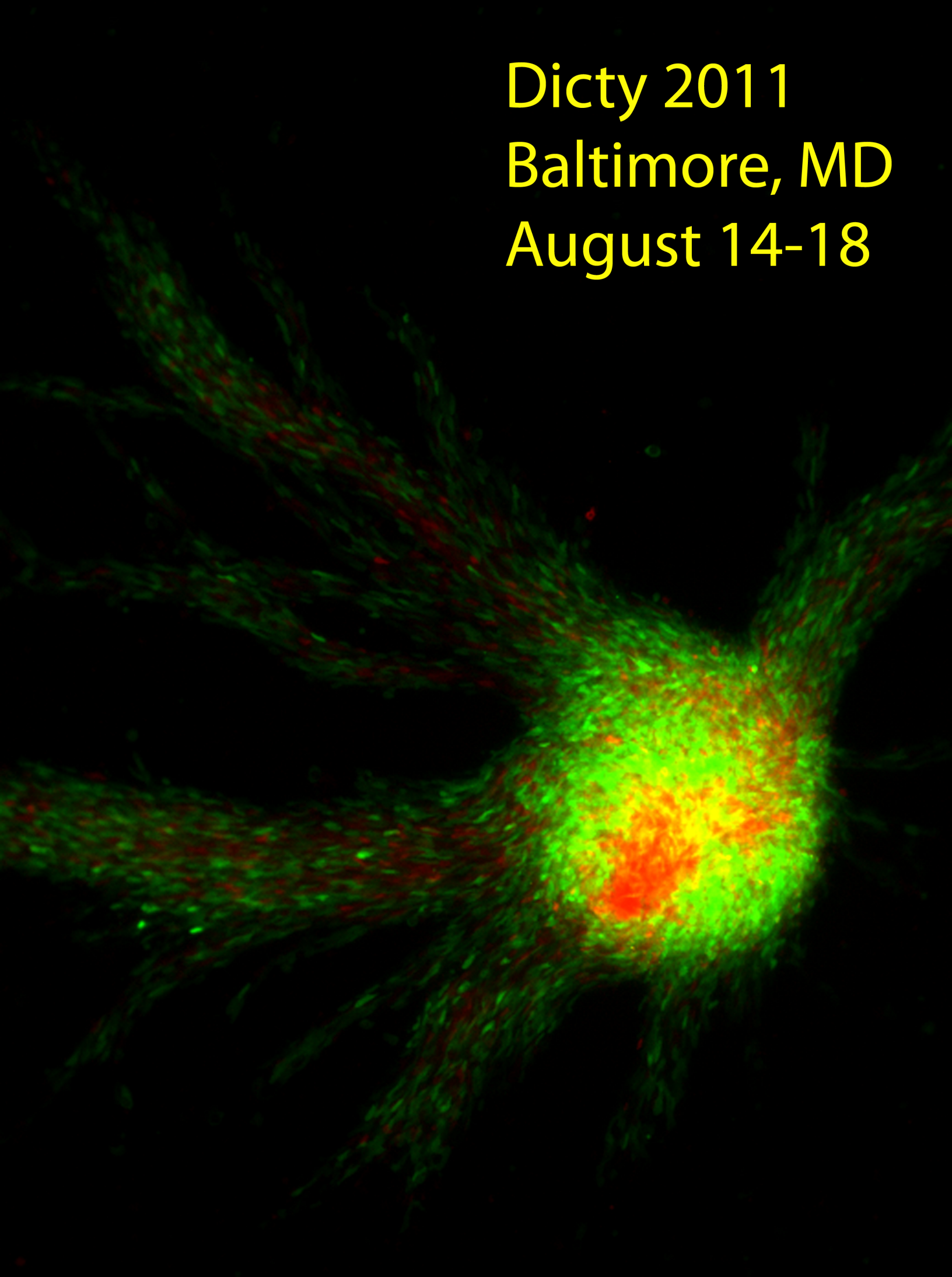
Dicty 2011
Baltimore, MD
August 14-18

# Welcome to

# Dicty 2011

## Radisson Cross Keys

## Baltimore, MD

<u>Organizers</u>
Douglas Robinson
Peter Devreotes
Miho Iijima

RADISSON HOTEL
AT CROSS KEYS

**We thank Ian Hodge and Formedium for sponsoring this meeting**

# Dicty 2011 Program

**Sunday, Aug. 14**

| | | |
|---|---|---|
| 5:00-7:00 p.m. | Registration and Reception | *Cross Keys Courtyard or White Oak Foyer (depending on weather)* |
| 7:00-8:00 p.m. | **Plenary Lecture by** ***James A. Spudich*** | *White Oak Ballroom* Abstract Page No.: vii |
| 8:00 p.m. - on | Open time, cash bar on the first floor | |

**Monday, Aug. 15**

| | | |
|---|---|---|
| **7:30-8:30 a.m.** | **Breakfast** | *Cross Keys Courtyard or White Oak Foyer* |
| | ***Genomics and Gene Expression*** *Alexandra Surcel, Moderator* | *White Oak Ballroom* |
| | | Abstract Page No**.** |
| 8:30-8:45 | Petra Fey | 2 |
| 8:55-9:10 | Gernot Gloeckner | 3 |
| 9:20-9:35 | Christian Hammann | 4 |
| 9:45-10:00 | James Platt | 5 |
| 10:10-10:30 | **Break** | *White Oak Foyer* |
| 10:30-10:45 | Mariko Katoh-Kurasawa | 6 |
| 10:55-11:10 | Tsuyoshi Araki | 7 |
| 11:20-11:35 | Linh Vu Hai | 8 |
| **12:00-1 p.m.** | **Lunch** *(DictyBase Scientific Advisory Board Meeting with lunch in the Mather Room, 12-2 p.m.)* | *Woodland and Dogwood Rooms* |
| 1:00-2:15 p.m. | Afternoon Break | |
| | ***Adhesion*** *Hoku West-Foyle, Moderator* | *White Oak Ballroom* |
| 2:15-2:30 | Bill Loomis | 10 |
| 2:40-2:55 | Erin Rericha | 11 |
| 3:05-3:20 | Pierre Cosson | 12 |
| 3:30-3:45 | Chi-Hung Siu | 13 |
| 3:55-4:10 | Gong Chen | 14 |

| | | |
|---|---|---|
| 4:20-4:35 | **Break** | *White Oak Foyer* |
| | ***Growth and Motility I***<br>*Kristen Swaney, Moderator* | *White Oak Ballroom* |
| 4:35-4:50 | Rob Kay | 16 |
| 5:00-5:15 | Robert Insall | 17 |
| 5:25-5:40 | Robert Cooper | 18 |
| **5:50-7 p.m.** | **Dinner Buffet** | *Woodland and Dogwood Rooms* |
| | ***Growth and Motility II***<br>*Sheil Kee, Moderator* | *White Oak Ballroom* |
| 7:30-7:45 | Wolfgang Losert | 19 |
| 7:55-8:10 | Laurent Golé | 20 |
| 8:20-8:35 | Jonathan Phillips | 21 |
| 8:45-9:00 | Wouter-Jan Rappel | 22 |
| 9:10-10:30 p.m. | **Posters and Cash Bar** | *Halls outside White Oak Ballroom*<br>67-106 |
| 10:30 p.m.-12 a.m. | Shuttles to Mt. Washington Tavern | |

**Tuesday, Aug. 16**

| | | |
|---|---|---|
| **7:30-8:30 a.m.** | **Breakfast** | *Woodland and Dogwood Rooms* |
| | ***Ensemble Behaviors***<br>*Tianzhi Luo, Moderator* | *White Oak Ballroom* |
| | | Abstract Page No. |
| 8:30-8:45 | Neil J Buttery | 24 |
| 8:55-9:10 | Shigenori Hirose | 25 |
| 9:20-9:35 | Pauline Schaap | 26 |
| 9:45-10:00 | Debbie Brock | 27 |
| 10:10-10:25 | Yekaterina Poloz | 28 |
| **10:45 a.m. – 2 p.m.** | **Inner Harbor's Spirit Lunch Cruise** | |
| 10:45 a.m. | Board Buses to Inner Harbor | |
| 11:30 | Board Cruise Ship | |
| 12 p.m. | Disembark | |
| 2 p.m. | Return | |
| | ***Chemomechanical Signaling***<br>*Yulia Artemenko, Moderator* | *White Oak Ballroom* |
| 3:00-3:15 | Arhana Chattopadhyay | 30 |
| 3:25-3:40 | Tianzhi Luo | 31 |

| | | |
|---|---|---|
| 3:50-4:05 | Yee Seir Kee | 32 |
| **4:15-4:30** | **Break** | *White Oak Foyer* |
| | ***Centrosomes, Kinesins, Regulators***<br>*Yulia Artemenko, Moderator* | *White Oak Ballroom* |
| 4:30-4:45 | Mike Koonce | 34 |
| 4:55-5:10 | Nao Shimada | 35 |
| 5:20-5:35 | Annette Müller-Taubenberger | 36 |
| **5:45-7 p.m.** | **Dinner Buffet** | *Woodland and Dogwood Rooms* |
| | ***G-proteins and cAMP***<br>*Vasudha Srivastava, Moderator* | *White Oak Ballroom* |
| 7:30-7:45 | Hidekazu Kuwayama | 38 |
| 7:55-8:10 | Jeff Hadwiger | 39 |
| 8:20-8:35 | Keita Kamino | 40 |
| 8:45-10 p.m. | **Posters and Reception** | *Halls outside White Oak Ballroom*<br>67-106 |
| 10 p.m.-12 a.m. | Shuttles to Mt. Washington Tavern | |

**Wednesday, Aug. 17**

| | | |
|---|---|---|
| **7:30-8:30 a.m.** | **Breakfast** | *Cross Keys Courtyard or White Oak Foyer* |
| | ***Chemotaxis I***<br>*Huaqing Cai, Moderator* | *White Oak Ballroom* |
| | | Abstract Page No. |
| 8:30-8:45 | Rick Firtel | 42 |
| 8:55-9:10 | Satarupa Das | 43 |
| 9:20-9:35 | Alan Kimmel | 44 |
| 9:45-10:00 | Herbert Levine | 45 |
| **10:10-10:35** | **Break** | *White Oak Foyer* |
| 10:35-10:50 | Pascale Charest | 46 |
| 11:00-11:15 | Yulia Artemenko | 47 |
| 11:25-11:40 | Michelle Tang | 48 |
| 11:50-11:55 | Petra Fey (*Update on Dicty 2012*) | |

## *Dictyostelium*: a marvelous model system for multifaceted molecular studies

**James A. Spudich**

It is a special honor to be asked to be the Plenary Speaker at this year's *Dictyostelium* meeting. I thank the organizers, especially Doug Robinson, for giving me the opportunity to share my experiences with *Dictyostelium* as a model system to address important cell biological problems.

I had just finished a postdoc in Cambridge, England working on aspects of muscle contraction with Hugh Huxley, and wanted to explore an appropriate model eukaryotic cell to understand the structure and roles of the cytoskeleton in cell biology. I knew the value of model systems, but nearly all of my early experiences, outside the two years studying the muscle actin-myosin system, were with bacteria. Prokaryotes were not suitable for my major question at the time – are there myosin-like molecules in non-muscle cells and, if so, what are their cellular roles?

During the first year of my assistant professorship at UCSF, we grew *Neurospora*, *Saccharomyces*, *Physarum*, and *Dictyostelium* (introduced to me by Bill Loomis and Rick Firtel when I visited UCSD as a potential faculty candidate). We made extracts of each cell type and searched for a myosin-like motor. Of primary concern was the ability to obtain large quantities of cell pellets for purifying proteins from cell lysates – *Dictyostelium* proved to be perfect for this purpose! In the early 1980s, I confess that I was considering shifting to a new model system due to the lack of good genetic approaches for *Dictyostelium*. A very interesting set of accidental circumstances led my sharp-eyed, superb graduate student Arturo De Lozanne to discover homologous recombination in this organism in 1987 when he knocked out the myosin II gene, which added a pivotal dimension to our research for the next couple of decades.

We did indeed find a *Dictyostelium* myosin with properties similar to conventional muscle myosin, and we developed methods for visualizing the cytoskeleton in this organism. Later, we showed that this *Dictyostelium* non-muscle myosin II is essential for cytokinesis but, surprisingly, not for cell migration. We then characterized what was important about the structure and function of this myosin II for cell division. Along the way, we discovered a number of other *Dictyostelium* myosin isoforms and explored their roles in the biology of the cell. Importantly, having made a null myosin II strain we were able to study the effects of genetically modified molecules to elucidate how the myosin molecular motor works to transduce the chemical energy of ATP hydrolysis into mechanical movement. It's no surprise that I am a huge *Dictyostelium* fan!

There is, however, no perfect model system. This fact has challenged researchers throughout history. We are now embarking on a new quest – understanding the molecular basis of hypertrophic and dilated cardiomyopathies that lead to severe illness and sudden death. So, from muscle to *Dictyostelium*, we now return to muscle, specifically human cardiac muscle. But I will always have fond memories of the terrific opportunities offered to us by *Dictyostelium* in our quest to understand the roles of molecular motors in biology.

# *Genomics and Gene Expression*

**Title:** Gene Model Curation at dictyBase

**Authors:** Petra Fey, Pascale Gaudet, Robert Dodson, Yulia Bushmanova, Siddhartha Basu, Warren A. Kibbe, and Rex L. Chisholm

**Presenter:** Petra Fey

**Address for correspondence:** Petra Fey, Northwestern University/NUBIC, 750 N. Lake Shore Drive, Chicago, IL 60611, USA

**Abstract:** In 2011 we completed the 'first-pass' gene model curation of the *Dictyostelium discoideum* genome. This was a seven-year effort that culminated in the manual inspection of all gene predictions [1] and resulted in the annotation of 12,257 protein coding genes. Nearly half of the gene models have been curated within the past year, thanks to a strong focus on the task. We will present the genome curation highlights from the *D. discoideum* AX-4 strain, including the accuracy of the automated pipeline predictions, the number of genes with corrected exon/intron junctions, and pseudogene characterization. We will show our new gene curation tool that allows curators to see a wide range of evidence for the gene model in one glance (ESTs, RNA expression, sequence similarity, etc.) and enables easy approval of a gene model in case no modifications are needed. We discuss possible causes of wrong automatic calls, and show the different kinds of corrections made. We present our guidelines for pseudogene annotations and highlight some genes that have evidence suggesting multiple transcripts. Finally we will present an updated assessment of average gene lengths, protein lengths, intron lengths, intron numbers, and the number of genes with introns.

[1] Eichinger *et al*. (2005) 'The genome of the social amoeba *Dictyostelium* discoideum.' Nature 435:43-57

dictyBase and the Dicty Stock Center are supported by grants from the NIH (GM64426, GM087371 and HG00022)

**Abstract type:** Talk

**Title:** Comparative genomics in social amoebae

**Authors:** Andrew J. Heidel, Hajara M. Lawal, Marius Felder, Christina Schilde, Nicholas R. Helps, Budi Tunggal, Francisco Rivero, Uwe John, Michael Schleicher, Ludwig Eichinger, Matthias Platzer, Angelika A. Noegel, Pauline Schaap, Gernot Glöckner

**Presenter:** Gernot Gloeckner

**Address for correspondence:** Leibniz-Institute of Freshwater Ecology and Inland Fisheries, IGB Müggelseedamm 301 D-12587 Berlin

**Abstract:** *Dictyostelium discoideum* (DD), an extensively studied model organism for cell and developmental biology, belongs to the most derived group 4 of social amoebas, a clade of altruistic multicellular organisms. To understand genome evolution over long time periods and the genetic basis of social evolution, we sequenced the genomes of *Dictyostelium fasciculatum* (DF) and *Polysphondylium pallidum* (PP), that represent the early diverging groups 1 and 2, respectively. In contrast to DD, PP and DF have conventional telomere organisation and strongly reduced numbers of transposable elements. The number of protein coding genes is similar between species, but only half of them comprise an identifiable set of orthologous genes. In general, genes involved in primary metabolism, cytoskeletal functions and signal transduction are conserved, while genes involved in secondary metabolism, export and signal perception underwent large differential gene family expansions. This most likely signifies involvement of the conserved set in core cell and developmental mechanisms, and of the diverged set in niche- and species-specific adaptations for defense and food, mate and kin selection. Several indicators such as protein divergence, high ratio of non-synonymous to synonymous mutations and extensive loss of synteny show that DF, PP and DD split from their last common ancestor at least 0.6 billion years ago.

**Abstract type:** Talk

**Title:** Sequence and Generation of Mature Ribosomal RNA Transcripts in *Dictyostelium discoideum*

**Authors:** Carsten Boesler, Janis Kruse, Fredrik Soderbom and Christian Hammann

**Presenter:** Christian Hammann

**Address for correspondence:** Heisenberg Research Group Ribogenetics, Technical University of Darmstadt, 64287 Darmstadt, Germany

**Abstract:** The amoeba *Dictyostelium discoideum* is a well-established model organism for studying numerous aspects of cellular and developmental functions. Its ribosomal RNA (rRNA) is encoded in an extrachromosomal palindrome that exists in approx. 100 copies in the cell. In this study, we have set out to investigate the sequence of the expressed rRNA. For this, we have ligated the rRNA ends and performed RT-PCR on these circular RNAs. Sequencing revealed that the mature 26 S, 17 S, 5.8 S, and 5 S rRNAs have sizes of 3741, 1871, 162, and 112 nucleotides, respectively. Unlike the published data, all mature rRNAs of the same type uniformly display the same start and end nucleotides in the analyzed AX2 strain. We show the existence of a short lived primary transcript covering the rRNA transcription unit of 17 S, 5.8 S, and 26 S rRNA. Northern blots and RT-PCR reveal that from this primary transcript two precursor molecules of the 17 S and two precursors of the 26 S rRNA are generated. We have also determined the sequences of these precursor molecules, and based on these data, we propose a model for the maturation of the rRNAs in *Dictyostelium discoideum* that we compare with the processing of the rRNA transcription unit of *Saccharomyces cerevisiae*.

**Abstract type:** Talk

**Title:** Functional and Gene Specificity of the CHD Chromatin Remodeling Family.

**Authors:** James L. Platt^*, Ben J. Rogers*, Nick A. Kent*, Adrian J. Harwood* and Alan R. Kimmel^

**Presenter:** James Platt

**Address for correspondence:** ^ NIDDK/NIH, Bethesda, Maryland, 20892, USA * Cardiff School of Biosciences, Cardiff University, Cf10 3AX, UK

**Abstract:** CHD (Chromo, Helicase, DNA binding domain) family proteins are one of the four major types of ATP-dependent, chromatin remodeling factors. CHDs utilize energy from the hydrolysis of ATP to alter nucleosome positioning and control access of transcription factors/polymerase to the DNA backbone. CHDs are linked to both activation and repression of gene expression, and aberrant CHD functions are associated with several human diseases, including dermatomyositis, Hodgkin's lymphoma, neuroblastoma, and CHARGE syndrome. However, it is not yet clear how the specificity of individual CHDs is directed to unique gene subsets. Employing *Dictyostelium discoideum*, we have begun to characterize targeted gene specificity and functions of individual CHD proteins. *Dictyostelium* has three CHD proteins (ChdA, ChdB, ChdC) that cluster with the CHD protein subfamilies I (ChdA) and III (ChdB,C) of humans. We have created gene disruption mutations for each CHD gene, and shown they have developmental phenotypes that correlate with their unique protein expression patterns and that are consistent with non-redundant developmental functions. Developmental mRNA deep sequencing (Illumina RNAseq) analyses indicate a role for ChdC in co-ordinated gene expression throughout development. Further, by coupling micrococcal nuclease digestions and high throughput sequencing, we have been able to map nucleosome positions at a genome-wide level in wild-type cells and shown specific changes in chromatin organization in *chdC*-null cells. These data provide mechanistic insight into the fundamentals of chromatin remodeling and developmentally regulated gene expression.

**Abstract type:** Talk

**Title:** Conserved regulation of developmental gene expression

**Authors:** Mariko Katoh-Kurasawa, Eryong Huang, Danyeal Heckard, Shaheynoor Talukder, Timothy Hughes, Tomaz Curk, Blaz Zupan, Adam Kuspa and Gad Shaulsky

**Presenter:** Mariko Katoh-Kurasawa

**Address for correspondence:** Baylor College of Medicine, One Baylor Plaza, Houston TX 77030

**Abstract:** Genome sequencing projects have yielded data on 5 different Dictyosteldis, which should enable us to perform comparative sequence analysis of developmental genes in these organisms. We have analyzed the developmental expression profiles of all the genes in *Dictyostelium* discoideum and *Dictyostelium purpureum* and revealed that as many as 50% of the genes are developmentally regulated in both species and the expression patterns of about 50% of the orthologs are conserved. Some of the outstanding questions in the field are how genes are regulated and what is the relationship between gene regulation and development. In most organisms, proper progression of cell differentiation and morphogenesis are regulated by transcription factors and chromatin remodeling elements. Surprisingly, the *Dictyostelium* genomes encode fewer transcription factors than any other sequenced eukaryotic genome. Moreover, genetic screens for developmental mutants have not yielded many transcription factors. These findings suggest that developmental gene expression in *Dictyostelium* is regulated by complex networks, which consist of many overlapping elements. We propose that the central elements of these networks are conserved in evolution. To find links between gene expression and specific developmental events, we focused on transcription factors whose coding sequences and developmental expression patterns are conserved in *D. discoideum* and *D. purpureum*. BzpF and GtaG are two examples. Protein Binding Microarray analysis revealed that BzpF binds the canonical cAMP response element (CRE) and CRE-like motifs, and GtaG binds to AGATCTAA. Transcription regulatory networks are established when one transcription factor regulates another. We found that bzpF is a potential targets of GtaG because its promoter contains a GtaG binding motif and its developmental expression is positively correlated with that of gtaG with a short delay. Mutant analysis has revealed that these factors regulate sequential processes in development. *gtaG*$^{-}$ mutants are arrested at the slug stage and fails to produce spores in a non-cell-autonomous manner. *bzpF*$^{-}$ mutants produce compromised spores, which are extremely unstable and germination defective. We propose that these transcription factors coordinately regulate developmental processes.

**Abstract type:** Talk

**Title:** Identification of the kinase that tyrosine phosphorylates and activates a non-metazoan STAT

**Authors:** Tsuyoshi Araki[1], Takefumi Kawata[2] and Jeffrey G. Williams[1]

**Presenter:** Tsuyoshi Araki

**Address for correspondence:** [1] College of Life Sciences, Welcome Trust Biocentre, University of Dundee, Dow St., Dundee DD1 5EH, United Kingdom, [2] Department of Biology, Faculty of Science, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan

**Abstract:** *Dictyostelium* is the only non-metazoan organism with functionally characterised SH2 domains but the cognate tyrosine kinases have not been identified. There are no identifiable orthologues of the animal tyrosine kinases but there are an exceptionally large number of tyrosine kinase-like (TKL) proteins. The Signal Transducer and Activator of Transcription STATc is phosphorylated on tyr992 when cells are treated with the prestalk inducer DIF-1 or exposed to hyper-osmotic stress. We show genetically that a TKL, Pyk2, is essential for DIF-1 induced tyrosine phosphorylation of STATc. We also present biochemical evidence that Pyk2 is the kinase that inducibly phosphorylates STATc in response to DIF-1 or stress. The fact that a TKL protein phosphorylates a non-metazoan STAT significantly extends our understanding of the evolutionary origins of SH2 domain-phosphotyrosine signaling.

