.*

K.Ishida and H.K.Urushihara

University of Tsukuba, Japan

Cells of *Dictyostelium discoideum* feed on bacteria as single amoebae and gather upon starvation to culminate in fruiting bodies. Under submerged and dark conditions, however, the cells become sexually mature and fuse with opposite mating-type cells to form zygote giant cells, which finally develop into dormant structures called macrocysts. This is a sexual reproduction process of *D. discoideum*. We recently observed that the multinuclear fused cells formed during this process do not behave as united cells but continue random independent movements toward different directions, which result in cytokinesis. The split cells were capable of re-fusion, and repeated cell division. This radical behavior continued till around 12 hours of sexual development, when giant cells and peripheral cells started aggregation. These observations suggest that gamete fusion and initiation of zygote development do not coincide in the mating of *D. discoideum.* Then, what are the signals for ultimate co-ordination of random and local movements within the fused cell? As the first step toward this question, we examined nuclear behaviour and the distribution of cytoskeletal components. Analysis by confocal microscopy showed that the giant cells contain many nuclei at the beginning, but just one or two at 8 hours of development, indicating that nuclear fusion occurs around 8 hours of sexual development. This was also confirmed by flow cytometric analyses. We also found that microtubule localization dramatically changed during macrocyst development, from uniformly cytoplasmic to exclusively peripheral. Moreover, microtubule organizing centers (MTOCs) abundant at the beginning gradually decreased and only one of them remained within a developed macrocyst. These results suggest that change in microtubule organization is closely associated with and probably responsible for nuclear fusion. In addition, genes known to control cell movement, such as rasGEFB and rasS, increase shortly before the cessation of repeated fusion-cytokinesis.

Abstract P23

## Genetic analysis of dedifferentiation mutants in *Dictyostelium discoideum*.

Mariko Katoh, Adam Kuspa, and Gad Shaulsky

Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

Using microarray analysis of gene expression, we have shown that *Dictyostelium* dedifferentiation is defined by three parameters: cell division, DNA synthesis and erasure event and is a genetically regulated process. When developing *Dictyostelium* cells are dissociated they can re-aggregate within less than an hour and finally form fruiting body within 12-14 hrs. The property of rapid re-development is lost after 12-16 hours of dedifferentiation (erasure). This rapid spore formation can be used for selection of mutations that are defective in dedifferentiation because these mutants should retain the ability to re-develop rapidly while non-erasure mutants lose that ability. We tried to perform the screen for mutants with altered dedifferentiation timing using the property of rapid spore formation. A large population of REMI mutants (Blasticidin resistant) were developed to the slug stage, dedifferentiated past the wild-type erasure point in HL5 and re-developed with disaggregated wild-type (Blasticidin sensitive) as a carrier to provide the critical number of cells for development. Mutant cells arising from spores that formed within 12-14 hrs were selected by Blasticidin, then grown and developed, and the process was repeated 2-4 times until defective dedifferentiation mutants took over the population. We will describe several mutated genes that have been cloned and characterized.

Abstract P24

## Determination of the elastic properties of *Dictyostelium discoideum* cells using the atomic force microscope.

B. J. Haupt[1], R. Spanhoff[1], M. Osbourn[1], S. de Keijzer[1,2], E. Snaar-Jagalska[2], and T. Schmidt[1]

[1] Physics of Life Processes, LION, Leiden University; [2] Molecular Cell Biology, IBL, Leiden University

Our knowledge about the coupling of chemotactic signalling to cell motility is still in its infancy. In the current study we used the atomic force microscope (AFM) to investigate the elastic properties of the slime mould *Dictyostelium discoideum.* The AFM is a scanning probe microscope that uses a sharp tip on a flexible cantilever as a force probe. It can be used to obtain topographic images as well as a sample's response to an applied force. Live wild type, and mutant, *Dictyostelium* cells were compared in both the vegetative and the pre-aggregate state while adsorbed to a glass substrate. We used force volume imaging to simultaneously obtain high resolution topographic and elastic maps of single cells. Differences in the Young's Modulus, which is a measure of stiffness, of polarized with respect to unpolarized cells were found. There is a tenfold increase in stiffness of polarized AX3 wild type cells and a twofold increase for the mutant lacking the actin-interacting protein 1 (DAip1). This suggests there is a relationship between elasticity and polarity of the cell during chemotactic movement. Further, the Young's Modulus of the front (pseudopod) and rear of polarized cells was found to be different.

Abstract P25

## Novel mode of cell growth regulation by *Tuberous sclerosis* protein 2 (TSC2); the role of *Dictyostelium* TSC2 and Rheb in phagocytosis.

D. Rosel and A.R. Kimmel

National Institutes of Health, USA

*Tuberous sclerosis* is an autosomal dominant disease, caused by inactivation of either the tsc1 or tsc2 gene. In mammals, TSC1/TSC2 acts downstream of the PI3K/PKB pathway to antagonize TOR, the key regulator of nutrient and growth factor activated cell growth. Here we report that inactivation of tsc2 in *Dictyostelium* decreases phagocytosis. The effect of TSC2 on phagocytosis is mediated through its GAP domain. Our data also suggest that the TSC2 GAP activity targets the small G-protein Rheb. In addition, tsc2 null cells are less responsive to folic acid, a bacterial product utilized by *Dictyostelium* as a chemoattractant. These findings suggest an interesting twist in the regulation of cell growth. Data from other systems show that inactivated TSC2 promotes cell growth, while our data shows that loss of tsc2 inhibits phagocytosis and decreases chemotaxis towards a source of nutrients.

Abstract P26

## A Rab21/LIM-only complex regulates phagocytosis by repressing the action of an associated CH-LIM protein.

T. Khurana and A.R. Kimmel

Laboratory of Cellular and Developmental Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892 USA

The LIM domain is a unique motif of two conserved, zinc finger-like modules, CX2CX16-23HX2CX2CX2CX16-21CX2C. Structural stability of LIM domains depends on the coordinated tetrahedral binding of two zinc atoms via cysteine and histidine residues. LIM proteins act as adapters, mediating protein-protein interactions in processes such as transcription regulation, cell fate determination and actin cytoskeleton organization. LIM-regulated cytoskeletal reorganization is required for cell motility, cell-cell interaction, cell-substratum attachment, development, and other dynamic processes. We have identified two novel LIM domain proteins, LimF and CH-LIM, from *Dictyostelium* that act as binding partners and that coordinate with Rab21 to regulate phagocytosis. LimF, a 23 kDa protein consisting of three tandem LIM domains, was used as a two-hybrid bait to screen for interacting proteins. CH-LIM and Rab21 were isolated from this screen and interaction was confirmed by GST pull-down binding assays. CH-LIM is a 76 kDa protein consisting of three LIM domains and an N-terminal Calponin Homology (CH) domain. The CH domain is a 100 amino acid motif which also serves as a protein binding interface for many structural and signaling molecules. Rab21 is a member of the small G protein family of signaling molecules often associated with membrane trafficking. Tagged versions of each protein, GFP-LimF, GFP-CH-LIM, and GFP-Rab21, were expressed in cells to identify their intracellular localizations. LimF, CH-LIM, and Rab21 all exhibited association with intracellular vacuoles.

To investigate in vivo function, we generated limF and ch-lim knock-out and overexpression cell lines and strains that express activating or inhibiting mutations of Rab21. Overexpression of LimF, loss of CH-LIM, or expression of the constitutively active Rab21 increases the rate of phagocytosis above that of wild-type. Conversely, overexpression of CH-LIM, loss of LimF, or expression of constitutively inactive Rab21 decreases the rate of phagocytosis. Studies using cells that carry mutations in multiple genes suggest that the activating function of Rab21-GTP requires LimF, and that, in turn, activated LimF represses the inhibiting function of CH-LIM on phagocytosis. We suggest that these components participate in a single complex to regulate the activity of the phagocytic cup.

Abstract P27

# Analysis of the spore matrix of the social amoebae, *Dictyostelium discoideum*.

K.Z. Kirmani[1], S. Kiev[1], V. Chow[1], M. Askew[1], E.K.M Squires[1], L. Sovran[1], M. Sameshima[2], and D.A. Cotter[1]

[1]Department of Biological Sciences, University of Windsor, Windsor, ON, Canada, N9B 3P4; [2]Electron Microscopy Center, The Tokyo Metropolitan Institute of Medical Science, Tokyo Metropolitan Organization for Medical Research, 3-18-22 Honkomagome, Bunkyoku, Tokyo 113-8613, Japan

The social amoeba, *Dictyostelium discoideum*, is a unique organism for its ability to sustain itself as a unicellular organism and as a multi-cellular organism. Since the biochemical and molecular makeup of this organism is homologous to many higher eukaryotic cells, and since there are no known pathogenic diseases associated with the social amoebae, *D. discoideum* makes for an excellent eukaryotic cell model. Although extensive research has been conducted on the spores and vegetative cells of *D. discoideum*, there has not been very much analysis of the extracellular spore matrix of *D. discoideum*. Research in our laboratory has focused recently on the spore matrix. We have found that 2-day old spore matrix from the wild-type strain *V12* expresses significantly less protein than 2-day old matrix from the spontaneous germinator strain *SG2* when viewed on SDS-PAGE. 2-day old spore marix from strain *SG2* showed a significant amount of protein in the range of 25-66 kDa, which were less visible in the spore matrix of strain *V12*. We suspect that the age of the spores from which the spore matrix was collected correlates with the protein patterns that are viewed on SDS-PAGE. Since spontaneous germinator strain *SG2* is ready to spontaneously germinate at 2 days of age and wild-type strain *V12* is not, we have hypothesized that dormant spores may secrete proteins on a continuous basis as they approach the age of spontaneous germination. Furthermore, we have also characterized the 18 kDa spore matrix cysteine protease, CP18 (North *et al*., 1996 and Cotter *et al*., 1997). CP18 has been regarded in the past as being a unique cysteine protease in that it is non-acid activatable, which is contrary to the rest of the cysteine proteases in *D. discoideum*. We have found that CP18 is in fact acid-activatable under conditions where SDS is absent from the PAGE-zymogram system used to view the acid activatability of the cysteine proteases. Furthermore, our laboratory group has observed that under non-acid treated conditions, there is low activity generated by the 18 kDa protein. We are examining the possibility that CP18 may serve as a timer for spore germination.

North MJ, Nicol K, Sands TW, Cotter DA. The Journal of Biological Chemistry. 27. 14462-67. 1996

Cotter DA, Cavallo D, Gale KE, Sands TW, North MJ. *Dictyostelium*-A Model System for Cell and Developmental Biology. Universal Academy Press, Inc. Chapter 23. 325-35. 1997.

# Poster Session II

Abstract P28

# An analysis of upstream sequences of *Dictyostelium discoideum* using a distributed computer system.

N.Kobayashi[1], M.Marin[2], T.Morio[1], Y.Tanaka[1] , and H.Urushihara[1]

[1]University of Tsukuba, Japan; [2]Johann Radon Institute for Computational and Applied Mathematics, Austria

We describe an analysis of the upstream sequences of *Dictyostelium discoideum* using a distributed system deployed on the Web. Our goal is the estimation of elements which are involved in temporal and spatial regulation of transcription. For this purpose, we have developed a prototype system which extracts statistical characteristic elements of upstream sequences. The realization of our system follows mainly three steps: development of an upstream sequence database, implementation of statistical analysis programs, and implementation of a Web environment for collaboration among those programs. The upstream sequence database was obtained by alignment between genome sequences published at DictyBase and cDNA contig sequences using the sequence alignment program GeneSeqer. These sequences are stored in a relational database system, PostgreSQL, and they can be accessed via our graphical viewer embedded in a Web page. Secondly, we have designed and implemented a program which extracts elements of sequences which have a structural characteristic. The program finds such elements in the following way: it prepares a candidate sequence, gets the positions of upstream sequences which are similar to the candidate sequence by homology search based on dynamic programming, and obtains a statistic distribution of these positions when the upstream sequences are grouped by life cycle stages and expression patterns. From a practical perspective, the computation, especially dynamic programming, requires huge computational resources. In addition, the computation can be divided into individual sub-computations for each candidate sequence. Therefore, we adopt a distributed approach: executing the computation by collaboration among computers deployed on the Web. The system mainly consists of two components: computation service providers which are individual processes running on each computer, and a coordinator which manages a user-defined collaboration among these providers. To achieve collaboration, we should rely on a standardized communication framework. In our current implementation, we adopt Jini and SOAP (Simple Object Access Protocol), and our entire database and programs are wrapped as service providers. Further, we have designed a user language which describes the collaboration. Finally, using our system, we obtained the distinctive elements that are 4-20 bases long as candidates of *cis*-elements. We have also evaluated the effectiveness of our system. As a result, we have realized a practicable upstream sequence analyzer which lists candidate elements involved in transcriptions by collaboration among distributed computers on the Web.

Abstract P29

## Vesicle trafficking is important for the proper cellular distribution and efficient activation of adenylyl cyclase in chemotaxing cells.

