Session I – Chemotactic Signaling I

Memory of Motility in Chemotaxis

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Dictyostelium cells migrate chemotactically up cAMP gradients when the concentration is increasing but ignore cAMP gradients when the concentration is decreasing. The direction of movement is maintained for several minutes in the back of a travelling wave although the direction of increasing cAMP is reversed as the concentration decreases. The direction of movement is determined by accumulation of Ras-GTP in a patch at the anterior as the result of an incoherent feedforward loop initiated by cAMP bound to CAR1 receptors that activates the opposing functions RasGEF and RasGAP (Takeda et al., 2012). GEF activity is greater than GAP activity at the anterior because the concentration of external cAMP is slightly higher than at the back and diffusion of GEF is limited by being membrane bound while GAP diffuses freely in the cytoplasm and is the same at the front and the back. The activation/ inactivation kinetics of GEF are faster than those of GAP and result in ultrasensitivity.

When the concentration of cAMP is decreasing in the back of the wave, the cells do not respond chemotactically because GAP is inactivated more slowly than GEF and the Ras-GTP patch disappears. Ultrasensitivity of the GEF/GAP opposing activities not only amplifies the cAMP spatial signal but also produces a temporal response to cAMP concentrations. Cells that have developed for at least 5 hrs not only ignore the reversed gradient of cAMP in the back of the wave but continue for a few minutes after the peak has passed in the same direction they had been going (Skoge et al., 2014). This memory of past motility is impaired when the TORC2-PKB R1 pathway is inhibited with caffeine. Likewise, memory is lost in mutants cells lacking essential components of this pathway, PiaA or PKB R1. While both PkbA and PkbR1 protein kinases activate downstream kinases that stimulate pseudopod extension, PkbR1 appears several hours later in development than PkbA and is somewhat more stable. It may be advantageous for cells to be able to immediately change their direction of movement between waves for the first few hours of aggregation and be more persistent later in development when centers are well established.

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Pulling-down the chemotaxis pathways

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Central to chemotaxis is the molecular mechanism by which a shallow spatial gradient of chemoattractant induces symmetry breaking of activated signaling molecules. Previously we have identified a basal signaling module in *Dictyostelium*, consisting of heterotrimeric and small G-proteins, that is sufficient to induce symmetry breaking, actin polymerization at the leading edge, and chemotaxis[1]. Here we have used an extensive and efficient proteomic approach to identify further components of this basal signaling pathway that collectively form the dynamic signaling network for chemotaxis. We have used these proteomic data to get more insight in the mechanism by which: (1) the heterotrimeric G protein cycle is regulated[2] (2) heterotrimeric G proteins induce symmetry breaking in small G protein signaling[3], and (3) symmetry breaking in G protein signaling induces cytoskeleton rearrangements and subsequently cell migration.

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GPCR-controlled membrane recruitment of C2GAP1 locally inhibits Ras signaling for adaptation and long-range chemotaxis

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Eukaryotic cells chemotax in a wide range of chemoattractant concentration gradients, and thus need inhibitory processes that terminate cell responses to reach adaptation while maintaining sensitivity to higher-concentration stimuli. However, the molecular mechanisms underlying inhibitory processes are still poorly understood. Here, we reveal a locally controlled inhibitory process in a GPCR-mediated signaling network for chemotaxis in *Dictyostelium discoideum*. We discover a novel negative regulator of Ras signaling, C2GAP1, which localizes at the leading edge of chemotaxing cells and is essential for chemoattractant cAMP-mediated Ras adaptation. The altered Ras activation results in impaired gradient sensing and excessive polymerization of F-actin in *c2gap1* knockout (*c2gap1*$^{-}$) cells, leading to chemotaxis defects. Remarkably, *c2gap1*$^{-}$ cells display altered cell response, impaired directional sensing, and chemotaxis defects in a chemoattractant concentration-dependent fashion. We show that both C2 and GAP domains are required for the membrane translocation of C2GAP1, and GPCR-triggered Ras activation recruits C2GAP1 from cytosol and retains it on the membrane to locally inhibit Ras signaling. Thus, we have uncovered a novel inhibitory mechanism required for cells to chemotax in a large range of chemoattractant concentrations.

GPCR-mediated imperfect adaption of Ras signaling guides chemotactic responses to a large range of chemoattractant concentrations

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A GPCR-mediated signaling network enables a chemotactic cell to generate adaptation responses to a large range of concentrations of a chemoattractant and polarized responses to a chemoattractant gradient. It is not clear how the signaling network mechanistically organizes at the molecular level, dynamically encodes information at each signaling step, and systematically produces these outputs during chemotaxis. Here, we applied a systems biology approach to investigate GPCR cAR1-mediated chemotactic signaling network in *D. discoideum*. Chemoattractant cAMP binding to cAR1 activates heterotrimeric G-proteins and Ras signaling, which regulate chemotaxis of *D. discoideum* cells. Using live cell imaging, we found that each signaling event has distinct kinetic patterns in response to stimuli. Activation of cAR1 induces a persistent G-protein activation and an imperfectly adapted Ras activation. Using these dynamics as the foundation, we constructed mechanistic diffusion models of cAR1-mediated Ras signaling network by incorporating potential molecular mechanisms of an activator (RasGEF) and an inhibitor (RasGAP), and simulated spatiotemporal dynamics of signaling events to computationally explore functions of RasGEF and RasGAP in generating Ras adaptation. Our computer simulations showed that a cAMP gradient induces imperfect Ras adaption around the cell surface, and the modeled cell is unable to produce significant spatial amplification at the Ras activation step. Together, our study showed that adaption models require additional mechanisms for a signaling network to amplify directional cues at the steps of G-protein activation and Ras activation into highly polarized responses of PIP_3 production and actin polymerization for chemotaxis. Our model construction and computer simulation approach can help to identify missing components that are required for adaptation and amplification responses in the GPCR-mediated signaling network for eukaryotic chemotaxis.

Session II – Mitochondrial Function

Does Parkinson's Disease really involve pathologically impaired mitochondrial function?

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Impaired mitochondrial respiratory function is believed to contribute to the cytopathology of Parkinson's Disease (PD). The main evidence for this is 3-fold: 1) Pharmacological or genetic damage to mitochondrial respiratory function (notably to Complex I) can cause PD 2) Post-mortem PD brains exhibit mitochondrial pathology with Complex I defects and 3) Reactive Oxygen Species production is elevated in various types of cell from living PD patients. However, there are alternative explanations for each of these types of evidence, while direct measurements of mitochondrial function in cells from living PD patients have produced contradictory and collectively inconclusive results. We have used Seahorse respirometry to measure mitochondrial respiratory function in *Dictyostelium* mitochondrial and PD mutant cells, as well as in immortalized human lymphocytes (lymphoblasts) from PD patients. *Dictyostelium* mutants lacking MidA/NDUFAF7, a Complex I methylase and assembly factor, exhibited a Complex I-specific defect in respiration. By contrast, exposing wild type *Dictyostelium* cells to oxidative stress (150 mM H_2O_2) caused an elevation of respiration and this was reversed by overexpression of DJ-1. DJ-1 is a PD protein that translocates to the mitochondria under oxidative stress conditions, where it protects cells from the cytopathological consequences of the stress. Mitochondrial respiratory activity was elevated in *Dictyostelium* cells lacking Roco4 (homologue of the human PD protein LRRK2). These results suggest that rather than respiration being impaired in PD, it is elevated. In accordance with this, the mitochondria in lymphoblasts from human PD patients were functionally normal, but hyperactive in respiration. This was accompanied by elevations of steady state ATP levels, ROS production and expression of indicative mitochondrial OXPHOS proteins. However mitochondrial mass, genome copy number and membrane potential were unaltered. Our results suggest a new understanding of the role of mitochondrial respiratory activity and elevated ROS production in PD.

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Determining the immunity functions of reactive oxygen species using *Dictyostelium discoideum*

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Reactive oxygen species (ROS) are key components of the immune response to intracellular pathogens. Deleterious mutations in the ROS---generating phagocyte NADPH oxidase (NOX) underlie chronic granulomatous disease, marked by severe, recurring bacterial and fungal infections. NOX comprises a heterodimer of transmembrane proteins: Nox2, the catalytic subunit, and $p22^{phox}$, which recruits cytosolic proteins required for activation. Vegetative *Dictyostelium* expresses NoxA, CybA, and NcfA, homologs of Nox2, of $p22^{phox}$, and of the NOX activator $p67^{phox}$, respectively. Two additional Nox2 homologs, NoxB and C, contribute to immunity during development.

Using fluorescence---based assays, we have observed that *Dictyostelium* amoebae produce ROS when exposed to bacterial products such as lipopolysaccharide (LPS) and that the rate of ROS production varies based on the type of LPS. ROS production is decreased in NOX---deficient mutants, which also exhibit delayed killing of bacteria after phagocytosis. Fluorescent protein fusions indicate that CybA localizes to the plasma membrane and phagosomes while NcfA is cytosolic and is enriched at macropinocytic and phagocytic cups. Enrichment of NcfA is independent of CybA and NoxA and requires a predicted Rac---binding domain in its N---terminus. These results suggest that Rac activity recruits NcfA to phagocytic/macropinocytic sites to coordinate NOX activation with uptake events.

We are currently engineering wildtype and NOX---deficient *Dictyostelium* strains to express redox---sensitive GFP2 (roGFP2) in the cytosol and targeted to NOX and mitochondria to identify the sources and extent of ROS production during infection. Similarly, we will use wildtype and mutant *Mycobacterium* expressing roGFP2 to track the oxidative stress encountered by bacteria.