**Abstract type:** Talk

**Title:** Identification of two tyrosine kinase-like (TKL) proteins that redundantly activate STATc in response to hyper-osmotic stress

**Authors:** Linh Vu Hai (1), Tsuyoshi Araki (2), Jianbo Na (1), Takefumi Kawata (3), Jeffrey G. Williams (2) and Ludwig Eichinger (1)

**Presenter:** Linh Vu Hai

**Address for correspondence:** (1) Center for Biochemistry, Medical Faculty, University of Cologne, Joseph-Stelzmann-Str. 52, 50931 Cologne, Germany (2) College of Life Sciences, Welcome Trust Building, University of Dundee, Dow St., Dundee DD1 5EH, Scotland (3) Department of Biology, Faculty of Science, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan

**Abstract:** *Dictyostelium* possesses effective response mechanisms to changes in the osmotic environment that allows it to adapt. Subjected to high osmolarity the cells react by changes in cell shape, the cytoskeleton, transport processes, metabolism and gene expression. Previously we found that STATc (Signal Transducer and Activator of Transcription c), one of four STAT-Proteins encoded in the *Dictyostelium* genome, is a key regulator of the transcriptional response to hyper-osmotic stress. Under these conditions STATc is activated by tyrosine phosphorylation, dimerises and translocates to the nucleus where it regulates the expression of a large number of target genes (Araki *et al.*, 2003, Na *et al.*, 2007). In vertebrates, STAT proteins are in most cases activated by an upstream Janus kinase (JAK) upon receipt of extracellular stimuli. In *Dictyostelium*, however, there are no recognisable JAK orthologues and the identity of the STATc kinase(s) is still unresolved. Based on microarray results and sequence analysis we identified four kinases that depend for their expression on STATc: Pyk3 (PkyA), MORN, Pyk1 (SplA) and Phg2, all members of the TKL (tyrosine kinase-like) group. We confirmed their STATc-dependent differential regulation in response to stress by real-time PCR and generated knock-out strains for Pyk3 and Phg2. Strikingly, we find that both, Pyk3 and Phg2, are required for optimal stress-induced activation of STATc, strongly implying a positive feed-back loop. Similar results were obtained using 8-bromo-cGMP, a membrane-permeable form of cGMP and known activator of STATc, as inducer. We also show, using an *in vitro* phosphorylation assay, that sorbitol and 8-bromo-cGMP treatment increases Pyk3 activity as a STATc tyrosine kinase, indicating that these are direct kinase-substrate interactions. A double knock-out strain is currently being generated and should tell us whether additional TKL proteins are required for stress-induced STATc phosphorylation. Our results imply that in *Dictyostelium* at least two TKL proteins, Pyk3 and Phg2, are responsible for stress-induced STATc activation.

**Abstract type:** Talk

# *Adhesion*

**Title:** Innate cell adhesion mediated by non-specific surface glycoproteins

**Authors:** Danny Fuller, Albert Bae, Monica Skoge, Edgar Gutierrez, Alex Groisman, Herbert Levine, Wouter-Jan Rappel and William F. Loomis

**Presenter:** Bill Loomis

**Address for correspondence:** Cell and Developmental Biology, University of California San Diego, La Jolla, CA 92093

**Abstract:** Cells of *Dictyostelium discoideum* do not appear to use an integrin-based form of adhesion to gain traction during chemotaxis since the genome does not carry genes encoding integrin homologs. Moreover, the cells are highly motile on both hydrophobic and hydrophilic surfaces in microfluidic devises where components of extracllular matrices would be washed away. Since adsorption to naked glass does not involve covalent or hydrogen bonds, Van der Waals attraction of closely apposed membrane components appears to be the likely adhesive force. We have quantitatively measured substratum adhesion in a microfluidic device that generates a range of fluid dynamic shear stress. We find that addition of either 50 mM glucose or 50 mM amino acids to the microfluidic buffer significantly reduces cell adhesion and together they reduce it further, suggesting that surface glycoproteins are chiefly responsible for the van der Waals attraction. Moreover, if we briefly treat cells with alpha-mannosidase or N-acetyl-glucosaminidase, the adhesion is significantly reduced. Since membrane glycoproteins coat the surface of almost all eukaryotic cells, it is likely that this innate adhesion mechanism is wide spread. It may be the predominant mechanism for rapidly migrating cells such as lymphocytes and may facilitate cancer cell metastasis.

**Abstract type:** Talk

**Title:** Impact of surface adhesiveness on amoeboid adhesion, migration, and aggregation patterns

**Authors:** Erin C. Rericha, Colin McCann, Wolfgang Losert, and Carole Parent

**Presenter:** Erin Rericha

**Address for correspondence:** Institute for Reseach in Electronics and Applied Physics University of Maryland College Park Paint Branch Dr., Bldg #223 College Park, MD 20895

**Abstract:** In the canonical model of amoeboid cell migration, the bounds of motility are set by cell-surface adhesion; surface attraction is required for actin mediated extension of pseuodopods to push the cell center of mass forward, yet it must be sufficiently low to allow cells to de-adhere and retract their rear [1]. When collective migration occurs, as in epithelial sheet migration during wound healing, dorsal closure in embryogenesis, or group metastasis, the balance of resistive adhesion forces with protrusive forces is presumably altered as cell-cell contact provides an additional mass to push against [2]. Specific integrin binding sites are known to impact focal adhesions during individual migration [3]. In the absence of specific binding sites such as in the social amoeba *Dictyostelium*, we envision that hydrophobic attraction and electrostatics works in a similar manner [4]. We studied the ability of *Dictyostelium discoideum*, which migrate individually and collectively, to move on surfaces of varying hydrophobicity and charge. We found that these cells actively regulate their surface contact such that individuals adhere and migrate equally well on surfaces of dramatically varying properties without changing cell shape. Cells in which the cytoskeleton was altered through treatment with Latrunculin A or knocking out myosin II, in contrast, show marked differences in cell-surface contact, indicating that the regulation of adhesion is actomyosin-dependent. Cells crossing from one surface to another quickly adapt to the new surface, however crossings from low adhesion to high adhesion surfaces are rare. Remarkably, we find the dynamics of collective cell motion are strongly affected by surface properties, with cells migrating in a fashion that minimizes cell-surface contact on highly adherent surfaces.

[1] Lauffenburger, D. A simple model for the effects of receptor-mediated cell--substratum adhesion on cell migration. Chemical Engineering Science 44, (1989) 1903-1914.
[2] Hutson, et al, Forces for Morphogenesis Investigated with Laser Microsurgery and Quantitative Modeling. Science. 300, 145-149 (2003)
[3]Barnhart EL, Lee K-C, Keren K, Mogilner A, Theriot JA, 2011 An Adhesion-Dependent Switch between Mechanisms That Determine Motile Cell Shape. PLoS Biol 9 (2011)
[4]Renkawitz, J., Schumann, K., Weber, M., Lammermann, T., Pflicke, H., Piel, M., Polleux, J., Spatz, J.P., and Sixt, M. Adaptive force transmission in amoeboid cell migration. Nat Cell Biol 11, (2009) 1438-1443.

**Abstract type:** Talk

**Title:** TM9 proteins control surface expression and stability of SibA adhesion molecules in *Dictyostelium*

**Authors:** Romain Froquet, Marion le Coadic, Nathalie Cherix, Sophie Cornillon, Pierre Cosson

**Presenter:** Pierre Cosson

**Address for correspondence:** Pierre Cosson Centre Medical Universitaire Dept of Cell Physiology and Metabolism 1 rue Michel Servet CH1211 Geneva 4, Switzerland Email:Pierre.Cosson@unige.ch Tel. (41) 22 379 5293 Fax (41) 22 379 5338

**Abstract:** TM9 proteins form a family of conserved proteins essential for cellular adhesion in many biological systems, but their exact role in this process remains unknown. Here we found that in *Dictyostelium* amoebae, genetic inactivation of the TM9 protein Phg1 dramatically decreases the surface levels of the SibA adhesion molecule. This is due to a decrease in SIBA mRNA levels, in SibA protein stability, and in SibA targeting to the cell surface. A similar phenotype was observed in cells devoid of SadA, a protein that does not belong to the TM9 family but exhibits 9 transmembrane domains and is essential for cellular adhesion. A csA-SibA chimeric protein comprising only the transmembrane and cytosolic domains of SibA with the extracellular domain of csA also showed reduced stability and relocalization to endocytic compartments in phg1 knockout cells. These results indicate that TM9 proteins participate in cell adhesion by controlling the levels of adhesion proteins present at the cell surface.

**Abstract type:** Talk

**Title:** Identification of TgrB1 as the heterophilic binding partner of the cell adhesion molecule TgrC1/LagC: protein-protein interaction mediated by IPT/TIG domains

**Authors:** Gong Chen, Jun Wang, XiaoQun Xu, Xiangfu Wu, Ruihan Piao, and Chi-Hung Siu

**Presenter:** Chi-Hung Siu

**Address for correspondence:** Banting and Best Department of Medical Research and Department of Biochemistry, University of Toronto, Toronto, Ontario M5S 1A8, Canada

**Abstract:** During *Dictyostelium* development, three cell adhesion systems (DdCAD-1, gp80/csA, TgrC1/LagC) play important roles in aggregate formation and the maintenance of multicellularity. In the post-aggregation stages of development, cell-cell adhesion is maintained primarily by the heterophilic cell adhesion molecule TgrC1. To investigate the mechanism by which TgrC1 mediates cell-cell adhesion, we used far western blot assays to detect TgrC1 binding proteins in the plasma membrane and mass spectroscopy led to the identification of TgrB1, a 130 kDa plasma membrane glycoprotein, as the adhesion partner of TgrC1. The interaction between TgrB1 and TgrC1 was confirmed by pull-down and co-capping studies. Both tgrB1 and tgrC1 gene are located on chromosome 3 and they show identical transcription patterns during development. Antibodies raised against TgrB1 inhibited the re-association of dispersed post-aggregation stage cells and impaired the formation of fruiting bodies and spores. Additionally, cell strains expressing either TgrB1 or TgrC1 ectopically at the vegetative stage formed chimeric aggregates when cultured together, thus providing *in vivo* evidence for TgrB1/TgrC1-mediated cell-cell adhesion. Both TgrB1 and TgrC1 contain three IPT/TIG domains in their extracellular region. Binding studies using various truncated fragments of TgrB1 and TgrC1 showed that the TIG-2 domain of TgrC1 interacts with the TIG-3 domain of TgrB1 to mediate cell-cell adhesion. Ectopic expression of TgrC1 carrying mutations in the TIG-2 domain in AK127 (tgrC1-) cells failed to rescue these cells. Over-expression of wild type TgrB1 or TgrC1 in the double knockout cells led to the formation of tipped mounds but development was arrested at the slug stage resulting in the formation of abnormal morphological structures. These results suggest that both TgrB1 and TgrC1 play crucial roles in cell differentiation and morphogenesis during *Dictyostelium* development.

Supported by the Canadian Institutes of Health Research.

**Abstract type:** Talk

**Title:** Assembly of the TgrB1/TgrC1 Heterophilic Cell Adhesion Complexes during Development of Dictysotelium discoideum

**Authors:** Gong Chen, Xiaoqun Xu, Xiangfu Wu, Alexander Thomson, Alvin Li, and Chi-Hung Siu

**Presenter:** Gong Chen

**Address for correspondence:** Banting and Best Department of Medical Research and Department of Biochemistry, University of Toronto, Toronto, Ontario M5S 1A8, Canada

**Abstract:** TgrB1 and TgrC1 are two Ca2+/Mg2+-independent heterophilic adhesion partners, which function in the post-aggregation stages of *Dictyostelium* development. Neither *tgrB1* nor *tgrC1* knockout strain can complete development and produce viable fruiting bodies. To investigate the assembly of the TgrB1 and TgrC1 adhesion complexes, we employed GFP complementation assays using TgrB1 and TrgC1 fused to either of the split halves of GFP which were expressed ectopically in the knockout strains. We found that the cis-homodimerization of TgrC1 occurs constitutively, whereas cis-homodimerization of TgrB1 is dependent on its trans interaction with TgrC1. However, complementation assays show that TgrB1 and TgrC1do not form cis-heterodimers. Nevertheless, FRET analysis showed weak signals of energy transfer between TgrC1-GFP and TgrB1-RFP, indicating that they are in the close vicinity of each other. Based on these results, a model of the assembly process is proposed. To investigate downstream signaling pathways initiated by TgrC1/TgrB1, pull-down experiments using recombinant proteins containing the cytoplasmic domain of TgrC1 fused to GST were carried. Analysis of the pulled down proteins by MALDI-TOF mass spectrometry led to the identification of myosin heavy chain kinase C (MHCK-C) as a TgrC1 intercellular binding partner. Substitution of the basic residues in the TgrC1 cytoplasmic domain inhibited its binding with MHCK-C. Cells expressing these mutant TgrC1 proteins showed compromised cell motility and reduction in the size of slugs and fruit bodies. The data suggest that the binding of MHCK-C to the TgrC1 cytoplasmic domain influences cell motility and size regulation during morphogenesis.

Supported by the Canadian Institutes of Health Research.

**Abstract type:** Talk

# *Growth and Motility*

**Title:** Bleb-driven motility in *Dictyostelium*

**Authors:** Evgeny Zatulovskiy[1], Richard Tyson[2], Till Bretschneider[2] & Robert Kay[1]

**Presenter:** Rob Kay

**Address for correspondence:** 1. MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK 2. University of Warwick Coventry CV4 7AL, UK

**Abstract:** Blebs are protrusions of the plasma membrane formed when it detaches from the underlying cortex and is driven outwards by hydrostatic pressure. Although actin polymerization is commonly thought to be the motor driving protrusion of the leading edge of moving cells, blebs provide an alternative mode of protrusion, largely independent of actin. We confirm the observations of Yoshida & Soldati (JCS 119, 3833, 2006) that small blebs are frequently formed at the leading edge of *Dictyostelium* cells migrating in standard conditions. The membrane is projected 3-4 times faster than in actin-driven pseudopods and the expansion phase is typically complete in less than 1 second. A bleb leaves behind an F-actin cortical scar and is initially free of F-actin; however a new cortex forms within seconds so that blebs are difficult to distinguish from F-actin driven projections, except in films taken faster than 1 frame per second. These characteristics may account for blebs frequently being over-looked in standard chemotaxis movies. In resistive conditions, where more force is required to extend the membrane, cells can switch to moving almost entirely in bleb mode. Blebbing also increases with more developed cells, possibly in preparation for movement in the multicellular stages. Cells moving in steady cyclic-AMP gradients mainly form blebs at the front. If they are forced to re-orientate by changing the direction of the gradient, blebs can be formed in the new direction within 30 seconds, showing that the site of bleb formation is closely regulated by the gradient, and thus by the cAMP signal transduction system. Blebs can frequently transform into actin-driven pseudopods, and in general the two modes of extending the membrane often co-exist in the same cell. Genetically, blebbing require myosin II function, but conversely, blebbing is actually increased when actin polymerization is impaired by mutation of the SCAR or Arp2/3 complexes. The signal transduction pathway controlling bleb formation appears at least partially distinct from that controlling actin polymerization: crucially, it does not require PKB/PKBR1, nor the PI4P5kinase, PikI. We suggest that bleb mode motility should be considered on a par with that driven by actin polymerization, and suggest that the need of cells to regulate both modes of movement may account for some of the complexity of the cyclic-AMP signal transduction system.

**Abstract type:** Talk

**Title:** Actin and pseudopod dynamics in the control of *Dictyostelium* chemotaxis

**Authors:** Douwe Veltman, Seiji Ura, Jason King, Peter Thomason, Andrew Davidson, Laura Park, Matt Neilson, John Mackenzie and Robert Insall

**Presenter:** Robert Insall

**Address for correspondence:** Beatson Institute for Cancer Research, Switchback Road, Bearsden, Glasgow G63 9AE, UK

**Abstract:** Chemotaxis and cell migration are driven by actin polymerization in pseudopods. This actin polymerization is catalyzed by the Arp2/3 complex and the Arp2/3 activator SCAR (also known as WAVE in some organisms). SCAR activity is known to be controlled in turn by Rac and a number of other activators. However, the pattern of Rac activation is far less well spatially defined and temporally dynamic than SCAR itself, indicating that other upstream signals are key drivers in migration. However, we find most candidate signals also cannot control the localisation or timing of SCAR. The proline-rich domains, which mediate activation through SH3-containing adaptors such as Nck, are essential for SCAR function but do not provide specificity - either scrambling their sequence (to destroy specific SH3 binding sites) or swapping in polyproline domains from other adaptors does not change the specificity or dynamics of SCAR activation. PI 3-kinases are also not essential for SCAR dynamics. Instead, it appears that a positive feedback loop centred on SCAR, whose activity is modulated by Rac, can explain nearly all features of pseudopod dynamics. Thus the behaviour of pseudopods shows many characteristics of an excitable medium driven by positive feedback loops, in agreement with our "pseudopod-centred" explanation of chemotaxis which puts the dynamics of pseudopod growth rather than external signals at the heart of cell migration. We have generated a computational model based on this type of cycle, and find it explains nearly every measured observation about chemotaxis, strongly supporting a pseudopod-centred scheme. We will also discuss the mechanisms through which SCAR's activity is controlled, and explanations for the extremely surprising result that SCAR mutants can still make pseudopods and chemotax.

**Abstract type:** Talk

**Title:** New pseudopod dynamics are predicted by an excitable cortex with memory model

**Authors:** Robert Cooper, Ned Wingreen, Ted Cox

**Presenter:** Robert Cooper

**Address for correspondence:** Dept of Molecular Biology Princeton University Princeton, NJ 08544

**Abstract:** Motile eukaryotic cells migrate with directional persistence, even in the absence of external cues. Such directional persistence arises from an alternating, zig-zag pattern of pseudopod extension in cells such as *Dictyostelium discoideum*. The mechanisms underlying this zig-zag pattern, however, remain unknown. To understand pseudopod zig-zagging, we propose a new model which considers pseudopods to be bursts from an excitable cortex. The key feature giving rise to zig-zagging is our hypothesis that previous pseudopod activity makes the local cortex temporarily more excitable. Simulations using this model reproduce known behavior, and make several quantitative new predictions about pseudopod dynamics and placement. We test these predictions using a new pseudopod-detection algorithm, and find that data from live cell-tracking experiments agree with model predictions. Furthermore, our assumption that pseudopods temporarily alter cortical structure is supported by changes in GFP-myosin localization over time.

**Abstract type:** Talk

**Title:** Cell Shape Dynamics: From Waves to Migration

**Authors:** Meghan Driscoll, Colin McCann, Xiaoyu Sun, John Fourkas, Carole Parent, Wolfgang Losert

**Presenter:** Wolfgang Losert

**Address for correspondence:** Wolfgang Losert IREAP, Paint Branch Dr Bldg 223 University of Maryland College Park, MD 20742

**Abstract:** We demonstrate the existence of wave-like dynamic shape changes during the migration of *Dictyostelium discoideum*. Cell shapes have regions of high boundary curvature that propagate from the leading edge toward the back. We find that curvature waves initiate during protrusive activity of the leading edge and propagate along alternating sides of the cell. To examine the role of the surface contact in turning such waves into motion, we analyze the correlation between cell shape changes and changes in measured surface contact area. We also guided cells to extend with most of the cell body over the edge of fabricated micro-cliffs, only able to maintain surface contact at their rear. These cells still exhibit curvature waves that travel along their sides with the high characteristic speeds of waves near the leading edge. Finally, we strongly reduce overall cell-surface adhesion and observe a decoupling of curvature waves from migration. Consistent with other recent studies our observation of persistence of the wave-like protrusive activity explains the zig-zag-like appearance of cell tracks and the directional persistence of cell motion during chemotaxis.

**Abstract type:** Talk

**Title:** A quorum-sensing molecule in vegetative *Dictyostelium* cells revealed by quantitative migration analysis

**Authors:** Laurent Golé , Charlotte Rivière ,Yoshinori Hayakawa and Jean-Paul Rieu

**Presenter:** Laurent Golé

**Address for correspondence:** Laboratory of condensed matter and nanostructures, University of Lyon 1, CNRS, UMR 5586, 43 Boulevard du 11 Nov. 1918, Villeurbanne Cedex, France

**Abstract:** Many cells communicate through the production of diffusible signal molecules that accumulate and once a critical concentration has been reached, can activate or repress a number of target genes in a process termed quorum sensing (QS). In the social amoeba *Dictyostelium discoideum*, QS plays an important role during development. However little is known about its effect on cell migration especially in the growth phase. To investigate the role of cell density on cell migration, we used multisite timelapse microscopy and automated cell tracking. This analysis revealed a high heterogeneity within a given cell population, and the necessity to use large data sets to draw reliable conclusions on cell motion. In average, motion was persistent for short periods of time ($t \leq 5$min), but normal diffusive behavior was recovered over longer time periods. The persistence times were positively correlated with the migrated distance, indicating a constant intrinsic velocity for cells, with a movement efficiency governed by pseudopod extension direction. The adaptation of cell migration to cell density highlighted the role of a secreted quorum sensing factor (QSF) on cell migration. Using a simple model describing the balance between the rate of QSF creation and the rate of QSF dilution, we were able to gather all experimental results into a single master curve, showing a sharp cell transition between high and low motile behaviors with increasing QSF. This unambiguously demonstrates the central role played by QSF on amoeboid motion.

**Abstract type:** Talk

**Title:** Evidence for a chemorepellant produced by *Dictyostelium discoideum* during vegetative growth

**Authors:** Jonathan E. Phillips and Richard H. Gomer

**Presenter:** Jonathan Phillips

**Address for correspondence:** Richard Gomer, Texas A&M, Department of Biology MS 3474, College Station, TX 77843-3474. Phone: 979-458-5745. Email rgomer@bio.tamu.edu

**Abstract:** AprA and CfaD are proteins secreted by vegetative *Dictyostelium* cells that inhibit cell proliferation in a concentration-dependent manner. As the cell density increases, the extracellular concentrations of AprA and CfaD increase, establishing a negative feedback mechanism that regulates cell number. We found that AprA, but not CfaD, is also a chemorepellant. Compared to wild type, plaques of *aprA*$^-$ cells expand less rapidly when grown on solid media with bacteria. Wild type, but not *aprA*$^-$ cells, show an average directed movement outward from a cell colony when grown axenically. Wild-type cells also show a directed movement away from a source of recombinant AprA or dialyzed conditioned media from high density wild-type cells, though dialyzed conditioned media from *aprA*$^-$ cells has no significant effect on the direction of cell movement. Cells lacking CfaD, the kinase QkgA, or the kinase PakD also show reduced cell colony size and do not show directed movement away from recombinant AprA, implicating these proteins in the mechanism by which AprA functions as a chemorepellant. We propose that, in addition to inhibiting the proliferation of cells at high density, AprA may also function in the dispersal of high density populations of cells by providing a chemorepellant gradient that directs the movement of cells to regions of low *Dictyostelium* cell density, where nutrient sources are more likely to be present.

