INTERNATIONAL
DICTYOSTELIUM CONFERENCE

Dictyostelium 2001

July 22 –July 26, 2001

La Jolla, California, USA

Organizers: Richard A. Firtel
William F. Loomis

MEETING OVERVIEW

Time	Sunday	Monday	Tuesday	Wednesday	Thursday
09:00-10:00		Expression Analyses			
09:00-10:20	Adhesion		Directional Responses	GSK3	Life and Death
10:00-10:20		Coffee Break			
10:20-10:40	Coffee Break		Coffee Break	Coffee Break	Coffee Break
10:20-12:00		Signaling II			
10:40-12:00	Signaling I		Signaling and Development	Signals	New Directions
11:40				Lunch	
12:00	Lunch	Lunch	Lunch		Lunch
12:30-16:30				Zoo Trip	
16:00-18:00	Poster Session I, nos. 1-16	Poster Session II, nos. 17-34	Poster Session III, nos. 35-53		End of Meeting
18:00				Banquet	
19:00-20:20	Genomics	Chemotaxis	Genes		
20:20-20:40	Coffee Break	Coffee Break	Coffee Break		
20:40	cDNA Project	Signaling III and Modeling	Regulating Development		

Dictyostelium 2001

Schedule of Oral Presentations:

Sunday, July 22, 2001

09:00-10:20 ADHESION, Chair: Peter Van Haastert

1. *SADA*, a novel *Dictyostelium* gene necessary for substrate adhesion. Petra Fey and Rex L. Chisholm

2. The myosin VII FERM domains are essential for its role in cell-substrate adhesion. Richard I. Tuxworth, Gregory C. Addicks, Stephen Stephens, and Margaret A. Titus

3. Involvement of RAFT-like structures in the assembly of gp80 adhesion complexes during *Dictyostelium* development. Chi-Hung Siu, Tony J. C. Harris, and Amir Ravandi

4. The novel anti-adhesive protein AMPA effects cell type specific differentiation and pattern formation. Timothy R. Varney, Chere Petty, Jayne Dolman, and Daphne D. Blumberg

10:20-10:40 Break

10:40-12:00 SIGNALING I, Chair: Pauline Schaap

5. Myosin IK, another myosin I plugging in the actin-polymerisation machinery!? Claudia Kistler, Eva C. Schwarz, and Thierry Soldati

6. Temperature-sensitive adenylyl cyclase activation due to a single point mutation in the gene encoding pianissimo. Barbara Pergolizzi, Barbara Peracino, James Silverman, Adriano Ceccarelli, Angelika Noegel, Peter Devreotes, and Salvatore Bozzaro

7. Role of protein carboxyl methylation in *Dictyostelium* development. Ying Chen, Edward C. Cox, and Jeffry B. Stock

8. Activation of cAMP relay in *Dictyostelium* discoideum by the calmodulin antagonist W7 is due to Ca^{2+}-release from Ca^{2+}-stores. Dieter Malchow, Daniel F. Lusche, and Christina Schlatterer

12:00 Lunch

19:00-20:20 GENOMICS, Chair: Jeffrey Williams

9. The *Dictyostelium* genome sequencing project at Baylor College of Medicine: Progress and prospects. Richard Sucgang, Brian Desany, Ryan Lindsay, Hermela Loulseged, Donna Muzny, Richard Gibbs, William Loomis, and Adam Kuspa

10. Sequence and analysis of chromosome 2 from the model organism *Dictyostelium discoideum.* L. Eichinger, G. Glöckner, K. Szafranski, P. Dear, J. Pachebat, K. Kumpf, R. Lehmann, J. Abril, G. Parra, R. Guig, B. Tunggal, E. Cox, M. A. Quail, M. Platzer, A. Rosenthal, and A. Noegel

11. Annotating *Dictyostelium* genes. Negin Iranfar, Christophe Anjard, Rolf Olsen, and William F. Loomis

12. A demonstration of phenotyping in mixed cultures for use in large-scale analyses of mutant. Adam Kuspa, Sijie Lu, Bin Wang, Guokai Chen, Miroslava Ibarra, and Gad Shaulsky

20:20-20:40 Break

20:40-21:40 cDNA PROJECT, Chair: Ludwig Eichinger

13. From EST data to genes: Assembling cDNA sequences with PHRAP program. Hideko Urushihara, Takahiro Morio, Yuji Kohara, Tamao Saito, Hiroshi Ochiai, Mineko Maeda, and Yoshimasa Tanaka

14. Generation and sequencing of full-length enriched cDNA library from developing *Dictyostelium* cells**.** Takahiro Morio, Hidekazu Kuwayama, Takao Isogai, Ai Wakamatsu, Sumio Sugano, Yuji Kohara, Tadasu Shin-i, Hideko Urushihara, Shinji Obara, Mariko Katoh, Jeffrey Williams, Mineko Maeda, Tamao Saito, Hiroshi Ochiai, Ikuo Takeuchi, and Yoshimasa Tanaka

15. PCR mediated, two-step procedure for construction of a gene disruption vector in *Dictyostelium discoideum.* Hidekazu Kuwayama, Takahiro Morio, Hideko Urushihara, Mariko Katoh, and Yoshimasa Tanaka

Monday, July 23, 2001

09:00-10:00 EXPRESSION ANALYSES, Chair: Rex Chisholm

16. Statistical analyses of microarray data on gene expression during *Dictyostelium* development. Roman Sasik, Negin Iranfar, Terry Hwa, and William F. Loomis

17. A transcriptional profile of *Dictyostelium* development. Nancy Van Driessche, Chad Shaw, Richard Sucgang, Miroslava Ibarra, Jeffrey Tollett, John Halter, Adam Kuspa, Gad Shaulsky, Mariko Katoh, Takahiro Morio, Hidekazu Kuwayama, Tamao Saito, Hideko Urushihara, Mineko Maeda, Ikuo Takeuchi, Hiroshi Ochiai, and Yoshimasa Tanaka

18. Genepath: An intelligent assistant for genetic data analysis and pathway discovery. Blaz Zupan, Peter Juvan, Janez Demsar, Ivan Bratko, John A. Halter, Adam Kuspa, and Gad Shaulsky

10:00-10:20 Break

10:20-12:00 SIGNALING II, Chair: Alan Kimmel

19. *Dictyostelium discoideum* as a host model system for *Legionella pneumophila* infection. Marcela Fajardo, Sonja Hägele, Carina Skriwan, Stephan Schwager, Michael Schleicher, Jörg Hacker, and Michael Steinert

20. 5'-Nucleotidase in *Dictyostelium*: Regulation, promoter analysis and localization. Muatasem Ubeidat, Can M. Eristi, Danielle F. Overall, Brad R. Joyce, and Charles L. Rutherford

21. A nuclear phosphatidylinositol phosphate kinase required for developmental gene expression. Kunde Guo, Richard Nichol, Paul Skehel, Jeffrey G. Williams, and Catherine Pears

22. Regulation of adenylyl cyclases by a region outside the minimally functional cytoplasmic domains. Carole A. Parent, Jane Borleis, and Peter N. Devreotes

23. G alpha 9: A heterotrimeric G protein that functions as an inhibitor of cAMP signaling pathways in *Dictyostelium discoideum.* Joseph A. Brzostowski, Cynthia Johnson, Carole Parent, and Alan R. Kimmel

12:00 Lunch

19:00-20:20 CHEMOTAXIS, Chair: Salvatore Bozzaro

24. Interpreting the role of a protein in locomotion and chemotaxis by mutant analysis. David R. Soll

25. Analysis of WASP-like proteins in *Dictyostelium.* Wendy Mahler, Deborah Wessels, Diana Caracino, Julia Steiner, Cheryl Jones, David Soll, and Charles L. Saxe

26. Regulation of F-actin assembly and chemotaxis by *Dictyostelium* WASP. Chang Y. Chung, Young-Hoon Han, and Richard A. Firtel

27. Recruitment of myosin heavy chain kinase activity to actin-rich cortical structures. Paul A. Steimle, Shigehiko Yumura, Graham P. Côté, Quint G. Medley, Mark V. Polyakov, and Thomas T. Egelhoff

20:20-20:40 Break

20:40-22:00 SIGNALING III AND MODELING, Chair: Cornelis Weijer

28. Phosphorylation of the myosin regulatory light chain plays a role in motility and polarity during *Dictyostelium* chemotaxis. Hui Zhang, Deborah Wessels, Petra Fey, Karla Daniels, Rex Chisholm, and David R. Soll

29. Regulation of the cytoskeleton by cyclic GMP signaling in *Dictyostelium*. Janet L. Smith, Jonathan M. Goldberg, and Ana C. Urbin-Reyes

30. Chemotactic response in *Dictyostelium*: A diffusive-inhibitor model. Wouter-Jan Rappel, Peter Thomas, Herbert Levine, and William F. Loomis

31. A computational study of ameboid polarity and chemotaxis. Dean C. Bottino, Noah Edelson, and Hans Othmer

Tuesday, July 24, 2001

09:00-10:20 DIRECTIONAL RESPONSES, Chair: Peter Newell

32. Imaging of individual cAMP-receptors on the plasma membrane of *Dictyostelium.* B.E. Snaar-Jagalska, P.H.M. Lommerse, S. de Keijzer, G.A. Blab, G. Lamers, H.P. Spaink, and T. Schmidt

33. The visualization of heterotrimeric G-protein activation in living *Dictyostelium.* Chris Janetopoulos, Tian Jin, Linnan Tang, Ning Zhang, Yi E. Huang, Miho Shimizu, and Peter N. Devreotes

34. Localized activation and deactivation of PI3K at the leading edge: Differential chemoattractant-mediated translocation of PI3K and PTEN to the plasma membrane. Satoru Funamoto, Ruedi Meili, and Richard A. Firtel

35. Visualizing cell-cell signaling during development using CRAC-GFP. Dirk Dormann, Carol Parent, Peter Devreotes, and Cornelis J. Weijer

10:20-10:40 Break

10:40-12:00 SIGNALING AND DEVELOPMENT, Chair: Margaret Titus

36. Searching for *Dictyostelium* cyclase functions. Marcel Meima, Elisa Alvarez-Curto, Irene Verkerke-Van Wijk, Jeroen Roelofs, Peter van Haastert, and Pauline Schaap

37. The *Dictyostelium* homologue of mammalian soluble adenylyl cyclase encodes a guanylyl cyclase. Jeroen Roelofs, Marcel Meima, Pauline Schaap, and Peter J.M. Van Haastert

38. Filactin is a vesicle-associated protein that contains conventional actin as a domain. Michael Schleicher , Francisco Rivero-Crespo, Lars Israel, Christian Schuberth, Ludwig Eichinger, Rolf Müller, and Angelika A. Noegel

39. The vacuolar H^+-ATPase in endocytosis and osmoregulation of *Dictyostelium* cells. Margaret Clarke, Quyen Arana, and Tongyao Liu

12:00 Lunch

19:00-20:20 GENES, Chair: Catherine Pears

40. Constitutively active G protein-coupled receptor mutants dominantly block *Dictyostelium* development. Minghang Zhang and Dale Hereld

41. The *Dictyostelium* RHO-GTPASE dissociation inhibitor regulates diverse RAC-dependent signalling pathways. Heidrun Dislich, Ann-Kathrin Mayer, Nicola Adam, Daria Illenberger, and Francisco Rivero

42. Valproic acid inhibits inositol trisphosphate signalling during *Dictyostelium* development. Robin S.B. Williams, W. Jonathan Ryves, and Adrian J. Harwood

43. Control of *Dictyostelium* development by a TRAP1 homologue. Tsuyoshi Morita, Kenji Saitoh, Aiko Amagai, and Yasuo Maeda

20:20-20:40 Break

20:40-22:00 REGULATING DEVELOPMENT, Chair: Julian Gross

44. Evidence that the DNA replication protein RPA is a regulator of one of the control elements within the cudA promoter. Christopher Sugden, Masashi Fukuzawa, and Jeff Williams

45. RAP1 is essential for *Dictyostelium discoideum* viability and is activated in response to osmotic stress. Rujun Kang, Hermia Ip, George B. Spiegelman, and Gerald Weeks

46. Phagocytosis mutants in *Dictyosteliu.* Mohammed Benghezal, Sophie Cornillon, Leigh Gebbie, Laurence Zulianello, Anna Marchetti, and Pierre Cosson

47. A “tolerance” range in cell type proportioning. Ismael Ràfols, Harry K. MacWilliams, Aiko Amagai, Yasuo Maeda, and Yasuji Sawada

Wednesday, July 25, 2001

09:00-10:20 GSK3, Chair: Richard Gomer

48. Antagonistic 7-TM receptor signaling regulates cell fate determination by differential tyrosine phosphorylation and activation of GSK3. Leung Kim, Jingchun Liu, and Alan R. Kimmel

49. The interaction between GSKA and AAR. Jon Reynolds, Juliet Coates, Trevor Dale, and Adrian J. Harwood

50. DIF functions as a repressor of the nuclear export of Dd-STATC and may act via a GSK-3 signalling pathway. Tsuyoshi Araki, Tomoaki Abe, Masashi Fukuzawa, Christina Schilde, Leung Kim, Adrian Harwood, Alan Kimmel, and Jeff Williams

51. DIF-1 and the proportioning mechanism for prestalk and prespore cells. Robert Kay and Christopher Thompson

10:20-10:40 Break

10:40-11:40 SIGNALS, Chair: Adam Kuspa

52. New components of the counting factor**.** Debra A. Brock and Richard H. Gomer

53. Possible new components of a cell number counting signal transduction pathway. Wonhee Jang, Tong Gao, and Richard H. Gomer

54. An autophagy gene, *Ddapg*6, is required for *Dictyostelium* development. Grant Otto, Hubert Hilbi, Herbert L. Ennis, Howard Shuman, and Richard H. Kessin

11:40 Lunch

12:30-16:30 Trip to the San Diego Zoo

18:00 Banquet

Thursday, July 26, 2001

09:00-10:20 LIFE AND DEATH, Chair: Margaret Clarke

55. Calcium signaling and *Dictyostelium* development : A role for Dd-ALG-2/Dd-ALIX. Laurence Aubry, Michel Satre, and Gérard Klein

56. Genetic and morphological analysis of *Dictyostelium* cell death. Jean-Pierre Levraud, Myrian Adam, Marie-Françoise Luciani, Vanessa Dubus-Bonnet, Céline Roisin-Bouffay, and Pierre Golstein

57. On the evolutionary conservation of the cell death pathways: Involvement of a mitochondrial apoptosis inducing factor as nuclear effector during *Dictyostelium discoideum* cell death. D. Arnoult, I. Tatischeff, J. Estaquier, M. Girard, F. Sureau, J.P. Tissier, A. Grodet, M. Dellinger, F. Traincard, A. Kahn, J.-C. Ameisen, and P.X. Petit

58. Hirano bodies in *Dictyostelium.* Marcus Fechheimer, Andrew Maselli, Rich Davis, Sonbol Shahid-Salles, and Ruth Furukawa

10:20-10:40 Break

10:40-12:00 NEW DIRECTIONS, Chair: Robert Kay

59. Cloning of a putative Trehalase gene from *Dictyostelium discoideum.* D.C. Mahadeo, C. Jaekhl, Y. Kishi, M. Sameshima, S.C. Kales, and D.A. Cotter

60. RNAi in *Dictyostelium.* Henrik Martens, Jindrich Novotny, Juergen Oberstrass, and Wolfgang Nellen

61. RNA interference in *Dictyostelium.* Hideshi Otsuka, Emily R. Cogill, Chu-Yun Kuan, Robert P. Dottin, and Julian D. Gross

62. Competition between clones in chimeras**.** David C. Queller, Angelo Fortunato, and Joan E. Strassmann

12:00 Lunch

POSTER SESSIONS

All posters can be displayed during the afternoon/evening of the designated session. Please mount your posters on poster boards according to the numbers indicated in the LIST OF POSTERS. Posters should be removed before the coffee break of the following day, so that others can put theirs up.

The presentations of the posters are subdivided into three sessions. It is recommended that presenters be present at their posters during the designated sessions.

Session I: Sunday, July 22, Posters 1-16
Session II: Monday, July 23, Posters 17-34
Session III: Tuesday, July 24, Posters 35-53

LIST OF POSTERS

Session I

1. Identification of a novel family of RGS domain-containing proteins in *Dictyostelium.* Mousumi Goswami, Minghang Zhang, and Dale Hereld

2. Novel expression pattern of *Dictyostelium* genes revealed by *in situ* hybridization. Keiko Nishio, Masako Yokoyama, Masatsune Tsujioka, Hidekazu Kuwayama, and Mineko Maeda

3. Peroxisomal β-oxidation is essential for cyclic AMP-relay, cell adhesion and multicellular development of *Dictyostelium.* Satomi Matsuoka, Hidekazu Kuwayama, Masakazu Oyama, Tamao Saito, Daisuke Ikeno, and Mineko Maeda

4. Relation between abnormal lipid metabolism and developmental defect of *Dictyostelium* mutant lacking peroxisomal multifunctional enzyme 2. Tamao Saito, Satomi Matsuoka, Naoki Morita, Hiroshi Ochiai, and Mineko Maeda

5. LimC and LimD: Two novel cytoskeleton-associated LIM proteins of *Dictyostelium discoideum.* Taruna Khurana, Bharat Khurana, and Angelika A. Noegel

6. The internal phosphodiesterase RegA and PKA lie in a regulatory pathway emanating from the front of the natural chemotactic wave and ending in the suppression of lateral pseudopod formation essential for chemotaxis. Paul J. Heid, Deborah J. Wessels, Hui Zhang, Karla Daniels, Adam Kuspa, William F. Loomis, and David R. Soll

7. A computational model for crawling in *Ascaris suum* sperm. Dean C. Bottino, Alexander Mogilner, Thomas Roberts, and George Oster

8. The behavioral phenotype of the myosin I mutants myoA-, myoB-, and myoA-/B- present a paradox in interpretation. David L. Falk, Deborah Wessels, Margaret A. Titus, and David R. Soll

9. Modeling spatial sensing in chemotactic cells. Jane H. Kim, Chris Janetopoulos, Peter N. Devreotes, and Pablo A. Iglesias

10. Myosin II is required for cell motility in a mechanically constrained environment. Gary Laevsky and David Knecht

11. Arachidonic acid attracts aggregation competent *Dictyostelium dicoideum* cells. Ralph Schaloske, Dagmar Blaesius, and Daniel F. Lusche

12. Functional analysis of *Dictyostelium discoideum* RHO-related proteins RACG and RACH. Baggavalli P. Somesh, Heidrun Dislich, and Francisco Rivero

13. The role of DdVASP, a *Dictyostelium* homologue of vasodilator-stimulated phosphoprotein in chemotaxis and filopodia formation. Young-Hoon Han and Richard A. Firtel

14. Paradoxical effect of phosphatidic acid on myosin heavy chain kinase B activity. Maribel Rico and Tom Egelhoff

15. A novel secreted factor that potentiates development. Joseph A. Brzostowski, Cynthia Johnson, and Alan R. Kimmel

16. TagA is required for the differentiation of a subset of spore cells. Matthew Cabral, J. Randall Good, and Adam Kuspa

Session II

17. AMP deaminase affects cell-type proportioning in *Dictyostelium.* Soo-Cheon Chae, Danny Fuller, and William F. Loomis

18. Identification of FbiA, a potential target of FbxA-mediated degradation, via yeast two-hybrid analysis. Jennifer Christman, Carla Moré, Sarah E. Petricca, Stacey C. Miller, Maryann Borsick, Seth Houwer, Kelly McFeaters, and Margaret K. Nelson

19. Transcriptional regulation of the 5'-nucleotidase gene of *Dictyostelium discoideum.* Can M. Eristi, Muatasem Ubeidat, Brad R. Joyce, Danielle F. Overall, and Charles L. Rutherford

20. Ammonia differentially suppresses the cAMP chemotaxis of anterior-like cells and prestalk cells in *Dictyostelium discoideum.* Ira N. Feit, Erika J. Medynski, and Michael J. Rothrock

21. Recombinant countin affecting *Dictyostelium* development. Tong Gao, Lei Tang, Hamam Alraaba, and Richard H. Gomer

22. Intercellular communication genes in *Dictyostelium* development. Kirsten Kibler and Gad Shaulsky

23. Identification and analysis of a gene that is homologous to mammalian Cdk8 in *Dictyostelium.* Sophia Hsiu-Hsu Lin, Hao-Jen Huang, Gerald Weeks, and Catherine Pears

24. cGMP-Phosphodiesterase-inhibitors block receptor-mediated Ca^{2+} -influx in *Dictyostelium discoideum* and bovine cone cyclic nucleotide gated channel activity. D.F. Lusche, S. Frings, D. Reuter, and D. Malchow

25. A pathway of ubiquitin-mediated proteolysis that influences cell type proportioning in *Dictyostelium.* David I. Ratner, Turgay Tekinay, Herbert L. Ennis, Mary Y. Wu, Margaret K. Nelson, and Richard H. Kessin

26. Role of pH regulation during the rapid patterning of *Dictyostelium* in two-dimensional culture. Satoshi Sawai, Takashi Hirano, Yasuo Maeda, and Yasuji Sawada

27. Vegetatively activated elements of the gp64 promoter of the cellular slime mold *Polysphondylium pallidum.* Naohisa Takaoka, Masashi Fukuzawa, Tamao Saito, and Hiroshi Ochiai

28. Investigation of biochemical parameters in phospho-relay (two-component) signaling. Peter Thomason, Ted Cox, and Jeff Stock

29. Rapid Ca^{2+} responses to DIF-1 during early development. David Traynor, Jaqueline Milne, and Robert R Kay

30. Expression of a *Dictyostelium* gene encoding multiple repeats of adhesion inhibitor-like domains has pleiotrophic effects on cell size and growth as well as cell adhesion, developmental progression and patterning. Timothy R. Varney, Hoa N. Ho, Chere Petty, Jayne Dolman, and Daphne D. Blumberg

31. Microarray analysis of the wild-type *Dictyostelium* developmental time course. Chad Shaw, Nancy Van Driessche, Gad Shaulsky, Adam Kuspa, Jeffrey Tollett, Bill Eaton, and Richard Sucgang

32. A role for YakA, cAMP and PKA in the nitrosoative/oxidative stress response of *Dictyostelium discoideum* cells. Raquel Bagattini, Renata Gorjão, Alexandre Taminato, Nancy Van Driessche, Guokai Chen, Thiago Teixeira Santos, Gustavo Tadao Okida, Roberto Hirata Jr. , João Eduardo Ferreira, Eduardo Jordão Neves, Junior Barrera, Adam Kuspa, Gad Shaulsky, and Glaucia Mendes Souza

33. Isoprenylcysteine protein carboxyl methyltransferase in *Dictyostelium.* Ying Chen, Jeff Stock, and Ted Cox

34. Deletion analysis and GFP-tagging of a *Dictyostelium* β-catenin. Jonathan P. Reynolds and Adrian J. Harwood

Session III

35. Characterization of a mutant strain that cannot make the transition from growth to development. Rui Fang, Olga Timofeevsky, and Charles K. Singleton

36. Mass cultivation of *Dictyostelium discoideum* in bioreactors. Erwin Flaschel, Yinghua Lu, Sang-In Han, and Karl Friehs

37. The cost of chimerism. Kevin Foster, Angelo Fortunato, Joan Strassmann, and David Queller

38. Identification and manipulation of novel targets that increase the efficacy of cisplatin mediated cell death. Christopher Foote, Guochun Li, Hannah Alexander, and Stephen Alexander

39. Exploring the role of filamin in phototactic and thermotactic migration of slugs. Nandkumar K. Khaire, Francisco Rivero, and Angelika A. Noegel

40. Recycling of the *Bsr* marker using Cre/*loxP*-mediated recombination. Lisa Kreppel, Gad Shaulsky, and Alan Kimmel

41. Production of the human FAS ligand by means of high cell density cultivation of *Dictyostelium discoideum.* Yinghua Lu, Jaco C. Knol, Maarten H.K. Linskens, Karl Friehs, Peter J.M. van Haastert, and Erwin Flaschel

42. Genetic selection for components of the phagocytosis pathway. Andrew Maselli, Gary Laevsky, and David Knecht

43. Control of cell proliferation and early differentiation by *dng1*, a *Dictyostelium ING1* homologue. Taira Mayanagi, Aiko Amagai, and Yasuo Maeda

44. *D. discoideum* expresses proteins containing multiple calpain DIII domains. Ronald L. Mellgren, Eric Czerwinski, and Xinhua Huang

45. LSD1, a lipid storage droplet protein in *Dictyostelium.* Shinji Miura, Joseph Brzostowski, Jai-Wei Gan, Michael Parisi, Constantine Londos, Brian Oliver, and Alan R. Kimmel

46. Characterization of calreticulin and calnexin, and dynamics of the endoplasmic reticulum in *Dictyostelium.* Annette Mueller-Taubenberger, Mary Ecke, Andrei N. Lupas, Hewang Li, Paul R. Fisher, Evelyn Simmeth, and Guenther Gerisch

47. Folate reception by vegetative *Dictyostelium discoideum* amoebae: Distribution of receptors and trafficking of ligand. Jared L. Rifkin

48. The role of a ubiquitin processing protease, UbpA, in the growth-to-development transition of *Dictyostelium* development. Michael A. Sanz and David F. Lindsey

49. A novel Cdc2-related kinase is required for transition from growth to differentiation in *Dictyostelium.* Kosuke Takeda, Tamao Saito, and Hiroshi Ochiai

50. Making ts mutants in essential genes in *Dictyostelium.* Chris Thompson and Mark S. Bretscher

51. Studies on a RelA/SpoT like-protein of *Dictyostelium.* Yan Zhang and Charles K. Singleton

52. Regulation of MEK1 by sumoylation and association with the RING finger protein MIP1 during chemotaxis. Alex Sobko, Hui Ma, and Richard A. Firtel

53. The role of a phospholipase D in quorum sensing and development in *Dictyostelium discoideum.* Vanessa Rodrick, Yi Yan, Tarek Abbas, and Derrick T. Brazill

ORAL PRESENTATIONS

SUNDAY, JULY 22, 2001

09:00-10:20 ADHESION

1. *SADA*, A NOVEL *DICTYOSTELIUM* GENE NECESSARY FOR SUBSTRATE ADHESION. Petra Fey and Rex L. Chisholm, Dept. of Cell and Molecular Biology, Northwestern University Medical School, 303 E. Chicago Ave, Chicago, IL 60611, USA.

Because we know little about cell-substrate adhesion, and how motile and adhesive forces work together in moving cells, we performed insertional mutagenesis in *Dictyostelium* and selected for adhesion deficient mutants. The resulting sad (substrate adhesion) mutants grew in plastic dishes without attaching to the substrate. The cells were generally larger than their wildtype parents and displayed a rough surface with many apparent blebs. One of these mutants, called sadA, carried an insertion in a gene predicted to encode a 952 amino acid protein with a calculated molecular weight of 105 kd. The insertion and surrounding sequences were recovered and introduced into wildtype cells to recapitulate the mutant phenotype (sadA-RC). We have also used flanking sequences to create an independent gene targeting vector, which produces cells with the same phenotype (sadA-KO). When stained with DAPI, the initial insertion line, as well as sadA-KO, was multinucleate, suggesting the cells were also defective in cytokinesis.
SadA has a predicted N-terminal signal sequence, and at least 9 additional transmembrane domains. Three EGF-like repeats are located, as is common, in a potential extracellular domain of the protein. EGF-like domains are observed in proteins of many organisms ranging from C. elegans to mammals and often occur in extracellular matrix or secreted proteins that are involved in cell adhesion. The 105 residues spanning the EGF-like repeats of sadA are similar to corresponding regions in many proteins, especially to mammalian tenascins (40% identical, 50% similar). However, outside this domain sadA has little similarity to tenascins or any other known proteins. Therefore, our data suggest that sadA is a novel *Dictyostelium* transmembrane protein necessary for cell-substrate adhesion and normal cytokinesis.

2. THE MYOSIN VII FERM DOMAINS ARE ESSENTIAL FOR ITS ROLE IN CELL-SUBSTRATE ADHESION. Richard I. Tuxworth, Gregory C. Addicks, Stephen Stephens, and Margaret A. Titus, Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis MN, USA.

Myosin VII (M7) is an actin-based motor protein that plays an essential role in hearing in humans, mice and zebrafish. The simple eukaryote *Dictyostelium* expresses a single M7 (DdM7). Null mutant analysis has revealed that DdM7 plays a critical role in cell-substrate adhesion required for phagocytosis and cell migration, as well as filopod extension, novel functions for a myosin (Tuxworth et al. 2001. *Curr. Biol.* 11:318-329). The available evidence suggests that DdM7 plays a role in organizing receptors into a high avidity complex capable of binding to and stabilizing initial contacts with substrata.

The tail region of M7 is postulated to interact either directly or indirectly with receptors. It contains a short region of predicted coiled-coil, along with a tandem repeat of combined MyTH4 and FERM domains separated by an SH3 domain. FERM domains are found in numerous cytoskeletal proteins that contribute to cell-substrate adhesion, but the function of these domains has not been clearly identified. Complementation and domain overexpression analyses employing GFP-tagged molecules were performed to determine the role of the DdM7 FERM domains. Our results demonstrate that deletion of either FERM domain results in a loss of DdM7 localization and function. However, the individual FERM domains themselves do not localize to the leading edge and filopodial tips of wild-type cells. They also do not act as dominant negative inhibitors of phagocytosis and filopod extension. Therefore, each of the FERM domains is necessary but not sufficient for DdM7 localization and function.

3. INVOLVEMENT OF RAFT-LIKE STRUCTURES IN THE ASSEMBLY OF gp80 ADHESION COMPLEXES DURING ***DICTYOSTELIUM*** DEVELOPMENT. Chi-Hung Siu, Tony J. C. Harris, and Amir Ravandi, Banting and Best Department of Medical Research, University of Toronto, 112 College Street, Toronto, Ontario, Canada M5G 1L6.

The cell adhesion molecule gp80 mediates EDTA-resistant cell-cell adhesion or contact sites A during the aggregation phase of ***Dictyostelium*** development. We have isolated and characterized a Triton-insoluble floating fraction (TIFF) from ***Dictyostelium*** membrane and found that gp80 is highly enriched in this membrane fraction. The sterol/phospholipid ratio of TIFF is 10-fold higher than that of the bulk plasma membrane. gp80 is also enriched in a low-density membrane fraction isolated by non-detergent methods. This membrane fraction contains most of the TIFF components. Chemical cross-linking studies have demonstrated the presence of gp80 oligomers in both low-density membrane fragments and TIFF. The results thus demonstrate the preferential association of gp80 clusters with raft-like domains of the plasma membrane. Mass spectrometry has demonstrated that TIFF and isolated contact regions (Ingalls et al. (1986) PNAS 83:4779-4783) share the same major protein components. Filipin staining revealed high sterol levels in both gp80-enriched cell-cell contacts and gp80 caps. Further experiments suggest that gp80-gp80 interactions may drive the assembly of adhesion complexes from the raft-like domains. The level of contact regions rises sharply from background only after gp80 expression, although substantial amounts of rafts exist before multicellular development. Moreover, gp80-null cells lack contact membranes despite the presence of raft-like domains. Finally, sterol sequestration by filipin and digitonin inhibits gp80-mediated cell-cell adhesion. Together, these results indicate that raft-like domains are required for optimal gp80-mediated cell-cell adhesion. (Supported by the Canadian Institutes of Health Research.)

4. THE NOVEL ANTI-ADHESIVE PROTEIN AMPA EFFECTS CELL TYPE SPECIFIC DIFFERENTIATION AND PATTERN FORMATION. Timothy R. Varney, Chere Petty, Jayne Dolman, and Daphne D. Blumberg, Department of Biological Sciences, University of Maryland, Baltimore County, 1000 Hilltop Circle Baltimore, Maryland 21250, USA.

The *ampA* gene, initially identified by the D11 cDNA clone, encodes a novel protein that modulates cell-cell and cell-substrate adhesions. During development expression of the *ampA* gene is localized to the anterior like cells and in mounds *ampA* expressing cells are largely localized to the mound periphery and base where they differentiate to form basal disc and lower cup structures. A few *ampA* expressing cells are scattered within the mound and localize to the upper cup. Here we demonstrate that insertional inactivation of the *ampA* gene results in patterning defects that define two distinct roles for the *ampA* gene product during development. Analysis of reporter gene expression during development of *ampA* null strains and chimeras formed with varying percentages of wild type cells mixed with the *ampA* null cells indicates that the AmpA protein is necessary in a non-cell autonomous manner to prevent expression of a prespore gene marker in cells at the mound periphery which will ultimately differentiate into prestalk cells. Additionally a cell autonomous function of *ampA* is needed to enable a subset of *ampA* expressing cells to localize to the interior of the mound. When these cells fail to localize correctly there is a reduction in the number of cells that activate *ecmAO* reporter gene expression and those that do express the *ecmAO* reporter remain at the mound periphery for a prolonged period of time. A model is presented for two distinct *ampA* functions during development.