The mitochondrial protein import aparatus in Dictyostelia

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The protein import into mitochondria is fundamental for the organelle proper functioning and consequently for eukaryotic cells. The import proceeds due to the presence of different pathways formed by import complexes located in all mitochondrial compartments.The complexes have been also proposed to contain subunits formed by proteins common to all eukaryotes and additional subunits regarded as specific for various eucaryotic lineages. Here we present data concerning the organization of the import complexes of slime molds represented by *Dictyostelium discoideum*, *Dictyostelium fasciculatum*, *Dictyostelium purpureum*, and *Polysphondyllium pallidum.* The data are based on biochemical assays as well as on bioinformatic analysis of available genome and transcriptome sequences. They concern: (i) the outer membrane complexes, namely the TOM complex (translocase of the outer membrane) and the TOB/SAM complex (topogenesis of the mitochondrial outer membrane β-barrel proteins/sorting and assembly machinery); (ii) the intermembrane space complexes, namely the MIA complex (mitochondrial intermembrane space assembly) and complexes of small Tim proteins and (iii) the inner membrane complexes, namely the TIM22 complex (translocase of the inner membrane 22), TIM23 complex (translocase of the inner membrane 23) and the PAM complex (presequence translocase - associated motor). The obtained results indicate differences in the organization of the TOM complex, appearing to be the most diversed. This is reflected by differences in the number of involved subunits and in similarities to the cognate proteins of representatives from different supergroups of eukaryotes. On the other hand, the organization of the other import complexes included most of the canonical subunits.

Session III – Bacterial Interactions

Characterization of a secreted antimicrobial protein in *Dictyostelium*

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We have described genetic and transcriptional evidence indicating that *D. discoideum* discriminates between different species of bacteria and that this is important for the amoeba to respond appropriately for optimal feeding and defense (1). We have continued to explore the genetic basis for bacterial discrimination by *D. discoideum* amoebae and have begun to characterize the proteins that mediate responses to bacteria. We have identified a number of genes and proteins involved in bacterial discrimination using REMI mutagenesis, and mass spectrometry of the *D. discoideum* secretome. We identified a REMI mutation in the *DDB_G0292236* gene that causes a severe growth defect on Gram(-) bacteria and have begun an initial characterization of its gene product. The predicted 38-kDa DDB_G0292236 protein appears to be a novel protein with a signal sequence. Although we have been able to identify the protein in the secretome of growing Ax4 cells by mass spectrometry, immunostaining of vegetative amoebae with anti-peptide polyclonal antibodies directed against DDB_G0292236 suggest that it is associated with the cell surface and cortical puncta. We have been able to purify a His-tagged version of this protein, after expression in insect cells, and this protein displays bactericidal activity against Gram(-) bacteria in an in vitro assay. Thus, DDB_G0292236 appears to represent a part of the complex response of *D. discoideum* to bacteria. Our results so far suggest that DDB_G0292236 is a secreted protein responsible for killing bacteria, possibly before they are ingested by amoebae.

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Chemotaxis to live bacteria

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In the wild, bacteria are one of the primary food sources to support *Dictyostelium* growth. *Dictyostelium* chemotax toward bacteria and eventually phagocytose the bacteria to fulfill nutritional requirements. However, the precision of *Dictyostelium* chemotaxis toward bacteria has not been well studied. We present a quantitative analysis of *Dictyostelium* chemotaxis towards bacteria and for the characterization of bacterially secreted chemoattractants. The present study also analyzes the separate signaling networks required for chemotaxis by growing *Dictyostelium.*

Identification of an orphan G protein-coupled receptor for folic acid that controls both chemotaxis and phagocytosis

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Eukaryotic phagocytes search and destroy invading microorganisms via chemotaxis and phagocytosis. The social amoeba *Dictyostelium discoideum* is a professional phagocyte that chases bacteria through chemotaxis and engulfs them as food via phagocytosis. G-protein-coupled receptors (GPCRs) are known for detecting chemoattractants and directing cell migration, but their roles in phagocytosis are not clear. Here, we developed a quantitative phosphoproteomic technique to discover signaling components. Using this approach, we discovered the long-sought-after folic acid receptor, fAR1, in *D. discoideum*. We showed that the seven transmembrane receptor fAR1 is required for folic acid-mediated signaling events. Significantly, we discovered that fAR1 is essential for both chemotaxis and phagocytosis of bacteria, thereby representing a chemoattractant GPCR that mediates not only chasing but also ingesting bacteria. We revealed that a phagocyte is able to internalize particles via chemoattractant-mediated engulfment process. We propose that mammalian phagocytes may also use this mechanism to engulf and ingest bacterial pathogens.

Session IV – Cytoskeleton I

CpnA Binds to Actin Filaments in a Calcium-dependent Manner *In Vitro* and has a Role in Chemotaxis

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Copines make up a multigene family of calcium-dependent, phospholipid-binding proteins. Copine proteins consist of two C2 domains at the N-terminus followed by an "A domain" similar to the von Willebrand A domain found in integrins. The C2 domain is a calcium-dependent membrane-binding motif, while the A domain is thought to be a protein-binding domain. We are studying copine protein function in the model organism, *Dictyostelium discoideum*, which has six copine genes, *cpnA- cpnF*. Previous research showed that *cpnA−* cells exhibit a cytokinesis defect, a developmental defect, and a defect in contractile vacuole function. To fully understand the role of CpnA in these cellular processes, we used column chromatography and mass spectrometry to identify proteins that interact with CpnA. One of the proteins identified was actin. To determine if CpnA associates with the actin cytoskeleton, we treated cells expressing GFP-CpnA or GFP-Ado (containing the A domain of CpnA) with Triton X-100 and spun down the insoluble cytoskeletal fraction. GFP-CpnA was found in the cytoskeletal pellet only in the presence of calcium, while GFP-Ado was found in the cytoskeletal pellet in the presence and absence of calcium. To determine if CpnA directly binds to actin, we performed F-actin binding assays with purified GST-CpnA and found that GST-CpnA bound to actin filaments in a calcium-dependent manner. In addition, we performed immunoprecipitations with cells expressing GFP, GFP-CpnA, and GFP-Ado. We then incubated the precipitated proteins with F-actin or G-actin in the presence or absence of calcium. We found that F-actin, but not G-actin, co-precipitated with GFP-CpnA in the presence of calcium. Our results indicate that CpnA binds directly to F-actin only in the presence of calcium. We also used *cpnA*- cells to investigate the function of CpnA in chemotaxis. Using both folate and cAMP as chemoattractants, we found that the *cpnA*- cells moved away from their original location in all directions, while the WT cells moved specifically towards the chemoattractant. These results suggest that CpnA plays a role in the ability of *Dictyostelium* cells to orient and move towards both cAMP and folate.

Chemical and Mechanical Stimuli Act on Common Signal Transduction and Cytoskeletal Networks

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Chemoattractant-mediated signal transduction pathways have been extensively studied, but little is known about the events that regulate responses to mechanical stimuli. We discovered that acute mechanical perturbation of cells triggered rapid and transient activation of every pathway we examined across the chemotactic signal transduction network, as well as actin polymerization. Similarly to chemoattractants, shear flow-induced signal transduction events displayed features of excitability, including the ability to produce a full response irrespective of stimulation length and a refractory period that is shared with that generated by chemoattractants. Disruption of calcium signaling, inhibition of multiple signal transduction pathways, and loss of heterotrimeric G-protein subunits attenuated the response to acute mechanical stimulation. Unlike the response to chemoattractants, an intact actin cytoskeleton was essential for the response to acute mechanical perturbation. Overall, these results suggest that chemotactic and mechanical stimuli activate a common signal transduction network that integrates external cues to regulate the activity of the actin cytoskeleton and drive cell migration.

Changes in PI(4,5)P2 levels are critical for phagocytosis, cell polarity, migration, and contribute to the metastatic properties of cancer cells

Dawit Jowhar, Gus Wright, Shanna Rucker, Max Housman, Hali Mohammed, Mariam Beshay, Nada Bawazir, BiChang Chang, Eric Betzig and Chris Janetopoulos

Cells have evolved to use fluctuations in plasma membrane (PM) phosphoinositide levels to regulate morphological changes. We propose that elevated PI(4,5)P2 levels contribute to actomyosin contraction, while areas where PI(4,5)P2 is used as a substrate and turns over, are largely associated with branching actin networks. At the onset of cytokinesis, cells become quiescent, PI(4,5)P2 levels elevate slightly and are uniform across the PM, and cells round up. The spindle orientation likely regulates symmetry breaking, starting feedback loops that both lower PI(4,5)P2 at the poles, and elevate them in the furrow. In order for cells to move directionally, receptor pathways evolved that bias PI(4,5)P2 levels so that the high side of the ligand gradient results in more rapid turnover and lowering of PM PI(4,5)P2. In Dictyostelium discoideum, we have found that confined cells are unable to switch directions and make a new leading edge until the local PI(4,5)P2 levels drop. High PI(4,5)P2 appears to inhibit Ras activity. PTEN and Ras activity are reciprocally regulated. Artificially depleting PI(4,5)P2 levels in breast cancer cells leads to PI3K and Rac activation, actin reorganization and cell spreading. PI(4,5)P2 depletion also results in the release of Aldolase A from sequestration with F-actin. We propose that the dropping of PI(4,5)P2 levels contributes to actin reorganization by releasing actin severing proteins. This, in turn, locally releases glycolytic enzymes bound to F-actin, including AldolaseA. This will create ATP and GTP locally and should fuel the machinery used to create branching actin networks used for protrusion. Regulation of Aldolase A through PI(4,5)P2 depletion is likely critical for the Warburg effect. This work suggests a mechanistic link between the local turning over of PM PI(4,5)P2, the regulation of actin networks controlling polarity, and glycolytic activity.