**Abstract type:** Talk

**Title:** Adaptation of activated Ras in a chemotaxis pathway of *Dictyostelium*

**Authors:** Kosuke Takeda, Danying Shao, Micha Adler, Pascale G. Charest, William F. Loomis, Herbert Levine, Alex Groisman, Wouter-Jan Rappel and Richard A. Firtel

**Presenter:** Wouter-Jan Rappel

**Address for correspondence:** Section of Cell and Developmental Biology, Division of Biological Sciences, Department of Physics and Center for Theoretical Biological Physics University of California San Diego La Jolla, CA 92093

**Abstract:** Adaptation in signaling systems, during which the output returns to a fixed base-level following a change in the input, often involves negative feedback loops and plays a crucial role in eukaryotic chemotaxis. We determined the dynamical response of the *Dictyostelium* chemotaxis pathway immediately downstream from G protein-coupled receptors following a uniform change in chemoattractant concentration. We found that the response of an activated Ras shows near perfect adaptation. Using mathematical models, these experimental results were used to determine the network topology that can provide perfect adaptation.

**Abstract type:** Talk

# *Ensemble Behaviors*

**Title:** Genetic drift in the non-social growth phase promotes the maintenance of cooperation in *Dictyostelium*

**Authors:** Neil J Buttery, Chandra N Jack, Boahemma Adu-Oppong, David C Queller and Joan E Strassmann

**Presenter:** Neil J Buttery

**Address for correspondence:** Department of Biology Washington University in St. Louis Campus Box 1137 One Brookings Drive St. Louis, MO 63130-4899

**Abstract:** The fitness and success of a group of cooperators is under constant threat of collapse by the infiltration and spread of selfish individuals cheaters that reap all the benefits of cooperation without paying any of the costs themselves. Kin selection theory predicts that cooperative behaviours can be selected for and maintained if relatives preferentially interact with one another, either by recognition or as a result of high population viscosity. However, another way for a gene that conveys low relative fitness to increase in a population is by genetic drift as a result of a founder effect. In small populations, genetic drift can be a larger evolutionary driving force than natural selection. We used the social amoeba *Dictyostelium discoideum* to investigate if founder effects and genetic drift can act as a possible mechanism for the formation of highly-related groups, maintaining cooperative interactions. We demonstrate that genetic drift during the non-social vegetative growth phase can act as a mechanism for the increase and maintenance of relatedness in *D. discoideum*. We also show for the first time in a real interacting population that in a group of cooperators and cheaters, cooperators can ‘escape’ the detrimental group effects of cheaters, whether present in the minority or majority, keeping relatedness high and promoting the evolution of altruistic behaviour.

**Abstract type:** Talk

**Title:** Self/non-self recognition in *Dictyostelium* is mediated by polymorphic tgrB1-tgrC1 alleles

**Authors:** Shigenori Hirose, Rocio Benabentos, Hsing-I Ho, Adam Kuspa and Gad Shaulsky

**Presenter:** Shigenori Hirose

**Address for correspondence:** Baylor College of Medicine, One Baylor Plaza, Houston TX 77030

**Abstract:** The aggregative nature of *Dictyostelium* development may lead to conflicts, such as cheating, between different genetic groups. Wild *Dictyostelium* strains can distinguish self from non-self and segregate from the latter. Developing within an isogenic population may reduce the conflict between genetically distinct strains. We have identified two polymorphic genes, *tgrB1* and *tgrC1*, which are necessary and sufficient for self/non-self recognition in *Dictyostelium*. *tgrB1* and *tgrC1* encode transmembrane proteins with multiple extracellular Ig-like domains. The genes are co-expressed at the loose aggregate stage, when self/non-self recognition is observed. The genes are highly polymorphic in natural populations. Gene replacement strains whose *tgrB1-tgrC1* alleles are replaced with different pairs of *tgrB1-tgrC1* alleles from wild isolates complete development in a pure population and segregate from the parental AX4 cells in mixed populations. They also recognize the donor strains as self. In addition, merodiploid strains that carry an additional polymorphic *tgrB1-tgrC1* pair of alleles from a wild isolate can recognize both the parental AX4 and the donor strain as self. We would like to examine the cellular responses to self/non-self recognition in terms of gene expression and cellular behavior. We have developed a system in which a small minority (~1%) of test cells, carrying a reporter construct and a certain *tgrB1-tgrC1* pair of alleles, is co-developed with majority of another strain that carries either an identical or a polymorphic *tgrB1-tgrC1* allele pair. Under these conditions, most of the test cells encounter only the designated cells (self or non-self), allowing us to monitor motility and gene expression at the single cell level. Using this system we found that intercellular interactions via compatible TgrB1-TgrC1 induces the postaggregative gene, *cotB*, but interactions between incompatibles Tgr pairs does not. We also observed that test cells, which are surrounded by other cells with an incompatible *tgrB1-tgrC1* pair of alleles, have decreased locomotion and compromised polarity during aggregation.

**Abstract type:** Talk

**Title:** Correlated evolution of size, complexity and cAMP signalling in the Dictyostelia.

**Authors:** Pauline Schaap, Yoshinori Kawabe, Maria Romeralo, Anna Skiba, Christina Schilde and Hajara Lawal.

**Presenter:** Pauline Schaap

**Address for correspondence:** College of Life Sciences, University of Dundee, UK

**Abstract:** Colony formation by their unicellular ancestors is the earliest event in the evolution of most multicellular life forms. This strategy has been used by Dictyostelid social amoebas for almost a billion years to form structures with increasing levels of morphological complexity and cell-type specialization. To understand how multicellular complexity evolved, we measured 33 phenotypic characters over 99 Dictyostelid taxa and we resolved conflicts between existing single gene Dictyostelid phylogenies with a novel 18 gene core phylogeny. We next used phylogeny-based statistical methods to retrace character history and assess co-evolution of characters. Our analyses show that the last common ancestor (LCA) of all Dictyostelia formed very small clustered fruiting structures. Its amoebas may have used folate or glorin to aggregate and first differentiated into prespore cells, of which some redifferentiated to form the stalk. This phenotype persisted up to the LCAs of three of the four major groups. The group 4 LCA evolved much larger solitary structures with novel cellular supports. It used cyclic AMP as attractant for aggregation and evolved an intermediate light-oriented migrating slug stage, which displayed regulated proportioning and patterning of future stalk, spore and supporter cells. Larger structures and light-oriented slug migration also co-evolved within the other groups, where twice independently, structures achieved stability by forming regularly branched whorls. SpcAMPS disruption of post-aggregative morphogenesis in almost all studied taxa suggest that the dictyostelid LCA and all its descendants used cyclic AMP signalling to coordinate the post-aggregative stages of development. This is supported by fact that lesions in the ACA, PdsA and cAR genes of the group 2 species P.pallidum all disrupt fruiting body morphogenesis without affecting aggregation.

**Abstract type:** Talk

**Title:** Non-nutritive bacteria carried by farmers harm non-farmers and enhance farmer proliferation

**Authors:** Debbie Brock, Silven Read, Alona Bozhchenko, David Queller, and Joan Strassmann

**Presenter:** Debbie Brock

**Address for correspondence:** Department of Biology Washington University in St. Louis Campus Box 1137 One Brookings Drive St. Louis, MO 63130-4899

**Abstract:** Some of the most enduring mutualisms involve organisms from different kingdoms, because the partners can contribute very different assets. Many of these mutualisms were established and fixed in the distant evolutionary past, so the selective processes favoring or disfavoring them are no longer available for study. We previously reported a novel facultative farming symbiosis between a eukaryotic soil amoeba, *Dictyostelium discoideum* and several different bacteria species. We observed farmer *Dictyostelium discoideum* clones carry bacteria that they do not use as food sources, and both farmer and non-farmer amoebae proliferate equally well, suggesting the non-nutritive bacteria carried by farmers do not negatively impact fitness. Here we investigate the ability of non-farming clones to produce spores from initial numbers of amoebae under the starving conditions that lead to the social stage. We find the presence of farming clones reduces spore production in non-farmers. Furthermore, this effect increases with the frequency of farming clones in the mixture suggesting non-farming clones may be vulnerable to bacteria carried by other *D. discoideum* clones and that farmers have evolved tolerance to these effects increasing their competitive ability against conspecifics. One common non-nutritive bacterial species isolated from farmer *D. discoideum* hosts is *Burkholderia xenovorans* LB400 and we found *Dictyostelium* clones are unable to complete the social stage when this bacteria species alone is used as the food source. We isolated host-specific *B. xenovorans* isolates from several *D. discoideum* farmer hosts and prepared supernatant from each of the clonally grown host specific bacterial isolates. We treated *Dictyostelium* clones at the beginning of the social stage to eliminate any feeding differences and found that the supernatant enhanced spore production of farming clones and reduced spore production of non-farming clones. This is consistent with the view that these non-food bacteria have a beneficial effect on the *Dictyostelium* clones that carry them.

**Abstract type:** Talk

**Title:** Puromycin-sensitive aminopeptidase A functions in spore cell differentiation in *Dictyostelium*

**Authors:** Yekaterina Poloz, Andrew Catalano and Danton H. O'Day

**Presenter:** Yekaterina Poloz

**Address for correspondence:** Department of Biology, University of Toronto Mississauga, 3359 Mississauga Road, Mississauga ON L5L 1C6, CANADA e-mail: danton.oday@utoronto.ca

**Abstract:** Aminopeptidases have been shown to regulate differentiation and morphogenesis in many organisms. Here we show that puromycin-sensitive aminopeptidase A (PsaA) functions in spore cell differentiation in *Dictyostelium discoideum*. Northern and western blotting analysis revealed that PsaA is expressed throughout development, with the highest expression observed at 20h, at the time of culmination. Western blotting has also revealed that PsaA protein expression is specifically induced by cAMP but repressed by DIF-1 and ammonia. Immunohistochemistry with anti-PsaA revealed that PsaA localizes to the nucleus and cytoplasm of both prestalk and prespore cells. Treatment of cells with bestatin methyl ester (BME), a cell permeable inhibitor of zinc-binding aminopeptidases altered spore cell differentiation as calcofluor-stained spores appeared round after encapsulation (600 μM) or did not encapsulate at all (900 μM). The timing of development and stalk cell differentiation were not affected by BME. Overexpression of PsaA as a PsaA-GFP fusion protein also altered spore cell differentiation, as the calcofluor stained spores appeared round. Otherwise development progressed normally to the fruiting body stage and stalk cell differentiation was not affected. In chimera slugs where these cells were mixed 20:80% with wildtype AX3 cells PsaA-GFP expressing cells preferentially differentiated into prestalk cells at the prestalk/prespore boundary and the posterior portion of the prespore zone. During culmination PsaA-GFP expressing cells differentiated into lower and upper cup cells and stalk cells of the basal disc but very few cells were observed in the stalk proper or the sorus. This suggests that cells overexpressing PsaA have a sorting defect and thus cannot sort to the anterior prestalk zone of the slug and that they also cannot differentiate into spores. Overexpression of PsaAΔNLS2-GFP, a PsaA deletion construct that does not localize to the nucleus, rescued normal spore cell differentiation. It remains to be analyzed whether PsaAΔNLS2-GFP expressing cells have a sorting defect similar to PsaA-GFP expressing cells and whether spore cell differentiation is inhibited by BME in these cells. Thus DdPsaA functions in spore cell differentiation in *D. discoideum* and its nuclear localization may be important for its function. We discuss the possible roles of DdPsaA in spore cell differentiation in the light of the role of this enzyme in differentiation of higher eukaryotes.

**Abstract type:** Talk

# *Chemomechanical Signaling*

**Title:** *D. discoideum* Integrate Chemical and Mechanical Signals to Achieve Directional Motility

**Authors:** Arhana Chattopadhyay, Natalie Andrew, Jeremy Gunawardena

**Presenter:** Arhana Chattopadhyay

**Address for correspondence:** achattop@post.harvard.edu, Natalie_Andrew@hms.harvard.edu 17 Windsor Drive, Foxboro, MA 02035 (508) 431-7943

**Abstract:** Little is known about how cells process mechanical cues from the environment, and less is known about how these cues are processed in the presence of simultaneous chemical cues. Using a microfluidic device, *Dictyostelium discoideum* cells were exposed to chemical gradients and orthogonally directed shear stress simultaneously. Time-lapse imaging of cells experiencing combined stimuli of 3 different chemical gradient regimes and 3 different flow regimes revealed: 1) cells directionally integrate the stimuli rather than switching between cues; 2) increasing the mechanical signal but not the chemical signal impacts the cell's direction. These findings support a selective rather than instructive model of cell motility in the presence of multiple directional cues.

**Abstract type:** Talk

**Title:** Engineering the Mechanosensitivity of *Dictyostelium* Cells

**Authors:** Tianzhi Luo and Douglas Robinson

**Presenter:** Tianzhi Luo

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

**Abstract:** Recently, we found that cytoskeletal proteins, such as myosin II and cortexillin I, cooperatively accumulate to highly deformed regions in *Dictyostelium* cells and the accumulation extent increases with increasing forces. Accompanying the protein accumulation, the cellular deformation decreases even as the external forces are kept constant. Besides myosin II and cortexillin I, other proteins such as actin cross-linking proteins, also bear the load but do not display comparable accumulations. The actin cytoskeleton can be structurally considered an apparatus consisting of parallel springs and each type of load-bearing proteins corresponds to one spring. The external load is shared by these springs and the amount of load on each spring is presumably proportional to the concentration of the corresponding protein and its binding affinity to actin filaments. Myosin II binding affinity to actin is known to increases with applied force, which is suggested to be one of the mechanisms for its accumulation. Another possible mechanism is the cooperative interaction between bound myosin heads. Theoretically, the cooperativity increases with local myosin II concentration. Therefore, we propose that the mechanosensitivity, *i.e*., the extent of myosin II accumulation, is determined by the relative ratio of myosin II concentration to the concentrations of other proteins and the applied forces. Using genetic tools, we show that mechanosensitivity does increase with the relative ratio and the external force in mutant cell lines. Consistently, the cell deformability that is supposed to be inversely proportional to the myosin II concentration when the concentrations of other proteins are fixed, shows positive correlation with the mechanosensitivity for the cells of the same type.

**Abstract type:** Talk

**Title:** A mechanosensory control system governs cytokinesis contractility

**Authors:** Y.-S. Kee, Y. Ren, D. Dorfman, M. Iijima, R. Firtel, P.A. Iglesias, D.N. Robinson

**Presenter:** Yee Seir Kee

**Address for correspondence:** Department of Cell Biology, Johns Hopkins School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205

**Abstract:** The mitotic spindle is generally considered the initiator of furrow ingression though recent studies have shown that furrows can form in the absence of spindles, particularly during asymmetric cell division. In *Dictyostelium*, myosin II and the actin crosslinker cortexillin I form a mechanosensor that responds to mechanical stress, which could account for spindle-independent contractile protein recruitment. Here, we show that contractility occurs through a control system where myosin II and cortexillin I accumulate and contract through a stress-induced amplification loop. Mechanotransduction is mediated by IQGAP2 through a kinesin-6 protein. Additionally, IQGAP2 competes with IQGAP1, defining mechanoresponsive and non-mechanoresponsive pools of cortexillin I, which suggests a possible mechanism for discriminating mechanical from biochemical inputs. Finally, the mitotic spindle is dispensable for the control system. Overall, we propose that such a control system is the essential component of cleavage furrow contractility that may be activated by mechanical stress. This self-tuning system also allows the cell to divide under diverse mechanical constraints such as that which may be experienced by cells in 3D matrix or tissue environments.

**Abstract type:** Talk

# *Centrosomes, Kinesins, Regulators*

**Title:** A kinesin-mediated linkage between centrosomes and the nuclear envelope

**Authors:** Tikhonenko, I., Magidson, V., Gräf, R., Khodjakov, A., and Koonce, M.P.

**Presenter:** Mike Koonce

**Address for correspondence:** Division of Translational Medicine, Wadsworth Center, Albany, NY, USA and Institute for Biochemistry and Biology, Dept. of Cell Biology, University of Potsdam, Potsdam-Golm, Germany

**Abstract:** Centrosomes in interphase *Dictyostelium* cells are located in the cytoplasm, adjacent to and tightly connected with the nucleus (hence the name, nuclear-associated bodies). Disruption of the M-type kinesin isoform, Kif9, severs the centrosome-nuclear linkage and enables the centrosome to move in the cytoplasm independent of the nucleus. This disruption leads to accumulation of supernumerary centrosomes and produces multiple mitotic defects. Kif9 null cells are viable but accumulate in suspension at an exceedingly slow rate, indicating that a physical association between these two organelles is required for normal cell growth. Kif9 contains a C-terminal transmembrane/tail domain that appears to function as a nuclear envelope anchor. Kif9 localization reveals that the motor is restricted to a perinuclear region close to the centrosome position. Removal of this C-terminal domain redistributes the truncated motor into the cytosol, whereas a Kif9 fragment of only the C-terminal tail is nuclear envelope localized similar to the wild type protein. The solubilities of expressed full length and truncated polypeptides are consistent with the localization data. These results indicate that the C-terminus of Kif9 contains an important nuclear targeting motif. Dual labeling of Kif9 and the nuclear envelope linker protein, Sun1, demonstrate significant overlap in polypeptide distribution. Similar to Kif9, Sun1 staining is enriched on the side of the nucleus facing the centrosome. Interestingly, in the absence of Kif9, Sun1 distribution is more uniform over the nuclear surface. Kif9 also shows ATP sensitive binding to microtubules. Taken together, we postulate that Kif9 functions as a KASH protein, and directly couples Sun1 to cytoplasmic microtubules. If Kif9 contains a microtubule depolymerase activity characteristic of other M-type kinesins, it would function to draw the centrosome toward the nucleus and maintain the normal tight association between these two organelles. A close proximity between these two organelles likely facilitates centrosome insertion into the nuclear envelope for division and minimizes multiple centrosome-nuclear interactions in multinucleated cells. We are currently testing this hypothesis.

**Abstract type:** Talk

**Title:** Roles of SunB in centrosome and kinetochore organization

**Authors:** Nao Shimada and Satoshi Sawai

**Presenter:** Nao Shimada

**Address for correspondence:** Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan

**Abstract:** For accurate separation of daughter chromosomes, the polar microtubles extended from opposing directions must each be attached to the sister kinetochores to provide their bi-orientation. Kinetochore is a large protein complex that forms at the centromeric region of DNA and provides sites for microtuble association for the chromosomes. Despite the importance of centromeric chromatin assembly for kintochore formation, its molecular basis is not entirely clear. Here, we report that *Dictyostelium* SunB is a protein required for microtubule-kinetochore association and centromeric chromatin assembly. SunB is a protein that contains a Sad1/UNC-84(SUN)-domain in the middle of the amino acid sequence and is enriched both in the centrosome and kinetochore during mitosis. The SunB knockdown mutant (KD7) exhibits irregular size and numbering of microtubules and centrosomes. Growth of the mutant cells is hypersensitive to nocodazole - an inihibitor of microtubule polymerization. Moreover, KD7 cells are aneuploid, *i.e.* the number of chromosomes is irregular and almost doubled in KD7. This was also true for wildtype cells overexpressing SunB. We discovered that centromere-specific histone CenH3 is scattered throughout nucleus in the KD7 cells. This is not due to failure for centromeric DNA to condensate, as these DNA loci appear correctly localized in the KD7 cells. We will discuss a model of SunB action on microtubule-kinetochore association and suggest its roles on force generation between the centrosome and chrmosomes and deposition of the histone variant.

**Abstract type:** Talk

**Title:** Functions of NDR/LATS kinases in *Dictyostelium*

**Authors:** Peter M. Kastner [1], Michael Schleicher [1], Parvin Bolourani [2], Gerald Weeks [2], and Annette Müller-Taubenberger [1]

**Presenter:** Annette Müller-Taubenberger

**Address for correspondence:** [1] Institute for Anatomy and Cell Biology, Ludwig Maximilian University of Munich, 80336 Munich, Germany [2] Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada

**Abstract:** The NDR (nuclear Dbf2-related)/LATS (large tumor suppressor) kinase family belongs to the AGC group of serine/threonine kinases. NDR/LATS kinases are highly conserved from lower eukaryotes to humans and are essential governors of pathways regulating cellular processes like mitotic exit, cytokinesis, cell proliferation, morphological changes and apoptosis. Loss of NDR/ LATS kinase function has been shown to cause the development of various human malignancies. Exploring the regulation of the NDR/LATS kinases and uncovering their effectors will help to understand their functions in normal and disease states. Mammals have four NDR/LATS kinases (Ndr1, Ndr2, Lats1, Lats2) reflecting a higher degree of diversification, while invertebrates and lower eukaryotes generally have only two NDR/LATS kinases. In contrast to other lower eukaryotes, *Dictyostelium discoideum* encodes four NDR/LATS kinases: NdrA–D. In an attempt to elucidate the functions of the *Dictyostelium* NDR/LATS kinases we have generated mutants and employed interaction studies and immunofluorescence microscopy. We could show that NdrA, NdrB and NdrC localize to centrosomes, but only NdrC/Lats2 was found to play a crucial role in the regulation of cell division. NdrC/Lats2-null cells are multinucleate and characterized by an excess number of centrosomes. While NdrC/Lats2-null cells undergo synchronous mitosis, they are unable to divide properly. Interaction studies revealed that NdrC/Lats2 function is controlled by Ras proteins that have been previously shown to be involved in the regulation of cytokinesis. We now present an updated model how NdrC/Lats2 and members of the oncogenic Ras family GTPases, RasB and RasG, may be implicated in the regulation of cell division. The localization of NdrA is regulated during the cell cycle. In prophase, NdrA disappears from the centrosome and forms a cloud-like structure around the spindle, which is totally absent during later stages until completion of mitosis. NdrA does not play a role in centrosome integrity or cytokinesis. Deletion of the *ndrA* gene resulted in reduced growth caused by defects in phagocytosis. EmpC, a member of the p24 family of cargo receptors of Golgi derived vesicles was identified as an interactor of NdrA by immunoprecipitation. The deficiency of NdrA-null cells may be caused by an impairment of vesicle trafficking which is required for providing new membrane at sites of particle uptake.

Kastner, P. M., Schleicher, M., and A. Müller-Taubenberger. 2011. The NDR family kinase NdrA of *Dictyostelium* localizes to the centrosome and is required for efficient phagocytosis. Traffic 12, 301-312.

**Abstract type:** Talk

# *G-proteins and cAMP*

**Title:** A rabGAP, regulating the life-span in *Dictyostelium discoideum*

**Authors:** Hidekazu Kuwayama, Yukihiro Miyanaga, Hideko Urushihara and Masahiro Ueda

**Presenter:** Hidekazu Kuwayama

**Address for correspondence:** Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Tennodai 1-1-1, Ibaraki 305-8572, Japan

**Abstract:** In this meeting, we show that a member of rabGAP gene family is relevant to the regulation of the life-span duration in *Dictyostelium discoideum*. The rabGAP was revealed to be a mammalian and *Caenorhabditis elegans* rbg-3 homologue and hence designated Dd rbg-3. The deletion mutant showed rapid growth as well as short developmental duration, while the over-expression mutant yielded the opposite effects. Furthermore, we found that the deletion mutant was caffeine resistant both in growth and in developmental phases, suggesting that Dd rbg-3 functions in the regulatory pathway for cAMP production. Interestingly, when the human and *C. elegans* homologous genes were expressed in the Dd rbg-3 deleted cells, they could perfectly rescued the *Dictyostelium* deletion phenotype. By FRET analysis and the pull-down assay, it was shown that Dd RBG-3 was directly interact with Gα2 subunit in cAMP dependent manner, which strongly suggests that the activity of Dd RBG-3 is regulated by extracellular cAMP ligand stimulation via direct interaction with Gα2.