10:40-12:00 SIGNALING I

5. MYOSIN IK, ANOTHER MYOSIN I PLUGGING IN THE ACTIN-POLYMERISATION MACHINERY!? Claudia Kistler, Eva C. Schwarz, and Thierry Soldati, Dept. of Molecular Cell Research, Max-Planck Institute for Medical Research, Jahnstrasse 29, 69120 Heidelberg, Germany.

Dictyostelium MyoK is the most divergent member of the class I of myosins. Like some other myosin Is, MyoK localizes to dynamic regions of the actin cortex. Surprisingly, despite the potential functional redundancy among the seven members of this class in *Dictyostelium*, *myoK* null cells are impaired in chemotactic motility and phagocytosis. Using a "pipette suction" assay, we showed that *myoK* null cells have diminished cortical tension, whereas MyoK overexpressers had increased tension compared to wild type. At the regulatory TEDS site, MyoK carries a Thr. In analogy to other class I myosins, phosphorylation of this residue is expected to be essential for activity. We suggested that MyoK functions as a regulated actin-crosslinker important for the modulation of peripheral activities, such as phagocytosis and motility.

MyoK is unique as it virtually lacks a tail domain but carries in a surface loop of its motor domain a 150 residues insertion rich in Gly, Pro and Arg, the GPR-loop. In addition, MyoK has a CAAX motif at its C-terminus (CLIQ), a classical farnesylation signal. The GPR-loop resembles an ATP-independent actin-binding domain often found in the tail of class I myosins. Indeed, a GST-GPR-loop fusion binds F-actin in a salt-dependent fashion. Potentially owing to the particular geometry of its two actin-binding sites, the interaction of MyoK with F-actin is both ATP-dependent and salt sensitive. Most strikingly, the GPR-loop shares 40 to 50% identity with the Pro-rich domain of WASp family members. Finally, it contains at least four Pro-rich motifs (pPPø) known to bind profilin, and an SH3 binding motif (RxxPxxP). Incubation of the purified GPR-loop with cytosol identified potential interacting partners including the profilin-G-actin complex. Using cells expressing profilin mutants having lost either their strong actin-binding site (K114E) or their poly-Pro-binding site (W3N), we demonstrated that the GPR-loop interacts directly with the poly-Pro binding site. The interaction with purified profilin was further analysed using an ELISA-based assay. We are using pull-down assays to define additional binding partners.

We constructed GFP chimeras with the GPR-loop and the C-terminal domain of MyoK. GFP-GPR-loop appears to be evenly distributed throughout the cytoplasm, indicating that it does not contain a major determinant for MyoK localization. In sharp contrast, GFP-C-term is found almost exclusively at the plasma membrane. Live recording of cells expressing GFP-C-term clearly showed how it is taken up during phagocytosis and recycled back to the surface. Expression and localization of the full length MyoK with its CAAX box as well as subcellular fractionation will be used to confirm and expand these data biochemically.

6. TEMPERATURE-SENSITIVE ADENYLYL CYCLASE ACTIVATION DUE TO A SINGLE POINT MUTATION IN THE GENE ENCODING PIANISSIMO. Barbara Pergolizzi, Barbara Peracino, James Silverman#, Adriano Ceccarelli, Angelika Noegel*, Peter Devreotes#, and Salvatore Bozzaro, Dip. Scienze Cliniche e Biologiche, Università di Torino, 10043-Orbassano (Italy), #Dept Biological Chemistry, JHU School of Medicine, Baltimore, MD 21205 (USA) and *Inst. für Biochemie I, Universität zu Köln, 50931-Köln, Germany.

G protein-mediated adenylyl cyclase activation requires the concomitant activity of two cytosolic proteins, CRAC and PIA. We reported last year that the temperature-sensitive nitrosoguanidine mutant HSB1 harbours a point mutation in the *piaA* gene, which results in the $G^{917}D$ substitution. As a result of this replacement, the local secondary structure of the protein is modified by abolishment of a putative turn and formation of a longer α-helix. HSB1 aggregates and forms fruiting bodies at temperatures below 17°C, but remains unicellular at or above 18°C. The temperature-sensitive developmental stage is aggregation; if cells are allowed to aggregate at permissive temperatures, they complete development upon shifting at 23°C, whereas a temperature shift at any time before or during aggregation inhibits further development. *In vivo* receptor-dependent and G protein-linked activation of adenylyl cyclase, not however guanylyl cyclase, is defective in the mutant, and the enzyme is not stimulated *in vitro* by GTPγS; stimulation is restored upon addition of cytosol from wild-type cells.

Transfection of the mutant with wild-type *piaA* (*piaA* ^{wt}) completely rescued the mutant phenotype. Overexpression of *piaA*^{HSB1} in HSB1 or AX2 cells partially rescued the HSB1 phenotype, but inhibited partially AX2 development, at 23°C. The rescuing effect on HSB1 development, not however the inhibitory effect on AX2 cell development, was abolished by further increasing the developmental temperature to 27°C. These results can be explained by assuming that PIA couples, directly or indirectly, the heterotrimeric G protein to adenylyl cyclase *via* two binding sites, only one of which is altered in a temperature-sensitive way by the $G^{917}D$ mutation. When overexpressed in the wild-type background, HSB1-PIA competes with WT-PIA for binding to the first substratum *via* the non-mutated binding site, resulting in a dominant-negative inhibition of cell development. Binding to the second substratum *via* the mutated domain is temperature-sensitive, and the remnant activity observed in overexpressors at 23°C is abolished at 27°C.

To investigate whether PIA activation of adenylyl cyclase requires translocation of the protein to the membrane, and whether this step is affected by the HSB1 mutation, WT- or HSB1-PIA fused to GFP were expressed in AX2 and HSB1 cells. Fusion with GFP did not alter the cellular activity of WT- or HSB1-PIA, which behaved as their counterparts free of GFP. Consistent with the cytosolic nature of the protein, a homogeneous fluorescent labeling of the cytosol was observed in confocal images of resting as well as actively chemotacting cells. We failed to observe recruitment of WT-PIA/GFP to the membrane in polarized cells moving toward cAMP diffusing from a capillary or in 4-h cells stimulated with a single shot of cAMP. Under these conditions rapid and transient recruitment of CRAC or actin to the cell membrane has been reported. It is possible that PIA, in contrast to CRAC which contains PH domains, does not translocate to the membrane during adenylyl cyclase activation; we are in any case unable to link the HSB1 PIA mutation with a potential defect in membrane translocation of the protein.

7. ROLE OF PROTEIN CARBOXYL METHYLATION IN ***DICYTOSTELIUM*** DEVELOPMENT. Ying Chen, Edward C. Cox, and Jeffry B. Stock, Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA.

Regulatory GTPases that mediate signal transduction in eukaryotic cells are generally subject to reversible methyl esterification at the alpha carboxylate of a conserved C-terminal isoprenylated cysteine residue. Biochemical and genetic results indicate that a single nonspecific methyltransferase is responsible for all of the prenylcysteine methylation. Previous studies have indicated that changes in Rho/Rac and G-protein carboxyl methylation may function to modulate signal transduction pathways that mediate inflammatory responses in neutrophils, macrophages, and platelets. A deletion of the mouse prenylcysteine methyltransferase leads to death at an early stage of development. To further investigate the role of methylation in signal transduction and development we have investigated the effect of deleting the ***Dictyostelium*** enzyme. The resulting strain exhibits an Ag- phenotype and is defective very early in the signaling response.

8. ACTIVATION OF CAMP RELAY IN *DICTYOSTELIUM DISCOIDEUM* BY THE CALMODULIN ANTAGONIST W7 IS DUE TO Ca^{2+}-RELEASE FROM Ca^{2+}-STORES. Dieter Malchow, Daniel F. Lusche, and Christina Schlatterer, University of Konstanz, Department of Biology 78457 Konstanz, FRG.

Quite some time ago Brenner and Thoms [Dev. Biol. 101 (84) 136-146] have shown that caffeine blocks the cAMP relay. We found that the calmodulin antagonist W7 augmented light scattering oscillations and cAMP synthesis. Caffeine inhibited the activation of light scattering oscillations by W7. In addition W7 induced Ca^{2+}-release from Ca^{2+} storage compartments *in vitro*. In the presence of caffeine, however, the amount of Ca^{2+} liberated by W7 was substantially reduced. Caffeine also abolished spontanous Ca^{2+}-bursts and calcium induced Ca^{2+}-release (CICR) suggesting that a large part of Ca^{2+} released by W7 was due to CICR. The primary target for W7 seems to be the acidic Ca^{2+} store as concanamycin A, an inhibitor of the V-type H^{+}-ATPase, blocked the Ca^{2+} response of W7. We, therefore, propose that the cAMP relay can be activated by a local rise of cytosolic Ca^{2+}.

19:00-20:20 GENOMICS

9. THE *DICTYOSTELIUM* GENOME SEQUENCING PROJECT AT BAYLOR COLLEGE OF MEDICINE: PROGRESS AND PROSPECTS. Richard Sucgang[1,2], Brian Desany[1], Ryan Lindsay, Hermela Loulseged[2], Donna Muzny[2], Richard Gibbs[2], William Loomis[4], and Adam Kuspa[1,2,3], [1]Department of Biochemistry and Molecular Biology, [2]Human Genome Sequencing Center, [3]Department of Human and Molecular Genetics, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030, [4]Section of Cell and Developmental Biology, Division of Biology, University of California, San Diego, La Jolla, CA 92093, USA.

The *Dictyostelium* genome sequencing effort at Baylor College of Medicine, in collaboration with Sanger Centre, is focussed on completing the sequencing and assembly of the smallest *Dictyostelium* chromosome, the 4.2 Mb C6. The Baylor team has developed and implemented changes in preparation of template DNA that has resulted in a dramatic improvement in sequence quality and production efficiency. Coverage of C6 is conservatively estimated at 5x with reads successfully generated at Baylor for chromosome-enriched random shotgun; all centers are pooling data to facilitate assembly of genomic data. The Baylor team has developed a hybrid whole genome shotgun and YAC-based binning strategy; the early stages of the process, a BLAST-based overlap graph, has been made available as a means for localized assembly of specific genes (http://dictygenome.bcm.tmc.edu/blast). To permit directed finishing of localized regions of the chromosome, we have isolated and mapped YAC-borne segments of the genome consistent with the published high-resolution HAPPY map of C6 (Konfortov et al, 2000), and will do the same for the rest of the genome as high-resolution markers become available. Progress on implementing assembly and finishing strategies, as well as results from the assembly and finishing of the 88 kb extrachromosomal ribosomal DNA palindrome, will also be presented.

10. SEQUENCE AND ANALYSIS OF CHROMOSOME 2 FROM THE MODEL ORGANISM *DICTYOSTELIUM DISCOIDEUM*. L. Eichinger, G. Glöckner, K. Szafranski, P. Dear, J. Pachebat, K. Kumpf, R. Lehmann, J. Abril, G. Parra, R. Guig, B. Tunggal, E. Cox, M. A. Quail, M. Platzer, A. Rosenthal, A. Noegel, and the *Dictyostelium* Genome Sequencing Consortium.

Chromosome 2 is the largest of the six *Dictyostelium discoideum* chromosomes. Sequencing and assembly are close to completion.
Chromosome 2 comprises approximately 7 megabases (Mb) of unique sequence, representing about 22% of the genome. Shotgun sequences were assembled at a 6-fold coverage which resulted in 500 contigs. Read pair information, a circular YAC and a HAPPY map were used to validate the assembly and to order the contigs along the chromosome. Efforts are under way to close the remaining gaps. The chromosome encodes app. 2,600 protein coding genes, 73 transfer RNA genes and several complex repeat elements. We observed a large number of intra- and inter-chromosomal duplications. The gene organisation with respect to intron abundance is comparable to that of other lower eukaryotes like *Plasmodium falciparum*. Yet, the gene density is one of the highest observed so far, only surpassed by *Saccharomyces cerevisiae*. We found several genes not present in *S. cerevisiae*, the model eukaryote cell, but in metazoa. This supports the value of *D. discoideum* as a model organism for the study of cellular processes absent from yeast but typical for animal cells.

11. ANNOTATING *DICTYOSTELIUM* GENES. Negin Iranfar, Christophe Anjard, Rolf Olsen*, and William F. Loomis, Section of Cell and Developmental Biology, Division of Biology *Department of Physics, University of California San Diego, La Jolla, CA 92093, U.S.A.

A Preliminary Directory of Dictyostelium Genes has been generated from all available contigs and can be access at http://dicty.sdsc.edu/annotationdicty.html to get a glimpse of the genetic complement of *Dictyostelium* and initiate the process of full annotation of the genome. Blast and Pfam annotation is provided for the genes in the Preliminary Directory with links to the databases.

Predicted ORFs were recognized by a version of the genefinder program HMMgene trained with a set of 435 individually annotated *Dictyostelium* genes. The program probabilistically defines the start, stop and splice patterns of exons and generates ORFs. A total of 8, 025 distinct ORFs were recognized but the number of genes in this dataset is likely to be lower since some genes are split between contigs and are counted more than once. We estimate that the Preliminary Directory represents >70% of the transcribed genes and that the *Dictyostelium* genome carries about 10, 000 genes. Efforts are now being made to generate longer contigs that will permit a more definitive estimation of the number of genes.

Using the raw sequences and contigs from the genome project, we assembled and analyzed genes encoding members of the histidine kinase and ABC gene families. These genes were used as a challenge set for the trained gene finder, HMMgene, to assess the limits of automated gene annotation in *Dictyostelium*.

Eukaryotic histidine kinases carry both a catalytic domain and a receiver domain which are often encoded by two different genes in prokaryotes. We found 15 hybrid histidine kinases in the *Dictyostelium* genomic data, two of which appear to be no longer functional kinases. Most *Dictyostelium* histidine kinases are more divergent from each other than from their prokaryotic homologs, indicating that multiple genes encoding histidine kinases were inherited from their prokaryotic ancestors and then proceeded to diverge. All but one has been lost in yeast.

A total of 63 complete or partial genes encoding **A**TP **B**inding **C**assette (ABC) proteins were identified using all the sequence available. In eukaryotes, ABCs have one or more transmembrane domains (TMDs) usually consisting of 6 transmembrane helixes and act as transporters for a wide variety of molecules including ions, lipids and polypeptides. The recently established classification based on gene organization and sequence relationships was used to position the *Dictyostelium* ABC genes in 7 families, from ABC A to ABC G. Phylogenic trees based on the conserved ATP Binding domains as well as the complete sequences of the *Dictyostelium* ABC genes confirmed the relationships within and between the families. It had previously been thought that the ABC A family was restricted to multicellular eukaryotes since it is absent in yeast. However, we found 11 genes clearly belonging to the ABC A family in *Dictyostelium*. This family appears to have been lost in fungi. Further evolutionary analyses of these genes families will be presented.

12. A DEMONSTRATION OF PHENOTYPING IN MIXED CULTURES FOR USE IN LARGE-SCALE ANALYSES OF MUTANTS. Adam Kuspa[1,2], Sijie Lu[1], Bin Wang[1], Guokai Chen[1], Miroslava Ibarra[2] and Gad Shaulsky[2]. Departments of [1]Biochemistry and Molecular Biology and [2]Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA.

In preparation for the analysis of a large number of *Dictyostelium* mutants we have tested one strategy for 'parallel' phenotyping using mixed cultures in which individual mutants are followed via their genomic DNA by using the mutated locus as a molecular tag. The mixed mutant culture is challenged through successive rounds of a physiologic test and the presence of each mutant's genome is monitored over time. The increase or decrease of a mutant's genome can be used as a relative measure of its capacity to carryout the function being tested. Our strategy is analogous to the "molecular barcoding" strategy developed for budding yeast by Davis and co-workers[1]. However, instead of adding a unique DNA tag to each insertion mutant as these workers did, we used the mutating vector within each strain as a unique PCR 'anchor' to amplify the DNA flanking the insertion sites using a vector-specific primer and a primer specific for each mutated gene.

To test the utility of this approach we carried out several pilot experiments. We tested the limit of detection by mixing the genomic DNA of a defined insertion mutant with a set of 480 randomly chosen insertion mutants mixed in equal proportions. We found that we could reliably detect the test mutant's genome when it was present at $\geq$0.002% of the total DNA (roughly 10-20 copies of the test mutant's genome under our standard PCR conditions for these assays). This suggests that assays utilizing mixtures of 1,000 different mutants would have a dynamic range of about 50-fold which should be sufficient for quantifying most phenotypes. Currently, we are converting our detection method to a DNA microarray platform with competitive hybridization between test and zero-time samples, utilizing DNA probes made by multiplex PCR.

For the phenotyping tests, we mixed 12 strains with insertion mutations in known genes, including 8 well-characterized mutations and 4 new mutations, and followed how their proportions changed through growth and development. A typical growth test consisted of 5 successive rounds of growth and subculture in liquid media, 3 generations per round, with samples taken at each round for preparation of genomic DNA. The filter development assay consisted of 5 successive rounds of spore selection (using detergent treatment) with intervening rounds of spore germination and growth in liquid. The previously characterized mutants displayed their published phenotypes through all of these tests, while new phenotypes were observed for two of the less well-characterized mutants. A mutant in the *gdtB* gene, a homolog of *gdt1*[2], was slowly lost from the populations subjected to the growth tests. A mutant in the *culB* gene, a cullin homolog[3], was slowly lost from the populations subjected to the sporulation test. Seperate phenotypic tests carried out on the *culB* and *gdtB* mutants confirmed the results obtained in the mixed culture experiments. The *gdtB* mutant clones displayed a small, but reproducible, growth deficit in liquid culture, while *culB* mutant cells displayed a marked propensity to form stalk cells in chimeras with wild-type cells. These results indicate that our strategy for parallel phenotyping can be used to discover defects in individual mutants. Of course, this method will not be useful for scoring phenotypes of groups of cells that require a clonal population, such as slug behavior.

We expect that subtle differences in the functional capacity of various mutants revealed by relative measures of phenotype in mixed cultures will allow more accurate predictions to be made about which genes carryout related functions or which genes comprise a functional unit. By combining direct biological measures of a mutant's function with its transcriptional profile derived from DNA microarrays, we hope to be able to more accurately classify genes into functional categories.

1. Shoemaker et al. (1996) Nature Genetics 14:450-456.
2. Zheng et al. (2000) Mol. Biol. Cell 11:1631-43.
3. Kipreos et al. (1996) Cell 85:829-839.

20:40-21:40 cDNA PROJECT

13. FROM EST DATA TO GENES: ASSEMBLING cDNA SEQUENCES WITH PHRAP PROGRAM. Hideko Urushihara[1], Takahiro Morio[1], Yuji Kohara[2], Tamao Saito[3], Hiroshi Ochiai[3], Mineko Maeda[4], and Yoshimasa Tanaka[1], [1]Institute of Biological Sciences, University of Tsukuba, Japan; [2]Center for Genetic Resource Information, National Institute of Genetics, Japan; [3]Division of Biological Sciences, Graduate School of Science, Hokkaido University, Japan; [4]Department of Biology, Graduate School of Science, Osaka University, Japan.

In consideration that cDNAs represent expressed *genes*, EST data must be extremely valuable to analyze genes in an organism. Thus we keep trying to make better use of sequence data generated by *Dictyostelium* cDNA Project in Japan. Here we report our efforts to convert them into *Dictyostelium* gene structures.

Clustering of EST data, the first thing to be done, is in most cases performed based on homology searches among themselves. There are mainly two problems in this approach. Firstly, false clustering occurs due to an arbitrary setting of the boundary between identical and non-identical sequences. Secondly, and fatally, homology searches do not generate contig sequences. That is, sequence information itself does not increase in value by this clustering procedure. To overcome these issues, we used Phrap program provided by Washington University. Since Phrap was designed for assembling genomic sequences, we first had to examine if it was applicable for cDNA sequences as well. After preliminary runs of Phrap on our EST data, and finding the resulted contigs enough appreciable, we optimized parameter and option settings. Plausibility of assembly was assessed by blast searching of known *D. discoideum* genes against the contig database. In addition to the standard assembly by the Phrap program, we introduced another processing step; when non-overlapping two (3' and 5') sequences derived from a cDNA clone were found in different contigs, the relevant contigs were merged with a fixed gap-signature (10 continuous Ns). The Phrap assembling followed by the post-Phrap merging process generated 5,392 contig sequences out of altogether 26,538 sequences from SL, SS, and VS libraries. The contig sequences will be included in the *Dictyostelium* cDNA database (Dicty_cDB) as its main components. We are currently trying to eliminate sequence-specific inaccuracies in the assembly as those caused by *dutA* and repeat sequences. We will also refer to the genome sequences released from the Genome Sequencing Consortium to correct possible base errors in contig sequences and to reveal exon-intron structures of individual genes.

14. GENERATION AND SEQUENCING OF FULL-LENGTH ENRICHED cDNA LIBRARY FROM DEVELOPING *DICTYOSTELIUM* CELLS. Takahiro Morio(1), Hidekazu Kuwayama(1), Takao Isogai(2), Ai Wakamatsu(2), Sumio Sugano(3), Yuji Kohara(4), Tadasu Shin-i(4), Hideko Urushihara(1), Shinji Obara(1), Mariko Katoh(1), Jeffrey Williams(5), Mineko Maeda(6), Tamao Saito(7), Hiroshi Ochiai(7), Ikuo Takeuchi(8), and Yoshimasa Tanaka(1), (1) Institute of Biological Sciences, University of Tsukuba, Japan, (2) Helix Research Institute, Japan, (3) Institute of Medical Science, University of Tokyo, Japan, (4) Center for Genetic Resource Information, National Institute of Genetics, Japan, (5) Department of Anatomy and Physiology, University of Dundee, UK, (6) Department of Biology, Graduate School of Science, Osaka University, Japan, (7) Division of Biological Sciences, Graduate School of Science, Hokkaido University, Japan, (8) Novartis Foundation for the Promotion of Science, Japan.

In an effort to understand the genetic networks involved in multicellular development, we are carrying out a *Dictyostelium* cDNA sequencing project with the aim of identifying and characterizing genes expressed during development. So far we have sequenced about 27,000 cDNA clones derived from the growth and slug stages and have identified about 5,400 non-redundant EST groups.

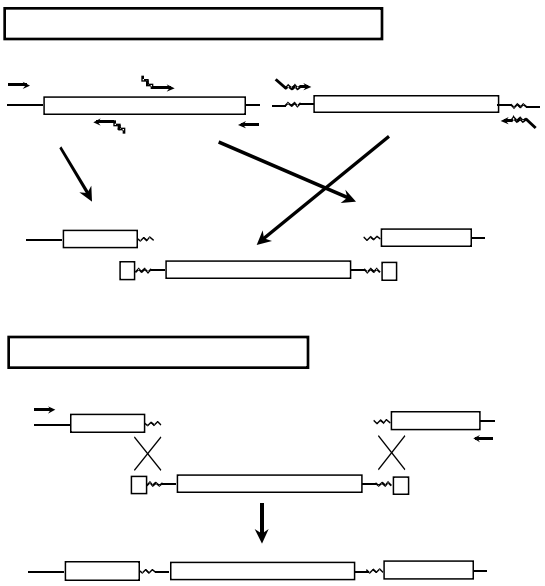
Although the EST libraries are of great value for discovering genes and analyzing their expression profile, there still remain difficulties in using them as the resource for functional analyses because individual cDNA clones often carry only a part of the protein coding sequence. Full-length cDNA sequences will reveal the structure of the coding region, the positions of promoters, exon-intron boundaries and the signals that determine mRNA stability and translational control. For these reasons, we have constructed a cDNA library which is enriched in full-length cDNAs using "Oligo-capping" method. By this method, mRNAs having a cap structure at their 5'-ends are selectively subjected to cDNA synthesis. We prepared size-fractionated cDNA libraries from four developmental stages: growth, aggregation, slug and early culmination. To evaluate their quality, we sequenced 100 to 200 clones from each library from their 5'- and 3'-ends. By comparing the sequences of the clones which encode known *Dictyostelium* genes, we estimate that about 90% of the cDNA clones should contain the entire protein coding region.

We plan to sequence 32,000 clones from the libraries and the summary of the sequencing analysis will be reported.

15. PCR MEDIATED, TWO-STEP PROCEDURE FOR CONSTRUCTION OF A GENE DISRUPTION VECTOR IN *DICTYOSTELIUM DISCOIDEUM.* Hidekazu Kuwayama, Takahiro Morio, Hideko Urushihara, Mariko Katoh and Yoshimasa Tanaka, Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan.

We report a method for a ligation-independent, PCR-based and rapid construction of a gene disruption vector in *Dictyostelium discoideum.* This method is free from cloning a particular DNA fragment in *E. coli* and using any restriction site.

In the first step, three primary PCR reactions are performed to amplify 5'- and 3'- fragments (ideally, longer than 1 kb long) of a gene allele and a selectable marker cassette sequence (see figure on the right). The amplification of the 5'- fragment is performed between a 5'-end primer (primer A) and a 3'-end tagged primer (primer B) which contains a 17 or 18 bp-long sequence tag at its 5'-end (twisted line in primer B). The tag sequence has the same sequence as the 5'-end sequence of the selectable marker cassette so as to join the 5'-flanking fragment to the 5'-end of the marker cassette in the second step PCR reaction. The same trick is applied in amplifying the 3'-fragment except using a 3'-end primer (primer D) and a 5'-end tagged primer (primer C) that contains a 17 or 18 bp tag sequence at the 5'-end (twisted line in primer C). The marker cassette sequence is amplified between two primers (primer B2 & C2) that are complementary to the tagged primers (primer B & C). In the second step, the three PCR fragments are mixed and a fusion PCR runs between the outermost primers (primer A & D), which results in the construction of the gene disruption vector as shown at bottom in the figure. The final PCR product can then be used directly to transform *Dictyostelium* cells.



In principle, this procedure can be used to any gene allele being already sequenced. As a model experiment, we applied this method to the disruption of the catalytic subunit of cAMP-dependent protein kinase gene, *pkaC*, and succeeded in obtaining many independent *pkaC* minus clones with high efficiency. Thus, as this method is suitable for mass-construction of gene disruption vectors because of its rapidity (just two reaction steps) and its relative inexpensiveness, we are now carrying out the construction of gene disruption vectors for all the transcription factor genes that appear in the cDNA and genome databases of *Dictyostelium discoideum*.

MONDAY, JULY 23, 2001

09:00-10:00 EXPRESSION ANALYSES

16. STATISTICAL ANALYSES OF MICROARRAY DATA ON GENE EXPRESSION DURING *DICTYOSTELIUM* DEVELOPMENT. Roman Sasik, Negin Iranfar, Terry Hwa*, and William F. Loomis, Section of Cell and Developmental Biology, Division of Biology, *Department of Physics, University of California San Diego, La Jolla, CA 92093, USA.

Dictyostelium cells proceed through a series of stereotyped stages as they aggregate, differentiate into prespore or prestalk cells, and culminate to form fruiting bodies. Several hundred developmental genes are known that are expressed at specific stages or affect the morphological transitions between stages. To determine their expression profiles, RNA was isolated at 2 hour intervals throughout development of wild type strain AX4 and analyzed on microarrays carrying genomic or cDNA targets from 690 genes encoding named products. The relevant transcriptional events, the times of onset and cessation of expression, were extracted from the kinetic data using a mathematical model that includes first-order decay of mRNA. The model assumes a single time at which transcription starts, a single time of cessation, constant rates of synthesis (S) between these times (t) and a constant half-life for the mRNA (D).

$$\frac{dA^i}{dt} = S^i_t - D^i * A^i$$ where A^i is a specific mRNA

mRNA from 50 genes was found to accumulate during development in a manner that fit the model with a p-value of 0.05 or better. The median half-life was found to be 4 hours in agreement with previous estimates. When the 50 genes are displayed on the basis of their time of onset, it can be seen that there are clusters of genes expressed at 3, 5, 7, 10 and 18 hours of development separated by statistically significant gaps. The possibility that expression of genes in early clusters is required for expression of genes in later clusters is being tested.

mRNA from 35 genes was found to be preferentially enriched in either prespore or prestalk cells. The expression profiles of 23 of these genes could be described by the simple equation with a p-value of 0.02 or better while the remaining mRNAs fit to some extent but appear to be subject to more complex transcriptional regulation. Both the cell type specificity and the developmental time courses were confirmed by Northern blot analyses. These studies have high-lighted 17 new genes not previously known to be cell type specific. The developmental roles of some of these genes are been genetically determined.

Genome-wide studies are now in progress using microarrays with over 5, 569 cDNAs provided by the Japanese cDNA Project together with the 690 previously characterized genes. The results from these studies should expand the number of genes in the clusters. Their expression profiles will be fit to the idealized curves and statistically analyzed.

17. A TRANSCRIPTIONAL PROFILE OF *DICTYOSTELIUM* DEVELOPMENT. Nancy Van Driessche[1,2], Chad Shaw[1], Richard Sucgang[3], Miroslava Ibarra[1], Jeffrey Tollett[1,4], John Halter[5], Adam Kuspa[1,2,3], Gad Shaulsky[1], Mariko Katoh[6], Takahiro Morio[6], Hidekazu Kuwayama[6], Tamao Saito[7], Hideko Urushihara[6], Mineko Maeda[8], Ikuo Takeuchi[9], Hiroshi Ochiai[7], and Yoshimasa Tanaka[6], [1]Department of Molecular and Human Genetics, [2]Graduate Program in Developmental Biology, [3]Department of Biochemistry and Molecular Biology, [4]DNA Array Core Facility, [5]Department of PM&R and Division of Neuroscience, Baylor College of Medicine, Houston TX 77030, [6]Institute of Biological Sciences, University of Tsukuba, Tsukuba, [7]Division of Biological Sciences, Hokkaido University, Sapporo, [8]Department of Biology, Osaka University, Osaka, [9]Novartis Foundation for the Promotion of Science, Takarazuka, Japan.

Analysis of the transcriptional profile of *Dictyostelium* was performed using an expression array of 7,744 targets that represent about 6,000 *Dictyostelium* genes. We analyzed the transcription of genes from strains NC4, AX4 and AX2 that were grown on bacteria and from axenically grown AX4 and AX2 cells. The most prominent feature in the data is a marked shift in the expression pattern of about 2,000 genes at 6-8 hours of development. This shift is coincident with the morphological transition from unicellular to multicellular development. In addition, expression of these 2,000 genes was insensitive to the genetic background or to the nutritional history of the cells. These genes represent the most basic transcriptional events during *Dictyostelium* development. Most of the previously described developmental genes are included in that group. We also found groups of genes whose expression pattern is characteristic of the cells' nutritional history or strain and groups of genes that were expressed in a cell-type specific manner.

To test the idea that transcriptional profiles can be used as molecular phenotypes, we applied a hierarchical clustering analysis to the data and found that the samples could be arranged in the correct temporal order based on the gene expression data alone. These findings indicate that the transcriptional profile is a useful reflection of the physiological state of the developing cells. Analyses of mutant strains and of cells treated with drugs support this notion.

18. GENEPATH: AN INTELLIGENT ASSISTANT FOR GENETIC DATA ANALYSIS AND PATHWAY DISCOVERY. Blaz Zupan[12], Peter Juvan[1], Janez Demsar[1], Ivan Bratko[1], John A. Halter[2], Adam Kuspa[2], and Gad Shaulsky[2], 1) University of Ljubljana, Slovenia, and 2) Baylor College of Medicine, Houston, TX, USA.

Genetic analysis often defines a framework for the elucidation of biological mechanisms. Mutations are the main tool used by geneticists to investigate biological phenomena. Initially, mutations help to define and catalogue genes that participate in a biological mechanism. Relations between genes are then determined using combinations of mutations in two or more genes. Information on gene relations obtained in this way is then used to construct genetic pathways or networks that outline the molecular details of a biological mechanism.

While it may be fairly straightforward for a geneticist to find the relation between two genes from experimental data, systematic search for all possible gene relations while accounting for all the data becomes complicated when the amount of data increases as many genes, mutations and phenotypes are analyzed. For the purpose of automating the task we have developed a program called GenePath that takes genetic data and any prior knowledge of the genetic network as input, and outputs a list of gene-to-gene and gene-to-outcome relations and a corresponding consistent genetic network.

