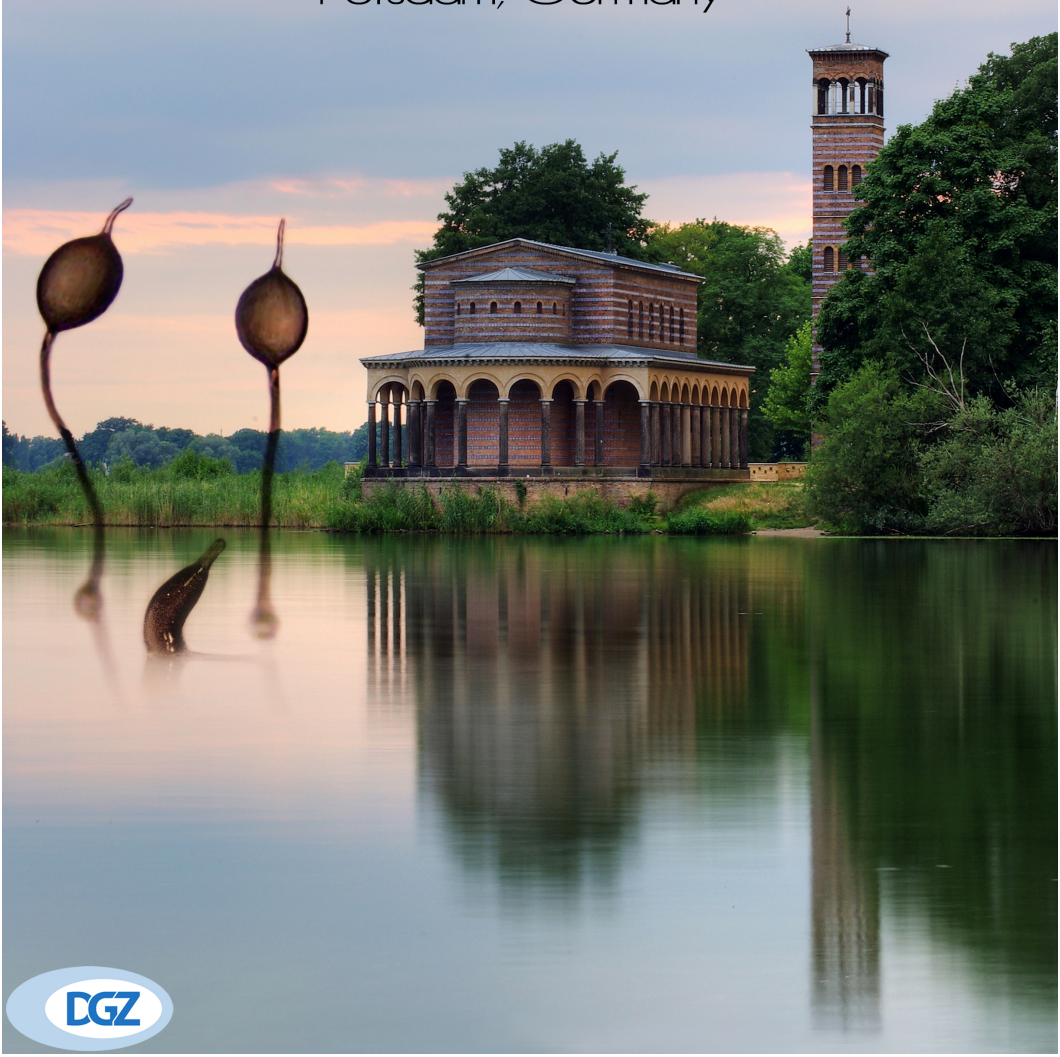
Dicty 2014

Annual International Dictyostelium Conference

August 3rd - August 7th

Potsdam, Germany



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Dicty 2014

Annual International Dictyostelium Conference

August 3 - 7, Potsdam, Germany

Venue:
Seminaris Hotel Potsdam
An der Pirschheide 40
14471 Potsdam

Organizing committee:
Ralph Gräf (Universität Potsdam)
Sascha Thewes (Freie Universität Berlin)
Carsten Beta (Universität Potsdam)