**Abstract type:** Talk

**Title:** Multiple G protein signaling pathways regulate developmental morphogenesis and chemotaxis through the down regulation of the phosphodiesterase RegA

**Authors:** Jeffrey A. Hadwiger and Hoai-Nghia Nguyen

**Presenter:** Jeff Hadwiger

**Address for correspondence:** Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, Oklahoma 74078, USA

**Abstract:** Heterotrimeric G proteins (Gαβγ) are known to regulate cAMP levels through the activation or inhibition of adenylyl cyclases but little is known about their regulation of cAMP-specific phosphodiesterases. The *Dictyostelium* Gα2, Gα4 and Gα5 subunit-mediated signaling pathways are known to regulate MAP kinases (MAPK) function and the MAPK ERK2 is known to down regulate the cAMP-specific phosphodiesterase RegA. To determine what role G protein subunits contribute to this regulation of RegA, a *regA* gene knockout was created strains lacking G protein subunits. The loss of RegA was found to rescue development and chemotaxis of $g\alpha 2^-$, $g\alpha 4^-$, and $g\alpha 5^-$ cells but not $g\beta^-$ cells. The rescued development of the Gα subunit mutants included a precocious elevation of intracellular cAMP, as indicated by STATa nuclear localization. The rescue of Gα mutants, but not the Gβ mutant, was not due to redundancy in Gα function. While individual Gα subunits are not essential for ERK2 phosphorylation these subunits can contribute to the efficiency of this activation and therefore play a role in the down regulation of RegA. The involvement of a diffusible intracellular signal, such as cAMP, in multiple G protein signaling pathways raises interesting questions about the mechanisms of pathway specificity.

**Abstract type:** Talk

**Title:** Single-cell level analysis of adaptive fold-change detection in cAMP relay response

**Authors:** Keita Kamino, Yohei Kondo, Koichi Fujimoto, Satoshi Sawai

**Presenter:** Keita Kamino

**Address for correspondence:** Graduate School of Arts and Sciences, University of Tokyo, Tokyo, 153-8902, Japan

**Abstract:** In *Dictyostelium discoideum*, a key feature of multicellular development is formation of chemoattractant cAMP gradients in the form of waves that direct chemotaxis. The underlying cAMP relay response has been shown to be adaptive *i.e*. cells are able to reset the response even when the extracellular cAMP level is kept at non-zero value. However, the exact nature of this adaptive response remains to be elucidated. Here, we revisit this problem at the single-cell level with a FRET-based cAMP sensor, applying well-defined time-varying extracellular cAMP stimuli to cells in a perfusion chamber. As was expected, cells are able to adapt to a constant level of extracellular cAMP and respond to its relative rise. Detailed investigations revealed that, to a step increase in input signal, the cells respond with intensities that depend precisely on the ratio of the input change. On the other hand, when the cells are released from the adapted state by washing away extracellular cAMP, it takes 5 minutes for the cells to recover full responsiveness, which is comparable to the oscillation period observed at the population level during early stage of cell aggregation. Simultaneous measurements of PH-domain protein translocation to the plasma membrane and cAMP production in individual cells revealed an extreme high sensitivity and nonlinear relation between the two events. Finally, we discuss minimal mathematical models that reproduce fold-change detection and propose a topology of the regulatory network responsible for the observed cAMP relay response.

**Abstract type:** Talk

# *Chemotaxis I*

**Title:** A systems approach to chemotaxis.

**Authors:** Verena Koelsch[1], Jesus Lacal Romero[1], Zhouxin Shen[1], Gadi Shaulsky[2], Jessica Chang[1], Susan Lee[1], Steve Briggs[1], and Rick Firtel[1]

**Presenter:** Rick Firtel

**Address for correspondence:** [1]Cell and Developmental Biology, UCSD and [2]Department of Molecular and Human Genetics, Baylor College of Medicine

**Abstract:** We have taken a systems approach to identify new regulatory loops that control chemotaxis using comparative phosphoproteomic analyses. We will discuss our progress and the analysis of Daydreamer (DydA), a newly identified leading edge regulator.

**Abstract type:** Talk

**Title:** Receptor desensitization modulates signal relay during *Dictyostelium* development

**Authors:** Satarupa Das, Erin C. Rericha, Anna Bagorda, and Carole A. Parent

**Presenter:** Satarupa Das

**Address for correspondence:** Carole A. Parent Laboratory of Cellular and Molecular Biology National Cancer Institute, National Institutes of Health 37 Convent Drive, Bldg.37/Rm2066 Bethesda MD 20892-4256 Tel: 301-435-3701 FAX: 301-496-8479 email: parentc@mail.nih.gov

**Abstract:** Upon starvation, individual *Dictyostelium discoideum* cells enter a developmental program that leads to collective group migration and the formation of a multicellular organism. The process is mediated by extracellular cAMP binding to the G protein-coupled cAMP receptor 1 (cAR1), which initiates a signaling cascade leading to the activation of Adenylyl Cyclase A (ACA), the synthesis and secretion of additional cAMP and an autocrine and paracrine activation loop. The release of cAMP allows neighboring cells to polarize and migrate directionally, a process known as chemotaxis, and form characteristic chains of cells called streams. We now report that cAMP relay can be measured biochemically by assessing ACA activity at successive time points after stimulating cells with sub-saturating concentrations of cAMP. We also find that the relay of cAMP signals can be measured by monitoring ERK2 or TORC2 activation. Using mutants exhibiting constitutive PKA activity, we further establish that the capability of cells to relay signals changes during development – a phenomenon that occurs coincidently with the streaming ability of cells during chemotaxis. We propose that as cells proceed through development, the cAMP induced desensitization and downregulation of cAR1 dramatically impacts chemotactic signaling cascades.

**Abstract type:** Talk

**Title:** Chemokine Stimulation of TORC2 is Regulated by Receptor/G protein Targeted Inhibitory Mechanisms that Function Upstream and Independently of an Essential GEF/Ras Activation Pathway in *Dictyostelium*

**Authors:** Xin-Hua Liao and Alan R. Kimmel

**Presenter:** Alan Kimmel

**Address for correspondence:** Laboratory of Cellular and Developmental Biology, NIDDK National Institutes of Health, Bethesda, MD 20892

**Abstract:** Global stimulation of *Dictyostelium* with different chemoattractants elicits multiple rapid, but transient signaling responses, including synthesis of cAMP and cGMP, actin polymerization, activation of kinases ERK2, TORC2, and PI3K, ion influx, and Ras-GTP accumulation; mechanisms that down-regulate these responses are still poorly understood. Here we examine the transient activation of TORC2 in response to chemically distinct chemoattractants, cAMP and folate, and suggest that TORC2 is regulated by adaptive, de-sensitizing responses to stimulatory ligands that function independently of direct kinase inactivation. We show that cells with acquired insensitivity to one chemoattractant remain fully responsive to activate TORC2 if stimulated with the other ligand. Thus, TORC2 responses to cAMP or folate are not cross-inhibitory. Furthermore, if the initial chemoattractant concentration is immediately diluted, the inhibitory signals subside rapidly and cells quickly regain full responsiveness. Finally, using a series of signaling mutants, we show that folate and cAMP utilize an identical GEF/Ras pathway but separate receptors and G protein couplings to activate TORC2. Data indicate that the common GEF/Ras pathway must lie downstream and function independently of adaptation to persistent ligand stimulation. We suggest that ligand adaptation functions in upstream inhibitory pathways that involve chemoattractant-specific receptor/G protein complexes and regulate multiple response pathways.

**Abstract type:** Talk

**Title:** Pseudopod Dynamics during Chemotactic Motion

**Authors:** Alexandre Colavin, Monica Skoge, Inbal Hecht, Wouter-Jan Rappel, and Herbert Levine

**Presenter:** Herbert Levine

**Address for correspondence:** Dept. of Physics, UCSD, La Jolla, CA 92093

**Abstract:** We compare simple models of pseudopod dynamics and the resulting cell motility with experimental data from cells chemotaxing in a microfluidics chamber. These models assume that the creation of new pseudopods is a random event which occurs with a probability distribution determined by a compass model which amplifies the external gradient. We show that commonly observed features of pseudopods, such as tip-splitting events and a tendency to alternate left-right protrusions, follow directly from such a model without the need for specific dynamical mechanisms.

**Abstract type:** Talk

**Title:** Tight temporal control of Ras and TORC2 during chemotaxis through negative feedback.

**Authors:** Pascale G. Charest, Zhouxin Shen, Pouya Lotfi, Steven P. Briggs, and Richard A. Firtel.

**Presenter:** Pascale Charest

**Address for correspondence:** University of California, San Diego 9500 Gilman Drive Natural Sciences Building, Rm 6315 La Jolla, CA 92093-0380

**Abstract:** The evolutionarily conserved Target of Rapamycin Complex 2 (TORC2) was recently found to play an important role in the control of directed cell migration in both *Dictyostelium* and neutrophils. In *Dictyostelium*, TORC2 modulates the activities of PKB and PKB-related PKBR1, which, in turn, lead to modulation of the actin cytoskeleton and activation of adenylyl cyclase A (ACA), thereby controlling both cell motility and relay of the chemoattractant signal (cAMP) to neighboring cells, respectively. TORC2 is partly regulated by the Ras protein RasC, which is activated downstream from the chemoattractant receptors, heterotrimeric G proteins, and the RasGEF-containing Sca1 complex that promotes RasC activation. We recently found that the RasC-TORC2 pathway regulates its own level of activity during chemotaxis through a negative feedback loop, in which TORC2-dependent PKB/PKBR1 phosphorylation of Sca1 inhibits the localization of the Sca1 complex to the leading edge membrane and RasC activation. We now have evidence that, in addition to this TORC2 and PKB/PKBR1-dependent negative feedback regulation, the cAMP-dependent Protein Kinase A (PKA) is also involved in the regulation of the RasC-TORC2 pathway during chemotaxis. PKA is a known regulator of chemotaxis in both *Dictyostelium* and neutrophils, but its exact role is undefined. We found that cells lacking ACA or the PKA catalytic subunit (pkaC) display increased and extended TORC2-mediated phosphorylation of PKB/PKBR1, PKB/PKBR1 kinase activity, and PKB/PKBR1-mediated Sca1 phosphorylation. Since the RasC-TORC2-PKB/PKBR1 pathway controls ACA activation and therefore cAMP production, these observations suggest the presence of another feedback regulatory mechanism. Together, our findings highlight the presence of multiple negative feedback loops involved in the temporal control of Ras and TORC2 signaling at the leading edge of chemotaxing cells. These feedback loops are likely to play an important role in the adaptation of cells to fluctuating concentrations of chemoattractant as the cells migrate towards the source of the signal, which is essential to directed cell migration.

**Abstract type:** Talk

**Title:** Identification of a novel cAMP-responsive negative regulator of *Dictyostelium* chemotaxis

**Authors:** Yulia Artemenko, Doriane Sanséau, Jane Borleis, Josephine Lee, Stacey Willard, and Peter N. Devreotes

**Presenter:** Yulia Artemenko

**Address for correspondence:** Johns Hopkins University, School of Medicine 725 N. Wolfe St., 116 WBSB Baltimore, MD 21205

**Abstract:** Chemotaxis, or cell migration along a chemical gradient, is a fundamental process that plays an important role in many physiological and pathological processes. Studies in the social amoeba *Dictyostelium discoideum* have contributed extensively to the current understanding of chemotactic signaling pathways. Since directed migration is important for many stages of the *Dictyostelium* life cycle, aberrant chemotaxis often contributes to defective multicellular development. Thus, to identify novel potential regulators of directed migration, we have recently conducted a screen of restriction enzyme mediated integration (REMI) mutants using multicellular development as a readout. Using this approach, we identified *krsB* as a novel gene important in *D. discoideum* multicellular development and chemotaxis. KrsB is a Ste20 family protein kinase homologous to the Drosophila Hippo and mammalian Mst1/2 kinases, and is predicted to consist of an N-terminal kinase domain, followed by four calpain III domains. Disruption of the *krsB* gene resulted in delayed initiation of aggregation on non-nutrient agar, and reduced fruiting body size compared to wild-type cells. Aggregation-competent *krsB* disruptants showed reduced chemotaxis to cAMP. Evidence suggests that this behavior is due at least in part to increased adhesion of *krsB*-null cells to the substrate. Consistent with this, vegetative *krsB*-null cells also showed decreased motility compared to wild-type cells. Expression of KrsB with mutation and/or deletion of its regulatory regions revealed that the kinase domain is essential, whereas calpain III domains are dispensable for KrsB function in regulating development and adhesion. Using an antibody against Mst1 phosphorylated on Thr183, we showed that cAMP treatment induces a transient increase in KrsB phosphorylation. We are currently examining the importance of chemoattractant-stimulated phosphorylation of KrsB in its role as a negative regulator of *D. discoideum* chemotaxis.

**Abstract type:** Talk

**Title:** Disruption of PKB signaling restores polarity to cells lacking tumor suppressor PTEN

**Authors:** Tang M, Iijima M, Kamimura Y, Chen L, Long Y, Devreotes P.

**Presenter:** Michelle Tang

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

**Abstract:** By limiting phosphotidylinositol 3,4,5-triphosphate(PIP3) levels, tumor suppressor PTEN not only controls cell growth but also maintains cell polarity required for cytokinesis and chemotaxis. To identify the critical targets of PIP3 that link it to the cytoskeleton, we deleted secondary genes to reverse the deficiencies of *pten*$^{-}$ cells in *Dictyostelium*. The polarity defects in *pten*$^{-}$ cells correlate with elevated phosphorylations of PKB substrates. Deletion of AKT orthologue, PkbA, or a subunit of its activator TORC2, reduced the phosphorylations and suppressed the cytokinesis and chemotaxis defects in *pten*$^{-}$ cells. In these double mutants, the excessive PIP3 levels and, presumably, activation of other PIP3-binding proteins had little or no effect on the cytoskeleton. In bands with increased phosphorylation in *pten*$^{-}$ cells, we found PKB substrates, PI5K, GefS, GacG, and PakA. Disruption of PakA in *pten*$^{-}$ cells restored a large fraction of the cells to normal behavior. Consistently, expression of phosphomimetic PakA in *pten*$^{-}$ cells exacerbated the defects but non-phosphorylatable PakA had no effect. Thus, among many putative PTEN- and PIP3-dependent events, phosphorylation of PKB substrates is the key downstream regulator of cell polarity.

**Abstract type:** Talk

# *Immunity and Disease Modeling*

**Title:** Bacterial discrimination in *Dictyostelium discoideum*

**Authors:** Waleed Nasser, Balaji Santhanam, Anup Parikh, Roshan Miranda, Chris Dinh, Rui Chen, Gad Shaulsky and Adam Kuspa

**Presenter:** Adam Kuspa

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

**Abstract:** We will describe genetic and transcriptional evidence indicating that *D. discoideum* discriminates between different species of bacteria and that such an ability is important for the amoeba to respond appropriately for optimal feeding and for defense. In particular, we are testing the hypothesis that the amoebae mount fundamentally distinct responses to Gram-negative bacterial species versus Gram-positive bacterial species. We will present evidence that a metabolite of glucose acts as an internal signal for the presence of Gram-positive bacteria and present preliminary data on specific promoter elements that control part of the transcriptional response to Gram-positive bacteria.

**Abstract type:** Talk

**Title:** Investigating the cellular processes that mediate LPS-induced bactericidal activity in *Dictyostelium discoideum*

**Authors:** Katherine Pflaum, Kimberly Gerdes, Kossi Yovo and Michelle D. Snyder

**Presenter:** Michelle Snyder

**Address for correspondence:** Towson University, Dept. of Biological Sciences 8000 York Rd Towson, MD 21252

**Abstract:** Cells of the innate immune system detect pathogen-associated molecular patterns (PAMPs) through highly conserved pattern-recognition machinery. The conservation of this machinery allows for its characterization in model organisms. Although *Dictyostelium discoideum* has long been used as a model for bacterial phagocytosis by immune cells, it has not been determined to what extent *D. discoideum* might use pattern-recognition machinery to detect and respond to PAMPs on their bacterial prey. Here we show that *D. discoideum* mounts a response against the bacterial cell wall PAMP, lipopolysaccharide (LPS). LPS treatment results in an increased clearance of phagocytized bacteria by *D. discoideum*. LPS-induced bactericidal activity appears to be dependent on MAPK and Tir domain-containing protein A (TirA)-mediated pathways. Recent results in mammalian cells suggest that PAMP recognition might mediate the induction and maturation of autophagosomal pathways. Our recent studies with *D. discoideum* suggest that the increased bactericidal activity we see upon treatment with LPS may involve the induction and maturation of autophagosomes. We show here, by following the delivery of the autophagosomal marker Atg8 to degradative compartments, that *Staphylococcus aureus* induces autophagosomal maturation in *D. discoideum* only upon addition of LPS. We are currently characterizing the signaling pathways underlying bacterial induction of autophagy in *D. discoideum*. Characterization of the cellular events induced upon microbial pattern recognition in *D. discoideum* should provide valuable insight into conversed cellular mechanisms underlying microbial detection and killing.

**Abstract type:** Talk

**Title:** Functions of CD36 homologues in *Dictyostelium* during infection with *Mycobacterium marinum*

**Authors:** Natascha Sattler, Navin Gopaldass and Thierry Soldati

**Presenter:** Natascha Sattler

**Address for correspondence:** Department of Biochemistry, Sciences II, University of Geneva, 30 quai Ernest-Arnsermet, CH-1211 Geneva-4, Switzerland

**Abstract:** *Mycobacterium tuberculosis* invades immune phagocytes and reprograms their otherwise bactericidal phagosome maturation pathway to establish a replication niche. Recent reports using murine and insect systems indicate that uptake of mycobacteria depends on the scavenger receptors class B (CD36 and SR-B1). We have established *Dictyostelium* as an alternative host model for pathogenic *Mycobacterium marinum*, a close cousin of *M. tuberculosis*. *Dictyostelium discoideum* possesses three CD36 homologs, the Lysosomal Membrane glycoProteins A, B and C. To assess the importance of these proteins during bacterial killing, we examined the growth of *lmp* knockout mutants on different bacteria. Knock out of *lmpA* leads to growth defects on various types of bacteria especially Gram+ and some mycobacteria. Interestingly, this growth fingerprint of *lmpA*$^-$ is similar to the knockout of several proteins such as the BEACH domain containing proteins LvsA and LvsB. Both are important during lysosome biogenesis and transport. Our results suggest a function of LmpA during phagocytosis and/or digestion of these microbes. To distinguish between these two processes we tested uptake of *M. marinum* in the *lmp* mutants. Phagocytosis of this bacterium is indeed specifically and severely decreased in *lmpA*$^-$ and *lmpB*$^-$ cells. Since LmpB was found at the plasma membrane it likely acts as a receptor. Interestingly only uptake of *M. marinum,* but not of other bacteria like *K. pneumoniae* or *B. subtilis,* is severely reduced. In addition, we could demonstrate that knockout of *lmpB* leads to only a small adhesion defect towards latex beads. Therefore, we suggest that LmpB is a mycobacteria-specific receptor. Further experiments will determine unequivocally whether LmpB is important for adhesion to mycobacteria. Contrary to LmpB, neither LmpA nor LmpC are found at the plasma membrane, as both reside mainly in phagolysosomes. Preliminary results showing lower surface levels of LmpB in *lmpA*$^-$ cells might explain their strong phagocytosis defect and might indicate a role for LmpA in the trafficking of receptors such as LmpB. Killing and digestion of bacteria is dependent on correct acidification, production of reactive oxygen species and proteolysis in the phagosome. Our studies show that these three processes are impaired in *lmpA*$^-$ cells. Additional preliminary results suggest a function of LmpA in the delivery of lysosomal enzymes, a finding reminiscent of the role of LIMP 2 (CD36 family member) in trafficking of hydrolases in mammalian cells. Altogether, these results suggested that the bactericidal capacity of the *lmpA*-null mutant might be impaired. Indeed, *lmpA*$^-$ cells show a delay in killing the Gram+ *Bacillus subtilis*. However, none of the mutants show attenuation in killing the Gram- *K. aerogenes*. Weak killing capacities might correlate with increased susceptibility to pathogens. This is indeed the case for the *lmpA*$^-$ cells that are more susceptible to infection by *M. marinum*. We therefore, suggest that LmpB at the cell surface is necessary for mycobacterial recognition and possibly adhesion, whereas LmpA at the endosomes regulates trafficking events such as the delivery of lysosomal hydrolases and maybe cell surface receptors to and from the phagosome.

**Abstract type:** Talk

**Title:** Dissecting the role of ROS-generating NADPH oxidases in bacterial sensing, killing and host-pathogen interactions.

**Authors:** Xuezhi Zhang and Thierry Soldati

**Presenter:** Thierry Soldati

**Address for correspondence:** Département de Biochimie, Sciences II, Université de Genève, 30 quai Ernest-Ansermet, CH-1211 Genève -4, Switzerland

**Abstract:** Reactive oxygen species (ROS) are highly reactive chemicals due to the presence of unpaired valence shell electrons. The membrane-bound NADPH oxidases (NOX) are considered as the major enzymes devoted to ROS generation. The observation of severe infections in chronic granulomatous disease (CGD) patients, which are deficient in NOX2, indicated that NOX2-generated ROS are essential for bactericidal activity of the innate immune system. The oxidative burst (a rapid release of ROS) from neutrophils plays a crucial role in the killing of microbes. However, the roles of NOX-generated ROS from macrophages and dendritic cells are less well understood. *Dictyostelium discoideum* has become a popular experimental model to study the cell-autonomous innate immune response, because *Dictyostelium* and human phagocytes share several unique functions, such as engulfment killing and digestion of microorganisms by phagocytosis. *Dictyostelium* has three NOX isoforms (A, B, C) and a homolog of p22phox (CybA) (Lardy et al (2005) Biochim Biophys Acta 1744:199-212). NoxA is mainly expressed in growing amoebae, whereas, NoxB and NoxC are expressed at various times during the starvation-induced differentiation cycle. Production of hydrogen peroxide ($H_2O_2$) by *Dictyostelium* was measured by several assays, including the visualisation of local production in the phagosome. $H_2O_2$ production is produced at a significant basal level and can be stimulated to various extents by different bacteria cultures and supernatants. The level of stimulation varies in a dose-dependent manner. Knockout strains of NoxA, B and C and of CybA were generated and exhibit impaired $H_2O_2$ production under stimulation. In addition, starvation decreases the basal level and increases the stimulation efficiency of bacteria supernatant in a time dependent manner. Preliminary data suggest that the autophagy pathways might be involved in NOX-related ROS production. Overexpression of Atg8 and knockout of p62-SQSTSM-1 in *Dictyostelium* cells altered the stimulated $H_2O_2$ production. We are studying how ROS production affects killing of specific bacteria, and how it impacts on the cross-talk of signalling and manipulation by mycobacterial pathogens.

