Annual International *Dictyostelium* Conference

2013

August 4th to 8th

DoubleTree by Hilton

Asheville, NC

Organizers:

Paul Steimle and Chris Janetopoulos

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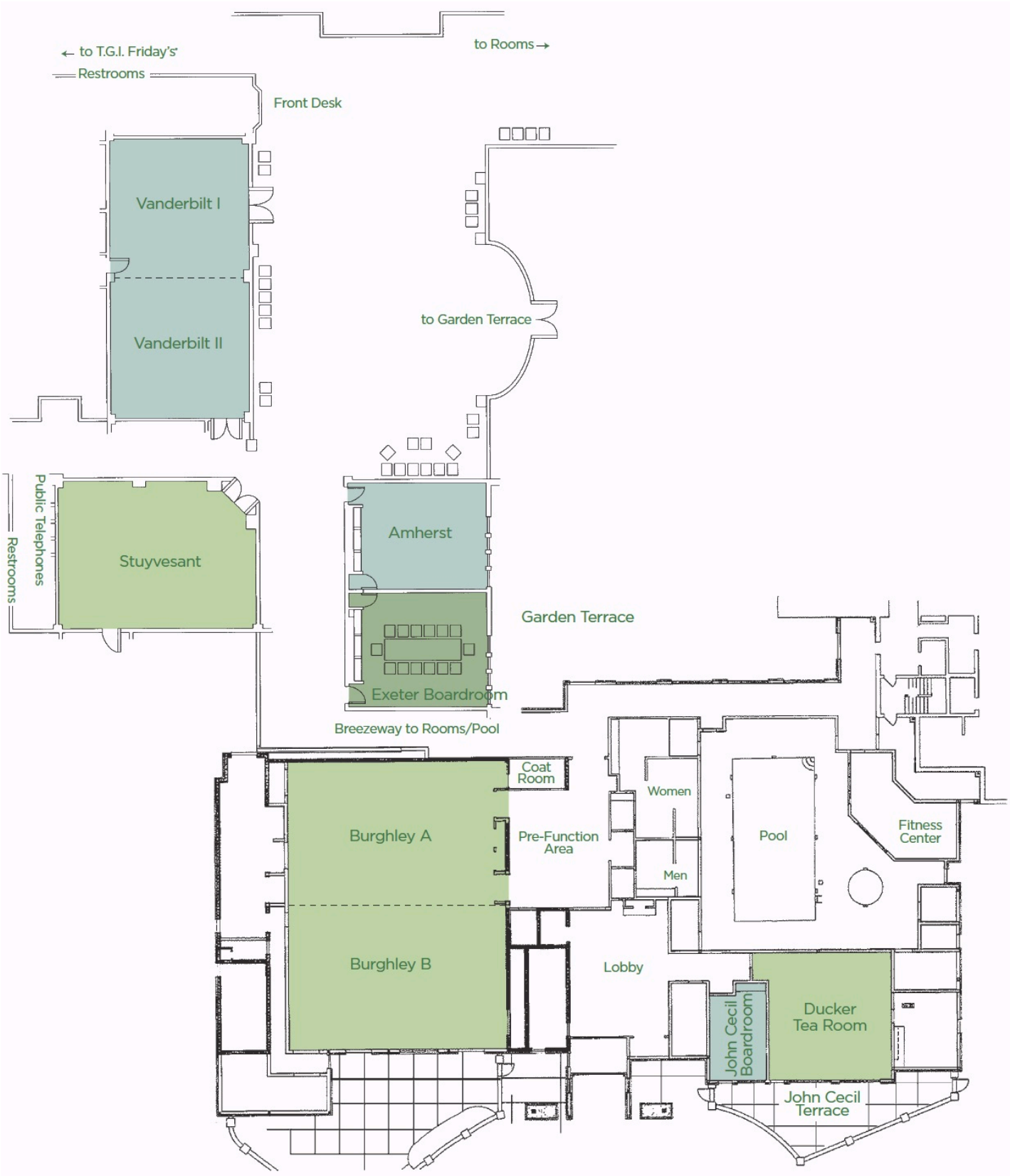
DoubleTree by Hilton Floor Plan



Dicty 2013 Meeting Program

Sunday, August 4th

4:00-6:30 PM	**Check-in**	***Main Lobby***
7:00-8:00 PM	**Keynote Seminar**	***Burghley Ballroom***

Michael W. Davidson

"*Recent Advances in Fluorescent Protein Technology* "
National High Magnetic Field Laboratory
Florida State University

8:00-10:00 PM	**Reception**	***Burghley Terrace***

Monday, August 5th

7:30-8:30 AM	**Breakfast**	***Garden Terrace***
9:00-10:15 AM	**Session I. Transcriptional regulation** Chair: Gad Shaulsky	***Burghley Ballroom***
Nicole Gruenheit	*Antisense transcription – just noise or another layer of regulation*	
Ludwig Eichinger	*Identification of the protein kinases Pyk3 and Phg2 as regulators of the STATc mediated response to hyperosmolarity*	
James Platt	*Chromatin re-modeling and Dictyostelium development*	
10:15-10:45 AM	**Coffee Break**	***Burghley Ballroom***
10:45 AM-12:00	**Session II. *Dictyostelium* Signaling I** Chair: Pascal Charest	***Burghley Ballroom***
Seido Nagano	*The role of G-protein dynamics in adaptation and spontaneous cAMP oscillation in Dictyostelium*	
Jeff Hadwiger	*G protein signaling pathways that regulate phosphodiesterase activity and development*	
David Ratner	*Interactions between Dictyostelium histidine kinases and the cAMP phosphodiesterase RegA*	
12:00-1:30 PM.	**Lunch** (dictyBase Scientific Advisory Board Meeting)	***Garden Terrace*** ***Exeter Boardroom***

1:30-2:45 PM **Session III. Chemotaxis I** ***Burghley Ballroom***
Chair: Jason King

Wouter-Jan Rappel *Dynamics of pseudopod competition in chemotaxing Dictyostelium cells*

Thomas Lampert *Utilizing cell-substrate adhesion to discover novel regulators of directed cell migration*

Pascale G. Charest *Tight regulation of TORC2 function in chemotaxis*

2:45-5:45 PM **Afternoon Break**

5:45-7:15 PM **Dinner** ***Garden Terrace***

7:20-8:15 PM **Session IV. Dynamic Movements** ***Burghley Ballroom***
Chair: Cynthia Damer

Annette Müller-Taubenberger *Organization and dynamics of microtubules in Dictyostelium*

Huaqing Cai *Nucleocytoplasmic shuttling of a GATA transcription factor functions as a developmental timer*

8:15-10:15 PM **Poster Session**(Odd numbered posters) ***Vanderbilt Room***

Tuesday, August 6th

7:30-8:30 AM **Breakfast** ***Garden Terrace***

9:00-10:15 AM **Session V. Chemotaxis II** ***Burghley Ballroom***
Chair: **Chris Janetopoulos**

Miho Iijima *Rho GTPases Orient Directional Sensing in Chemotaxis*

Douwe Veltman *PIP3-dependent macropinocytosis is incompatible with chemotaxis*

Chuan-Hsiang Huang *Cell migration is mediated by coupling an excitable signaling network to an oscillatory cytoskeletal network*

10:15-10:45 AM **Coffee Break** ***Burghley Ballroom***

10:45 AM-12:00 **Session VI. Membrane Dynamics, Lysosomes, And Symbiosis** ***Burghley***
Chair: Paul Kreibel

Kari Naylor *Mitochondrial Fission and Fusion in Dictyostelium discoideum: a search for proteins involved in membrane dynamics*

Jason King *WASH is required for lysosomal recycling and efficient autophagic and phagocytic digestion*

Debra A Brock *Food or defensive symbiont: How a stop codon can change your fate*

12:00-1:30 PM	**Lunch**	***Garden Terrace***
1:30-3:25 PM	**Session VII. Dicty And Disease** Chair: Robin Williams	***Burghley Ballroom***
	Robin Williams	*Dictyostelium as a pharmacological model system in medical research*
	Anna Frej	*Development of a first-choice non-animal model for bipolar disorder research*
	Coffee Break (2:20-2:35 PM)	
	Alexandra Surcel	*Dicty on drugs: Characterizing a novel small molecule regulator of myosin-II dynamics*
	Michael Myre	*Mutant full length human huntingtin confers an in vivo gain-of-function on the ordered extension of pseudopodia in Dictyostelium*
3:25-5:45 PM	**Afternoon Break**	
5:45-7:15 PM	**Dinner**	***Garden Terrace***
7:20-8:15 PM	**Session VIII. *Dictyostelium* Signaling II** Chair: Derrick Brazill	***Burghley Ballroom***
	Adam Kuspa	*TirA signaling and the biochemical characterization of bacterial "extracellular traps" produced by Sentinel cells in D. discoideum*
	Allyson Sgro	*Understanding the dynamical origins of collective cAMP oscillations in Dictyostelium discoideum*
8:15-10:15 PM	**Poster Session**(Even numbered posters)	***Vanderbilt Room***

Wednesday, August 7th

7:30-8:30 AM	**Breakfast**	***Garden Terrace***
9:00-10:15 AM	**Session IX. Cytoskeleton and Adhesions** Chair: **Alexandra Surcel**	***Burghley Ballroom***
	Yidai Yang	*Dictyostelium PakB is involved in Maintenance of Cortical Tension Through Regulation of Myosin I*
	Derrick Brazill	*Dictyostelium PakD mediates quorum sensing by regulating the actin cytoskeleton*
	Paul Kriebel	*The Isolation, Proteomic Analysis and Function of Dictyostelium Exosomes*
10:15-10:30 AM	**Coffee Break**	***Burghley Ballroom***

	Bill Loomis	*Changes in cell-substrate adhesion during early development*
	Marco Tarantola	*Dictyostelium discoideum* substrate adhesion
11:20 AM-4:30 PM.	**Lunch and Excursions** Whitewater Rafting – Packed Lunches. Bus leaves at 11:45 AM. Biltmore Estate Tour – Buffet Lunch (Garden Terrace). Bus departs at 12:30 PM	
6:00-9:00 PM	**Banquet at Antler Hill Barn on Biltmore Estate** Bus to the banquet will leave at 5:30 PM	

Thursday, August 8th

7:30-8:30 AM	**Breakfast**	***Garden Terrace***
9:00-9:50 AM	**Session X. Evolution and Development** Chair: Susanne DiSalvo	***Burghley Ballroom***
	Tracy Douglas	*Selection on multiple mating types in Dictyostelium: more than two but fewer than many*
	Shashi Prakash Singh	*Molecular characterization of thyroxine 5' deiodinase (DIO3) in the cellular slime mold Dictyostelium discoideum*.
9:50-10:15 AM	**Coffee Break**	***Burghley Ballroom***
10:15-11:30 AM	**Session XI. Chemotaxis III** Chair: **Paul Steimle**	***Burghley Ballroom***
	Arjan Kortholt	*Essential roles for small G-proteins in the regulation of directional cell movement*
	Monica Skoge	*Chemotaxis in spatiotemporal waves: memory in Ras activation and the back-of-the-wave problem*
	Netrapal Meena	*A Novel Secreted Protein Potentiates Dictyostelium Aggregation at Low Cell Density*
11:30 AM	**DEPARTURE**	

ORAL PRESENTATIONS

Title: Antisense transcription – just noise or another layer of regulation

Authors: Nicole Gruenheit, William Salvidge, Suzanne Battom, Lauren Harkin, Christopher Thompson

Presenter: Nicole Gruenheit

Address for correspondence: Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester UK M13 9PT

Abstract: The discovery of non-coding RNAs has revealed intriguing new insights into the specificity and complexity of gene regulation. One type of long non-coding RNAs are the natural antisense transcripts (NATs), which for decades have been considered as transcriptional noise. Recently, however, a small number of NATs have been identified that regulate cell differentiation and development throughout the tree of life. One problem with these studies, however, is that they tend to focus on a single transcript. This is because whole genome studies, and especially cell type specific analyses, have proven to be difficult, due to genome size and complexity of the respective organisms (e.g. human, mouse, Arabidopsis). Furthermore, other tractable small single celled organisms like yeast lack different developmental stages. We therefore decided to use *Dictyostelium*, combining a small genome, different developmental stages, and cell differentiation, to conduct a whole genome study elucidating the amount and potential regulatory role of NATs.