Using adhesion to discover novel regulators of cell migration

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Cell migration is a fundamental biological process and plays a critical role in numerous pathological states, particularly inflammation and cancer metastasis. Recent studies in *Dictyostelium* show that deletion of orthologues for tumor suppressors, or expression of oncogene cognates, cause cell flattening, decreased motility, and increased adhesion. A newly developed genetic screening procedure using REMI mutagenesis and increased cellular adhesion as a selection method have revealed eighteen genetic loci that regulate adhesion and motility. Many of the mutations have relevant domains and orthologues in humans implicated in disease. The functions of a subset of the eighteen novel genes have been studied with a combination of disruptions, GFP-tagged expression, atomic force microscopy, cell morphology, in addition to migration and chemotaxis. The GFP-tagged localizations have demonstrated a diverse array of intracellular localizations regulating these processes. Two of the newly discovered genes that have a strong effect on adhesion and motility are a putative actin binding (ABN) protein and a ring-finger/zinc-finger (RNF) protein. Newly created knockouts for these two genes have demonstrated functions in regulating multicellular development and migration. Specifically, the RNF protein has a direct human ortholog that is not well studied. Future work will continue to elucidate the functions of these cell migration relevant genes.

Session V – Development I

Regulation of Metabolic Pathways by the Nutrient Sensor mTORC1

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The TOR kinase functions in two protein complexes, mTORC1 and mTORC2. mTORC1 is a key regulator for cell growth, sensing signals from nutrients and energy levels. In contrast, mTORC2 does not regulate cell growth, but is crucial for developmental pathways. While mTORC1 activity is inhibited by nutrient removal, it is also inactivated by the immunosuppressive drug rapamycin. Thus, withdrawal of nutrients from or addition of rapamycin to growing cells leads to a rapid (<5min) de-phosphorylation of the mTORC1 mediated growth regulator p4EBP1. In parallel, the energy status regulator AMPK is phosphorylated/activated. Even in the presence of nutrients, rapamycin suppression of mTORC1 promotes mRNA unloading from polysomes, similar to that of nutrient depleted cells, and inhibition of protein synthesis.

To distinguish metabolic pathways that are separately sensitive to nutrient depletion or mTORC1 inhibition, we removed nutrients from or added rapamycin to growing cells and assayed metabolic pathways by Mass Spectrometry. We will discuss regulation of certain metabolic pathways, including glycolysis, lipolysis, and protein synthesis and degradation, by nutrient sensor mTORC1 and relate these to the energy deficit state in control of growth/development.

Strategic investment and the tragedy of the commons in a microbe

Balint Stewart, Philip G. Madgwick, Laurence Belcher, Christopher R.L. Thompson and Jason B. Wolf

Common goods are resources or assets that are costly to produce but benefit all members of a group. Individuals are thus faced with a dilemma over whether to invest in common goods since they can gain the benefits whether they contributed or not. If individuals have a high stake in the success of the group, they should invest heavily in common goods. In contrast, individuals with a low stake should free ride and exploit the investments of others. However, if no individual has a large enough stake, the group will be entirely composed of free-riders and suffer the tragedy of the commons. Such scenarios are widespread in nature, but the extent to which individuals act as savvy investors into common goods –and whether they suffer the tragedy of the commons – is unknown. To address these questions we derive a novel 'Collective Investment' (CI) game to model stake-dependent strategies in the social microbe *Dictyostelium discoideum*. Because mixtures of *D. discoideum* strains collectively build a stalk (a common good) to facilitate spore dispersal, the relative frequency of each strain will determine its relative stake in the success of the group. We find that experimental measurements of natural strains of *D. discoideum* reveal stalk investment increase as a function of stake while relative success declines, matching predictions made by the CI game. However, when a small number of different strains are mixed they avert the tragedy of the commons, by strategically deploying segregation behaviour to achieve a controlling interest. The recognition of individuals as savvy investors transforms our perspective on cooperation and cheating in nature by replacing fixed strategies ('cooperate' or 'defect') with continuously-variable strategies shaped by conflict over collective (not cooperative) investment. Because conflicts between selfish interests of individuals and the good of group are widespread in nature, the Collective Investment Game provides a powerful new framework for understanding strategic behaviour of selfish individuals.

Allorecognition signaling in *Dictyostelium* development

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TgrB1 and TgrC1 are polymorphic trans-membrane proteins that mediate allorecognition and post-aggregative development in *D. discoideum (1-3)*. We hypothesize that TgrB1 and TgrC1 binding at the cell surface triggers signal transduction cascades that integrate allorecognition and developmental responses. Genetic suppressor screens using REMI mutagenesis suggested that TgrB1 and TgrC1 have distinct functions, but have failed to identify components of the relevant signal transduction pathways (*4, 5*). We therefore utilized whole-genome sequencing as a means of characterizing chemically-induced mutations and used the method to identify candidate components of the TgrB1-TgrC1 signal transduction mechanism (*6*). We also analyzed merodiploid strains in which we expressed different alleles of *tgrB1* and *tgrC1* along with downstream reporters to measure several aspects of development and allorecognition. Our findings suggest that TgrC1 acts as a ligand and TgrB1 acts as a receptor during the interaction between adjacent compatible cells in the process post-aggregative tissue formation. The mechanisms that transduce the signals downstream of TgrB1 involve several small GTPases and their regulators.

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The multicellularity genes of dictyostelid social amoebas

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The evolution of multicellularity enabled specialization of cells, but required novel signalling mechanisms for regulating cell differentiation. Early multicellular organisms are mostly extinct and the origins of these mechanisms are unknown. Here, using comparative genome and transcriptome analysis across eight uni- and multicellular amoebozoan genomes, we find that 80% of proteins essential for development of the multicellular Dictyostelia are already present in their unicellular relatives. This set is enriched in cytosolic and nuclear proteins and protein kinases. The remaining 20%, unique to Dictyostelia, mostly consists of extracellularly exposed and secreted proteins, with roles in sensing and recognition, while several genes for synthesis of signals that induce cell-type specialization were acquired by lateral gene transfer. Across Dictyostelia, changes in gene expression correspond more strongly with phenotypic innovation than changes in protein functional domains. We conclude that the transition to multicellularity required novel signals and sensors rather than novel signal processing mechanisms.

Session VI – Chemotactic Signaling II

Dicty World Race – a Competition for Highly Motile Cells

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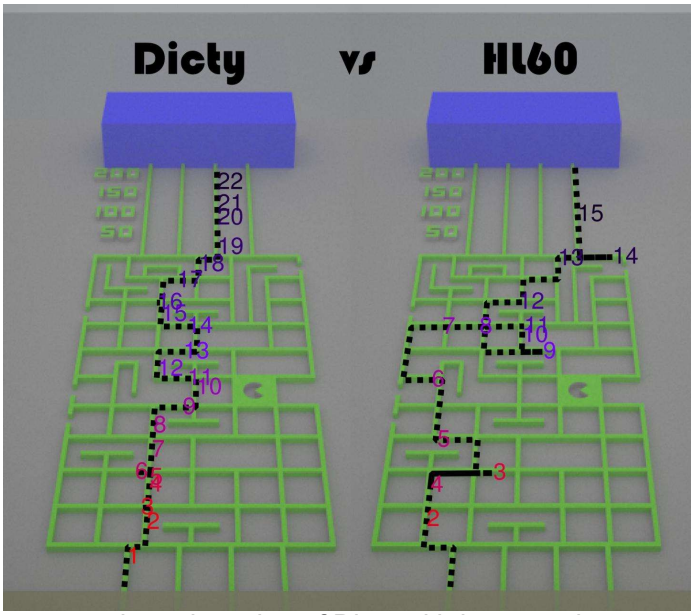

Dicty World Race is a fun-spirited competition that aims to bring together a diverse research community and to reveal aspects of cell motility not available through traditional research efforts. The race employs new microfluidic technologies and enables large-scale analyses of cell motility and chemotaxis in complex environments. The 2014 Dicty World Race uncovered an intriguing balance of speed and accuracy of the two model systems for human neutrophils, *Dicty* and HL60. In the competition, a total of 428 cells finished the race in the 3-hour observation time. The winner had the highest representation (48%) of their team's cells within the first 100 to cross the finish line. While teams used a variety of engineering strategies to enhance their cells' chemotactic ability, the most successful cells were an adapted version of Dicty with increased activity of a particular protein, Ric8, engineered in the laboratory of A. Kortholt and P. van Haastert at the University of Groningen. The consequent enhanced G-protein signaling helped the cells navigate better in shallow chemical gradients. The runner up with 19% representation was an HL60 cell line engineered by the group of G. Charras at the University College London, to overexpress the regulatory light chain to enhance actomyosin contractility. In comparing speed with accuracy, HL60 cells were overall more than twice as fast (v_{avg} = 18 μm/min) as Dicty cells (v_{avg} = 8 μm/min). However, Dicty cells were better at finding shortcuts, giving them a slightly enhanced combination of chemotactic accuracy and speed in this maze. At every junction in the maze, cells were forced to choose among multiple paths and Dicty cells had a significantly higher probability of making the optimal choice than HL60 cells for small differences in gradient. When unbiased by a chemical gradient, both Dicty and HL60 cells showed no persistence with equal probability of going straight or turning. When biased by the presence of a larger gradient along one of the edges, Dicty cells were significantly more likely to choose the edge with the larger gradient than HL60 cells. Chemotactic performance of cells correlated to their speed, but the relationship was different in Dicty and HL60 cells. Individual Dicty cells tended to slow down as the gradient increased ($p = 0.01$) and HL60 cells sped up slightly with increasing concentration ($p = 0.01$).

The differences in performance between Dicty and HL60 will be explored further during the Dicty World Race 2016. Details regarding the registration and rules for the event planned for Oct 26, 2016 are presented at www.dictyworldrace.com.