**Abstract type:** Talk

**Title:** Oxygen regulation of *Dictyostelium* development is mediated via prolyl hydroxylation and glycosylation of the E3SCFubiquitin ligase subunit Skp1

**Authors:** Christopher M West, Zhuo A Wang, Hanke van der Wel, Yuechi Xu, Dongmei Zhang, Jennifer M Johnson, Geert-Jan Boons, Carol M Taylor, Brad Bendiak

**Presenter:** Chris West

**Address for correspondence:** Dept. of Biochemistry & Molecular Biology, University of Oklahoma Health Sciences Center, 975 NE 10th St., BRC 417, Oklahoma City, OK 73104

**Abstract:** In the social soil amoeba *Dictyostelium*, the E3SCFUb-ligase subunit Skp1 is modified at a specific proline residue by a novel pentasaccharide. This modification is assembled by an HIFα-like prolyl 4-hydroxylase (P4H1) and a novel cytoplasmic glycosylation pathway that appears to be conserved in many other protists including the agent for human toxoplasmosis, Toxoplasma gondii. Genetic perturbations of *Dictyostelium* P4H1 affect specific developmental checkpoints, including culmination and sporulation, in a way that suggests this enzyme is an $O_2$-sensor as implied in animals. Analysis of the glycosyltransferase genes, whose actions depend on P4H1, show that their activities modulate P4H1 signaling suggestive of hierarchical control of development via successive sugar additions. Manipulations of Skp1 expression levels have inverse effects on $O_2$-dependence. Together with evidence for genetic interactions between Skp1 and the modification genes, effects of proline mutations, and biochemical findings that Skp1 is the only substrate of these enzymes, Skp1 is strongly implicated as the target of P4H1-signaling during development. Whereas $O_2$-dependent hydroxylation of the animal transcription factor HIFα triggers its polyubiquitination and degradation, P4H1 does not affect Skp1 stability. However, the consequence of Skp1 hydroxylation/glycosylation on cellular protein degradation may be similar if E3SCFUb-ligase assembly or activity is affected. Assays indicate that the enzymes recognize highly conserved features of Skp1 that are blocked by partial SCF assembly, potentially restricting effects of environmental parameters to nascently synthesized Skp1. Recent evidence that Skp1 from Toxoplasma is similarly modified in a regulated manner suggests that $O_2$-regulation of SCF Ub-ligases may be a general process that predates the association with HIFα and transcription that occurs in metazoa.

**Abstract type:** Talk

**Title:** *Dictyostelium discoideum*: a model system to study LRRK2-mediated Parkinson disease

**Authors:** Bernd Gilsbach[1], Wouter van Egmond[1], Yiu Fung Ho[1], Alfred Wittinghofer[2], Peter J.M. van Haastert[1] and Arjan Kortholt[1]

**Presenter:** Arjan Kortholt

**Address for correspondence:** [1] Department of Molecular Cell Biology, 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. Since all attempts to purify mammalian LRRK2 have failed so far, detailed biochemical and structural understanding of LRRK2 is very limited. We focus on *Dictyostelium* Roco proteins which have very similar domain structure and characteristics as LRRK2, but are biochemical more tractable. Our results show that *Dictyostelium* Roco proteins are excellent models for LRRK2 and can thus be used to answer key questions for the intramolecular regulation of LRRK2 and give insight in the function and mechanism of LRRK2 activation and how the PD-linked missense mutations alter the interactions between the different domains.

**Abstract type:** Talk

**Title:** Deficiency of Huntingtin Has Pleiotropic Effects in the Social Amoeba *Dictyostelium discoideum*

**Authors:** Myre MA, Lumsden AL, Thompson MN, Wasco W, Macdonald ME, Gusella JF

**Presenter:** Michael Myre

**Address for correspondence:** myre@chgr.mgh.harvard.edu

**Abstract:** Huntingtin is a large HEAT repeat protein first identified in humans, where a polyglutamine tract expansion near the amino terminus causes a gain-of-function mechanism that leads to selective neuronal loss in Huntington's disease (HD). Genetic evidence in humans and knock-in mouse models suggests that this gain-of-function involves an increase or deregulation of some aspect of huntingtin's normal function(s), which remains poorly understood. To facilitate studies aimed at detailed knowledge of huntingtin's normal function(s), we generated a null mutant of hd, the HD ortholog in *Dictyostelium discoideum*. We found that *Dictyostelium* cells lacking endogenous huntingtin were viable but display a conditional defect in cytokinesis evident by the formation of a long, thin cytoplasmic bridge between proportions of dividing cells. However, growth in suspension appears unaffected. When induced to develop submerged in KK2, the cells round up and do not exhibit the typical polarized morphology of *Dictyostelium* cells, fail to stream, yet form loose aggregates by accretion rather than chemotaxis. However, starvation for longer periods of time in the presence of specific ions, but not timed pulses of cAMP, restores cell shape and partially rescues chemotactic streaming. Upon closer examination we further found that huntingtin is required for osmoregulation. Although *hd*$^-$ cells completed development, it was delayed and proceeds asynchronously under conditions of low osmolarity, producing small fruiting bodies with round, defective spores that germinated spontaneously within a glassy sorus. In contrast to wild type cells, development on KK2 agar supplemented with EGTA blocked development. In *Dictyostelium*, huntingtin deficiency is compatible with survival of the organism and produces pleiotropic cell autonomous defects that affect cAMP signaling and as a consequence development. Thus, *Dictyostelium* provides a novel haploid organism model for genetic, cell biological, and biochemical studies to delineate the functions of the HD protein.

**Abstract type:** Talk

**Title:** Leveraging *Dictyostelium* for Small Molecule Modulators of Cytokinesis

**Authors:** Alexandra Surcel, Kirsten Meyer, Win Pin Ng, Danielle Dorfman, and Douglas N. Robinson

**Presenter:** Alexandra Surcel

**Address for correspondence:** 725 North Wolfe Street Baltimore, MD

**Abstract:** The search for anti-cancer drugs has been hampered in part by an incomplete understanding of the mechanics and biochemical pathways involved in cytokinesis. We have previously demonstrated the ability to combine chemical and genetic approaches to identify novel components of cell division. Here, we have expanded on this two-prong approach by establishing a large-scale, small-molecule chemical screen using *Dictyostelium discoideum* and by developing CIMPAQ (Cytokinesis Image Processing Analysis Quantification) – an analytical segmentation program used to identify both mitotic and cytokinesis inhibitors, as well as lethal compounds. We have screened over 22,000 small molecules and identified a number of inhibitors which have been recapitulated in secondary screening. Of these, we have begun to identify target pathways and proteins – one hit compound appears to modulate the dynamics of the mechanoenzyme, myosin II, at the cortex, while preliminary data suggests that another compound affects the ATP synthase beta subunit. We are currently investigating how these compounds affect cytokinesis and are testing their cross-reactivity in mammalian cell lines.

**Abstract type:** Talk

# *Chemotaxis II*

**Title:** A PIP3 signalling circuit active during *Dictyostelium* chemotaxis

**Authors:** Regina Teo, Jonathan Ryves & Adrian J Harwood

**Presenter:** Adrian Harwood

**Address for correspondence:** School of Biosciences, Cardiff University, Museum Ave, Cardiff, CF10 3AX

**Abstract:** We have recently discovered two unusual mutant chemotaxis phenotypes that provide new insights into the regulation of PIP3 signaling and chemotaxis. During chemotaxis, cAMP simultaneously stimulates the production of two inositol-based signal molecules, PIP3 and IP3 from the same substrate PIP2. However, whilst genetic ablation of PIP3 synthesis has a significant effect on chemotactic behaviour, loss or over-expression of phospholipase C (PLC), which produces IP3, or loss of the IP3 receptor (*iplA*) has no apparent effect. Remarkably, we have discovered that combining PLC over-expression in an *iplA* null mutant background creates a novel synthetic phenotype that closely matches loss of PIP3. We have used this mutant to reveal a set of feedback loops that control the build-up and degradation of PIP3. Key features of this circuit are: an amplifier, via PIP3-mediated regulation of PIP2 synthesis; a buffer via suppression of the PIP3 phosphatase PTEN by a PIP2 mediated negative feedback loop and a suppressor limiting formation of PIP2. We have isolated a second mutant that is able to chemotax in low cAMP, but is blocked in high concentrations. This mutant arises from loss of *arp8*, a subunit of the Ino80 chromatin re-modelling complex. We show that this mutant has elevated inositol synthesis, leading to over-activity of the PIP3 oscillator.

**Abstract type:** Talk

**Title:** A Novel Protein Identified at the Lagging Edge of Chemotaxing Cells Regulates Polarity

**Authors:** Kristen F. Swaney, Peter N. Devreotes

**Presenter:** Kristen F. Swaney

**Address for correspondence:** 116 WBSB, 725 N. Wolfe Street, Baltimore, MD

**Abstract:** Chemotaxis, or the directed migration of cells up chemical gradients, is an essential process for development of the embryo, tissue maintenance and the immune response in the adult organism, and the pathogenesis of many diseases. The social amoeba *Dictyostelium discoideum* has been used to elucidate many of the signaling pathways that mediate chemotaxis. Studies of *Dictyostelium* have shown that directed migration depends on the localization of signaling components specifically to the leading or lagging edge of cells. Therefore, a screen was conducted to identify novel chemotactic regulators based on protein localization. To do this, PH domain-containing proteins were tagged with GFP, expressed in *Dictyostelium* cells, and assessed for intracellular localization during migration. Like known PIP3-binding PH domain-containing proteins, several of the screened fusion proteins localized to membrane protrusions at the leading edge. However, one protein, PH21, was found at the lagging edge. While other lagging edge proteins become confined to the rear only in differentiated cells, PH21 is restricted to the back of randomly migrating cells prior to differentiation, suggesting that PH21 may promote polarity. The N-terminal portion of this protein, which contains its PH domain plus a 150 amino acid extension, is sufficient for localization to the lagging edge. FRAP and preliminary biochemical analyses suggest that the localization of PH21 is stabilized in polarized cells through potentially direct interactions with the cytoskeleton. To assess the function of this protein in the regulation of chemotaxis, null mutants were generated. Cell polarity is reduced in these mutants, resulting in delayed streaming and aggregation behaviors during development on non-nutrient agar. Therefore, PH21 is a novel protein at the lagging edge that may play a role in initiating polarity, an important component of normal chemotactic behavior.

**Abstract type:** Talk

**Title:** Ipℓ A Is Necessary For $Ca^{++}$, But Not For cAMP Chemotaxis, And Plays A Role In Natural Aggregation.

**Authors:** Daniel F. Lusche, Deborah Wessels, Amanda Scherer, Karla Daniels, Spencer Kuhl, David R. Soll

**Presenter:** Daniel F Lusche

**Address for correspondence:** The W.M. Keck Dynamic Image Analysis Facility, Department of Biology, The University of Iowa, Iowa City, Iowa 52242

**Abstract:** Cells of the deletion mutant *ipℓA*$^{-}$, a putative $Ca^{++}$ channel, are defective in at least one aspect of $Ca^{++}$ -induced polarity and motility, but normal in $K^{+}$ facilitation, as we expected given that Nhe1, a putative $Na^{+}$-$H^{+}$ exchanger, mediates $K^{+}$ effects (Lusche *et al.*, 2011). Although *ipℓA*$^{-}$ cells undergo chemotaxis in a spatial gradient of cAMP generated in a facilitating concentration of $K^{+}$ as Traynor *et al.* (2000) previously reported, and respond normally to temporal gradients of cAMP mimicking the temporal dynamics of natural cAMP waves, they are incapable of undergoing chemotaxis in a spatial gradient of $Ca^{++}$. We show, however, that when a minority of *ipℓA*$^{-}$ cells are mixed with a majority of parental control cells in natural aggregation territories, they respond abnormally to the relayed chemotactic waves. They undergo normal surges in motility in response to the increasing temporal gradient of cAMP in the front of each wave, but exhibit a reduced ability to orient towards the aggregation center at the onset of each relayed natural cAMP wave. These results support our previous suggestion (Scherer *et al.*, 2010) that transient $Ca^{++}$ gradients augment orientation in the front of a natural cAMP wave.

**Abstract type:** Talk

**Title:** Myosin Heavy Chain Kinase A and Myosin Heavy Chain Kinase C Are Necessary for Calcium Facilitation of Cell Motility, Calcium Enhancement of cAMP Chemotaxis and Calcium Chemotaxis

**Authors:** Deborah Wessels, Amanda Scherer, Daniel Lusche, Spencer Kuhl, Kristin Wood, Paul Steimle, Thomas Egelhoff and David R. Soll

**Presenter:** Deborah Wessels

**Address for correspondence:** deborah-wessels@uiowa.edu

**Abstract:** We previously reported that aggregation competent *Dictyostelium discoideum* amoebae not only chemotax up spatial gradients of cAMP, but also up spatial gradients of $Ca^{2+}$. We have also shown that extracellular $Ca^{2+}$ facilitates cell motility and enhances chemotaxis to cAMP at least in part by inducing the polarized cortical localization of myosin II. Furthermore, we have shown that extracellular $Ca^{2+}$ exerts these effects through pathways that are independent of $K^{+}$ facilitation. cAMP has also been shown to induce rapid uptake and then release of $Ca^{2+}$. Taken together, these data suggest a model in which a transient gradient of $Ca^{2+}$, released by aggregating cells in response to the increasing phase of a natural cAMP wave, activates a receptor mediated signal transduction pathway in neighboring cells that ultimately regulates myosin II localization in the cortex. To explore this hypothesis at the level of myosin regulation, we tested $Ca^{2+}$ facilitation of motility, $Ca^{2+}$ enhancement of cAMP chemotaxis and $Ca^{2+}$ chemotaxis in amoebae lacking myosin heavy chain kinase A (*mhckA*), myosin heavy chain kinase B (*mhckB*) and myosin heavy chain kinase C (*mhckC*). These kinases phosphorylate residues in the tail region of the myosin heavy chain molecule resulting in depolymerization of myosin thick filaments. Chemotaxis up spatial gradients of cAMP is normal in *mhckA*$^{-}$, *mhckB*$^{-}$ and *mhckC*$^{-}$ mutants. However, *mhckA*$^{-}$ and *mhckC*$^{-}$ mutants do not exhibit $Ca^{2+}$ facilitation of motility, $Ca^{2+}$ enhancement of cAMP chemotaxis and fail to chemotax up spatial gradients of $Ca^{2+}$. We also show that neither $Ca^{2+}$ nor $Ca^{2+}$/calmodulin directly regulate these kinases, a further indication that $Ca^{2+}$ effects are receptor-mediated.

**Abstract type:** Talk

**Title:** Identification and Characterization of Two Putative Myosin II Heavy Chain Kinases in *Dictyostelium*

**Authors:** Carrie Richardson, Almagul Kanafina, Paul Steimle

**Presenter:** Paul Steimle

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

**Abstract:** Contraction-dependent processes such as cytokinesis and cell migration rely on the proper assembly and localization of myosin II bipolar filaments. In human cells, defects in myosin II bipolar filament turnover can lead to platelet malformation, defects in kidney function, among other pathologies. These defects have been shown to arise from mutations in the human nonmuscle myosin II heavy chain-A gene, and are collectively called MYH9 disorders. In *Dictyostelium* cells, as well as in mammalian cells, myosin II filament disassembly can be driven by phosphorylation of the myosin II heavy chain (MHC) “tail”. MHC phosphorylation in *Dictyostelium* is catalyzed by at least three kinases (MHCK-A, -B, and -C) that share homologous alpha-kinase catalytic and WD-repeat domains. In the current study, we examined the cellular and biochemical characteristics of another *Dictyostelium* alpha kinase, MHCK-D (GenPept accession XP_640080), that possesses the same domain organization as the other MHCKs; and thus is predicted to function as a MHCK. We found that over-expression of MHCK-D slows suspension growth, with cells becoming large and multinucleated over time; a phenotype consistent with a role for MHCK-D in driving myosin II filament disassembly. A GFP-tagged truncation of MHCK-D exhibits a uniform distribution throughout the cytoplasm in both nonmotile and chemotaxing cells, and remains unchanged upon uniform stimulation of cells with cAMP. RT-PCR analysis of expression revealed that MHCK-D, unlike the other MHCKs, is induced during development (~16h). Moreover, MHCK-D phosphorylates MHC to a stoichiometry of ~0.6 mol/mol MHC and in manner that leads to myosin II filament disassembly. Based on these results, we conclude that MHCK-D is a bone fide MHCK that is likely to function in myosin II filament turnover during development. In a related set of studies we found that another *Dictyostelium* alpha kinase, AK1 (GenPept accession XP_629868.1), phosphorylates a peptide substrate (MH-1) that contains one of the MHC regulatory phosphorylation sites, suggesting the potential for this enzyme to use MHC as a substrate. Studies are in progress to further characterize MHCK-D and AK1 with the goal of identifying additional mechanisms by which myosin II function can be controlled.

**Abstract type:** Talk

**Title:** *Dictyostelium* huntingtin controls chemotaxis and cytokinesis through the regulation of myosin II phosphorylation

**Authors:** Yu Wang, Paul A. Steimle, Yixin Ren, Christopher A. Ross, Douglas N. Robinson, Thomas T. Egelhoff, Hiromi Sesaki, and Miho Iijima

**Presenter:** Miho Iijima

**Address for correspondence:** Department of Cell Biology, Johns Hopkins School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205; miijima@jhmi.edu

**Abstract:** Abnormalities in the huntingtin protein (Htt) are associated with Huntington's disease, yet despite its importance, the function of Htt is largely unknown. Here, we show that Htt is required for normal chemotaxis and cytokinesis in *Dictyostelium discoideum*. Cells lacking Htt showed slower migration towards the chemoattractant cAMP and contained lower levels of cortical myosin II, likely due to defects in its dephosphorylation mediated by protein phosphatase 2A (PP2A). *htt*$^-$ cells also failed to maintain myosin II in the cortex of the cleavage furrow, generating unseparated daughter cells connected through a thin cytoplasmic bridge. Furthermore, similar to *Dictyostelium htt*$^-$ cells, siRNA-mediated knockdown of human HTT also decreased the PP2A activity in HeLa cells. Our data indicate that Htt regulates the phosphorylation status of myosin II during chemotaxis and cytokinesis through PP2A.

**Abstract type:** Talk

**Title:** Open Microfluidic Devices for Quantitative Studies of *Dictyostelium* Chemotaxis.

**Authors:** D. Jowhar, G. Wright, L. Costa, A. Terekhov, W. Hofmeister, C. Janetopoulos

**Presenter:** Chris Janetopoulos

**Address for correspondence:** Department of Biological Sciences Vanderbilt University Nashville, TN 37235

**Abstract:** The social amoeba *Dictyostelium discoideum* has played a critical role in elucidating the signaling mechanisms regulating directed cell migration. These eukaryotic cells exhibit chemotaxis toward folic acid during vegetative growth and respond to cyclic adenosine monophosphate (cAMP) during their developmental cycle. The standard method for visualizing subcellular responses in these migrating cells has been to image them with an inverted microscope as they respond to a chemoattractant gradient set up by a micropipette attached to a micromanipulator. In recent years, numerous groups have developed microfluidic assays with varying degrees of success. To further our understanding of the mechanisms regulating cell migration in response to both cAMP and folic acid, we have taken these two basic techniques and combined them into a simple method. A micropipette system, which produces a passive gradient, is coupled with microfluidics to assay *D. discoideum* cells under a variety of experimental paradigms. The micropipette is connected to a micromanipulator and pump, which provides the experimenter with on-the-fly capabilities to change the positioning and profile of the chemoattractant gradient. At the same time, the microfluidic channels, formed in polydimethylsiloxane (PDMS), provide 3-dimensional constraints on the cell. PDMS is optically clear, non-toxic and is permeable to oxygen and have allowed us to successfully perform quantitative chemotaxis assays on cells expressing a variety of signaling and cytoskeletal markers. To further our capabilities, we are now using femtosecond laser micromachining (FLM) to develop even more sophisticated chemotaxis assays. FLM has been demonstrated to be a viable, fast and flexible direct-write method of fabricating microfluidic devices. FLM offers unprecedented capability when it comes to machining micrometer and nanometer scale features that cannot be attained using conventional lithography techniques. We have created devices containing both micro and nanofluidic channels in bulk glass and cover slips. These microfluidic devices are unique since the chemical concentration gradients are established exclusively by passive diffusion, which provides a chemical profile typically seen by migrating cells. These devices deliver the experimenter additional platforms not currently available using other microfluidic techniques.

**Abstract type:** Talk

**Title:** A GATA transcription factor is a key regulator of early development and chemotaxis

**Authors:** Huaqing Cai, Yu Long, Peter Devreotes

**Presenter:** Huaqing Cai

**Address for correspondence:** hcai6@jhmi.edu

**Abstract:** *Dictyostelium* undergoes a developmental program when starved. During the first few hours after starvation, cells gradually gain the ability to sense and migrate towards the chemoattractant cAMP. In a forward genetic screen for chemotaxis and developmental mutants, we identified a loss-of-function mutation in a GATA transcription factor (TF). Cells lacking this TF fail to express genes that are normally upregulated during early stages of development and the whole developmental program is significantly delayed. This TF accumulates in the nucleus at the beginning of development and shifts to the cytosol at later time point, which is accompanied by serine/threonine phosphorylation. The change of cellular localization and phosphorylation do not occur in cells lacking the cAMP receptor. Consistently, extracellular cAMP stimulates both events. Through mass spectrometry analysis, we identified several cAMP-dependent phosphorylation sites that are important for the nucleus-to-cytosol translocation. Furthermore, we found that GskA, a homologue of the mammalian glycogen synthase kinase-3, is partially responsible for the phosphorylation. Cells expressing this TF with alanine substitutions at the GskA phospho-sites exhibit aberrantly accelerated development and acquire chemotactic ability about 2 hours before wild-type cells. These results indicate that the spatiotemporal regulation of this GATA TF is a key component of the genetic program that equips *Dictyostelium* to aggregate and chemotax. Experiments are carried out to identify genes that are targeted by this TF.