We compared whole transcriptomes of vegetative cells and whole slugs by strand-specific RNA-seq to determine 1) if there are NATs in *Dictyostelium* and 2) if there are changes in their expression between developmental stages. As expected, for the majority of the genes we found only sense transcription. Importantly, for a significant proportion - 698 genes - we also detected antisense transcription. To rule out annotation errors and 'run-over transcription' from an adjacent gene, we analysed these genes further with respect to their orientation and location in the genome, which indeed led to the exclusion of some genes. Importantly though, we were able to identify 348 genes, which not only had an NAT, but also significant changes in sense and antisense transcription between the stages. We then also compared expression levels in prespore and prestalk cells, which led to the identification of additional 562 genes with NATs. Finally, we are now employing molecular genetic techniques to our progress in understanding the role and regulation of NATs.

This study is the first to show that not only do NATs exist in *Dictyostelium* but they also play an important role in regulating development as well as cell differentiation. These studies thus establish *Dictyostelium* as a key model system to understand the regulation of NATs and their role in determining dynamic gene expression profiles during multicellular development.

Title: Identification of the protein kinases Pyk3 and Phg2 as regulators of the STATc mediated response to hyperosmolarity

Authors: Linh Hai Vu, Tsuyoshi Araki, Jianbo Na, Jeffrey G. Williams and Ludwig Eichinger

Presenter: Ludwig Eichinger

Address for correspondence: Ludwig Eichinger, Center for Biochemistry, Medical Faculty, University of Cologne, Joseph-Stelzmann-Str. 52, 50931 Cologne, Germany

Abstract: The cellular adaptation to changes in environmental osmolarity is crucial for cell survival. In *Dictyostelium discoideum*, STATc is a key regulator of the transcriptional response to hyperosmotic stress. Its phosphorylation and activation is controlled by two signaling branches, the cGMP- and the Ca2+-dependent branch, of which many signaling components have not yet been identified.
Based on microarray studies and BLAST searches, we chose two tyrosine-kinase like proteins, Pyk3 and Phg2, as possible modulators of STATc phosphorylation and generated single and double knock-out mutants. Transcriptional regulation of STATc and STATc dependent genes was disturbed in pyk3-, phg2-, and pyk3-/phg2- cells. The absence of Pyk3 and/or Phg2 resulted in diminished or completely abolished differential transcription of STATc dependent genes in response to sorbitol, 8-Br-cGMP and BHQ. Phospho-STATc levels were significantly reduced in pyk3- and phg2- cells and further decreased in pyk3-/phg2- cells. The reduced phosphorylation in these strains was mirrored by a significant delay in nuclear translocation of GFP-STATc. The protein tyrosine phosphatase 3 (PTP3), which dephosphorylates and inhibits STATc, is regulated by phosphorylation on S448 and S747. Use of phosphoserine specific antibodies showed that Phg2 but not Pyk3 is involved in the phosphorylation of PTP3 on S747. In pull-down assays Phg2 and PTP3 interacted directly suggesting that Phg2 phosphorylates PTP3 on S747 in vivo. Phosphorylation of S448 was unchanged in pyk3- as well as phg2- cells.
We show that Phg2 and an as yet unknown protein kinase are responsible for PTP3 phosphorylation and inhibition and that Pyk3 is involved in the regulation of STATc by either directly or indirectly activating it. In summary, our results add to the complex regulation of STATc which presumably ascertains its optimal activation in response to different environmental cues.

Title: Chromatin re-modelling and *Dictyostelium* development

Authors: James Platt, Mark Robinson, Alan Kimmel and Adrian J Harwood

Presenter: James Platt

Address for correspondence: Cardiff School of Biosciences Cardiff University Museum Avenue Cardiff CF10 3AX

Abstract: Nucleosomes are the basic unit of chromatin structure, and their relative positions determine higher order genome structure. Nucleosome positions are not randomly distributed, but instead are conserved between individual cells and preserved during DNA replication. Changes of nucleosome position and chromatin structure have been correlated with regulation of gene expression at the individual gene level. Furthermore, mutations in chromatin-remodelling genes are associated with several human diseases, including dermatomyositis, Hodgkin's lymphoma, neuroblastoma, and CHARGE syndrome. However, it remains to be established whether these individual changes can be extrapolated to the genome-wide scale, and how regulation of chromatin structure relate to developmental gene expression.

Dictyostelium offers a promising system in which to investigate the relationship between chromatin re-modelling and development. Both its pattern of nucleosome positions within coding regions and spectrum of chromatin re-modelling proteins are conserved with mammals, unlike that seen in yeast. However, its compact, gene-rich genome focuses epigenetic studies on gene rather than intra-genic regions. Here, we describe gene disruption and resulting phenotypes for each *Dictyostelium* chromatin re-modelling factors. These results indicate gene and phenotypic specificity of different chromatin re-modellers. Focussing on the mechanism of action of ChdC, a sub-family III CHD (Chromodomain-Helicase-DNA binding) protein, we show that for this re-modeller controls nucleosome spacing in a subset of genes, but only approximately 20% of these show altered gene expression. Nonetheless, there is a significant, but small, correlation between changes in nucleosome position and transcriptional control, suggesting chromatin structure influences genes expression, but may not be its primary function.

Title: The role of G-protein dynamics in adaptation and spontaneous cAMP oscillation in *Dictyostelium*

Authors: Seido Nagano and Shunsuke Sakurai(*)

Presenter: Seido Nagano

Address for correspondence: Department of Bioinformatics, Ritsumeikan University, 1-1-1 Nojihigashi, Shiga 525-8577, Japan,(*)Life Science Production Div., NOF Corporation, 5-10 Tokodai, Tsukuba 300-2635, Japan

Abstract: We propose a molecular network that controls the spontaneous cAMP oscillations in *Dictyostelium discoideum*. The double negative feedback by PKA and G-protein dynamics produced sustained cAMP oscillations. We clarify the relationship between G-protein dynamics and adaptation. Janetopoulos et al observed that G-protein activation steadily increased and reached a dose-independent steady-state level during continuous stimulation, and the activation did not decline as long as cAMP receptors were stimulated. Unless there is a constant supply of cAMP from outside, external cAMP density declines due to its degradation by PDE and diffusion. Our G-protein dynamics is completely consistent with such experimental observations. However, very low extracellular cAMP density (cAMPe) was observed in our numerical investigations. This low cAMPe density originated in the reported dissociation constant (Kd) value. When Kd value is increased by eight times or association rate constant is deceased, cAMPe density is enhanced six times. Although the reported Kd value was for the equilibrium state, the association of the ligand to the receptor is likely to be reduced due to the enhanced entropy when the pulsatile cAMP is being produced. Our cellular dynamics study with cAMP diffusion also confirmed enhanced cAMPe density when the cell density is high. However, the effect of cAMP association rate constant was more influential. Thus, we hope that our hypothesis of decreased association rate constant can be confirmed experimentally for the further quantitative modeling study.

Title: G protein signaling pathways that regulate phosphodiesterase activity and development

Authors: Jeffrey Hadwiger, David Schwebs, and Hoai-Nghia Nguyen

Presenter: Jeff Hadwiger

Address for correspondence: Department of Microbiology and Molecular Genetics Oklahoma State University Stillwater, OK 74078-3020

Abstract: *Dictyostelium* has a least two G protein-mediated signaling pathways that regulate cAMP levels in response to external signals. The stimulation of these pathways can result in the activation of ERK2, a MAP kinase (MAPK) that down regulates the phosphodiesterase RegA and thereby increases the levels of cAMP. We have analyzed the role of RegA in these G protein-mediated signaling pathways by disrupting the regA gene in G protein and MAPK mutants and then characterizing developmental morphology and gene expression. In most strains, the regA gene disruption had little impact on developmental morphology but did promote the localization of cells to the pst O/prespore border region and precocious prestalk gene expression. The regA gene disruption accelerated and greatly increased spore production of gα4- mutants but not in cells overexpressing the Gα4 subunit. Sporulation of gα2- mutants was not rescued by the disruption of the regA gene suggesting a requirement for Gα2 function in sporulation that cannot be bypassed through the loss of RegA. Disruption of the regA gene corrected the delayed development of gα5- mutants but resulted aberrant fruiting body morphology. These findings suggest that G protein signaling pathways activate multiple cellular responses in addition to the regulation of cAMP signaling.

Title: Interactions between *Dictyostelium* histidine kinases and the cAMP phosphodiesterase RegA

Authors: James Murithi, Mizuho Ota, Sooyoung Lim, and David Ratner; Department of Biology, Amherst College, Amherst MA

Presenter: David Ratner

Address for correspondence: Dr. David Ratner, Department of Biology, Amherst College, Amherst MA, USA, 01002

Abstract: RegA, a developmentally regulated cAMP phosphodiesterase of *Dictyostelium discoideum*, is activated by phosphorylation of an aspartic acid residue[1]. The histidine kinases that initiate activation are thought to vary over the course of development. While experimental evidence suggests roles for both DhkA[2] and DhkC[3], among others, the nature of their effect is open to question. Since His-Asp phosphorelay is reversible, with these enzymes and/or other response regulator domain proteins acting also as phosphatases, a variety of potentially interactive pathways are possible[4, 5]. Previously we showed that dhkA null mutation, like that of regA, partially suppresses the severe sporulation deficiency of an fbxA knockout mutant[2]. In the present work, we ask if mutation of dhkC similarly rescues the loss of the FbxA E3 ubiquitin ligase. By examination of developmental morphology, sporulation efficiency, and developmental gene expression in fbxA/kinase single and double null mutants, we infer that DhkA (as opposed to DhkC) is primarily responsible for RegA activation. Direct measurement of RegA's phosphodiesterase activity immunoprecipitated from crude cell extracts confirms the need for activation by DhkA throughout much of development. Preliminary measurements of the effects of fbxA and rdeA mutation upon RegA enzymatic activity will also be presented.

1. Thomason, P.A., et al., Embo J., 1998. 17: p. 2838-2845.
2. Tekinay, T., et al., Eukaryotic Cell, 2003. 2(3): p. 618-626.
3. Singleton, C.K., et al., Dev. Biol., 1998. 203: p. 345-357.
4. Anjard, C. and W.F. Loomis, Proc. Natl. Acad. Sci. USA, 2005. 102: p. 7607-7611.
5. Schaap, P., Development, 2011. 138(3): p. 387-96.

Title: Dynamics of pseudopod competition in chemotaxing *Dictyostelium* cells

Authors: Alexandre Colavin, Monica Skoge, Albert Bae, Herbert Levine, William F. Loomis and Wouter-Jan Rappel

Presenter: Wouter-Jan Rappel

Address for correspondence: Department of Physics and Center for Theoretical Biological Physics, UC San Diego, La Jolla, CA

Abstract: In order to achieve persistent and directed movement during chemotaxis, pseudopod extensions in moving cells must be restricted to the front of the cell and inhibited elsewhere. It has been proposed that the required inhibitory communication can be mediated through either the establishment of an internal compass biasing the placement of otherwise independent pseudopods or through a fast-acting global inhibitor actively produced by competing pseudopods. Here we report quantitative observations of the dynamics of pseudopods in chemotaxing *Dictyostelium* cells. We show that coexisting pseudopods often exhibit oscillatory behavior, suggesting a global inhibitory mechanism. Using a novel moving-boundary cell motility simulator, we show that the observed oscillatory dynamics is consistent with pseudopod competition mediated either by a diffusible inhibitor or by membrane tension.

Title: Utilizing cell-substrate adhesion to discover novel regulators of directed cell migration

Authors: Thomas J. Lampert, Peter N. Devreotes

Presenter: Thomas Lampert

Address for correspondence: Department of Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA

Abstract: The model organism *Dictyostelium* has greatly facilitated our understanding of the signal transduction and cytoskeletal pathways that govern cell migration. As cell-substrate adhesion is a target of many chemotaxis signaling events, it can be used to screen for cells that have defects in cell migration. In fact, cells lacking PTEN, a negative regulator of cellular extensions, adhere strongly to the surface. Reasoning that other regulators of migration would also affect adhesion, I devised a screening method and isolated overly adherent mutants from a pool of REMI insertions strains. Over 100 REMI transformations comprising about 50000 insertions yielded about 40 mutant strains with the desired phenotypes. The insertion sites in 18 of the strains have been mapped and many of the phenotypes are similar to those of PTEN knockout cells. With few exceptions, the strains from the REMI screen have increased adhesion, decreased motility, and increased filamentous actin at their periphery. Some of the targeted genes are actin binding, ring finger, kinase, and phosphatase domain containing proteins. Many have human homologs with unknown functions. Therefore, the future study of this new group of regulators of adhesion and motility in *Dictyostelium* will not only advance the knowledge of cell migration in amoeboid cells but elucidate the functions of novel human genes with potential disease relevance.