P.W. Kriebel, V.A. Barr, and C.A. Parent

Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health, 37 Convent Drive, Bethesda, Maryland 20892

We are interested in understanding the signaling mechanisms that control cell polarization and chemotaxis. These responses are important in many processes such as wound healing, neuronal growth and leukocyte cell migration. In *Dictyostelium*, the binding of cAMP to G protein-coupled cAMP receptors (cARs) activates a variety of effectors including the adenylyl cyclase ACA, which converts ATP to cAMP. cAMP acts both internally, to alter gene expression through PKA, and externally to relay the chemoattractant signal. We have shown that ACA is enriched at the back of chemotaxing cells and proposed that this provides a compartment from which cAMP is secreted allowing cells to orient themselves head to tail and form streams. Interestingly, we also found that ACA labels rapidly moving intracellular vesicles. We investigated whether these vesicles are involved in the trafficking of ACA using Fluorescence Recovery After Photobleaching (FRAP). As a control we followed the fate of cAR1. These studies show that in nonpolar cells, bleached cAR-1 recovers first in areas near unbleached membranes, consistent with recovery by diffusion. In contrast ACA recovers uniformly across the bleached area, consistent with vesicle delivery to the plasma membrane (PM). Moreover, in polarized chemotaxing cells specifically bleached at the tail, ACA also recovers in a manner consistent with vesicle delivery. These results show that ACA is replenished via vesicle fusion to the PM and implicate vesicle trafficking as a mechanism for ACA tail enrichment in polarized cells. When polarized cells are treated with latrunculin A, which depolymerizes the actin cytoskeleton, the enrichment of ACA at the tail is lost and the internal ACA labeled vesicles disappear. Under these conditions FRAP of ACA now occurs first near unbleached membranes. This is consistent with the requirement for the actin cytoskeleton for ACA vesicle delivery to the PM. Finally, when latrunculin A treated cells are stimulated with chemoattractant, ACA activity levels are reduced, suggesting that vesicle trafficking is also important for cyclase activation. Together, these results establish a role for vesicle trafficking in adenylyl cyclase localization and activation. Since, intracellular compartmentalization of cAMP and localization of adenylyl cyclase to cellular domains has been observed in mammalian cells this novel pathway may also play a role in the regulation of mammalian adenylyl cyclases.

Abstract P30

## Exploiting new terrain: an advantage to sociality in the slime mould *Dictyostelium discoideum*.

J.J.Kuzdzal, K.R.Foster, D.C.Queller, and J.E.Strassmann

Rice University, USA

When starving, the normally solitary amoebae of the slime mould *Dictyostelium discoideum* aggregate in their thousands and form a differentiated multicellular organism known as a slug. The slug migrates towards the soil surface where it metamorphoses into a fruiting body of hardy spores held up by a dead stalk containing one fifth of the cells. Multicellularity in *D. discoideum* is thought to have evolved to provide safe passage of amoebae to the soil surface for long distance dispersal as spores. Here we show that multicellularity has another advantage: local dispersal. We made *D. discoideum* slugs migrate across a narrow field of bacteria and show that this migration results in removal of the bacteria. Furthermore, using time lapse, we show that the removal of bacteria is not due to slugs breaking up and dedifferentiating on contact with the food source. Instead, cells that are shed from migrating slugs consume the bacteria. We propose that the exploitation of local food patches is an important selective benefit favouring multicellularity in *D. discoideum*.

Abstract P31

# Using *Dictyostelium discoideum* to understand how different functions of the Adenomatous Polyposis tumour suppressor protein are co-ordinated.

H.-H. S. Lin, I.P. Newton, and I.S. Näthke

University of Dundee, UK

Mutations in the tumour suppressor gene Adenomatous Polyposis Coli (APC) are common to most colon cancers. APC is a multifunctional protein involved in cell migration, proliferation, and differentiation. APC is best known as a scaffold in a large protein complex that regulates beta-catenin in a Wnt-responsive manner. In addition, APC is an important cytoskeletal regulator. Little is known about how different interactions of the APC protein are co-ordinated and how these different functions relate to each other. To this end we aimed to establish a system that would allow us to study the relative contribution of different functions of APC using the simple eukaryotic organism, *Dictyostelium discoideum*.

*Dictyostelium* do not appear to express an APC-like protein and do not express Wnt proteins. However, they express a number of components of the Wnt-signalling pathway that usually bind to APC in mammalian cells, including the regulatory kinase GskA and a beta-catenin homologue called Aardvark (Aar). These homologues are sufficiently similar to their mammalian counterparts to predict their ability to interact with human APC proteins. Furthermore, cytoskeletal regulation in *Dictyostelium* is highly related to that in other organisms.

Initially, we established that expressing different fragments of APC results in different phenotypes in *Dictyostelium*.

A fragment of APC that can participate in Wnt-signalling in mammalian cells genetically interacts with GskA and Aar in *Dictyostelium*. Expressing this fragment of APC in *Dictyostelium* rescues a number of defects in *gskA* null (*gskA*$^-$) and *aar* null (*aar*$^-$) cells: gene expression and substrate adhesion were restored to normal, and growth defects were partially rescued in *aar* null cells.

Expressing the N-terminal domain of APC produces defects in directed cell migration and phago- and pinocytosis whereas the C-terminal third of APC leads to compromised centrosomes.

These data establish *Dictyostelium* as a suitable system to investigate the role of APC in GskA- and Aar-mediated processes. Furthermore, we are now in a position to investigate how the different functions of APC may be co-ordinated by comparing the effect of expressing multiple domains of APC in *Dictyostelium*.

Abstract P32

# Fascinated behaviors of *Dictyostelium* homologues of TRAP1 and GRP94 during development, revealed by immuno-electron microscopy.

Hitomi Yamaguchi*, Tsuyoshi Morita**, Aiko Amagai, and Yasuo Maeda

Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Aoba, Sendai 980-8578, Japan; Present address: *Department of Cell Genetics, National Institute of Genetics, Mishima, Shizuoka-ken 411-85

TRAP1 (tumor necrosis factor receptor-associated protein 1) is a member of the molecular chaperone HSP90 (90-kDa heat shock protein) family. In this study, we mainly examined the behavior of *Dictyostelium* TRAP1 homologue, Dd-TRAP1 during *Dictyostelium* development by immuno-electron microscopy. In vegetatively growing *D. discoideum* Ax-2 cells, Dd-TRAP1 locates in nucleolus and vesicles in addition to the cell cortex including cell membrane. A good many Dd-TRAP1 molecules moved to the mitochondrial matrix in response to differentiation, though Dd-TRAP1 on the cell membrane seems to be retained. Some Dd-TRAP1 was also found to be secreted to locate outside the cell membrane in Ax-2 cells starved for 6 hrs. At the multicellular slug stage, Dd-TRAP1 was primarily located in mitochondria and cell membrane in both prestalk and prespore cells. More importantly, in differentiating prespore cells a large number of Dd-TRAP1 in the PSV (prespore-specific vacuole), a sole cell type specific organelle and is essential for spore wall formation, whereas some Dd-TRAP1 in the cell cortical region of prestalk cells. These findings strongly suggest importance of Dd-TRAP1 regulated temporally and spatially during *Dictyostelium* development. Incidentally, we also have certificated that the glucose-regulated protein 94 (Dd-GRP94) is predominantly located in Golgi vesicles and cisternae, followed by its colocalization with Dd-TRAP1 in PSV, thus suggesting the coordinated function of the two proteins in PSV formation.

Abstract P33

## A new late step in spore coat biogenesis in *Dictyostelium*.

Talibah Metcalf, Hanke van der Wel, Yunyan Zhang, and Christopher M. West

Dept. of Biochemistry & Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, 73104 USA

A terminal event in sporulation in *Dictyostelium* is formation of the spore coat at the surface of the differentiating prespore cell. Spore coat formation involves a sequence of steps, including 1) early synthesis of coat protein precursors and a Gal-rich polysaccharide by prespore cells and storage in intracellular prespore vesicles (PSVs), 2) delivery of these stored precursors to the cell surface by exocytosis of the PSVs, 3) cell shrinkage, and 4) deposition of fibrils of cellulose via a plasma membrane associated cellulose synthase. An additional step is suggested by the finding that two new spore coat proteins, SP65 and SP55, are synthesized late, contemporaneously with SpiA. SP65 and SP55 were initially detected by SDS-PAGE analysis of density gradient purified spore coats and Edman sequencing of CNBr-fragments. Their genes were reconstructed using PCR approaches guided in part by genome sequence data. Northern blot analysis carried out by R. Escalante and L. Sastre (Univ. of Madrid) revealed that SP65 is expressed coordinately with the plasma membrane protein SpiA in a SrfA-independent fashion based on analysis of an SrfA-null mutant. Based on gene array expression analyses (UCSD & Baylor groups), SP55 is also expressed late but is dependent on SrfA. SP65 was expressed recombinantly in the growth medium of growing *Dictyostelium* cells, purified chromatographically, and used to induce polyclonal and monoclonal antisera. Western blot analysis of a developmental time course confirmed expression of SP65 late in development. An SP65-mutant strain was constructed by homologous recombination and exhibits abnormally permeable spore coats. Previous studies had shown that SP85 from spores complexes with SP65 from sori and Avicel cellulose based on pull-down studies, and new studies confirm that SP65 fails to incorporate into the coats of SP85-null spores. Therefore, each of the three components of the previously described SP65-SP85-cellulose complex exhibits distinct temporal regulation of expression and possibly distinct pathways of delivery to the cell surface. Binding studies on renatured SP55 derived from urea-extracted spore coats shows it to possess intrinsic cellulose-binding activity. In contrast to the other known cellulose-binding protein SP85, which is made by prespore cells and stored in PSVs, SP55 is synthesized late suggesting that the coat derives from separate pools of proteins that potentially do not mix until they are released to the cell surface.

Abstract P34

## Reverse genetic analyses of gamete-enriched genes revealed a novel regulator of cAMP signaling pathway in *Dictyostelium discoideum*.

T. Muramoto and H. Urushihara

University of Tsukuba, Japan

Sexual development in *Dictyostelium discoideum* is initiated by fusion of opposite mating type cells to form zygote giant cells, which subsequently gather surrounding cells and phagocytose them for nutrition to form macrocysts. We constructed a gamete-specific subtraction library and selected 67 gamete enriched genes, 24 of which were highly (> 5 x) enriched. In the present study, we attempted to target all of those 24 genes in KAX3. The gene disruption was also performed in V12, an opposite mating-type strain, to avoid complementation in zygotes. We successfully generated the knockout mutants for 16 genes. The knock-down mutants were generated by RNAi technique for 20 genes including 6 genes without knockout mutants. Some of the mutants showed decreased competency for sexual cell fusion, but not to a complete loss. One of the knockdown mutants was growth-defective. In two knockout mutants, cell aggregation toward the giant cells was much less extensive and many surrounding cells remained. Since these phenotypes resembled the REMI mutant we previously reported, the relevant gene being *tmc1* (tiny macrocysts 1; should be renamed *tmcA*), we named the relevant genes *tmcB* and *tmcC*. Both structure and expression pattern of *tmcB* and *tmcC* are mutually similar, being high at the gamete and the aggregation stages of both asexual and sexual development. However, they seem to be functionally distinct. Namely, *tmcB* disruptant formed macrocysts in the presence of higher concentrations of cAMP, where wild type cells and *tmcC* disruptants were unable to do so. We analyzed the expression of genes involved in the cAMP signaling pathway in the *tmcB* disruptants, and found that mRNA level of phosphodiesterase (*pde*) was higher and that of its inhibitor (*pdi*) was lower compared to the parental strains. Interestingly, these effects were specific to sexual development and expression patterns of *pde* and *pdi* in the asexual development were unaltered. These results indicate that *tmcB* gene is a novel regulator of cAMP signaling pathway specific to the sexual development, and suggest that *Dictyostelium* cells make efficient and differential use of the gene repertoire for their survival strategy. Functions of *tmcC* as well as the remaining 21 genes are currently unknown.

Abstract P35

## Role of the WH1 domain of WASP in the regulation of F-actin polymerization and chemotaxis.

S.A. Myers, J. Moon, and C.Y. Chung

Department of Pharmacology, Vanderbilt University Medical center, Nashville, TN, U.S.A

Wiskott-Aldrich Syndrome (WAS) is a human X-linked immunodeficiency characterized by recurrent infections, hematopoietic malignancies, eczema, and thrombocytopenia. These defects are caused by mutations in the gene encoding the Wiskott-Aldrich Syndrome protein (WASP), a key downstream "adaptor" protein that connects multiple signaling pathways to F-actin polymerization, a process essential for the formation of lamellipodia and filopodia during chemotaxis. The goal of this study is to understand the role of the WASP Homology 1 (WH1) domain in the activation of WASP during chemotaxis.