**Abstract type:** Talk

# Poster Abstracts

## Posters may be displayed throughout the meeting and will be presented on Monday and Tuesday evenings

**Title:** *Dictyostelium* as a model for motor neurone disease

**Authors:** Sarah J. Annesley and Paul R. Fisher (Department of Microbiology, La Trobe University, Bundoora, VIC, Australia)

**Presenter:** Sarah Annesley

**Address for correspondence:** S.Annesley@latrobe.edu.au

**Abstract:** MND (Motor Neurone Disease) or ALS (Amyotrophic Lateral Sclerosis) is the third most common neurodegenerative cause of adult death. The disease is characterised by degeneration of the motor neurons which inevitably leads to paralysis and death generally within 1-5 years since onset of symptoms. About 10% of cases are familial and of these 20% are due to mutations in the sod1 gene (Cu/Zn superoxide dismutase). Over 100 mutations to the sod1 gene have been reported and these mutations do not affect the catalytic activity of the enzyme rather they result in a toxic gain of function. There is accumulating evidence that oxidative stress and mitochondrial dysfunction play an important role in the pathogenesis of MND. Progress in understanding mitochondrial dysfunction is hampered by the complexity of mitochondrial biology in animals and humans. To overcome this, our laboratory has created a model for mitochondrial disease in the organism *Dictyostelium*. Mitochondrial disease has been created in this organism through the down regulation or knockout of genes encoding various mitochondrial proteins (11, 12, 13) and all resulted in an array of characteristic aberrant phenotypes. These disease phenotypes are faithfully reproduced by overexpression of an active form of AMPK and are suppressed by knock down of AMPK expression in mitochondrially diseased cell lines (strains). This shows that the cytopathologies are due to chronic activation of AMPK rather than insufficiency of ATP per se. To determine if mitochondrial dysfunction is causal in MND we have created a *Dictyostelium* model of MND by overexpressing mutant SodE and SodF (homologs of Sod1). The MND strains display some phenotypes characteristic of mitochondrial disease such as short thick stalks. These preliminary results support the hypothesis that Sod mutations that cause MND are cytotoxic because they cause mitochondrial dysfunction. To determine if the phenotypes are a result of chronic AMPK activation AMPK will be downregulated in cells overexpressing mutant sodE or sodF. Future experiments will attempt to elucidate how SodE/SodF cause mitochondrial dysfunction focussing particularly on the localisation of mutant Sod and the calcium responses in MND strains.

**Abstract type:** Poster

**Title:** *Dictyostelium* Nramp proteins are involved in resistance to invasive bacteria, regulation of development and iron homeostasis

**Authors:** Peracino Barbara, Simona Buracco, Alessio Sillo and Salvatore Bozzaro

**Presenter:** Salvatore Bozzaro

**Address for correspondence:** Department of Clinical and Biological Sciences, University of Turin, AOU S. Luigi, 10043 Orbassano, Italy

**Abstract:** *Dictyostelium* cells harbour two genes encoding members of the Nramp superfamily, which is widely distributed from bacteria to humans. Nramp's are proton-driven metal ion transporters with a preference for iron and manganese. The Nramp prototype, Nramp1 (Slc11a), is specifically expressed in phagocytes and confers resistance to infection by invasive bacteria. Mammals possess also Nramp2, which is detected in most tissues, is found on the plasma membrane and in recycling early endosomes and is the major iron transporter, together with the transferrin receptor. Nramp2 is essential for life and mutations results in deficient intestinal iron uptake and severe microcytic anemia. *Dictyostelium* Nramp1, like its mammalian homolog, is expressed in phagosomes and macropinosomes, and gene disruption results in increased susceptibility to infection by legionella and mycobacteria (Peracino et al, 2006). In wild type cells, legionella inhibits V-H+ ATPase, but not Nramp1, recruitment to phagosomes, a process controlled by phosphoinositide metabolism (Peracino et al, 2010). In contrast to Nramp1, *Dictyostelium* Nramp2 expression is confined to the membrane of the contractile vacuole (CV). Nramp2 gene disruption also results in increased sensitivity to infection by L. pneumophyla. The double KO mutant additionally displays delayed development and forms large multi-tipped aggregates. Differential effects of iron overload or iron depletion are detected in single and double mutants, suggesting that both Nramp proteins regulate iron homeostasis.

**Abstract type:** Poster

**Title:** *Dictyostelium discoideum* as a model to evaluate the virulence of the swine pathogen Streptococcus suis

**Authors:** Laetitia Bonifait [1,2], Steve J. Charette [3,4,5], Geneviève Filion [3,4], Marcelo Gottschalk [2,6], and Daniel Grenier [1,2]

**Presenter:** Steve Charette

**Address for correspondence:** [1]Groupe de Recherche en Écologie Buccale (GREB), Faculté de médecine dentaire, Université Laval, Quebec City, Quebec, Canada; [2]Centre de Recherche en Infectiologie Porcine (CRIP), Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT), Quebec City, Quebec, Canada; [3]Institut de Biologie Intégrative et des Systèmes (IBIS), Quebec City, Quebec, Canada; [4]Centre de recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de Québec (IUCPQ), Hôpital Laval, Quebec City, Quebec, Canada; [5]Département de Biochimie, de Microbiologie et de Bio-Informatique, Faculté des sciences et de génie, Université Laval, Quebec City, Quebec, Canada; [6]Groupe de Recherche sur les Maladies Infectieuses du Porc (GREMIP), Faculté de médecine vétérinaire, Université de Montréal, Ste-Hyacinthe, Quebec, Canada

**Abstract:** *Streptococcus suis*, a Gram-positive bacterium, is a major swine pathogen worldwide that causes meningitis, septicemia, arthritis, and endocarditis. *S. suis* produces a variety of cell-associated and secreted virulence factors. We investigated whether the amoeba *Dictyostelium discoideum*, which is employed as an alternative host model mainly for Gram-negative pathogenic bacteria, could be also used to study the virulence of *S. suis*. The amoeba model made it possible to detect major differences between wild-type virulent strains of *S. suis* and attenuated mutants that lacked either a capsule or the subtilisin-like protease and that are less virulent in animal models. *D. discoideum* was able to grow and form plaques on lawns of the *S. suis* mutants but not on lawns of the two wild-type virulent strains. In addition, *D. discoideum* was also able to grow on a non-typeable *S. suis* strain that has been shown by transmission electron microscopy to be naturally devoid of capsule. Our results indicated that a non-metazoan model can be used to assess the virulence of *S. suis*. This is the first comprehensive study of a Gram-positive pathogenic bacterium using the *D. discoideum* plaque assay.

**Abstract type:** Poster

**Title:** Role of Class I Myosin in Phagocytosis through PtdIns(3,4,5)P3 Signaling

**Authors:** Chun-Lin Chen, Yu Wang, Miho Iijima

**Presenter:** Chun-Lin Chen

**Address for correspondence:** Department of Cell Biology, The Johns Hopkins University School of Medicine, Baltimore, MD

**Abstract:** Phagocytosis and chemotaxis play key roles in immune responses and their mechanism are conserved in mammalian leukocyte and *Dictyostelium* amoebae. PH-domain-containing proteins which specifically bind to PtdIns(3,4,5)P3 mediated intracellular signaling in chemotaxis. However, it is largely unknown PtdIns(3,4,5)P3 signaling mechanisms also function in phagocytosis. It has been shown that *Dictyostelium* cells that lack myosin I are impaired in normal phagocytosis and cell aggregation during development. Here, we show that Myo1D, Myo1E, and Myo1F function in phagocytosis through interactions with PtdIns(3,4,5)P3. These myosin I molecules contain PH-domain-related sequence and bind to PtdIns(3,4,5)P3 in lipid dot-blot assays. Mutations in this sequence block their function in phagocytosis. Suggesting a role of myosin I at early stages of phagocytosis, MyoE is transiently recruited to phagocytic cups before completion of internalization in a PtdIns(3,4,5)P3-dependent manner. When we analyzed single, double, and triple knockout cells for MyoD, MyoE, and MyoF, all the single mutants showed different degree of growth defects in suspension culture, and the double and triple mutants showed more severe defects. Interestingly, single mutants showed no defects in phagocytosis while combined loss of these myosin I molecules displayed strong inhibition of phagocytosis. Our data suggest that MyoD, E, and F have partially overlapping function in phagocytosis through PtdIns(3,4,5)P3 signaling.

**Abstract type:** Poster

**Title:** A Possible Role for Copine A in Actin Filament Disassembly

**Authors:** Hanqian Mao, Helena E Lucente, Torrin L McDonald, Kristen M Karasiewicz, Paul A Steimle, David Loiselle, Timothy AJ Haystead, and Cynthia K Damer

**Presenter:** Cynthia K Damer

**Address for correspondence:** 217 Brooks Hall, 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 consists 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 several methods to identify proteins that interact with CpnA. We first used column chromatography and mass spectrometry to isolate proteins that bound to a CpnA-linked agarose column. One of the proteins that eluted from the CpnA-linked column and not the control column was actin. We also carried out immunoprecipitations using a polyclonal antibody to GFP with *Dictyostelium* cells expressing GFP, GFP-CpnA, or GFP-fused to the VWA domain of CpnA (GFP-Ado). Actin co-precipitated with GFP-Ado, but not GFP or GFP-CpnA. Using the A domain of CpnA as bait we screened a *Dictyostelium* developmental cDNA library for genes that code for binding partners of CpnA. Preliminary screening results included the genes, *aip1* and *corA*. Both of these genes code for actin-binding proteins that regulate actin disassembly. Fluorescence microscopy studies using rhodamine-phalloidin to label actin filaments indicated that *cpnA*$^{-}$ cells have more actin filaments than wild-type cells. In addition, *cpnA*$^{-}$ cells exhibited a smaller increase in actin polymerization in response to cAMP stimulation than wild-type cells, possible due to increased actin polymerization in resting cells. Preliminary studies show the *cpnA*$^{-}$ cells are more adhesive than wild-type cells and scanning electron microscopy images indicate that *cpnA*$^{-}$ cells are flatter with more lamellipodia than wild-type cells. These studies indicate that CpnA may act as a regulator of actin disassembly.

**Abstract type:** Poster

**Title:** The Effect of AmpA on Actin Polymerization

**Authors:** Elizabeth Ford, Chere Petty, Yuchao Zheng, Katherine Lannon, Stephanie Steiner, Sudeshna Arramraju, Daphne Blumberg

**Presenter:** Elizabeth Ford

**Address for correspondence:** Dept. of Biological Sciences, University of Maryland, Baltimore County, Baltimore Md. 21250

**Abstract:** The *ampA* gene encodes a novel secreted protein that modulates cell adhesion, actin polymerization and developmental patterning. The AmpA protein is necessary in a non-cell autonomous manner to prevent premature differentiation of prespore cells. In *ampA* null cells a prespore marker is expressed in cells at the mound periphery that will normally differentiate into prestalk cells. A supernatant source and synthetic peptides from the AmpA protein can prevent this misexpression indicating a function of secreted AmpA. Expression in growing cells, where AmpA is not secreted in wild type cells, reveals a second function for AmpA. AmpA loss results in an increase in cell adhesion, and a reduction in F actin with a concomitant increase in G actin. Overexpression of AmpA reduces adhesion and increases F actin. As a result of these changes in the cytoskeleton and in adhesion, AmpA also influences cell migration. In comparison to wild type cells, *ampA* null cells are defective in migration on top of agarose. AmpA overexpressing cells show the opposite behavior, migrating well on top of agarose. Research is now focused on two objectives. The first is to determine how AmpA affects the cytoskeletal dynamics of actin polymerization. The second is to determine the localization of AmpA in the cell. In order to determine how AmpA affects actin polymerization, wild type, AmpA KO and AmpA over-expressing strains have been created containing an Actin binding domain fused to GFP. This has allowed us to visualize actin dynamics in living cells. Results suggest that AmpA over expressing cells polymerize significantly more actin than wild type and the excess actin appears to concentrate at the rear of the migrating cells. In *ampA* null cells, the F-actin in significantly reduced at the cell periphery but found in punctuate spots in the cell interior. In feeding cells, over expressers make larger, more actin rich endosomal cups, which tend to form repeatedly in the same area of the membrane as though the site of actin polymerization cannot be disassembled. Also in AmpA over expressing cells, unlike wild type cells, the AmpA is found both inside the cell and extracellularly, but the affects of AmpA on actin polymerization appear to be due to the intracellular fraction of AmpA. However recent data suggests that extra-cellular AmpA may play a role in cell size and membrane blebbing. Strains containing AmpA fused to a TAP tag and AmpA fused to mRFP have been created. Immunofluorescence studies show that AmpA is localized in possible vesicles throughout the cell as well as at the cell periphery. Co-localization studies indicate isolated sites of localization of the vesicles with actin, and localization with calnexin, an ER marker, at discrete sites surrounding the nucleus.

**Abstract type:** Poster

**Title:** The Effect of AmpA on Actin Polymerization and its Localization in the Vesicle Trafficking Pathway

**Authors:** Elizabeth Ford, Stephanie Steiner, Sudeshna Arramraju, Daphne Blumberg

**Presenter:** Elizabeth Ford

**Address for correspondence:** Dept. of Biological Sciences, University of Maryland, Baltimore County, Baltimore Md. 21250

**Abstract:** The *ampA* gene encodes a novel secreted protein that modulates cell adhesion, actin polymerization and developmental patterning. The AmpA protein is necessary in a non-cell autonomous manner to prevent premature differentiation of prespore cells. In *ampA* null cells a prespore marker is expressed in cells at the mound periphery that will normally differentiate into prestalk cells. A supernatant source and synthetic peptides from the AmpA protein can prevent this misexpression indicating a function of secreted AmpA. Expression in growing cells, where AmpA is not secreted in wild type cells, reveals a second function for AmpA. AmpA loss results in an increase in cell adhesion, and a reduction in F actin with a concomitant increase in G actin. Overexpression of AmpA reduces adhesion and increases F actin. As a result of these changes in the cytoskeleton and in adhesion, AmpA also influences cell migration. In comparison to wild type cells, *ampA* null cells are defective in migration on top of agarose. AmpA overexpressing cells show the opposite behavior, migrating well on top of agarose. Research is now focused on two objectives. The first is to determine how AmpA affects the cytoskeletal dynamics of actin polymerization. The second is to determine the localization of AmpA in the cell. In order to determine how AmpA affects actin polymerization, wild type, AmpA KO and AmpA over-expressing strains have been created containing an Actin binding domain fused to GFP. This has allowed us to visualize actin dynamics in living cells. Results suggest that a subset of AmpA over expressing cells polymerize significantly more actin than wild type and the excess actin appears to concentrate at the rear of the migrating cells. In stationary cells, over expressers make larger, more actin rich endosomal cups, which tend to form repeatedly in the same area of the membrane. Also in AmpA over expressing cells, the AmpA is found both inside the cell and extracellularly, but the effects of AmpA on actin polymerization appear to be due to the intracellular fraction of AmpA. In order to determine how AmpA affects actin polymerization, localization analyses have been done. Strains containing AmpA fused to a TAP tag and AmpA fused to mRFP have been created. Immunofluorescence studies show that AmpA is localized in possible vesicles throughout the cell. Co-localization studies indicate isolated sites of localization of these vesicles with actin, and localization with calnexin, an ER marker, at discrete sites surrounding the nucleus. We postulate that cellular AmpA is also an autocrine factor associated with the cell surface and are trying to visualize surface associated AmpA and trying to determine if the AmpA in vesicles is post-golgi or endosomal in origin. Fractionation experiments with both mRFP and TAP tagged AmpA indicate that AmpA is found in both the cytoplasm and the vesicles of vegetative cells.
**Abstract type:** Poster

**Title:** Comparative Analysis of Gene Families in Acytostelium subglobosum

**Authors:** Kensuke Fukuhara, Hidekazu Kuwayama, Hideko Urushihara

**Presenter:** Kensuke Fukuhara

**Address for correspondence:** Graduate school of Life and Environmental Sciences, University of Tsukuba

**Abstract:** We have been performing the genome and transcriptome analyses of *Acytostelium subglobosum*, a group 2 species that forms fruiting bodies with acellular stalks. Since cell-type differentiation does not occur in this species, its genomic differences from *Dictyostelium* and *Polysphondylium* species should contain key information related to the history for the system of somatic cell differentiation to have been established. We previously reported that *A. subglobosum* possessed a nearly equal number of genes to *D. discoideum* and that the most of the gene families were shared between the two species. In the present study, we performed detailed comparative analysis of *D. discoideum* gene families, which fell into three categories. The first one contains conserved gene families in which all or most of the members have orthologous genes in *A. subglobosum*. The second one represents gene families that expanded in the lineage of *D. discoideum* after split from the *A. subglobosum* lineage. This category includes pks, act, psi families and so on. The last category contains few gene families in which all or most of the family members were absent in *A. subglobosum*, such as the pon family. Although orthologous genes are not necessarily involved in the same cellular processes, their presence or absence may reflect the similarity or dissimilarity in developmental properties of the two species. Additional genomic properties of *A. subglobosum* will also be discussed.

**Abstract type:** Poster

**Title:** The Use of Quantum Dots for the tracking of G Protein-Coupled Receptors in *Dictyostelium*

**Authors:** Justin Galloway, Meghdad Radhar, Kwan Hyi Lee, Jeaho Park, Peter Searson and Peter Devreotes

**Presenter:** Justin Galloway

**Address for correspondence:** 3400 North Charles Street Maryland Hall 205 Baltimore, MD 21218

**Abstract:** Live cell imaging at the single molecule level presents several problems that are difficult to overcome, even when employing more recent technologies. Selectively targeting molecules of interest using fluorophores typically presents some level of non-specific binding and photobleaching within 10 – 15 seconds can limit results. Fluorescent proteins are imperfect for use in single molecule due to the inability to control expression. To target the G protein-coupled receptor cAR1 of *D. discoideum*, we use the HaloTag Technology System that selectively binds chlorinated alkane chains. By coupling a chlorinated alkane chain to the surface of a lipid coated quantum dot, we are able to target cAR1 with a fluorophore that does not photobleach. In this manner, we are able to track fluorophores for times much longer than that allowed by organic fluorophores.

**Abstract type:** Poster

**Title:** Mechanistic studies of upstream signaling events during *Dictyostelium* gradient sensing

**Authors:** Noelle Holmes, Gus Wright, Chris Janetopoulos

**Presenter:** Noelle Holmes

**Address for correspondence:** Janetopoulos Lab U5215 MRB III 465 21st Ave South Nashville, TN 37232 tel 615 936 8907 fax 615 343 6707

**Abstract:** Metastasis is responsible for as much as 90% of cancer-associated mortality, yet it remains the most poorly understood component of cancer pathogenesis. By understanding the mechanisms that lead to physical translocation, early stage cancer metastasis could be prevented. Cancer cells become migratory and can upregulate their chemokine receptors and migrate directionally. Chemokine receptors are G-protein coupled receptors (GPCRs) and are critical for chemotaxis. *Dictyostelium discoideum* is a model organism for studying GPCRs and contains ~55 GPCRs based on sequence homology. During cAMP-mediated chemotaxis, the bound cAR1 receptor triggers the dissociation of the heterotrimeric G proteins which signal to a number of downstream effectors. These include a number of small G proteins like Ras C and G, the enzymes that regulate PI(4,5)P2 and PI(3,4,5)P3, and members of the TOR complexes. In addition, cAR1 is essential for aggregation and subsequent development because it transiently activates adenylyl cyclase to mediate cAMP relay, and it plays a direct role in aggregation as it mediates chemotaxis of cells up the cAMP gradient. We have made numerous mutations in cAR1 that we predict will alter the dynamic range of the cAMP chemoattractant and have already obtained some interesting results. Further testing of the ability of cells to adapt and de-adapt to cAMP will be tested in a number of these cAR1 mutants. These experiments will give us further insight into how the GPCRs modulate gradient sensing during cellular chemotaxis.

**Abstract type:** Poster

**Title:** The role of G-proteins in adaptation to chemotactic signaling

**Authors:** Chuan-Hsiang Huang, Peter N. Devreotes

**Presenter:** Chuan-Hsiang Huang

**Address for correspondence:** 725 N Wolfe St, WBSB 116 Baltimore MD 21209

**Abstract:** Chemotaxis is the directed migration of cells in chemical gradients and is fundamental to the survival of many types of cells. In *Dictyostelium*, the chemoattractant cAMP triggers G-protein coupled receptor signaling mediated by the heterotrimeric Gα2/Gβ/Gγ complex. Activation of G protein can be monitored in real time using FRET between Gα2-CFP and Gβ-YFP as a readout. cAMP stimulation leads to a rapid decrease in FRET due to the dissociation of Gα2-CFP and Gβ-YFP, whereas removal of stimulus causes a slow recovery of FRET. The different kinetics of FRET loss and recovery is explained by a G-protein cycle model. However, if FRET loss reflects G-protein activation, the slow recovery would cause signal delocalization and compromise gradient sensing during chemotaxis. Moreover, expression of a constitutively active mutant of G-protein leads to persistent activation of downstream signaling. These results suggest that G-protein may be inactivated in the dissociated state.

**Abstract type:** Poster

**Title:** Investigating the intracellular targets of DdEGFL1-enhanced cell movement in *Dictyostelium discoideum*

**Authors:** Robert J. Huber*[1] and Danton H. O'Day[1,2]

[1]Department of Cell and Systems Biology, University of Toronto, Toronto, Ontario, Canada; [2]Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada

**Presenter:** Robert J. Huber

**Address for correspondence:** Robert J. Huber University of Toronto Mississauga Department of Biology 3359 Mississauga Road North Mississauga, Ontario L5L 1C6 Canada

**Abstract:** The Epidermal Growth Factor (EGF) regulates a number of cell processes in mammalian cells, including cell movement and chemotaxis. EGF-like (EGFL) repeats share sequence similarity with EGF. Ten14, an EGFL repeat from the extracellular matrix protein tenascin C, has been shown to enhance mammalian cell movement by binding to the EGF receptor (EGFR) and activating EGFR-dependent signalling. In *Dictyostelium discoideum*, a synthetic EGFL peptide (DdEGFL1) based on an 18 amino acid sequence from the extracellular calmodulin-binding protein CyrA, has been shown to enhance cell movement via a CarA and CarC independent mechanism involving intracellular calcium release, calmodulin activity, actin polymerization, and myosin II heavy chain (MHC) assembly. Our current research investigates the intracellular targets of DdEGFL1 enhanced movement, specifically the threonine phosphorylation of a ~200 kDa protein whose phosphorylation is sustained during DdEGFL1 treatment. Immunoprecipitation of the phosphothreonine protein followed by an LC/MS/MS analysis has identified the ~200 kDa protein as vinculin B (VinB). VinB was also detected in DdEGFL1 pull-down fractions supporting its involvement in the mechanism regulating DdEGFL1-enhanced cell movement. VinB-GFP localizes to the cytoplasm and co-immunoprecipitates with MHC, actin, and α-actinin supporting its function in mammalian cells as a linker protein that anchors the actin cytoskeleton to the cell membrane. Together, our research provides new insight into the mechanism mediating EGFL repeat/peptide enhanced cell movement in *Dictyostelium* and may ultimately identify *Dictyostelium* as a model system for studying EGFL repeat/peptide function in higher organisms.

**Abstract type:** Poster

**Title:** Conserved action of adenosine and its antagonist caffeine on aggregation pattern in cellular slime molds.

**Authors:** Pundrik Jaiswal, Thierry Soldati, Sascha Thewes and Ramamurthy Baskar

**Presenter:** Pundrik Jaiswal

**Address for correspondence:** Department of Biotechnology, Indian Institute of Technology-Madras, Chennai-600036, India E-mail: pundrik_jaiswal@yahoo.co.in

**Abstract:** Multicellularity in cellular slime molds is achieved by aggregation of several hundreds of cells. It is well known that adenosine induces large aggregates. Caffeine, an adenosine antagonist favours compact and small-sized aggregates. However, it is not clear whether adenosine and caffeine actions are evolutionarily conserved among cellular slime molds. By choosing slime molds from different evolutionary groups known to make use of diverse chemoattractants for their aggregation, we find that adenosine and caffeine induces large and small aggregate formation, respectively. We found that changes in aggregate size are caused by effects of the compounds on several parameters such as cell number and size, cell-cell adhesion, cAMP signal relay and counting mechanism. Adenosine acts like a nutrient supplement similar to glucose, increasing cell number and size, whereas caffeine acts as a cell growth inhibitor. Both compounds strengthen cell-cell adhesion via increased Cad-1 and CsaA expressions. Both compounds also inhibit synthesis of cAMP phosphodiesterase (PdsA) and activation of adenyl cyclases thus weakening the relay of extracellular cAMP signal. Finally, both rescue mutants impaired in stream formation (pde4-, pdiA-), and restore their parental aggregate size. We propose that PdsA and PDI are the key proteins affecting the aggregation pattern and sizes. The aggregation process is conserved among *Dictyostelia* despite their use of unrelated signaling molecules for aggregation.