Title: Tight regulation of TORC2 function in chemotaxis

Authors: Nieves Montano, Pouya Lotfi, Zhouxin Shen, Steve Briggs, Arjan Kortholt, and Pascale G. Charest

Presenter: Pascale G. Charest

Address for correspondence: University of Arizona, Department of Chemistry and Biochemistry, 1041 E. Lowell Street, Tucson, AZ 85721-0088.

Abstract: The Target of Rapamycin Complex 2 (TORC2) has evolutionarily conserved roles in regulating the cytoskeleton and cell migration, and TORC2 is central to *Dictyostelium* and neutrophil chemotaxis. In *Dictyostelium*, chemoattractant stimulation leads to TORC2 activation at the leading edge of chemotaxing cells, in part, through the small GTPase RasC. Genetic analyses suggest that TORC2 is then involved in the integration and coordination of multiple processes underlying chemotaxis, including cell motility, cell polarity and relay of the chemoattractant signal cAMP. To better understand how cells achieve efficient directed migration, my group investigates the regulation and function of TORC2 in chemotaxis. Here, I will present data suggesting that, in addition to RasC, the small GTPase Rap1 and Protein Kinase A (PKA) coordinately regulate TORC2 activity during chemotaxis and may be part of a mechanism by which TORC2 integrates cell motility, cell polarity and signal relay.

Title: Organization and dynamics of microtubules in *Dictyostelium*

Authors: Matthias Samereier, Sabrina Schuller, Annette Müller-Taubenberger

Presenter: Annette Müller-Taubenberger

Address for correspondence: Department of Anatomy and Cell Biology, Medical Faculty, Ludwig Maximilian University of Munich, 80336 Munich, Germany

Abstract: Microtubules are dynamic cytoskeletal polymers with essential functions in cell division, shape generation, motility and signaling. While the actin cytoskeleton and associated proteins have been analyzed in detail in *Dictyostelium discoideum*, little is known about the regulators that determine and modulate the organization of the microtubule network. The microtubule network together with the centrosome polarize towards the leading edge of cells and microtubules elongate into newly formed protrusions, and thus microtubules are also important for the organization of the actin cytoskeleton. We are interested in the coordination of the cellular cytoskeleton and of microtubule functions during directed cell migration in fast moving cells such as *Dictyostelium* and neutrophils by exploring both systems under comparable experimental conditions.

Furthermore we have characterized the kinesin-7-like motor protein Kif11 as a slow motor that moves towards the plus-ends of microtubules. Our data suggest that a subclass of CENP-E motors have functions outside of mitosis.

Current work concentrates on the characterization of a spastin-homologous protein in *Dictyostelium*. In humans, mutations of the spastin gene cause hereditary spastic paraplegia (HSP), a neurodegenerative disease that is characterized by a progressive weakness of the lower limbs due to degeneration of the upper motoneurons. Spastin is a AAA-ATPase with microtubule-severing activity, but the mechanism how spastin severs microtubules is elusive. In order to investigate the biochemical activities of the *Dictyostelium* spastin, we have expressed and purified Flag-tagged spastin constructs and analyze their in-vitro properties.

Title: Nucleocytoplasmic shuttling of a GATA transcription factor functions as a developmental timer

Authors: Huaqing Cai, Mariko Katoh-Kurasawa, Yu Long, Balaji Santhanam, Gad Shaulsky, and Peter N. Devreotes

Presenter: Huaqing Cai

Address for correspondence: Johns Hopkins, Department of Cell Biology

Abstract: Self-organized propagated waves of extracellular cAMP observed in *Dictyostelium discoideum* represent one of the first known examples of biological oscillations. Periodic cAMP signals serve as chemoattractant and, by an unknown mechanism, time the acquisition of chemotactic ability and development of *Dictyostelium*. We show that a zinc-finger GATA transcription factor translates the information encoded in the oscillatory cAMP signaling into a distinct gene expression program that brings about robust chemotaxis and timely development. The transcription factor exhibits rapid nucleocytoplasmic shuttling during early development. This behavior requires the coordinated action of the nuclear localization signal and reversible receptor-mediated phosphorylation that promotes nuclear exit. Altering shuttling genetically or chemically predictably changes the transcriptional program and developmental phenotypes. In addition, cAMP generates an activation signal so that proper gene expression occurs only when cells detect repeated fold changes in stimuli. This mechanism may allow cells to count the number of stimuli they experience and tune gene expression accordingly.

Title: Rho GTPases Orient Directional Sensing in Chemotaxis

Authors: Miho Iiijima

Presenter: Miho Iijima

Address for correspondence: Department of Cell Biology, Johns Hopkins University School of Medicine

Abstract: During chemotaxis, cells sense extracellular chemical gradients and position Ras GTPase activation and PIP3 production toward chemoattractants. These two major signaling events are visualized by biosensors in a crescent-like zone at the plasma membrane. Here, we show that a *Dictyostelium* Rho GTPase, RacE, and a guanine nucleotide exchange factor, GxcT, control the orientation of the signaling crescent, but not its formation, independently of the actin cytoskeleton and cell polarity. Cells lacking RacE and GxcT fail to persistently direct Ras activation and PIP3 production toward chemoattractants, leading to lateral pseudopod extension and impaired chemotaxis. Active forms of RacE and human RhoA are located on the portion of the plasma membrane that faces lower concentrations of chemoattractants, opposite of Ras activation. Mechanisms that control the localization of active RacE require its effector domain, but not PIP3. Our findings reveal a critical role for Rho GTPases in positioning Ras activation and thereby establishing the accuracy of directional sensing.

Title: PIP3-dependent macropinocytosis is incompatible with chemotaxis

Authors: Douwe M. Veltman, David A. Knecht, Robert H. Insall and Rob R. Kay

Presenter: Douwe Veltman

Address for correspondence: MRC Laboratory of Molecular Biology Francis Crick Avenue Cambridge Biomedial Campus Cambridge CB2 0QH

Abstract: Pseudopods are the major drivers of cell motility and chemotaxis. New actin filaments in pseudopods are nucleated by the Arp2/3 complex, which in turn is activated by the SCAR/WAVE complex. We have examined the link between the SCAR/WAVE complex and its putative regulators: Rac, PIP3 and SH3-domain proteins using a cell biological approach. To maximize the significance of any findings we broadened the experimental scope by using NC4 as an additional cell strain and folic acid as an alternative chemoattractant. Important differences were observed between AX3 and NC4 and also between the different chemoattractants, especially regarding the relation between PIP3 and the SCAR complex in pseudopods. Moreover, colocalisation of active Rac and SCAR/WAVE at the leading edge does not comply with a simple 1:1 recruitment of SCAR/WAVE by active Rac. Combined results shed new light on the role signal amplification in cell migration and chemotaxis.

Title: Cell migration is mediated by coupling an excitable signaling network to an oscillatory cytoskeletal network

Authors: Chuan-Hsiang Huang, Ming Tang, Changji Shi, Pablo A. Iglesias, and Peter N. Devreotes

Presenter: Chuan-Hsiang Huang

Address for correspondence: Department of Cell Biology, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA

Abstract: It is generally thought that cytoskeletal activity drives random cell migration and that signal transduction events initiated by receptors can bias the motility apparatus to guide cells. However, we find that the cytoskeletal network, involving Scar/Wave or Wasp, Arp 2/3, and actin binding proteins is only capable of generating rapid oscillations, which cause small undulations of the cell boundary, but not large protrusions that lead to cell migration. Instead a slower signal transduction network, comprising multiple pathways that include Ras and PI3K, is required to generate large protrusions by engaging the cytoskeletal network through Rac. The signal transduction network is excitable, displaying wave propagation, refractoriness, and maximal response to suprathreshold stimuli, even in the absence of the cytoskeleton. We suggest that various chemical, mechanical or electrical cues that guide cell migration are integrated to modulate the threshold for excitability, and that protrusions result from coupling of "pacemaker" signal transduction and "idling motor" cytoskeletal networks. Our findings represent a novel view of cell migration that helps to explain many apparently disparate observations, including the ultrasenstivity of migrating cells to guidance cues.

Title: Mitochondrial Fission and Fusion in *Dictyostelium discoideum*: a search for proteins involved in membrane dynamics

Authors: Brixey Schimmel, Gregory Berbusse, Lakin Woods, Kari Naylor

Presenter: Kari Naylor

Address for correspondence: University of Central Arkansas 201 Donaghey Ave Conway AR, 72035

Abstract: Mitochondrial morphology is maintained by two distinct membrane events -fission and fusion. Altering these conserved processes can disrupt mitochondrial morphology and distribution, thereby disrupting the organelle's functionality and impeding cellular function. In higher eukaryotes, these processes are mediated by a family of dynamin-related proteins (DRP's). In the lower eukaryotes, for instance *Dictyostelium discoideum*, mitochondrial fission and fusion have been implicated but not yet established. To understand the overall mechanism of these dynamics across organisms, we developed an assay to identify fission and fusion events in *Dictyostelium* and to assess the involvement of the mitochondrial proteins, MidA, CluA, and two DRPs, DymA and DymB. Using laser scanning confocal microscopy to observe real time movement of fluorescently labeled *D. discoideum* mitochondria, we show that these lower eukaryotes do indeed mediate mitochondrial fission and fusion. In *Dictyostelium*, these processes are balanced, occurring approximately 1 event/minute. Quantification of the rates of fission and fusion in midA-, cluA-, dymA-, or dymB- strains established that MidA appears to play an indirect role in the regulation of fission and fusion, while the DRP's are not essential for these processes. Rates of fission and fusion were significantly reduced in cluA- cells, indicating that CluA is necessary for maintaining both fission and fusion.
Mitochondrial dynamics are intimately linked to mitochondrial motility thus we have also assessed the motility of the mitochondria in these mutants to ensure there is no underlying motility defect. Our preliminary data suggests that there is no change in the rate of mitochondrial movement in any of these strains. In conclusion, we have successfully demonstrated that *Dictyostelium* mitochondria undergo the dynamic processes of fission and fusion. The classical mediators of membrane dynamics, the DRP's, are not necessary for these dynamics, whereas CluA is necessary for both processes and the loss of the DRPs, CluA, nor MidA affect the rate of mitochondrial movement. This work contributes to our overall understanding of mitochondrial dynamics and ultimately will provide additional insight into mitochondrial disease.

Title: WASH is required for lysosomal recycling and efficient autophagic and phagocytic digestion

Authors: Jason S. King, Aurélie Gueho, Monica Hagedorn, Navin Gopaldass, Florence Leuba, Thierry Soldati and Robert H. Insall

Presenter: Jason King

Address for correspondence: Beatson Institute for Cancer Research, Glasgow, UK.

Abstract: WASH is an important regulator of vesicle trafficking. By generating actin on the surface of intracellular vesicles, WASH is able to directly regulate endosomal sorting and maturation. Here we report that in *Dictyostelium*, WASH is also required for the lysosomal digestion of both phagocytic and autophagic cargo. Consequently, *Dictyostelium* cells lacking WASH are unable to grow on many bacteria, or digest their own cytoplasm to survive starvation. WASH is required for efficient phagosomal proteolysis, and proteomic analysis demonstrates that this is due to reduced delivery of lysosomal hydrolases. Both protease and lipase delivery are disrupted, and lipid catabolism is also perturbed. Starvation-induced autophagy therefore leads to phospholipid accumulation within WASH null lysosomes. This causes the formation of multilamellar bodies typical of many lysosomal storage diseases. Mechanistically, we show that in cells lacking WASH, cathepsin D becomes trapped in a late endosomal compartment, unable to be recycled to nascent phagosomes and autophagosomes. WASH is therefore required for the maturation of lysosomes to a stage where hydrolases can be retrieved and reused.