The particular feature of GenePath is that it mimics expert geneticists by using the reasoning patterns they would otherwise employ manually. For instance, to determine if one gene precedes another, a pattern "*IF mutation of GeneA changes the phenotype P (compared to the wild type) and adding the mutation of GeneB reverses the phenotype P THEN gene A precedes gene B in a pathway for an outcome P*" is used. GenePath currently incorporates about fifteen such patterns, and can determine which genes influence (or do not influence) the outcomes, and which genes block (or do not block) other genes. GenePath uses these patterns to systematically search over the data and obtain all possible gene relations. These serve as constrains for the genetic network that is generated by GenePath as a final result of the analysis.

The particular advantage of GenePath is its explanation mechanism. Users can query on any relation or part of the network discovered, and GenePath can provide the corresponding evidence in terms of experiments and patterns that were required for its inference. The program also facilitates storage and communication of data from genetic experiments in a consistent manner.
GenePath was implemented in the Prolog logic-based programming language, and has an easy-to-use web-based interface (`magix.fri.uni-lj.si/genepath`). GenePath was developed around a genetic pathway that regulates the transition from growth to development in the social amoeba *Dictyostelium discoideum*. It has been tested successfully on other *Dictyostelium* pathways as well as on two *C. elegans* pathways.

10:20-12:00 SIGNALING II

19. *DICTYOSTELIUM DISCOIDEUM* AS A HOST MODEL SYSTEM FOR *LEGIONELLA PNEUMOPHILA* INFECTION. Marcela Fajardo[1], Sonja Hägele[1], Carina Skriwan[1], Stephan Schwager[2], Michael Schleicher[2], Jörg Hacker[1], and Michael Steinert[1], [1]Institut für Molekulare Infektionsbiologie, Universität Würzburg, Röntgenring 11, D-97070 Würzburg, Germany. [2]Institut für Zellbiologie, Ludwig-Maximilians-Universität, Schillerstrasse 42, D-80336 München, Germany.

Legionella pneumophila, the etiologic agent of Legionnaires´ disease, is an ubiquitous microorganism inhabiting natural and man-made freshwater biotopes. Upon transmission to humans via *Legionella*-containing aerosols the bacteria invade alveolar macrophages and multiply within an endosomal-maturation blocked phagosome. New insights into the complex interplay of this bacteria-host interaction are expected from model systems where host factors can be manipulated. *D. discoideum* is such a host model system since cellular markers, cell signaling pathways, and different genetic tools are well established. The infection of single-cell stages of *D. discoideum* with different *Legionella* species and well characterized *Legionella* mutants parallels the infection in human macrophages and the natural amoebal host *Acanthamoeba castellanii*. After 96h co-culture the inoculum of virulent bacteria increased 150 - 1500 fold, as measured by colony forming units while avirulent bacteria exhibited decreasing counts. Electron micrographs confirmed the intracellular growth of the virulent bacteria. In order to analyze host factors involved in the infection process, different *D. discoideum* mutants were infected with *Legionella*. In these experiments profilin-minus mutant cells showed higher rates of infection than wild type cells. Comitin-minus mutants also showed a positive effect on bacterial growth. In contrast synexin-minus mutants did not show differences when compared to infections of wild type cells. In conclusion our study demonstrated that *Dictyostelium* is a representative model system to study the cellular pathogenesis of Legionnaires´disease.

20. 5’-NUCLEOTIDASE IN *DICTYOSTELIUM*: REGULATION, PROMOTER ANALYSIS AND LOCALIZATION. Muatasem Ubeidat, Can M. Eristi, Danielle F. Overall, Brad R. Joyce and Charles L. Rutherford, Biology Department, Molecular and Cellular Biology Section, Virginia Polytechnic Institute and State University, Blacksburg, VA 24060-0406, USA.

5'-Nucleotidase (5NT) is a ubiquitous enzyme found in wide variety of species and in several different cell types. In *Dictyostelium*, this enzyme shows substrate specificity for 5'AMP. The enzyme has received considerable attention because of the critical role played by cAMP. Formation and maintaining of cell specific levels of cAMP may be the result of a concerted interaction of: (1) adenylate cyclase, (2) cAMP phosphodiesterase, and (3) 5'-nucleotidase, the enzyme that degrades 5'AMP to adenosine. During the time course of development, the enzyme activity of 5NT increases and become restricted to a narrow band of cells that form the interface between the prestalk/ prespore zones. A peptide associated with 5NT activity was purified and sequenced. PCR amplification of genomic DNA using degenerate oligonucleotides and a search of sequences of the cDNA project yielded a DNA fragment with sequence corresponding to the peptide sequence of 5NT. Northern blot analysis showed that the gene is developmentally regulated. Southern analysis showed a single form of the 5nt. Knockout mutagenesis using 5nt sequences produced five strains in which the gene was disrupted. These strains formed multi-tipped mounds delayed at the early culmination stage before finally completing development. In order to analyze 5’-nucleotidase (*5nt*) gene expression we made a fusion construct in which the *5nt* promoter directed the expression of β-galactosidase, green fluorescent protein (*gfp*), or luciferase. Reporter gene activity during the time course of development showed that the fusion genes were first expressed in the early aggregation stage, in agreement with the temporal expression of the authentic *5nt* gene. Cells expressing β-galactosidase as a reporter gene were concentrated in the upper 75% of the tight aggregate while the lower 25% were devoid of fusion gene activity. At the slug stage, *5nt* was highly expressed in a group of cells that form a funnel-shaped core in the front of the slug, probably in pstAB cells. We also observed that as the slug moved along the substratum, high activity of β-galactosidase was detected in cells that were left behind the moving slug in the slime sheath. At the early culmination stage, the *5nt*-fusion gene was expressed in a group of cells at the interface of the prespore/prestalk regions, a region previously shown to contain active 5NT. In the completed fruiting body, *5nt* was expressed in the lower cup, in the slime sheath, and the basal disc. Analysis of a series of promoter-deletion constructs fused to luciferase gene showed a decreasing activity when deletions downstream -547bp were tested. No luciferase activity was detected when -365bp fragment fused to luciferase nor to any other reporter gene. This result showed that the -365bp construct is not an effective promoter because of the lack of element(s) that drive the expression of the fusion gene in a specific cell type. Internal deletions of the promoter were generated by PCR to define a region of about 182bp that could contain the core promoter.

21. A NUCLEAR PHOSPHATIDYLINOSITOL PHOSPHATE KINASE REQUIRED FOR DEVELOPMENTAL GENE EXPRESSION. Kunde Guo(1), Richard Nichol(1), Paul Skehel(2), Jeffrey G. Williams(3), and Catherine Pears(1). 1. Department of Biochemistry, Oxford University, South Parks Road, Oxford, OX1 3QU, UK. 2. Department of Neuroscience, University of Edinburgh, 1 George Square, Edinburgh, EH8 9JZ UK. 3. Department of Anatomy and Physiology, MSI/WTB Complex, University of Dundee, Dow Street, Dundee DD1 5EH, UK.

The generation of diacylglycerol in response to receptor stimulation is a well-documented signalling mechanism that leads to activation of protein kinase C (PKC). Putative alternative effectors contain sequences which interact with diacylglycerols but the mechanisms of signal transduction are unknown. We have identified a *Dictyostelium* gene encoding a novel protein which contains a domain with high identity to the diacylglycerol binding domain of PKC. It does not encode a PKC homologue as the conservation does not extend outside this region. We confirm biochemically that the proposed diacylglycerol binding domain is sufficient to mediate interaction of a fusion protein with vesicles containing diacylglycerol. The protein also shows significant homology to mammalian phosphatidylinositol phosphate (PIP)kinases and we show that this domain has PIPkinase activity. The protein, PIPkinA, is concentrated in the nucleus and abrogation of gene function inhibits early developmental gene expression and blocks development at an early stage. Thus we have identified a PIPkinase from *Dictyostelium* which is required for development, is a candidate effector for diacylglycerol and has the ability to synthesise nuclear PIP2.

22. REGULATION OF ADENYLYL CYCLASES BY A REGION OUTSIDE THE MINIMALLY FUNCTIONAL CYTOPLASMIC DOMAINS. Carole A. Parent[#], Jane Borleis*, and Peter N. Devreotes*, # Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health; *Department of Anatomy and Cell Biology, The Johns Hopkins School of Medicine, USA.

The highly conserved topological structure of G protein activated adenylyl cyclases seems unnecessary since the soluble cytoplasmic domains retain regulatory and catalytic properties. Yet, we previously isolated a constitutively active mutant of the *D. discoideum* adenylyl cyclase harboring a single point mutation in the region linking the cytoplasmic and membrane domains (L394). We show here that multiple amino acid substitutions at L394 also display constitutive activity. The constitutive activity of these mutants is not dependent on G proteins or cytosolic regulators, although some of the mutants can be activated to higher levels than wild type. Combining a constitutive mutation such as L394T with K482N, a point mutation that renders the enzyme insensitive to regulators, restores an enzyme with wild type properties of low basal activity and the capacity to be activated by G proteins. Thus regions located outside the cytoplasmic loops of adenylyl cyclases are not only important in the acquisition of an activated conformation, they also have impact on other regions within the catalytic core of the enzyme.

23. G ALPHA 9: A HETEROTRIMERIC G PROTEIN THAT FUNCTIONS AS AN INHIBITOR OF cAMP SIGNALING PATHWAYS IN *DICTYOSTELIUM DISCOIDEUM*.

Joseph A. Brzostowski, Cynthia Johnson, Carole Parent*, and Alan R. Kimmel, Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health and National Cancer Institute*, Bethesda, MD 20892-8028, USA.

Dictyostelium, like mammalian cells, use 7-transmembrane receptor (7-TMR)/G protein-coupled pathways to mediate cellular signaling. During development, *Dictyostelium* initiates a pulsatile, extracellular release of cAMP that directs cell migration and activates downstream effectors via specific cAMP 7-TMRs (cARs). Adenylyl cyclase (AC) is transiently activated by the cAMP signal and then rapidly adapts. While Gβγ and the cytosolic factor CRAC are implicated in AC activation, adaptation mechanisms for AC remain unknown. We identified a novel Gα (Gα9) in *Dictyostelium* that functions as a negative regulator of cAR signal transduction.

gα9-null cells develop faster, form more aggregation centers, and chemotax faster to cAMP than wild-type cells, consistent with the loss of an inhibitory protein. Additionally, *gα9*-null cells initiate cAMP pulses more frequently, suggesting that these cells become resensitized (de-adapted) more rapidly to the cAMP signal. Our data suggest Gα9 does not directly inhibit AC, but functions to inhibit the signaling events upstream of AC. In contrast, cells expressing constitutively activated Gα9 are developmentally delayed, form significantly fewer signaling centers, and are restricted in their ability to activate AC and other downstream effectors, again, consistent with Gα9 functioning in an inhibitory signaling pathway.

In mammalian cells, phosphorylation of 7-TMRs is linked to adaptation. Although cAR phosphorylation is contemporaneous with AC adaptation in *Dictyostelium*, the results of experiments with mutant cARs suggested that phosphorylation is not required for adaptation. We provide strong evidence to support a functional synergism between Gα9 and receptor phosphorylation to adapt AC. When phosphorylation-defective cAR1 is expressed in *car1/car3/gα9*-null cells, AC remains constitutively active in response to cAMP stimulation. These are the first mechanistic studies to demonstrate a loss of AC adaptation in *Dictyostelium*. Our data also support a role for Gα9 in a global inhibitory network and the potential for Gα-mediated sensory adaptation in other organisms.

19:00-20:20 CHEMOTAXIS

24. INTERPRETING THE ROLE OF A PROTEIN IN LOCOMOTION AND CHEMOTAXIS BY MUTANT ANALYSIS. David R. Soll, W.M. Keck Dynamic Image Analysis Facility, Department of Biological Sciences, University of Iowa, Iowa City, IA 52242, USA.

Using 2D and 3D computer-assisted motion analysis systems, we have developed a framework for defining the roles of specific regulatory and cytoskeletal elements in locomotion and chemotaxis through mutant analysis. Cells exhibit basic motile behavior in buffer, which is manipulated by the spatial and temporal characteristics of each phase of the natural wave (onset, front, peak, back) in the process of chemotaxis. These manipulations result in very specific behavioral responses to the different spatial, temporal and concentration components of the natural wave, which have been dissected by characterizing wild type cell behavior in a) a standing spatial gradient of cAMP, which mimics the increasing spatial gradient in the front of the wave, b) increasing and decreasing temporal gradients of cAMP, which mimic the temporal dynamics of the front and back of natural waves, c) after the rapid addition of 10^{-6} M cAMP, which mimics the peak of the natural wave, and, finally, d) in natural waves. Using the above wild type arsenal of behavioral phenotypes for comparison, we have analyzed the following mutants supplied by collaborators (Loomis, Knecht, Condeelis, Luna, Kuspa, Shaulsky, Egelhoff, Chisholm, Saxe, Titus, Firtel, Devreotes, Spudich, O'Halloran): 1) myosin II null mutants; 2) the myosin heavy chain phosphorylation mutant 3XALA; 3) the myosin regulatory light chain phosphorylation mutant S13A; 4) the internal phosphodiesterase null mutant *reg*A$^-$; 5) the PKA regulatory subunit null mutant *pka*R$^-$; 6) the myosin I null mutants *myo*A$^-$, *myo*B$^-$, *myo*C$^-$, *myo*F$^-$, *myo*AB$^=$, *myo*BC$^=$, *myo*AC$^=$, *myo*AF$^=$; 7) the Wiscott Aldrich Syndrome protein null mutant Scar; 8) the MAP kinase kinase null mutant *ddmek*1$^-$; 9) the null mutant *tor*A$^-$; 10) ponticulin$^-$; 11) ABP120$^-$; 12) migA$^-$; and 13) clathrin$^-$. The results of these mutant analyses distinguish two major categories of proteins: 1) proteins that play a role in the basic motile behavior in buffer and 2) proteins that play exclusive roles in particular responses to one of the four phases of the natural wave. Proteins in the first category are often the downstream targets of the regulatory pathways composed of proteins in the second category. The results have led to a unique model of chemotactic regulation that defines independent pathways emanating from the different phases of the wave (i.e., different spatial and temporal dynamics of receptor occupancy) which terminate in very specific behavioral responses. These results and the emerging model suggest that beginning a pathway simply with "cAR1" and ending it with "chemotaxis" is now insufficient.

25. ANALYSIS OF WASP-LIKE PROTEINS IN *DICTYOSTELIUM*. Wendy Mahler[1], Deborah Wessels[1], Diana Caracino[1], Julia Steiner[1], Cheryl Jones[1], David Soll[2], and Charles L. Saxe[1], [1]Department of Cell Biology, Emory University School of Medicine, Atlanta, GA, 30322, [2]Department of Biological Sciences, University of Iowa, Iowa City, IA 52242, USA.

Scar and related proteins of the WASp (Wiskott-Aldrich Syndrome protein) family have been shown to facilitate the polymerization of actin in vitro. The mechanism of action involves direct binding to actin and components of the arp2/3 complex. In vivo Scar and WASp have been implicated in regulating the formation of lamellipodia and filopodia, respectively, and we previously showed that the absence of Scar in *Dictyostelium* cells leads to defects in F-actin organization.

We now report that Scar$^-$ cells are unable to form lateral pseudopods and a localized increase of F-actin polymerization associated with new pseudopod formation is absent. Chemotaxis is severely impaired in these cells and receptor and GTPγS-stimulated F-actin polymerization are significantly reduced. Reintroduction of Scar reverts these phenotypes. Introduction of the C-terminal, actin and arp2/3 interacting regions of Scar, does not revert any of the phenotype. On the other hand introduction of the N-terminal, SHD, region of Scar allows the production of pseudopods and F-actin polymerization in response to receptor activation or GTPγS. Chemotaxis is not recovered and development is abnormal. Similar results are seen when DdWASp is introduced into Scar null cells. We propose that Scar and WASp differentially regulate actin dynamics in *Dictyostelium*.

26. REGULATION OF F-ACTIN ASSEMBLY AND CHEMOTAXIS BY *DICTYOSTELIUM* WASP. Chang Y. Chung, Young-Hoon Han, and Richard A. Firtel, Section of Cell and Developmental Biology and Center for Molecular Genetics, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0634, USA.

The Wiskott-Aldrich syndrome protein (WASP) and related proteins have emerged as key downstream components converging multiple signaling pathways to F-actin polymerization. We used molecular genetic approaches to define the function of WASP in controlling F-actin assembly during *Dictyostelium* chemotaxis. There are two alleles encoding WASP and we have not obtained a null mutant (double knockout) using standard approaches, possibly because such a strain may exhibit growth defects and thus be selected against using the standard protocol. However, we were able to disrupt one of the copies, and analysis of this hemizygous strain has already provided us with direct evidence of a role for WASP in the *in vivo* regulation of F-actin assembly during chemotaxis. The WASP hemizygous strain moves more slowly and exhibits altered pseudopodia extension, reflecting aberrant regulation of F-actin assembly. Moreover, these cells have a very significant motility defect in the multicellular mound, as WASP hemizygous cells stay at the mound stage three times longer than wild-type cells, indicating a role for WASP in controlling morphogenesis. When mixed with wild-type cells, the WASP hemizygous cells initially distribute randomly in the mound, but they are then excluded to the periphery of the mound in the late stage, probably due to the motility defect. To obtain a strain with lower WASP expression levels than WASP hemizygous cells, we took advantage of the antisense RNA approach. Cells were transformed with a vector encoding RNA complementary to WASP mRNA under the control of the *discoidin* promoter. Cells expressing antisense RNA are unable to protrude pseudopodia with a high persistency and their directional motility appears to be greatly impaired.

We also created a series of WASP subdomain deletion mutations that are expected to abrogate the function of specific domains. Cells overexpressing WASP or the VA domain, which is known to have a F-actin nucleation activity in association with the Arp2/3 complex, are not well-polarized compared to wild-type cells and they do not exhibit a prominent actin-rich cortical region with phalloidin staining. This might result from the unregulated nucleation of F-actin due to the overexpression of WASP or the VA domain. Cells expressing a WASP lacking the whole polyproline domain (WASPΔPro) or the last two polyproline repeats and the V domain (WASPΔPstI) have a virtually opposite F-actin organization. They exhibit very intense and up-regulated cortical actin filaments along the periphery of cells. Cells expressing WH1 and CRIB domains show a similar up-regulation of F-actin assembly, although in these cells, the F-actin is found throughout the cells. The up-regulation of F-actin assembly might be due to the inhibition of the dynamic regulation of F-actin assembly by these mutants in a dominant negative fashion. Cells expressing WASP mutants appear to have severe defects in chemotactic movement. Cells exhibiting up-regulated cortical F-actin assembly have substantial defects in the regulation of cell shape and motility, probably due to cytoskeletal defects. For example, cells overexpressing WASPΔPro are not polarized and cannot extend pseudopodia effectively, probably because they lack dynamic regulation of the F-actin cytoskeleton. These results suggest that these deletion mutants exert a different effect on the machinery of F-actin assembly and that WASP activity plays a very important part in the regulation of the actin cytoskeleton during *Dictyostelium* chemotaxis.

27. RECRUITMENT OF MYOSIN HEAVY CHAIN KINASE ACTIVITY TO ACTIN-RICH CORTICAL STRUCTURES. Paul A. Steimle*, Shigehiko Yumura[†], Graham P. Côté[‡], Quint G. Medley[‡§], Mark V. Polyakov*, and Thomas T. Egelhoff*, *Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH 44106, USA; [†]Department of Biology, Yamaguchi University, Yamaguchi 753 8512, Japan; [‡] Department of Biochemistry, Queens University, Kingston, Ontario K7L 3N6, Canada; [§] Department of Cancer Immunology and AIDS, Dana-Farber Cancer Institute, Boston, MA 02115, USA.

In ***Dictyostelium discoideum***, myosin II filament assembly is controlled primarily through myosin heavy chain phosphorylation. The phosphorylation of sites near the tip of the myosin tail domain by myosin heavy chain kinase A (MHCK A) drives disassembly of myosin filaments ***in vitro*** and ***in vivo***. MHCK A has a novel domain structure consisting of a helical coiled-coil domain, a catalytic domain unrelated to conventional protein kinases, and a WD-repeat domain. Two other structurally related kinases, MHCK B and C, have been identified in ***Dictyostelium***. In order to better understand the cellular regulation of MHCK A activity, and thus the regulation of myosin II filament assembly, we studied the localization of native and green fluorescent protein (GFP)-tagged MHCK A in a variety of cellular settings. We have shown that MHCK A is dynamically recruited to the cell cortex in response to chemoattractants, and is preferentially recruited into leading edge actin-rich protrusions. Truncation analysis has revealed that a GFP construct containing only the coiled-coil domain of MHCK A translocates to the cell cortex upon cAMP stimulation; this construct also shows frequent enrichment in actin-rich cellular protrusions. In related studies, we have found that MHCK A co-sediments with actin-filaments in a manner that requires the presence of the coiled-coil domain of the kinase. Taken together, these results indicate that cortical recruitment of MHCK A may occur by a mechanism involving a direct interaction between the coiled-coil domain of the kinase and actin filaments. In a broader context, we propose that MHCK recruitment to forming and established actin-rich protrusions may represent a fundamental mechanism that prevents stabilization of the actomyosin cytoskeleton in leading edge protrusions.

20:40-22:00 SIGNALING III AND MODELING

28. PHOSPHORYLATION OF THE MYOSIN REGULATORY LIGHT CHAIN PLAYS A ROLE IN MOTILITY AND POLARITY DURING *DICTYOSTELIUM* CHEMOTAXIS. Hui Zhang[a], Deborah Wessels[a], Petra Fey[b], Karla Daniels[a], Rex Chisholm[b], and David R. Soll[a] , [a]Dept. Biol, Sciences, Univ. of Iowa, Iowa City, IA 52242 and [b]Dept. Cell and Mol. Biol., Northwestern Univ. Med. School, Chicago, IL 60611, USA.

The myosin regulatory light chain (RLC) of *Dictyostelium discoideum* is phosphorylated at a single serine site in response to chemoattractant. To investigate the role of the phosphorylation of RLC in both motility and chemotaxis, a mutant was generated in which the single serine was replaced with a nonphosphorylatable alanine, and subjected to high resolution motion analysis under the following five conditions: in buffer in the absence of a chemotactic signal, in a spatial gradient of cAMP in the absence of a temporal gradient, in a temporal gradient of cAMP in the absence of a spatial gradient, after the rapid addition of 10^{-6} M cAMP, and in natural waves of cAMP generated in aggregation territories. We demonstrate that the phosphorylation of RLC plays a role in basic motility in buffer as well as in chemotaxis, and is specifically involved first in the frequency of lateral pseudopod formation, second in the velocity of cellular translocation, and third in cell shape changes leading to the loss of polarity that are induced by the peak and back of natural cAMP waves. These observations led us to formulate the following model: The RLC is phosphorylated and dephosphorylated in equilibrium during basic motile behavior in buffer. However, in a natural chemotactic wave, this equilibrium is shifted to the phosphorylated state at the peak and in the back of the wave, resulting in loss of polarity and the maintenance of the depolarized state, respectively. Rearrangement of myosin II in the cortex at the peak of the wave may be facilitated by the increase in motor function resulting from RLC phosphorylation. The role of increased RLC phosphorylation in response to the peak and back of the wave, therefore, is to increase the efficiency of chemotaxis. That is, the loss in cell polarity induced at the peak and the maintenance of the apolar state in the back of natural cAMP waves that are the consequences of the shift of RLC to the phosphorylated state serve to increase the efficiency of chemotaxis by assuring that cells do not turn around and chemotax in the wrong direction in response to the reversed spatial gradient in the back of the wave.

29. REGULATION OF THE CYTOSKELETON BY CYCLIC GMP SIGNALING IN *DICTYOSTELIUM*. Janet L. Smith, Jonathan M. Goldberg, and Ana C. Urbin-Reyes, Boston Biomedical Research Institute, 64 Grove St., Watertown, MA 02472, USA.

Intracellular cGMP levels transiently increase in response to chemoattractants in *Dictyostelium*, and this increase appears to be essential for cytoskeletal rearrangements during chemotaxis. Cyclic GMP is required for phosphorylation of the myosin II regulatory light chain during chemotaxis, and we have shown that myosin light chain kinase A is activated by cGMP-dependent phosphorylation. It follows that cGMP-binding proteins in the cell directly or indirectly regulate key cytoskeletal components after treatment with chemoattractants, but to date, no cGMP-binding proteins have been purified or cloned in *Dictyostelium*. The current *Dictyostelium* genomic sequence database contains sequence information for >97% of the genes. Using a proteomics approach, we have determined that there are three genes encoding proteins that are strongly predicted to bind cGMP. We have designated these three cGMP-binding proteins GBP-A, B, and C. GBP-A and B are homologous to each other, and have a Zn^{2+}-binding domain, followed by two tandem cGMP-binding sites. The presence of tandem cyclic nucleotide binding sites is a hallmark of cyclic nucleotide dependent protein kinases, suggesting that GBP-A and B are regulatory subunits of a novel cGMP-regulated protein kinase. GBP-A is expressed throughout development, with a maximum at 4 hours after starvation is initiated, a time when cells are beginning to form aggregates. GBP-B is also expressed throughout development, but with a slight peak of expression in vegetatively growing cells. GBP-C contains a MAP kinase kinase kinase (MAPKKK) domain, a Ras guanine nucleotide exchange factor (RasGEF) domain, and a single cGMP-binding domain, suggesting that cGMP may regulate one or both of these activities. GBP-C expression increases dramatically at 8 hours after starvation is initiated, when aggregation is completed. We have recently isolated a gene disruption for GBP-B, and are currently engineering gene disruption strains for GBP-A and C, in order to determine their biological roles. The identification of GBP-A, B, and C represents the first step in a molecular approach to studying cGMP signaling in *Dictyostelium*. Moreover, the novel domain structures of these proteins challenge the idea that all cGMP signaling is mediated by cGMP-dependent protein kinase, cGMP-regulated phosphodiesterases, and cGMP-gated ion channels.

30. CHEMOTACTIC RESPONSE IN DICTYOSTELIUM: A DIFFUSIVE-INHIBITOR MODEL. Wouter-Jan Rappel, Peter Thomas[‡], Herbert Levine, and William F. Loomis*, Department of Physics, [‡] Salk Institute, and *Section of Cell and Developmental Biology, Division of Biology, University of California, San Diego, La Jolla, CA 92093, USA.

The chemotactic response of *Dictyostelium* to a gradient in cAMP requires translocation of the PH-domain protein CRAC from the cytoplasm to the cell membrane. Release of cAMP from a pipette elicits CRAC membrane localization on the near side of the cell (the front) but not on the far side (the back). Inasmuch as the signal diffuses rapidly around the cell and that the imposed cAMP concentration is far above threshold, this requires the rapid action of an inhibitor that can suppress activation at the back.

We propose a simple scheme for how cGMP produced by localized activation of guanyl cyclase at the front can rapidly diffuse through the cell interior to inhibit the response at the back. Our model makes specific predictions for the necessary rate of cGMP production and its inhibitory action. Finally, simulations of our equations demonstrate how a new "two-pipette" experimental protocol should be able to discriminate between our scheme and more exotic possibilities for the inhibitory signal. The model also makes specific predictions on the behavior of certain mutant strains.

31. A COMPUTATIONAL STUDY OF AMEBOID POLARITY AND CHEMOTAXIS.
Dean C Bottino[1,2], Noah Edelson[2], and Hans Othmer[3], [1]Physiome Sciences, 307 College Road East, Princeton, NJ 08540 (contact address), [2]University of California, College of Natural Resources, Berkeley, CA 94720, [3]University of Minnesota, Department of Mathematics, Minneapolis, MN 55455.

Recent experimental results indicate that sensing and amplification of the external gradient occurs upstream of cytoskeletal activation; for example, rounded ***Dictyostelium discoideum*** (Dd) cells incapable of actin polymerization still exhibit a sharp gradient of pleckstrin homology (PH) binding sites in the direction of cAMP stimulation (Devreotes, Science 1999,2000). However, actin polymerization does appear to play a role in the formation of a sensing gradient in strongly polarized cells; G-beta-null cells rescued with G-beta-GFP perform normal chemotaxis, but both the G-beta-GFP gradient and motion are blocked when actin polymerization is abolished by latrunculin-A. This suggests the existence of an important feedback loop: cAMP sensing → sharp PH binding site gradient → asymmetric actin polymerization and locomotion → formation of sensing gradient → asymmetric sensitivity to cAMP. Notably, a similar actin polymerization dependent feedback loop (apparently independent of G-proteins, however) seems to occur in human neutrophils as well.

We present a computational model incorporating the suggested feedback loop, with individual functional "modules" corresponding to different steps in the loop. The model predicts 2D cell traces and difference images that can be compared to the time-series centroid and shape traces of living cells exposed to chemoattractant. The model will attempt to explain how the basic properties of the individual functional modules determine the chemotactic behavior of Dd. For example, what determines whether a moving Dd cell exposed to a lateral cAMP source turns its dominant pseudopod toward the new cAMP source rather than extending a new lateral pseudopod? These same modeling techniques can also be applied to neutrophil chemotaxis.

TUESDAY, JULY 24, 2001

09:00-10:20 DIRECTIONAL RESPONSES

32. IMAGING OF INDIVIDUAL cAMP-RECEPTORS ON THE PLASMA MEMBRANE OF *DICTYOSTELIUM*. B.E. Snaar-Jagalska[1], P.H.M. Lommerse[1,2], S. de Keijzer[1], G.A. Blab[2], G. Lamers[1], H.P. Spaink[1], and T. Schmidt[2], [1]Institute of Molecular Plant Science, and [2]Department of Biophysics, Leiden University, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

Single-molecule microscopy is a novel approach to monitor the behavior of signaling molecules *in vivo.* The lateral and rotational mobility of all membrane constituents is believed to play an important role in various signal transduction processes. Using fluorescence labeled lipids, and proteins fused to the autofluorescent proteins, we are able to perform single-molecule experiments on the plasma membrane of various cell lines under physiologically conditions. Our experimental setup consists of an inverted microscope using laser excitation and image detection on an ultrasensitive CCD-camera. The setup allows to image individual fluorophores within a time-frame of a few milliseconds at a signal-to-background ratio of up to 30. Individual autofluorescent proteins were characterized by typical photon emission rates of ~3000 photons/ms, saturation intensities of 10 kW/cm^2, and photobleaching yields of ~10^{-4} (Harms, et al., Biophysical J., 2001, 80, 2396). The major obstacle for their detection on living cells concerns cellular autofluorescence, photobleaching of the autofluorescent proteins, and phototoxicity in the excitation field of the laser. Those aspects will be discussed in depths. For the *in vivo* studies in *Dictyostelium* we showed that fusion proteins using the yellow-fluorescent protein mutant eYFP are superior compared to all the other autofluorescent proteins. Optimization of the detection parameters allowed us to study the dynamics of individual fusion proteins and lipid-anchored proteins (eYFP-CAAX, cAMPR-eYFP) in the plasma membrane of *Dictyostelium*. The results will be compared with the results obtained on eYFP-CAAX and various lipids in a human cell line.

33. THE VISUALIZATION OF HETEROTRIMERIC G-PROTEIN ACTIVATION IN LIVING *DICTYOSTELIUM*. Chris Janetopoulos, Tian Jin, Linnan Tang, Ning Zhang, Yi E.Huang, Miho Shimizu, and Peter N. Devreotes, Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD 21201, USA.

Free-living amoeba, like mammalian leukocytes, sense chemoattractant gradients with serpentine G-protein coupled receptors. GFP-tagged chemoattractant receptors are evenly distributed along the membrane while the G-protein βγ-subunits and α-subunits are uniformly distributed along the cell surface. Labeled PH domains are selectively recruited to the cell's leading edge. The visualization of dynamic events in chemotactic cells has helped elucidate how responses in the signaling pathway become localized. The activation of heterotrimeric G-proteins in living *D. discoideum* cells can now be visualized by monitoring fluorescence resonance energy transfer (FRET) between α- and β- subunits fused to cyan and yellow fluorescent proteins. Upon addition and removal of chemoattractant, the G-protein heterotrimer rapidly dissociated and reassociated. When cells were continuously stimulated, G-protein activation reached a dose-dependent steady state level. The activation remained steady even after physiological responses had subsided. Thus, adaptation occurs at another point in the signaling pathway and occupied receptors, whether or not phosphorylated, catalyze the G-protein cycle.

34. LOCALIZED ACTIVATION AND DEACTIVATION OF PI3K AT THE LEADING EDGE: DIFFERENTIAL CHEMOATTRACTANT-MEDIATED TRANSLOCATION OF PI3K AND PTEN TO THE PLASMA MEMBRANE. Satoru Funamoto, Ruedi Meili, and Richard A. Firtel, Section of Cell and Developmental Biology and Center for Molecular Genetics, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0634, USA.