**Abstract type:** Poster

**Title:** Base Excision Repair System is developmentally modulated in *Dictyostelium discoideum*

**Authors:** Hyun-Ik Jun[+], Chan-Woong Wang[+], Sun Young Goo, Eugene Noh, Jung-Suk Sung*, and Wonhee Jang*

[+]H-IJ and C-WW contributed equally to this work.
*J-SS and WHJ are co-corresponding authors.

**Presenter:** Wonhee Jang

**Address for correspondence:** Department of Life Science, Dongguk University, 3-26 Pil-dong, Jung-gu, Seoul, Korea 100-715

**Abstract:** Every organism is constantly exposed to the variety of DNA-damaging agents in the environment. If the damaged DNA is left unrepaired, it can lead to DNA mutations or cell death. In order to maintain genomic integrity, cells have various DNA repair mechanisms, including the base excision repair (BER) pathway. There was a considerable hardship in studying the roles of DNA repair during development of higher organisms due to frequent embryonic lethality in mouse model systems with disrupted DNA repair gene functions. Thus, *Dictyostelium* whose development is separated from cell division may offer a very unique organism to look at the process of DNA repair during development. We found that the levels of transcripts and the levels of enzyme activities involved in BER pathway are developmentally modulated in *Dictyostelium*. A further study on the roles of BER enzymes in *Dictyostelium* during development may give us a clue in the developmental roles of the enzymes in higher organisms.

**Abstract type:** Poster

**Title:** Spontaneous activation of chemotactic TORC2-PDK-PKB pathway

**Authors:** Yoichiro Kamimura, Hauqing Cai, Masahiro Ueda, and Peter Devreotes

**Presenter:** Yoichiro Kamimura

**Address for correspondence:** Laboratory for Cell Signaling Dynamics, Quantitative Biology Center (QBiC), RIKEN 6-2-3, Furuedai, Suita, Osaka, Japan, 565-0874

**Abstract:** During chemotaxis transduction of signals from cell surface receptors leads to directional motility along chemotactic gradients. *Dictyostelium discoideum* is one of the most well-characterized organisms for studies of the signaling events involved in chemotaxis. Stimulation of cells with the chemoattractant cAMP activates multiple signaling pathways including those leading to production of PIP3 or cGMP or the TORC2- and PDK-mediated activation of PKBs. The PIP3 and TORC2-PDK-PKB pathways converge on two PKB kinases, PIP3-dependent PKBA and PIP3–independent PKBR1. These two kinases are regulated through phosphorylation of their hydrophobic motifs (HM) by TORC2. PKBA also depends on recruitment to membrane PIP3 via its PH domain while myristoylated PKBR1 does not. Recent studies have shown that PIP3 patches are produced in a self-organized fashion in the absence of chemoattractant, suggesting the importance of this activity in spontaneous motility. To study self-organization of the other chemotactic signaling pathways, we have assessed the TORC2-PDK-PKB activation by using R1-AKT-HA, a reporter where the N-terminus of PKBR1 is fused to the kinase and HM domains of human AKT, along with the AKT S473 phosphorylation dependent antibody. In vegetative cells harboring the reporter, the TORC2-PDK-PKB pathway is spontaneously activated at plausible pseudopods. Furthermore, cytoskeletal abrogation by Latrunculin B eliminates this activity. These features are similar to those previously observed for the spontaneous accumulations of PIP3. Furthermore, the activation of the TORC2-PDK-PKB pathway is enhanced by expressing constitutively active RasC(Q62L), also independently of PIP3. Here, we will discuss mechanisms and biological meanings of self-organized activities of chemotactic pathways.

**Abstract type:** Poster

**Title:** The Characterization of Novel Migration Modulators and Possible Involvement in the AmpA Pathway

**Authors:** Jessica Kelsey, Nathan Fastman, Elizabeth Ford, and Daphne Blumberg

**Presenter:** Jessica Kelsey

**Address for correspondence:** Biological Sciences Dept. University Of Maryland Baltimore County Baltimore Maryland,21250

**Abstract:** The *ampA* gene is critical for *Dictyostelium discoideum* cell migration. To help understand the elusive ampA migrational pathway, second site suppressors were created by REMI mutageneis. Three novel genes were identified as suppressors of the AmpA overexpressing increased migration phenotype. In order to understand and characterize the novel suppressor gene functions mRFP fusion proteins were created and knockout cell lines were established. Characterization results of two of the suppressors will be presented. One is lmbd2b and belongs to the evolutionarily conserved LMBR1 protein family, some of whose known members are transmembrane endocytic receptors. Our findings suggest LMBD2B likely also functions as a plasma membrane endocytic receptor. It colocalizes with proteins key in endocytic events such as clathrin, coronin, and F-actin. LMBD2B exists as dispersed puntacte signal on the periphery of cells in suspension. But with prolonged substrate contact, LMBD2B begins to concentrate and likely undergoes large-scale endocytosis via micropinocytosis. Vegetative *lmbd2B*$^{-}$ cells display defects in migration. These cells have difficulty sensing a chemoattractant as indicated by a decrease in their directionality, productive distance movement, and most significantly their chemotactic index. *Lmbd2B*$^{-}$ cells appear to extend excessive pseudopods and have difficulty establishing a front/back orientation to facilitate migration. The second suppressor is a multi-domain F-BAR containing protein. The protein associates with PI3P phospholipids. It exists in vesicles that change in cellular localization from dispersed throughout the cell to more nuclear based on cell density. It inhibits macropinocytosis and is involved in clathrin-mediated endocytosis. The drastic increase in endocytosis exhibited by null cells appears to interfere with migration. Its association with PI3P and its domain similarities (it contains BAR and CAST domains, both known to be involved in vesicle trafficking) suggest the suppressor protein may be involved in vesicle trafficking and endocytosis.

**Abstract type:** Poster

**Title:** *Dictyostelium* Chemotaxis: Essential Ras activation and accessory signaling pathways for amplification

**Authors:** Arjan Kortholt, Rama Kataria, Ineke Keizer-Gunnink, Wouter N. Van Egmond, Ankita Khanna and Peter J.M. Van Haastert

**Presenter:** Arjan Kortholt

**Address for correspondence:** Department of Molecular Cell Biology, University of Groningen, The Netherlands

**Abstract:** Central to chemotaxis is the molecular mechanism by which cells exhibit directed movement in shallow gradients of chemoattractant. Recent studies have shown that chemotaxis does not depend on a single molecular mechanism, but rather depends on several interconnecting pathways. So far four major signaling enzymes have been implicated in chemotaxis, PI3K, TorC2, PLA2 and sGC. Surprisingly, small G-proteins of the Ras subfamily appear to play essential roles in all these pathways. Thus far, Ras is the most upstream component of the signaling pathway that shows stronger activation at the leading edge than the steepness of the gradient, suggesting that symmetry breaking and/or amplification of the gradient occurs at or before Ras activation. To understand the molecular mechanisms of chemotaxis we have investigate the Ras signaling pathway in more detail. Here we present an update on our latest research.

**Abstract type:** Poster

**Title:** The Isolation and Proteomic Analysis of *Dictyostelium* Exosomes

**Authors:** Kriebel P., Jenkins L., Zhang G., and Parent C.

**Presenter:** Paul Kriebel

**Address for correspondence:** Kriebelp@mail.nih.gov

**Abstract:** In *Dictyostelium*, the binding of the chemoattractant cAMP to its G protein coupled receptor activates a variety of effectors including the adenylyl cyclase, ACA, which converts ATP into cAMP. A large portion of synthesized cAMP is secreted to relay signals to neighboring cells and allow the formation of aggregates. We have previously shown that vesicles containing ACA are specifically trafficked to the back of migrating *Dictyostelium* cells, where they are essential for cells to align in a head to tail fashion during chemotaxis. We proposed that this pool of ACA containing vesicles provided a compartment from which cAMP is locally released. Electron microscope analysis revealed that ACA is enriched on multi-vesicular bodies that coalesce at the back of cells and fuse with the plasma membrane to release their vesicular content (called exosomes) which also contain ACA, to the outside to form trails. We have developed methods to isolate ACA containing exosomes from cell culture supernatant. These experiments employed a combination of differential centrifugations sucrose density gradients and western blot analysis to characterize the various membrane fractions. The membrane fractions were further analyzed by reverse phase HPLC followed by LTQ mass spectrophotometry. These results indicate that exosomes are a conserved organelle in both *Dictyostelium* and mammals. By exploring the protein and lipid composition of *Dictyostelium* exosomes we wish to gain more insight into the mechanisms that regulate cAMP secretion and the role of exosomes in cell-cell signaling during chemotaxis.

**Abstract type:** Poster

**Title:** Exploring the phosphoproteome of *Dictyostelium discoideum* when responding towards cAMP

**Authors:** Lacal, J., Kölsch V., Shen, Z., Charest, P.G., Shaulsky, G., Briggs, S.P., Firtel, R.A.

**Presenter:** Jesús Lacal

**Address for correspondence:** DIV BIOLOGICAL SCI. Natural Sciences Bldg. 9500 Gilman Drive # 0380. La Jolla, CA 92093-0380

**Abstract:** A major and longstanding interest of our laboratory is the elucidation of the cellular and molecular mechanisms by which cAMP regulates chemotaxis in *Dictyostelium discoideum*. We have carried out phosphoproteomics with high-resolution qualitative mass spectrometry and bioinformatics to analyse the effects of cAMP on the phosphoproteome and analysis of signal transduction pathways in *Dictyostelium discoideum*, focusing on the changes that take place at the leading edge of the cells when responding to cAMP. These studies allow for the identification of regulated phosphorylation sites in the cellular proteome following exposure to cAMP and the molecules that interact with cellular kinases or phosphatases. We present here an analysis of the screen and the most relevant proteins that may be involved in the signaling pathway for the chemotaxis towards cAMP.

**Abstract type:** Poster

**Title:** Functional Genomics and Glycomics of Cooperative Behavior in *Dictyostelium discoideum*

**Authors:** Si Li, Kanoelani Pilobello, Lara Mahal, Michael Purugganan

**Presenter:** Si Li

**Address for correspondence:** Center for Genomics and Systems Biology, New York University, 12 Waverly Place, New York, NY 10003

**Abstract:** Confronting starvation, solitary *Dictyostelium discoideum* can co-aggregate with cells of different genetic backgrounds to form chimeric multicellular fruiting bodies. The fact that only the spores are viable in the fruiting body while the stalk eventually dies raises questions on the evolution and ecology of this social interaction and its underlying genetic and biochemical bases. As a single-celled organism, direct cell-cell contact largely mediated by glycoproteins is considered as a major mechanism for social recognition and interaction in *D. discoideum*. To unravel the transcriptomic effects of cell-cell recognition and adhesion, a system is established to study global gene expression during chimeric development using two natural strains known to exhibit social dominance. Labeled cells of focal strain were collected at multiple developmental stages from different conditions using FACS. A customized Agilent gene expression microarray that recognizes 10858 genes was designed to perform transcriptomic profiling. Comparisons across different conditions are expected to suggest specific molecular mechanisms associated with cooperative behavior. To survey the variation and alteration in cell-surface glycosylation, a lectin array is employed to identify the species and amounts of glycans that are present on cell surface. Time-course membrane samples were isolated from various natural strains with known social dominance hierarchy. A clear shift in glycome was observed over time, and we are examining the data for strain-specific glycosylation patterns.

**Abstract type:** Poster

**Title:** Expression of the prestalk and prespore marker homologues during the development of Acytostelium subglobosum

**Authors:** Kurato Mohri, Hitoshi Saitoh, Hidekazu Kuwayama and Hideko Urushihara

**Presenter:** Kurato Mohri

**Address for correspondence:** Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Tennodai 1-1-1, Ibaraki 305-8572, Japan

**Abstract:** Somatic cell differentiation is a crucial event for the development of the multicellular organisms. Separation of mortal stalk cells from spores during fruiting body development of *Dictyostelium discoideum* is a simple model for the somatic cell differentiation. On the other hand, an evolutionarily older species of the dictyostelids, *Acytostelium subglobosum* does not produce somatic cells. It produces acellular stalks and all aggregated cells turn into spores. To understand how somatic cell differentiation systems were acquired during evolution of the dictyostelids, we have been performing comparative genomic analyses between these two species. In this study, to understand the cellular details of fruiting body formation of *A. subglobosum* and compare with those of *D. discoideum* at the molecular level, we isolated *A. subglobosum* homologues of marker genes for prestalk and prespores in *D. discoideum* and studied their spacio-temporal expression patterns during *A. subglobosum* development. These molecular markers of *A. subglobosum* were not detected in cell aggregation but became detectable in the protruding sorogens when they started to produce sorocarps. Unlike *D. discoideum* pseudoplasmodia, *A. subglobosum* prespore marker was expressed in the anterior region of sorogens whereas prestalk markers are restricted in the posterior region, the root of protrusion. These results suggest that when cells in aggregates enter into the root of the protruding sorogens, they start to express genes for producing stalks, and then they move to the anterior region and switch gene expression for sporulation.

**Abstract type:** Poster

**Title:** Frequency switch in cAMP waves and its relation to cell migration

**Authors:** Akihiko Nakajima, Shigenori Hirose, Daisuke Taniguchi, Gad Shaulsky, Adam Kuspa, Satoshi Sawai

**Presenter:** Akihiko Nakajima

**Address for correspondence:** Graduate School of Arts and Sciences, University of Tokyo, Tokyo, 153-8902, Japan

**Abstract:** What are the features of cAMP waves that cells read out? It has long been proposed that *Dictyostelium* cells can read both spatial and temporal gradient of extracellular cAMP. However, how the modes of movement and cell shape changes are dictated by the endogenous chemoattractant field of cAMP and how they coordinate with one another have been difficult to address quantitatively. With the aid of a FRET-based cAMP reporter, we show phase and frequency relation between the cycles of the cAMP wave propagation and cell migratory behaviors. From in-vivo imaging of cytosolic cAMP level during the aggregation and the mound stage, we demonstrate that the periodicity of the wave propagation switches from slow (6-9 min) to fast (3-4 min) oscillations and that this transition coincides with the onset of directional cell migration. In the deletion mutants of the cell adhesion molecules tgrB1 and tgrC1, we found that the fast frequency cAMP wave propagation takes place for an extended period of time and that the waves become unstable. This instability causes random appearance and disappearance of the signaling centers in a mound and results in the well-known dispersal of cells. Our observations indicate that wave frequency provides a signal for the onset of aggregation and that the cell-cell contact effectively acts to stabilize the frequency of wave propagation. We will discuss how this behavior can be explained from the cAMP-relay response at the single-cell level.

**Abstract type:** Poster

**Title:** Functional analysis of Rpb4, a subunit of RNA Pol II, in *Dictyostelium discoideum*

**Authors:** Aruna Naorem, Parag P. Sadhale

**Presenter:** Aruna Naorem

**Address for correspondence:** Department of Genetics,Bachhawat Block Basement, University of Delhi South Campus,Benito Juarez Road, New Delhi-110021

**Abstract:** Few genes of a particular genome are expressed at any given time in a cell and these expressed genes, in turn, provide specific character to the cell. This control of gene expression occurs at multiple levels targeting general transcription factors, gene-specific transcription factors and RNA polymerase II (RNA Pol II), the transcription machinery. Among the 12 subunits in yeast RNA Pol II, the dissociable Rpb4/Rpb7 subcomplex is an interesting to investigate as they play important regulatory role during stress response besides transcription. In *S. cerevisiae* and *H. sapiens*, function of Rpb4 is required for proper functioning of its interacting partner, Rpb7. Thus, study of Rpb4, RNA polymerase II subunit, is of special interest in understanding the protein network of gene expression during cell differentiation and development. Being one of the simplest organisms to show differentiation and development in response to starvation, study of *D. discoideum* Rpb4 was initiated to understand its function during growth and development. Computational studies showed sequence conservation of Rpb4 with known homologs in *S. cerevisiae* and *H. sapiens*. In consistent with the computational studies, genetic complementation in *S. cerevisiae* and biochemical studies showed functional conservation with known homologs as well as distinct features which were not reported before. The expression profiles of Rpb4 were found to be regulated temporally and spatially during *D. discoideum* development. In addition, overexpression studies showed the existence of regulatory mechanism operating in cell to regulate the Rpb4 protein level in cell. Results of these genetic and biochemical studies carried out to understand the function of Rpb4 in growth and starvation-induced development of *D. discoideum* will be presented. The poster will also feature the work to be initiated in the laboratory on transcription factors and other associated factors of transcription machinery in order to understand the network of proteins involved in regulation of transcription during growth and development of *D. discoideum*.

**Abstract type:** Poster

**Title:** The roles of two *Dictyostelium* protein homologues in cell-type proportioning

**Authors:** E. M. Frick, K. J. McGough, C. L. DiNinno, R. J. Beichner, and M. K. Nelson

**Presenter:** Margaret Nelson

**Address for correspondence:** Department of Biology, Allegheny College, Meadville, PA 16335 USA

**Abstract:** FbiA is an evolutionarily-conserved protein identified via its interaction with the WD-40 repeat region of FbxA, a component of an SCF E3 ubiquitin ligase. This interaction, as well as the phenotypes of null mutants, suggests that FbxA-mediated ubiquitination of FbiA plays a role in cell-type proportioning. The C-terminal region of FbiA is homologous to proteins in humans, mice, *Drosophila*, *C. elegans*, *Arabidopsis*, *S. pombe*, *S. cerevisiae*, *N. crassa*, and *P. falciparum*. The function of these FbiA homologues is, however, unknown. Hence, an understanding of FbiA's role in *Dictyostelium* development may shed light on the function of this evolutionarily conserved protein family. We have previously reported alternations in the prestalkO and prespore populations (increased and decreased, respectively) in the null mutant. Since such a proportioning shift could arise from an alteration in DIF production or response, which, in the case of mutants such as *stlB*$^-$ and *dimB*$^-$, alters the pstB-lacZ staining pattern and the size of the basal disc and lower cup, we have investigated these populations in the *fbiA*$^-$ mutant. We also report our initial characterization of the *fbiB*$^-$ mutant. The FbiB protein is the closest FbiA homologue in *Dictyostelium*; in a T-Coffee alignment the C-terminal ~300 amino acids of each protein are 32% identical and 47% similar. DictyExpress data indicate both a similar developmental expression profile and comparable enrichment in the prespore population. We therefore sought to determine to what extent the two mutants would phenocopy one another.

**Abstract type:** Poster

**Title:** EGF-like repeat function in regular and inhibited folic acid-mediated chemotaxis in axenically and bacterially-grown *D. discoideum*

**Authors:** Ina Nikolaeva [2], Robert J. Huber [1] and Danton H. O'Day [1,2]

[1] Department of Cell and Systems Biology, University of Toronto, Toronto, Ontario, Canada; [2] Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada

**Presenter:** Ina Nikolaeva

**Address for correspondence:** Robert J. Huber University of Toronto Mississauga Department of Biology 3359 Mississauga Road North Mississauga, Ontario L5L 1C6 Canada

**Abstract:** Epidermal Growth Factor-like (EGFL) repeats have been shown to regulate cell motility, adhesion and proliferation in mammalian cells. Recent research on cyrA, a calmodulin-binding, extracellular matrix protein from *Dictyostelium discoideum* that is secreted during development contains 4 such repeats (EGFL1-4) (Suarez et al, 2011). A synthetic peptide (DdEGFL1) equivalent to the first 18 amino acids of EGFL1 has previously been shown to enhance random cell motility and cAMP chemotaxis (Huber and O'Day, 2009). Here, we used a radial bioassay to evaluate the effects of DdEGFL1 on folic acid (FA)-mediated chemotaxis during *D. discoideum*'s vegetative stage and to compare the responses of axenically and bacterially-grown cells. Unlike cAMP chemotaxis, DdEGFL1 did not significantly enhance FA chemotaxis in bacterially or axenically grown cells. In both axenically and bacterially grown cells, W-7 (calmodulin antagonist) and Genistein (tyrosine kinase inhibitor) significantly inhibit folic acid chemotaxis, as reported previously (Gauthier and O'Day, 2001, Browning et al, 1995). DdEGFL1 abrogated the inhibitory effect of Genistein on folic acid chemotaxis for both types of cells. It also rescued the inhibition of W-7 on FA chemotaxis, but for axenically grown cells only. The significance of these and other results on the effects of DdEGFL1 on FA chemotaxis will be discussed.

**Abstract type:** Poster

**Title:** Novel interactors of *Dictyostelium* RapA

**Authors:** K.M. Plak, A. Kortholt, P.J.M. Van Haastert

**Presenter:** K.M. Plak

**Address for correspondence:** k.m.plak@rug.nl

**Abstract:** RapA is one of many small GTPase family proteins found in *Dictyostelium* genome. It is known to have a function in divergent cellular processes like cell adhesion, phagocytosis and chemotaxis. It is also suspected to be an essential gene as many attempts to create a stable RapA-null cell line have failed. In this study we used *E. coli* purified GST fusion RapA protein as a bait in a pull down experiment with cell lysate of vegetative *Dictyostelium* wild type cells, aiming at identifying new RapA interaction partners. Potential interacting proteins were further identified using LC-MS. Among the identified proteins we found those that are involved in cellular processes like cell division, cellular adhesion, regulation of cytoskeletal formation and chemotaxis. True nature of the interaction was further confirmed by direct binding assays and *in vivo* studies.

**Abstract type:** Poster

**Title:** Colchicine affects cell motility, pattern formation and stalk cell differentiation in *Dictyostelium* by altering calcium signaling

**Authors:** Yekaterina Poloz and Danton H. O'Day

**Presenter:** Yekaterina Poloz

**Address for correspondence:** Department of Biology, University of Toronto Mississauga, 3359 Mississauga Road, Mississauga ON L5L 1C6, CANADA e-mail: danton.oday@utoronto.ca

**Abstract:** Previous work, verified here, showed that colchicine affects *Dictyostelium* pattern and polarity, disrupts morphogenesis at multicellular stages, inhibits spore differentiation and induces terminal stalk cell differentiation. Here we show that colchicine specifically induces *ecmB* gene expression and enhances accumulation of ecmB-expressing cells at the posterior end of multicellular structures. It also inhibits *ecmA*, *cotB* and *spiA* gene expression while altering the pattern of expression of *ecmA*, *ecmO* and *cotB* in multicellular stages. Unlike differentiation inducing factor 1 (DIF-1), a stalk cell morphogen, colchicine did not induce stalk cell differentiation *in vitro*, suggesting colchicine requires multicellularity to exert its effects. Also, colchicine induced terminal stalk cell differentiation in a mutant strain that does not produce DIF-1 (*dmtA*$^-$) and after the treatment of cells with DIF-1 synthesis inhibitor cerulenin (100 μM). This suggests that colchicine induces the differentiation of ecmB-expressing cells that are not regulated by DIF-1. Depending on concentration, colchicine enhanced random cell motility, but not chemotaxis, by 3-5 fold (10-50 mM colchicine, respectively) through a calcium-mediated signaling pathway involving phospholipase C, calmodulin and heterotrimeric G proteins. Colchicine's effects were not due to microtubule depolymerization as other microtubule-depolymerizing agents did not have these effects. Finally normal morphogenesis and stalk and spore cell differentiation of cell treated with 10 mM colchicine were rescued through chelation of calcium by BAPTA-AM and EDTA and calmodulin antagonism by W-7 but not PLC inhibition by U-73122. Morphogenesis or spore cell differentiation of cells treated with 50 mM colchicine could not be rescued by the above treatments but terminal stalk cell differentiation was inhibited by BAPTA-AM, EDTA and W-7, but not U-73122. Thus colchicine disrupts morphogenesis, inhibits spore cell differentiation and induces stalk cell differentiation through a calcium-mediated signaling pathway involving specific changes in gene expression and cell motility. Based on these results, colchicine could be used as a tool to study the differentiation of ecmB-expressing cells that are not regulated by DIF-1.