Title: Food or defensive symbiont: How a stop codon can change your fate

Authors: Debra A Brock, Pierre Stallforth, Alexandra M. Cantley, Xiangjun Tian, David C. Queller, Joan E. Strassmann, and Jon Clardy

Presenter: Debra A Brock

Abstract: Agricultural crops are valuable investments that could be exploited by others. We have previously shown farmer clones of the social amoeba *Dictyostelium discoideum* carry bacteria through the dispersing spore stage to establish new food populations, but they also carry other non-food bacteria. Why carry food they cannot eat? Here we investigate two strains of *Pseudomonas fluorescens* associated with one farmer clone. Only one of the *Pseudomonas* strains serves as a food source for the farmer. We show the non-food strain over-produces two diffusible small molecules: chromene and pyrrolnitrin. Small molecules can regulate beneficial interactions between bacterial symbionts and their eukaryotic hosts as seen in other systems such as fungus-farming ants and bark beetles. We find chromene increases the farmer's spore production and harms spore production of non-farmers suggesting a beneficial association for the farmer. We report similar results with pyrrolnitrin. Thus, these two farmer-associated *P. fluorescens* strains, though genetically similar, have different functional roles. Next we used whole genome sequences and phylogenetic analyses to further investigate why one strain is an inedible defensive symbiont and the other strain is an edible food. We identified a premature stop codon in GacA, the response regulator of a bacterial GacA-GacS two-component regulatory system as a candidate for the functional role difference between these two strains. We generated a knockout in the non-food strain of this same regulatory gene and determined this deletion is sufficient to alter the chemical profile of the non-food bacteria and convert it into an edible food source for the host farmer. We also examined how closely related these two strains carried by a single host farmer may be. Phylogenetic evidence suggests that a single mutation in an inedible ancestral strain that served a protective role converted it to a 'domesticated' food source. Therefore, taken together, we show the farmer/host-associated bacteria symbiosis displays some of the same multipartite qualities of other farming symbioses.

Title: *Dictyostelium* as a pharmacological model system in medical research.

Authors: Pishan Chang, Steven Robery, Marthe H R Ludtmann, Nicholl Pakes, Richard Tyson, Christopher Dinh, Abdul Waheed, Angelika A Noegel, Till Bretschneider, Debbie Baines, Mark A Carew, Paul LR Andrews, Matthew C Walker, Adam Kuspa, Robin SB Williams

Presenter: Robin SB Williams

Address for correspondence: Centre for Biomedical Sciences, School of Biological Sciences, Royal Holloway University of London, Egham TW20 0EX, UK

Abstract: Our understanding of how many widely used medicines or dietary components function in the human body is often poor, since their specific targets and/or molecular mechanisms remain unknown. The initial identification of how these medicines or chemicals regulate cell behaviour is extremely difficult in animal models, due to limited approaches for mutant screening, and difficulties in gene ablation. These problems have held back the development of more potent or improved treatments. Understanding the molecular mechanism of such chemicals may therefore allow both the development of improved treatments, and increase our understanding of the illness, condition or disease that is being treated.

We have had recent success in using *Dictyostelium* to better understand the underlying mechanisms and targets of several poorly characterised chemicals including: valproic acid, a commonly used epilepsy treatment; phenylthiourea, a well-accepted standard in bitter tastant research; and naringenin, a dietary flavonoid. Here we summarise the use of *Dictyostelium* in understanding the mechanism of action of these chemicals and the successful translation of these discoveries to mammalian models. We first describe the effect these compounds on *Dictyostelium*, and the discovery of a mechanism of action for each compound in *Dictyostelium*. We then describe the successful translation of these mechanisms to mammalian models, to identify how valproic acid functions in seizure control, to show a novel phenylthiourea target provided by a human GABA receptor subunit, and to illustrate the role of naringenin in regulating a mammalian calcium channel.

These projects highlight a growing niche for *Dictyostelium*, as a simple and malleable model system for pharmacological investigations, yielding directly translatable outcomes for medical research.

Title: Development of a first-choice non-animal model for bipolar disorder research

Authors: Anna Frej, Sergio Lilla, Robert Insall, Grant Churchill and Robin SB Williams

Presenter: Anna Frej

Address for correspondence: School of Biological Sciences Royal Holloway, University of London Egham Hill Egham, Surrey TW20 0EX UK

Abstract: Bipolar disorder is a neuropsychiatric condition characterised by cyclic mood changes. Current mood stabilising therapies are not curative however a design of improved treatments is hindered by the unknown aetiology of the disease.
Inositol depletion is a proposed mechanism of action for bipolar disorder treatments. In *Dictyostelium*, bipolar disorder treatments, valproic acid (VPA) and lithium, function to reduce inositol (1,4,5)-trisphosphate (IP3) levels, leading to a block in development. This inositol depletion effect has been also verified for three structurally distinct bipolar disorder treatments (including VPA) in a mammalian model. Although the mechanism by which VPA causes inositol depletion remains unknown, it has been suggested to indirectly inhibit inositol-3-phosphate synthase (INO1) in de novo inositol biosynthesis.
In this study, we investigate a role for INO1 as a potential target for VPA. We ablate and overexpress ino1, identify potential INO1 binding partners, and develop a model for the analysis of the human INO1 protein. We show that a cell line with ablated ino1 is unable to grow or develop unless supplemented with inositol. *Dictyostelium* ino1 overexpression rescues these phenotypes, confirming a critical requirement for INO1 in growth and development. Cell lines with either ablated or overexpressed ino1 remain sensitive to VPA during development, suggesting that VPA does not act via INO1 in *Dictyostelium*.
We also identify, via immunoprecipitaton, a range of potential INO1 binding partners that may provide VPA-sensitive targets that alter INO1 activity. These targets are currently being analysed. To examine if the human protein may specifically provide the VPA target we are presently producing a tagged version of the human INO1 in *Dictyostelium*.
This project is developing *Dictyostelium* as a model for bipolar disorder neuropharmacology research to advance the understanding of the disorder and to aid the development of effective bipolar disorder treatments without the animal use.

This project is founded by The Dr Hadwen Trust for Humane Research, the UK's leading medical research charity funding exclusively non-animal research techniquesto replace animal experiments.

Title: Dicty on drugs: Characterizing a novel small molecule regulator of myosin-II dynamics

Authors: Alexandra Surcel, Win-Pin Ng, Hoku West-Foyle, Namandje Bumpus, David Meyers, Caren Meyers, Douglas Robinson

Presenter: Alexandra Surcel

Address for correspondence: Johns Hopkins School of Medicine, 100 Physiology 725 North Wolfe Street Baltimore, MD 21205

Abstract: Changing mechanical properties of cells underlie a variety of normal cellular processes such as cytokinesis and embryogenesis, as well as diseased states, most notably cancer. Identifying small molecules that can alter cellular mechanical properties has far reaching implications for therapeutic advancement. Here we have developed and performed a large-scale, small molecule chemical screen for mechanical modulators. We have characterized one such identified compound, whose breakdown products lead to cytokinesis defects by affecting the racE and myosin-II pathways. With these compounds we can alter the recruitment of the mechanoenzyme myosin-II to the cell cortex in a myosin-II assembly-independent manner. This recruitment leads to changes in the mechanical properties of the cell. Parallel observations have been observed in mammalian cells, suggesting that this compound may be further explored pharmacologically.

Title: Mutant full length human huntingtin confers an in vivo gain-of-function on the ordered extension of pseudopodia in *Dictyostelium*

Authors: R. Vijayvargia1†, R.S. Atwal1†, I.S. Seong1, M.E. Macdonald1, J.F. Gusella1, M.A. Myre1

Presenter: Michael Myre

Address for Correspondence: 1Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA. †These authors contributed equally to the work.

Abstract: Huntington's disease (HD) is caused by a polyglutamine repeat in the pleiotropic protein huntingtin (Htt). Several lines of evidence suggest that the HD mutation confers a CAG length-dependent 'gain-of-function' causing a distinct neuropathology through some functional feature of Htt. In *Dictyostelium* extension of pseudopods is comprised of two highly ordered events: pseudopod splitting and de novo formation. Pseudopod formation is impaired in htt- cells when submerged in various developmental buffers including KK2 and SB which greatly reduces the rate of chemokinesis. Introduction of normal full length human huntingtin (Fl-hHtt) (Q23) rescues pseudopod formation in htt- cells and accentuates the process in wild type cells. Disease-causing human mutant huntingtin alleles (fl-mHtt) (Q67 or Q78) primarily promote de novo pseudopod formation in either strain. These effects appear to involve the localization of F-actin at the leading edge of the pseudopod and actin Tyr-phosphorylation. Despite the wide evolutionary distance between hHtt and DdHtt our data supports human genetic studies that suggest that polyQ expansion is a gain-of function and that *Dictyostelium* can be used as an in vivo tool to study polyQ-dependent hHtt structure-function studies.

Title: TirA signaling and the biochemical characterization of bacterial "extracellular traps" produced by Sentinel cells in *D. discoideum*

Authors: Olga Zhuchenko, Zhiyi Liu, Eddie Nam, Tim Farinholt, Gadi Shaulsky, and Adam Kuspa

Presenter: Adam Kuspa

Address for correspondence: Department of Biochemistry and Human and Molecular Genetics, Baylor College of Medicine, Houston, TX, USA

Abstract: The Toll/interleukin receptor-1 (TIR) domain protein, TirA, is required for optimal growth of *D. discoideum* amoebae on Gram(-) bacteria (1), the transcriptional response of amoebae to Gram(-) bacteria (2), and for lipopolysaccharide(LPS)-stimulated bacterial killing (3). To dissect the TirA signaling pathway we have identified potential TirA-interacting proteins by immuno-precipitation of GFP-tagged TirA protein followed by mass spectrometry. Among the proteins identified in TirA complexes isolated from cells growing on Gram(-) bacteria we have focused on an extracellular polysaccharide (EPS) depolymerase related protein that we have called EpdR (encoded by DDB_G0271970), a class of proteins known to bind and hydrolyze the O-antigen polysaccharide of LPS. Interestingly, the presence of epdR appears to be the result of a lateral gene transfer into the *D. discoideum* genome from a Gram(-) enterobacterium. We have confirmed the binding of EpdR to TirA in vitro, and disrupting the epdR gene results in defective growth on Gram(-) bacteria. Thus, EpdR may be a receptor or effector subunit of the TirA pathway.

Sentinel (S) cells appear to provide innate immunity in the slug during development (1). We have also described at previous *Dictyostelium* conferences that S cells form extracellular DNA nets that appear analogous to "extracellular traps" (ETs), produced by neutrophils and eosinophils, that are known to trap and kill bacteria. Purified S cells are able to elaborate DNA-containing nets upon stimulation with LPS. Bacteria bind to these nets and appear to be actively killed, similar to what is seen with mammalian ETs. We have begun to characterize the proteins associated ETs in an effort to determine how ETs kill bacteria and we will describe some of these preliminary results. We have also found that TirA is required for net formation by S cells. Thus, TirA is critically required both for *D. discoideum*'s response to Gram(-) bacteria during growth and in innate immune function during development.

1. Chen, G., O. Zhuchenko, and A. Kuspa, Immune-like phagocyte activity in the social amoeba. Science, 2007. 317: p. 678-681.
2. Walk, A., et al., Lipopolysaccharide enhances bactericidal activity in *Dictyostelium discoideum* cells. Dev Comp Immunol, 2011. 35(8): p. 850-856.
3. Nasser, W., et al., Bacterial discrimination by Dictyostelid amoebae reveals the complexity of ancient interspecies interactions. Current Biology, 2013. 23(10): p. 862-872.

Title: Understanding the dynamical origins of collective cAMP oscillations in *Dictyostelium discoideum*

Authors: Allyson Sgro, David Schwab, Javad Noorbakhsh, Pankaj Mehta, and Thomas Gregor

Presenter: Allyson Sgro

Address for correspondence: Joseph Henry Laboratories of Physics, Princeton University, Princeton, NJ 08544

Abstract: *Dictyostelium discoideum* is a classic model system for probing collective behaviors such as those that are observed during the development phase. However, development in this system is controlled by a complex biochemical network and while many of the network constituents have been identified, the relationships between them are still poorly understood. Thus, it is challenging to model and predict phenomena resulting from this network using an approach that takes into account each component and interaction. We have observed that individual cells display a qualitative change (bifurcation) in their behaviors with increasing external cAMP concentrations. As only a few qualitative behaviors exist near bifurcations, it is possible to use a universal, top-down approach to phenomenologically model the network's dynamics to understand how single-cell internal signaling dynamics control population-level behaviors. Here, we combined such an approach with experimental data to study the emergence of collective internal oscillations of the signaling molecule cAMP. Our results show that the internal cAMP production and relay network in single cells is dominated by an as-yet-unknown feedback mechanism and that the model can describe both short time scale (5 min) cAMP network behaviors. Finally, we use this model to predict novel population level behaviors that are then experimentally confirmed.