The functional importance of the WH1 domain has been revealed by the frequency of mutations found to cause WAS. Despite comprising only one-fifth of the whole protein, more than half of the known missense mutations causing WAS are present in the WH1 domain. The WASP interacting protein (WIP), known to be essential for filopodia formation, has been shown to interact with human N-WASP, a ubiquitously expressed homolog, in this region. We found that overexpression of the WH1 domain alone led to a mislocalization and upregulation of F-actin polymerization with subsequent defects in chemotaxis and early development. Based upon the crystal structure of the poly-proline peptide bound to a signaling domain highly homologous to the WASP WH1 domain, we selected four highly conserved amino acids for targeted mutation to alanine. The WH1 overexpression phenotype was relieved by mutation of these four aromatic residues. Ectopic expression of full length WASP containing these WH1 mutations in a *Dictyostelium* cell line expressing very low level of WASP revealed that these mutations cause defects in early development, chemotactic speed, and cAMP-induced F-actin polymerization. We are currently investigating the hypothesis that this phenotype is caused by the activation of a *Dictyostelium* homolog of WIP (WASP interacting protein) by the WH1 domain.

Abstract P36

## FbiA, a potential target of ubiquitin-mediated degradation, regulates cell-type proportioning in *Dictyostelium discoideum*.

C.J. Mason, E.E. Staley, J.T. Maxwell, M.J. Niederst, B.L. Lubawy, D.S. McGill, K.A. McFeaters, C. Moré, J.A. Christman, T. Abe, and M.K. Nelson

Allegheny College, Meadville, PA USA

The FbiA protein of *Dictyostelium discoideum* was identified via yeast two-hybrid analysis in a search for proteins capable of interacting with the WD-40 repeat region of the F-box/WD-40 repeat-containing protein FbxA. Based on analogy to FbxA homologues in other systems, this FbiA-FbxA interaction suggests that FbiA is likely to be targeted for FbxA-dependent, ubiquitin-mediated degradation. The C-terminal region of FbiA is homologous to proteins in humans, mice, *Drosophila*, *C. elegans*, *Arabidopsis*, *S. pombe*, *S. cerevisiae*, *N. crassa*, and *P. falciparum*. The function of these FbiA homologues is, however, unknown. Hence, further characterization of FbiA's role in *Dictyostelium* development may shed light on the function of this evolutionarily conserved protein family. We have taken a genetic approach to analysis of FbiA's function. An *fbiA*$^-$ null mutant develops more slowly than wild-type cells, but is able to undergo culmination. The resulting fruiting bodies, however, are unusually tall, with longer stalks and smaller sporeheads than wild-type culminants. Furthermore, transformation with cell-type specific *lacZ* reporter constructs reveals that the *fbiA*$^-$ null mutant produces structures with an unusually high prestalk:prespore cell ratio. Hence it appears that FbiA normally plays some role in either inhibiting the prestalk fate or promoting the prespore fate. This finding is consistent with a model in which the unusually low prestalk:prespore ratio of *fbxA*$^-$ null mutants results from the accumulation of abnormally high levels of FbiA in the absence of FbxA-mediated ubiquitination and degradation. One prediction of such a model is that an *fbxA*$^-$ *fbiA*$^-$ double null mutant should phenocopy the *fbiA*$^-$ null mutant with respect to the elevated prestalk:prespore ratio. We report here the creation of an *fbxA*$^-$ *fbiA*$^-$ mutant and our initial analysis of the double mutant's developmental phenotype. In addition, we describe our use of epitope-tagged FbiA derivatives to determine whether the stability of FbiA varies in an FbxA-dependent fashion, as our model would predict. (This research was supported in part by NSF CAREER Grant IBN-9985265.)

Abstract P37

## Cloning and characterization of rnoA, a gene coding for a putative guanine nucleotide exchange factor in *Dictyostelium*.

Liem Nguyen and Derrick Brazill

Department of Biological Science, Hunter College, City University of New York, Graduate Center, 695 Park Avenue New York, NY 10021

The mammalian phospholipase D (PLD) and ADP- ribosylation factor (ARF) GTPase are believed to play an integral role in cancer metastasis. The regulator of ARF activity is ARNO (ARF nucleotide-binding-site opener), a guanine nucleotide exchange factor (GEF). However, very little is known about their interactions with each other or downstream effectors. To address these issues, we are studying an ARNO ortholog in *D.discoideum*, designated as *rnoA*, that contains 2 conserved Sec7 (yeast Sec7p) and PH (pleckstrin homology) domains found in members of the ARF-GEF family across species. A tetracycline-regulated overexpression system and a conventional gene knockout technique were employed to generate overexpression and knockout constructs of the *rnoA* gene. We are currently in the process of examining *rnoA* functions in cell motility, cell aggregation and chemotaxis; these are the underlying forces in tissue formation, wound healing and cancer metastasis in higher eukaryotes. In addition, we are studying the role of RnoA in *Dictyostelium* developmental morphogenesis, including cell-type specification, cell sorting and cell patterning. Lastly, RnoA activity will be monitored for the activation of PldB, a component that we found may be involved in CMF (Conditioned Medium Factor) signaling pathway during early development.

Abstract P38

## A PKB/AKT related kinase is essential for both slug migration and ecmB expression in core region of slug.

H. Ochiai, S. Watanabe, K. Naito, T. Saito, K. Takeda, and A. Kato

Hokkaido University, Japan

Our results show the critical function of pkbR gene in slug movement and morphogenesis during *Dictyostelium* multicellular development. PKBR has a kinase and a C-terminal domain related to those of protein kinase B (PKB/AKT), but no PH domain. Instead, it has an N-terminal myristoylation site, which is required for its constitutive membrane localization (Meili *et al*. (2000) Cur. Biol. 10: 708-717). A half of PKBR-null multicellular organisms arrest development at the tight mound stage, but the residual ones develop further with pleiotropy during about 48 hours. The developing organisms however never migrate at the slug stage, and do not express the ecmB gene at the core region of the slug, although there is expression of the ecmB gene at the basal disc region. In finally pkbR null cells form irregular fruiting bodies (or sometimes seemingly normal fruiting bodies) with a few percentages, but its stalk is fragile, easily could be collapsed. We also observed that a large number of irregular fruiting bodies have been collapsed like fallen trees after the passing of a Typhoon. This type of collapse may be due to lack of a stalk matrix protein (cellulose formation is normal in pkbR- cells), namely lack of ordered arrangement of matrix proteins and cellulose and other constituents.

The organisms express the prestalk-specific ecmA and ecmO genes and prespore-specific SP60 gene with normal spatial patterning. In addition, the PKBR-null cells in chimeras with wild type cells (9:1 and 1:9) preferentially form spores. Accordingly the pkbR null cells resemble the mutant cells lacking an F-box /WD 40 repeat protein, which in chimeras is preferentially forms spores rather than stalk cells (Ennis *et al*.(2000) PNAS: 3292-3297; Nelson *et al*.(2000) Dev. Biol. 224: 42-59). Finally the lack of ecmB gene at the core region of the slug and later stages bring unordered process of stalk formation and finally may result in fragile structure of originally tough stalk.

Abstract P39

## *Dictyostelium discoideum* disrupted in sepiapterin reductase provides a model system to investigate cellular functions of tetrahydrobiopterin.

Yong Kee Choi, Jeong Soon Park, Jin Seon Kong, and Young Shik Park

School of Biotechnology and Biomedical Science, Inje University Kimhae 621-749, KOREA

L-erythro-tetrahydrobiopterin (BH4) is a well-known cofactor for aromatic amino acid hydroxylation and nitric oxide synthesis in higher animals. BH4 is also ubiquitous in lower eukaryotes including fungi. *Dictyostelium discoideum* is peculiar in its synthesis of a D-threo isomer of biopterin, named dictyopterin. The tetrahydro form of dictyopterin (DH4) is synthesized during aggregation of spores together with a lower amount of L-erythro form and both are known to interfere with GTP binding to G protein. However, the biosynthesis of DH4 as well as the cellular function have not been clearly defined. We created a *Dictyostelium discoideum* transformant deficient in sepiapterin reductase (SR), a crucial enzyme for BH4 synthesis, by gene targeting. The knockout transformant, which completely lacks of SR gene expression, generated aberrant fruiting bodies of smaller sizes. The cellular content of BH4 and DH4 decreased much but not completely, supporting the synthesis by salvage pathway as suggested in human SR deficiencies. We could also identify both sepiapterin and its D-isomer, oxidized forms of the immediate precursors for BH4/DH4 synthesis, in the medium: the immediate precursors are L- or D-lactoyltetrahydropterin generated from 6-pyruvoyltetrahydropterin. These results suggest that the SR knockout mutant will be useful for elucidating the synthesis of DH4 and the putative physiological role of BH4/DH4 related to nitric oxide, which might be applicable to neurological problems caused by SR deficiency. [supported by KOSEF grant R05-2003-000-11206-0]

Abstract P40

# A peptide based *in vivo* inhibitor of cyclic-AMP dependent protein kinase in *Dictyostelium.*

W.J. Ryves and A. J. Harwood

MRC-Laboratory for Molecular Cell Biology, University College London, Gower Street, London, WC1E 6BT.

In *Dictyostelium*, starvation of free living amoebae induces a development program that begins with the cells aggregating and proceeds, through coordinate cell type differentiation and morphogenesis, to a final fruiting body that allows for the dispersal of spores. The cyclic AMP / protein kinase A (cAMP / PKA) pathway is central to this development both at the early stages of aggregation through pulsatile secretion of cAMP, and for later transcriptional activation of both prespore and prestalk genes to regulate the relative timing of terminal differentiation and cell patterning

Penetratin sequences, such as that discovered within the *Drosophila* protein antennapedia (ANT), form peptides that can pass through the plasma membrane. The penetratin sequence can be used to carry psuedosubstrate peptide inhibitors into the cell. We report the design and use of such a strategy to inhibit the *Dictyostelium* cAMP dependent protein kinase (PKA) *in vivo*.

We have established that a penetratin based peptide sequence (ANT) can enter *Dictyostelium* cells. When the ANT peptide is coupled to a PKA psuedosubstrate from PKI, a heat stable PKA inhibitor protein, it blocks aggregation. This effect is only associated with the ANT and PKI peptides in fusion. To test specificity, the *rdeA Dictyostelium* mutant was treated with ANT-PKI peptide. This mutant has elevated intracellular cAMP leading to constitutive PKA activity and aberrant morphogenesis. Titration of ANT-PKI peptide into developing *rdeA* cells rescued the morphogenetic defect at lower concentrations, before further addition of the peptide blocked aggregation. These experiments suggest that the ANT-PKI peptide is an effective *in vivo* inhibitor of PKA.

Abstract P41

## Looking for social genes in the social mold *Dictyostelium discoideum.*

L. Santorelli[1], D. Queller[1], J. Strassmann[1], C. Thompson[2], and G. Shaulsky[2]

[1]Rice University, USA; [2]Baylor College of Medicine, USA

During the unique life cycle of the social amoeba, *Dictyostelium discoideum*, cells propagate as free unicellular organisms, but under starving condition they undergo a process of aggregation which leads to the formation of a multicellular organism. The cells differentiate into spores that will give rise to the next generation, and to stalk cells, which are dead. The stalk cells are sacrificed to help the spores disperse and survive, much like somatic cells help to spread the germline of a metazoan multicellular organism. When the cells that form a *Dictyostelium* aggregate are not uniclonal, a conflict may arise in deciding which cells will become spores and survive and which will become stalk and die. In the case of a mix between two or more genetically different clones, the one that differentiates more spores is called “cheater” and the other “loser”. We hypothesize that some genetic mechanism(s) can regulate and modulate this social behavior. To test this hypothesis, we generated a large pool of mutants by REMI and selected for cheaters. To simulate evolutionary selection the mutant population was subjected to several rounds of development, spore germination and growth in a mixed population. The spores were collected, germinated and grown again. Cheaters should become over-represented in the evolving population. The process was repeated 20 times. At the end of 10 and 20 cycles, we were able to isolate 11 strong cheaters that were not morphologically different from the wild type. In all cases, the mutants make more than their fare share of spores in chimera with wild type cells but appear to develop normally in pure populations. We are cloning the mutated genes in order to decipher the mechanisms that regulate cheating.

Abstract P42

## Analyses of srfA-dependent gene promoters.

Patricia Torija, Carmen Valenzuela, Juan-Jesús Vicente, Ricardo Escalante, and Leandro Sastre

Instituto de Investigaciones Biomédicas CSIC/UAM, Madrid, Spain

The transcription factor SrfA, homologous to animal Serum Response Factor (SRF), serves several functions during *Dictyostelium* development. Mutant strains where srfA has been interrupted show a developmental delay, defects in slug migration, stalk formation and spore differentiation. srfA- spores are more rounded and less resistant to stress than wild type spores. Ultrastructural analysis has shown that actin rods initially form but do not mature in srfA- spores. Besides, the external coat layer is not well structured and degenerates in a few hours (1).

Comparative gene expression analyses, either through subtractive hybridization (2) or DNA microarrays (3), have identified 24 genes whose expression is dependent on srfA. These genes are expressed at late developmental stages in wild type but not in srfA- structures. The functional analyses of the promoter regions of five of these genes has been approached to better understand SrfA function in the regulation of gene expression at late developmental stages.