Phosphatidylinositol 3' kinase (PI3K) produces $PI(3,4)P_2$ and $PI(3,4,5)P_3$ upon stimulation of ligand binding. Activation of PI3K regulates various biological processes, including apoptosis, cell metabolism, and cell polarity through downstream effectors. Several labs, including our own, have shown that several proteins are recruited to the leading edge due to their affinity to 3-phosphoinositides. Recently, we demonstrated that downstream effectors PKB and PhdA are required for proper cell polarity by regulating 1) PAKa and myosin assembly and 2) actin polymerization, respectively, during chemotaxis (Meili et al., 1999, 2000; Chung et al., 2001; Funamoto et al., 2001). PKB and PhdA transiently and rapidly bind to PI3K products on the plasma membrane via their PH domains upon cAMP stimulation. This finding suggests that translocation of PH-domain-containing proteins may occur by localized activation of PI3K on the membrane.

To examine the temporal and spatial localization of PI3K after cAMP stimulation, we made cell lines expressing PI3K-GFP and PI3K-GFP deletion mutants. PI3K-GFP translocated to the membrane upon stimulation and localized on the leading edge during chemotaxis. Expression of PI3K-CFP and PhdA-YFP enabled us to demonstrate simultaneous co-translocation of both proteins to the membrane. Interestingly, the kinetics of release from the plasma membrane do not coincide, suggesting that the adaptation pathways controlling these two processes may be distinct. Deletion analysis revealed that only the N-terminal region (500 aa) of PI3K was required for the translocation. Translocation of N-terminal region was observed in *pi3k* null cells and in cells treated with LY294001, a PI3K inhibitor, suggesting translocation of PI3K does not require PI3K activity. The N-terminal region may have a binding partner for localization, since mammalian PI3Ks (α, β and γ) contain a regulatory subunit binding site on their N-terminal region. We found two *Dictyostelium* ORFs that show some homology to p101, the regulatory subunit of PI3Kγ, suggesting that such PI3K subunits may exist in *Dictyostelium*.

Cell polarization of phosphoinositides takes place at the leading edge, while initial establishment of a leading edge might involve local down-regulation of such a phosphatase activity. We show that the phosphoinositide 3' phosphatase PTEN, best known as a tumor suppressor, attenuates downstream PI3K signaling (e.g. PKB/Akt activation) and that its localization during chemotaxis and its redistribution in response to cAMP are consistent with a role in establishing and maintaining a phosphoinositide patch at the leading edge.

35. VISUALIZING CELL-CELL SIGNALING DURING DEVELOPMENT USING CRAC-GFP. Dirk Dormann, Carol Parent*, Peter Devreotes*, and Cornelis J. Weijer, School of Life Science, University of Dundee, Wellcome Trust Biocentre, Dow Street, Dundee, DD1 5EH, UK, *Department of Biological Chemistry, Johns Hopkins University, School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205.

During early aggregation, in mounds and in slugs cell movement is co-ordinated by propagating waves of extracellular cAMP. cAMP stimulation leads to the activation of PI3-Kinases and the generation of phosphatidyl inositol lipids, like PtdIns(3,4,5)P_3 (PIP3) in the plasma membrane. CRAC translocates transiently from the cytosol to the membrane by binding to PIP3 via its pleckstrin-homology (PH) domain. By measuring the membrane translocation of a CRAC-PH-domain-GFP fusion protein we have studied the in-vivo dynamics of cAMP relay during different stages of the life cycle. There exists a close correlation between dark field waves and CRAC-GFP translocation. The binding of CRAC-GFP to the membrane remains transient in late aggregation and in the mound stage, however the duration of membrane binding decreases during the course of development. In streams and in mounds the cells are polarized showing membrane association of CRAC-GFP only at their leading edges. The CRAC translocation can be seen to propagate from cell to cell. We have also analysed CRAC-PH-GFP translocation in slugs and in prespore and prestalk cells and we will discuss our findings.

10:40-12:00 SIGNALING AND DEVELOPMENT

36. SEARCHING FOR ***DICTYOSTELIUM*** CYCLASE FUNCTIONS. Marcel Meima[1], Elisa Alvarez-Curto[1], Irene Verkerke-Van Wijk[2], Jeroen Roelofs[3], Peter van Haastert[3] and Pauline Schaap[1], [1]School of Life Sciences, University of Dundee, UK, [2]Department of Biology, University of Leiden, Netherlands, [3]Department of Biochemistry, University of Groningen, Netherlands.

Extracellular cAMP induces prespore differentiation in ***Dictyostelium*** and is required for maintenance of the differentiated state (Wang et al., 1988). One of the three adenylyl cyclases ACA, ACB and ACG may therefore be expected to be active in the posterior prespore region of the slug. ACG mRNA was only detected at low levels in spores (Pitt et al., 1992); ACB is active in slugs, but its null mutant shows normal prespore differentiation (Meima and Schaap, 1999; Soderbom et al., 1999). We recently found that ACA is only expressed at the slug tip (Verkerke-van Wijk et al., 2001).

In search for a fourth adenylyl cyclase, we identified a homologue of the mammalian soluble adenylyl cyclase from the ***Dictyostelium*** genome databases. Subsequent disruption of the gene indicated that the ***Dictyostelium*** gene encoded a guanylyl cyclase (sGC), which most likely functions in chemotaxis (see abstract Roelofs et al.). With Mn^{2+}/GTP as substrate most of sGC activity is soluble, but with the more physiological Mg^{2+}/GTP the activity is detected in membranes and stimulated by GTPγS.

Since sGC was unlikely to play a role in prespore differentiation, we examined the regulation of ACG in more detail. Cells transformed with an ACG promoter-lacZ fusion already show ACG promoter activity in the prespore region of newly formed slugs. ACG specific antibodies confirm this localization and show that ACG protein colocalizes with prespore vesicles in prespore cells and becomes located at the periphery of the cell during spore maturation. Spore-specific genes, such as ***spiA*** are not expressed in the spore maturation deficient ACB null mutant (Soderbom et al., 1999). ACG is expressed in this mutant, which further emphasizes that ACG is a prespore rather than a spore gene with putative functions in the induction of prespore differentiation.

Wang, M., Van Driel, R., and Schaap, P. (1988). Cyclic AMP phosphodiesterase induces dedifferentiation of prespore cells in ***Dictyostelium discoideum*** slugs: evidence that cyclic AMP is the morphogenetic signal for prespore differentiation, Development 103, 611-618.

Pitt, G. S., Milona, N., Borleis, J., Lin, K. C., Reed, R. R., and Devreotes, P. N. (1992). Structurally distinct and stagespecific adenylyl cyclase genes play different roles in ***Dictyostelium*** development, Cell 69, 305-315.

Meima, M. E., and Schaap, P. (1999). Fingerprinting of adenylyl cyclase activities during ***Dictyostelium*** development indicates a dominant role for adenylyl cyclase B in terminal differentiation, Dev Biol 212, 182-190.

Soderbom, F., Anjard, C., Iranfar, N., Fuller, D., and Loomis, W. F. (1999). An adenylyl cyclase that functions during late development of ***Dictyostelium***, Development 126, 5463-5471.

Verkerke-van Wijk, I., Fukuzawa, M., Devreotes, P. N., and Schaap, P. (2001). Adenylyl cyclase a expression is tip-specific in ***Dictyostelium*** slugs and directs ***StatA*** nuclear translocation and ***CudA*** gene expression, Dev Biol 234, 151-60.

37. THE *DICTYOSTELIUM* HOMOLOGUE OF MAMMALIAN SOLUBLE ADENYLYL CYCLASE ENCODES A GUANYLYL CYCLASE. Jeroen Roelofs[1*], Marcel Meima[2*], Pauline Schaap[2], and Peter J.M. Van Haastert[1], [1]GBB, Department of Biochemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands, [2]Department of Biochemistry, University of Dundee, Dundee DD1 5EH, UK, *These authors contributed equally to this work.

A new *Dictyostelium* cyclase (sGC) was identified; the deduced protein sequence suggests that sGC consists of three segments. The N-terminal region (~1000 amino acids) shares no similarity with other proteins. The central segment of ~400 amino acids contains two cyclase domains. These cyclase domains and the C-terminal ~1400 amino acids are 35% similar to the corresponding cyclase domains and C-terminal region of mammalian soluble adenylyl cyclase (sAC). Gene disruption of sGC has no effect on adenylyl cyclase activity and results in more then ten-fold reduction of guanylyl cyclase activity. The *scg-* null mutants show reduced chemotactic sensitivity and aggregate poorly under stringent conditions. For further biochemical characterisation see abstract Meima et al..

Unexpectedly, orthologs of sGC and sAC are present in bacteria and vertebrates, but absent from *Drosophila, C. elegans, Arabidopsis* and *S. cerevisiae*. This suggests either horizontal gene transfer from bacteria to *Dictyostelium* and vertebrates, or multiple and independent gene losses in several lineages. We will present a phylogenetic analysis demonstrating that sGC and human sAC evolved from the bacterial orthologs by conventional vertical evolution, implying that the gene was lost independently from the plants, yeast, worms and flies lineages. The complete sequence of the human genome has resulted in the identification of 113 genes that supposedly are derived by horizontal gene transfer from bacteria to the vertebrates, because no orthologs are present in yeast, worm, fly and plant (Lander *et al*., 2001). Besides sGC, we identified another 15 unambiguous and 17 potential orthologs of this group of human genes in *Dictyostelium*, thereby questioning horizontal gene transfer and suggesting that gene loss in multiple organisms is not a rare event.

Lander, E.S., et al. (2001) Initial sequencing and analysis of the human genome. *Nature*, **409**, 860-921.

38. FILACTIN IS A VESICLE-ASSOCIATED PROTEIN THAT CONTAINS CONVENTIONAL ACTIN AS A DOMAIN. Michael Schleicher[1], Francisco Rivero-Crespo[2] , Lars Israel[1], Christian Schuberth[1], Ludwig Eichinger[2], Rolf Müller[2], and Angelika A. Noegel[2], [1]Inst. f. Zellbiologie der Ludwig-Maximilians-Universität München, [2]Inst. f. Biochemie I, Med. Fak., Universität zu Köln.

The structural backbone of the microfilament system is the actin molecule which has a molecular mass of about 42 kDa and belongs to the most conserved proteins in eukaryotic evolution. In recent years several actin-related proteins (ARPs) have been identified that are in general of similar size as cytoskeletal actin, but do not polymerize into long filaments and often form very specific complexes. Here we describe a yet unknown type of actin-related protein with a molecular mass of 105 kDa. The most intriguing feature is the presence of an essentially complete actin that shows higher homology to cytoskeletal actin than to actin-related proteins from the ARP family. The gene has a size of approximately 3.2 kb and is present in a single copy in the genome of *Dictyostelium discoideum*. The N-terminal 577 amino acids contain two repeats of about 100 amino acids each, which are similar to the Ig repeats in filamin. The C-terminal third of the protein harbors the actin-domain. Based on the homologies to filamin repeats in the N-terminal region and its similarity to cytoskeletal actin we refer to the protein as "filactin". The protein is present in soluble and particulate fractions. GFP-filactin shows colocalization with filamentous actin in moving fronts, in phagocytic cups, and in crown-like structures that appear during macropinocytosis. Filactin codistributes with nuclear actin paracrystals induced by DMSO treatment of cells, and is found in the triton-insoluble cytoskeleton upon stimulation with chemoattractant. In addition, monoclonal antibodies against the N- and C-terminal regions stain vesicular structures. We propose from these results that the unique N-terminus redistributes the whole protein to vesicles where it might function as a novel connection between a vesicle coat and the microfilamentous network via a conventional actin domain.

39. THE VACUOLAR H^+-ATPase IN ENDOCYTOSIS AND OSMOREGULATION OF *DICTYOSTELIUM* CELLS. Margaret Clarke, Quyen Arana, and Tongyao Liu, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

All eukaryotic cells contain a highly conserved multi-subunit enzyme, the vacuolar proton pump or V-ATPase, a rotary motor that transports protons across membranes at the expense of ATP. The V-ATPase serves many functions, among them energizing transport and acidifying intracellular organelles. In *Dictyostelium*, a single isoform of this enzyme is found in membranes of the contractile vacuole (CV) system and the endo/lysosomal system. We have created a mutant of *Dictyostelium* in which expression of the 100-kDa transmembrane V-ATPase subunit, VatM, is conditional upon food source. Under restrictive conditions (growth on bacteria), VatM levels drop, the number of proton pumps declines to about one-third of normal, and VatA, a peripheral V-ATPase subunit, is overproduced and mislocalized. The mutant cells remain capable of osmoregulation, but they grow slowly and their endosomal transit time increases two to three-fold. We have transformed wild type and mutant cells with a plasmid expressing a fusion of VatM with GFP. The fusion protein is correctly targeted to CV and endosomal membranes, and, in mutant cells, it restores proper localization of VatA. Proteolytic digestion of isolated endosomes has shown that the GFP tag, which is associated with the carboxy-terminus of VatM, lies on the cytosolic side of the endosomal membrane. With VatM-GFP, we are visualizing the dynamics of the CV system and monitoring the trafficking of proton pumps in living cells. Brightest labeling is observed in the vacuolar and reticular elements of the CV system. The extensive reticular network of the CV system lies mostly on the cytoplasmic face of the plasma membrane that is attached to the substratum; reticular elements are incorporated into vacuoles as they swell. Phagosomes and endosomes, found higher in the cell, are also labeled with VatM-GFP; these organelles can be identified by feeding the cells yeast particles or fluid phase markers such as TRITC-Dextran. Pulse-chase experiments have shown that phagosomal membranes acquire VatM-GFP within a few minutes after particle uptake and that membranes of late endosomes and phagosomes are devoid of GFP, suggesting retrieval of proton pumps. The timing of these events is under study.

Live cell imaging was performed with the assistance of John Heuser for contractile vacuole dynamics, and in collaboration with Guenther Gerisch and Jana Koehler for endocytosis and phagocytosis. These studies are supported by a grant from the National Science Foundation.

19:00-20:20 GENES

40. CONSTITUTIVELY ACTIVE G PROTEIN-COUPLED RECEPTOR MUTANTS DOMINANTLY BLOCK *DICTYOSTELIUM* DEVELOPMENT.
Minghang Zhang and Dale Hereld, Department of Microbiology and Molecular Genetics, Univ. of Texas Houston Health Science Center, Houston, TX 77030, USA.

G protein-coupled receptors including cAMP receptor 1 (cAR1) play important roles in the multicellular development of *Dictyostelium*. cAR1 mediates chemotaxis to cAMP and plays essential roles in aggregation. Upon binding to cAMP, cAR1 activates the heterotrimeric G protein $G\alpha_2\beta\gamma$, which in turn activates a variety of downstream effectors including adenylyl cyclase (AC). Many of these responses, including the activation of AC, are only transient. It has been suggested that cAR1, upon binding cAMP, sends out an excitatory signal which activates AC, as well as a subsequent inhibitory process (termed adaptation) which terminates AC activation. Prior studies [Pupillo *et al*. (1992) MBC 3:1229] suggest that adaptation is mediated by a $G\alpha_2$-independent pathway that originates from cAR1 and acts at a downstream site to negatively regulate the activation of AC by G protein. Little else is known about the mechanism of adaptation.

Towards uncovering this unknown pathway, we undertook a genetic screen for dominant negative cAR1 mutants. We randomly mutagenized the transmembrane region of cAR1, expressed the library of cAR1 mutants in wild-type cells, and screened for mutants that impair aggregation and development. Four mutants, named dn1-4, have been identified through this screen. All of them are defective in aggregation. Interestingly, all of these mutants are phosphorylated in the absence of cAMP, suggesting that the mutations cause the receptors to adopt a conformation resembling the ligand-bound state which can be recognized by the receptor kinase. Accordingly, these mutants were found to have an increased affinity for their ligand, which is characteristic of constitutively active G protein-coupled receptors. The extent to which adenylyl cyclase can be activated by GTPγS is reduced in mutant cell lysates, suggesting that this pathway is adapted to some extent in these cells. Taken together, our results suggest that all four dominant negative receptor mutants are constitutively active and block development by persistently causing adaptation of the adenylyl cyclase pathway. Sequence analysis revealed that most of the mutations are clustered at the cytoplasmic end of the third transmembrane helix, indicating that this region is important for receptor activation. A screen for genetic suppressors of the dominant negative mutants is currently underway and might reveal components of the adaptation pathway.

41. THE *DICTYOSTELIUM* RHO-GTPASE DISSOCIATION INHIBITOR REGULATES DIVERSE RAC-DEPENDENT SIGNALLING PATHWAYS. Heidrun Dislich(1), Ann-Kathrin Mayer(1), Nicola Adam(2), Daria Illenberger(2), and Francisco Rivero(1), Institut für Biochemie I, Medizinische Fakultät, Universität zu Köln, Joseph-Stelzmann-Str. 52, 50931 Köln, Germany (1) and Department of Pharmacology and Toxicology, Universität Ulm, Albert-Einstein-Allee 11, D-89081 Ulm, Germany (2).

Small GTPases of the Rho family of Ras-related proteins are molecular switches involved in the reorganization of the actin cytoskeleton in a variety of organisms. Up to 15 genes coding for Rho related proteins have been identified in *Dictyostelium discoideum*. Most of these Rho proteins are largly uncharacterized. To better understand the role of signal transduction cascades involving Rho GTPases in cytoskeleton-dependent processes, we are currently characterizing a *Dictyostelium* Rho-GTPase dissociation inhibitor (RhoGDI). Using protein sequences of human RhoGDIs as query, we identified several EST clones encoding two *Dictyostelium* homologues. DdRhoGDI1 shares 35% identity to several known RhoGDIs from animal species. DdRhoGDI2 is more divergent (22% identity) and lacks the N-terminal regulatory arm characteristic of RhoGDI proteins. Both are cytosolic proteins and do not display any particular relocalization upon reorganization of the actin cytoskeleton.

To gain insight into the function of DdRhoGDI1, we generated a knockout strain using homologous recombination with a vector carrying a blasticidin resistance cassette. Cells deficient in DdRhoGDI1 are multinucleate, grow slowly in axenic medium and display a pinocytosis defect, but rates of phagocytosis are unaffected. Giant multinucleate cells present large vacuoles that are continuous with the contractile vacuole system. The actin polymerization response upon stimulation with cAMP was reduced in the mutant cells.

Reintroduction of DdRhoGDI1 or complementation with mammalian RhoGDI, but not with mammalian LyGDI, compensates the cytokinesis and other defects. Using a two-hybrid approach we identified Rac1a/b/c, RacB, RacC and RacE as interacting partners for DdRhoGDI1, and confirmed this interaction in an in vitro translocation assay. Our results indicate that RhoGDIs play a pivotal role in the regulation of diverse signal transduction cascades in which Rho GTPases are involved.

42. VALPROIC ACID INHIBITS INOSITOL TRISPHOSPHATE SIGNALLING DURING *DICTYOSTELIUM* DEVELOPMENT. Robin S.B. Williams, W. Jonathan Ryves, and Adrian J. Harwood, MRC Laboratory for Molecular Cell Biology, University College London, Gower St, London, WC1E6BT, UK.

Valproic acid (VPA) is a short chain fatty acid that is used in the teatment of epilepsy and manic depression. It has been reported to inhibit GSK-3 and have teratogenic effects on *Dictyostelium* (Tillner et al 1998). We have re-examined the action of VPA on *Dictyostelium* development. VPA inhibits aggregation, in a similar manner to that observed for lithium. In contrast to lithium treatment, however, VPA treatment does not cause the pattern formation effects seen with a mutant that lacks glycogen synthase kinase (GSK-A). Consistent with this observation, we find that VPA inhibition of GSK–A is only seen at concentrations 200 times greater than the biological effects. We have previously shown that lithium affects aggregation through depletion of inositol trisphosphate (IP_3). We have now established that VPA also alters IP_3 levels and that LisA, a lithium-resistant mutant with elevated IP_3, is cross resistant to VPA during aggregation.

These results provide the first mechanism for VPA action and, as both lithium and VPA are anti-manic drugs, implicates IP_3 signalling as the therapeutic target for the treatment of manic depression.

Tillner, J., Nau, H., Winckler, T., and Dingermann, T. (1998). Evaluation of the Teratogenic Potential of Valproic Acid Analogues in Transgenic *Dictyostelium discoideum* Strains. *Toxicology in Vitro* **12,** 463-469.

43. CONTROL OF *DICTYOSTELIUM* DEVELOPMENT BY A TRAP1 HOMOLOGUE. Tsuyoshi Morita, Kenji Saitoh, Aiko Amagai, and Yasuo Maeda, Biological Institute, Graduate School of Life Sciences, Tohoku University, Aoba, Sendai 980-8578, Japan.

Dd-TRAP1 is a homologue of TRAP1 (tumor necrosis factor receptor-associated protein 1) identified by the yeast two hybrid system as a human protein that binds to the intracellular domain of the type1 TNF receptor. TRAP1 has a putative mitochondiral localization sequence and shows significant homology to members of heat shock protein 90 (HSP90) family. Although TRAP1 is localized to mitochondria in several cell lines, Dd-TRAP1 is mainly located in the cell membrane/cortex of *Dictyostelium* cells at the vegetative growth phase. Following starvation, however, Dd-TRAP1 translocates to cytoplasmic organelles that are probably mitochondria. Dd-TRAP1-overexpressing cells ($TRAP1^{OE}$) became prematurely adhesive after starvation, and this may result from the precocious expression of the cell adhesion molecule contact sites A (CsA). Surprisingly, however, a major effect of Dd-TRAP1 overexpression was to suppress expressions of several differentiation-associated genes such as *car1* and *dia3* during development, thus resulting in impaired cAMP signaling and morphogenesis. In this connection, $TRAP1^{OE}$ cells also exhibited the precocious and enhanced expression of *dia1* gene, which is specifically expressed in response to the growth/differentiation transition (GDT) and suppresses the GDT coupling with reduced expressions of early genes (*car1, aca, mybB*). Accordingly, it is likely that the delayed differentiation as observed in $TRAP1^{OE}$ cells may be closely related to the overproduction of DIA1 protein.

20:40-22:00 REGULATING DEVELOPMENT

44. EVIDENCE THAT THE DNA REPLICATION PROTEIN RPA IS A REGULATOR OF ONE OF THE CONTROL ELEMENTS WITHIN THE cudA PROMOTER. Christopher Sugden, Masashi Fukuzawa, and Jeff Williams, University of Dundee, MSI/WTB Complex, Dow Street, Dundee DD1 5EH.

The cudA gene has a complex pattern of gene expression at the slug stage. It is expressed in the prestalk cells that comprise the extreme slug tip and also in the prespore cells. Expression in tip cells is positively regulated by Dd-STATa but expression in prespore cells is under a more complex form of control; one region of the promoter has the potential to direct expression in all slug cells but a negatively acting promoter element restricts its actual activity to the prespore cells (1). The group of Adrian Tsang has shown that the promoter of the small subunit of the ribonucleotide reductase gene (rnrB) displays a similar form of control (2) and, based on this similarity, we previously noted an apparent homology between the repressor elements in the cudA and rnrB promoters (1). We have now purified a protein that specifically binds to the cudA prespore repressor element. Mass spectrometric analysis shows that one component of the purified protein complex is the 70Kda subunit of RPA. The RPA protein was initially identified because of its role in the initiation of DNA replication but recent studies have revealed a role in regulating eukaryotic gene transcription. The genes that are regulated are involved with DNA repair and DNA metabolism and one of the yeast genes that is regulated is rnr2, the ribonucleotide reductase gene. Also, Charles Rutherford has shown that *Dictyostelium* RPA binds to a promoter region of the Dictyostelium glycogen phosphorylase gene (3). In support of a role for RPA we find that the promoter elements in genes regulated by yeast and mammalian RPA show homology to the cudA and rnrB regulatory elements. We are now attempting to obtain more direct evidence for the in vivo binding of RPA to the cudA regulatory element using molecular genetic approaches.

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45. RAP1 IS ESSENTIAL FOR *DICTYOSTELIUM DISCOIDEUM* VIABILITY AND IS ACTIVATED IN RESPONSE TO OSMOTIC STRESS**.** Rujun Kang, Hermia Ip, George B. Spiegelman, and Gerald Weeks, Department of Microbiology and Immunology, University of British Columbia, 300 - 6174 University Blvd., Vancouver, BC, V6T 1Z3, Canada.

Dictyostelium encodes a single *rapA* gene encoding the Rap1 monomeric G protein. Attempts at generating *rapA* null cells were unsuccessful, suggesting the possibility that *rapA* is an essential gene. The expression of antisense *rapA* from the folate repressible discoidin promoter was therefore tried as a method to ablate *rapA* gene function. In transformants carrying a *rapA* antisense construct, Rap1 levels gradually decreased following folate removal, and this was accompanied by a decrease in growth rate and cell viability, a result is consistent with the idea that *rapA* is an essential gene. The Rap1 depleted cells were smaller and more rounded than wild type cells, and exhibited reduced viability in response to osmotic shock. The accumulation of cGMP in response to osmotic shock was reduced in the *rapA* antisense transformants and enhanced in cells over expressing the constitutively activated Rap1(G12V) protein, suggesting that Rap1 is important for the regulation of guanylyl cyclase. In addition, Rap1 was activated in response to osmotic shock with similar initial kinetics to that observed for the accumulation of cGMP. These results suggest a direct role for Rap1 in the activation of guanylyl cyclase during osmoregulation.

46. PHAGOCYTOSIS MUTANTS IN *DICTYOSTELIUM*. Mohammed Benghezal, Sophie Cornillon, Leigh Gebbie, Laurence Zulianello, Anna Marchetti, and Pierre Cosson, Université de Genève, Centre Médical Universitaire, Département de Morphologie, 1 rue Michel Servet, CH-1211 Genève 4, Switzerland.

To identify the molecular mechanisms involved in phagocytosis, we used a random insertion mutagenesis approach to isolate *Dictyostelium discoideum* mutants defective in phagocytosis. Characterisation of these initial four mutants and the affected genes is currently underway. From preliminary results it appears that all of the mutants exhibit similar selective defects in their ability to phagocytose different phagocytic substrates due to a defect in cell adhesion. To date most progress has been made in characterising the phg1 mutant which shows a 90% defect in phagocytosis of latex beads. The affected gene encodes a polytopic membrane protein with an N-terminal lumenal domain and nine potential transmembrane segments which may act as a cell surface receptor. Interestingly, PHG1 homologues have been identified in many species. Although functions have not be assigned to any of these PHG1-like genes, the identification of homologues in non-phagocytic cells such as yeast suggests that the role of the *Dictyostelium* PHG1 protein may not be strictly limited to phagocytosis. The latest results from studies on phg1 and other phagocytosis mutants will be presented and discussed.

47. A “TOLERANCE” RANGE IN CELL TYPE PROPORTIONING. Ismael Ràfols[1], Harry K. MacWilliams[2], Aiko Amagai[2], Yasuo Maeda[2], and Yasuji Sawada[1], [1]Research Institute of Electrical Communication, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan, [2]Zoologisches Institut, Ludwig-Maximilians-Universität, Luisenstrasse 14, 80333 München, Germany, [3]Biological Institute, Graduate School of Science, Tohoku University, Aoba, Sendai 980-8578, Japan.

The regulation of the proportion of prestalk and prespore cells in *Dictyostelium discoideum* slugs is often considered to be "almost perfect". However, by using a reporter construct containing a whole ecmAO promoter driving a labile beta-galactosidase, we have found that the regulation is intrinsically imprecise. The prestalk proportion decreases from an average of 24±5% in slugs of 10^3 cells to 10±3% when 10^5 cells are present. Regeneration experiments suggest that this difference is not due to a shift in the set-point proportion with size, as one might have expected; instead there appears to be a regulatory "tolerance zone" at all sizes. After amputation of the whole posterior region, transdifferentiation from prestalk to prestalk stops when the proportion reaches 28±2%, well above the initial value of 10±3%, while after anterior amputation the transdifferentiation ends around 10%. Moreover, no regulation at all occurs after partial amputation of the prespore region. Any proportion seems to be stable between a 10% lower threshold and 30% upper threshold.

To explain this finding we present a model based on: (i) a global negative feedback for the regulation of proportion. (ii) a cell-autonomous positive feedback represented by a hysteresis-like behaviour (bistability of cell type). This assumption guarantees the robustness of the differentiation. The regulated proportion is indeed found to span over a range of values as a consequence of the bistability. We conclude that the model illustrates a general conflict between the precision of proportion regulation and the robustness of the differentiation of cell types.

WEDNESDAY, JULY 25, 2001

09:00-10:20 GSK3

48. ANTAGONISTIC 7-TM RECEPTOR SIGNALING REGULATES CELL FATE DETERMINATION BY DIFFERENTIAL TYROSINE PHOSPHORYLATION AND ACTIVATION OF GSK3. Leung Kim (1), Jingchun Liu (1), and Alan R. Kimmel (1) LCDB/NIDDK, National Institutes of Health, Bethesda, MD 20892, USA.

A paradigm to establish cell fate patterns and axis formation in development involves morphogen signaling through 7-transmembrane (7-TM) receptors to regulate GSK3. Once considered a simple on-off switch subject only to inhibition, data now indicate that GSK3 regulation involves activating, as well as, inhibiting circuits, and the differential formation of multi-protein complexes. We have previously described the regulation of anterior-posterior axis formation in *Dictyostelium* by the antagonistic actions of 7-TM cAMP receptors CAR3 and CAR4 on GSK3. GSK3 is required for posterior cell fates while it inhibits anterior differentiation. Stimulation of the high affinity CAR3 will activate tyrosine kinase ZAK1 that phosphorylates and consequently activates GSK3. In contrast, stimulation of the low affinity CAR4 has an antagonistic effect on GSK3 and cell patterns. We now show that CAR4 inhibition of GSK3 appears to depend on a loss of tyrosine phosphorylation. car4-nulls have a persistent increase in both tyrosine phosphorylation and activity of GSK3 compared to wild-type. However, ZAK1 is not persistently activated in car4-nulls, suggesting that inhibition of GSK3 tyrosine phosophorylation is mediated by a CAR4-activated Protein Tyrosine Phosphatase. To identify such additional components in the CAR/GSK3 signaling circuits, we have performed a series of suppressor screens and have isolated and are characterizing two new *Dictyostelium* genes that appear to function antagonistically in the CAR3/GSK3 activating pathway for posterior fate patterning. We are also mapping the tyrosine phosphorylation sites that regulate GSK3 activity. Phosphorylation of Y214 within the activation loop is absolutely required for activity in vitro and in vivo. Although the Y214F mutant of GSK3 lacks activity, it is still subject to developmentally-regulated tyrosine phosphorylation. Therefore, ZAK1-mediated phosphorylation at other tyrosine residues appears necessary for the CAR3-dependent activation of GSK3 during development.

49. THE INTERACTION BETWEEN GSKA AND AAR. Jon Reynolds, Juliet Coates, Trevor Dale*, and Adrian J. Harwood, MRC-Laboratory for Molecular Cell Biology, University College London, Gower St, WC1E 6BT, UK, *Institute of Cancer Research, 237 Fulham Rd., London SW3 6JB, UK.

The protein kinase GskA is required for patterning during *Dictyostelium* multicellular development and acts through a regulatory pathway that possesses both similarities to the Wnt signalling pathway and some novel features. Loss of GskA switches cell fate from prespore to the pstB class of prestalk cells. Aardvark (Aar) is a second *Dictyostelium* protein in common to the Wnt pathway. This β-catenin homologue is both a structural component of actin associated cell junctions and acts downstream of GSK-3 to regulate prespore gene expression.

We have constructed an "activated" GskA protein which causes both over-expression of the prespore marker PsA, and aberrant cell shape and motility. The same effect is also seen when Aar is over-expressed. These observations provide further evidence that GskA acts positively on Aar during *Dictyosteliuim* development, and raises questions about other Aar functions.