Title: *Dictyostelium* PakB is involved in Maintenance of Cortical Tension Through Regulation of Myosin I

Authors: Yidai Yang, Scott Crawley, Emilia Furmaniak-Kazmierczak and Graham P. Côté

Presenter: Yidai Yang

Address for correspondence: Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario, Canada K7L 3N6

Abstract: In this study we show that *Dictyostelium discoideum* amoeba that lack PakB, a p21-activated kinase (PAK) that activates myosin-I, are missing the cortical layer of actin filaments that is normally associated with the plasma membrane. Loss of the cortical layer of actin filaments correlates with the inability of PakB-null (PakB-) cells to resist external stresses. PakB-l cells form large blebs at a significantly higher rate than parental JH10 cells when placed under a coverslip or subjected to electroporation. When placed under a sheet of 0.5% agarose, PakB- cells chemotax much more slowly than JH10 cells and cannot maintain an elongated morphology. These defects are rescued by expression of full-length PakB but not by an N-terminal fragment of PakB that binds actin filaments and dAbp1. Immunoblot analysis carried out using an antiphospho-antibody raised against the TEDS phosphorylation site of MyoD, showed that MyoD phosphorylation levels were 5-fold lower in PakB-null cells than JH10 cells. The results suggest a model in which PakB promotes the integrity of the cortical actin cytoskeleton through activation of myosin I.

Title: *Dictyostelium* PakD mediates quorum sensing by regulating the actin cytoskeleton

Authors: Garcia, M., Ray, S. and Brazill, D.

Presenter: Derrick Brazill

Address for correspondence: Hunter College Department of Biological Sciences, 927N 695 Park Ave New York, NY 10065

Abstract: Quorum sensing, the ability of cells to determine the relative number of cells in a given area, is crucial for proper tissue development and maintenance. Although cell density sensing (quorum sensing) pathways have been described for bacteria, much remains to be understood about eukaryotic quorum sensing. In the eukaryote *Dictyostelium discoideum*, the CMF quorum-sensing pathway ensures that development will initiate only when enough starving cells are present by regulating cAMP signaling as well as by priming the cytoskeleton for cAMP chemotaxis. Here we describe a putative protein kinase, PakD, which regulates quorum sensing and the cytoskeleton in *D. discoideum*. We found that cells overexpressing pakD aggregate at low cell density, as if in the presence of CMF. Conversely pakD^{-} cells are unable to aggregate even at high cell density. pakD^{-} cells chemotax poorly towards cAMP, but show normal chemotaxis towards folate, suggesting that PakD mediates some but not all chemotaxis responses. Compared to wild-type, cells lacking PakD have decreased polarity when placed in a cAMP gradient, indicating that the chemotactic defects of the pakD^{-} cells may be due to an impaired cytoskeletal response to cAMP. In addition, while wild-type cells polymerize actin in response to global stimulation by cAMP, pakD^{-} cells exhibit F-actin depolymerization under the same conditions. Taken together, the results suggest that PakD is part of the CMF quorum sensing pathway, and is specifically involved in coordinating F-actin organization during development.

Title: The Isolation, Proteomic Analysis and Function of Dictyoselium Exosomes

Authors: P.W. Kriebel, L. Jenkins, L. Liu, S. Ammu, G. Zhang and C.A. Parent*

Presenter: Paul Kriebel

Address for correspondence: kriebelp@mail.nih.gov

Abstract: We have demonstrated in *Dictyostelium* that adenylyl cyclase A (ACA) is localized in multivesicular bodies (MVBs) docked at the back of polarized migrating cells. The MVBs fuse with the plasma membrane to release their vesicular content (exosomes) containing ACA, extracellularly in trails. These processes are essential for the efficient chemotaxis of cells to create aggregates by forming head to tail alignments of cells termed streams. We purified exosomes from cell culture supernatants, employing differential centrifugations and sucrose gradients. The resulting preparation was characterized and subjected to LTQ mass spectrophotometry. We found that Dicytostelium exosomes are cup shaped vesicles 50-100nm in diameter. They have a density of 1.17-1.19mg/ml which is comparable with density of mammalian exosomes. Mass spectrophotometry analysis identified many canonical exosome proteins in our preparation including chaperone, small G, cytoskeletal, signaling, ATPase, and translational proteins. In fact we determined that 85% of the proteins identified by mass spectrophotometry were orthologues of proteins found in mammalian exosomes. We next tested whether isolated exosomes contain cAMP and can attract nearby cells using ELISA, EZ-TAXIScan and transwell chemotaxis assays. We found that exosomes isolated from ACA-YFP expressing cells contain cAMP and can attract cells in a cAMP dependent manner. We are now studying the mechanisms that regulate cAMP production and secretion from exosomes. By using the composition of *Dictyostelium* exosomes to guide biological studies we wish to gain further insight into the regulation of cAMP secretion and the role of exosomes in cell-cell signaling during chemotaxis.

Title: Single Cell Force Spectroscopy of *Dictyostelium discoideum* substrate adhesion

Authors: Marco Tarantola, Christian Westendorf, Eberhard Bodenschatz

Presenter: Marco Tarantola

Address for correspondence: Max Planck Institute for Dynamics and Self-Organization (MPIDS), Laboratory for Fluid Dynamics, Pattern Formation and Biocomplexity, Am Fassberg 17, 37077 Goettingen, Germany

Abstract: *Dictyostelium discoideum* does not possess the cell adhesion molecule class of integrins. Over the last dozen years, *Dictyostelium*-specific transmembrane proteins have been identified, which are involved in substratum adhesion of vegetative cells, in particular SibA and SadA. Their involvement in substratum adhesion at later stages of development, however, remains unclear.
We address this question by applying Single Cell Force Spectroscopy (SCFS). We used *Dictyostelium* cells attached to a cantilever as a probe to quantitatively measure substrate adhesion. We find that substratum adhesion of *Dictyostelium* Ax3 wildtype cells is dramatically reduced during the first 6 hrs of development resulting in a decreased adhesion force, reduced work of substrate release, and diminished number of tether-like steps.
We compared null mutants of sibA and sadA to wild type cells after 6 h of development (6h) and find no difference in the adhesion properties. This suggests that sibA and sadA do not play a significant role in the adhesion of developing *Dictyostelium* cells.

Title: Changes in cell-substrate adhesion during early development

Authors: William F. Loomis, Danny Fuller, Edgar Gutierrez, Alex Groisman, and Wouter-Jan Rappel
Departments of Biology and Physics, University of California San Diego, La Jolla, CA

Presenter: Bill Loomis

Address for correspondence: William F. Loomis; Department of Biology 0116; UCSD, La Jolla CA 92093

Abstract: *Dictyostelium* cells can move rapidly on naked glass in the absence of extracellular matrices. Their innate adhesion is equally strong on either hydrophilic or hydrophobic materials and may involve van der Waals attraction of their surface glycoproteins. Using microfluidic devices that generate a range of hydrodynamic shear stress we have found that the strength of adhesion decreases dramatically during the first 6 hours of development. This decrease in substratum adhesion fails to occur in carA- mutant cells lacking the cAMP receptor, CAR1, and so can be considered a developmentally regulated event.
As the laboratories of Pierre Cosson and Rex Chisholm have shown, vegetative cells of strains carrying null mutations in either sibA or sadA are much less adhesive than wild type vegetative cells. However, after 5 hours of development, adhesion of the mutant cells does not decrease further and there is no significant difference in adhesion between wild type and mutant cells at this stage. Therefore, it seems that neither Sib1 nor SadA play a significant role in substratum adhesion when cells are streaming into aggregates and the cell types are sorting out. Cytoskeletal dynamics may play a more critical role at this stage of development.

Title: Selection on multiple mating types in *Dictyostelium*: more than two but fewer than many

Authors: Tracy Douglas, Katherine Geist, David Queller, Joan Strassmann

Presenter: Tracy Douglas

Address for correspondence: Department of Biology, Washington University in St. Louis, St. Louis, MO

Abstract: In the expansion of sexual systems from two mating types to multiple, theory indicates that the number of mating types expressed in a system should tend towards infinity. This means it is a puzzle that dictyostelids often have only 3 or 4 mating types, a question that has yet to be addressed in these species. In the model organism *Dictyostelium discoideum*, there are four known mating types: one self-compatible type and three self-incompatible types, which cannot mate with themselves but can mate with any other type. Because so little is known about the characteristics of the *D. discoideum* mating types, we predict that many factors could contribute to the maintenance of this system. To start, we investigated common measurements for evidence of selection on mating systems: mating type distribution, gamete size and gene orthology. So far, we have yet to find evidence for selective constraints on the evolution of further mating types in *D. discoideum* using these traditional methods. Mating type distribution and gamete size measurements revealed no evidence of differing selective pressures within *D. discoideum*, showing that mating types are evenly distributed both within and across populations and gametes across mating types are homologous in size. Gene orthologs identified in *D. discoideum* relatives confirmed the persistence of the mating type genes across species, providing further evidence for the maintenance of this system over evolutionary time. While these results do not explain the low numbers of mating types in dictyostelids, we will discuss future efforts to identify conflicts between mating types caused by differential contributions to macrocyst production and insight into how these conflicts could be lead to constraints on the evolution of many mating types in *D. discoideum*.

Title: Molecular characterization of thyroxine 5' deiodinase (DIO3) in the cellular slime mold *Dictyostelium discoideum*.

Authors: Shashi Prakash Singh, Pundrik Jaiswal and Ramamurthy Baskar

Presenter: Shashi Prakash Singh

Address for correspondence: C/o, Dr. R. Baskar, Dept. of Biotechnology, Indian Institute of Technology Madras, Chennai- 600 036, India

Abstract: The genome of *Dictyostelium discoideum*, has many homologues of human genes and one among them is the gene encoding for a putative selanoprotein-type III thyroxine 5'deiodinase gene (DIO3) which inactivates thyroxine hormone. DIO3 is known to be involved in Selanocysteine metabolism (Lobanov et al 2007). We observed that in the presence of caffeine this gene gets up-regulated by 1.53 fold (Jaiswal et al unpublished). DIO3 gene may be involved in pathways other than thyroxine inactivation because thyroxine is not reported in *Dictyostelium* so far. To investigate the role of DIO3 gene in Dicty, we have disrupted this gene with BlasticidinR gene cassette and characterized the mutant. Absence of this gene manifests in large aggregates, delayed growth and development. ACA and cAR1 transcripts levels are downregulated and cAMP levels also go down during early development in this mutant.

Reference:

1. Lobanov AV, Fomenko DE, Zhang Y, Sengupta A, Hatfield DL, Gladyshev VN (2007) Evolutionary dynamics of eukaryotic selenoproteomes: large selenoproteomes may associate with aquatic life and small with terrestrial life. Genome Biol. 8(9):198.

Title: Essential roles for small G-proteins in the regulation of directional cell movement

Authors: Ineke Keizer-Gunnink, Peter J.M. van Haastert, and Arjan Kortholt

Presenter: Arjan Kortholt

Address for correspondence: Department of Cell Biochemistry, University of Groningen, the Netherlands

Abstract: A central problem in cell biology lies in understanding how small-scale biochemical interactions generate large-scale organization and cellular structure. Eukaryotic cells move and navigate in gradients of diffusive molecules. This process, called chemotaxis, the directional movement towards a chemical compound, is an essential property of many cells and has been linked to the development of several diseases. Chemotaxis is a complex cellular process involving a multitude of signalling pathways and molecules. In a recent studies we have identified an essential basal signaling module of chemotaxis in *Dictyostelium*, which consists of heterotrimeric and small G-proteins [1]. The next challenge will be to discover additional components of this basal pathway and to determine the mechanism by which heterotrimeric G-proteins induce Ras activation. Here we present a novel mass-pull-down proteomic strategy to isolate these effectors and regulators [2]. This approach together with the advantages of *Dictyostelium* as model system give new insights in the molecular mechanisms underlying regulation of G-protein signaling and chemotaxis.