Putative promoter regions of the genes sigA-D and rpnA between 0.6 and 2 kb in length were cloned in unstable b-Galactosidase expression vectors. Putative promoter regions from the genes sigB, sigD and rpnA directed b-Gal expression to spores at late developmental stages (22-24 hours). Promoter activity was srfA-dependent since no b-Gal activity was detected in srfA- strains. The putative promoter region of the sigA gene, coding for the malic enzyme, showed a more complex pattern of expression. b-Gal expression was observed in vegetative cells, decreased during aggregation and increased again in prespore and spore cells. Spore expression, but not expression in vegetative or prespore cells, was dependent on srfA. Both sigA and sigC promoters showed a graded b-Gal expression in the sorus, decreasing from the upper to the lower cup, as previously described for the spiA promoter (4). The putative promoter region of sigC drived b-Gal expression to isolated cells scattered through the structure in the slug and at mid culmination.

Functional regions are being determined at the present time through the analyses of the activity of different fragments of the promoters. These studies might allow to determine promoter regions that direct cell-type specific gene expression during late development. Some of the functional regions could represent srfA regulatory elements.

Bibliography
1. Escalante, R., Yamada, Y., Cotter, D., Sastre, L. and Sameshima, M. (2004) The MADS-box transcription factor SrfA is required for actin cytoskeleton organization and spore coat stability during Dictyostelium sporulation. Mech. Dev., 121/1, 51-56.
2. Escalante, R., Moreno, N. and Sastre, L. (2003) Dictyostelium discoideum developmentall

y regulated genes whose expression is dependent on the MADS-box transcription factor SrfA. Eukaryotic Cell, 2, 1327-1335.
3. Escalante, R., Iranfar, N., Sastre, L. and Loomis, W.F. (2004) Identification of genes dependent on the MADS-box transcription factor SrfA in Dictyostelium development. Eukaryotic Cell, 3, 564-566.
4. Richardson, D.L., Loomis, W.F. and Kimmel, A.R. (1994) Progression of and inductive signal activates sporulation in Dictyostelium discoideum. Development 120, 2891-2900.

Abstract P43

# RasG affects early developmental adhesion in *Dictyostelium* through the phosphorylation of DdCAD-1.

D. M. Secko[1], C. H. Siu[2], G. B. Spiegelman[1], and G. Weeks[1]

[1]Department of Microbiology and Immunology, University of British Columbia, 300-6174 University Blvd., Vancouver, British Columbia, V6T 1Z3, Canada; [2]Banting and Best, Department of Medical Research, and Department of Biochemistry, University of Toronto,Toronto, Ontario M5G 1L6, Canada

*Dictyostelium* RasG signal transduction has been linked to a variety of processes including the regulation of early development, but the molecular components involved in these processes are only now beginning to be uncovered. We previously identified the cell adhesion molecule DdCAD-1 as a putative protein whose phosphorylation state is RasG-regulated. Here we show that a *cadA* null strain lacks all three phosphoproteins identified previously as DdCAD-1, indicating it was the putative phosphoprotein. Investigation of the link between RasG signaling and DdCAD-1 phosphorylation revealed that cells expressing RasG(G12T) have increased cell-to-cell cohesion. This cohesion was DdCAD-1 dependant and correlated with the membrane localization of DdCAD-1 and DdCAD-1 dephosphorylation. DdCAD-1 phosphorylation also decreased as starving *Dictyostelium* cells began to aggregate, consistent with the same correlation between DdCAD-1 dephosphorylation, cell surface localization and DdCAD-1-mediated cell adhesion. These results suggest that adhesion is dependant on the phosphorylation state of DdCAD-1 and that RasG may regulate this phosphorylation and hence affect early developmental adhesion.

Abstract P44

## Progress of the *Dictyostelium* DNA Microarray Resource.

J. Skelton[1], G. Bloomfield[2], N. Nikolaidou-Katsaridou[1], B. Barrell[1], R. Kay[2], and A. Ivens[1]

[1]Wellcome Trust Sanger Institute, UK; [2]MRC Laboratory of Molecular Biology, UK

In this poster we describe a DNA microarray resource set up at the Sanger Institute, UK, with funding from the Wellcome Trust. Its aim has been to produce comprehensive arrays for the use of *Dictyostelium* researchers by making use of data made available by the *Dictyostelium* Sequencing Project. We have put together a series of arrays successively increasing in coverage, the latest of which contains over 9000 features printed in duplicate. Interested parties are welcome to apply for use of our arrays or begin collaborative projects with our group. Issues regarding the design of microarray experiments are discussed, and example projects illustrated.

Abstract P45

## Importance of myosins, actin dynamics and membrane trafficking in phagocytosis and cell motility.

Y. von Heyden[2], M. Saha[1], K. Yoshida[1], and T. Soldati[1,2]

[1]Department of Biological Sciences, Imperial College, London, UK; [2]Department of Biochemistry, University of Geneva, Switzerland

The machinery used by cells to deform their plasma membranes and produce a protrusion, as necessary during cell motility and phagocytosis, is extremely well conserved. The role of actin dynamics in such processes is well established and has recently overshadowed two other contributing mechanisms, focal exocytosis and contraction mediated protrusion. Using *Dictyostelium* as a model system, we have found evidence for a contribution of all three mechanisms to overall motility and phagocytic uptake and egestion.

First, actin dynamics has been visualised during uptake and particle egestion, using GFP-ABD and GFP-Arp3 as probes. In addition, immunofluorescence assays revealed the presence of class I myosins MyoK and MyoB and their major effectors, Abp1 and CARMIL, at different stages of phagosome formation and maturation. Second, our data combined to results from other labs strongly support a role for focal insertion of endosomal membranes during phagocytic cup formation. There is supportive evidence that MyoB plays a role in membrane recycling and contributes to this focal exocytosis. Third, cell tracking by video-enhanced microscopy with high temporal and spatial resolution, including confocal and TIRF microscopy, illustrated the contribution of contraction-mediated cell protrusion to the advance of the pseudopode. Use of GFP and GFP-ABD revealed the formation of local spherical protrusions (blebs) that become “consolidated” by F-actin polymerisation. Computational analysis of velocity of wild-type and MHC null cells showed that in normal conditions, actin-mediated and contraction-mediated processes both contribute to cell centroid motility. Importantly, higher buffer osmolarity opposed contraction-mediated motility.

In parallel with the in vivo studies, we are analysing the biochemical properties of the class I myosins involved in cell motility and phagocytosis. We present evidence in support of a model where these myosins act mainly through the recruitment/regulation of the actin nucleation machinery. MyoK is at the centre of a network of interacting proteins that bridge the actin dynamics and membrane trafficking machineries. MyoK binds to the profilin-actin complex and to Abp1, an F-actin binding protein that is proposed to bind and activate the Arp2/3 complex. In addition, we found that profilin binds to Abp1 and Dynamin, and Abp1 also binds Dynamin directly. Finally, we propose that the role of MyoB in phagocytosis is exerted at least in part through the Capping protein-Arp2/3-MyosinI linker (CARMIL) adapter protein.

Abstract P46

## Proteomic analysis of the GSKA-ZAK1 signalling pathway.

L. Strmecki[1], T. Araki[2], C. Schilde[2], D.M. Greene[1], A.J. Harwood[3], J.G. Williams[2], and C. Pears[1].

[1]Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK; [2]School of Life Sciences, University of Dundee, Wellcome Trust Biocentre, Dow Street, Dundee, DD1 5EH, UK; [3]MRC Laboratory for Molecular Cell Biology & Dept. of Biology, University College London, Gower Street, London WC1E 6BT, UK

Genetic and biochemical experiments suggest that ZAK1, and GSKA function in the same regulatory pathway with GSKA as a direct target for phosphorylation and activation by ZAK1 (Kim *et al*. 1999). ZAK1 was the first identified tyrosine kinase described to be required for cell fate determination in *Dictyostelium* (Kim *et al*. 1999). Loss of ZAK1 function does not phenotypically affect early development, but does alter subsequent processes. The severe reduction in spore cell production and the loss of cAMP-dependent inhibition of stalk cell formation in zak1-nulls are similar to the phenotypes exhibited by *Dictyostelium* cells that lack GSKA (Harwood *et al*., 1995).

We wish to identify proteins whose expression is dependent on both ZAK1 and GSKA as potential targets of the signalling pathway. We have performed a 2D proteomic comparison between parental, zak1-null and gska-null whole cell lysates from cells which have been developed for five hours in shaken suspension, with cAMP pulsing to ensure that the strains all reach the same developmental stage. We have identified candidate proteins that are differentially expressed in both zak1-null and gska-null cells (DdCAD-1, putative single-stranded RNA binding protein, protein H5, discoidinI, aldo-keto reductase) as well as proteins which are only altered in one or other mutant strain (Rho GDP dissociation inhibitor, glutamine amidotransferase, transketolase). Analysis of the mechanism of regulation of the protein expression level will reveal the signalling pathways involving both of these proteins and those which are specific to one.

Kim, L., Liu, J. and Kimmel, A.R. (1999). The novel tyrosine kinase ZAK1 activates GSK3 to direct cell fate specification. Cell 99, 399-408.

Harwood A., Plyte, S., Woodgett, J., Strutt, H. and Kay, R. (1995) Glycogen synthase kinase 3 regulates cell fate in *Dictyostelium*. Cell 80, 139-148

Abstract P47

## Collosin: a new giant protein.

G. Chen, J. Song, The *Dictyostelium* Genome Sequencing Consortium, R. Sucgang, and A. Kuspa

Department of Biochemistry and Molecular Biology, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030, USA

We describe the assembly and characterization of a gene in the *Dictyostelium* genome predicted to encode a novel protein of over 11,100 amino acids in size, making it one of the largest proteins described in nature. We have dubbed it “colossin” in homage to its size. The sequence consists primarily of a series of CnaB repeats, a motif that is typically found in certain bacterial cell wall proteins, connected to a 500 amino acid tail segment. Three shorter genes in the genome share the same C-terminal segment, indicating that a family of colossins may have arisen through a series of duplication events, and diverged from each other through tandem duplication of the intragenic motifs. The colossin A transcript is developmentally regulated, peaking between 6-8 hours of development, and we will present the effects of mutating the gene.

Abstract P48

# Aphidicolin addition just before electroporation increases the transformation efficiency in *Dictyostelium discoideum.*

K.S.K. Uchida[1], S. Kuhara[2], and Y. Tanaka[1]

[1]University of Tsukuba, Japan; [2]Kyushu University, Japan

Preparation of gene knockout strains is essential for the study of gene function. High transformation efficiency is required to obtain such strains efficiently. In NIH/3T3 and HeLa cells, aphidicolin treated synchronized cells show that frequency of transformation increases during S phase in the cell cycle. As aphidicolin is an inhibitor for DNA polymerase alpha, addition blocks cell cycle in the S phase. In this study, we examined whether transformation efficiency in *Dictyostelium* cells is also increased by the addition of aphidicolin.

After aphidicolin was added to the mixture of cell and knockout construct, electroporation was performed. As a result, transformants were obtained more than in the case of no aphidicolin. The results were found to be consistent with the previous study showing that, in synchronized *Dictyostyelium* cells by temperature shift treatment, transformation efficiency is 2-3 folds higher during S or early G2 phase. For improvement, we examined suitable time for the aphidicolin addition. When aphidicolin was added just before electroporation, average efficiency of transformation was found to be 2.5 fold higher than that of control (no aphidicolin). On other hand, addition just after electroporation had no effect. It is assumed that electroporation will introduce aphidicolin into cells more effectively. After electroporation, the mixture of cell/vector/aphidicolin was kept for more than 2 hours at room temperature. The result showed that the average efficiency of transformation was 7 fold higher than that of control (no aphidicolin, no incubation). These results infer that 1) addition of the aphidicolin just before electroporation and 2) incubation of the mixture more than 2 hours after electroporation, increase the transformation efficiency in *Dictyostelium* cells.

Based on the above results, we tried to obtain knockout strains for a myb-related gene. Consequently, 28 transformants were obtained, with one of them being a myb-null strain. In the case of no aphidicolin, only three transformants and no knockout strains were obtained.

Abstract P49

# The two RasGEF containing proteins GbpC and GbpD have opposite effects on cell polarity and chemotaxis in *Dictyostelium discoideum*.