**Abstract type:** Poster

**Title:** Cysteine proteinase associated to secreted multivesicular bodies produced by *Dictyostelium discoideum*

**Authors:** A., SEDIGHI [1,2], V.E., PAQUET [1,2], G., FILION [1,2], S.J., CHARETTE [1,2]

**Presenter:** AHMADREZA SEDIGHI

**Address for correspondence:** [1]Institut de Biologie Intégrative et des Systèmes (IBIS), Université Laval; [2]Centre de Recherche de l'Institut Universitaire de Cardiologie et de Pneumologie de Québec (CRIUCPQ)

**Abstract:** *Dictyostelium discoideum* produces abundant multivesicular bodies when grown in presence of bacteria, which are used as food by the amoeba. Previous results demonstrated that these multivesicular bodies (MVB) are exocytosed in the medium when cells are starved. Here we show that these structures are in fact continually secreted by amoeba when grown on a bacterial lawn. To understand the role of these secreted MVB in the physiology of *D. discoideum*, we used different antibodies against amoeba's membrane-associated proteins to identify at least one present on these structures. Immunofluorescences have been done using H72, H161 and H36 antibodies. Immunoprecipitation was performed with H36 antibody and two different approaches using either protein A sepharose and protein G sephorose on cell lysate. Proteins were separated by SDS-PAGE. The band corresponding to the protein specifically immunoprecipitated by the antibody was excised and identified by mass spectrometry. The antigen recognized by H36 antibody is present on secreted MVB but it was not the case for antigens recognized by H72 and H161 antibodies. The identity of the H36 antigen needed to be determined. The analysis of the mass spectrometry results revealed that the protein immunoprecipitated by H36 antibody is the Cysteine Proteinase 7 (cprG). It is a glycoprotein of 47 kDa, usually associated to the lysosomes of the phagocytic pathway. This protein also has a signal peptide and quaternary structure involving disulfide bridges. The identification of the H36 antigen was confirmed by western blot analyses where an antibody specific to cprG was used in parallel with H36 antibody. It appeared that both antibodies recognized the same specific bands. This study demonstrated that secreted MVB contain an amoebal protease (cprG). This is the first description of the association of a cysteine proteinase with secreted membrane structures in *D. discoideum*.

**Abstract type:** Poster

**Title:** Modeling cell migration using the LEGI-BEN model

**Authors:** Changji Shi[1], Flori Yellin[1], Peter N. Devreotes[2], Pablo A. Iglesias[1]

**Presenter:** Changji Shi

**Address for correspondence:** [1]Department of Electrical & Computer Engineering, The Johns Hopkins University, 3400 N. Charles Street, Baltimore, MD 21218; [2]Department of Cell Biology, Johns Hopkins School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205. shichangji@gmail.com

**Abstract:** The chemotactic response of *Dictyostelium* cells can be conceptually divided into three separate processes: gradient sensing, motility and polarization. Recently, we put forward a theoretical model, the *local-excitation, global-inhibition-biased excitable network* (LEGI-BEN), that explains how cells that migrate randomly can be steered by the gradient sensing mechanism in the direction of a cAMP gradient. In the absence of an external chemoattractant stimulus, the LEGI-BEN model recreates spontaneous regions of high signaling around the cell perimeter. When stimulated by a spatially uniform dose of chemoattractant, the model generates a uniform initial high level of activity, followed by smaller, more localized second peaks of activity. In a gradient, the model directs the activity towards the side of the cell with highest chemoattractant concentration. Here we test how the LEGI-BEN model drives cell shape changes during chemotaxis. To simulate changes in morphology, we use a viscoelastic model of *Dictyostelium* cells using a level set framework. We assume that the patches of activity generate force, for example, through actin polymerization. We then model both the migration of cells, both in the absence and presence of chemoattractant gradients. We also consider the effects of complex stimuli, such as changes in the location of a gradient. Our results faithfully recreate many aspects of the chemotactic behavior of cells.

**Abstract type:** Poster

**Title:** The role of actin filaments in mechanosensing

**Authors:** Vasudha Srivastava, Tianzhi Luo, Douglas N. Robinson

**Presenter:** Vasudha Srivastava

**Address for correspondence:** Department of Cell Biology, Johns Hopkins School of Medicine, 725 N. Wolfe St., Baltimore, MD 21205

**Abstract:** Cells can sense and respond to mechanical stresses during many cellular processes such as cytokinesis and tissue morphogenesis. In *Dictyostelium discoideum*, the mechanoenzyme myosin II and actin crosslinker cortexillin I form the core of a mechanosensory system. The active role of the actin filaments, through which these proteins interact, is less characterized. Recently, we have developed a computational model for mechanosensing that couples cooperative interactions between myosin II and cortexillin I with myosin bipolar thick filament assembly dynamics to predict stress-derived accumulation of these proteins. The simulations predict that F-actin concentration is a critical parameter in affecting the assembly rate of myosin thick filaments. To experimentally characterize the sensitivity of mechanosensory response to F-actin amounts, we use F-actin affecting drugs latrunculin-A and jasplakinolide to alter cellular levels of filamentous actin, which significantly alter the cellular F-actin level over a 3-fold range while having little impact on actin turnover rates. We are currently testing the effect of these compounds on the myosin II accumulation at the deformation site during micropipette aspiration. This study will reveal the role of actin filaments in mechanosensing.

**Abstract type:** Poster

**Title:** Time-Lapse Microscopy Reveals the Dynamic Behavior of *D. discoideum* Mitochondria

**Authors:** Sarah Stark and Kari Naylor

**Presenter:** Sarah Stark

**Address for correspondence:** Kari Naylor University of Central Arkansas 201 Donaghey 180 Lewis Science Center/Biology Dept Conway AR 72035

**Abstract:** The powerhouses of the cell, mitochondria, are extremely dynamic. Imaging of these organelles in yeast and mammalian cells demonstrates that the steady state tubular structure undergoes numerous fission and fusion events. The balance of these events ensures that the mitochondria can exchange vital components, as well as remain distributed throughout the cell. Mitochondrial dynamics have been studied in many cell types, and thus far all the mitochondria studied have this tubular, highly branched structure. Interestingly, *Dictyostelium discoideum* has spherical mitochondria. Our goal is to determine if these spherical mitochondria also undergo fission and fusion events. Through our analysis of time-lapse confocal images we found that *D. discoideum* mitochondria do indeed undergo both fission and fusion events. Quantification of these rates indicates that these events are balanced and occur roughly once per minute. In an effort to understand the molecular fission mechanism in *D. discoideum*, we have begun to identify the proteins involved in this process, by analyzing mitochondrial morphology mutants. We have identified several proteins that play a role in mitochondrial structure and are currently quantifying the rate of fission and fusion in these mutant strains. By analyzing these events in live *D. discoideum* cells, we will further our understanding of the mechanism regulating fission in this organism. Ultimately, we hope to gain insight into mitochondrial dynamics of all eukaryotes, as well as increase our understanding of mitochondrial evolution.

**Abstract type:** Poster

**Title:** Mass-spectroscopic analysis of autophosphorylation sites in *Dictyostelium discoideum* Myosin Heavy Chain Kinase A

**Authors:** Vandana Rai, Thomas Egelhoff, Paul Steimle

**Presenter:** Paul Steimle

**Address for correspondence:** University of North Carolina at Greensboro, Department of Biology, 321 McIver St., Greensboro, NC 27402

**Abstract:** *Dictyostelium* myosin heavy chain kinase A (MHCK-A) catalyzes the disassembly (and inactivation) of myosin II by catalyzing the phosphorylation of three specific threonine residues in the “tail” region of the myosin II heavy chain. Studies of purified MHCK-A have revealed that the kinase is activated up to 50-fold by an autophosphorylation reaction that results in the incorporation of up to 10 moles of phosphate/mole of kinase. Results presented here demonstrate that MHCK-A autophosphorylation is detectable by SDS-PAGE/Western blotting as a shift in molecular mass from 130 kDa to about 140 kDa. Lysates of growth-phase cells contain about 4% of the total MHCK-A in the 140 kDa (autophosphorylated) form. As cells enter into the developmental cycle the proportion of MHCK-A in the autophosphorylated form increases; representing about 25% of the total MHCK-A by 12 hours of development, a period during which cells are undergoing chemotaxis and aggregation. To understand the mechanism by which autophosphorylation activates MHCK A, and the *in vivo* roles of this autophosphorylation, we performed mass spectroscopy studies to map the autophosphorylation sites. MS analysis of purified MHCK-A revealed eleven sites that were phosphorylated and of these, only three became phosphorylated (T164, T513, T634) with increasing time of autophosphorylation; the other sites were already phosphorylated at the time of purification. We generated individual T->A and T->D mutant versions of T164, T513, and T634 and then analyzed cells over-expressing the mutant proteins for defects in cytokinesis and development. In short, the cellular activities of the T164 and T513 mutants, were enhanced in by the T->D conversion and reduced upon replacing the T->A. Biochemical analysis and further *in vivo* studies are in progress to assess the behavior of these mutant kinase constructs.

**Abstract type:** Poster

**Title:** Spatio-temporal Expression of the Heterophilic Pair of Adhesion Partners TgrB1-TgrC1 Defines Two Sub-types of Cells in the Mound Structure during Development of *Dictyostelium discoideum*

**Authors:** Alexander Thomson, Jun Wang, Gong Chen, and Chi-Hung Siu

**Presenter:** Alex Thomson

**Address for correspondence:** Department of Biochemistry and the Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada

**Abstract:** During development of the social amoeba *Dictyostelium discoideum*, cell sorting and morphogenetic movement are regulated by several cell adhesion systems. In the post-aggregation phase, multicellularity is maintained primarily by a pair of heterophilic binding proteins, TgrB1 and TgrC1. To investigate the spatio-temporal expression patterns of TgrB1 and TgrC1, morphological structures were collected from different developmental stages, fixed and immunostained for confocal microscopy. Intense staining of TgrC1 was observed in the peripheral layer of cell aggregates, coincident with extracellular matrix components, indicating that TgrC1-positive cells are actively involved in the synthesis of the slime sheath. When the common promoter shared by these two genes was fused to either TgrB1-RFP or TgrC1-GFP for ectopic expression in cells, low levels of these proteins were first detected during cell streaming while strong expression was observed in loose mounds. TgrB1 and TgrC1 express at high levels among cells that arrive at the aggregate last. These cells then move from the basal region upward to form an outer layer of TgrB1/TgrC1-positive cells. Three-dimensional structures reconstructed from confocal images reveal a one-cell layer of TgrB1/C1-expressing cells, thus defining an epithelium-like structure which envelopes the inner cell mass. Soon afterwards, cells expressing high levels of TgrB1 and TgrC1 begin to appear in the apex of mounds, defining the “tip” structure which adopts a cylindrical shape. Serial confocal images show that these cells migrate from the mound periphery to the emerging tip. Recent studies also show that cells in the tip region also express an abundance of DdCAD-1 in the cell-cell contact regions, suggesting that both DdCAD-1 and TgrB1/TgrC1 play an important role in the morphogenesis of the tip structure.

(Supported by the Canadian Institutes of Health Research.)

**Abstract type:** Poster

**Title:** Analysis of *in vivo* consequences of a disease-relevant point mutation in p97/VCP

**Authors:** Sze Man Tung[1], Khalid Arhzaouy[1], Karl-Heinz Strucksberg[2], Karthikeyan Tangavelou[1], Maria Stumpf[1], Rolf Schröder[2], Christoph S. Clemen[1] and Ludwig Eichinger[1]

**Presenter:** Sze Man Tung

**Address for correspondence:** [1]Institute of Biochemistry I, Medical Faculty, University of Cologne, 50931 Cologne, Germany, ludwig.eichinger@uni-koeln.de ; [2]Institute of Neuropathology, University Hospital Erlangen, Erlangen, Germany

**Abstract:** p97 (VCP or valosin containing protein in mammals and Cdc48p in yeast) is a ubiquitously expressed and evolutionarily highly conserved hexameric member of the magnesium-dependent Walker P-loop AAA-ATPases. p97 has been associated with various essential cellular processes including ubiquitin-proteasome protein degradation. Point mutations in the human *p97* gene on chromosome 9p13.3 cause a late-onset form of autosomal dominant Inclusion Body Myopathy with early-onset Paget disease and Frontotemporal Dementia or IBMPFD. Most prevalent with 80% of the cases is the R155C mutation. We study the function of p97 in *Dictyostelium* an established model for autophagic processes. *D. discoideum* strains that ectopically express mutant p97R155C fused to RFP in AX2 wild-type (K4) and autophagy 9 (ATG9) knock-out (A6) cells have been generated. The former strain mimics the situation in heterozygous patients while ATG9 knock-out cells allow the investigation of the point mutation in the background of macroautophagy deficiency (Tung *et al.*, 2010). Co-immunoprecipitation studies using RFP and p97 antibodies showed that endogenous p97 and the p97R155C-RFP mutant associate with one another. All three mutant strains display a clear growth defect in shaking culture with the A6 mutant showing the strongest defect. Immunofluorescence analysis revealed that p97 containing aggregates co-localize with ubiquitin in ATG9 knock-out cells but not in aggregates of strains that co-express p97R155C. We also detected an increase in the amount of ubiquitinated proteins as well as autophagy proteins ATG8 and ATG9 in all mutant strains.

References: Tung, S. M., Unal, C., Ley A., *et al.*, (2010). Cell. Microbiol. 12, 765-780.

**Abstract type:** Poster

**Title:** Live-cell-imaging of fruiting body formation

**Authors:** Toru Uchikawa, Masahiro Ueda and Kei Inouye

**Presenter:** Toru Uchikawa

**Address for correspondence:** Laboratories for Nanobiology, Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan

**Abstract:** Over the course of fruiting body formation, prestalk cells in its apex consecutively enter the stalk tube and then differentiate into mature stalk cells while the stalk tube is elongated vertically. Prespore cells climb the stalk, following the prestalk cells, and differentiate into spores within a short period. This picture of fruiting body formation is, however, mostly based on histological observations of fixed samples, and the behavior of individual cells and changes in cellular structure in living tissues have not been investigated. To analyze the behavior and structural changes of individual cells during fruiting body formation, we continuously observed immature fruiting bodies under confocal microscope using transformants in which the cytosol, F-actin, or nuclear is visualized by GFP-fusion proteins. The main results can be summarized as follows. (1) Prestalk cells in the apical region of a fruiting body have numerous large vesicles. (2) A small fraction of apical prestalk cells became less motile, left behind, and consequently trapped in the stalk tube. (3) In the stalk tube, prestalk cells developed a single large vacuole and a cell wall to become mature stalk cells. We will discuss a possible mechanism for the formation of a continuous stalk based on these and published observations.

**Abstract type:** Poster

**Title:** Are aquaporin water channels required for cell migration?

**Authors:** von Bülow, J., Beitz, E.

**Presenter:** Julia von Bülow

**Address for correspondence:** Julia von Bülow, University of Kiel, Gutenbergstr. 76, 24118 Kiel, Germany

**Abstract:** Cell migration contributes to several important physiological processes, such as embryogenesis, wound healing and immune response as well as to pathophysiological processes, including osteoporosis, mental retardation and cancer [1]. There is evidence that water influx into cells is required for the formation of lamellipodia, *i.e*. a prerequisite of motility. Aquaporin (AQP)-dependent cell migration has been described on the phenotypical level, *e.g*. deletion of AQP1 reduces endothelial cell migration, limits tumor angiogenesis and growth likewise, AQP4 deletion slows asterocyte migration [2]. To elucidate the role of aquaporins in cell migration on the molecular level we set up a model using *Dictyostelium discoideum*. The slime mould has a cellular organization typical of higher eukaryotes. As the entire 34 Mb genome has been sequenced, molecular genetic tools are easily applicable, such as PCR amplifications and gene replacements. We identified, cloned and functionally characterized the first aquaporin (DdAQP) in *Dictyostelium* amoeboid single cells. To our surprise, expression of DdAQP in *Xenopus laevis* oocytes produced a non-functional channel. Closer inspection of the protein sequence revealed an unusually long intracellular loop D. Deletion of 12 amino acids from loop D yielded water permeability 5-fold above background. The presence of multiple putative phosphorylation sites in this region hints at channel gating. Regulated opening of DdAQP at the cell front and closing at the flanks may allow for directed cell migration. We will investigate this hypothesis by targeted disruption of the DdAQP gene and cell motiliy assays.

[1] Ridley, A.J., Schwartz, M.A., Burridge, K., Firtel, R.A., Ginsberg, M.H., Borisy, G., Parsons, J.T., Horwitz, A.R. Science 2003
[2] Saadoun, S., Papadopoulos, M.C., Hara-Chikuma, M., Verkman, A.S. Nature 2005

**Abstract type:** Poster

**Title:** *DICTYOSTELIUM* HUNTINGTIN CONTROLS CHEMOTAXIS AND CYTOKINESIS THROUGH THE REGULATION OF MYOSIN II PHOSPHORYLAION

**Authors:** Yu Wang, Paul A. Steimle, Yixin Ren, Christopher A. Ross, Douglas N. Robinson, Thomas T. Egelhoff, Hiromi Sesaki, and Miho Iijima

**Presenter:** YU WANG

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

**Abstract:** Abnormalities in the huntingtin protein (Htt) are associated with Huntington's disease, yet despite its importance, the function of Htt is largely unknown. Here, we show that Htt is required for normal chemotaxis and cytokinesis in *Dictyostelium discoideum*. Cells lacking Htt showed slower migration towards the chemoattractant cAMP and contained lower levels of cortical myosin II, likely due to defects in its dephosphorylation mediated by protein phosphatase 2A (PP2A). *htt*$^-$ cells also failed to maintain myosin II in the cortex of the cleavage furrow, generating unseparated daughter cells connected through a thin cytoplasmic bridge. Furthermore, similar to *Dictyostelium htt*$^-$ cells, siRNA-mediated knockdown of human HTT also decreased the PP2A activity in HeLa cells. Our data indicate that Htt regulates the phosphorylation status of myosin II during chemotaxis and cytokinesis through PP2A.

**Abstract type:** Poster

**Title:** Five species of social amoebae display qualitatively unique N-glycomes that may support developmental autonomy in soil environments

**Authors:** Christa L. Feasley, Jennifer M. Johnson, Christopher M. West

**Presenter:** Chris West

**Address for correspondence:** Dept. of Biochemistry & Molecular Biology, University of Oklahoma Health Sciences Center, 975 NE 10th St., BRC 417, Oklahoma City, OK 73104 USA

**Abstract:** Cellular slime molds (CSMs) exist as free amoebae in overlapping subterranean environments near the soil surface. Despite similar life-styles, individual species form independent starvation-induced fruiting bodies whose spores can renew the life cycle. We have hypothesized that a glycan shroud associated with the cell surface glycocalyx contributes to intercellular recognition and avoidance. N-glycans from five CSM species whose genomes have been sequenced were generated by pepsin digestion followed by PNGase A, fractionated into neutral and acidic pools by anion exchange, and profiled by MALDI-TOF-MS. As reported recently by the Iain Wilson group and us, growing amoebae of *Dictyostelium discoideum* (*Dd*) express modestly trimmed high mannose N-glycans that can be peripherally decorated with 1-2 GlcNAc's and 1-2 fucose's, as well as 1-2 methylphosphates and/or sulfates. Comparative analyses of *D. purpureum*, *D. fasciculatum*, *Polysphondylium pallidum*, and *Actyostelium subglobosum* reveal that each CSM displays a characteristic signature of high-mannose species with quantitative and qualitative differences relative to *Dd*. Differences include reduction or absence of peripheral GlcNAc and core fucose, increased fucosylation, increased Man-trimming, novel mannose methylation, and increased or decreased anionic modifications. Starvation-induced developmental changes include further mannose-trimming, increased fucosylation, and decreased abundance of methylphosphates and sulfates. Glycan structure models derived from the MALDI-TOF-MS analyses were tested using linkage specific antibodies, exoglycosidase digestions, MS/MS, and chromatography studies. Bioinformatics analyses point to the identities of genes responsible for mediating peripheral GlcNAc and core-fucose additions, which will enable future reverse genetic studies to eliminate N-glycomic differences to test their functions in interspecific relations.

**Abstract type:** Poster

**Title:** γ-aminobutyric acid (GABA) regulates the process of aggregation during the early development of *Dictyostelium discoideum*

**Authors:** Yuantai Wu, Chris Janetopoulos

**Presenter:** Yuantai Wu

**Address for correspondence:** Department of Biological Sciences, Vanderbilt University, Nashville TN 37232

**Abstract:** γ-aminobutyric acid (GABA) has been intensively studied as a neurotransmitter in mammals, whereas it is recognized merely as a metabolite in protozoan. The function of GABA in the social amoeba *Dictyostelium discoideum* was investigated and it was revealed that GABA is utilized as a signaling molecule in the early development of this simple pre-metazoan organism. Similar to mammals, two glutamate decarboxylases (GAD) exist in *Dictyostelium*. Based on previous mRNA expression profile and measurements of extracellular GABA using HPLC in different gad mutants, it was determined that GABA is generated and secreted in both vegetative and developmental stages. During vegetative growth and in the early developmental stage, gadB is the major generator of GABA. *gadB* null mutant exhibited delayed aggregation during early development, suggesting GABA is involved in early developmental events. Furthermore, a GABAB receptor like protein grlB and [3H]GABA binding assay showed that grlB is the major GABA receptor in vegetative cells in *Dictyostelium*. *grlB* null mutant also exhibited delayed aggregation, and showed weak chemotactic capacity toward cAMP during aggregation. Western blot analysis revealed that cAMP receptor cAR1 expression is delayed in the *grlB* null mutant. In accordance with delayed aggregation, the expression of adhesion molecule contact site A (csA) was also delayed. Moreover, discoidin I expression was altered in *grlB* nulls, indicating disruption of *grlB* abolished the pre-starvation response. Altogether, these results suggest GABA is an important signaling molecule in *Dictyostelium*, and this may help us to understand the origins and evolution of GABA signaling mechanism.

**Abstract type:** Poster