50. DIF FUNCTIONS AS A REPRESSOR OF THE NUCLEAR EXPORT OF Dd-STATC AND MAY ACT VIA A GSK-3 SIGNALLING PATHWAY. Tsuyoshi Araki, Tomoaki Abe, Masashi Fukuzawa, Christina Schilde, Leung Kim*, Adrian Harwood+, Alan Kimmel*, and Jeff Williams, University of Dundee, MSI/WTB Complex, Dow Street, Dundee DD1 5EH, UK, *NIH/NIDDK, Laboratory of Cell and Developmental Biology, Building 6, Room B1-12, Bethesda, MD 20892, USA, +MRC Laboratory for Molecular Cell Biology, University College, Gower Street, London WC1E 6BT, UK

The *Dictyostelium* STAT protein, Dd-STATc, is tyrosine phosphorylated and translocates to the nucleus in response to DIF, a chlorinated hexaphenone produced by the developing cells. Photobleaching analysis of a Dd-STATc:GFP fusion protein shows that DIF functions to inhibit export of Dd-STATc from the nucleus. Furthermore, deletion of a 50 amino acid, centrally located region of Dd-STATc (region A) causes the protein to accumulate in the nucleus constitutively. Again, nuclear accumulation occurs because the mutant protein fails to be exported from the nucleus. Furthermore, this region acts as a weak, leptomycin B sensitive export signal when coupled to GFP. In combination these observations suggest that conditions within the cell are permissive for the nuclear import of Dd-STATc and that DIF induces the nuclear accumulation of Dd-STATc by repressing its nuclear export. There is a second short region near the N terminus (region B) that, when deleted, also results in constitutive nuclear accumulation. A peptide with the sequence of this region serves as an in vitro substrate for GSK-3 and so we determined the effect of genetically modifying the GSK-3 signalling pathway. Mutations in cAR3, ZAK and GSK-3 inhibit the DIF induced nuclear translocation of Dd-STATc, while a mutant form of GSK-3 that is constitutively active causes elevated basal nuclear accumulation of Dd-STATc. We are currently testing a model for DIF action whereby interaction of regions A and B creates a functional nuclear export signal that is disrupted when a DIF/GSK-3 signalling pathway is activated.

51. DIF-1 AND THE PROPORTIONING MECHANISM FOR PRESTALK AND PRESPORE CELLS. Robert Kay and Christopher Thompson, MRC Laboratory of Molecular Biology, Hills Rd, Cambridge, CB2 2QH, England.

We have performed two experiments that bear on the mechanism of cell type proportioning in *Dictyostelium*.

It has been shown by many people that prestalk and prespore cells first appear intermingled with each other at the mound stage of development, suggesting that their differentiation cannot be governed by a global mechanism of positional information. However prestalk-A cells are a potential exception, as they first become detectable at the periphery of the mound. A difficulty with all these marker studies is that cells move rapidly in the mound and so prestalk and prespore cells will have moved far from their place of origin by the time their marker gene becomes detectable. We have found that mound cells can be completely paralyzed by latrunculin treatment, yet cell differentiation continues on its normal schedule. In these conditions of no cell movement, both prestalk-A and prestalk-O cells differentiate at random in structures blocked at the early mound/stream stage. Thus prestalk cells can differentiate without positional information and it seems unlikely that gradients of DIF-1 (or any other prestalk-inducing signal) need to be generated in the mound.

DIF-1 has been shown genetically to be the inducer of prestalk-O cell differentiation in normal development. Our working hypothesis is that DIF-1 levels in the mound govern the ratio of prespore to prestalk-O cells that differentiate and we have therefore investigated which cells make DIF-1. Several lines of evidence indicate that DIF-1 is made by prespore cells and hence that prespore cells cross-induce the differentiation of prestalk-O cells. This gives a regulatory loop which will tend to fix the prespore/prestalk-O ratio.

10:40-12:00 SIGNALS

52. NEW COMPONENTS OF THE COUNTING FACTOR. Debra A. Brock and Richard H. Gomer, Howard Hughes Medical Institute and Department of Biochemistry and Cell Biology, Rice University, Houston, Texas, 77005-1892, USA.

The mechanisms that determine the size of a group of cells are mostly unknown. We previously found that disrupting the *smlA* gene caused aggregation streams to break into small groups and subsequently develop into very tiny fruiting bodies due to oversecretion of an unknown factor. We partially purified this "counting factor," and found that it behaves as a complex of polypeptides. One of the polypeptides is a novel protein we named countin. When the *countin* gene was disrupted, the developing cells aggregated into large, non-breaking streams resulting in the formation of abnormally large aggregates and fruiting bodies. This supported the hypothesis that "counting factor" was involved in a size-sensing process.

We have partially sequenced and cloned eleven additional candidate components of the partially purified "counting factor" complex. We have identified the complete sequence for two of these genes, *cf50* and *cf45-1*, and found they are 30% identical to lysozyme, contain distinctive serine-glycine motifs, and are 64% identical to each other. Transformants with a disrupted *cf50* or *cf45-1* gene, like *countin*$^-$ cells, have abnormally large aggregates and fruiting bodies indicating involvement of these proteins in the counting factor complex. Addition of *smlA*$^-$ or WT conditioned medium decreases the *cf50*$^-$ and *cf45-1*$^-$ group size, and adding recombinant cf50 protein to developing *cf50*$^-$ cells rescues their phenotype. A wide variety of abnormalities seen in aggregating *countin*$^-$ cells (such as high cell-cell adhesion and low motility) are also observed in the *cf50*$^-$ and *cf45-1*$^-$ cells. Although the *countin*$^-$, *cf50*$^-$, and *cf45-1*$^-$ fruiting bodies are very large compared to WT, the *cf50*$^-$ and *cf45-1*$^-$ appear more aberrant. Aggregates are irregularly shaped, slug/finger length can be amazingly extended, and some fingers appear to fruit horizontally. Antibodies have been raised against unique regions of cf50 and cf45-l. Western blot analysis of conditioned medium sieve column fractions showed that the cf50 protein is present in the same fraction as the 450 KD counting factor complex. In the absence of cf50, countin is degraded in the conditioned medium, suggesting that the function of cf50 in the counting factor complex may be to protect countin from degradation.

53. POSSIBLE NEW COMPONENTS OF A CELL NUMBER COUNTING SIGNAL TRANSDUCTION PATHWAY. Wonhee Jang*, Tong Gao[+] and Richard H. Gomer*,[+]
[+]Howard Hughes Medical Institute and *Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77005-1892, USA.

It is unclear how an organism can regulate the size of its components during development. ***Dictyostelium*** cells form relatively evenly sized groups of about $2x10^4$ cells during aggregation. We have two transformants with defects in a size determination mechanism. One (***smlA***$^-$) oversecretes a diffusible factor known as counting factor (CF), and forms very small groups. The other (***countin***$^-$) does not express one of the components of CF, and forms large groups. Other workers in the lab have found that CF increases motility and decreases adhesion. Computer simulations indicated that this then allows CF to regulate group size. To identify additional components of size determination pathways, ***countin***$^-$ cells were subjected to REMI mutagenesis to produce second-site enhancers and suppressors. One of the enhancers (***icdh***$^-$) formed fruiting bodies which were larger than those of ***countin***$^-$. This was successfully recapitulated in ***countin***$^-$ and Ax2. ***icdh***$^-$ has an insertion in the promoter region of one of 3 isocitrate dehydrogenase genes. The predicted amino acid sequence encoded by the disrupted gene shares over 60% homology to the NAD+ dependent isocitrate dehydrogenase alpha subunit in ***C.elegans***, rats and humans. To characterize ***icdh***$^-$, synergy experiments, motility assays, and adhesion assays were performed. Synergy experiments showed that this mutant is resistant to CF. In addition, the transformant has a higher cell-cell adhesion and a lower motility compared to wild-type cells. Citrate is known to negatively regulate glycolysis, and previous work done by Garrod and Ashworth (1972) showed that growth in the presence of high glucose resulted in bigger fruiting bodies. We also have found that aldose reductase is regulated by CF, and another successfully recapitulated second-site enhancer of ***countin***$^-$ has a disruption of a gene encoding a protein with a sugar transporter motif. Therefore the effect of glucose in various cell lines was studied. Addition of 1 mM glucose at 1, 2 or 6 hours of starvation increased the group size in Ax2 and ***smlA***$^-$, however, other sugars and glucose analogues such as mannose, 3-O-methylglucose and pyruvate did not have any effect. During development, ***smlA***$^-$ cells had lower levels of internal glucose than Ax2, while ***countin***$^-$ had higher levels. Addition of recombinant countin decreased the internal glucose level in Ax2 cells. In ***smlA***$^-$ cells, glucose increased adhesion during the first 4 hours of starvation. Sugars and glucose analogues which did not affect group size did not alter adhesion in ***smlA***$^-$ cells. Motility assays showed reduced motility in wild-type cells when glucose was added after starvation. These results indicate that glucose is a possible downstream component in the CF signaling pathway.

54. AN AUTOPHAGY GENE, *Ddapg*6, IS REQUIRED FOR *DICTYOSTELIUM* DEVELOPMENT. Grant Otto[1], Hubert Hilbi[2], Herbert L. Ennis[1], Howard Shuman[2], and Richard H. Kessin[1], [1]Department of Anatomy and Cell Biology, [2]Department of Microbiology, Columbia University, 630 West 168th St., New York, NY 10032, USA.

When cells starve they initiate a program called autophagy, which serves to recycle cellular components. Bulk cytoplasm, and even organelles, are sequestered in double-membraned vesicles called autophagosomes, which fuse with lysosomes, resulting in degradation of cargo by lysosomal enzymes. Autophagy is a universal process, occurring in yeast, plants, animals and *Dictyostelium*. Despite its universality, autophagy is a poorly understood process. A number of autophagy mutants have been isolated in yeast, and they define a set of genes required for this process. We have recovered the homologue of an autophagy gene, *Ddapg*6, in a screen for suppressors of a *cht*A/*fbx*A mutant.

The *Ddapg*6 mutant has defective development when grown on bacterial lawns and developed *in situ*, forming multi-tipped aggregates that struggle to complete development. Suprisingly, axenically-grown mutant cells develop normally on filters, making fruiting bodies with spore masses, yet they produces many fewer spores compared to wild-type fruiting bodies. If mutant cells are grown in HL/5 with reduced levels of glucose prior to development on filters, the phenotype once more resembles that seen for mutants on bacterial lawns. This result is consistent with a failure to supply the energy necessary for development. The exact function of *apg*6 in autophagy is unknown. The mammalian *apg*6 homologue, beclin1, is a *Bcl*2-interacting protein that plays a role in defence against neuronal viral infection in mice, and is mutated in many human breast, ovarian and prostrate cancers. We have identified many of the *Dictyostelium* homologues of the 14 known yeast autophagy genes. We are isolating these genes and mutating them to study autophagy.

The causative agent of Legionnaires' disease, the bacterium *Legionella pneumophila*, infects macrophages and is hypothesised to replicate in autophagosomes. To test this, we are examining *L. pneumophila* survival in *Dictyostelium* autophagy mutants.

THURSDAY, JULY 26, 2001

09:00-10:20 LIFE AND DEATH

55. CALCIUM SIGNALING AND *DICTYOSTELIUM* DEVELOPMENT: A ROLE FOR Dd-ALG-2/Dd-ALIX. Laurence Aubry, Michel Satre, and Gérard Klein, CEA-Grenoble, DBMS/BBSI, 17, rue des Martyrs, 38054 Grenoble Cedex, France.

During *Dictyostelium* development, prestalk cells undergo a programmed cell death process that shares some morphological features with mammalian apoptosis. However, the number of proteins known in higher Eukaryotes to control programmed cell death and for which homologues could be identified in *Dictyostelium* genome is fairly limited. We focused our attention on a Ca^{2+}-dependent pathway recently identified in higher Eukaryotes as part of the apoptotic machinery: the ALG-2/Alix pathway.

Two isoforms of the ALG-2 protein are present in *Dictyostelium*, Dd-ALG-2A and -2B, which are expressed throughout development. Recombinant proteins are able to form homodimers and heterodimers in the presence of Ca^{2+}. Ca^{2+} induces a conformational change of both protens and exposure of hydrophobic regions. Surprisingly, the double knock-out strain Dd-*alg2a*$^-$/Dd-*alg2b*$^-$ does not harbor a developmental phenotype.

The protein Alix is described as a direct partner of ALG-2. In *Dictyostelium*, deletion of Dd-*alx* leads to an abnormal development past aggregation with a lower expression of cell-type specific markers. Using surface plasmon resonance techniques, we showed that Dd-Alix is able to interact with Dd-ALG-2A in a Ca^{2+}-dependent manner, but not with Dd-ALG-2B.

Taken together, our results suggest that Dd-Alix is likely to act downstream of Dd-ALG-2A, with Dd-ALG-2A being a negative regulator of Dd-Alix. Dd-ALG-2B might regulate Dd-ALG-2A by modulating Dd-ALG-2A availability or its ability to interact with Dd-Alix.

56. GENETIC AND MORPHOLOGICAL ANALYSIS OF *DICTYOSTELIUM* CELL DEATH.
Jean-Pierre Levraud, Myriam Adam, Marie-Françoise Luciani, Vanessa Dubus-Bonnet, Céline Roisin-Bouffay, and Pierre Golstein, Centre d'Immunologie INSERM-CNRS de Marseille Luminy, 13288 Marseille Cedex 9, France.

Dictyostelium is one of the simplest organisms able of developmental cell death, which makes it an attractive model to study putatively simple and conserved mechanisms of programmed cell death. We investigate programmed cell death using the cell line HMX44 which is unable to produce DIF but is highly sensitive to the effect of exogenously added DIF-1, allowing efficient differentiation into stalk cells in vitro.

We are going on investigating the corresponding phenomenology. When subjected to DIF, HMX44 cells become committed to death (i.e. unable to regrow when put back into rich medium) long before their metabolic activity stops. This commitment also seems to precede visible stalk cell differentiation events (vacuolization). Time-lapse recordings have shown mobility and cell shape changes concomitant to this early cell death commitment.

To determine genes involved, we have performed REMI mutagenesis followed by repeated cycles of in vitro stalk cell differentiation followed by regrowth of surviving cells, in order to select cell death mutants . So far, this approach has yielded 16 mutants. Phenotypically, some of these mutants appear to be arrested at early steps of starvation-induced differentiation, while others differentiate into stalk cells yet are still able to regrow. The plasmid insertion of some of these mutants appear to be irrelevant to the cell death phenotype, when reconstituted by knockout experiments, probably because of spontaneous mutations acquired during the rather long selection process. Nevertheless, at least two new genes involved in the differentiation process have been identified by this approach. The first one, *dlrA*, codes for a Leucine-Rich Repeat bearing molecule, the first one described in *Dictyostelium*. *DlrA* mutants are arrested at a very early stage of development and are unable to aggregate. This phenotype is probably due to the lack of PKA-C mRNA, the expression of which appears to be controlled by *dlrA*. The second gene seems also to be involved in very early differentiation steps: knockouts are unable to aggregate, and, despite the fact that they are resistant to a DIF-induced cell death assay, they have been found to die more rapidly than wild-type cells when starved.

57. ON THE EVOLUTIONARY CONSERVATION OF THE CELL DEATH PATHWAYS: INVOLVEMENT OF A MITOCHONDRIAL APOPTOSIS INDUCING FACTOR AS NUCLEAR EFFECTOR DURING *DICTYOSTELIUM DISCOIDEUM* CELL DEATH. D. Arnoult, I. Tatischeff, J. Estaquier, M. Girard, F. Sureau, J. P. Tissier, A. Grodet, M. Dellinger, F. Traincard, A. Kahn, J.-C. Ameisen, and P. X Petit, Department of Genetics, Development and Molecular Pathology, Institut Cochin de Génétique Moléculaire, 24 rue du Faubourg Saint-Jacques, 75014 Paris, France, E-mail: pxpetit@icgm.cochin.inserm.fr.

Mitochondria play a pivotal role in apoptosis in multicellular organisms by releasing apoptogenic factors such as cytochrome *c*, that activate the caspases effector pathway, and apoptosis-inducing factor (AIF) that is involved in a caspase-independent cell death pathway. Here we report that cell death in the single-celled organism *Dictyostelium discoideum* involves early disruption of mitochondrial transmembrane potential ($\Delta\Psi m$), that precedes the induction of several apoptosis-like features, including exposure of the phosphatidyl residues at the external surface of the plasma membrane, an intense vacuolization, a fragmentation of DNA into large fragments, an autophagy and the release of apoptotic corpses that are engulfed by neighboring cells. Theses death processes are depicted both in photoinduced death and in DIF-induced developemental cell death of *Dictyostelium*.

We have cloned for the first time a *Dictyostelium* homologue of mammalian AIF, that is localized into mitochondria and is translocated from the mitochondria to the cytoplasm and the nucleus after the onset of cell death. Cytoplasmic extracts from dying *Dictyostelium* cells trigger the breakdown of isolated mammalian and *Dictyostelium* nuclei in a cell-free system, and this process is inhibited by a polyclonal antibody specific for DdAIF suggesting that DdAIF is involved in DNA degradation during *Dictyostelium* cell death.

Our findings indicate that the cell death pathway in *Dictyostelium* involves mitochondria and an AIF homologue, suggesting the evolutionary conservation of at least part of the cell death pathway in unicellular and multicellular organisms.

58. HIRANO BODIES IN *DICTYOSTELIUM*. Marcus Fechheimer, Andrew Maselli, Rich Davis, Sonbol Shahid-Salles, and Ruth Furukawa, Department of Cellular Biology, University of Georgia, Athens, GA 30602, USA.

Senile plaques, fibrillary tangles, Lewy Bodies, and Hirano bodies are abnormal structures consistently reported in brains of patients with neurodegenerative diseases. Aggregation and deposition of the proteins b-amyloid in plaques, tau in neurofibrillary tangles, and a-synuclein in Lewy bodies are presently under intensive investigation. By comparison, Hirano bodies have received little attention. Hirano Bodies are paracrystalline actin filament containing structures that have been reported repeatedly over a period of 30 years, but characterized almost exclusively by studies of fixed material using light and electron microscopy. Hirano bodies have been described in normal or pathological material associated with neurodegenerative disease, muscle disease, diabetes, alcoholism, and cancer.

We have developed the first cultured cell model for studies of Hirano bodies. Expression in *Dictyostelium* of altered forms of the *Dictyostelium* 34 kDa actin bundling protein that exhibit activated actin binding and calcium-insensitive actin filament cross-linking activity results in formation of paracrystalline inclusions. The structures are termed *Dictyostelium* Hirano bodies since they bear numerous similarities to mammalian Hirano bodies including: 1) elliptical cross-section; 2) juxtaposition of ordered and disordered regions; 3) filaments of 9-12 nm arranged in a broad range of spacings around 20 nm; 4) ultrastructural appearance dependent on the plane of section ranging from a herringbone pattern in oblique sections to paired sheets of filaments in transverse or longitudinal sections; 5) immunocytochemical staining reveals enrichment for actin filaments, cofilin, and a-actinin, but not tubulin. *Dictyostelium* cells with Hirano bodies grow, endocytose, and develop either normally, or slightly more slowly than wild type cells. These findings contradict the view in the literature that Hirano bodies are manifestations of cell death. Further, our findings suggest that formation of Hirano bodies is a general cellular response to or a consequence of aberrant function of the actin cytoskeleton. This model provides facile approaches to investigation of the biochemistry, dynamics, mechanism of formation of Hirano bodies, and relation to disease. Present efforts are directed at: 1) biochemical purification of Hirano bodies to identify major structural components; 2) elucidation of the mechanism of formation of Hirano bodies; 3) attempts to induce formation of Hirano bodies in cultured mammalian cells; 4) studies of pathological material to assess the predictive value of the *Dictyostelium* model.

This work was supported by grants from the National Science Foundation and the Alzheimer's Association.

10:40-12:00 NEW DIRECTIONS

59. CLONING OF A PUTATIVE TREHALASE GENE FROM ***DICTYOSTELIUM DISCOIDEUM.*** D.C. Mahadeo*, C. Jaekhl*, Y. Kishi+, M. Sameshima+, S.C. Kales*, and D.A. Cotter*, *Dept. of Biological Sciences, University of Windsor, Windsor, Ontario Canada, +Electron Microscopy Center, The Tokyo Institute of Medical Science, Tokyo Metropolitan Organization for Medical Research, Bunkyo-ku, Tokyo, Japan.

The major storage carbohydrate in dormant ***Dictyostelium*** spores is trehalose. It can make up to 5.5% of the dry weight of spores. Upon stimulation of germination, the trehalose stores are rapidly mobilized to provide energy for production of vegetative amoebae. The hydrolytic enzyme responsible for trehalose mobilization is trehalase. This enzyme is present during all stages of the life cycle of ***Dictyostelium***. During development, cells dump their lysosomal contents including trehalase into the surrounding media. At late culmination when stalk cells are dying, trehalase can also be detected within the extracellular matrix that bathes the spores within the sorus. The presence of this enzyme in the extracellular matrix represents a 20 fold purification compared to the enzyme present within the cells. The inherent stability of the enzyme through elevated temperatures, ionic conditions, denaturing detergents also aid in the ability to study and purify this enzyme. Previous studies have localized this protein to the lysosomal compartment within cells. This designates the ***Dictyostelium*** trehalase as a member of the acid trehalases, which are not regulated by phosphorylation and exhibit activity in a low pH buffer. Also, immunoprecipitation studies implicate N-linked glycosylation of this enzyme. Using size fractionation and affinity chromatography with Concanavalin A sepharose we have attempted to purify this enzyme to homogeneity for protein sequencing.

Another attempt to clone the trehalase has identified a small cDNA fragment that encodes a trehalase-like peptide. Using the sequence identified from the cDNA we have attempted to isolate the full length gene by anchor PCR from a cDNA library. Preliminary investigation into the ***Dictyostelium*** genome sequencing project has identified a full length putative trehalase including the cDNA fragment first identified. We have cloned this full length putative trehalase gene and characterized the genomic locus as well as expression studies. The predicted gene product from the 800 bp gene is 24 kDa with three putative N-linked glycosylation sites. The protein has an apparent molecular weight of 40 kDa when analyzed by partially denatured gel electrophoresis. This may represent dimer formation or the result of added hindrance due to the N-linked glycosylation.

60. RNAi IN *DICTYOSTELIUM*. Henrik Martens, Jindrich Novotny, Juergen Oberstrass, and Wolfgang Nellen, Abt. Genetik, Kassel University, Heinrich-Plett-Str. 40, 34132 Kassel, Germany.

We have established RNAi mediated gene silencing in *Dictyostelium* and show that expression of a transgene as well as of an endogenous gene can be efficiently reduced to undetectable levels. rrpA, one out of three RNA dependent RNA polymerases (RdRp) is required for the RNAi effect and the detection of sequence specific products of RNA interference (~23mers). ~23mers are not detected when only the RNAi expressing gene construct, but not the target gene is introduced into the cell. This suggests an RdRp dependent amplification mechanism which requires the target gene. Gene silencing by dsRNA depends on growth conditions and is developmentally regulated.

61. RNA INTERFERENCE IN *DICTYOSTELIUM*. Hideshi Otsuka[1], Emily R. Cogill[1], Chu-Yun Kuan[1], Robert P. Dottin[2], and Julian D. Gross[1], Department of Biochemistry, University of Oxford, Oxford, OX1 3QU, UK[1], Center for Gene Structure and Function, Hunter College, The City University of New York, New York, N.Y. 10021, USA[2].

RNA interference (RNAi) refers to sequence-specific silencing of a gene by homologous double-stranded RNA (dsRNA). It has been suggested that RNAi is a defence mechanism against virus infection. dsRNA is thought to be digested into ~22bp fragments that are used as "guide RNA's" to direct an RNase complex to homologous mRNA. RNA interference has been employed in functional genomic studies in several organisms, including *Neurospora*, *C.elegans*, *Drosophila* and *Arabidopsis*. In the case of *C. elegans,* dsRNA can be introduced by microinjection, by expression of constructs containing inverted repeat (IR) sequences or by feeding with bacteria that express dsRNA.

Gene silencing by antisense RNA has been shown to occur in *Dictyostelium* (Davies et al. 1996; Nellen and Sczakiel 1996), but RNA interference has not yet been reported. We have examined the effect of dsRNA by transforming wild type cells with constructs expressing inverted repeats of regions of the *acaA* or *mybB* genes. All three constructs examined, *acaA*-IR1, *acaA*-IR2 and *mybB*-IR1, rendered the host strain (Ax3) completely aggregation-minus. RNAi appears to be much more effective than antisense, as transformants expressing control *acaA* antisense or sense fragments developed normally. Since expression of inverted repeats can be placed under the control of inducible or cell-type specific or promoters, this method of gene-silencing may be useful for studying the function of essential genes, and for preferentially silencing genes in particular developmental cell types.

Davies, L., N.A. Farrar, M. Satre, R.P. Dottin, and J.D. Gross. 1996. Vacuolar H(+)-ATPase and weak base action in Dictyostelium. *Mol Microbiol* **22**: 119-26.

Nellen, W. and G. Sczakiel. 1996. In vitro and in vivo action of antisense RNA. *Molecular Biotechnology* **6**: 7-15.

62. COMPETITION BETWEEN CLONES IN CHIMERAS. David C. Queller, Angelo Fortunato, and Joan E. Strassmann, Department of Ecology and Evolutionary Biology MS-92, Rice University, P.O. Box 1892, Houston TX 77251-1892, USA.

Previous work in our lab has shown that different *D. discoideum* clones collected from the field will mix to form chimeric fruiting bodies, and that clones often contribute differently to stalk and spores in chimeras. Here we report on two further issues: the frequency of mixing in nature and the transitivity of competitive interactions.

The evolutionary importance of competition in chimeras depends on the clonal diversity in the field, particularly on the very small scale at which aggregation occurs. To study the population genetic structure of wild *D. discoideum*, we collected numerous small (6 mm across, mean less than 0.2 g) soil samples at Mountain Lake, Virginia, and reared out clones from all the viable *D. discoideum* cells. Most of the soil samples that yielded *D. discoideum* yielded multiple isolates. To establish whether they were the same clones or not, we genotyped these isolates at 5 highly variable microsatellite repeat loci. The genotypes showed that the majority of these samples included more than one clone. The soil samples were small enough to make it probable that co-occurring clones would mix if provided with growth conditions. The fact that clones frequently are in a position to mix in nature suggests that there may have been a long evolutionary history of competition in chimeras.

As a first step toward understanding competition in chimeras, we formed 50:50 chimeric mixtures of all 21 pairwise combinations of 7 field-collected clones. If there is a single mechanism regulating competition, such as DIF, then there should be a hierarchy, with the top clone dominating all, the second-best dominating all clones except the top one, and so on. When we measured success as a clone's percentage in spores, there was a hierarchy. However, when we measured success as percentage in the slug's prespore region minus its percentage in prestalk, there was no hierarchy. This suggests that success at getting into the prespore region rather than prespores involves multiple mechanisms. It also shows that considerable further competition occurs after the slug stage that we assayed.

POSTER SESSIONS

POSTER SESSION I, SUNDAY, JULY 22, 2001

1. IDENTIFICATION OF A NOVEL FAMILY OF RGS DOMAIN-CONTAINING PROTEINS IN *DICTYOSTELIUM.* Mousumi Goswami, Minghang Zhang, and Dale Hereld, Dept. of Microbiology & Molecular Genetics, Univ. of Texas Houston Health Science Center, Houston, TX 77030, USA.

Dictyostelium discoideum makes extensive use of G protein-mediated signaling throughout its starvation-induced developmental program. Nine G protein α subunits have been extensively analyzed and, for the most part, yield demonstrable phenotypes when their genes are either disrupted or over-expressed. Additional Gα genes are also being revealed by ongoing cDNA and genome sequencing efforts. This complement of Gα subunits, in association with Gβ and Gγ subunits, presumably transduce signals from specific seven-transmembrane-spanning receptors to appropriate cellular targets. The active state of the G protein, characterized by dissociation of the Gβγ dimer and exchange of Gα−bound GDP with GTP, is short-lived due to the Gα's intrinsic GTPase activity. In recent years, a family of so-called RGS proteins (regulators of G protein signaling) has been delineated which negatively regulate G protein function by accelerating GTP hydrolysis. Numerous examples from diverse species ranging from mammals to yeast have been described. Common to all is a ~120 amino acid RGS domain which binds active Gα subunits.

To determine if *Dictyostelium* possesses RGS proteins, we searched the databases of the *Dictyostelium* cDNA Project in Japan and the multinational Genome Sequencing Project and, thereby, identified a family of six RGS domain-containing proteins, designated RgsA-F. In an effort to define their functions, we have presently disrupted the genes encoding three of the putative RGS proteins (RgsA, D, and E). Among these is one (RgsA) whose expression is restricted to the aggregation stage and coincides with that of Gα2, which is critical for cAMP signaling at this stage. Elimination of an RGS protein is expected to result in increased activity of G proteins under its control and in other genetic systems, namely yeast and worms, has resulted in outward phenotypic effects. The independent disruption of RgsA, D, and E, however, produced no gross defect in growth or development. As a further test, each of the three RGS null cells lines was marked with actin15::LacZ so that their developmental fates could be followed in co-mixtures with wild-type cells. In each case, mutant cells were found evenly distributed throughout the resulting multicellular structures, indicating that loss of these RGS proteins does not compromise their ability to differentiate into the predominant developmental cell types. These findings suggest that the functions of RgsA, D, and E are either subtle or masked by redundant function. RGS over-expression experiments are now underway and might reveal function despite redundancy by attenuating the activity of the relevant G proteins.

2. NOVEL EXPRESSION PATTERN OF *DICTYOSTELIUM* GENES REVEALED BY *IN SITU* HYBRIDIZATION. Keiko Nishio, Masako Yokoyama, Masatsune Tsujioka, Hidekazu Kuwayama, and Mineko Maeda, Department of Biology, Graduate school of Science, Osaka University, Machikaneyama 1-16, Toyonaka, Osaka 560-0043, Japan, mmaeda@bio.sci.osaka-u.ac.jp.

One of the purposes of the *Dictyostelium* cDNA project is to catalog spatial expression patterns by *in situ* hybridization to get insight into the mechanism how dynamically and coordinately spatio-temporal gene expression is regulated by. Our previous *in situ* hybridization study revealed that spatial expression of myosin-function related genes is regulated in quite striking manner (M. Maeda *et al*., 2000). In addition to this, we analyzed the spatial expression patterns of the genes involved in cAMP response, membrane trafficking and lipid metabolism during morphogenesis. The genes encoding Ca2+-binding protein CBPs, CAFs or PEFs (penta EF-hand protein) were also analyzed. Based on such analyses, we identified four basic expression patterns at the first finger or slug stage and found one novel type of spatial expression pattern.

Currently ongoing *Dictyostelium* genome project will provide valuable upstream sequence information of the genes with particular expression pattern. Systematic analyses of the promoter region of these cataloged genes will contribute to identification of *cis*-elements and our understanding of gene expression network in *Dictyostelium*.

3. PEROXISOMAL β-OXIDATION IS ESSENTIAL FOR CYCLIC AMP-RELAY, CELL ADHESION AND MULTICELLULAR DEVELOPMENT OF *DICTYOSTELIUM*.

[1)]Satomi Matsuoka, [1)]Hidekazu Kuwayama, [2)]Masakazu Oyama, [3)]Tamao Saito, [1)]Daisuke Ikeno, and [1)]Mineko Maeda*, 1) Department of Biology, Graduate School of Science, Osaka University, Machikaneyama-cho 1-16, Toyonaka, Osaka 560-0043; 2) Department of Life Science, Faculty of Science, Himeji Institute of technology, Shosha 2107, Himeji, Hyogo 671-2201; 3)Division of Biological Sceinces, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan, mmaeda@bio.sci.osaka-u.ac.jp.

We reported previously that defect in *mfe2a* encoding *Dictyostelium* counterpart of human MFE2 which catalyzes peroxisomal β-oxidation exerted severe effects on both aggregation and multicellular development of bacterially grown *mfe2a* -null cells. However, when mixed with wild-type cells, GFP-labeled mutant cells formed chimeric fruiting bodies, in which the mutant cells differentiated into spores, stalk and anterior-like cells, indicating that such defects of the mutant are not cell-autonomous. Since the defects first appear in the aggregation stage, we presumed that such mutant phenotype might be caused by suppression of cAMP signaling. Thus, we tested this possibility by monitoring cAMP level after stimulation with exogenously added cAMP-analog, 2'-deoxy cAMP. Interestingly, the stimulant elicited the first rise in cAMP level in bacterially grown mutant, but never induced the second rise as observed in the wild type, suggesting that bacterially grown mutant can not generate cAMP oscillation. Consistently, a cell adhesion molecule, gp80, that is known to be cAMP pulse-inducible was not induced in the mutant, though the other cell adhesion molecule, gp24, was normally expressed. Thus, the development of the mutant might be rescued by the emission of cAMP pulses from the wild type. Such impaired cAMP signaling might be related to a decrease in the ratio of dienoic fatty acids (FAs) to monoenoic FAs in phospholipid fraction of the mutant. These results suggest that peroxisomal MFE2a is indispensable for the development of *Dictyostelium*, probably by optimizing cellular lipid constituents.

4. RELATION BETWEEN ABNORMAL LIPID METABOLISM AND DEVELOPMENTAL DEFECT OF *DICTYOSTELIUM* MUTANT LACKING PEROXISOMAL MULTIFUNCTIONAL ENZYME 2. Tamao Saito[1], Satomi Matsuoka[3], Naoki Morita[2], Hiroshi Ochiai[1], and Mineko Maeda[3], [1]Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan, [2]Research Institute of Biological Resources, National Institute of Advanced Industrial Science and Technology (AIST), Toyohira-ku, Sapporo 062-8517, Japan, [3]Department of Biology, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan.