Leonard Bosgraaf[1], Arjen Waijer[1], Ruchira[2], Ton Visser[2], Deborah Wessels[3], David Soll[3], and Peter J.M. van Haastert[1]

[1]Department of Biochemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, the Netherlands; [2]MicroSpectroscopy Centre, Laboratory of Biochemistry, Wageningen University Dreijenlaan 3, 6703 HA Wageningen, The Netherlands; [3]W.M. Keck Dyn

The regulation of cell polarity plays an important role in chemotaxis. In *Dictyostelium discoideum*, occasional interruption of polarity leading to direction change is important to achieve optimal cell aggregation. However, the mechanism that underlies the control of polarity is currently poorly understood. We have previously identified two proteins, GbpC and GbpD that contain RasGEF and cyclic nucleotide binding domains. Previously we have shown that GbpC is the major cGMP-target. A gbpC / gbpD double knock out cell has lost the ability to polarize and displayed diminished chemotaxis towards cAMP (Bosgraaf *et al*. (2002) EMBO J. 21: 4560-4570). We have ivestigated the effect of separate disruption of the gbpC and gbpD genes on chemotaxis. We found that gbpC null cells display the same aberrancies as the gbpC -/gbpD – mutants: chemotaxis is severely diminished and cells are unable to polarize in a chemotactic gradient. Furthermore, cAMP-induced myosin II translocation to the cortex is abolished, which could explain the inability to polarize. To our surprise, gbpD null mutants exhibit quite the opposite phenotype: the cells display improved chemotaxis and appear hyperpolar in a chemotactic gradient due to a reduced tendency to make lateral pseudopodia. Furthermore, the speed of chemotaxing cells is greatly increased, which might be related to the decreased adhesion to the substratum that was observed. Overexpression of GbpD results in severely reduced chemotaxis due to an increased tendency to make lateral pseudopodia and enhanced attachment to the substratum. This phenotype was not dependent on the presence of cGMP or cAMP, indicating that GbpD can function without cyclic nucleotides. We conclude that GbpC is needed for polarity during chemotaxis, whereas GbpD disrupts polarity. Understanding the regulation and interplay of these two proteins might reveal important clues on the regulation of cell polarity.

Abstract P50

# The phosducin-like protein phlp1 is essential for Gbeta-gamma dimer formation in *Dictyostelium*.

Jaco C. Knol[1], Ruchira Engel[2], Mieke Blaauw[1], Antonie J.W.G. Visser[2], and Peter J.M. van Haastert[1]

[1]Department of Biochemistry, University of Groningen, Groningen, The Netherlands; [2]MicroSpectroscopy Centre, Laboratory of Biochemistry, Wageningen, The Netherlands

Phosducin proteins are thought to inhibit G protein signalling by sequestering Gbeta-gamma subunits, serving cellular adaptation processes. We have recently characterised three *Dictyostelium* phosducin-like proteins (PhLP1-3), and shown that they are members of three distinct phosducin subfamilies (Blaauw *et al*. (2003) EMBO J. 22: 5047). *Dictyostelium* PhLP1 groups with the classical mammalian phosducin and phosducin-like proteins. Unexpectedly, cells lacking PhLP1 display defective rather than enhanced G protein signalling, and exhibit a phenotype which is very similar to that of Gbeta null cells (no starvation-induced aggregation, no chemotaxis towards cAMP or folic acid, no receptor-G protein interaction, no intracellular cAMP and cGMP responses to stimulation with cAMP). Here, we show that GFP-tagged Gbeta and Ggamma subunits exhibit drastically reduced steady state levels in phlp1_ cells and are absent from the plasma membrane. Triton X-114 partitioning suggests that lipid attachment to GFP-Ggamma occurs in wild-type, but not in phlp1_ and Gbeta null cells, indicating that a processing-competent Gbetagamma complex is not formed. Indeed, Gbetagamma dimer formation could not be detected in co-immunoprecipitation assays with phlp1_ cells. In addition, diffusion measurements using fluorescence correlation spectroscopy showed that GFP-Ggamma proteins diffuse as free monomers in phlp1_ and Gbeta null cells, and as Gbetagamma heterodimers in wild-type cells. Collectively, our data strongly suggest the absence of Gbetagamma dimer formation in *Dictyostelium* cells lacking PhLP1. We propose that PhLP1 serves as a co-chaperone assisting the assembly of Gbeta and Ggamma into a functional Gbetagamma complex. Thus, phosducin family proteins may fulfill hitherto unsuspected biosynthetic functions.

Abstract P51

## Dock180 homologues and their roles in regulation of *Dictyostelium* behaviour.

Jun Wang and Kees Weijer

Wellcome Trust Biocentre, School of Life Sciences, University of Dundee, Dundee DD1 5EH UK

Aggregation competent *Dictyostelium* cells respond by chemotaxis to cAMP gradients. cAMP stimulation causes a transient localized increase in PIP3 concentration in the membrane. PIP3 binding domain containing Rac GEF's are thought to mediate the cAMP signal through the activation of Racs to actin polymerization. Inhibition of PIP3 formation however results only in a partial defect of chemotaxis, implying that there may exist other signaling pathways that mediate the cAMP signal. Members of the Dock family have been show to form another class of Rac activators and deletion of Dock in *Drosophila* and *C.elegans* results in chemotaxis and phagocytosis defects.

We have identified the 8 Dock genes in *Dictyostelium*, which we named as DockA, B, C, D, E, F, G and H. *Dictyostelium* cells in which the genes for DockA and DockB were disrupted develop into small fruit bodies. Slugs formed by mutant cells show poor phototaxis. DockB mutant grow slowly in the axenic medium cells and are defective in pinocytosis and phagocytosis. DockA null mutants grow normally but are defective in pinocytosis. DockA/B double knockout mutant cells show reduced pinocytosis, phagocytosis, and phototaxis and grow slowly in medium. Interestingly, DockA/B mutant cells formed tiny fruiting bodies but preferentially sort to the prestalk region in synergy experiments. DockA/B null cells show reduced chemotaxis which compared to wildtype cells both in the absence and presence of a PI 3 kinase inhibitor LY42008. DockA, B&A/B null mutants all show altered cAMP stimulated actin polymerization responses

Abstract P52

## How TRE5-A retrotransposons select tRNA genes as integration targets – an update.

Oliver Siol, Theodor Dingermann, and Thomas Winckler

Institut für Pharmazeutische Biologie, Universität Frankfurt (Biozentrum), D-60439 Frankfurt am Main, Germany

Almost every organism carries along a multitude of molecular parasites known as transposable elements (TEs). TEs influence their host genomes in many ways by expanding genome size and complexity, rearranging genomic DNA, mutagenizing host genes, and altering transcription levels of nearby genes. *Dictyostelium discoideum* has a highly compacted, haploid genome with less than 1 kb of genomic DNA separating coding regions. Thus, mobile elements can hardly move within the *D. discoideum* genome without causing gene disruption upon reintegration into the genome. Nevertheless, the *D. discoideum* genome is loaded with 10% TEs that managed to settle and survive in this inhospitable environment. In depth analysis of *D. discoideum* genome project data has provided intriguing insights into the evolutionary challenges that mobile elements face when they invade compact genomes. Two different mechanisms are used by *D. discoideum* TEs to avoid disruption of host genes upon retrotransposition. TEs that lack integration specificities show a strong bias to nested integration, thus forming large TE clusters at certain chromosomal loci that are hardly resolved by bioinformatics approaches. By contrast, several TEs have invented the specific targeting of tRNA gene-flanking regions as a means to avoid integration into coding regions. These elements have been dispersed on all chromosomes, closely following the distribution of tRNA genes. We have shown in previous experiments that TRE5-A is actively retrotransposing and the only active one of the seven TRE elements present in the *D. discoideum* genome. We summarize our current view of the molecular mechanism that allows the retrotransposon TRE5-A to integrate ca. 50 bp upstream of tRNA genes. We discuss new data showing that it is the tRNA gene itself that determines an integration site for TRE5-A, and that tRNA gene-specific transcription factors are most likely involved in this process.

Abstract P53

## Phylogenetic analysis of the *Dictyostelium* Ras subfamily.

Michelle Wyse[1], Matthew Rogers[2], Patrick Keeling[2], George Spiegelman[1], and Gerry Weeks[1]

[1]Department of Microbiology and Immunology, University of British Columbia, 6174 University Boulevard, Vancouver, BC, Canada, V6T 1Z3; [2]Department of Botany, University of British Columbia, 6270 University Boulevard, Vancouver, BC, Canada, V6T 1Z4

Previously, five Ras proteins and one Rap protein have been characterized as members of the *Dictyostelium* Ras subfamily. To fully define the *Dictyostelium* Ras subfamily, we examined the genome as presented by the Dictybase database for putative Ras subfamily proteins. Eight new protein sequences were identified, all of which are Ras subfamily members as defined by conserved residues of the G boxes and 50% identity with known Ras subfamily members. A complete phylogenetic analysis revealed that six of the eight newly-identified Ras sequences form a distinct group, designated the RasX group. The remaining two sequences were shown to associate with the Rap group of proteins.

In this study, phylogenetic analysis of all known human Ras subfamily proteins yielded seven groups: H-Ras, R-Ras, Ral, Ric/Rit/Rin, Rap1, Rap2 and Di-Ras. Analysis of the previously characterized *Dictyostelium* Ras proteins, RasG, RasD, RasB and RasC, demonstrated that these proteins form a group that is distinct from RasS. None of these proteins clearly cluster with any of the seven human groups. Conversely, the *Dictyostelium* Rap1 protein strongly associated with the human Rap1 group. The RasX group is also distinct from all seven human groups and does not cluster with the previously characterized *Dictyostelium* Ras proteins; however, its members do appear to be weakly related to the fungal Ras2 proteins.

These results suggest that *Dictyostelium* possesses extensive Ras signaling networks, which may prove to be important in the dissection of Ras function.

Abstract P54

## Transcriptional transitions in *Dictyostelium* spore germination.

Q. Xu[1], M. Ibarra[1], D. Mahadeo[2], C. Shaw[1], E. Huang[1], A. Kuspa[1], D. Cotter[2], and G. Shaulsky[1]

[1]Baylor College of Medicine, USA; [2]University of Windsor, Canada

The soil amoeba *Dictyostelium discoideum* forms asexual spores in response to starvation. The spores germinate in favorable conditions to restore vegetative growth. The germination process is characterized by two morphological transitions: the swelling phase and the amoeba emergence. We used cDNA microarray technology to study the physiological changes during spore germination. Genome-wide transcriptional transitions corresponding to the morphological transitions were found. Genes having invariant expression pattern regardless of activation treatment were filtered out and divided into different groups. Gene Ontology analysis on these groups provided some insights into the biological processes involved during spore germination. Mutant analysis on genes that are specifically induced during germination identified myoI as a target that controls *Dictyostelium* germination process. Further study of the myoI mutant spores suggests that gene participates in a novel germination pathway.

Abstract P55

## GOAT: A Gene Ontology Analysis Tool Package.

Q. Xu, C. Shaw, N. Whitehouse, and G. Shaulsky

Baylor College of Medicine, USA

Gene Ontology (GO) provides a universal set of hierarchically structured terms for describing gene functions. In microarray data analysis, we often get a list of genes that are differentially expressed or have a common expression pattern. Examining the GO term enrichment in the list may help to understand the biological functions that correlate with the gene expressions. Here we present a GO term enrichment analysis tool, GOAT, based on the R statistical software. Given a gene list, the tool can recursively traverse all three ontologies (biological process, molecular function and cellular component) and return those GO terms that are significantly enriched in the gene list. This tool can be applied to any organism that has a genome scale annotation as those listed on the Gene Ontology website (http://www.geneontology.org). It also allows the user to easily update the ontologies and annotations. The package also contains a plotting tool that can be used to make a graphical presentation of the enrichment analysis result. Since the package is written in R, it can be seamlessly incorporated into microarray data analysis packages like BioConductor. The prototype of this tool has been successfully applied in some of our research work.

Abstract P56

## Identify novel functions of DNA Mismatch Repair (MMR) protein using *D. discoideum* as a model system.

X.S. Xu, Y. Zhang, and G. Wang

Wayne State University, USA

DNA repair plays an important role in maintaining genetic integrity. Deficiency in DNA repair has been linked to many human diseases including cancer. Understanding the molecular mechanism on how the DNA repair deficiency leads to the disease development is important for the disease prevention and treatment. However, due to the complexity of repair pathways in human cells, it is difficult to determine the specific roles of individual DNA repair proteins in the disease development process. Therefore, simpler model systems that can represent the human counterpart will be greatly appreciated. *D. discoideum* carries many genes that exhibit greater homology to human genes than those found in other model systems. Its short life cycle shares many developmental processes with mammalian cells. In addition, *D. discoideum* is resistant to many DNA damaging agent, such as ionizing radiation and Cisplatin, the cancer chemotherapeutic drug. Several DNA repair genes, including the Nucleotide Excision Repair (NER) genes XPB (repB) and XPD (repD), and the Base Excision Repair (BER) genes, AP endonuclease and UDP-glycosylase, have been cloned from the *D. discoideum*. However, none of the Mismatch Repair (MMR) genes, which play a critical role in the prevention of many types of cancer, especially for hereditary non-polyposis colorectal cancer (HNPCC) and prostate cancer, have been investigated. Recently, we have cloned two major human homologue repair genes in the MMR pathway (Msh2 and Mlh1) from this model system. Sequencing analyses indicate that these two genes have 40% identity and 55% similarity to their human counterparts. Preliminary data indicate that the expressions of Msh2 and Mlh1 gene are up regulated during development, which suggests that these two genes may play an important role during *Dictyostelium* development. This work allows us to study the processes involved in modulating the initiation phase of carcinogenesis and in modulating genetic stability/instability, both of which are relevant to the processes involved in tumor progression. In addition, this work will allow us to identify the roles that the MMR proteins play in the cancer cell drug resistance and provide a model system to screen new therapeutic drugs and environmental agents that cause DNA damage.