Disrupting a novel ubiquitin ligase of the HERC1 family restores chemotaxis and development in a *Dictyostelium* PIA-deficient mutant

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Cyclic AMP binding to G protein-coupled receptors orchestrates chemotaxis and development in *Dictyostelium.* Among the multiple signalling pathways activated upon cAMP binding to serpentine receptors, a major role is played by the Ras-TORC2-AKT/PKB module. TORC2-dependent AKT/PKB phosphorylation regulates cell polarization during chemotaxis. PIA, the ortholog of the TORC2 subunit Rictor, is also essential for GPCR-dependent stimulation of adenylyl cyclase A (ACA), thus regulating chemotaxis and developmental gene expression. Two PIA mutants have been described, a PIA-null mutant obtained by homologous recombination (Chen et al., 1977), and HSB1, a temperature-sensitive, nitrosoguanidine mutant harbouring a point mutation in the *piaA* gene (Pergolizzi et al., 2002). Consistent with PIA function, both mutants are defective in cAMP relay and development.

Being a nitrosoguanidine mutant, HSB1 is amenable to mutagenesis by random insertion of the blasticidin resistance. Near-saturation mutagenesis of HSB1 led to selection of two suppressor mutants, in which spontaneous chemotaxis and development were restored. TORC2-depepndent PKB phosphorylation and chemotactic cell polarization were rescued, whereas PIA-dependent ACA stimulation was not restored but bypassed, leading to spontaneous chemotaxis and cAMP-dependent developmental gene expression. Knocking out the gene encoding the adenylylcyclase B (ACB) in the parental strain showed ACB to be essential for this process. In both suppressor mutants the same gene was disrupted in two different, but very close insertion sites. The tagged gene encodes a giant HECT ubiquitin ligase, homologous to mammalian HERC1, but harbouring a pleckstrin homology domain. Expression of the isolated $HECT^{wt}$, but not $HECT^{C5185S}$, domain was sufficient to reconstitute the parental phenotype. We suggest that the novel HECT ubiquitin ligase regulates cell sensitivity to cAMP signalling and TORC2-dependent PKB phosphorylation.

Copines translocate to the plasma membrane in response to cAMP stimulation in *Dictyostelium*

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Copines are calcium-dependent phospholipid binding proteins found in many eukaryotic organisms. Copines are characterized by having tandem C2 domains at the N-terminus accompanied by an A domain at the C-terminus. Copines have been suggested to be involved in calcium-dependent signaling pathways that regulate a wide variety of cellular processes. Six copine genes have been identified in the *Dictyostelium* genome, *cpnA – cpnF*. Cell lines expressing CpnA, CpnB, CpnC, CpnE, or CpnF tagged with green fluorescent protein (GFP) at the N-terminus (GFP-Cpn) or the C-terminus (Cpn-GFP) have been created as tools to study copine protein membrane-binding and localization. In general, the GFP-tagged copine proteins were localized to the cytoplasm when cells were fixed with 3.7% formaldehyde in phosphate-buffered saline (PBS) or in live cells. However, when cells were fixed with 1% formaldehyde in methanol or when live cells were treated with calcium ionophore, all of the GFP-tagged proteins, expect CpnF, localized to the plasma membrane and vesicular membranes. CpnF was found mostly in the cytoplasm and in the nucleus, with some membrane association. CpnB and CpnC were also found in the nucleus. Aggregate competent cells were stimulated with cAMP, which causes a transitory increase in the intracellular calcium concentration. All of the copines translocated to the plasma membrane in response to cAMP signaling, but with distinct on and off times, suggesting each of the copines has different calcium-sensitivities and membrane-binding properties. *In vitro* membrane binding assays showed that all of the GFP-tagged copines pelleted with cellular membranes in the presence of calcium; yet, each copine displayed distinct calcium-independent membrane binding in the absence of calcium. An overlay assay of purified GFP-tagged copine proteins was used to screen for specific phospholipid-binding targets. Similar to other proteins that contain C2 domains, GFP-tagged copines bound to a variety of acidic phospholipids but with varying affinities. Our studies suggest copines are soluble cytoplasmic and nuclear proteins that have the ability to bind intracellular membranes in response to an increase in intracellular calcium. Copine homologs display different membrane-binding properties suggesting they play distinct roles. The transient translocation of copines to the plasma membrane in response to cAMP in aggregation competent cells suggests copines may have a specific role in chemotaxis signaling.

Function of Rho GTPase in Directional Sensing

Hiroshi Senoo & Miho Iijima

A fundamental question in chemotaxis is how cells translate unstable extracellular cues into robust intracellular signalling? During *Dictyostelium* chemotaxis, gradients of the chemoattractant cAMP are converted to the local activation of Ras GTPase and the production of phosphatidylinositol 3,4,5 triphosphate (PIP3) at the leading edge of migrating cells. The conversion of extracellular chemical gradients to intracellular polarised signalling is called directional sensing and serves as an internal compass to determine the direction of cell movements. However, the mechanism underlying directional sensing in chemotaxis is largely unknown.

We have previously shown that a Rho GTPase, RacE, regulates directional sensing in *Dictyostelium* cells. Recently, using affinity purification and mass spectrometry, we identified a novel RacE-binding protein, which regulates directional sensing.

Identification and characterisation of RacE binding partners will likely help reveal how RacE spatially restricts Ras activation and PIP3 production.

Session VII – Development II

Understanding the origins of cell type differences using single cell transcriptomics

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The appropriate control of gene expression is central to the generation of differences between cells during differentiation. Standard population approaches to monitor gene expression take population and time-averaged snapshots, and hide the very differences between cells that we are trying to understand, in addition to losing dynamic information. Until recently, single cell alternatives have only been able to monitor expression of a few genes at the same time. We have therefore introduced single cell transcriptomics for the study of the gene expression changes occurring during *Dictyostelium* development. Our data reveal early regulators of cell type specialization, and the genome-wide mechanisms that generate divergence in the expression programs of individual cells. We also use these new approaches, in combination with live imaging of transcription dynamics, to reveal the dynamic processes of co-regulation within gene families.

Robust cell type proportioning achieved via a complex noise dampening system acting upon the cell cycle

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The formation of differentiated cell types from a population of genetically identical cells is one of the most fundamental processes in biology. In situations where "salt-and-pepper" patterning mechanisms apply (such as the early mammalian embryo) cells acquire different identities in a heterogeneous manner, whilst maintaining strict proportions of each cell type. Whilst seeking the sources of such heterogeneity we have previously described a molecular mechanism in *Dictyostelium* where the specification of pre-stalk and pre-spore cells depends on stochastic Ras-GTPase activity and heterogeneous nutritional status to achieve lineage-specific responses to differentiation signals (Chattwood et al., 2013).

Further analysis of this system by RNAseq has identified inverse gene expression profiles during growth that result in the same eventual phenotypic outcome – i.e. employment of different strategies during vegetative growth to achieve the same developmental cell fate. Through characterisation of the cell cycle and tracing mitotic history we find the cell cycle is differentially affected upon variation of Ras-GTPase activity and nutritional status, with subsequent impact on developmental cell fate. Furthermore we find that this system acts in a density-dependent manner, whereby cells at a given density are more likely to adopt a particular genetic strategy, thus employing a density-sensing mechanism that adds a further element of control over correct oscillatory cell type proportioning that is buffered against extrinsic variability.

MAPK and phosphodiesterase regulation in G protein signaling pathways

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Dictyostelium has two MAPKs (mitogen activated protein kinases), ERK1 and ERK2, that are important for development and several studies have shown that ERK2 is phosphorylated in response to the stimulation of G protein signaling pathways with cAMP or folate. We recently discovered that ERK1 is also phosphorylated in a secondary response to these external signals. The phosphorylation of ERK1 is regulated by cell-cell signaling and can occur in *erk2*$^-$ mutants. The loss of ERK1 rescues the slow tip formation of *gα5*$^-$ mutants implying that Gα5 might negatively regulate ERK1. However, no major changes in the phosphorylation ERK1 are observed in *gα5*$^-$ mutants. Others have shown that ERK2 negatively regulates the phosphodiesterase RegA, presumably through the phosphorylation of a threonine residue on RegA. We are currently examining potential ERK2/RegA interactions through the analysis of a putative MAPK docking site in RegA. Using tandem mass spectrometry we have discovered other phosphorylation sites on RegA that suggest PKA (cAMP dependent protein kinase) and other kinases might regulate RegA in *Dictyostelium*.

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All-cell 3D tracking in “mini” slugs

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Cell movement within the slug has been studied mostly by tracking the small fraction (~2%) of cells that had been labelled. However, these methods may not detect cell movement patterns exhibited by a small minority of cells. Bonner (1998), on the other hand, developed a method to make tiny, 2-dimensional “mini-slugs” with which all the cells could be traced during migration, and made several important observations. We extended Bonner's method to achieve 3-dimensional cell tracking by making very small but ordinary 3-dimentional slugs with a strain expressing histone-GFP. Using the laboratory-made software based on the method of Yasui, *et al.* (2014), we were able to obtain 3-D coordinates of almost all nuclei from time-lapse z-stack images of migrating mini-slugs. Some of the known properties of the migrating slug, such as the difference in the cell movement pattern between prestalk and prespore cells, have been confirmed. Also, twisting movement of cells was observed in the posterior region of mini-slugs during spontaneous turning. We are presently trying to apply this method to trace all cells over the entire course of development using a small population of starved cells. By doing so, we expect to see a possible correlation between the cell behaviour during the preaggregation stage and later cell differentiation and morphogenesis.