1. Kortholt A., Kataria R., Keizer-Gunnink I., van Egmond W.N., Khanna A., and van Haastert P.J. (2011). *Dictyostelium* chemotaxis: essential Ras activation and accessory signalling pathways for amplification. EMBO Rep. 12: 1273-1279.
2. Kataria R., Xu X., Fusetti F., Keizer-Gunnink I., Jin T., van Haastert P.J., and Kortholt A. (2013). *Dictyostelium* Ric8 is a nonreceptor guanine exchange factor for heterotrimeric G proteins and is important for development and chemotaxis. Proc. Natl. Acad. Sci. U. S. A. 110(16):6424-9.

Title: Chemotaxis in spatiotemporal waves: memory in Ras activation and the back-of-the-wave problem

Authors: Monica Skoge, Haicen Yue, Herbert Levine, Wouter-Jan Rappel, and William Loomis

Presenter: Monica Skoge

Address for correspondence: Departments of Biology and Physics, University of California San Diego, La Jolla, CA

Abstract: As part of the aggregation process, *Dictyostelium* chemotax to the source of natural waves of cAMP. To do so, cells must sense both spatial and temporal gradients to distinguish the front of the wave, which has a spatial gradient increasing towards the source, from the back of the wave, which has a spatial gradient in the opposite direction. How cells orient during the front of the wave, but not reverse direction in the back of the wave is not well understood. We study this problem using a novel microfluidic spatiotemporal wave generator. By varying the period and velocity of the wave (while keeping the spatial profile constant), we find that cells maintain directionality towards the source in the back of the wave for 6-minute periods, but increasingly reverse direction in the back of the wave as the period is increased (and velocity is decreased). These experiments suggest cells have a limited memory (~2 min) of the direction of the source, giving rise to maximal chemotaxis towards the source for 6-minute wave periods.

We further connect the ability of cells to maintain direction in natural waves to the localization of activated Ras at the single-cell level. Using microfluidic devices designed to rapidly switch between two linear static gradients, we found evidence for memory in Ras activation: (1) when static spatial gradients are suddenly replaced with lower uniform concentrations, activated Ras patches at the cell front disappear immediately and return with a delay dependent on the drop in concentration and (2) cells do not reverse their direction of movement or Ras-GTP localization when the gradient is reversed if the new gradient is sufficiently weaker. We propose a simple model of Ras activation, based on incoherent-feedforward regulation coupled to a transient memory module via positive feedback, which captures many aspects of the observed memory and response to natural waves.

POSTER ABSTRACTS

Posters will be available for viewing both Monday and Tuesday in the Vanderbilt Room.

Authors of odd-numbered abstracts should be at their posters Monday evening, and those with even-numbered abstracts should be at their posters on Tuesday.

Poster #1

Title: CpnA Depolymerizes Actin Filaments in a Calcium-dependent Manner

Authors: Andrew A. Banas, Mingxi Han, Hanqian Mao, David Loiselle, Timothy A.J. Haystead, and Cynthia K. Damer

Presenter: Andrew Banas

Address for correspondence: Department of Biology, Central Michigan University, Mount Pleasant, MI 48859

Abstract: Copines make up a multigene family of calcium-dependent, phospholipid-binding proteins. Copine proteins consist of two C2 domains at the N-terminus followed by an "A domain" similar to the von Willebrand A domain found in integrins. The C2 domain is a calcium-dependent membrane-binding motif, while the A domain is thought to be a protein-binding domain. We are studying copine protein function in the model organism, *Dictyostelium discoideum*, which has six copine genes, cpnA- cpnF. Previous research showed that cpnA− cells exhibited a cytokinesis defect, a developmental defect, and a defect in contractile vacuole function. To fully understand the role of CpnA in these cellular processes, we used column chromatography and mass spectrometry to identify proteins that interact with CpnA. One of the proteins identified was actin. Rhodamine-phalloidin staining of cpnA- cells revealed that cpnA- cells have more actin filaments than wild-type cells. To determine if CpnA associates with the actin cytoskeleton, we treated cells expressing GFP-CpnA or GFP-Ado (containing the A domain of CpnA) with Triton X-100 and spun down the insoluble cytoskeletal fraction. GFP-CpnA was found in the cytoskeletal pellet only in the presence of calcium, while GFP-Ado was found in the cytoskeletal pellet in the presence and absence of calcium. To determine if CpnA directly binds to actin, we performed F-actin binding assays with purified GST-CpnA and found that GST-CpnA was able to cause depolymerization of F-actin in a calcium-dependent manner. In addition, we did immunoprecipitations with a GFP antibody and found that both the full-length CpnA and the A domain of CpnA were able to bind F-actin, but not G-actin. Our results indicate that CpnA can bind to F-actin in a calcium-dependent manner and act as an F-actin depolymerizing protein.

Poster #2

Title: Do the traits that allow amoebae to harbor beneficial bacteria leave them vulnerable to pathogenic bacteria?

Authors: Susanne DiSalvo, Debra A. Brock, David C. Queller, Joan E. Strassmann

Presenter: Susanne DiSalvo

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Abstract: Bacteria-eukaryote interactions are ubiquitous, diverse, and dramatic. Understanding microbial relationships along a wide spectrum of outcomes has the potential to yield novel insight into the cause and consequences underlying these interactions. *Dictyostelium discoideum*, displays differential long-term associations with bacterial species. "Farmer" isolates carry bacteria through the social stage, while "non-farmers" do not. Here, we investigate whether farming is associated with differential sensitivity to bacterial pathogens by challenging farmer and non-farmer amoebas to a panel of variably pathogenic Escherichia coli strains. Our preliminary studies did not reveal striking differences between farmers and non-farmers for their efficiency of grazing on the *E. coli* strains, although non-farmers generally grow more efficiently in our assays. Interestingly, amoebas are able to overcome growth inhibition when plated at high densities on toxic *E. coli*, suggesting positive interactions during the solitary stage.

Poster #3

Title: Literature curation at dictyBase

Authors: Petra Fey, Robert Dodson, Kerry Sheppard, and Rex L. Chisholm

Presenter: Robert Dodson

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Abstract: Literature curation is a central task at dictyBase. As of July 2013, the dictyBase literature corpus is 7,419 of which 1,940 have been annotated. While these numbers are small compared to larger research fields, we only have 2 curators whose responsibilities are manifold: user requests, HTML page updates, grant and paper writing, supervising Dicty Stock Center (DSC) operations, literature, and gene curation. In order to keep up with new literature we needed to make curation more efficient.

From literature we primarily annotate gene names, gene products, strains, phenotypes, and Gene Ontology (GO) terms. Strain annotations are of importance at dictyBase because of our close integration with the DSC. Strain annotation is often done in conjunction with the DSC staff who request newly published strains for deposit. Strains are annotated with several controlled vocabularies, such as strain descriptors, characteristics, and mutagenesis types. Phenotypes are then attached to the strains, which in turn are linked to genes. We have our own phenotype ontology, a pre-composed ontology from *Dictyostelium* anatomy terms. Strain curation is very time consuming because information is often not well represented in papers. Therefore we recently initiated a community curation trial, in which researchers annotate their newly published papers prior to curator review. This has been very helpful particularly for strain and phenotype curation and we will present preliminary results.

As members of the Gene Ontology consortium, GO annotations also represent an important part of literature curation. Gene ontology is annotated using the Protein2GO tool provided by the EBI, which is now becoming the universal GO tool for all model organism databases. In collaboration with Wormbase we began to integrate a textpresso pipeline to assist cellular component GO annotations, making some GO annotations semi-automatic. As a small database in which each person has many obligations, literature curation is becoming more dependent on users, collaborators and efficient tools.

dictyBase is funded by NIH GM64426, GM087371 and HG0022.

Poster #4

Title: Cyclic AMP mediates stress induced encystation in social and pathogenic amoebas.

Authors: Qingyou Du, Christina Schilde, Elin Birgerson, John Sinclair, Zhi-hui Chen, Stuart McElroy and Pauline Schaap*

Presenter: **Qingyou Du**

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Abstract: Many protists survive stress by differentiating into resilient cysts. Cysts are resistant to immune clearance and biocides, preventing effective treatment of protist borne disease Lack of gene manipulation procedures applicable to protists, has left the mechanisms that control encystation largely unexplored. Dictyostelid social amoebas aggregate and form spores in multicellular fruiting bodies in response to nutrient stress. Spore formation and dormancy are triggered by elevated intracellular cAMP levels, requiring activation of the adenylate cyclase, AcrA and inhibition of the cAMP phosphodiesterase, RegA. Here we show that RegA and AcrA are deeply conserved in amoebozoa and that deletion of *regA* in the encysting amoeba *Polysphondylium pallidum* causes premature encystation and loss of cyst germination. From a panel of 32 compounds, we identified two RegA-specific inhibitors, which increased cAMP levels and induced encystation of the pathogen *Acanthamoeba castellani*. Conversely, inhibition of AcrA in *A.castellani* prevented encystation. Our results indicate that the role of cAMP in Dictyostelid spore formation is evolutionary derived from a role as signaling intermediate for stress-induced encystation and we identified AcrA as a target for therapeutic intervention with encystation.

Poster #5

Title: Characterization of Nudix mRNA decapping enzymes in *Dictyostelium discoideum*

Authors: Kirsten Bickford, Catherine O'Keeffe, Marlee Nelson, and Susan Parrish

Presenter: Susan Parrish

Address for correspondence: McDaniel College, Department of Biology, 2 College Hill, Westminster, MD 21157

Abstract: The *D. discoideum* genome encodes 19 putative proteins that contain a Nudix hydrolase signature, a hallmark of enzymes that cleave substrates comprised of nucleoside diphosphates linked to another moiety X. A subset of Nudix enzymes has been shown to cleave the mRNA cap, thereby promoting mRNA turnover and proper regulation of gene expression. In *D. discoideum*, the putative DDB_ G0283315 Nudix protein shares sequence similarity to the yeast DCP2 mRNA decapping enzyme. Likewise, the putative *D. discoideum* DDB_G0278957 Nudix protein shares sequence similarity to the Mimivirus L534 enzyme, which has been shown to cleave the mRNA cap in vitro. To characterize the functions of these two proteins in *D. discoideum*, we sought to knockout each gene independently. First, we constructed two Blasticidin S knockout cassettes containing the respective flanking regions of each gene. Following transformation and selection with Blasticidin S, viable *D. discoideum* mutant colonies were obtained for each gene, suggesting that these genes are not required for viability. The two genetic knockout mutants were subsequently purified and analyzed to confirm gene disruption. Future studies will determine if these Nudix proteins modulate mRNA turnover and development in *D. discoideum*.

Poster #6

Title: The new dictyBase Genome Browser

Authors: Petra Fey, Siddhartha Basu, Robert Dodson, and Rex L Chisholm

Presenter: Petra Fey

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Abstract: dictyBase genomic annotations are graphically displayed in the Generic Genome Browser (GBrowse 2.4), a versatile and customizable tool developed by the Generic Model Organism Database project (GMOD; http://gmod.org/wiki/GBrowse). GBrowse is accessible from the top bar of each Dictyostelid database, and also from individual gene pages.

The genomic position of both manually curated and automatically predicted genes and gene models is based on their chromosomal coordinates on the genome sequence. Other annotations such as GenBank records, ESTs, and interspecies BLAST hits (TBLASTN) between Dictyostelids are shown as alignments to the genome sequence. RNA sequence data represents individual nucleotide reads from multiple developmental time points that are available for view as single tracks or compiled into one track. Different tracks can easily be chosen in the 'Select Tracks' interface and each track is individually customizable. The genome browser allows retrieval of any section of DNA sequence determined by the genome segment viewed, as either a plain or a decorated Fasta file. Navigation in the Overview, Region, or Details areas is very intuitive; any size window can simply be drawn with a mouse up to the allowed size of 100 kb. When zooming in to 100 bp or less, one can see the actual nucleotide sequence. Furthermore, the compiled RNA track from all developmental time points allows semantic zooming where each actual RNA sequence read can be viewed. Inspect intron/exon boundaries, retrieve sequence, view restriction maps, evaluate expression status or check interspecies alignments – all are available in the dictyBase Genome Browser.

BRING YOUR COMPUTER!