Abstract P57

## Dynamics of chemotactic gradient sensing in *Dictyostelium* revealed by quantitative live cell imaging.

Xuehua Xu[1], Martin Meier-Schellersheim[2], Lauren E. Nelson[1], and Tian Jin[1]*

[1]Laboratory of Immunogenetics; [2]Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, NIH Twinbrook II Facility, 12441 Parklawn Drive, Rockville, Maryland 20852, USA

The central question driving chemotaxis research is how an external chemoattractant gradient is translated into a steep intracellular gradient of certain signaling components leading to morphological cell polarization and directional cell movement. Determining the detailed spatiotemporal patterns of intracellular localization and activation of the components of the chemotactic signaling pathway is essential for answering this question. Here, we have reported advances in live-cell fluorescence microscopy that allowed us to measure the applied chemoattractant concentration, the degree of G-protein activation by FRET imaging, and changes in the level and distribution of PIP3 in single living cells with the needed high temporal and spatial resolution. Our study confirmed the expectation that the extent of G-protein activation in different regions of the cell surface reflects the local extracellular cAMP concentration. We found that a higher level of uniformly applied cAMP stimulation triggers not only a stronger G-protein activation but also a faster adaptation. Using a new method that allowed us to abruptly expose naïve cells (which have not experienced chemoattractant) to stable cAMP gradients in a controlled manner, we found that G-proteins were persistently activated at the entire cell surface under this condition, whereas PIP3 accumulation in the front of the cell displayed an unexpected biphasic temporal pattern, an initial transient and asymmetrical PIP3 accumulation around the cell membrane, followed by a second phase producing a highly polarized distribution of PIP3 only in the front. This latter observation is not consistent with the predictions of the prevailing “local excitation, global inhibition” model, and we instead propose a modified model involving signal-dependent locally recruited inhibitors to account for the observed dynamics of PIP3.

# Participant List

**Tomoaki, Abe**
Ishinomaki Senshu University
1 Shinmito, Minami-Sakai
Ishinomaki, Miyagi, 986-8580, Japan
Tel: 81-225-22-7713 ex.3108
Fax: 81-225-22-7710
Email: tabe@isenshu-u.ac.jp

**Kathryn Adley**
University College London
Darwin Building Gower St
London, Wc1 E6BT, UK
Tel: 44 20 7679 7268
Fax: 44 20 7679 7069
Email: K.gregory@ucl.ac.uk

**Steve Alexander**
University of Missouri
Division of Biological Sciences, 303 Tucker Hall
Columbia, MO, 65211-7400, USA
Tel:1 573-882-6670
Fax: 1 573-884-0123
Email: alexanderst@missouri.edu

**Aiko Amagai**
Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University
Aoba, Sendai 980-8578, Japan
Tel: 81-22-217-6710
Fax: 81-22-217-6710
Email: aiamgai@mail.tains.tohoku.ac.jp

**Christophe Anjard**
University of California, San Diego
9500 Gilman drive
La Jolla, CA, 92093, USA
Tel : 1 858 534 2465
Fax : 1 858 822 2094
Email : canjard@biomail.ucsd.edu

**Tsuyoshi Araki**
Wellcome Trust Biocentre, School of Life Science, University of Dundee
Dow Street
Dundee, DD1 5EH, UK
Tel: 44-1382-344586
Fax: 44-1382-345386
Email: t.araki@dundee.ac.uk

**Deenadayalan Bakthavatsalam**
Institute of Biochemistry I, University of Koeln
Joseph Stelzmann Str, 52
Koeln, Nordrhein-Westfalen, 50931, Germany
Tel: 49 2214786981
Fax: 49 2214786979
Email: ake65@uni-koeln.de

**Peter Beech**
Deakin University
221 Burwood Hwy
Melbourne, Victoria, 3125, Australia
Tel: 61 3 92517341
Fax: 61 3 92517328
Email: plbeech@deakin.edu.au

**Gareth Bloomfield**
MRC Laboratory of Molecular Biology
Hills Road
Cambridge, Cambridgeshire, CB2 2QH, UK
Tel: +44 1223 834244 Ext. 7123
Fax: +44 1223 412142
Email: garethb@mrc-lmb.cam.ac.uk

**Daphne Blumberg**
University of Maryland, Baltimore County
Department of Biological Sciences
Baltimore, Maryland, 21250, USA
Tel: 1-410-455-2242
Fax : 1-410-455-3875
Email : blumberg@umbc.edu

**Bethany Bodwell**
University of Maine
5735 Hitchner Hall
Orono, Maine, 04469-5735, USA
Tel : 1-207-581-2802
Fax : 1-207-581-2801
Email : Bethany_Bodwell@umit.maine.edu

**Katrina Boeckeler**
FU Berlin – Microbiology
Koenigin-Luise Str. 12-16
Berlin, 14195, Germany
Tel: 49-30-83853373
Fax: 49-30-83857773
Email: kboeck@zedat.fu-berlin.de

**Salvatore Bozzaro**
University of Turin
Department of Clinical and Biological Sciences, Ospedale S. Luigi
Orbassano, Torino, 10043, Italy
Tel: 39-0116705406
Fax: 39-0119038639
Email: salvatore.bozzaro@unito.it

**Derrick Brazill**
Hunter College, City University of New York
695 Park Ave
New York, NY, 10021, USA
Tel: 1 212-650-3144
Fax: 1 212-772-5227
Email: brazill@genectr.hunter.cuny.edu

**Till Bretschneider**
Max-Planck Institute of Biochemistry
Am Klopferspitz 18A
Martinsried, D-82152, Germany
Tel: 49-89-8578 2329
Fax: 49-89-8578-3885
Email: bretschn@biochem.mpg.de

**Debbie Brock**
HHMI/Rice University
Biochemistry Dept., Ms-140 Rice University
Houston, Texas, 77005-1892, USA
Tel: 1 713-348-2394
Email: dbrock@bioc.rice.edu

**Matthew Cabral**
Baylor College of Medicine
One Baylor Plaza
Houston, Texas, 77030, USA
Tel: 1 713 798-4532
Fax: 1 713 798-8597
Email: mcabral@bcm.tmc.edu

**Mathieu Cellier**
INRS-Institut Armand-Frappier
531, Blvd des Prairies
Laval, QC, H7V 1B7, Canada
Tel: 1 450-687-5010 Ext. 4681
Fax: 1 450-686-5301
Email: mathieu.cellier@inrs-iaf.uquebec.ca

**Steve Charette**
Dept. de Physiologie cellulaire et métabolisme, CMU
1 rue Michel Servet
Geneve, 1211, Switzerland
Tel: 41 22 379 5294
Fax: 41 22 379 5338
Email: charett2@etu.unige.ch

**Yi Chen**
Hunter College, City Univeristy of New York
695 Park Ave.
New York, NY, 10021, USA
Tel: 1-212-650-3862
Fax : 1-212-772-5227
Email : ychen@genectr.hunter.cuny.edu

**Lingfeng Chen**
Johns Hopkins School of Medicine/Department of Cell Biology
WBSB 116, 725 N. Wolfe st
Baltimore, MD, 21205, USA
Tel: 1-410-955-4699
Fax: 1-410-614-9461
Email: lchen5@jhmi.edu

**Rex Chisholm**
Northwestern University
303 E. Chicago Ave.
Chicago, IL , 60611, USA
Tel: 1-312-503-3209
Fax: 1-312-503-3210
Email: r-chisholm@northwestern.edu

**Jonathan Chubb**
Albert Einstein College of Medicine
1300 Morris Park Avenue
New York, NY, 10461, USA
Tel:1 718 430 8597
Email: jchubb@aecom.yu.edu

**Barrie Coukell**
York University
4700 Keele St.
Toronto, ON, M3J 1P3, Canada
Tel: 416-736-2100 Ext. 33554
Fax: 416-736-5698
Email: bcoukell@yorku.ca

**Chang Chung**
Vanderbilt University Medical Center
468 RRB 1215 21st Ave. South @ Pierce
Nashville, TN, 37232, USA
Tel: 1-615-322-4956
Fax : 1-615-343-6532
Email : chang.chung@vanderbilt.edu

**Edward C. Cox**
Princeton University
Department of Molecular Biology, 333 Moffett Lab
Princeton, NJ, 08544-1014, USA
Tel : 1 609-258-1604
Fax :1 609-258-1343
Email : swinslow@molbio.princeton.edu

**Margaret Clarke**
Oklahoma Medical Research Foundation
825 N.E. 13th Street
Oklahoma City, OK, 73104, USA
Tel: 1-405 271-7664
Fax: 1-405 271-3153
Email: clarkem@omrf.ouhsc.edu

**Cynthia Damer**
Vassar College
124 Raymond Ave. Box 566
Poughkeepsie, NJ, 12603, USA
Tel : 1-845-437-7115
Fax : 1-845-437-7315
Email : cydamer@vassar.edu

**Margaret Clotworthy**
MRC Laboratory of Molecular Biology
Hills Road
Cambridge, Cambridgeshire, CB2 2QH, UK
Tel: 441223402271
Fax: 441223412142
Email: clotworm@mrc-lmb.cam.ac.uk

**Sandra de Keijzer**
Leiden University
Wassenaarseweg 64
Leiden, 2333 Al, Netherlands
Tel: 31-71-5274980
Fax: 31-71-5274999
Email: keijzer@physics.leidenuniv.nl

**Laetitia Cortes**
INRS- Institut Armand-Frappier
531 boulevard des Prairies
Laval, QC, H7V 1B7, Canada
Tel: 1 450 687 50 10
Email: laetitia.cortes@inrs-iaf.uquebec.ca

**Kimchi Doquang**
Concordia University
L-SP 510
7141 Sherbrooke St. West
Mtl. QC, H4B 1R6, Canada
Tel: 1 514 848 2424 Ext. 8654
Fax: 1 514 848 4504
Email: kimchi@gene.concordia.ca

**David Cotter**
University of Windsor
401 Sunset Avenue
Windsor, ON, N9B 3P4, Canada
Tel: (519)253-3000 Ext. 5042
Fax: (519) 971-3609
Email: cotter1@unwindsor.ca

**Dirk Dormann**
Wellcome Trust Biocentre, Division of Cell and Developmental Biology
University of Dundee
Dundee, DD1 5EH, UK
Tel: 44 1382345891
Fax: 441382345386
Email: d.dormann@dundee.ac.uk

**Robert Dottin**
Hunter College City University of New York
695 Park Ave
New York, NY, 10021, USA
Tel: 1 212 772 5171
Fax:1 212 772 5466
Email: dottin@genectr.hunter.cuny.edu

**Maria-Berenice Duran**
Hunter College, City University of New York
695 Park Ave.
New York, NY, 10021, USA
Tel: 1 212 650 3862
Fax: 1 212 772 5227
Email: duran@genectr.hunter.cuny.edu

**Ludwig Eichinger**
University of Cologne, Institute for Biochemistry
Joseph-Stelzmann-Str. 52
Cologne, D- 50931, Germany
Tel: 49 221 4786928
Fax: 49 221 4786979
Email: ludwig.eichinger@uni-koeln.de

**Okyay Ezgi**
Baylor College of Medicine
One Baylor Plaza, S432
Houston, Texas, 77030, USA
Tel:1 713-798-8308
Fax:1 713-798-8142
Email: eo135456@bcm.tmc.edu

**Jan Faix**
Ludwig-Maximilians University
Schillerstr. 42
Munich, 80336, Germany
Tel: 49-89-5996-873
Fax: 49-89-5996-882
Email: hfaix@bio.med.uni-muenchen.de

**Patrick Farbrother**
Institute for Biochemistry I
Joseph-Stelzmann-Str. 52
Cologne, D- 50931, Germany
Tel: 492214786928
Fax: 492214786979
Email: patrick.farbrother@uni-koeln.de

**Ira Feit**
Franklin snd Marshall College
Department of Biology
Lancaster, PA, 17601, USA
Tel:1 717 291-2959 or 291-4118
Fax:1 717 358-4548
Email: ifeit@fandm.edu

**Petra Fey**
Northwestern University
303 E Chicago Ave, Ward 9-142
Chicago, IL, 60611, USA
Tel: 1-312-503-4372
Fax:1-312-503-3210
Email: pfey@northwestern.edu

**Rick Firtel**
Cell and Development Biology, University of California, San Diego
9500 Gilman Drive
La Jolla, CA, 92093-0634, USA
Tel : 1-858-534-2788
Fax : 1-858-822-5900
Email : rafirtel@ucsd.edu

**Paul Fisher**
La Trobe University
Bundoora
Melbourne, Victoria,3086, Australia
Tel: 61 3 9479 2496
Fax: 61 3 9479 1222
Email: P.Fisher@latrobe.edu.au