Defect in peroxisomal multifunctional enzyme 2 (MFE2) which catalyses β-oxidation of both very long chain fatty acids and branched chain fatty acids causes a fatal disease known as Zellweger Syndrome (ZS) (van Grunsven et al. 1998). Although these fatty acids (FAs) are enormously accumulated as the plasma and cellular lipid constituents of the ZS patients, causal relationship between such abnormal lipid accumulation and ZS remains to be investigated. *Dictyostelium* might be a useful model organism to get some clues for this relation. We found that disruption of *Dictyostelium* gene homologous to human MFE2 caused developmental arrest at the loose mound stage when grown on bacteria for 6-8 hours and then allowed to develop on non-nutrient agar. Terminal cell differentiation into spores and stalk cells was also inhibited. When the mutant cells (mfe2-null) were grown for longer than 48 hours, they failed to enter the multicellular stage. On the other hand, when grown axenically, mfe2-null performed almost normal development. Biochemical analyses revealed that cholesterolesters and triacylglycerides, which exist at very low level in wild type cells, were greatly accumulated in bacterially grown but not in axenically grown mfe2-null cells. These lipids contained a large amount of cyclic fatty acids (CFAs) derived from bacteria, suggesting that CFAs are metabolized by peroxisomal β-oxidation but not by mitochondrial one. The ratio of CFAs to the total FAs gradually increased as feeding prolonged, which might be responsible for the abnormal development of mfe2-null. Similar developmental arrest was observed when mfe2-null cells were cultured in the presence of purified bacterial phosphatidylethanolamine with high CFAs contents in the axenic medium HL5. All these results strongly suggest that the abnormal accumulation of bacterial CFA-enriched lipids causes developmental arrest of mfe2-null. The ratio of dienoic FAs to monoenoic FAs was extremely low in bacterially grown mfe2-null compared to that of bacterially grown wild type, which was caused by the inhibition of Δ5 desaturase activity. The Northern analysis revealed that this inhibition did not occur at the transcriptional level. We conclude that the defect of MFE2 elicits abnormal accumulation of bacterial CFA-enriched lipids and then results in developmental arrest of mfe2-null probably by affecting membrane physiology.

5. LimC AND LimD: TWO NOVEL CYTOSKELETON-ASSOCIATED LIM PROTEINS OF *DICTYOSTELIUM DISCOIDEUM*. Taruna Khurana*[¶], Bharat Khurana*[‡], and Angelika A. Noegel[§], [¶]Laboratory of Cellular and Developmental Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892-2715, USA, [‡]Laboratory of Viral Diseases, NIAID, National Institutes of Health, Bethesda, MD 20892-2715, USA, [§]Institute of Biochemistry I, Medical Faculty, University of Cologne, Joseph-Stelzmann-Str. 52, D-50931 Cologne, Germany, *These authors contributed equally to this work.

LIM domain has been identified in diverse class of proteins in many different species as a conserved cysteine and histidine rich sequence motif. The LIM domain acts as a binding interface mediating specific protein-protein interactions, thereby acting as a crucial protein structure. We have identified two novel LIM proteins of *Dictysostelium discideum*, LimC and LimD. LimC is comprised of two LIM domains whereas LimD consists of single LIM domain. To study the cellular dynamics of both the proteins their GFP-tagged versions were used. Fluorescence studies reveal that GFP-LimC and GFP-LimD fusion proteins preferentially accumulate at the distinct areas of cell cortex where they co-localize with actin and transiently associate with cytoskeleton dependent dynamic structures like phagosomes, macropinosomes and pseudopods. Our in-vitro studies indicate that both LimC and LimD directly interact with actin. To assess the in-vivo functions of both the genes $LimC^-$, $LimD^-$ and $LimC^-/LimD^-$ mutants were generated. Under optimal growth conditions all the mutants show normal growth with mild cytokinesis defect, however, stress conditions (high temperature and high osmolarity) severely impaired their growth. In addition all the mutant strains are highly sensitive to acute osmotic shock indicating reduced cortical strength of the mutant strains. All the mutant strains exhibit a delay in aggregation when developed under a layer of starvation buffer. Moreover, the aggregation streams of the mutant strains often fractured along their length forming smaller aggregates, possibly because of a defect in cAMP signaling. Our results indicate that both LimC and LimD may act as adapter proteins coupling cortical cytoskeleton to intracellular signaling pathways.

6. THE INTERNAL PHOSPHODIESTERASE REGA AND PKA LIE IN A REGULATORY PATHWAY EMINATING FROM THE FRONT OF THE NATURAL CHEMOTACTIC WAVE AND ENDING IN THE SUPPRESSION OF LATERAL PSEUDOPOD FORMATION ESSENTIAL FOR CHEMOTAXIS. Paul J. Heid,* Deborah J. Wessels,* Hui Zhang,* Karla Daniels,* Adam Kuspa,[‡] William F. Loomis,[§] and David R. Soll*, *Dept. of Biological Sciences, University of Iowa, Iowa City, IA 52242, [‡]Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, [§]Dept. of Biology, University of California-San Diego, La Jolla, CA 92037.

It has been proposed that occupancy of the cAMP receptor cAR1 activates ERK2, which inhibits RegA, leading to a transient increase in internal cAMP. Previous studies suggested that the exclusive role of the REGA —| [cAMP] → PKA pathway was in the regulation of developmental gene expression. Under the assumption that this pathway plays a role in chemotaxis through the regulation of lateral pseudopod formation, and in light of the observation that *ERK2* plays a role in chemotaxis, we characterized *RegA* and *PkaR* mutants. In buffer, regA$^-$ cells were indistinguishable from wild type cells. However, regA$^-$ cells formed small aggregates on agar, suggestive of a defect in chemotaxis. In natural aggregation territories regA$^-$ cells did not exhibit normal cyclic behavior. In mixing experiments with a majority of wild type cells, regA$^-$ cells did not respond normally to natural waves of cAMP. To pin point the defect in chemotactic responsiveness, we analyzed single cell motility in a spatial gradient of cAMP and found that regA$^-$ cells were capable of relatively normal chemotaxis. We next analyzed the responsiveness of regA$^-$ cells to sequential temporal waves generated in the absence of spatial gradients. Although regA$^-$ cells exhibited normal velocity changes in the front, at the peak and in the back of simulated waves, the centroid tracks revealed unusual zigzagging in the front of the wave which disrupted persistent translocation. When cell perimeters were examined in temporal wave experiments, it was discovered that regA$^-$ cells formed lateral pseudopods 5 times more frequently than wild type cells in the front of waves. Behavior at the peak and in the back of waves was normal. An analysis of myosin II localization in RegA$^-$ cells revealed that in the front of the wave, myosin II was not restricted to the cortex as it was in wild type cells. These results demonstrated that this pathway functioned to suppress lateral pseudopod formation in the front of the natural wave in response to the increasing temporal gradient of cAMP. This leads to the tentative pathway: increased CAR1 occupancy → ERK1 —| REGA—| [cAMP] → PKA ⋯→ myosin II dephosphorylation → decreased lateral pseudopod formation. If REGA functions upstream of PKA, then disruption of PKA-R should result in the same behavioral defect exhibited by regA$^-$ cells. We, therefore, subjected pkaR$^-$ cells to the same analysis, and have shown that these cells exhibit the same defect as regA$^-$ cells in the suppression of lateral pseudopod formation in the front of simulated temporal and natural waves of cAMP, which supports the REGA—| [cAMP] → PKA portion of the pathway. These mutants also appear similar to RegA$^-$ cells in their behavior in mixing experiments.

7. A COMPUTATIONAL MODEL FOR CRAWLING IN ***ASCARIS SUUM*** SPERM. Dean C. Bottino[4,5], Alexander Mogilner[6], Thomas Roberts[7], and George Oster[2], [1] Physiome Sciences, 307 College Road East, Princeton, NJ 08540 (current contact address), [2] University of California, College of Natural Resources, Berkeley, CA 94720 (research done here), [3] University of California, Institute for Theoretical Dynamics, Davis, CA 95616, [4] Florida State University, Department of Biological Science, Tallahassee, FL 32306.

Despite recent advances in cell biology and the biophysics of the motile cell, we still do not have a complete picture of how animal cells move across surfaces. One reason for this is that a huge variety of molecular mechanisms are involved in locomotion, which leads to a multiplicity and redundancy in force generation machineries and regulatory pathways. The current research is aimed at dissecting the complex processes of motility into simpler phenomena that can be more easily analyzed. Nematode sperm discard their actin-based cytoskeleton and deploy an entirely new motility machinery based on a major sperm protein (MSP). These cells offer at least one advantage for investigating principles of cell crawling: they are remarkably simple and dedicated entirely to locomotion, yet their migrating behavior is essentially indistinguishable from that of actin-based cells.

We present a 2-D computational model of the ***Ascaris suum*** nematode sperm. The model has submodels for (i) polymerization, (ii) gel depolymerization and contraction, (iii) electrostatic adhesion to the substratum, and (iv) dynamics of the pH gradient that determines the zones of polymerization and de-swelling. Each of these submodels determines a local behavior that are described with PDEs, incorporated into a computational subroutine and assembled into the code that describes the global behavior of the crawling sperm. These simulations provide new insights into the basic mechanism of amoeboid cell motility.

8. THE BEHAVIORAL PHENOTYPE OF THE MYOSIN I MUTANTS MYOA$^-$, MYOB$^-$, AND MYOA$^-$/B$^-$ PRESENT A PARADOX IN INTERPRETATION. David L. Falk,[a] Deborah Wessels, [a] Margaret A. Titus,[b] and David R. Soll[a], [a] Department of Biology, University of Iowa, Iowa City, Iowa 52242; [b] Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, Minnesota 55455, USA.

The myosin Is represent a family that includes short tailed molecules, like myoA, which lack the proline-rich and SH3 domains, and long tailed molecules , like myoB, which contain both domains, and can bind Arp2/3. In our original analysis of myoA$^-$ and myoB$^-$, we found that both mutants exhibited a similar defect, a 2 to 3 fold increase in the frequency of lateral pseudopod formation during basic motile behavior in buffer. This resulted in an increase in turning and a related decrease in instantaneous velocity. The increase in frequency occurred almost exclusively for pseudopods formed on the substrate, hence the dramatic increase in turning. Recent analyses have demonstrated that myoA$^-$ and myoB$^-$ cells respond normally to the peak and back of simulated waves and natural waves of cAMP, losing polarity in the former phase and maintaining the depolarized state in the latter phase. However, myoA$^-$ and myoB$^-$ cells have trouble suppressing lateral pseudopod formation in the front of the wave, resulting in aberrant chemotaxis. The paradox arises when one considers how two quite different members of the same family provide the same mutant phenotype. This paradox is heightened when one considers the recent observation that they don't colocalize (myoB is in the pseudopod, while myoA is in the posterior cell cortex). The paradox is heightened even farther by the results of the characterization of the double mutant. One might expect the double mutant myoA$^-$/B$^-$ to be the same as the single mutants. In fact, the double mutant phenotype was extreme, including loss of polarity, a major depression in translocation, formation of wimpy pseudopods around the entire cell perimeter, and loss of chemotaxis in a spatial gradient a combined phenotype analogous to that of myosin II heavy chain and myosin II regulatory light chain null mutants. The myoA$^-$/B$^-$ double mutant, but not the single mutants, also exhibits a major decrease in cortical tension, just like the myosin II heavy chain null mutant, implicating myoA/myoB and myosin II in the genesis of cortical tension and the suppression of lateral pseudopods. But none of this really solves this paradox. Suggestions?

9. MODELING SPATIAL SENSING IN CHEMOTACTIC CELLS. Jane H. Kim[1], Chris Janetopoulos[2], Peter N. Devreotes[2], and Pablo A. Iglesias[1], [1] Department of Electrical and Computer Engineering, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD, 21218, USA, [2] Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, 725 N. Wolfe St., Baltimore, MD 21201, USA.

Chemotactic cells migrate directionally in response to concentration gradients of certain chemoattractants or chemorepellants. While moving up the concentration gradient, cells must demonstrate directional sensing and then localize the response to the side facing the gradient. Although some of the components have been isolated and studied during sensing, an understanding of the signaling transduction network involved in eukaryotic chemotaxis has yet to be fully understood. *Dictyostelium discoideum* is ideal for modeling eukaryotic chemotaxis because of its ability to demonstrate: (1) perfect adaptation to homogeneous changes in ligand concentration, and (2) polarization while motionless. Due to the complexity of the proposed problem, the use of the Virtual Cell interface provides an accommodating platform for further investigation and analysis. The Virtual Cell is a user interface that provides a general framework for the spatial modeling and simulation of cellular physiology. The ability of the Virtual Cell to specifically resolve spatial simulations via the PDE simulator facilitates the creation and analysis of physiological models in the context of complex cellular structure. It also allows the generation of specific simulations, the control and modeling of simulations, and data analysis. The goal of our current research is to use the tools of control engineering and dynamical systems theory to model the regulatory network controlling eukaryotic chemotaxis and then test what the model predicts in a living cell.

10. MYOSIN II IS REQUIRED FOR CELL MOTILITY IN A MECHANICALLY CONSTRAINED ENVIRONMENT. Gary Laevsky and David Knecht, University of Connecticut, Department of Molecular and Cell Biology, USA.

We have shown that mutants lacking Myosin II heavy chain (MHC-) cells are unable to enter wild type aggregation streams. We hypothesized that these mutant cells have insufficient cortical integrity to create force to push against a resisting medium (adhered wild-type cells). Cells lacking the essential light chain of the Myosin II (ELC-) lack motor function but are able to move normally in streams. We hypothesized that the cross-linking function of myosin, and not the motor activity, is sufficient to maintain cortical integrity in this situation. To refine these observations, we have now developed an under-agarose assay for vegetative amoebae undergoing folate chemotaxis. Cells exit the troughs, by flattening first their leading edge, and subsequently the entire cell body, and at the same time deforming the agarose upward. Increasing concentrations of agarose up to 2.5% decrease the ability of wild-type cells to exit the troughs and decrease the speed of the cells once they are under the agarose. MHC- cells can exit the troughs and move chemotactically when placed in 0.5% agarose. However, the cells have difficulty retracting their uropods, often leaving fragments behind and eventually cease movement a short distance from the trough edge. At higher concentrations of agarose, MHC- cells are unable to exit the troughs. Consistent with the chimeric streaming assay, mutants lacking ELC show no defect in UA motility. Confocal imaging of myosin-GFP has shown transient increases in myosin at the leading edge, as well as in the prominent “C-ring” in the posterior. We hypothesize that myosin II acts as a cross-linker providing structural support to the actin cytoskeleton allowing the cell to both apply and resist compression forces during motility. Surprisingly, mutants deficient in actin cross-linking proteins show no comparable defects in cell movement under agarose.

11. ARACHIDONIC ACID ATTRACTS AGGREGATION COMPETENT *DICTYOSTELIUM DISCOIDEUM* CELLS. Ralph Schaloske*, Dagmar Blaesius, and Daniel F. Lusche, University of Konstanz, Department of Biology 78457 Konstanz, FRG, *present adress University of California, San Diego, Department of Chemistry and Biochemistry, La Jolla, CA 92093-0601, USA.

Cells of *Dictyostelium discoideum* are known to chemotax to folate, pterins, cAMP and lysophosphatidic acid (LPA). cAMP is the natural signaling molecule secreted by the cells that initiates and maintains aggregation. In the absence of cell surface receptors ion channels could mediate orientation of the cells. To address this question we analysed whether arachidonic acid (AA) is a chemoattractant for *Dictyostelium discoideum* cells. Previously, we have shown that AA induces Ca^{2+}-influx and releases Ca^{2+} from Ca^{2+} -stores [Schaloske et al. Biochem. J. 327 (1997) p. 233 and Biochem. J. 332 (1998) p. 541]. AA contained in a glass capillary attracted aggregation competent cells to the tip of the capillary at a distance of 40µm within about 5 min. The attraction was abolished in the presence of EGTA in the medium although the cells still responded to a cAMP source. This indicates that influx of Ca^{2+} is required for the AA-mediated chemotaxis. The response to cAMP involves a transient upregulation of cGMP. We found that AA did not induce an elevation of the cGMP level as was recently shown for LPA.

12. FUNCTIONAL ANALYSIS OF *DICTYOSTELIUM DISCOIDEUM* RHO-RELATED PROTEINS RACG AND RACH. Baggavalli P. Somesh, Heidrun Dislich, and Francisco Rivero, Institut für Biochemie I, Medizinische Fakultät, Universität zu Köln, Joseph-Stelzmann-Str. 52, 50931 Köln, Germany.

Rho family proteins have been implicated in regulating various cellular processes, including actin cytoskeleton organisation, endocytosis, cell cycle and gene expression. Up to 15 genes coding for Rho related proteins have been identified in Dictyostelium discoideum, a eukaryotic microorganism whose cells exhibit actin based cell motility comparable to cells of higher eukaryotes like the leukocytes, are chemotactically active and are able to efficiently perform phagocytosis. Except for Rac1a/b/c, RacC, RacE and RacF1, the data available to date are insufficient to assign a functional role for each of the *Dictyostelium* Rac proteins. Rac1 proteins control cell morphology, chemotaxis, endocytosis, cytokinesis and development. RacE appears to be essential for cytokinesis, but not for other processes like phagocytosis, chemotaxis or development. A role in actin cytoskeleton organization, pinocytosis and phagocytosis has been proposed for RacC, based on a study carried out with RacC overexpressor cells lines. Finally, RacF1 appears to be involved in pinocytosis, phagocytosis and formation of cell-to-cell contacts.

In this study, we are investigating the function of two novel *Dictyostelium* Rho family proteins, RacG and RacH. The racG and racH genes encode proteins of about 200 amino acids and are constitutively expressed throughout the *Dictyostelium* life cycle. To investigate the role of these proteins in cytoskeleton-dependent processes, we have fused them at their amino-terminus with green fluorescent protein (GFP) and studied the dynamics of subcellular redistribution using a confocal laser-scanning microscope. GFP-RacG is distributed predominantly in the plasma membrane whereas GFP-RacH is apparently homogeneously distributed in the cytosol. GFP-RacG is also localized transiently to phagocytic cups. Furthermore, we have generated cell lines that overexpress constitutively active or dominant negative RacG or RacH, using a tetracycline-regulated expression system. In addition, we have made constructs for disruption of the gene by introducing a blasticidin resistance cassette. All these cell lines are being investigated currently.

13. THE ROLE OF DdVASP, A *DICTYOSTELIUM* HOMOLOGUE OF VASODILATOR-STIMULATED PHOSPHOPROTEIN IN CHEMOTAXIS AND FILOPODIA FORMATION. Young-Hoon Han and Richard A. Firtel, Section of Cell and Developmental Biology and Center for Molecular Genetics, University of California, San Diego, La Jolla, California 92093-0634, USA.

We investigated the role of DdVASP, a *Dictyostelium* homologue of vasodilator-stimulated phosphoprotein, in chemotaxis and filopodia formation in *Dictyostelium*. VASP null cells lack filopodia and have a defect in cell adhesion on the substratum during chemotaxis resulting in inefficient chemotaxis. VASP overexpressors are rich in filopodia and, unexpectedly, show slightly faster movement toward the chemoattractant cAMP. Both *in vivo* and *in vitro* data indicate that DdVASP induces actin assembly and the activity resides in proline-rich domain (PRD)-Ena/VASP homology (EVH) 2 domain structure. The EVH2 domain is essential for actin assembly inducing activity, having about 10% activity of full-length protein, while central PRD, which does not induce actin assembly by itself, recovers the full activity when combined with the EVH2 domain. The EVH2 domain is also responsible for the localization of DdVASP to the leading edge and cAMP-stimulated sites; EVH1, which was previously reported to mediate targeting of the Ena/VASP family by interaction with proline-rich motifs, does not mediate the localization of DdVASP. PRD-EVH2 expression in VASP null cells completely restores the ability to form filopodia and adhere on the substratum, indicating that EVH1-mediated interaction of DdVASP with other proteins is not required for these processes. However, the chemotaxing speed of VASP null cell expressing PRD-EVH2 is much slower than that of the DdVASP overexpressor, although the morphology of these two cell lines is quite similar during chemotaxis. This finding suggests that EVH1-mediated interaction of DdVASP with other signaling molecules plays an important role during chemotaxis of *Dictyostelium*.

14. PARADOXICAL EFFECT OF PHOSPHATIDIC ACID ON MYOSIN HEAVY CHAIN KINASE B ACTIVITY. Maribel Rico and Tom Egelhoff, Department of Physiology and Biophysics, School of Medicine, Case Western Reserve University, 10900 Euclid Ave., Cleveland, OH 44106, USA.

Myosin Heavy Chain Kinase B (MHCK B; 83 KDa) is a member of a novel family of threonine-specific protein kinases that is unrelated to the conventional Ser/Thre-Tyr protein kinases or to the histidine kinases. MHCK A; MHCK C and eEF-2 Kinase are other examples.

In order to investigate the physiological role of this kinase we assessed myosin II function in MHCK B over-expressing cell lines. These cell lines lose the ability to grow in suspension to a high density. They also show partial defects in development, consistent with possible disassembly of myosin filaments by the over-expressed MHCK B. Cytoskeletal ghosts from over-expressing MHCK B cell lines contain less myosin than those of control cells, further indicating that MHCK B is a bona-fide myosin heavy chain kinase *in vivo*. Additionally, MHCK B phosphorylates myosin *in vitro* and this phosphorylation disassembles myosin II filaments.
Several acidic phospholipids as well as other bioactive lipids have been tested *in vitro* searching for potential physiological modulators of MHCK B activity. Among others -phosphatidylserine (PS); phosphoinositol bis-phosphate (PIP2); lysophosphatidic acid (LPA); diacylglycerol (DAG); etc.- Phosphatidic acid (PA) resulted the most potent activator of MHCK B. Up to 20-fold increase in MHCK B activity was found towards the synthetic peptide MH-1 throughout an increasing series of PA concentrations. Paradoxically, *in vitro* assays using PA as activator and myosin as a substrate show a slight decrease in MHCK B activity. One of the two major routes by which PA is biosynthesized consists of the phosphorylation of diacylglycerol (DAG) molecules by diacylglycerol kinases (DGKs). In earlier studies in *Dictyostelium*, a knock out of an enzyme (MHC-PKC) that contains a highly conserved DGK catalytic domain was made. That cell line over-assembles myosin in the cytoskeleton, suggesting a connection between PA biosynthesis and myosin heavy chain phosphorylation. Work is in progress to determine whether PA is in fact a physiological activator of MHCK B.

15. A NOVEL SECRETED FACTOR THAT POTENTIATES DEVELOPMENT. Joseph A. Brzostowski, Cynthia Johnson, and Alan R. Kimmel, Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-8028, USA.

Dictyostelium is sensitive to a variety of secreted factors that regulate chemotaxis and development. We identified a new aggregation promotion factor (APF) complex secreted by starving cells that augments development. We determined that APF is distinct from other secreted factors that regulate early development, such as the secreted cAMP phosphodiesterase (PDE), prestarvation factor, conditioned media factor and counting factor. Cells starved at densities lower than 50,000 cells/cm2 do not aggregate; this affect is abrogated by the addition of APF to the starvation buffer, prompting cells to form signaling centers and aggregate into mounds. In addition, cells starved at high density will form territories within six hours in the presence of APF compared to ten hours in its absence.

We found that cells null for a particular Gα subunit, Gα9, (see abstract by Brzostowski *et al.*) are hypersensitive to AFP. We utilized *gα9*-null cells in APF-bioassays to purify APF to homogeneity. APF purifies from wild-type cells as a glycosylated, 250 kDa complex, made up of four distinct proteins. Each protein was sequenced and identified by mass spectrometry. The complex includes a novel 150 kDa protein (p150), a cysteine protease, a novel oxidase-related protein (OxyA), and PDE. When purified from *pde*-null cells, the molecular weight of APF shifts to 150 kDa and the peak APF activity correlates only with the presence of p150. OxyA and the cysteine protease do not co-fractionate with APF activity in conditioned buffer from *pde*-nulls. We disrupted the gene encoding OxyA and confirmed that OxyA does not contribute APF activity. Interestingly, *oxyA*-null cells form extremely large aggregation territories and have defects in the production of spores. A full-length p150 gene has been isolated and experiments to disrupt the gene are in progress.

16. TagA IS REQUIRED FOR THE DIFFERENTIATION OF A SUBSET OF SPORE CELLS. Matthew Cabral, J. Randall Good, and Adam Kuspa, work carried out in part under grant R25GM56929, Department of Biochemistry, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030, USA.

Dictyostelium discoideum grows as a single-celled organism until starvation triggers a developmental pathway that results in the aggregation of cells and their development into a fruiting body. Two main cell types, stalk and spore, contribute to the mature fruiting body. *Dictyostelium* have developed a robust mechanism for assuring correct proportioning of the fruiting body, roughly twenty percent stalk and eighty percent spore cells, even in the face of cell loss early in development. The protein TagA, contains a putative serine protease and ATP-binding cassette (ABC) transporter domains, and is expressed in a subset of prespore cells during early aggregation, and in most mature spores of the fruiting body. TagA RNA is detected as early as two hours following aggregation, making it the earliest known cell specific marker in *Dictyostelium*. Fruiting bodies comprised of cells in which TagA function is lost, result in a changed cell type proportion such that a greater percentage of cells become stalk cells. Furthermore, when tagA null cells are mixed with wild type cells to make fruiting bodies, they show a marked preference for becoming prestalk cells. Current work is focused on determining TagA's role in specifying prespore cell fate.

POSTER SESSION II, MONDAY, JULY 23, 2001

17. AMP DEAMINASE AFFECTS CELL-TYPE PROPORTIONING IN *DICTYOSTELIUM*. Soo-Cheon Chae, Danny Fuller, and William F. Loomis, Cell and Developmental Biology Section, Division of Biology, UCSD, La Jolla, CA 92093, USA.

One of the REMI strains encountered in our saturation mutagenesis project, DG1114, forms short, thick stalks and glassy sori with less than 5% the normal number of spores. The gene disrupted in these cells, *amdA*, encodes a 89 kDa protein with 60% identity to AMP deaminases of humans, *Arabidopsis* and yeast. This enzyme catalyzes the conversion of AMP to IMP and is a key component of the adenylate catabolic pathway. We found that there is considerable AMP deaminase activity throughout development of wild type strains of *Dictyostelium* and that it is completely missing at all stages of development of the mutant cells. Transformation of the mutant cells with a construct that overexpresses AMP deaminase rescued the phenotype and resulted in high levels of the enzyme activity.

The levels of expression of the prestalk-specific markers, *ecmA* and *tagC*, were increased in *amdA*$^-$ null strains relative to those in wild type strains, while the levels of expression of prespore-specific markers, *cotB* and *spiA*, were reduced in *amdA*$^-$ cells. The levels were returned to normal in the rescued strains overexpressing *amdA*.

Using a ecmA::lacZ construct, we found that the anterior prestalk region was expanded more than two fold in *amdA*$^-$ null slugs. The number of cells expressing a ecmO::lacZ construct was not affected by loss of AmdA indicating that the mutation results in an increase in PST-A prestalk cells rather than PST-O cells. The alteration in cell-type proportioning appears to be a cell autonomous consequence of the loss of AMP deaminase since mutant cells developed together with wild type cells still produced excess prestalk cells and wild type cells carrying the ecmA::lacZ construct formed normal numbers of prestalk cells even when developed together with an equal number of *amdA*$^-$ mutant cells.

Most of the AMP that accumulated in *amdA*$^-$ cells appeared to be secreted since it was not significantly higher in the mutant cells than in wild type cells but was released into the surrounding buffer at 8 time the the normal rate during early development. Of the purine metabolites, only inosine accumulated within the cells to abnormal levels, reaching values that were 3 times higher than in wild type cells during aggregation. Some of the AMP that accumulated within the mutant cells appeared to be hydrolysed to adenosine and subsequently converted to inosine by adenosine deaminase (ADA).

We have found that cell type proportioning is unaffected by additon of AMP, IMP, adenosine or inosine to the developmental environment. The cell autonomous nature of the phenotype resulting from loss of AMP deaminase indicates that this enzyme affects how the cells respond to cell type proportioning signals.

18. IDENTIFICATION OF FbiA, A POTENTIAL TARGET OF FbxA-MEDIATED DEGRADATION, VIA YEAST TWO-HYBRID ANALYSIS. Jennifer Christman, Carla Moré, Sarah E. Petricca, Stacey C. Miller, Maryann Borsick, Seth Houwer, Kelly McFeaters, and Margaret K. Nelson, Department of Biology, Allegheny College, Meadville, PA 16335, USA.

Mutants lacking the FbxA protein display several developmental defects, including ammonia hypersensitivity, a tendency to remain as slugs under conditions that would normally induce culmination, a decreased prestalk:prespore ratio, and the formation of aberrant culminants. [1,2] FbxA is a member of a family of proteins that contain an F-box and WD-40 repeats and that target specific proteins for degradation via proteasomes. As a strategy to detect targets of FbxA-mediated degradation, we have employed yeast two-hybrid analysis, using as our bait the WD-40 repeat region of FbxA. Approximately 7.0 x 10^7 yeast transformants containing the FbxA bait plasmid and a slug-stage cDNA prey library were analyzed. Prey plasmids were isolated from the six colonies that displayed a galactose-dependent Leu^+ blue phenotype and retransformed into yeast containing a variety of bait plasmids to verify reproducibility and specificity of the interaction with the WD-40 region of FbxA. Two plasmids, which were subsequently shown to contain identical inserts, passed the tests for reproducibility and specificity. We have given the protein encoded by this insert the name FbiA (for FbxA-interacting protein). A tblastx search of the NCBI database using partial sequence data from *fbiA* reveals three stretches (each 40-60 amino acids in length) of homology to proteins in humans, mice, *Drosophila*, *C. elegans*, *Arabidopsis*, *S. pombe*, *S. cerevisiae*, *N. crassa*, and *P. falciparum*. These other proteins all appear to be conceptual translations from genome sequencing projects and, as of yet, have no assigned function. Hence, FbiA may be the founding member of a new protein family.

[1]Nelson, M.K., A. Clark, T. Abe, A. Nomura, N. Yadava, C.J. Funair, K.A. Jermyn, S. Mohanty, R.A. Firtel, and J.G. Williams. (2000) *Dev. Biol.* 224: 42-59.

[2]Ennis, H.L., D.N. Dao, S.U. Pukatski, and R.H. Kessin. (2000*) Proc. Natl. Acad. Sci. USA* 97:3292-3297.

19. TRANSCRIPTIONAL REGULATION OF THE 5'-NUCLEOTIDASE GENE OF *DICTYOSTELIUM DISCOIDEUM*. Can M. Eristi, Muatasem Ubeidat, Brad R. Joyce, Danielle F. Overall, and Charles L. Rutherford, Molecular and Cellular Biology Program, Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0406, USA.

A 5'AMP-degrading activity appears during the time course development in *Dictyostelium discoideum* and the activity becomes restricted to a narrow band of cells that form the interface between the prespore and prestalk zones. The enzyme has received considerable attention in the past because of the critical role played by cyclic AMP in cell differentiation in this organism. The gene is referred as *5' nucleotidase* (*5nt*). A peptide associated with 5NT activity was purified and sequenced. PCR amplification of genomic DNA using degenerate oligonucleotides and a search of sequences of a cDNA project yielded DNA fragments with sequence corresponding to the peptide sequence of 5NT. Analysis of the expression of *5nt* by Northern blotting indicated that the gene is developmentally regulated. Southern blot analysis showed a single form of the gene. In order to identify regulatory sequences, a genomic fragment containing about 1.2 kb upstream flanking region has been cloned. A series of 5' deletions have been generated and fused to a luciferase reporter gene. The reporter gene activity was high and relatively the same for all deletion constructs that contained 547 bp or longer promoter region. No luciferase activity was detected using 365 bp or shorter promoter. A gradual decrease in activity was observed when three deletion constructs between –547 and –365 were tested suggesting the presence of at least two *cis*-regulatory elements within this region. Numerous internal deletions were generated by PCR in order to more precisely define the region of 182 bp and to specifically localize the binding site(s) for potential *trans*-acting factor(s). It was observed that the reporter activity started at early aggregation stage, in agreement with the temporal expression of the authentic *5nt* gene. It was also found that the expression of the luciferase gene driven by *5nt* promoter sequences was induced by exogenous cAMP. Analysis of the full-length *5nt* promoter fused to a *LacZ* gene enabled us to observe the localization of the β-galactosidase activity during development. The expression occurred as soon as the cells begin moving towards aggregation centers. At the slug stage, the activity was found in a group of cells in the front of the slug, probably in pstAB cells. High activity was also detected in cells that were left behind the moving slug in the slime sheath. During culmination, staining was at the interface of the prespore/prestalk regions. In the completed fruiting body, the expression was in the lower cup, in the slime sheath and the basal disc.