Acknowledgements: We thank Gad Shaulsky and Adrian Tsang and their colleagues for the RNAseq data; dictyBase is funded by NIH GM64426 and HG0022.

Poster #7

Title: REMI-Seq - a step change in genetic studies of *Dictyostelium discoideum*

Authors: Nicole Gruenheit, Thomas Keller, Adrian Harwood, Christopher Thompson

Presenter: Nicole Gruenheit

Address for correspondence: Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, Manchester UK M13 9PT

Abstract: Over 80 years of research has established *Dictyostelium discoideum* as a simple cell system in which to investigate many cell and developmental biology processes that are seen in more complex animals or associated with human disease. A strength of *Dictyostelium* is its advanced molecular genetics, and this took a large step forward with the completion of the whole *Dictyostelium* genome sequence in 2005. However, a key challenge remains: understanding the function of each gene. One way to address this is to study mutant *Dictyostelium* strains in which a single gene has been removed. However, the creation of a genome-wide mutant bank has, to date, been impossible. We propose to overcome this limitation with a new technique, REMI-seq. REMI-seq combines Restriction Enzyme Mediated Insertional (REMI) mutagenesis with Next Generation Sequencing (NGS) to generate large numbers of mutants and then identify the disrupted gene by NGS. Each mutation position will be mapped onto the genome sequence, and be searchable on-line via the existing *Dictyostelium* genomic resource, dictyBase. Individual, groups and even large pools of mutants will be available to the research community via the linked Dicty Stock Center. This new mutant resource will produce a step change in *Dictyostelium* genetics.

Poster #8

Title: Phosphoinositides regulate GbpD mediated Rap activation

Authors: Ankita Khanna, Ineke Keizer-Gunnink, Peter J.M. Van Haastert, and Arjan Kortholt

Presenter: Ankita Khanna

Address for correspondence: Department of Cell Biochemistry, University of Groningen, The Netherlands.

Abstract: Rap proteins are members of the Ras superfamily of small GTPases. GbpD is by far the only characterized *Dictyostelium* Rap specific Guanine exchange factor (GEF) , and is implicated in cell adhesion, cell polarity and chemotaxis (1). GbpD consists of a catalytic GEF domain, two cyclic nucleotide binding domains and a GRAM domain. Here we show that despite the presence of two putative cyclic nucleotide binding domains, GbpD is not activated by cyclic nucleotides. However, GbpD activity is regulated by phosphoinositides; PI(4,5)P2 binding to the GRAM domain regulates the dynamic localization of GbpD at the membrane, whereas PIP3 binding to the regulatory domain of GbpD is essential for its activation. Previously we have shown that GbpD/Rap1 activates PI3K (2), suggesting a full-circle positive feedback loop of GbpD/Rap1/PI3K/PIP3/GbpD, which may explain the very strong phenotype of GbpD overexpression on pseudopod formation and cell polarity.

Poster #9

Title: *Dictyostelium* Roco proteins to study LRRK2-mediated Parkinson disease

Authors: Bernd Gilsbach1, Susanne Terheyden1, Katharina Rosenbusch, Alfred Wittinghofer2, and Arjan Kortholt1

Presenter: Arjan Kortholt

Address for correspondence: 1 University of Groningen, The Netherlands 2 Max-Planck-Institut für Molekulare Physiologie, Dortmund, Germany

Abstract: Parkinson Disease (PD) is a neurodegenerative disorder affecting more than five million people worldwide. Recently a number of genetic factors causing PD have been discovered. Mutations in human leucine-rich-repeat kinase 2 (LRRK2) have been found to be thus far the most frequent cause of late-onset PD. LRRK2 belongs to the Roco family of proteins, which are characterized by the presence of a Ras-like G-domain, called Roc and a kinase domain. Importantly, pathogenic mutations in LRRK2 result in decreased GTPase activity and enhanced kinase activity, suggesting a possible PD-related gain of abnormal function. Here we show that *Dictyostelium discoideum* Roco4 is an excellent model to study the structural and biochemical characteristics of the LRRK2 kinase and can be used for optimization of the current and identification of new LRRK2 inhibitors [1].

1. Gilsbach B.K., Ho F.Y., Vetter I.R., van Haastert P.J., Wittinghofer A., and Kortholt A. (2012). Roco kinase structures give insights into the mechanism of Parkinson disease-related leucine-rich-repeat kinase 2 mutations. Proc. Natl. Acad. Sci. U. S. A. 109(26):10322-7.

Poster #10

Title: Development of a RacF2DN-RFP Expression Vector for Rab8 Colocalization Studies in the Social Amobae, *Dictyostelium discoideum*

Authors: Lamont, Brittany; Angeloni, Joseph; Goldston, Amanda; Palazola, Brennan; Powell, Rhonda; Temesvari, Lesly; Bruce, Terri

Presenter: Brittany Lamont

Address for correspondence: Terri Bruce 132 Long Hall Clemson, SC 29631

Abstract: The small GTPase, Rab8, has been implicated in cellular adhesion events and actin cytoskeleton restructuring in mammalian cells and in the lower eukaryote, *Dictyostelium discoideum*. When constitutively activated Rab8 (Rab8CA) was expressed in *D. discoideum*, it caused changes in cell morphology including a reduction in the cell adhesion molecule, gp24, and development of actin-rich protrusions. Although Rab8 has been shown to affect actin restructuring, no specific pathway for this action has been identified. It is believed that the protein RacF2 may lie upstream of Rab8 in the actin pathway. The goal of this work was to provide further evidence for the interaction between Rab8 and RacF2 by determining if the two proteins colocalize within the cell. A new RacF2-DN-RFP plasmid was constructed in order to determine subcellular localization of RacF2-DN-RFP and Rab8-CA-GFP using fluorescence microscopy.

Poster #11

Title: Cellular studies of *Dictyostelium* myosin II heavy chain kinase D

Authors: Emily Lehman, Cariss Hill, David Banks, and Paul Steimle

Presenter: Emily Lehman

Address for Correspondence: University of North Carolina at Greensboro, Department of Biology, Greensboro, NC 27410

Abstract: *Dictyostelium* is a simple eukaryote with a small genome (34Mb) that encodes many genes with structural and functional homologues in higher eukaryotes. Previous studies have shown that myosin II turnover via phosphorylation is regulated by at least three myosin heavy chain kinases (MHCKs) –A, -B, and –C; all of which share homologous catalytic and WD-repeat domains. A fourth kinase, MHCK-D, shares the ability to phosphorylate and drive myosin II filament disassembly in vitro; however, the localization properties of MHCK-D, its ability to affect myosin II turnover in vivo, and its effects on *Dictyostelium* slug activity have not been explored. Our studies using fluorescence microscopy to examine live cells expressing GFP-tagged MHCK-D revealed that the kinase undergoes robust translocation to the cell cortex in response to stimulation with the chemoattractant cyclic-AMP. Our findings from cell fractionation studies indicate the MHCK-D indeed plays a role in myosin II filament turnover in vivo, especially during the early stages of multicellular development. Further studies revealed that a cell line lacking MHCK-D expression, while still able to form slugs, exhibited a measurable increase in migration towards light (phototaxis). Taken together, our data indicate that MHCK-D, presumably through its ability to drive myosin II disassembly in the cell, plays a central role in controlling the tightly regulated and highly specific changes in shape required for the movement of cells in the contexts of chemotaxis and slug migration.

Poster #12

Title: Studies of the cellular functions of diacylglycerol kinase and alpha kinase 1 in the social amoeba *Dictyostelium discoideum*

Authors: Jonah Nikouyeh, Kyle Burgett, and Paul Steimle

Presenter: Jonah Nikouyeh

Address for Correspondence: University of North Carolina at Greensboro, Department of Biology, Greensboro, NC 27410

Abstract: In *Dictyostelium discoideum*, four related myosin II heavy chain kinases (A, B, C, and D) regulate myosin II-mediated contraction of actin filaments during cell division (cytokinesis) and cell migration. In addition, there is evidence suggesting that two other proteins, diacylglycerol kinase (DgkA) and alpha kinase I (AK1), may also function in regulating myosin II filament turnover in the cell. DgkA phosphorylates diacylglycerol to produce phosphatidic acid, a potential regulator of signal transduction pathways in the cell. Our localization studies indicate that DgKA translocates to the cell cortex in response to chemoattractant (cyclic-AMP) stimulation of *Dictyostelium* cells. We also found that forced over-expression of DgkA has no effect on the ability of *Dictyostelium* cells to divide, indicating that DgKA does not regulate myosin II activity during cytokinesis. In contrast to DgkA, the AK1 protein shares sequence homology with the catalytic domains of the MHCK family, and thus may also play a role in regulating myosin II function in the cell. To explore this possibility, we have engineered a recombinant plasmid for the inducible expression of the AK1 catalytic domain in bacterial cells. Protein expression has been confirmed by SDS-PAGE/Western blot analysis and attempts are being made to purify the AK1 protein for biochemical studies. Collectively, these studies have the potential to impact our understanding of the basic cellular functions of these two novel proteins in a variety of cellular contexts, including cell division and cell migration, both of which are impaired in cancer cells that exhibit uncontrolled multiplication and metastasis.

Poster #13

Title: The evolution of the roles of cell adhesion molecules TgrB1 and TgrC1 in multicellularity and allorecognition

Authors: Cheng-Lin Li, Adam Kuspa and Gad Shaulsky

Presenter: Cheng-Lin Li

Address for correspondence: Baylor College of Medicine, One Baylor Plaza S930, Houston, Texas 77030, USA

Abstract: Cellular interactions through heterotypic cell adhesion molecules (CAMs) play essential roles in cell recognition and development. One interesting unresolved question is how CAMs were able to diversify within species given that their essential functions would be expected to impose severe functional constraints. Here we attempt to better understand this puzzle in *Dictyostelium discoideum* by studying the heterotypic cell adhesion and allorecognition proteins, TgrB1 and TgrC1, by addressing the function and evolution of CAMs from two aspects at once: multicellular development and allorecognition.

Previous studies have shown that TgrB1/TgrC1 mediates allorecognition and cell differentiation during aggregating development. Firstly, cells lacking tgrB1 or tgrC1 are developmentally arrested at the loose aggregation stage and fail to undergo cell differentiation. Secondly, they function as a receptor pair for allorecognition. Cells with different tgrB1/tgrC1 alleles segregate from one another during streaming. Moreover, tgrB1 and tgrC1 genes evolved rapidly and are highly polymorphic in natural populations. tgrB1 and tgrC1 alleles of one strain are considered as a matching allelic tgrB1/tgrC1 pair (e.g., tgrB1AX4/tgrC1AX4 or tgrB1QS45/tgrC1QS45) and alleles in two divergent strains are considered as mismatching allelic pairs (e.g., tgrB1AX4/tgrC1QS45 or tgrB1QS45/tgrC1AX4). Cells carrying a mismatched pair of tgrB1/tgrC1 have severe developmental defects, similar to tgrB1-null or tgrC1-null cells. Therefore, tgrB1/tgrC1 genes must co-evolve to maintain optimum heterotypic interactions since mutations in one gene could disrupt these interactions and lead to failure of sporulation. The tgrB1/tgrC1 system presents a novel model to study the rapid evolution of CAMs and may help us to understand how adhesion molecules can evolve in the context of strong functional constraints.

To elucidate the evolvability of this system, we have to dissect the signal transduction pathways of TgrB1/TgrC1. tgrC1-null cells and single gene replacement strains (e.g., tgrB1AX4/tgrC1QS45) fail to develop beyond the loose aggregation stage. We have devised screens for genetic suppressors that rescue these developmental defects. Such genetic suppressors allow us to elucidate the underlying signaling pathways. In addition, those molecular mechanisms may play roles in buffering novel tgrB1/tgrC1 recognition mutations and allow the organism to tolerate fitness disadvantages during evolution. Thus, activation of such alternate pathways may promote allelic evolution. In our preliminary results, we discovered several candidate genes, including glycosyltransferases and protein kinases, which may compensate for suboptimal function of TgrB1/TgC1. We are continuing the genetic screens and analyzing the function of the genetic suppressors in various genetic backgrounds.

This study is using a novel system that could broaden our understanding of signaling components of CAMs and how allelic repertoires expand despite the risk of selective disadvantages. It might also shed light on the roles of CAMs in the transition to multicellularity and allelic evolution of allorecognition systems.