**Robert Fitzhenry**
Columbia University
630 West 168th Street
New York, NY, 10032, USA
Tel: 1 212 305 1512
Fax: 1 212 305 3970
Email: rjf2105@columbia.edu

**Elizabeth Ford**
University of Maryland, Baltimore County
1000 Hilltop Circle
Baltimore, Maryland, 21250, USA
Tel: 1-410-455-3474
Email: elf2@umbc.edu

**Jonathan Franca-Koh**
John Hopkins University
8314 Silver Trumpet Drive
Columbia, MD, 21045, USA
Tel:1 410 964 2523
Fax:1 410 614 9461
Email: jonnyfrancakoh@yahoo.co.uk

**Jakob Franke**
Columbia University
P&S 12-442, 630 W 168 Street
New York, NY, 10032, USA
Tel: 1-212-342-0178
Fax: 1-212-305-3970
Email: jf31@columbia.edu

**Danny Fuller**
University of California, San Diego
Division of Biology
9500 Gilman Dr. MC0368
La Jolla, CA, 92093-0368, USA
Tel : 1 858-534-2465
Fax:1 858-822-2094
Email: dfuller@ucsd.edu

**Pascale Gaudet**
Northwestern University
303 E. Chicago Ave, Ward 1-142
Chicago, IL, 60611, USA
Tel: 1-312-503-4665
Fax: 1-312-503-3210
Email: pgaudet@northwestern.edu

**Gernot Gloeckner**
Institute for Molecular Biotechnology
Beutenbergstr. 11
Jena, 07745, Germany
Tel: 49-3641-656440
Fax: 49-3641-656255
Email: gernot@imb-jena.de

**Pierre Golstein**
Centre d'Immunologie de Marseille-Luminy
Case 906, avenue de Luminy
Marseille, 13, France
Tel : 33 491 26 94 68
Fax : 33 491 26 94 30
Email : golstein@ciml.univ-mrs.fr

**Richard Gomer**
HHMI/ Rice
Dept. Biochem MS-140 6100 S. Main
Houston, Texas, 77005-1892, USA
Tel: 1 713 348-4872
Fax: 1 713 348-5154
Email: richard@rice.edu

**Scott Gruver**
Vanderbilt University
1215 21st Ave. South, RRB 468
Nashville, TN, 37232, USA
Tel : 1-615-322-5037
Fax : 1-615-343-6532
Email : Scott.Gruver@Vanderbilt.edu

**Adrian Harwood**
University College London
Gower St
London, WC1E 6BT, UK
Tel: 44 207 679 7257
Fax: 44 207 679 7805
Email: a.harwood@ucl.ac.uk

**Paul Heid**
University of Iowa
Department of Biological Sciences, Rm. 14 BBE
Iowa City, Iowa, 52242, USA
Tel:1 319 335-2883
Fax:1 319 335-2772
Email: paul-heid@uiowa.edu

**Dale Herald**
The University of Texas Medical School at Houston
6431 Fannin Street, JFB 1.765
Houston, Texas, 77030, USA
Tel: 1-713-500-5444
Fax: 1-713-500-5499
Email: dhereld@uth.tmc.edu

**David Hilbert**
Columbia University
630 W. 168th St. P & S 12-517
New York, NY, 10032, USA
Tel:1 212-305-1512
Fax:1 212-305-3970
Email: dwh2102@columbia.edu

**Negin Iranfar**
University of California, San Diego
9500 Gilman Drive, MC 0368
La Jolla, CA, 92093, USA
Tel : 1 858-534-3021
Fax : 1 858-822-2094
Email : niranfar@ucsd.edu

**Kentaro, Ishida**
Graduate School of Life Environmental Sciences, University of Tsukuba
1-1-1 Tennoudai
Tsukuba, Ibaraki, 305-8572, Japan
Tel: 81 29 853 4910
Fax: 81 29 853 4910
Email: ikentaro@sakura.cc.tsukuba.ac.jp

**Tian Jin**
NIAID/NIH
12441 Parklawn drive, TwinbrookII
Rockville, MD, 20852, USA
Tel : 301-480-1430
Fax : 301 480-2168
Email : tjin@niaid.nih.gov

**Eric Just**
Northwestern University
303 E. Chicago Ave. Ste 9-142
Chicago, IL, 60611, USA
Tel:1 312.503.3218
Fax:1 312.503.3210
Email: e-just@northwestern.edu

**Helmut Kae**
University of British Columbia
5645 College St
Vancouver, BC, V5R 3Z6, Canada
Tel :1 604-822-0997
Fax :1 604-822-6041
Email : hkae@interchange.ubc.ca

**Mariko Katoh**
Baylor College of Medicine
One Baylor Plaza, S426
Houston, Texas, 77030, USA
Tel: 1 713-798-4347
Fax: 1 713-798-8142
Email: mkatoh@bcm.tmc.edu

**Yoshinori Kawabe**
University of Tsukuba
1-1-1 Tennodai
Tsukuba, Ibaraki, 305-8572, Japan
Tel: 81-29-853-7725
Fax: 81-29-853-7723
Email: favonius@sakura.cc.tsukuba.ac.jp

**Robert Kay**
MRC Laboratory of Molecular Biology
Hills Rd,
Cambridge, Cambridgeshire, CB2 2QH, UK
Tel: 44 1223 402298
Fax: 44 1223 412142
Email: rrk@mrc-lmb.cam.ac.uk

**Richard Kessin**
Columbia University
630 West 168th St
New York, NY, 10032, USA
Tel: 1 212 305 5653
Fax : 1 212 305 3671
Email : rhk2@Columbia.edu

**Leung Kim**
Florida International University
11200 SW 8th Street
Miami, Florida, 33199, USA
Tel: 1 305-348-7315
Fax: 1 305-348-1986
Email: kiml@fiu.edu

**Alan Kimmel**
NIH
Building 50/ Room 5521
Bethesda, MD, 20892, USA
Tel: 1 301-496-3016
Fax: 1 301-496-5239
Email: ark1@helix.nih.gov

**Jason King**
MRC-LMCB, University College London
Gower Street
London, WC1E 6BT, UK
Tel: 44 0 20 7679 2122
Fax: 44 0 20 7679 7805
Email: jason.king@ucl.ac.uk

**Kashif Kirmani**
University of Windsor
401 Sunset Ave.
Windsor, ON, N9B3P4, Canada
Tel: 1 519-253-3000 Ext.2701
Fax: 1 519-971-3609
Email: kashifzkirmani@hotmail.com

**Janet H. Kirsten**
Vanderbilt University
Dept. Biological Sciences; VU Station B, Box35-1634
Nashville, TN, 37235-1634, USA
Tel : 1 615-322-3274
Fax : 1 615-343-6707
Email : jan.kirsten@vanderbilt.edu

**Norio Kobayashi**
Graduate School of Life Environmental Sciences, University of Tsukuba
1-1-1 Tennoudai
Tsukuba, Ibaraki, 305-8572, Japan
Tel: 81 29 853 4910
Fax: 81 29 853 4910
Email: nori@nori.tv

**Michael Koonce**
Wadsworth Center
Empire State Plaza, PO Box 509
Albany, NY, 12201-0509, USA
Tel: 1 518-486-1490
Fax: 1 518-486-4901
Email: koonce@wadsworth.org

**Arjan Kortholt**
University of Groningen
Nijenborgh 4
Groningen, 9747AG, Netherlands
Tel: 31 50 363 4172
Fax: 31 50 363 4165

**Paul Kriebel**
National Cancer Institute
2712 Emmet Rd
Silver Spring, Maryland, 20902, USA
Tel: 1 301-962-5333
Fax : 1 301-496-8479
Email : kpaul@helix.nih.gov

**Adam Kuspa**
Baylor College of Medicine
One Baylor Plaza, S338
Houston, Texas, 77030, USA
Tel: 1 713-798-8278
Fax: 1 713-798-4086
Email: akuspa@bcm.tmc.edu

**Jennie Kuzdzal**
Rice University, Ecology and Evolutionary Biology, MS-170
P O Box 1892
Houston, Texas, 77251-18, USA
Tel: 1 713 348 4919
Fax: 1 713 348 5232
Email: jkuzdzal@rice.edu

**Denis Larochelle**
Clark University
950 Main St.
Worcester, MA, 01610, USA
Tel: 1 508 793-7631
Fax: 1 508 793-8861
Email: dlaroche@black.clarku.edu

**Andrea Lasagni**
Rice University
6100 Main Street
Houston, Texas, 77005, USA
Tel: 1 713 348 4913
Email: lasagni@rice.edu

**Hsiu-Hsu Sophia Lin**
WTB/MSI complex, School of Life Sciences, University of Dundee
Dow Street
Dundee, DD1 5EH, UK
Tel: 44-1382-345079
Fax: 44-1382-345386
Email: h.z.lin@dundee.ac.uk

**David Lindsey**
Walla Walla College
204 S. College Ave
College Place, WA, 99324, USA
Tel: 1 509-526-5731
Email: lindda@wwc.edu

**William Loomis**
University of California, San Diego, Division of Biology, 9500 Gilman Dr. MC0368
La Jolla, CA, 92093-0368, USA
Tel : 1 858-534-2543
Fax : 1 858-822-2094
Email : wloomis@ucsd.edu

**Natasha Mehdiabadi**
Rice University
Ecology and Evolutionary Biology, MS 170,
Main Street, Houston, Texas, 77005-1892, USA
Tel: 1-713-348-2762
Fax: 1-713-348-5232
Email: njum@rice.edu

**Yasuo Maeda**
Tohoku University, Graduate School of Life Sciences, Department of developmental Biology and Neurosciences
Aoba, Sendai, 980-8578, Japan
Tel : 81-22-21706709
Fax : 81-22-217-6710
Email : taira@biology.tohoku.ac.jp

**Rudolf Meili**
University of California, San Diego
Biology 0634 CMG Rm220
La Jolla, CA, 92093, USA
Tel : 1 858 534 4825
Fax : 1 858 822 5900
Email : rmeili@biomail.ucsd.edu

**Sebastian Mana-Capelli**
Clark University
950 Main St., Worcester, MA, 01610, USA
Tel : 1 508 793-7664
Fax : 1 508 793-8861
Email : smanacapelli@clarku.edu

**Sohel Merchant**
Northwestern University
303 E. Chicago Ave
Chicago, IL, 60611, USA
Tel: 1 312 503 4820
Fax: 1 312 503 3210
Email: merchantsohel@yahoo.com

**Andrew Maselli**
Chicago State University
9501 S. King Drive SCI310
Chicago IL, 60628, USA
Tel: 1 773-995-3298
Fax: 1 773-995-3759
Email: amaselli@csu.edu

**Talibah Metcalf**
University of Oklahoma Health Sciences Center, 940 Stanton L. Young Blvd., Rm. 937
Oklahoma City, OK, 73104, USA
Tel: 1 405-271-2227 x1247
Fax : 1 405-271-3139
Email : Talibah-metcalf@ouhsc.edu

**Taira Mayanagi**
Tohoku University
Department of Developmental Biology and Neuroscience, Graduate School of Life Science, Aramaki, Aoba
Sendai, Miyagi, 980-8578, Japan
Tel : 81-22-217-6710
Fax : 81-22-217-6710
Email: taira@biology.tohoku.ac.jp

**Takahiro Morio**
Graduate School of Life and Environmental Sciences, University of Tsukuba
1-1-1 Tennoudai
Tsukuba, Ibaraki, 305-8572, Japan
Tel: 81-29-853-7725
Fax: 81-29-853-7723
Email: morio@sakura.cc.tsukuba.ac.jp

**Kyle McQuade**
Princeton University
333 Moffett Labs
Princeton. NJ, 08544, USA
Tel : 1 609 258 3571
Email : kmcquade@molbio.princeton.edu

**Annette Muller-Taubenberger**
Max Planck Institute for Biochemistry
Am Klopferspitz 18, Martinsried, 82152, Germany
Tel: 49 89 85782339
Fax: 49 89 8578-3885
Email: amueller@biochem.mpg.de

**Tetsuya Muramoto**
Graduate School of Life Environmental Sciences, University of Tsukuba
1-1-1 Tennoudai
Tsukuba, Ibaraki, 305-8572, Japan
Tel: 81 29 853 4910
Fax: 81 29 853 4910
Email: tetsuya@nenkin.gene.tsukuba.ac.jp

**Scott Myers**
Vanderbilt University
2200 21st Ave South, RRB Room 468
Nashville, TN, 37232, USA
Tel : 1 615-322-5037
Email : scott.myers@vanderbilt.edu

**Lauren Naliboff**
Vassar College
124 Raymond Ave
Poughkeepsie, NY, 12604, USA
Tel : 1 207-491-6704
Email : lanaliboff@vassar.edu

**Margaret Nelson**
Allegheny College
520 North Main Street
Meadville, PA, 16335, USA
Tel : 1-814-332-2788
Fax : 1-814-332-2789
Email : mnelson@allegheny.edu

**Liem Nguyen**
Hunter College, City University of New York
695 Park Avenue
New York, NY, 10021, USA
Tel: 1 212-650-3862
Email: liem2001@earthlink.net