20. AMMONIA DIFFERENTIALLY SUPPRESSES THE cAMP CHEMOTAXIS OF ANTERIOR-LIKE CELLS AND PRESTALK CELLS IN *DICTYOSTELIUM DISCOIDEUM*. Ira N. Feit, Erika J. Medynski, and Michael J. Rothrock, Department of Biology, Franklin and Marshall College, Lancaster, PA 17603, USA.

A drop assay for chemotaxis to cAMP confirms that both anterior-like cells (ALC) and prestalk cells (pst cells) respond to cAMP gradients. We present evidence that the chemotactic response of both ALC and pst cells is suppressed by ammonia, but a higher concentration of ammonia is required to suppress the response in pst cells.

We also confirm that ALC show a chemotactic response to cAMP when moving on a substratum of prespore cells in isolated slug posteriors incubated under oxygen. ALC chemotaxis on a prespore cell substratum is suppressed by the same concentration of ammonia that suppresses ALC chemotaxis on the agar substratum of the drop assays. Chemotaxis suppression is mediated by the unprotonated (NH3) species of ammonia.

The observed suppression, by ammonia, of ALC chemotaxis to cAMP supports our earlier hypothesis that ammonia is the tip-produced suppressor of such chemotaxis. We discuss implications of ammonia sensitivity of pst cells and ALC with regard to the movement and localization of ALC and pst cells in the slug and to the roles played by ALC in fruiting body formation. In addition, we suggest that a progressive decrease in sensitivity to ammonia is an important part of the maturation of ALC into pst cells.

21. RECOMBINANT COUNTIN AFFECTING *DICTYOSTELIUM* DEVELOPMENT. Tong Gao[1], Lei Tang[2], Hamam Alraaba[1], and Richard H. Gomer[1, 2], [1]Howard Hughes Medical Institute and [2]Department of Biochemistry and Cell Biology, MS-140, Rice University, 6100 S. Main Street, Houston, TX 77005-1892, USA.

Very little is known about how a multicellular organism or a tissue controls the size of a group of cells. *Dicyostelium discoideum* is a simple but excellent system to study group size control. Previous work showed that *smlA*$^-$ cells form small fruiting bodies due to over-secretion of counting factor (CF), a ~450kDa complex of polypeptides. CF also affected signal transduction by increasing cAMP induced cAMP pulse size and decreasing cAMP induced cGMP pulse size. CF enhanced cell motility, stimulated F-actin polymerization, increased myosin phosphorylation and decreased cell-cell adhesion. CF also appeared to regulate the expression of at least 6 specific genes during development. When the gene encoding countin (a component of CF) was disrupted, the aggregation streams of *countin*$^-$ cells did not break up, and thus formed large fruiting bodies. To further analyze the biological activities of countin, we expressed countin protein in *E.coli*. Our data showed that 0.2 μg/ml of purified recombinant countin caused developing cells to form small fruiting bodies, and affected signal transduction similar to the effect of 0.1 μg/ml purified CF. Recombinant countin increased cell motility, decreased cell-cell adhesion, and regulated gene expression in a manner similar to the effect of CF. Like CF, recombinant countin increased myosin phosphorylation. When developing cells were exposed to recombinant countin for 1 minute, it caused an increase in F-actin polymerization. These results suggest that recombinant countin has the same activities as the counting factor complex. Since the CF preparation contains multiple polypeptides, one possibility is that the CF activity is due to a 450kDa polymer of countin, and the other proteins are simply contaminants. We ran recombinant countin on a sieve column and found the molecular weight of the active fraction is ~40kDa, the molecular mass of a dimer of recombinant countin. Ultracentrifugation of 100, 50 and 25 μg/ml recombinant countin indicated that it behaves in solution as a dimer. From these studies of recombinant countin, we suggest that the 40kDa countin protein is the major effector in the CF complex; that countin might exist as a dimer in the 450kDa complex, and that the other proteins within the complex may modify or enhance the activity of countin.

22. INTERCELLULAR COMMUNICATION GENES IN *DICTYOSTELIUM* DEVELOPMENT. Kirsten Kibler and Gad Shaulsky, Program in Developmental Biology and Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA.

Integration of individual cells into a multicellular organism requires intercellular communication. Developmental aberrations that result from mutations in communication genes can be overcome by generating chimerae of mutant and wild-type cells. We have performed a large scale mutagenesis screen, disrupting genes with restriction enzyme mediated introduction of a linearized plasmid. After selecting for strains capable of bearing spores when mixed with wild-type cells, we isolated 123 mutants with distinct developmental phenotypes. We have cloned and sequenced 48 of these genes. The mutant strains exhibit one or more of the following traits: defective aggregation, improper cell type proportioning, and delayed or asynchronous development. We are focusing on mutant strains unable to generate spores when developed in a pure population but capable of bearing spores when co-developed with wild-type cells. Wild-type cells supply these mutant cells with a non cell-autonomous factor that rescues the mutant's defect in spore production. Our initial sequence analysis indicates that the mutant strains are deficient in secreted or cell contact-dependent intercellular signaling molecules required for proper development and differentiation. We are characterizing the developmental role of these communication factors, with emphasis on cellular adhesion, motility, and cell-type differentiation. These experiments may help to elucidate cell-autonomous and non cell-autonomous factors important in intercellular communication in *Dictyostelium* and in other organisms.

23. IDENTIFICATION AND ANALYSIS OF A GENE THAT IS HOMOLOGOUS TO MAMMALIAN Cdk8 IN *DICTYOSTELIUM*. Sophia Hsiu-Hsu Lin, Hao-Jen Huang, Gerald Weeks*, and Catherine Pears, Department of Biochemistry, Oxford University, South Parks Road, Oxford, OX1 3QU, United Kingdom, *Department of Microbiology and Immunology and Department of Medical Genetics, University of British Columbia, Vancouver V6T 1Z3, Canada.

A cDNA clone which encodes a 380 amino acid protein, Ddcdk8, was isolated. Database analysis indicated that the encoded protein is homologous to mammalian Cdk8, which is involved in transcriptional regulation by phosphorylating the carboxyl-terminal domain (CTD) of RNA polymerase II. We have shown DdCdk8 to be a CTD kinase. Disruption of the DdCdk8 gene caused severe defects in both growth and development. Ddcdk8 null (Ddcdk8$^-$) cells show reduced growth in shaking suspension. Most of Ddcdk8$^-$ cells fail to aggregate, and northern analysis of Ddcdk8 null cells is consistent with a role for Ddcdk8 in regulating transcription of early developmental genes. When plated at a higher density, some multicellular structures form, and eventually produce abnormal fruiting bodies that have distorted stalk tubes and lack proper spore heads. When mixed with wild type cells, Ddcdk8$^-$ cells show a tendency to become stalk cells. Taken together, our results show that DdCdk8 play an important role in regulating both growth and morphogenesis in *Dictyostelium.*

24. cGMP-PHOSPHODIESTERASE-INHIBITORS BLOCK RECEPTOR-MEDIATED Ca^{2+} -INFLUX IN *DICTYOSTELIUM DISCOIDEUM* AND BOVINE CONE CYCLIC NUCLEOTIDE GATED CHANNEL ACTIVITY. D.F. Lusche[1], S. Frings[2], D. Reuter[2], and D. Malchow[1], [1]University of Konstanz, Department of Biology 78457 Konstanz, FRG, [2]Institut fur Biologische Informationsverarbeitung, Forschungszentrum 52425 Julich, FRG.

Chemotaxis and light scattering oscillations in *Dictyostelium* involve changes in the intracellular concentrations of cyclic GMP and Ca^{2+}. When starved *Dictyostelium* cells are exposed to cAMP, the intracellular cGMP concentration rises 10-fold and Ca^{2+} -influx causes an increase of the cytsosolic Ca^{2+} concentration. We used known antagonists of cGMP-phosphodiesterases to examine the role of cGMP in these processes. We found that SCH51866 and Sildenafil are poor inhibitors of cGMP-hydrolysis in *Dictyostelium*. However, SCH51866 blocked cAMP-induced Ca^{2+} -influx, suggesting that SCH51866 interacts directly with Ca^{2+}-permeable channels which are activated during a rise in cytosolic cGMP concentration. K^{+} -efflux was not blocked by SCH51866. SCH58166 and Sildenafil are known to compete with cGMP for the cGMP binding site of type V phosphodiesterases. We, therefore, tested whether the drugs act in a similar way on other cGMP-regulated proteins. The activity of cGMP-dependent kinase I was not altered by SCH51866. In contrast, bovine cone cGMP-gated channels (CNCα2), stably expressed in HEK 293 cells, are blocked by both SCH51866 and Sildenafil, but not by UK 114,542, another phosphodiesterase inhibitor, as measured using the patch-clamp technique. Channel inhibition could be relieved by excess cGMP, indicating competitive inhibition by the drugs. The sensitivity of highly Ca^{2+}-permeable cGMP-gated channels to SCH51866 and Sildenafil raises the possibility that *Dictyostelium* cells also express cGMP-gated channels which are acivated upon a rise of cytosolic cGMP. This notion is supported by our finding that LY83583, a compound known to inhibit several types of cyclic nucleotide-gated channels as well as guanylyl cyclase, inhibits cAMP-induced Ca^{2+} -influx in *Dictyostelium*, but does not affect cAMP-induced cGMP accumulation. Our data support the hypothesis that cGMP-gated channels play a role in the chemotactic response of *Dictyostelium* cells to cAMP stimulation by mediating Ca^{2+} -influx and generating a cytosolic Ca^{2+} signal.

25. A PATHWAY OF UBIQUITIN-MEDIATED PROTEOLYSIS THAT INFLUENCES CELL TYPE PROPORTIONING IN *DICTYOSTELIUM*. David I. Ratner (1,2), Turgay Tekinay (1), Herbert L. Ennis (1), Mary Y. Wu (1), Margaret K. Nelson (3), and Richard H. Kessin (1), (1) Department of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, New York, NY, (2) Department of Biology, Amherst College, Amherst, MA, 3. Department of Biology, Allegheny College, Meadville, PA, USA.

Mutants of *Dictyostelium discoideum* were selected on the basis of their capacity to "cheat" in an evolutionary sense. Amoebae mutagenized by the random integration of plasmid DNA were allowed to develop together with wild type cells so as to produce stalk and spores. Successive rounds of spore selection resulted in the isolation of the *chtA* strain, which in a mixed aggregate coerces wild type neighboring cells to differentiate into dead stalk cells while the *chtA* amoebae preferentially differentiate into viable, propagating spores. Recovery of the disrupted DNA revealed that the underlying *chtA* mutation inactivates the gene encoding the F-box component of a presumptive SCF ubiquitinating complex (1,2).

Because *chtA* cells are themselves developmentally deficient in pure culture, we were able to select suppressor mutations which promote sporulation of the original mutant. In one suppressor, there has been a disruption of the gene *dhkA*, a "two component" histidine kinase known previously to influence *Dictyostelium* development. A second suppressor mutation resides within the gene *regA* which encodes a cyclic AMP phosphodiesterase linked to an activating "response regulator" domain subject to phosphorylation. The final gene isolated from the screen (though one which seemingly does not suppress effectively when disrupted) encodes a protein of unknown function but with limited homology to ubiquitin hydrolase enzymes. By means of immunoprecipitation and mass spectrometry, we are examining the interactions between these various proteins in order to elucidate the role of targeted proteolysis in establishing the proportion of cell types (spore vs. stalk) in *Dictyostelium*.

(1) Ennis, H. L., Dao, D. N., Pukatzki, S.U., and Kessin, R. H. (2000) *Dictyostelium* amoebae lacking an F-box protein form spores rather than stalk in chimeras with wild type, Proc. Natl. Acad. Sci. USA, 97, 3292-3297.

(2) Nelson, M.K., Clark, A. Abe, T., Nomura, A., Yadava, N., Funair, C.J., Jermyn, K.A., Mohanty, S., Firtel, R.A., and Williams, J.G. (2000) An F-Box/WD40 repeat-containing protein important for *Dictyostelium* cell-type proportioning, slug behaviour, and culmination. Develop. Biol., 224, 42-59.

26. ROLE OF pH REGULATION DURING THE RAPID PATTERNING OF ***DICTYOSTELIUM*** IN TWO-DIMENSIONAL CULTURE. Satoshi Sawai[1*], Takashi Hirano[2], Yasuo Maeda[2] and Yasuji Sawada[3], Graduate School of Information Sciences[1], Biological Institute, Graduate School of Science[2] and Research Institute of Electrical Communication[3], Tohoku University, Sendai, Japan, *Present address: Princeton University, Department of Molecular Biology.

Recently it was demonstrated that a rapidly forming self-organizing pattern which emerges within confined ***Dictyostelium discoideum*** cell mass [1-3] could later give rise to stripes of distinct zones each comprised of different cell-types [4]. In order to elucidate the nature of a possible chemical gradient present under the two-dimensional culturing condition, we studied the kinetics of the initial rapid patterning event. It was found that as temperature is lowered the characteristic length of the pattern increases. From this dependency, we obtained the activation energy of the reaction (~67kJ/mol). Fluorescence of cells loaded with fluorescein dextran-conjugate revealed that the cytosolic pH of cells in the inner-zone becomes lower than the cells in the outer-zone; a pattern similar to the one reported by Azhar and Nanjundiah [5] using neutral-red. The pattern could be extinguished by preincubating cells with plasma membrane proton pump inhibitors diethylstilbestrol (DES) or miconazole. Also, preincubation of cells with weak-acid such as propionate or DMO delayed the onset of the patterning, whereas weak-base such as ammonia and methylamine fastened it. When a drop of pH indicating dye boromocresol purple is placed next to the cell mass, it changed its color from orange to purple possibly due to accumulation of ammonia. The same change was only observed in the extra-cellular space of the outer-zone and not in that of the inner-zone.

Using a simple mathematical model describing reaction and diffusion of a weak-acid and base pair, we demonstrate how oxygen gradient could set the polarity and select between different observed patterns. The observed pH gradient may partly explain why cells could differentiate position-dependently under this culturing condition.

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27. VEGETATIVELY ACTIVATED ELEMENTS OF THE gp64 PROMOTER OF THE CELLULAR SLIME MOLD POLYSPHONDYLIUM PALLIDUM. Naohisa Takaoka, Masashi Fukuzawa[a], Tamao Saito, and Hiroshi Ochiai, Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo, 060-0810 Japan; a, Wellcome Trust Building, Department of Anatomy and Physiology, University of Dundee, Dow Street, Dundee, DD1 4HN, UK.

The mRNA of cysteine-rich membrane protein gp64 of *Polysphondylikum pallidum* is expressed extensively under the growth and then decreases rapidly at the onset of development (1). A deletion reporter analysis of the gp64 promoter revealed the presence of two vegetative activated elements (2). We further analyzed the vegetative activated elements by deletion and point mutations, showing that it is important for promoter activity in response to growth conditions.

When an A1 region (-190 to -156 bp containing a GATTTT inverted repeat) was coupled to a gp64 minimal promoter, its construct showed elevated ß-galactosidase reporter activity. A DR1 region (-216 to -190 bp containing a TTTGTTGTG direct repeat) upstream of the A1 also showed a similar degree of activation. Interestingly, the sequence containing both the DR1 and A1 showed a strong promoter activity, suggesting a synergistic effect of these two elements. Furthermore, the data suggest that the inverted repeat in the A1 region confers the gp64 gene activation at the vegetative phase judging from deletion and point mutation analyses.

Food depended activation of the gp64 promoter at the vegetative growth phase was analyzed in the shaking culture with bacteria, liquid medium (A-medium) or mixed medium (A-medium with bacteria). In the presence of only bacteria as a food the enhanced reporter activity was observed. However, in the presence of both bacteria and A-medium the activity was decreased to the level of A-medium alone. This suggests that specific receptors for endocytosis and/or soluble components present in the medium might regulate signaling pathways of the vegetative gp64 promoter.

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28. INVESTIGATION OF BIOCHEMICAL PARAMETERS IN PHOSPHO-RELAY (TWO-COMPONENT) SIGNALING. Peter Thomason, Ted Cox, and Jeff Stock, Molecular Biology Department, Princeton University, Princeton, NJ 08544 USA.

Dictyostelium has a large gene family for proteins involved in phosphorelay (two-component) signaling. Genome studies reveal that *Dictyostelium* has at least 15 histidine kinases, one or more histidine phosphotransfer (HPt) proteins, and four or more response regulators. So far we have little information on which of these twenty components interact with each other biochemically or genetically. The disparity in numbers between the three different kinds of proteins also raises many basic questions regarding how (if?) signaling specificity is achieved: by differences in expression of the proteins spatially and/or temporally; by formation of distinct signaling complexes; and/or by differences in biochemical parameters such as stability of protein-protein interactions and efficiency of phosphotransfer? It is known that the HPt protein RdeA and the response regulator RegA interact biochemically and genetically. But many studies also show that the HPt family of proteins can substitute for one another in different organisms, thus it is hard to understand how this component can impart any specificity to phosphotransfer. We have studied the biochemical parameters of the phosphotransfer reaction between RdeA and RegA, and also between these proteins and CheA and CheY, bacterial proteins belonging to the same families as RdeA and RegA, respectively. We will present results of these studies and suggest explanations on the mechanisms of specificity in phosphorelay signaling.

29. RAPID Ca^{2+} RESPONSES TO DIF-1 DURING EARLY DEVELOPMENT. David Traynor, Jaqueline Milne*, and Robert R Kay, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK, *Present address: Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda Maryland 20892, USA.

DIF-1 is required for the induction of prestalk-O cells during normal development. The signal transduction pathway(s) that mediate DIF-1 action are poorly understood. Relatively long term responses to DIF-1 such as transcriptional activation, gene induction and cellular differentiation have been well documented. Recently DIF-1 has been shown to cause rapid cellular responses such as an increase in intracellular Ca^{2+} within two minutes of its addition to cells from early in development and a transient accumulation of the STATc protein in the nucleus of cells within 5 minutes of DIF-1 addition. During stalk cell formation however, no rise in cytoplasmic was detected until 1 hour after DIF-1 addition when a slow sustained elevation of cytoplasmic Ca^{2+} levels was observed.

We show that DIF-1 rapidly and transiently stimulates Ca^{2+} influx into amoebae throughout early development and this influx is shown to be dependent on the *iplA* gene product which resembles the inositol 1,4,5-trisphosphate receptor from higher eukaryotes. In aggregation competent cells, Ca^{2+} influx is stimulated within 10 seconds of DIF-1 addition and terminates after 50 seconds. The half maximal concentration of DIF-1 for stimulated Ca^{2+} influx is 70nM in these cells. DIF-1 analogues were used to test the specificity of the Ca^{2+} response. The effect of DIF-1 on the MAP kinase DdERK2 and the second messengers cAMP and cGMP were also investigated.

Stalk cell formation in monolayers is largely dependent on the presence of extracellular Ca^{2+} and drugs that can elevate intracellular Ca^{2+} levels stimulate stalk cell differentiation. We show that DIF-1 induced stalk cell formation in monolayers is impaired in the *iplA* null strain whereas spore cell formation is unaffected.

We hypothesise that DIF-1 stimulated Ca^{2+} influx is triggered by the DIF-1 receptor.

30. EXPRESSION OF A *DICTYOSTELIUM* GENE ENCODING MULTIPLE REPEATS OF ADHESION INHIBITOR-LIKE DOMAINS HAS PLEIOTROPHIC EFFECTS ON CELL SIZE AND GROWTH AS WELL AS CELL ADHESION, DEVELOPMENTAL PROGRESSION AND PATTERNING. Timothy R. Varney, Hoa N. Ho, Chere' Petty, Jayne Dolman, and Daphne D. Blumberg, Department of Biological Sciences, University of Maryland, Baltimore County, 1000 Hilltop Circle Baltimore, Maryland 21250, USA.

The *Dictyostelium* protein AmpA (Adhesion modulation protein A) is encoded by the gene originally identified by the D11 cDNA clone. AmpA contains repeated domains homologous to a variety of proteins that influence cell adhesion. The protein accumulates during development, reaching a maximal level at the finger stage. 30% of the AmpA protein is secreted at the initiation of development, and in culminants AmpA is localized to Anterior-Like Cells. Characterization of an *ampA*$^-$ strain generated by gene replacement reveals pleiotrophic phenotypes that effect growth, development and patterning. Axenically growing *ampA*$^-$ cells are smaller, demonstrate an increase in cell-cell clumping and grow logarithmically to higher densities. They also display defects in phagocytosis but not pinocytosis. Cells overexpressing *ampA* grow more rapidly. The increase in *ampA*$^-$ cell-cell adhesion becomes more dramatic when cells are starved in non-nutrient buffer suspensions. Developing *ampA*$^-$ cells are also more adhesive to the underlying substrate. Finally, *ampA*$^-$ cells are delayed in developmental progression, with the severity of the delay increasing as cells are grown for longer periods of time in the presence of bacteria. Reintroduction of the *ampA* gene rescues the growth and developmental defects of *ampA*$^-$ cells however expression of additional copies of the gene in wild type cells results in more severe developmental delays, increased cell substratum adhesion but decreased clumping in suspension culture indicating 2 distinct modes of action of the AmpA protein during development. We propose that AmpA functions as an anti-adhesive protein to limit cell adhesion during growth and to facilitate cell migration during morphogenesis.

31. MICROARRAY ANALYSIS OF THE WILD-TYPE *DICTYOSTELIUM* DEVELOPMENTAL TIME COURSE. Chad Shaw, Nancy Van Driessche, Gad Shaulsky, Adam Kuspa, Jeffrey Tollett, Bill Eaton, and Richard Sucgang, Baylor College of Medicine, USA.

We present data and analysis from cDNA microarray studies of wild-type *Dictyostelium* developmental time courses. To generate the time course information, independent wild-type *Dictyostelium* cell cultures are subjected to starvation and subsequent transition to multi-cellularity. These independent cultures are harvested at successive 2 hour increments throughout 24 hours of development. The result are13 data sets with expression information pertinent to the 0, 2, 4,…24 hour time points of development

Often lost in the excitement surrounding microarray studies is the variability to be expected of this complicated data. We take a statistical approach to consider the center and spread of data values generated in microarray experiments. We survey the literature in this area and present our own approach. We present results from microarray data generated at the BCM Microarray Core facility through the efforts of the *Dictyostelium* Development group. Among the results shown are the impact of data normalization procedures and variant image processing techniques.

The time course data show a repeatable array phenotype which can be productively viewed from at least 2 perspectives. The first view of the *Dictyostelium* time course differentiates developmental time points using joint expression information. In this view, each time point is represented by a large vector of by-gene expression values derived from the microarray experiments. This analysis clearly separates the unicellular phase of the *Dictyostelium* life-history from the multi-cellular phase. The ability to distinguish unicellularity from multi-cellularity is an important first step in validating the application of microarray technique to *Dictyostelium* development.

Another view of the time course data attempts to categorize genes by their differential expression across the time course. Analyses to classify genes demonstrate that biologically relevant modes of expression are recapitulated in the microarray data. In particular, genes known to be important in late development show clear patterns of increased expression.

32. A ROLE FOR YakA, cAMP AND PKA IN THE NITROSOATIVE/OXIDATIVE STRESS RESPONSE OF ***DICTYOSTELIUM DISCOIDEUM*** CELLS. Raquel Bagattini[#], Renata Gorjão[#], Alexandre Taminato[#], Nancy Van Driessche*, Guokai Chen*, Thiago Teixeira Santos[+], Gustavo Tadao Okida[+], Roberto Hirata Jr.[+], João Eduardo Ferreira[+], Eduardo Jordão Neves[+], Junior Barrera[+], Adam Kuspa*, Gad Shaulsky*, and Glaucia Mendes Souza[#¥], [#]Inst. Química, Dep. Bioquímica, USP. [+]Inst. Matemática e Estatística - USP, *Baylor College of Medicine, Houston, Texas, 77030, USA. [¥](glmsouza@iq.usp.br)

YakA is a protein kinase required for the transition from growth to development of ***Dictyostelium*** cells, decreasing vegetative mRNAs and inducing the up-regulation of ***pkaC*** that occurs when cells are starved. YakA also controls the cell cycle, regulating the intervals in between cell divisions. In this work we report a role for this kinase in the response that follows nitrosoative/oxidative stress of ***Dictyostelium*** cells. Growth curves of cells from ***yakA*** null, ***pkaC*** null, ***acaA*** null and ***pkaC*** over-expressing cells submitted to several stresses indicate a role for YakA, PKA and cAMP in the induction of the growth arrest that follows nitrosoative/oxidative stress. ***yakA*** null cells are extremely sensitive to stress generated by nitric oxide and hydrogen peroxide and a second-site mutation in ***pkaC*** or ***acaA*** suppresses this sensitivity. An increase in cAMP levels and PKA activity is observed when cells are submitted to sodium nitroprusside and H_2O_2 treatment. The analysis of this phenomenum through the use of DNA microarray technology indicate several candidate effectors of the growth arrest induced by SNP treatment that are differentially regulated in ***yakA*** and ***pkaC*** null cells. (This work is supported by FAPESP, Fundação de Amparo à Pesquisa do Estado de São Paulo).

33. ISOPRENYLCYSTEINE PROTEIN CARBOXYL METHYLTRANSFERASE IN *DICTYOSTELIUM.* Ying Chen, Jeff Stock, and Ted Cox, Dept. of Molecular Biology, Princeton University, Princeton, NJ 08544, USA.

Reversible protein modification is a fundamental strategy for intracellular communication. In eukaryotes, protein phosphorylation is common in signal transduction pathways. Recently, protein methylation has also been identified as playing a role in intracellular signaling.

Small G proteins such as Ras or Rho subfamilies are subject to CAAX-tail modification, whose last step is the prenylcysteine carboxyl methylation (1). Here, we use cellular slime mold *Dictyostelium discoideum* as a model system to study the role of prenylcysteine carboxyl methyltransferase in signaling processes.

Our data showed that the methylation was increased transiently when the cells were exposed to 1 mM cAMP. The gene was cloned and the Northern analysis showed that the methyltransferase is expressed at the highest level in the early development stage. We did the methyltransferase knockout by homologous recombination, using Blasticidin S as a selective marker. The phenotype of the knockout mutant appears to be cell-density dependent and cells show defects in chemotaxis and differentiation. In short, our study showed that prenylcysteine carboxyl methylation of small G proteins plays an important role in cAMP signal transduction processes in *Dictyostelium*.

34. DELETION ANALYSIS AND GFP-TAGGING OF A *DICTYOSTELIUM* β-CATENIN. Jonathan P Reynolds and Adrian J Harwood, MRC-Laboratory for Molecular Cell Biology, University College London, London, WC1E 6BT, UK

β-catenin is a dual function protein, important for both signalling and adhesion within the cell. It acts as a downstream effector of Gsk-3β in the Wnt signalling pathway as well as bridging cadherin molecules to the actin cytoskeleton at adherens junctions. Inappropriate activation of the Wnt pathway is associated with various human cancers, whilst loss of β-catenin from adherens junctions can lead to increased invasiveness of the cell.

Aardvark is a recently cloned *Dictyostelium* homologue of β-catenin (Grimson *et. al.*, 2000). Aardvark acts downstream of GskA to activate prespore gene expression and is also found at *Dictyostelium* adherens junctions.

We have made a series of N-terminal and C-terminal deletions of Aardvark, removing key regions known to be essential for either the signalling or adhesive properties of its mammalian counterpart. GFP-tagging has allowed us to observe the differences in the localisation of these truncated proteins within the cell.

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POSTER SESSION III, TUESDAY, JULY 24, 2001

35. CHARACTERIZATION OF A MUTANT STRAIN THAT CANNOT MAKE THE TRANSITION FROM GROWTH TO DEVELOPMENT. Rui Fang, Olga Timofeevsky, and Charles K. Singleton, Department of Biological Sciences, Vanderbilt University, USA.

An interesting mutant was found during an attempt to disrupt the two GCN2-like genes of *Dictyostelium*. A vector was constructed containing the blasticidin resistance cassette flanked by a fragment that encodes the kinase domain and one that encodes the RS domain of the *Dictyostelium* version of GCN2. The digested vector was transformed into KAx3 by calcium phosphate precipitation. From the transformants, a mutant strain, designated BS151, was found that was unable to develop when grown on bacteria. When BS151 cells were placed on filters with starvation buffer, either no development occurred or in some instances scattered, very small fruiting bodies formed after about 60 hours. Unfortunately, Southerns and PCR results proved that neither of the two GCN2-like genes were disrupted in this strain. However, BS151 had some interesting properties. The defect was autonomous as development was not rescued in mixed cultures with KAx3. Examination of genes that are differentially expressed upon initiating development suggested that BS151 cells were not undergoing the growth to development transition. Vegetative-specific genes of the V gene class were not inactivated under starvation conditions, and discoidin, cAR1, acaA and pkaC were not induced. The cell density-sensing factor CMF was made and was present in growing BS151 cells, but interestingly CMF was not secreted under starvation conditions. CMF secretion is among the first events of development as its accumulation is used to ensure that there are enough starved cells present to proceed along the developmental program. The lack of CMF secretion was not the only problem with BS151 cells as exogenously added recombinant CMF did not rescue the inability to develop. We hope soon to identify the gene that has been mutated in BS151 as it appears to play a key role in the initiation of development and as the strain provides a new tool to investigate the regulation of CMF secretion.

36. MASS CULTIVATION OF ***DICTYOSTELIUM DISCOIDEUM*** IN BIOREACTORS. Erwin Flaschel, Yinghua Lu, Sang-In Han, and Karl Friehs, University of Bielefeld, Faculty of Technology, D-33594 Bielefeld, Germany, E-mail : efl@fermtech.techfak.uni-bielefeld.de.

Dictyostelium discoideum represents an interesting host organism for the production of heterologous proteins. However, its application is seriously affected by slow growth rates in the presence of axenic (liquid) media with complex components (doubling time of 8-10 h) as well as low maximal cell densities ($<2 \cdot 10^7$ mL^{-1}), when grown on complex axenic media or bacteria. Therefore, strategies have been investigated, which have led to - at least partially - alleviate these restrictions. These strategies consisted in feeding ***Dictyostelium discoideum*** with a completely synthetic medium as well as by cultivating the cells in the presence of porous supports in order to immobilize them.

The completely synthetic medium of Franke and Kessin (FM) led to much higher cell densities than the usual complex media. The kinetics of amino acid consumption has been studied in detail and this information was used for adjusting the medium composition. This medium led to maximum cell densities of $5 \cdot 10^7$ mL^{-1} in common batch cultivation processes. Several strains of ***Dictyostelium discoideum*** AX2 and AX3 have been adapted to grow on this novel synthetic medium. The application of such media should facilitate looking for the elusive factor thought to limit the cell density of ***Dictyostelium*** cultures. In addition, the production of secreted heterologous proteins would be facilitated a lot by using such a synthetic medium.

Cells of ***Dictyostelium discoideum*** are relatively fragile due to the absence of a cell wall. However, they grow in conventional bioreactors, when certain precautions are taken. The main shear stress is induced by stirring. Therefore, the oxygen supply is best established by starting the cultivation with a low stirrer frequency and automatically adjusting it, when the oxygen demand increases. However, a maximum stirrer rate should not be exceeded.

A general strategy for obtaining high cell densities consisted in growing ***Dictyostelium discoideum*** in the presence of supports. Certain ceramic materials and pumice lead to cell densities in excess of $2 \cdot 10^8$ mL^{-1} with respect to the pore volume.

Growth of ***Dictyostelium discoideum*** in both suspension culture and in the immobilized state have been used to produce a surface antigen of ***Plasmodium falciparum*** as well as the human FasL in a secreted form.

37. THE COST OF CHIMERISM. Kevin Foster, Angelo Fortunato, Joan Strassmann, and David Queller, Ecology and Evolutionary Biology, Rice University, PO Box 1892, Houston, TX 77251-1892, USA.

The resolution of reproductive conflict is considered central to the evolution of biological complexity, including the origin of multicellularity. It is assumed that reproductive conflict is costly and must be minimised for stable group existence. However, there are few data on just how costly reproductive conflict is. *Dictyostelium discoideum* is an ideal system to investigate this question. When starving, the amoebae aggregate to form a differentiated multicellular organism, which may be clonal or chimeric (multiple clones). Furthermore, recent molecular work suggests that conflict occurs in chimeras with some clones cheating and over-contributing to reproduction. By comparing group functions in clonal (no conflict) versus chimeric (conflict) groups, therefore, we can assess the effect of reproductive conflict and ask the question, does chimerism cost the group?