References

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Session VII – Cytoskeleton II

Multiscale Analysis of Microtubule Arrays in Dictyostelium

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Interphase microtubule (MT) arrays in most eukaryotic animal cells grow radially from the centrosome and display a self-centering capacity in the cytosol due to dynamics at the distal MT ends. If two vertebrate tissue cells are artificially fused, the two MT arrays typically converge to form a single network. D. discoideum cultures frequently contain multinucleated cells; a striking feature of these cells is that the MT arrays generally remain separate from one another. This organization suggests a higher level of spatial control than just MT tip dynamics. To address how multiple arrays minimize spatial overlap in a common cytoplasm, we focused on components that engage interdigitating MTs of opposite polarity, and targeted the MAP65/Ase1/PRC1 protein family. D. discoideum contains two isoforms of Ase1 (A,B); knockout of either gene is viable and produces distinct phenotypes. As predicted by fungal studies, Ase1A disruption results in a reduction of spindle midzone MTs during mitosis. In addition, there is a novel interphase defect whereby the spacing between multiple MT arrays is reduced and MTs appear intertangled. These results indicate that Ase1A functions to manage the spacing between multiple MT arrays. Ase1B disruption does not perturb spindle MTs or centrosome spacing. Instead, there is an enhanced frequency of hyperpolar cell extensions. Our current work lies in generating a mechanistic understanding of how Ase1A functions, with the idea that it partners with Kinesin-4 (Dd Kif8) to minimize opposite polarity MT overlap during interphase. Supported in part by the NSF.

Topology of Dictyostelium centrosome proteins

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The Dictyostelium centrosome consists of a core structure build by three plaque-like layers, which are surrounded by a matrix, called corona, containing microtubule nucleation nodules. Meanwhile, 34 centrosomal Dictyostelium proteins have been identified in database searches through their homology to known proteins of other species, or by analysis of the centrosomal proteome followed by expression of GFP-fusions of the respective proteins and their localization at the centrosome. Some of them have known functions in cytokinesis or chromosome cohesion and may use the centrosome only as a distribution station during their intracellular traffic, while others are required for integral centrosomal functions.

By integrating data from advanced light microscopy, electron microscopy, functional studies and protein-protein interaction studies we have designated all proteins with a known function in centrosome biogenesis, centrosome integrity or microtubule nucleation to either the corona or the layers of the core structure. We present a model in which the novel core proteins CP39, CP75 and CP91 make up the central layer of the core, while CP55 and Cep192 (as the major component) build up the two outer layers. Cep192 and CP55 interact with Cep161 (=CDK5RAP2). The latter, in turn, binds CP148 and most likely through its conserved gamma-tubulin complex binding domain the nucleation complex consisting of gamma-tubulin, Spc97 and Spc98, which is assisted by microtubule elongation promoters TACC and CP224(=XMap 215). The interactions and role of the corona protein CP248 (=CP250) remain to be elucidated. Our assays using the biotin-identification (BioID) proximity assay indicate that we meanwhile know all major structural components of the core structure. As the corona dissociates prior to duplication of the core structure this also means that we know all structural components involved in the actual process of centrosome duplication.

Regulation of Dictyostelium macropinocytosis

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The axenic *Dictyostelium discoideum* strains are able to grow in liquid media due to a large increase in non-specific fluid uptake (macropinocytosis) compared to wild isolates. Macropinocytosis has also been implicated in host invasion by certain pathogens (e.g. *Salmonella*, some viruses), antigen sampling by immune cells, prion spreading and proliferation of ras-driven cancers. Despite its wide biological and clinical importance, very little is known about it.

Recent work has shown some of the genetic and molecular underpinnings of macropinocytosis in *D. discoideum*. The main causative mutation allowing axenic growth was deletion of the RasGAP NF1, of which heterozygous mutation in humans causes neurofibromatosis 1. In its absence, a large patch of active ras and phosphoinosine-3,4,5-trisphosphate (PIP3) organises a circular ring of scar around its rim, which directs actin polymerization and cup formation. The cup then closes to complete macropinocytosis.

Here, we look at how macropinocytosis is regulated. We developed an assay to look at fluid uptake in a high throughput manner and show that macropinocytosis is regulated rather than constitutive. We identified nutrients that cause upregulation of macropinocytosis and demonstrate that macropinocytosis itself is required for the upregulation of macropinocytosis through an increase in the formation of active ras/PIP3 patches. Finally, we screened many naturally occurring compounds for their ability to affect macropinocytosis and identified regulatory pathways.

These findings increase the control we have over macropinocytosis and open up avenues of research into molecular aspects of macropinocytosis that were not previously possible.

The impact of myosin I on macropinocytosis

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Macropinocytosis is an efficient way for cells to take up large volumes of medium into intracellular vesicles, from which they can extract nutrients and other useful molecules. It's important for a wide spectrum of human biology, including antigen sampling by immune cells, uptake of drugs and has been hijacked by pathogens as a major route of entry. Recent data suggest that macropinocytosis is a widely used method for feeding by cancer cells and may be implicated in the spread of neurodegenerative disease within the brain.

Considering its importance, macropinocytosis is poorly understood. Driven by the actin cytoskeleton it can be studied advantageously in axenic *Dictyostelium* cells, which use it for feeding. Myosin I proteins form linkers between the plasma membrane and the actin cytoskeleton, and recently we found that a myosin I inhibitor at very low concentrations could strongly inhibit both the uptake of liquid by macropinocytosis and particles by phagocytosis. To get a better understanding of how the inhibitor causes these dramatic effects we have analysed the subcellular changes in inhibited cells. In parallel we have GFP---tagged all seven of the *Dictyostelium* myosin I proteins and found that five are recruited to macropinosomes. While the PIP3 binding myosin Is are present in the whole cup, MyoIB and MyoIC localise to the rim, which is also the site of actin polymerization and SCAR recruitment.

Currently we are investigating in detail which myosin I proteins are of particular importance for macropinocytosis and are trying to understand how they regulate this process.

Session IX – Biomedical Models

Extracellular polyphosphate inhibits proliferation in an autocrine negative feedback loop in *Dictyostelium discoideum*

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Polyphosphate is a polymer of phosphate residues linked by high-energy phosphoanhydride bonds. Despite being highly conserved throughout nature, its function is poorly understood. Here we show that *Dictyostelium* cells accumulate extracellular polyphosphate, and this acts to inhibit proliferation at high cell densities. In shaking culture, extracellular polyphosphate concentrations increase as cell density increases, and if the concentration of polyphosphate observed at stationary phase is added to cells at mid-log, proliferation is halted. Two different enzymes appear to mediate the synthesis of extracellular polyphosphate, polyphosphate kinase (DdPpk1) and inositol hexakisphosphate kinase (I6kA). In agreement with an increase in multinucleate cells observed at stationary phase or upon polyphosphate treatment, proteomics analysis showed that extracellular polyphosphate causes a decrease in proteins involved in cytoskeletal rearrangment and cytokinesis, including PakA and myosin heavy chain kinase. Multiple subunits of the 20S proteasome were also downregulated, suggesting a decrease in proteasomal activty, which occurs in some differentiating cells. Interestingly, cells at stationary phase or cells treated with polyphosphate showed an increase in phosphorylated Adrm1 and Trap1, which are involved in the transition of growth to differentiation. The accumulation of extracellular polyphosphate thus appears to allow proliferating cells to anticipate the situation in which a high density of cells outgrows its food source and starves, acting as a pre-starvation factor to help trigger differentiation. Activated human platelets also secrete polyphosphate, and this is also mediated by inositol hexakisphosphate kinase. Although much is known about blood clotting, little is known about what triggers the formation of scar tissue. We found that polyphosphate released by activated platelets also triggers the differentiation of human monocytes into fibroblast-like cells called fibrocytes, which mediate scar tissue formation. Extracellular polyphosphate may thus have conserved roles in some stress responses.

Investigation of *Dictyostelium discoideum* protein quality control network

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The loss of proteostasis leading to an increase in protein aggregation is a hallmark of many neurodegenerative diseases. The nine polyglutamine diseases are caused by the expansion of a CAG trinucleotide repeat within the coding region of specific genes. CAG codes for the amino acid glutamine, resulting in the translation of a polyglutamine-expanded, aggregation-prone protein. In model organisms, including yeast, worms, flies, mice, rats, as well as in human cells, expression of proteins with the long repetitive amino acid tracts associated with these diseases recapitulates the protein aggregation that occurs in human disease. *Dictyostelium discoideum* is unique in that it normally encodes long polyglutamine tracts, and we have shown that it is highly resistant to polyglutamine aggregation. Here, we have performed an initial survey of the role of various protein quality control pathways in suppressing polyglutamine aggregation in *Dictyostelium discoideum*.

Tetrahydropteridine study in *Dictyostelium discoideum* Ax2

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L-erythro-tetrahydrobiopterin (BH4) is a multifunctional molecule acting as an antioxidant as well as a cofactor for aromatic amino acid hydroxylases and nitric oxide synthases in mammals. *Dictyostelium discoideum* Ax2 is notorious for the synthesis of two different isomers of tetrahydrobiopterin, BH4 and D-threo-tetrahydrobiopterin (tetrahydrodictyopterin, DH4). Our study in the organism suggested that BH4 is a preferential cofactor for phenylalanine hydroxylase (PAH) in vivo, while DH4 functions mainly as an antioxidant. Since PAH may be essential for *Dictyostelium* growth in nature, it appears that the organism has evolved a strategy to maintain BH4 level via regeneration pathway at the expense of DH4 under oxidative stress conditions. In this presentation we would like to concentrate on two recent findings: one is about aldose reductases involved in the biosynthesis of DH4 and the other is about human mutant PAH study performed in *Dictyostelium*, which may be useful for mass-screening of pharmacological chaperones for missense PAH mutations.