**Clement Nizak**
Rockefeller University, Leibler Lab
1230 York Avenue box 34
New York, NY, 10021, USA
Tel: 1 212 327 7642
Email: clement.nizak@mail.rockefeller.edu

**Angelika A. Noegel**
Centre for Biochemistry, Medical Faculty, University of Cologne
Joseph-Stelzmann-Str. 52, Building 44
Cologne, NRW, D- 50931, Germany
Tel: 49-221-4786981
Fax : 49-221-4786979
Email : ake01@uni-koeln.de

**Hiroshi Ochiai**
Hokkaido University
Chuo-ku, Kita 3 Jo, Nishi 13 Chome, Churisu Kita 3 Jo, 602 Go
Sapporo, Hokkaido, 060003, Japan
Tel: 81-11-261-3873
Fax: 81-11-261-3881
Email: hochiai@sci.hokudai.ac.jp

**Akiko Oohata**
Biological Laboratory, Kansai Medical University, 18-89, Uyamahigasimachi
Hirakata, Osaka, 573-1136, Japan
Tel: 81-72-856-2121
Fax: 81-72-850-0733
Email: oohata@makino.kmu.ac.jp

**Young Shik Park**
School of Biotechnology and Biomedical Science, Inje University, Obangdong 607
Kimhae, 621-749, South Korea
Tel: 81-55-320-3263
Fax: 81-55-336-7706
Email : mbyspark@inje.ac.kr

**Catherine Pears**
Oxford University, Biochemistry Department
South Parks Road
Oxford, OX1 3QU, UK
Tel: 44 1865 275737
Fax: 44 1865 275259
Email: pears@bioch.ox.ac.uk

**Karen Pilcher**
Northwestern University
303 E. Chicago Ave.
Chicago, IL, 60611, USA
Tel: 1-312-503-5392
Fax: 1-312-503-3210
Email: kpilcher@northwestern.edu

**David Queller**
Rice University
Dept. of Ecology and Evolution, MS-170, Rice University, P.O. Box 1892
Houston, Texas, 77005-1892, USA
Tel: 1 713-348-5220
Fax: 1 713-348-5232
Email: queller@rice.edu

**David Ratner**
Amherst College, Department of Biology
Amherst, Massachusetts, 01002, USA
Tel: 1-413-542-2248
Fax: 1-413-542-7955
Email: diratner@amherst.edu

**Jared Rifkin**
Queens College
65-30 Kissena Boulevard
Flushing, NY, 11367-1597, USA
Tel: 1-718-997-3432
Fax: 1-718-997-3445
Email: jared_rifkin@qc.edu

**Francisco Rivero**
Center for Biochemistry, Medical Faculty, University of Cologne
Joseph-Stelzmann-Str. 52
Cologne, D- 50931, Germany
Tel: 49-221-4786987
Fax : 49-221-478-3660
Email : francisco.rivero@uni-koeln.de

**Jonathan Ryves**
MRC-LMCB and CBU, UCL
Gordon Street, London, WC1E 6BT, UK
Tel: 44 0 2076793517
Fax: 44 0 2076797805
Email: dmcbwjr@ucl.ac.uk

**Lorenzo Santorelli**
Rice University
6100 Main Street
Houston, Texas, 77005, USA
Tel: 1 713 348 4913
Email: lasanto@rice.edu

**Leandro Sastre**
Instituto de Investigaciones Biomédicas
CSIC/UAM, Arturo Duperier, 4
Madrid, 28029, Spain
Tel: 34 91 585 4437
Fax: 34 91 585 4401
Email: lsastre@iib.uam.es

**Satoshi Sawai**
Princeton University
Department of Molecular Biology, 333 Moffett Laboratory, Princeton, NJ, 08544, USA
Tel : 1-609-258-3571
Fax : 1-609 258-1343
Email : ssawai@molbio.princeton.edu

**Charles Saxe**
Emory University School of Medicine
Department of Cell Biology, Whitehead Research Building, Room 405F, 615 Michael Street, Atlanta, Georgia, 30322-3030, USA
Tel: 1-404-727-6248
Fax: 1-404-727-6256
Email: karl@cellbio.emory.edu

**Pauline Schaap**
School of Life Sciences, University of Dundee
Dow Street, Dundee, Angus, DD15EH, UK
Tel: 44 1382 348078
Fax: 44 1382 345386
Email: p.schaap@dundee.ac.uk

**Michael Schleicher**
Adolf-Butenandt-Institut/Zellbiologie,
University of Munich, Schillerstrasse 42
Munich, Bavaria, 80336, Germany
Tel: 49895996876
Fax: 49895996882
Email: schleicher@lrz.uni-muenchen.de

**David Secko**
University of British Columbia
University Blvd.,
Vancouver, BC, V6T 1Z3, Canada
Tel : 1-604-822-0997
Fax : 1-604-822-6041
Email : dmsecko@interchange.ubc.ca

**Gad Shaulsky**
Baylor College of Medicine
One Baylor Plaza, S430
Houston, Texas, 77030, USA
Tel: 1 713-798-8082
Fax: 1 713-798-1021
Email: gadi@bcm.tmc.edu

**Philip Silverman**
Oklahoma Medical Research Foundation
825 NE 13th Street
Oklahoma City, OK, 73104, USA
Tel: 1 405 271 7663
Fax: 1 405 271 3153
Email: silvermanp@omrf.ouhsc.edu

**Chi-Hung Siu**
University of Toronto
112 College Street
Toronto, ON, M5G 1L6, Canada
Tel: 416-978-8766
Fax: 416-978-8528
Email: chi.hung.siu@utoronto.ca

**Jason Skelton**
Wellcome Trust Sanger Institute
Wellcome Trust Genome Campus
Cambridge, Cambridgeshire, CB10 1SA, UK
Tel: 44 1223 834244
Fax: 44 1223 494919
Email: jps@sanger.ac.uk

**Janet Smith**
Boston Biomedical Research Institute
64 Grove St.
Watertown, MA, 02461, USA
Tel:1 617-658-7783
Fax:1 617-972-1761
Email: smith@bbri.org

**Ewa Boguslawa Snaar-Jagalska**
Leiden University
Wassenaarseweg 64
Leiden, NL, 2333 AL, Netherlands
Tel: 00315274980
Fax: 00315274999
Email: jagalska@rulbim.leidenuniv.nl

**Thierry Soldati**
Department of Biological Sciences, Imperial College London, Sir Alexander Fleming Building, South Kensington Campus
London, SW7 2AZ, UK
Tel: 44-20-75945360
Fax: 44-20-75842056
Email: t.soldati@imperial.ac.uk

**Loling Song**
Cornell University, Department of Physics
117 Clark Hall
Ithaca, NY, 14853, USA
Tel: 1-607-255-5522
Fax: 1-607-255-6428
Email: LS99@cornell.edu

**Edward Squires**
University of Windsor
401 Sunset Drive
Windsor, ON, N9B 3P4, Canada
Tel: 1 519 253-3000
Fax: 1 519 971-3609
Email: edofficial@hotmail.com

**Joan Strassmann**
Rice University
Dept. of Ecology and Evolution, MS-170, Rice University, P.O. Box 1892
Houston, Texas, 77005-1892, USA
Tel:1 713-348-4922
Fax:1 713-348-5232
Email: strassm@rice.edu

**Lana Strmecki**
University of Oxford, Department of Biochemistry
South Parks Road
Oxford, OX1 3QU, UK
Tel: 44 1865 275786
Fax: 44 1865 275 259
Email: lana.strmecki@bioch.ox.ac.uk

**Teresa Suarez**
Centro de Investigaciones Biológicas
Ramiro de Maeztu, 9
Madrid, 28040, Spain
Tel: 34 91837 3112
Fax: 34 91 360 432
Email: teresa@cib.csic.es

**Richard Sucgang**
Baylor College of Medicine
1 Baylor Plaza 307B
Houston, Texas, 77030, USA
Tel:1 713 798 7657
Fax: 1 713 796 9438
Email: rsucgang@bcm.tmc.edu

**Peter Thomason**
University of Birmingham
Edgbaston, Birmingham, B15 2TT, UK
Tel: 44 121 414 2508
Email: P.Thomason@bham.ac.uk

**Chris Thompson**
School of Biological Sciences, University of Manchester
Oxford Road
Manchester, M13 9PT, UK
Tel: 44 (0)161 275 5632
Email:christopherthompson@man.ac.uk

**Adrian Tsang**
Concordia University
7141 Sherbrooke Street West
Mtl., QC, H4B 1R6, Canada
Tel 1 514 848 2424 Ext.3405
Fax: 1 514 848 4504
Email: tsang@vax2.concordia.ca

**Kazuhiko Uchida**
Institute of Biological Sciences, University of Tsukuba
Tsukuba, Ibaraki, 305-8572, Japan
Tel: 11-81-29-853-7725
Email: amx02193@mail2.accsnet.ne.jp

**Hideko Urushihara**
Univesity of Tsukuba
111 Tenoudai
Tsukuba, Ibaraki, 305-8572, Japan
Tel: 81 29 853 4664
Fax: 81 29 853 6614
Email: hideko@biol.tsukba.ac.jp

**Peter Van Haastert**
University of Groningen
Nijenborgh 4
Groningen, Nl-9747AG, Netherlands
Tel: 31-50-3634172
Fax: 31-50-3634165
Email: haastert@chem.rug.nl

**Douwe Veltamn**
University of Groningen
Nijenborgh 4
Groningen, N1-9747AG, Netherlands
Tel: 31-50-3634172
Fax : 31-50-3634165
Email : Veltman@chem.rug.nl

**Jun Wang**
Wellcome Trust Biocentre
Dow street
Dundee, DD1 5EH, UK
Tel: 44-1382-345889
Fax: 44-1382-345386
Email: j.u.wang@dundee.ac.uk

**Gerald Weeks**
Department of Microbiology and Immunology,
University of British Columbia
300-6174, University Boulevard
Vancouver, BC, V6T 1Z3, Canada
Tel : 1 604-822-6649
Fax : 1 604-822-6041
Email : gerwee@interchange.ubc.ca

**Kees Weijer**
Wellcome Trust Biocentre
Dow Street
Dundee, DD1 5EH, UK
Tel: 44-1382-345191
Fax: 44-1382-345386
Email: c.j.weijer@dundee.ac.uk

**Barbara Weissenmayer**
FU Berlin – Microbiology
Koenigin-Luise Str. 12-16
Berlin,14195, Germany
Tel: 49-30-83853373
Fax :49-30-83857773
Email : bweiss@zedat.fu-berlin.de

**Deborah Wessels**
University of Iowa
210 E Iowa Ave. Rm 14 BBE
Iowa City, IA, 52242, USA
Tel: 1 319 335-2883
Fax: 1 319 335-2772
Email: deborah-wessels@uiowa.edu

**Chris West**
Dept. of Biochemistry & Molecular Biology,
University of Oklahoma Health Sciences
Center, 940 Stanton L. Young Blvd., Rm. 937
Oklahoma City, OK, 73104, USA
Tel: 1 405-271-2227 Ext.1247
Fax: 1 405-271-3139
Email: Christopher-west@ouhsc.edu

**Jeffrey Williams**
University of Dundee
MSI/WTB University of Dundee
Dundee, DD1 5EH, UK
Tel: 44 1382 345823
Fax: 44 1382 344211
Email: j.g.williams@dundee.ac.uk

**Robin Williams**
University College London
Darwin Building Gower st
London, WC1 E6BT, UK
Tel: 44 20 7679 7268
Fax: 44 20 7679 7069

**Thomas Winckler**
University of Frankfurt, Institute of
Pharmaceutical Biology
Marie-Curie-Strasse 9
Frankfurt, 60439, Germany
Tel: 49-69-79829648
Fax: 49-69-79829662
Email: winckler@em.uni-frankfurt.de

**Danica Wyatt**
Cornell Univerisity
117 Grove School Road
Brooktondale, NY, 14817, USA
Tel : 1-607-592-1547
Email : dwyatt@gw.physics.cornell.edu

**Michelle Wyse**
University of British Columbia
6174 University Boulevard
Vancouver, BC, V6T 1Z3, Canada
Tel : 1 604 822-0997
Fax : 1 604 822-6041
Email : mwyse@interchange.ubc.ca

**Qikai Xu**
Baylor College of Medicine
One Baylor Plaza, S432
Houston, Texas, 77030, USA
Tel: 1 713-798-8308
Fax: 1 713-798-8142
Email: qx139921@bcm.tmc.edu

**Xiaoxin Susan Xu**
Institute of Environmental Health Sciences,
Wayne State University
MCHT Room 4000, 2727 Second Ave.
Detroit, MI, 48201, USA
Tel: 1 313 964 8107
Fax: 1 313 577 0082
Email: x.xu@wayne.edu

**Xuehua Xu**
National Institutes of Health
12441 Parklawn Drive, Twinbrook II
Rockville, MD, 20852, USA
Tel : 1 301-451-9789
Fax : 1 301-402-0259
Email : xxu@niaid.nih.gov

# Author Index