Poster #14

Title: A Novel Tauopathy Model Utilizing *Dictyostelium Discoideum*

Authors: K.E. Miller, A.L. Erwin, S.L. Hall, M.L. Steinhilb, and C.K. Damer

Presenter: Kristi Miller

Address for correspondence: Department of Biology, Central Michigan University, Mount Pleasant, MI 48858, USA

Abstract: Alzheimer's disease is a chronic, progressive brain disorder, affecting approximately 35 million people worldwide. Pathologically, Alzheimer's disease is characterized by the accumulation of two types of brain lesions: senile plaques and neurofibrillary tangles. Neurofibrillary tangles are found within neurons and are formed by the aggregation of paired helical filaments, in which the main component is tau, a microtubule associated protein. The mechanisms by which tau aggregates into filaments remains uncertain, but studies have shown that phosphorylation and calpain proteolysis are major contributors to tau-mediated toxicity. Cleavage of tau by calpain is thought to result in a highly toxic form of tau called the 17kD fragment. In this study, we are using *Dictyostelium discoideum* as a novel tauopathy model to investigate how these post-translational modifications of tau lead to its cellular toxicity. *Dictyostelium* cells were transformed with plasmids to express wild-type (tauWT) and mutant forms of human tau (phosphorylation-incompetent, tauAP; calpain-resistant, tauCR; tau 17kD fragment, tau17kD). The constitutive expression of tauWT, tauCR, and tau17 was sufficiently toxic to induce cell death. Expression of tauAP was not toxic to *Dictyostelium* wild-type cells. These results suggest that phosphorylation plays an important role in tau toxicity. Because the expression of tau under a constitutive promoter rapidly induces cell death, we are now using an inducible vector system. Thus far, tauWT and tauAP has been cloned into the inducible vector and these plasmids were transformed into wild-type *Dictyostelium* cells. TauAP expression has been induced, while the expression of tauWT has not been observed. Once all of the inducible vector plasmids are constructed, we will characterize the effect of tau and mutant tau expression on *Dictyostelium* survivability and cell division. Development of *Dictyostelium* as a tauopathy model may provide a unique system for high-throughput screening of new therapeutic molecules for the treatment of Alzheimer's disease.

Poster #15

Title: Characterization of a homologue of the Batten disease protein CLN3 in the model eukaryote *Dictyostelium*

Authors: Robert J. Huber[1], Susan L. Cotman[1], and Michael A. Myre[1]

Presenter: Michael A. Myre

Address for Correspondence: Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA

Abstract: Batten disease is a fatal childhood neurodegenerative disease that belongs to a common group of disorders known as neuronal ceroid lipofuscinosis (NCLs) and is caused by mutations in the *cln3* gene. CLN3 protein function has been studied in a number of systems however the precise function for this protein remains unknown. In this study, we have initiated characterization of the CLN3 homologue in the social amoeba *Dictyostelium* (DdCLN3). This organism has been an excellent model system for studying a variety of cell and developmental processes and has been successfully used in basic biomedical research in the study of a number of human diseases, including neurodegenerative disorders. We have generated *Dictyostelium cln3*-null cells and are currently assessing how *cln3*-deficiency impacts *Dictyostelium* during growth and development. Generation of cells expressing DdCLN3:GFP and human CLN3:GFP fusion proteins co-localize to the endomembrane system. Furthermore, overexpression of either *Dictyostelium* or human CLN3 rescues developmental phenotypes. The significance of these results is discussed.

Poster #16

Title: Investigating the Membrane-Binding Activity and Localization of Copine Proteins in *Dictyostelium*

Authors: Janet E. Price, Dexter R. McKellar, Jordan D. Dix, Bria N. Graham, and Cynthia K. Damer

Presenter: Janet Price

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Abstract: Copines are a family of membrane-binding proteins found in many diverse organisms and are hypothesized to be involved in cell signaling pathways. Copines have two C2 domains, which typically confer calcium-dependent lipid-binding activity. The C2 domains are followed by an A domain, which is similar to the VWA domain found in integrins and is thought to be a protein-binding domain. We are using *Dictyostelium discoideum* to study copine (cpn) function and have focused our work on cpnA. To study the other five copine genes, cpnB-cpnF, we obtained full-length cDNA clones of cpnB and cpnE and partial cDNA clones of cpnD and cpnF. We used PCR to create full-length cDNA clones for cpnD and cpnF by adding the missing bases to the primers. We are using RT-PCR to create the cDNA for cpnC. To determine the intracellular location of each of the Copine proteins, we are tagging each protein with GFP. We have created constructs to express CpnB and CpnE tagged with GFP at either the N or C-terminus. Using fluorescence microscopy, we observed GFP-tagged CpnB and CpnE in the cytoplasm and associated with intracellular organelles. CpnB was also observed in the nucleus in some cells. Membrane binding assays indicated that CpnB and CpnE with GFP at the C-terminus pelleted with membranes in a calcium-dependent manner; however, when the GFP was at the N-terminus, the GFP-copine proteins pelleted with membranes in a calcium-independent manner.

Poster #17

Title: Cloning a stable *Dictyostelium discoideum* genomic DNA library

Authors: Rafael D. Rosengarten, Pamela R. Beltran, and Gad Shaulsky

Presenter: Rafael D. Rosengarten

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Abstract: The social amoeba *Dictyostelium discoideum* is a haploid eukaryote long established as a genetic model for numerous biological phenomena and processes. These include studies of chemotaxis, cell differentiation and development, allorecognition, and signal processing and pattern formation. One of the most powerful and widely used methods for probing *Dictyostelium* genetics is Restriction Enzyme-Mediated Integration (REMI), in which gene function is disrupted by selectable marker insertion, and the resultant lesions identified by plasmid recovery. However, no method exists for complementation that allows recovery of other classes of haploid mutations, such as point mutations or marker-less indels. One major hurdle in *Dictyostelium* molecular biology has been difficulty in faithfully cloning its genomic DNA—notoriously AT-rich, prone to recombination, secondary structure formation and recalcitrant to in vivo cloning and in vitro amplification. This challenge precludes any attempt to test whether a library of genes in their native genomic contexts can complement haploid mutations. We report building a medium-insert *D. discoideum* genomic library in *E. coli*. Using the N-15 phage derived vector pJAZZ-OK (Lucigen), we cloned partial restriction fragments of three to ten kilobases. Fifty-four percent of end-sequenced clones mapped to the six gene-containing chromosomes, 34% derived from ribosomal repeats and the rest were ambiguous. The chromosomal positions of mapable inserts were statistically unbiased. Critically, we demonstrated that inserts are stable over multiple liquid culture dilutions. We subsequently have modified the vector to harbor a blasticidin-S deaminase selectable marker, and are rebuilding the library to test in mutants generated by chemical mutagenesis. We are currently testing several approaches for complementation using a known *Dictyostelium* mutant (tgrC1-) and defined dilutions of a pJAZZ-tgrC1construct. In addition to complementation, the ability to stably clone large genomic fragments should facilitate whole-genome sequencing efforts of additional strains and species, and expands our toolkit for genetic engineering.

Poster #18

Title: Gene regulatory networks in *Dictyostelium*

Authors: Balaji Santhanam, Mariko Katoh-Kurasawa, Huaqing Cai, Gregor Rot, Blaz Zupan, Peter Devreotes, Adam Kuspa, Gad Shaulsky

Presenter: Balaji Santhanam

Address for correspondence: Gad Shaulsky, Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX

Abstract: Gene regulation and transcriptional control are essential for cells to perform a variety of functions including cell division, response to signaling pathways, cell differentiation and development. Interactions between transcription factors (TFs) and their DNA binding sites are necessary to achieve transcriptional control. *D. discoideum* has about 200 predicted TFs, however, their binding site preferences and their downstream target genes, both direct and indirect, are not well understood.
D. discoideum cells lacking gtaC, one of 25 belonging to the GATA family of transcription factors, are defective in aggregation and do not form any multicellular structures. Using RNA-seq, we are trying to understand the effects of the mutation (gtaC–) at the mRNA level. In order to understand the genome-wide binding preferences of GtaC and to identify its direct targets, we performed ChIP-seq. We are trying to identify specific DNA motifs that are recognized and bound by GtaC by analyzing the promoter regions that show enriched binding. We are in the process of developing novel data fusion methodologies to integrate gene expression data from RNA-seq experiments, TF binding preferences from ChIP-seq experiments and DNA binding motifs from promoter analyses to identify key components of this transcriptional network in *Dictyostelium*.

Poster #19

Title: The role of microbial pattern recognition in bacterial-induced autophagy in *Dictyostelium discoideum*

Authors: Michelle Dykstra Snyder1, Marc Fink1, Katherine Pflaum1, Kierra Stephens1,2, Kossi Yovo1,2

Presenter: Michelle Dykstra Snyder

Address for correspondence: 1Towson University, Dept. of Biological Sciences, 8000 York Rd., Towson, MD 21252, 2Baltimore City Community College, 2901 Liberty Heights Ave., Baltimore, MD 21215

Abstract: Pattern recognition receptors found on innate immune cells allow for the detection of conserved molecular patterns found on microbial invaders. Recent evidence suggests that the social amoeba *Dictyostelium discoideum* may use conserved pattern recognition machinery to detect bacterial prey and to mount immune defenses against potential pathogenic bacteria they might encounter. Among cellular responses downstream of microbial pattern recognition in mammalian innate immune cells is the induction of autophagy, a process by which newly formed autophagosomes engulf intracellular contents, including damaged organelles and invading bacteria, and deliver them to acidic lysosomes for degradation. We have found that autophagosomal maturation is stimulated in *D. discoideum* cells upon incubation with bacteria or with the microbial pattern lipopolysaccharide (LPS). Our finding that exposure of cells to the autophagy-inducing drug rapamycin results in an increase in the rate of bacterial clearance points to a potential function of microbial-induced autophagy in *D. discoideum*. Further evidence linking pattern recognition and autophagy comes from studies showing a decrease in LPS-enhanced lysosomal transport of bacteria in *D. discoideum* cells lacking the autophagy protein, Atg1. We are currently investigating the effect of the proposed *D. discoideum* pattern recognition protein, TirA (toll/interleukin-1 receptor), on bacterial induction of autophagy by assessing both the expression of autophagy-related genes and the induction of autophagosomal maturation in TirA-deficient cells exposed to bacteria. Our preliminary results suggest an alteration in transcription of autophagy-related genes in TirA-deficient cells exposed to bacteria. In addition, we have found that bacterial-induced generation of reactive oxygen species, a potential stimulant of autophagy, is decreased in cells lacking TirA. These studies are aimed at linking microbial pattern recognition and the induction of autophagy in this model organism in order to provide greater insight into conserved mechanisms underlying pathogen detection.

Poster #20

Title: Developmental lineage priming by heterogeneous Ras activation

Authors: Wouter van Zon[1], Alex Chattwood[1,§], Koki Nagayama[1,§], Lauren Harkin[1], Marzieh Kamjoo1, Parvin Bolourani2, Gerald Weeks[2] and Christopher R.L. Thompson[1]*

Presenter: Wouter van Zon

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Abstract: In cell culture, genetically identical cells often exhibit heterogeneous behavior, with only 'lineage primed' cells responding to differentiation inducing signals. It has recently been proposed that such heterogeneity exists during normal embryonic development to allow position independent patterning based on 'salt and pepper' differentiation and sorting out. However, the molecular basis of lineage priming and how it leads to reproducible cell type proportioning is poorly understood.

To address this, we employed a novel forward genetic approach in the model organism *Dictyostelium discoideum*. This screen allowed us to identify genes involved in the nutritional control of cell fate decisions. Follow-up studies revealed the Ras-GTPase regulator, *gefE*, to be required for normal lineage priming and salt and pepper differentiation. We find that this is because Ras-GTPase activity sets the intrinsic response threshold to lineage specific differentiation signals. Importantly, we show that although *gefE* expression is uniform, transcription of its target, *rasD*, is both heterogeneous and dynamic, thus providing a novel mechanism for heterogeneity generation and position independent differentiation.

Currently, we are analysing other key components downstream of the *gefE* pathway, identified by comparative RNA sequencing, together with other genes found in the genetic screen described above. In the future, we aim to apply similar genetic approaches to other biases which could influence cell fate, such as cell cycle position. This may allow us to give a better understanding of the mechanisms underpinning heterogeneous lineage priming.

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