Session X – Cytoskeletal Signaling

WASP maintains cell polarity by preventing aberrant accumulation of active Rac

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WASP is a highly conserved actin nucleation-promoting factor, and one of its best-characterised roles is the internalisation of clathrin- coated pits (CCPs) during endocytosis. The vast majority of WASP within the cell is auto-inhibited, and Rho GTPases have been proposed to play a key role in its localisation and activation by interacting with its CRIB motif and releasing the auto-inhibition.

However, we found that WASP CRIB mutants that cannot bind active Rac are able to localise normally on CCPs and recruit the Arp2/3 complex, meaning that Rho GTPases are dispensable for WASP activation.

Instead, we found that the interaction between WASP and active Rac is required for the cells to maintain front–rear polarity, since cells expressing WASP CRIB mutants show aberrant accumulation of active Rac at the trailing edge during migration.
Moreover, we found that cells expressing WASP CRIB mutants share some of the distinctive features of cells with high levels of active Rac.

In summary, our data turn current dogma on its head - placing active Rac downstream of WASP- and point out an unexpected and fascinating role for WASP in the maintenance of active Rac homeostasis.

Protein Kinase C and Phospholipase D cooperatively regulate *Dictyostelium discoideum* development

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Protein kinase C (PKC) signaling is crucial to regulating nutrient sensing, cell growth and proliferation, development, cell motility, and the organization of the cytoskeleton. A putative PKC orthologue has recently been described in *Dictyostelium discoideum*, which has been shown to regulate chemotaxis and development. We found that PkcA regulates CMF quorum sensing and the actin cytoskeleton; as such it may interact with other proteins known to regulate quorum sensing and the actin cytoskeleton. Here we examine the interaction between PkcA and PldB, an orthologue of PLD, during development. We find that PkcA and PldB work together to regulate CMF quorum sensing, developmental timing, and spore formation. They also work together to regulate the actin based processes of adhesion and cytokinesis. Epistasis analysis suggests that in all cases, PkcA appears to function upstream of PldB. This relationship may be explained by the ability of PkcA to regulate PldB activity. Taken together, these results suggest that PkcA regulates development and organization of the cytoskeleton by regulating PldB activity. Interestingly, this relationship mimics the well-known interaction between PKC and PLD, suggesting an evolutionarily conserved mechanism.

Posters

Abstract 1

The small GTPases Ras and Rap1 bind to and control TORC2 activity

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Target of Rapamycin Complex 2 (TORC2) has conserved roles in regulating cytoskeleton dynamics and cell migration and has been linked to cancer metastasis. However, little is known about the mechanisms regulating TORC2 activity and function in any system. In *Dictyostelium*, TORC2 functions at the front of migrating cells downstream of the Ras protein RasC, controlling F-actin dynamics and cAMP production. Here, we report the identification of the small GTPase Rap1 as a conserved binding partner of the TORC2 component RIP3/SIN1, and that Rap1 positively regulates the RasC-mediated activation of TORC2 in *Dictyostelium*. Moreover, we show that active RasC binds to the catalytic domain of TOR, suggesting a mechanism of TORC2 activation that is similar to Rheb activation of TOR complex 1. Dual Ras/Rap1 regulation of TORC2 may allow for integration of Ras and Rap1 signaling pathways in directed cell migration.

Abstract 2

Transcriptional networks in *Dictyostelium* development

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Dictyostelium development, accompanied by cell differentiation and morphogenesis, is regulated by complex transcriptional networks, which consist of many overlapping transcriptional elements. We are focusing on evolutionarily-conserved transcription factors in Dictyostelids to unravel these transcriptional networks using next-generation sequencing techniques.

GtaC and MybB control the expression of early developmental genes. Loss-of-function mutants of those transcription factors exhibit aggregation-minus phenotypes. We performed RNA-seq analysis of the knockout and complemented strains as well as another aggregation-minus mutant, *acaA*$^{-}$, during development to compare their transcriptomes. We found differences between the transcriptomes of *gtaC*$^{-}$ and *mybB*$^{-}$ even though they exhibit similar morphological phenotypes, indicating that transcriptome analysis is a more precise phenotyping tool and pointing at the complexity of transcriptome regulation during development. We also studied the regulatory role of the GATA transcription factor, *gtaG*, which is essential for terminal differentiation in *Dictyostelium discoideum*. Transcriptome analyses of *gtaG* strains reveal that GtaG regulates prestalk gene expression during cell differentiation before culmination and is required for progression into culmination. We will discuss the coordinated transcriptional regulations in *Dictyostelium* developmental processes.

Abstract 3

Analysis of Human Tau Expression in a Novel Tauopathy Model System

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Alzheimer's disease (AD) is the sixth leading cause of death in the United States. This neurodegenerative disorder impairs brain regions associated with learning and memory. AD is characterized by two brain features: amyloid plaques and neurofibrillary tangles. Neurofibrillary tangles are intracellular aggregates of the protein tau, a microtubule binding protein. Abnormal phosphorylation and proteolysis of tau in AD brains is thought to be toxic. We are using the eukaryotic singled-celled model organism *Dictyostelium discoideum* to study tau toxicity. The goal of this study is to investigate the progression of tau toxicity using four different tau constructs: full-length human wild-type tau (tau^{WT}), incompetent phosphorylation tau (tau^{AP}), calpain resistant tau (tau^{CR}), and a proteolytic17kDa fragment of tau (tau^{17kD}). Previous results showed that constitutive expression of tau^{AP} in *Dictyostelium* cells was not toxic, while expression of tau^{WT}, tau^{CR}, and tau^{17kD} resulted in cell death within 2-3 weeks. Therefore, we are now using a doxycycline-inducible expression system in *Dictyostelium*. We have induced expression of tau^{AP} and tau^{17kD} in *Dictyostelium* thus far. Our results indicated that both tau^{AP} and tau^{17} can be expressed in a doxycycline dose-dependent manner. In addition, tau^{AP} is transiently expressed with one dose of doxycycline, peaking at 24-48 hours after induction, and is then rapidly degraded. In contrast, tau^{17kD} is still detected in cells seven days after one dose of doxycycline. These data suggest that *Dictyostelium* cells are able to rapidly degrade tau^{AP}, most likely through proteasomes, but not tau^{17kD}. When tau^{17kD} expression is induced with doxycycline continuously, cells slowly began to die over a 2-3 week period. Ultimately, we plan to use this new tauopathy model to reveal which posttranslational modifications cause tau toxicity and explore the mechanism of toxicity.

Abstract 4

Iron homeostasis in phagocytosis and resistance to invasive bacteria

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Dictyostelium amoebae are professional phagocytes that grow by engulfing bacteria, thus they are also natural host of pathogenic bacteria. Due to these features and amenability to genetic manipulation, Dictyostelium is a valuable model organism for phagocytosis and innate immunity.
By using fluorescence biosensors we have followed the recruitment in phagosomes of proteins involved in Fe^{3+} reduction and Fe^{2+} efflux. The *Dictyostelium* genome encodes three putative ferric reductases, one of which is expressed in the plasma membrane, in phagosomal and macropinosomal cups and vesicles, where it remains localized for 3 to 5 minutes after engulfment. Within 30 seconds from phagosome formation, concomitantly with the PI3P marker GFP-FYVE, the V-H^+ ATPase is recruited to the phagosome, inducing rapid acidification. Shortly thereafter the Fe^{2+} transporter Nramp1 is also recruited to the phagosomal membrane, thus allowing iron efflux from the phagosome. The phago-lysosome undergoes pH neutralization after about 20 min from phagosome formation, presumably due to gradual V-H^+ ATPase retrieval.

By using quenched Fe^{3+}-loaded calcein, which is taken up by macropinocytosis, we have shown that fluorescence de-quencing in Nramp1-coated vesicles occurs in 15 minutes, suggesting that iron is being reduced and released gradually from phagosomes within this time period. No de-quenching occurs in Nramp1-KO mutants, indicating that Nramp1 is essential for iron efflux.
By expressing Nramp1 in Xenopus oocytes, we have shown that Nramp1 transports Fe^{2+}, to a lower extent Mn^{2+}, but not Fe^{3+} or Cu^{2+}. Transport is electrogenic and pH-dependent, similar to the mammalian iron transporter DMT1.

Iron efflux from the phagosome has both a protective and a nutritive function, as it leads to iron accumulation in the cytosol and in mitochondria, where it is used for formation of heme and iron-sulphur groups, while depriving engulfed bacteria of an important element. Consistent with this hypothesis, pathogenic bacteria, such as *Legionella pneumophila*, proliferate better in Nramp1-KO mutants than in wild-type cells. Legionella intracellular growth is also favoured upon infection of *Dictyostelium* cells previously grown for 24 hours in iron-rich, rather than iron-depleted, FM medium.

Abstract 5

Heterotrimeric G protein Activation and Regulation monitored by Bioluminescence Resonance Energy Transfer (BRET)

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Chemotaxis, the directed migration of cells in response to chemical cues (chemoattractants), is central to normal physiology and is often implicated in the onset and progression of disease, including cancer metastasis. Therefore, the ability to therapeutically target the chemotaxis machinery would be highly beneficial. However, the signaling networks and molecular mechanisms underlying chemoattractant gradient sensing and directional migration of cells are not understood. Key to our understanding of the mechanisms underlying the directed migration of cells is a better knowledge of chemoattractant signaling dynamics. *Dictyostelium* has proven to be a great tool to decipher chemoattractant signal transduction, but the methods available to quantitatively measure signaling dynamics in this organism are limited. We have developed a protocol to quantitatively study chemoattractant signal transduction in *Dictyostelium* by monitoring protein-protein interactions and conformational changes using Bioluminescence Resonance Energy Transfer (BRET). We have used BRET to analyze the kinetics and dose-dependency of heterotrimeric G protein subunit (Gα2 and G$\beta\gamma$) dissociation, in response to chemoattractant stimulation in *Dictyostelium*. We show that BRET allows obtaining quantitative data with high temporal resolution, well suited for the study of chemoattractant signal transduction in *Dictyostelium* and the quantitative modeling of chemotactic responses.