38. IDENTIFICATION AND MANIPULATION OF NOVEL TARGETS THAT INCREASE THE EFFICACY OF CISPLATIN MEDIATED CELL DEATH. Christopher Foote, Guochun Li, Hannah Alexander, and Stephen Alexander, Division of Biological Sciences, University of Missouri, Columbia, MO 65211, USA.

Cisplatin is a commonly used antineoplastic agent for the treatment of a variety of cancers. However, its initial efficacy becomes limited due to selection of cancer cells resistant to the drug. Determining the mechanism(s) of resistance is an important step in discovering pharmacological targets that can potentiate cells to respond to the drug. To identify novel pathways of resistance, we utilized the REMI mutagenesis technique, and were able to identify six distinct genes that, when knocked out independently, conferred specific resistance to cisplatin. Our study was the first to identify these genes as important mediators of cisplatin resistance.

One of these gene products, sphingosine-1-phosphate lyase, is involved in cell fate decisions, and was chosen for further study. The sphingomyelin catabolism pathway produces two important metabolites: ceramide and sphingosine-1-phosphate (S-1-P). Elevated levels of ceramide activate cell death. In contrast, elevated S-1-P levels stimulate cell proliferation and growth. Sphingosine-1-phosphate lyase catalyzes the degradation of S-1-P to hexadecanal and phosphoethanolamine. Thus, we predict that the sphingosine-1-phosphate lyase null mutant has elevated S-1-P levels, and that S-1-P plays an important role in mediating cisplatin resistance. We are currently modulating endogenous levels of S-1-P to determine if an increase in S-1-P correlates with cisplatin resistance and, conversely, if decreases in S-1-P levels correlate with cisplatin sensitivity.

To determine if increased levels of S-1-P confer cisplatin resistance, we are constructing a sphingosine kinase I overexpressor strain. To date we have identified two distinct sphingosine kinase genes in *Dictyostelium*. Sphingosine kinase catalyzes the formation of S-1-P from sphingosine. Thus, the overexpressor is predicted to have an elevated level of S-1-P and an increased resistance to cisplatin. This is supported by our experimental results that show that the addition of the sphingosine kinase activator, forskolin, to wild type cells makes them more resistant to the drug.

To determine if decreasing S-1-P levels sensitize cells to cisplatin, we have constructed a sphingosine kinase I null strain, and it is slightly more sensitive than wild type to the drug. We are in the process of constructing a double mutant which lacks both the sphingosine kinase I and II genes. We predict that the double null mutant will be more sensitive to the drug due to its inability to synthesize S-1-P. In accordance with this prediction, treatment of wild type cells with N, N-dimethylsphingosine, a sphingosine kinase inhibitor, makes the cells more sensitive to cisplatin.

This study is important with respect to cancer therapy, as it has identified novel pathways involved in mediating cisplatin resistance, and moreover it has identified pharmacological targets that can be manipulated to sensitize cells to cisplatin.

39. EXPLORING THE ROLE OF FILAMIN IN PHOTOTACTIC AND THERMOTACTIC MIGRATION OF SLUGS. Nandkumar K. Khaire, Francisco Rivero, and Angelika A. Noegel, Institute of Biochemistry I, Medical Faculty, University of Cologne, 50931 Cologne, Germany.

In their natural habitat *Dictyostelium* slugs migrate to the soil surface to disperse their spores to a more favorable environment. The orientation of the slug is a critical requirement for this function. Wild type slugs migrate straightforward towards the source of light. In this oriented movement the product of at least 20 genes and all of the common eukaryotic second messengers may participate in transducing the signals. The loss of filamin (gelation factor/ABP120), an F-actin crosslinking protein, leads to severe impairment in phototaxis, without altering other physiological processes. Mutation in other genes for proteins having a similar actin binding domain, like α-actinin and fimbrin, does not affect the normal phototaxis. The related protein, human filamin (ABP280) mediates direct or indirect interaction with a number of regulatory proteins which are involved in signal transduction through its C-terminal rod domains. This led to the suggestion that filamin in *Dictyostelium* is involved in the regulation of normal phototactic and chemotactic migration of slugs by mediating interactions with regulatory proteins. To test this hypothesis we expressed full-length filamin and C-terminal rod domains in filamin deficient mutant cells. We studied the phototaxis rescue and the intracellular localisation of the GFP fusion protein in phagocytosing and developing cells. Further we plan to find out possible interactions between rod domains and Ras super family proteins, the molecular switches which control most of the cellular activities, by using the yeast two hybrid system. This system is also being used to identify the binding partner(s) of the *Dictyostelium* filamin.

40. RECYCLING OF THE *Bsr* MARKER USING Cre/*loxP*-MEDIATED RECOMBINATION. Lisa Kreppel[1], Gad Shaulsky[2] and Alan Kimmel[1,] [1]Laboratory of Cellular and Developmental Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892; [2]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA.

Although, genes can be easily disrupted in *Dictyostelium* by homologous recombination the creation of multiple gene disruptions in *Dictyostelium* is severely limited by the very small number of selectable markers available. To allow for the creation of multiple disruptions in any strain we have adapted the Cre/*loxP*– mediated recombination system for use in *Dictyostelium.* The Bacteriophage P1-derived Cre/*loxP* system is used for chromosomal engineering in a number of systems including mice and plants. Here we use the system to recycle the Blasticidin resistance selectable marker (*Bsr*) for reuse in the generation of double, and potentially triple or quadruple, knock-out strains. This method obviates the often used method of recycling ura+/ura- strains via 5-FOA.

A search of the *Dictyostelium* genome databases for *lox*-like sites found no endogenous *loxP* sites. Thus, the Cre-recombinase activity will be directed only at targeted sites within the genome. To create a disruption cassette *Bsr* was flanked by *loxP* sites, with stop codons in all six reading frames engineered 5' of *Bsr* and outside of the *loxP* sites. The presence of the stop codons ensures that after Cre-mediated excision of *Bsr*, the target gene will not encode a full-length functional protein. The disruption cassette is located within a SmaI fragment for simple blunt-end ligation into nearly any gene of interest for disruption and subsequent Cre-mediated excision of *Bsr* for recycling.

Using this cassette we disrupted Presenilin 2 (dPS2) in Ax3 cells within it's putative cytoplasmic loop. After confirming the initial disruption, these cells were transiently transfected with a plasmid encoding the Cre-recombinase. The resulting clonally-isolated colonies were screened for Cre-mediated recombination using PCR and were tested for blasticidin sensitivity. Cre-mediated chromosomal recombination will result in the excision of *Bsr* and will leave behind a single *loxP* site and the engineered stop codons. The presence of this recombination product was confirmed by sequence analysis. Additionally, we found that the plasmid encoding the Cre-recombinase was not retained.

The resulting dPS2-disrupted, blasticidin sensitive strain is currently being used to generate a second disruption in the dPS1 gene by reusing the *Bsr* marker. Thus, the Cre/loxP system can be applied to the recycling of the Bsr marker in the creation of multiply disrupted strains.

41. PRODUCTION OF THE HUMAN FAS LIGAND BY MEANS OF HIGH CELL DENSITY CULTIVATION OF *DICTYOSTELIUM DISCOIDEUM*.

Yinghua Lu[1], Jaco C. Knol, Maarten H.K. Linskens, Karl Friehs[1], Peter J.M. van Haastert, and Erwin Flaschel[1], [1]University of Bielefeld, Faculty of Technology, D-33594 Bielefeld, Germany; [2]University of Groningen, Department of Biochemistry, Nijenborgh 4, NL-9747 AG Groningen, The Netherlands, E-mail: ylu@fermtech.techfak.uni-bielefeld.de.

The social amoeba *Dictyostelium discoideum* is a promising host for the expression of heterologous proteins requiring post-translational modification[1]. However, an essential problem of this expression system is the limited cell density which can be achieved. It is reported that the maximal cell density of *D. discoideum* in submerged culture, either on axenic medium or on bacteria, is only in the range of 1-2x10^7 ml^{-1} [2]. For the production of high-value products like proteins in substantial quantities it would represent a considerable advantage, if *D. discoideum* could be cultivated at high cell density. Here, we report about achieving relatively high cell densities of *D. discoideum* by means of cultivation on a modified fully synthetic medium – in suspension culture and in an immobilized form. An AX3 strain, (AX3-pCESFL95G2) transformed with plasmid pCESFL95, an MB12NEO-based plasmid encoding human sFasL (AA 141-281) fused to a hCG-β signal peptide, was selected as a model system. The AX3 transformant produces a soluble (secreted) version of the human Fas ligand, a protein that induces programmed cell death, or apoptosis, in cells that express its receptor, the Fas protein.

The *D. discoideum* AX3 strain was cultured on HL-5C medium to a maximal cell density of 1.5-2x10^7 ml^{-1} with a doubling time of 8-10 h. Accordingly, 60-70 µg/l sFasL was produced in the stationary phase. After long-time adaptation the cells grew with a generation time of 14-16 h on a modified FM medium, which is based on the formula of Franke and Kessin,[3] with slightly changed amino acid composition. Although the cells grew slower on modified FM medium than on HL-5C medium, higher cell densities of 3-5x10^7 ml^{-1} were reached. Fas ligand was produced during growth phase. After 200 h of cultivation 100-110 µg/l Fas ligand was secreted into the medium – about twice that produced during growth on HL-5C medium.

Immobilization of the cells in batch mode of operation was carried out by growth in the presence of inorganic porous materials like CeramTec and pumice. Cultivation in HL-5C medium with CeramTec or pumice led to cell densities of more than 3x10^8 ml^{-1} or 2x10^8 ml^{-1} pore volume, respectively, which were up to 15-20 times higher than the maximum cell densities in suspension cultures (1.5-2x10^7 ml^{-1}). Fas ligand was produced by the immobilized cells in the medium. The production rate of Fas ligand amounted to 20-25 $\mu g \cdot h^{-1} \cdot l^{-1}$ pore volume of CeramTec. The productivity was increased by repeated batch fermentation.

A continuous fermentation with immobilized cells was carried out on 50 g pumice packed in a glass column. Fresh HL-5C medium was continuously fed to the reactor at the same rate at which medium was withdrawn. The medium in the reactor was circulated by a peristaltic pump through the column (from top to bottom) functioning as a co-current trickle bed. Cell densities as high as those in the batch experiments were reached, and a volumetric Fas ligand productivity of 20 $\mu g \cdot h^{-1} \cdot l^{-1}$ pore volume was attained.

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42. GENETIC SELECTION FOR COMPONENTS OF THE PHAGOCYTOSIS PATHWAY. Andrew Maselli, Gary Laevsky, and David Knecht, Department of Molecular and Cell Biology, University of Connecticut, Storrs CT 06269, USA.

We have used the molecular genetic tools available to search for genes that are required for the two known pathways of phagocytosis in *Dictyostelium* The kinetics of binding, uptake, and degradation of *E. coli* expressing the recombinant fluorescent protein DsRed were characterized. Stable expression of the fluorescent protein provides living bacteria with a bright internal fluorescent signal that is degradable in the phago-lysosomal pathway. *Dictyostelium* cells phagocytose DsRed bacteria with kinetics comparable to latex beads. Pulse chase experiments show that the fluorescent signal derived from DsRed is degraded with a half-life of approximately 45 minutes. Washing cells in sodium azide was found to release bacteria bound to the cells surface allowing us to distinguish bound from internalized bacteria or beads. Surprisingly, surface particle release by azide is independent of myosin II function. When bound in the cold, all of the bacteria can be released with azide.

We have used this assay in a search for mutants affecting phagocytosis in populations created by chemical mutagenesis and REMI gene disruption. Cells were subjected to mutagenesis and the resultant population was selected in successive rounds of FACS sorting for cells unable to bind or internalize *E. coli* or beads. Washing bound bacteria off the surface with azide allows us to identify mutants that are defective in the internalization pathway which would be masked by normal surface binding. In order to identify genes that are required for viability, the mutagenized cells were screened for temperature sensitive defects in phagocytosis. Thus far, $8x10^6$ chemically derived mutants have been screened and 54 putative mutants identified. Further characterization of these mutants and the search for additional mutants is underway. In order to isolate the phagocytosis genes, mutant cell lines are being complemented by transformation with a wild-type cDNA expression library and screened for recovery of phagocytic ability.

43. CONTROL OF CELL PROLIFERATION AND EARLY DIFFERENTIATION BY *dng1*, A *DICTYOSTELIUM ING1* HOMOLOGUE**.** Taira Mayanagi, Aiko Amagai, and Yasuo Maeda, Biological Institute, Graduate School of Science, Tohoku University, Aoba, Sendai 980-8578, Japan.

ING1 (Inhibitor of Growth 1) has been shown to be involved in control of cell growth (Garkavtsev, I. et al. 1996) as a putative tumor suppressor in human cells, in cooperating with *p53*, but its precise functions remain to be solved. We report here the structure and developmental functions of *dng1*, a *Dictyostelium ING1* homologue. The DNG1 protein shares significant sequence similarity with ING1 as a whole. The PSORT II program has predicted that the DNG1 localizes in the nucleus and has a putative PHD (plant homeodomain)-type zinc-finger domain in its highly conserved C terminal region, indicating that it may be a transcription co-factor. To analyze the *dng1* function in *Dictyostelium* cells, transformants (*dng1*) overexpressing the *dng1* cDNA under the control of Actin 6 promoter were prepared. As was expected, the *dng1* cells grew more slowly in nutrient medium as compared to vector control cells, and exhibited accelerated differentiation after starvation, as realized by earlier acquisition of chemotactic activity to cAMP. In this connection, the *dng1* cells were found to express the *car1* (cAMP receptor 1) even during the vegetative growth phase, and expressed the *car1* mRNA precociously after starvation. Incidentally, the expression of *car1* mRNA has been shown to increase specifically in response to differentiation from the PS-point, a checkpoint of growth/differentiation transition in cell cycle. Taken together these results suggest that the *dng1* may play crucial roles in regulation of cell cycle progression as well as in the transition of cells from growth to differentiation.

44. *D. DISCOIDEUM* EXPRESSES PROTEINS CONTAINING MULTIPLE CALPAIN DIII DOMAINS. Ronald L. Mellgren, Eric Czerwinski, and Xinhua Huang, Department of Pharmacology and Therapeutics, Medical College of Ohio, 3035 Arlington Avenue, Toledo, Ohio 43614-5804, USA.

Calpains comprise a family of non-lysosomal cysteine proteases that have increasingly recognized regulatory and developmental roles in many organisms. Examples include function of *A. nidulans* Pal B protease in adaptation to an alkaline environment, control of nematode sex determination by Tra 3 protease, and development of the *Drosophila* optic lobes (Sol protease). There is considerable interest in pathologic consequences of perturbed calpain function in man: limb girdle muscular dystrophy type 2A is caused by inactivating mutations in *Capn*3, and recently the NIDDM1 gene associated with type 2 diabetes in some populations has been identified as *Capn*10. The DIII domain present in many calpain family members is a C2-like domain that has been thought to be responsible for binding of calpains to phospholipids in membranes, or to other proteins. An understanding of its targeting function could help sort out the physiologic roles of the calpains by defining subcellular localizations or protein substrates for the different calpain gene family members. Two *Dictyostelium* proteins have been identified that contain multiple copies of DIII domains. CLP (calpain-like protein) contains tandem DIII domains and most closely resembles Capn 10 in Blast searches. DIII-X4 has four DIII repeats. Neither of these proteins appears to possess a calpain cysteine protease catalytic domain; therefore, they may be the first examples of non-calpain proteins containing DIII domains. Current studies include development of CLP gene knockout strains, to study phenotypic changes associated with loss of CLP, and isolation of CLP to determine protein binding partners and potential interactions with phospholipids. These studies in the experimentally tractable *Dictyostelium* system may facilitate understanding of calpain function in higher organisms, or, at the least, they should help define the targeting role of the DIII domains in these enzymes.

45. LSD1, A LIPID STORAGE DROPLET PROTEIN IN *DICTYOSTELIUM*. Shinji Miura, Joseph Brzostowski, Jai-Wei Gan, Michael Parisi, Constantine Londos, Brian Oliver, and Alan R. Kimmel, Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-2715, USA.

Most animal cells contain intracellular lipid droplets that store triacylglycerols and cholesteryl esters which, upon hydrolysis, give rise to compounds essential for energy metabolism, steroid hormone synthesis, membrane biosynthesis, and cell signaling. In vertebrates, the droplets in most cells are coated with ADRP, whereas in adipocytes and steroidogenic cells, the droplets are coated with Perilipins. Studies in cultured cells and "knock-out" animal models have implicated these proteins in the direct deposition and breakdown of neutral lipid storage droplets and in the uptake of free fatty acids. Perilipin and ADRP exhibit sequence similarities in their N-terminal ~300 amino acids. We now show that *Dictyostelium* and *Drosophila* express related LSD (Lipid Storage Droplet) proteins, that have ~25% identity and ~45% similarity to the ADRP/Perilipin family of proteins over their N-termini. These proteins, tagged with GFP, target exclusively to lipid droplets when expressed in mammalian cells. We speculate that the *Dictyostelium* proteins serve as essential regulators for lipogenesis, lipolysis, or packaging and trafficking of neutral lipid storage droplets. To identify critical elements for cellular function and targeting, we have initiated a series of expression and mutagenesis studies in *Dictyostelium.*

46. CHARACTERIZATION OF CALRETICULIN AND CALNEXIN, AND DYNAMICS OF THE ENDOPLASMIC RETICULUM IN *DICTYOSTELIUM.* Annette Mueller-Taubenberger, Mary Ecke, Andrei N. Lupas[*], Hewang Li, Paul R. Fisher[+], Evelyn Simmeth, and Guenther Gerisch, Cell Dynamics Group, Max-Planck-Institute for Biochemistry, Am Klopferspitz 18A, D-82152 Martinsried, Germany; [*]SmithKline Beecham Pharmaceuticals UP1345, 1250 South Collegeville Road, Collegeville, PA 19426-0989, USA; [+]Microbiology Department, La Trobe University, Bundoora VIC 3083, Australia.

Calreticulin and calnexin are ubiquitous Ca^{2+}-binding proteins, located in the endoplasmic reticulum (ER). Both proteins have well-recognized physiological roles in the ER as molecular chaperones and regulators of intracellular Ca^{2+}-homeostasis. In addition to that a variety of functions have been ascribed to calreticulin, both in and outside of the ER. *Dictyostelium* is the only known microorganism that contains both, calreticulin and calnexin. A comprehensive phylogenetic analysis comparing the sequences from protozoans, metazoans, *Dictyostelium* and plants revealed that the two proteins are monophyletic, although they share related sequence elements.

Calnexin-GFP and GFP-calreticulin proved to be brilliant markers of the ER membrane system. We use them to investigate shape changes of the ER in live *Dictyostelium* cells at different stages of development, in particular during mitotic cell division and in response to chemoattractant.

To characterize the function of calreticulin and calnexin in vivo we produced single and double mutants. *Dictyostelium* single mutants deficient for either calreticulin or calnexin reveal only subtle defects. On the contrary, calreticulin/calnexin double mutants are severely impaired in growth and development. Furthermore, deficiency of both calreticulin and calnexin causes a loss of phototactic and thermotactic orientation.

47. FOLATE RECEPTION BY VEGETATIVE *DICTYOSTELIUM DISCOIDEUM* AMOEBAE: DISTRIBUTION OF RECEPTORS AND TRAFFICKING OF LIGAND. Jared L. Rifkin, Biology Department, Queens College of CUNY, Flushing, NY 11367-1597, USA, jared-rifkin@qc.edu.

We report the first explicit demonstration of post-reception processing of a *Dictyostelium* chemoattractant. Folic acid stimulates reorganization of the cytoskeleton of vegetative amoebae of *D. discoideum*. In particular, folic acid is a potent chemoattractant and it causes enlargement of the filopodial array. The distribution of folic acid receptors and the fate of bound folate was investigated by presenting an agonist consisting of the conjugate, folic acid-lactalbumin-FITC (Folate*), to these amoebae. This novel probe was specifically bound to folic acid receptors of these amoebae and it stimulated chemotaxis and enlargement of their filopodial array. Hence, Folate* is a physiologically competent probe. The probe sans-folate moiety was not bound anywhere to living or fixed amoebae. Since Folate* did not bind to amoebae after incubation with equimolar folic acid, this probe is a receptor-specific agonist. We report here the first description, by confocal visualization of a competent agonist, of the distribution of folate receptors of *D. discoideum* vegetative amoebae and of the fate of this ligand. Examination of fixed amoebae revealed that bound Folate* was distributed generally over their entire surface including their filopodia. However, in living amoebae, Folate* was bound only at the cell body and this bound Folate* was almost completely internalized as concentrated packets into vacuoles. This endocytosis of the probe and the clustering of endocytosed Folate* is consistent with receptor-mediated internalization of a ligand. Possible routes for internalization of the folate probe and the implications of this endocytosis for signal molecule processing and temporal sensing are discussed.

48. THE ROLE OF A UBIQUITIN PROCESSING PROTEASE, UbpA, IN THE GROWTH-TO-DEVELOPMENT TRANSITION OF *DICTYOSTELIUM* DEVELOPMENT. Michael A. Sanz and David F. Lindsey, Department of Biological Sciences, Walla Walla College, College Place, WA, USA.

The *Dictyostelium* ubiquitin processing protease, UbpA, is required for the growth-to-development transition, and may be involved in the down-regulation of biosynthetic genes or repression of the cell cycle. UbpA is a functional homolog of yeast Ubp14 and human isopeptidase T, enzymes which appear to function largely, if not exclusively, in the disassembly of free ubiquitin chains. Control of cellular ubiquitin chain levels by UbpA may provide a mechanism for modulating the rates of protein degradation by the proteasome during changing developmental conditions.

We investigated whether specific genes implicated in the growth-to-development transition are controlled by *ubpA*-dependent mechanisms. Neither a null mutation in *pufA* nor overexpression of *yakA* suppress the *ubpA* defect. Upon starvation, the PKA-C mRNA level in *ubpA*-null cells appears as normal; however, the up-regulation of genes downstream of PKA activity and required for early development does not occur. These results suggest that UbpA is important for some stage between activation of PKA-C and up-regulation of developmental genes. However, when starved, *ubpA*-null cells fail to decrease the level of transcripts for CprD, a growth-stage cysteine proteinase, and growing cells have barely detectable levels of *lmcA* (V4) RNA. In addition, *rtoA* is expressed at much higher levels in *ubpA*-null cells. These results suggest that UbpA may be involved in the down-regulation of biosynthetic genes or repression of the cell cycle. It is possible that these processes must occur for efficient upregulation of genes required for development. We have isolated several putative second site suppressors of the *ubpA* mutation that allow formation of aberrant finger or culminate structures. The deduced amino acid sequence of one gene was similar to Ras-like proteins and another had similarity to histidine kinases.

49. A NOVEL Cdc2-RELATED KINASE IS REQUIRED FOR TRANSITION FROM GROWTH TO DIFFERENTIATION IN *DICTYOSTELIUM*. Kosuke Takeda, Tamao Saito, and Hiroshi Ochiai, Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo, 060-0810, Japan.

Using a novel terminator-REMI method, we have isolated 30 aggregateless mutants from 30,000 transformants. We recovered the flanking sequences of these mutants by 3'RACE and determined their sequences. Out of these clones whose the flanking sequences were recovered, 9 clones were mutant clones inserted into the known genes such as acaA, pka-C, erkB, and piaA. As one of the residual aggregateless clones, we are analyzing the mutant, which has a defect in cdc2-related kinase gene which are expressed in growing cells and also during late development. Analyses of the genomic DNA and the cDNA indicate that the gene codes for a predicted protein consisting of 380 amino acids with an intron (77bp). Homology search with BlastP algorithym revealed that deduced amino acids sequence of the gene has 46.2% identity with human cdk8. Cdk8 is known as a member cdc2-related kinases that are a conserved group of eukaryotic serine/threonine protein kinases and involved in the control of cell division and of development. We call this gene as Ddcdk8. The Ddcdk8-null cell grows somewhat more slowly in a shaking axenic medium and on bacterial plates than Ax2 cells. Interestingly Ddcdk8-null cell does not aggregate on bacterial plates. We confirmed recapitulation of this phenotype in wild-type Ax2 cells, the result indicating that the putative Ddcdk8 is required for transition from growth to differentiation.

50. MAKING ts MUTANTS IN ESSENTIAL GENES IN *DICTYOSTELIUM*. Chris Thompson, and Mark S Bretscher, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England.

We decided to try to make a ts mutant in an essential gene in *Dictyostelium discoideum* Ax2, and selected the single gene encoding NSF (the NEM-sensitive factor which appears to be required in all membrane fusion processes) that exists in Dicty (Weidenhaupt et al, 1998) as a target. Our strategy was basically to adapt the current procedure used for gene knockouts, hoping to replace the endogenous gene with one putatively encoding a temperature sensitive allele. There are several temperature-sensitive mutants in NSF in *Drosophila* and two of these, *comatose 4* and *6*, occur in highly conserved regions. We therefore constructed a Dicty NSF vector which contained, in the linear mutagenic portion: about 300bp upstream of the gene, the gene itself (including a 100 bp intron, ~2200bp in all) containing the *comatose 4* site (at position1270 from the 5' end of the vector) or *6* site (position1640), followed by 200bp trailer, a blasticidin casette (1350bp) and a further 1300bp of 3' genomic sequence.

These constructs were electroporated into Ax2 in the same manner as for gene knockouts. The cells were then parcelled out into wells and clones selected for drug resistance. These were recloned, grown up and the DNA from 18 of each mutant subjected to Southern blotting. This showed that in each case, 17/18 clones had arisen by homologous recombination. In the case of comatose 4, none of the clones contained the desired mutation; conversely, in the case of comatose 6, all of them contained the desired site. Furthermore, in clones in which an in-frame termination codon had been placed just after the comatose 6 site, all 12 clones examined had recombined homologously, but none now carried the comatose 6 mutation. This shows that the gene is essential and suggests that when it contains the *comatose 4* site, the encoded NSF is non-functional. The *comatose 6* clones had, unfortunately, no new phenotype.

We therefore made a mutagenic library in which the last 1000bp of coding sequence was made by PCR mutagenesis. Ax2 cells were transformed as before and positive wells recloned. About 1500 such clones were generated and each was screened for growth at 22˚C and 26.5˚C. From this, 11 ts mutants were recovered; they all arose by homologous recombination. Their properties are under study.

Reference: Weidenhaupt,M., Bruckert,F. and Satre, M. (1998) Gene 207, 53-60.

51. STUDIES ON A RelA/SpoT LIKE-PROTEIN OF *DICTYOSTELIUM*. Yan Zhang and Charles K. Singleton, Department of Biological Science, Vanderbilt University, VU Station B 35163, Nashville, TN 37235-1634, USA.

Bacteria shut down a wide range of activities when they find themselves in poor growth conditions that lack a sufficient supply of amino acids to sustain protein synthesis(the stringent response). In normal protein synthesis, the presence of aminoacyl-tRNA in the A site is a signal for peptidyl transferase to transfer the polypeptide chain. Under amino acid starvation, the presence of unchanged tRNA in the A site of the ribosome activates the RelA protein. The gene *relA* encodes a (p)ppGpp synthetase. Activated RelA causes the accumunation of (p)ppGpp which, among other things, inhibits the transcription of stable RNA by binding to target proteins to alter their activities. When amino acids become avaible, ppGpp is removed. The enzyme that catalyzes the degradation of ppGpp is called SpoT.

Under the environment of nutrient deprivation(amino acid starvation) and high cell density, *Dictystelium discoideum* cells will aggregate to form a fruiting body to resist various environmental assaults. The amino terminus of the protien predicted from the cDNA clone SSA601(*Dictyostelium* cDNA project in Japan) encompasses a region of about 150 amino acids that shows around 25% identity to the sequence of various bacterial RelA/SpoT proteins. Most of highly conserved residues are found in the *Dictyostelium* protien, including key residues required for catalysis and (p)ppGpp synthesis. We wondered if a “stringent-like response”, mediated by this RelA/SpoT-like protein, might be involved in sensing starvation and initiation development.

We will report on our attempts to disrupt the RelA/SpoT-like gene and on a possible stringent-like response in *Dictyostelium.*

52. REGULATION OF MEK1 BY SUMOYLATION AND ASSOCIATION WITH THE RING FINGER PROTEIN MIP1 DURING CHEMOTAXIS. Alex Sobko, Hui Ma, and Richard A. Firtel, Section of Cell and Developmental Biology and Center for Molecular Genetics, University of California, San Diego, 9500 Gilman drive, La Jolla, CA 92093-0634, USA.

SUMO, or small ubiquitin-like modifier, is a protein homologous to ubiquitin that post-translationally modifies target proteins by covalent conjugation of a single SUMO moiety. Sumoylation is thought to control a variety of protein functions, including subcellular localization. Previous work in our laboratory demonstrated that MEK1, a MAP kinase kinase, is required for proper chemotaxis. Here we demonstrate that MEK1 is modified by SUMO and that this modification is involved in MEK targeting. In vegetative and aggregation-competent cells, MEK1 resides predominantly in the nucleus and is not sumoylated. In contrast, MEK1 becomes sumoylated and localizes to actin-enriched cortex in response to cAMP stimulation and in the lamellipodia of chemotaxing cells. We have identified the lysine residue in MEK1 that is the site of SUMO conjugation. Expression of the MEK1 in which this lysine residue is mutated to arginine results in aggregation defects. Moreover, this protein remains nuclear in response to chemoattractant stimulation and thus does not effectively translocate to the plasma membrane. These results suggest that sumoylation may be part of the mechanism regulating MEK1 relocalization in response to chemoattractants.

Through a two-hybrid screen, we identified a new RING finger protein that is thought to be a member of the E3 ubiquitin ligase family of proteins. The non-RING finger portion of MIP1 interacts with MEK1 on a domain that lies upstream from the kinase domain. In addition to being sumoylated, MEK1 is ubiquitinated. Ubiquitination of MEK1 is reduced in *mip1* null cells, which also exhibit chemotaxis defects. Moreover, MEK1 shows a significantly reduced nuclear localization in *mip1* null cells, suggesting an interplay between ubiquitination and sumoylation in the control of MEK1 function and that MIP1 may function to localize MEK1 to the nucleus in unstimulated cells. Overexpression of MIP1 or the RING domain of MIP1 results in an inhibition of MEK1 translocation from the nucleus to the cytoplasm. This negative regulatory role of MIP1 on MEK1 is also consistent with the aggregation defects observed upon MIP1 overexpression. Our current data support the model in which MEK1-MIP1 association and cAMP-mediated sumoylation are involved in the dynamic subcellular targeting of MEK1 during chemotaxis and aggregation.

53. THE ROLE OF A PHOSPHOLIPASE D IN QUORUM SENSING AND DEVELOPMENT IN *DICTYOSTELIUM DISCOIDEUM.* Vanessa Rodrick, Yi Yan, Tarek Abbas, and Derrick T. Brazill, Department of Biological Sciences, Hunter College, 695 Park Avenue, New York, NY 10021, USA.

Quorum sensing, the ability to measure the local density of a population of cells, plays an important role in the initiation of development in *Dictyostelium discoideum*. Starving *Dictyostelium* are able to calculate the concentration of other starving cells by simultaneously sensing and secreting a glycoprotein called conditioned medium factor (CMF). When the density of starving cells is high, the corresponding high density of CMF allows signal transduction through the chemoattractant cAMP receptor cAR1 to occur, and only then will aggregation proceed. Binding of cAMP to cAR1 activates a heterotrimeric G protein whose alpha subunit is Gα2. We have shown previously that CMF regulates cAMP signal transduction in part by decreasing the cAMP stimulated GTPase activity of Gα2. CMF accomplishes this regulation by activating a signal transduction cascade involving Gα1, phospholipase C (PLC) and protein kinase C (PKC).

To discover other proteins involved in CMF regulation of Gα2, we have examined proteins that might act downstream of phospholipase C and upstream of Gα2. In mammals, PKC is known to stimulate a number of enzyme activities including phospholipase D (PLD). Because of this, we decided to examine the role of PLD in quorum sensing and general *Dictyostelium* development. A search of the genomic database revealed a gene with high homology to mammalian PLD1. We have found that this gene is developmentally regulated with a peak of expression at 18 hours. Using homologous recombination, we created a mutant strain disrupted for this gene. While these cells are able to form fruiting bodies, they do so at a highly accelerated rate and are able to complete development in 14 hours, arguing the PLD may be involved in regulating the timing of development. In addition, these cells are able aggregate at very low cell densities, suggesting that PLD may play a role in quorum sensing.

INDEX AND LIST OF PARTICIPANTS

INDEX

Yan, Y 15, 132

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