Abstract 6

Regulation of the Phosphodiesterase RegA in *Dictyostelium* During cAMP Signaling.

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Many eukaryotic signaling pathways use cAMP as a secondary messenger to evoke specific responses to different external stimuli. In many eukaryotes, localized levels of cAMP can be controlled by phosphodiesterases, which are sometimes regulated by phosphorylation. *Dictyostelium* offers an excellent system to study the regulation of phosphodiesterases as it contains relatively few cAMP-specific phosphodiesterases compared to mammals. The cAMP-specific phosphodiesterase, RegA, regulates important steps in *Dictyostelium* development and is negatively regulated by the MAP kinase, ERK2. Mammalian studies have suggested that cAMP-specific phosphodiesterases can also be regulated by cAMP-dependent protein kinase, PKA. The possible regulation of PKA on the activity of RegA has not been fully characterized in *Dictyostelium*. Mass spectrometry was used to detect potential phosphorylation sites on RegA after external stimulation. Two sites of interest have been identified, including a putative PKA phosphorylation site and another site that could be regulated by other kinases. Site directed mutagenesis was used to replace the residues at these sites and a previously described MAPK phosphorylation site to mimic or prevent phosphorylation events. The phenotypes of cells carrying these mutations are being analyzed through developmental analysis. Changes to the phosphorylation of RegA are being analyzed by phosphospecific antibodies to determine RegA regulation in response to external stimuli.

Abstract 7

Phosphodiesterase RegA D-motif Mutation Leads to Developmental Phenotype Defect.

Nirakar Adhikari, Nick Kuburich & Jeff Hadwiger

In *Dictyostelium* the MAP kinase (MAPK), ERK2, down regulates the phosphodiesterase, RegA. RegA lowers the level of the second messenger cAMP, an important regulator of cell aggregation and differentiation. Loss of RegA increases cAMP levels and accelerates developmental morphogenesis. RegA has a putative MAPK docking site (D-motif) that might be important in interactions with MAPKs. While MAPK-phosphodiesterase interactions have been observed in mammalian cells, characterizing the function of this D-motif in RegA will likely provide insights on the interactions between RegA and MAPKs during the developmental processes and these findings can lead to a better understanding of MAPK-phosphodiesterase regulation and function.It is expected that without the D-motif, a RegA D-motif mutant ($RegA^{D-}$), little or no interaction will occur between $RegA^{D-}$ and Erk2, resulting in low cAMP levels and delayed development. Expression of $RegA^{D-}$ in a regA- knockout mutant resulted in developmental defects in comparison cells expressing wild type regA. The $regA^{D-}$ clones were slower in overall rate of development. The expression of regA D-mutant in $regA^{-}$/$erk2^{-}$ double mutant strains is also being analyzed. Physical associations between RegA and ERK2 are being examined using immunoprecipitations and western blot study.

Abstract 8

Annotating the *Dictyostelium* Developmental Gene Regulatory Network

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Despite substantial genetic divergence among the various dictyostelid species, the timing and structural changes of the developmental program have remained intact across many species. Two species in particular, *Dictyostelium discoideum* and *Dictyostelium purpureum,* share similar developmental timelines and morphological phenotypes, though their protein sequences differ as much as do humans' from boney fish. This developmental conservation is likely due to the fact that both species of *Dictyostelium* have highly conserved transcriptomes, in which large gene expression changes correspond to distinct developmental stages. Such strong conservation suggests the presence of crucial and stable regulatory mechanisms that remain a mystery.

Because transcription factors play a powerful role in gene regulation and are often conserved, I hypothesize that a particular set of transcription factors are the key regulators of development. To identify these key regulators, I have isolated the transcription factors that are most important in *Dictyostelium* development by identifying those with strong sequence and expression-pattern conservation between *D. discoideum* and *D. purpureum*. I am characterizing the chosen transcription factors by creating knockout null mutant strains in the more genetically tractable *D. discoideum*, and I have been testing the mutant strains' developmental phenotypes. I will then determine the effects of transcription factor loss on gene expression and regulation via RNA-seq. I will finally characterize the transcription factor target genes by ChIP-seq. I will use the data from the developmental phenotypes and RNA-seq to identify the direct targets regulated by each transcription factor, and will use data mining methods to identify the relevant developmental processes. Characterizing these key *Dictyostelium* developmental regulators will provide novel insights into general principles governing eukaryotic development. Furthermore, by comprehensively annotating the *D. discoideum* developmental gene regulatory network I will set the foundation necessary to introduce dictyostelids into the emerging realm of synthetic biology. Evolutionarily determined mechanisms for broad body structures revealed through synthetic biology will be subsequently introduced into novel species, thereby enabling the study of evolution on an incredibly reduced and workable timescale. By determining genes responsible for sweeping structural changes in *Dictyostelium,* specifically, and confirming these genes through synthetic evolution, we will better understand the genetic establishment of body plans in the evolutionary switch from unicellular to multicellular organisms.

Abstract 9

Diversity of the mitochondrial TOB/SAM complex organization in different stages of *Dictyostelium discoideum* life cycle

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The TOB/SAM (Topogenesis of the Mitochondrial Outer Membrane β-Barrel Proteins/Sorting and Assembly Machinery) complex is responsible for insertion of specific proteins that have β-barrel structure into the mitochondrial outer membrane and assembly of the TOM (Translocase of the Outer Membrane) complex, which is termed the entry gate of imported proteins into mitochondria. Thus, the complex has fundamental meaning for mitochondria and consequently cell functioning. The organization of the *D. discoideum* TOB/SAM complex was studied in the following stages of the slime mold life cycle: unicellular, early aggregation, aggregation and cumulation.

Two forms of the complex were detected for the studied stages, namely ~650 kDa and ~160 kDa ones. The main subunit of the complex, Tob55/Sam50 protein, occurred in three forms (~40 kDa, ~45 kDa, ~47 kDa) expressed differently in the studied life cycle stages. Thus, it can be assumed that the presence of the forms may correlate with multicellularity. Another subunit of the TOB/SAM complex (~45 kDa) displayed distinct homology with human metaxin1 and was present in only one form in all studied life cycle stages. Moreover, proteins being subunits of other mitochondrial complexes and probably interacting with the TOB/SAM complex were detected. The both forms of the complex may interact with subunits of the TOM complex and MICOS complex (Mitochondrial Inner Membrane Complex Involved in Maintenance of Crista Junctions) whereas only 160 kDa form may interact with subunits of the ERMES complex (Endoplasmic Reticulum–Mitochondria Encounter Structures), TIM23 (Translocase of the Inner Membrane 23) and small Tim proteins (proteins assisting in transport of hydrophobic precursors across the mitochondria intermembrane space).

Summing up, the obtained results provide new data concerning the organization of the TOB/SAM complex and its interaction with other mitochondrial complexes. Observed differences between life cycle stages may bring some light to changes in *D. discoideum* cells leading to multicellularity.

Abstract 10

Mechanism for the formation of beaded fruiting bodies in *Dictyostelium rosarium*

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The fruiting body of the cellular slime mold varies in shape from simple, unbranched to more complex. However, previous studies on non-simple fruiting body shapes have been mostly limited to the group 2 species Polysphondylium pallidum, which produces regularly branched fruiting bodies.

Dictyostelium rosarium belongs to group 4 and shares the basic features of development in common with D. discoideum, such as aggregation by periodic cAMP signals and the clear prestalk-prespore pattern in the migrating slug. The fruiting body of D. rosarium is characterized by its beads-on-a- string structure, which is generated by pinching off of the posterior part of the slug at regular intervals, like Polysphondylium, but without forming stalks. Thus, elucidation of this "pinching off" mechanism would help understand the diversity of fruiting body architecture in the cellular slime mold.

We found that under certain conditions, the pinched off cell mass gave rise to a tip, which usually soon disappeared but sometimes went on to make a stalk. Cell tracking experiments showed that during the process of pinching-off of the slug some cells moved backwards to enter the region to be left behind. These results suggest a secondary organizer to appear near the posterior end of the slug to cause separation of the posterior cells from its main part. To test this possibility, we are investigating the expression of the adenylate cyclase and some prestalk-specific genes by whole mount in situ hybridization. Alternative models for the pinching-off mechanism of the sorogen will be discussed.

Abstract 11

Spatiotemporal different localizations of multiple signaling molecules mediating chemotaxis

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Chemotaxis of *Dictyostelium* cells is mediated by multiple parallel signaling pathways including phosphatidylinositol 3,4,5-triphosphate (PIP3) pathway, soluble guanylate cyclase (sGC) pathway and so on. These signaling molecules localize to the anterior pseudopods of the cell and regulate the polymerization and stabilization of F-actin. It has been shown that the localization of signaling molecules has an excitable feature to amplify cAMP signal inputs. A theoretical analysis shows that such excitability can regulate efficient unidirectional movement of chemotactic cells. Although the previous works have demonstrated the excitability for the PIP3 pathway, it remains to be clarified in terms of the sGC pathway.

Here, we have shown that the sGC pathway is also an excitable system by analyzing the temporal dynamics of sGC localization. Upon pulsed cAMP stimulation, sGC response follows the all-or-none law and has the refractory period, both of which are the important features of the excitable system. In particular, the refractory period of sGC localization is shorter than that of PIP3. This data indicates that sGC pathway has the inherent short inactivation time, which is consistent with more frequent localization of sGC than PIP3 at the pseudopods of spontaneously migrating cells. Moreover, cells lacking PI3K1/2 still show normal sGC localization, suggesting that chemotaxis is regulated by several independent pathways with unique excitable characteristics.

Abstract 12

Dictyostelium Roco proteins to study LRRK2-mediated Parkinson's disease

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Parkinson's Disease (PD) is a neurodegenerative disorder affecting more than five million people worldwide. There is still no treatment for PD and the exact cause is yet unknown. With the advancement in genomics, a number of genetic factors causing PD have been discovered. Mutations in leucine-rich-repeat kinase 2 (LRRK2) are thus far the most frequent cause of late-onset PD, and mutation have been found in both familial and sporadic cases. Despite intensive research much remains unknown about its activity and the effect of PD associated mutations. 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. We use orthologous Roco proteins from all kingdoms of life to elucidate the function and regulation of LRRK2 activity. As in LRRK2, PD-analogous mutations in *Dictyostelium* Roco proteins decrease the GTPase reaction and increase the kinase activity. The structure of *D. discoideum* Roco4 kinase was obtained for wild-type and PD mutants, and explains the G2019S related increased LRRK2 kinase activity. Here we show that *in vivo* both Roco4 and LRRK2 activity rely on a functional N-terminus, dimer/oligomerization and translocation. Furthermore, our data link PD-mutated Roco4 to mitochondrial 'dys'/function. Together our studies with Roco4 give new insights in the complex intra- and intermolecular LRRK2 activation mechanisms.

Abstract 13

CpnA is involved in post-lysosomal trafficking in *Dictyostelium discoideum*

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Copines are a family of well-conserved cytosolic proteins found in many eukaryotic organisms, including humans. Copines contain two C2 domains and are proposed to be involved in an array of cellular processes including cell signaling and vesicular transport. *Dictyostelium discoideum* has six copine genes and cells lacking *cpnA* have been shown to have defects in contractile vacuole function, cytokinesis, and development. GFP-tagged CpnA has been shown to associate with the plasma membrane, endosomes, lysosomes, phagosomes, and contractile vacuoles. To compare endolysosomal vesicles of WT and *cpnA*- cells, we used immunofluorescence to stain p80, an endosomal-membrane protein found on endosomes, lysosomes, contractile vacuoles, and post-lysosomes. We counted the number of post-lysosomes per cell and found that *cpnA*- cells had 38% fewer post-lysosomes than WT. In addition, we co-stained cells with rhodamine phalloidin and p80, which revealed that WT have fewer actin coats associated with post-lysosomes as compared to *cpnA*-. We also conducted a feed-chase experiment by treating live cells with fluorescein isothiocyanate (FITC)- and tetramethylrhodamine (TRITC)-labeled dextrans and imaged cells every 15 minutes over a 2-hour period. The FITC is only fluorescent in the neutral post-lysosome, so that lysosomes appear red and post-lysosomes appear yellow. The number of post-lysosomes per cell were counted at each time interval. Both *cpnA*- and WT cells appeared to develop post-lysosomes at a similar rate with similar numbers of post-lysosomes; however, the *cpnA*- cells had smaller post-lysosomes. These results indicate that CpnA may have a role in post-lysosomal maturation.

Abstract 14

Nuclear envelope organization in the amoebozoan *Dictyostelium discoideum*

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The nuclear envelope (NE) consists of the outer and inner nuclear membrane (INM), whereby the latter is bound to the nuclear lamina. With NE81 we have identified a nuclear lamina protein in the amoebozoan Dictyostelium clearly evolutionarily related to metazoan lamins. Microscopic data indicate that tagged versions of full length NE81 as well as NE81 lacking the nuclear localization signal and/or the lipid modification CaaX-signal are capable of cell cycle-dependent assembly of higher order structures. Field emission scanning EM of NE81 expressed in Xenopus reveales formation of filamentous structures at Xenopus nuclei that are very reminscent of Xenopus lamin B2 expressed in the same system. In parallel His-Myc-tagged NE81 was purified from Dictyostelium. The purified protein was soluble at high-salt conditions, but assembled to paracrystalline higher order structures at low-salt conditions as shown by negative staining EM.

Though proximity-dependent biotin identification (BioID) we could identify two protein interactors of NE81 at the INM, Sun1 and Src1. Src1 is a Dictyostelium homologue of the helix-extension-helix family of proteins, which also includes the human lamin-binding protein MAN1. Both endogenous Src1 and GFP-Src1 localized to the NE during the entire cell cycle and was enriched at regions of nucleolar attachment to the NE. Electron microscopy and light microscopy after differential detergent treatment indicated that Src1 resides in the INM. FRAP experiments with GFP-Src1 cells suggested that at least a fraction of the protein could be stably engaged in forming the nuclear lamina together with the Dictyostelium lamin NE81. Src1 interaction with NE81 could also be confirmed in a mis-localization assay where soluble, truncated mRFP-Src1 localized to artificial clusters consisting of an intentionally mis-localized mutant of GFP-NE81. Expression of GFP-Src1_1-646, a fragment C-terminally truncated after the first transmembrane domain, disrupted interaction of nuclear membranes with the nuclear lamina, as cells formed protrusions of the NE that were dependent on cytoskeletal pulling forces. Protrusions were dependent on intact microtubules but not actin filaments. Our results indicate that Src1 is required for integrity of the NE and highlight Dictyostelium as a promising model for the evolution of nuclear architecture.

Abstract 15

Towards a Mathematical Understanding of Cell Motility using *Dictyostelium discoideum*

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Proper cell motility is vital for organism survival. Dysfunctions in cell motility in unicellular organisms lead to foraging deficiencies and reduced predator evasion. For the slime mold *Dictyostelium discoideum*, dysfunctions in cell motility also interfere with aggregation and morphogenesis. Unfortunately, our understanding of cell motility is based upon studies where cells were free from environmental stressors. In actual biological environments, cells must crawl through cells and endure increased hydrostatic pressure from compaction. Under these conditions, the major mode of cell motility is directed by blebbing. Blebbing involves the membrane detaching from the cortex in the direction of the chemoattractant to facilitate movement. However, the mechanisms behind bleb-driven motility have not been elucidated. In order to better understand this, we intend to create a model of bleb-driven motility based on our hypothesis that bleb formation is induced by the negative curvature of the cell membrane. To measure membrane and cortex curvature during bleb formation, an under agarose cAMP chemotaxis assay was used. The membrane position was visualized by the addition of RITC-dextran to the agarose while the cortex was visualized using wild-type cells expressing LifeAct-GFP. Under our experimental conditions, we were able to induce bleb formation, which we confirmed through ImageJ intensity plots. In addition, we were able to simultaneously determine the positions of both the membrane and cortex throughout bleb formation in real-time. We can now quantify curvature, eventually leading to the generation of a mathematical model and simulations of bleb-based motility.

Abstract 16

The contractile vacuole polarizes to the rear of migrating *Dictyostelium*

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Many cells show distinct changes in morphology as cells polarize and migrate up chemoattactant gradients. *Dictyostelium discoideum* cells are no exception, and reorganize both their actin and microtubule cytoskeletons. In many cell types, the microtubule organizing center and nucleus take up distinct localizations relative to one another in polarized cells. We have discovered that the contractile network (CV) also polarizes during migration, with the CV moving rearward. Highly polarized cells localize the CV to the very rear of the cell. We have confirmed light microscopy observations with fluorescent labeling of the CV by visualizing cells expressing GFP-Dajumin. GFP-Dajumin-labeled CVs clearly redistribute and localize to the rear of cells exposed to gradients of chemoattractant. We hypothesized that this localization might be critical for cell streaming during cAMP-mediated chemotaxis during aggregation. Mutants lacking the Huntingtin protein (Htt nulls) fail to make a contractile vacuole. These Htt null cells have previously been shown to have an inability to regulate their osmotic pressure. Cells lacking a CV make very weak cAMP waves and also don't stream, suggesting that the CV is involved in the release of cAMP from the cell. Adenylyl cyclase (ACA) has been shown to be critical for streaming and has been shown to be concentrated at the rear of the cell. Surprisingly, the cytosolic regulator of ACA (CRAC) is required for cAMP synthesis, but is localized to the leading edge. We are testing whether cAMP is made mostly at the leading edge, then diffuses through the cytosol to the CV and is pumped out. Our data suggest that the CV redistributes to the rear of migrating cells and plays an important role in streaming and may be critical for cAMP secretion.

Abstract 17

Analysis of the effects of the natural product, sulforaphane, on *Dictyostelium* cell proliferation, viability and development.

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Sulforaphane is a natural isothiocyanate compound found in many cruciferous vegetables. Sulforaphane has been studied for its potential anticancer properties working through antioxidant and anti-inflammatory pathways. Our studies of the cellular effects of sulforaphane on *Dictyostelium discoideum* cells (strain AX2) revealed that this compound slows cell proliferation in suspension culture at concentrations above 5 μM. This effect is concentration dependent with further reduction in growth rates exhibited at 20 μM sulforaphane and higher. It is noteworthy that this decrease in proliferation is not associated with either a reduction in cell viability or an increase in multinuclearity. Moreover, cells recovered to normal rates of growth upon removal of sulforaphane from the culture medium. Exposure of cells to sulforaphane also led to delays in multicellular development, with the production of fruiting bodies with shorter stalks than fruiting bodies from untreated cells. Interestingly, development of sulphoraphane-treated cells sometimes resulted in the formation of multicellular intermediate structures that are donut shaped. The cells within these structures actively migrated in a directional manner within the circle. The observed effects of sulforaphane on growth and development were specific, with little or no effect on these processes in cells treated with structurally-related isothiocyanate organosulfur compounds. Further investigations are in progress to identify the specific mechanisms by which sulforaphane exerts its effects on cells, with the goal of gaining insight into the potential for this compound (or derivatives) to be used in cancer prevention or treatment.