Dicty 2014 - Programme

Sun 3rd	Mon 4th	Tue 5th	Wed 6th	Thu 7th
	9:00 - 10:40 1st morning session Development	9:00 - 10:40 1st morning session Chemotaxis and cell migration	9:00 - 10:40 1st morning session Phagocytosis	9:00 - 10:40 1st morning session Evolution
	coffee break	coffee break	coffee break	coffee break
	11:10 - 12:50 2nd morning session Signaling	11:10 - 12:50 2nd morning session Biophysics of cell migration	11:10 - 12:50 2nd morning session Pathogen interactions I (4)	11:10 - 12:50 2nd morning session Cell Biology
	13:00 - 14:30 lunch break	13:00 - 14:30 lunch break	12:50 - 13:30 luxury coffee break	13:00 - 14:30 lunch
	14:30 - 16:10 1st afternoon session Signaling II/Membrane-associated proteins	14:30 - 16:10 1st afternoon session Nucleus and chromatin	13:30 - 14:20 short afternoon session Pathogen interactions II (2)	Departure
	coffee break	coffee break		
16:00 Registration	16:40 - 18:20 2nd afternoon session Disease models	16:40 - 18:20 2nd afternoon session Gene expression and genomics	Tour to Sanssouci castle and park (busses leave at 14:30 pm)	
18:30 Keynote lecture/Dicty Race				
20:00 Welcome Dinner	18:30 - 20:00 Dinner	18:30 - 20:00 Dinner	19:30 pm Conference Dinner at the Sanssouci castle	
	20:00 Poster Session I	20:00 Poster Session II		

Sunday, August 3

16:00 Registration

18:30 1 Keynote lecture by **Günther Gerisch**

19:30 2 The Dicty World Race
Daniel Irimia

20:00 Welcome Dinner

Monday, August 4

1st morning session: Development; Chair: Tian Jin

9:00 3 Cell signaling during development of Dictyostelium **William F. Loomis**

9:25 4 Allorecognition mediates the transition from unicellularity to multicellularity in D. discoideum **Adam Kuspa**

9:50 5 Genetic analysis of Dictyostelium cell death control **Pierre Golstein**

10:15 6 Investigating 'rhomboid' membrane proteases' roles in development and signalling in Dictyostelium **Mehak Rafiq**

10:40 - 11:10 Coffee break

2nd morning session: Signaling I; Chair: Bill Loomis

11:10 7 The Actions of Chemoattractants Folate and cAMP during Adaptive and Non-Adaptive Signaling Response **Netra Pal Meena**

11:35 8 A GPCR-controlled imperfectly adaptive Ras signaling guides directional responses to a chemoattractant gradient **Tian Jin**

12:00 9 Essential roles for small G-proteins in the regulation of directional cell movement **Rama Kataria**

12:25 10 A novel heterotrimeric G-protein interacting protein (Gip1) and its function in chemotaxis **Yoichiro Kamimura**

13:00 - 14:30 Lunch break

1st afternoon session: Signaling II / Membrane-associated proteins; Chair: Ludwig Eichinger

14:30 11 A putative protein kinase C- like protein affects signaling, cell adhesion, mound morphogenesis and cell type differentiation in Dictyostelium **Wasima Mohamed**

14:55 12 The Dictyostelium discoideum RACK1 orthologue has roles in growth and development **Napoleon N. Omosigho**

15:20 13 The inverse BAR-domain protein IBARa drives membrane remodelling to control osmoregulation, phagocytosis and cytokinesis **Jan Faix**

15:45 14 Of lean and fat Dictyostelium cells: Going through a genetically forced yo-yo diet **Markus Maniak**

16:10 - 16:40 Coffee break

2nd afternoon session: Disease models; Chair: Annette Müller-Taubenberger

16:40 15 Dictyostelium a model system to study LRRK2-mediated Parkinson's disease **Arjan Kortholt**

17:05 16 The Role of Mitochondrial Dysfunction in Dictyostelium Parkinson's Disease Models **Paul R. Fisher**

17:30 17 Tip genes, autophagy and human diseases **Ricardo Escalante**

17:55 18 Dissecting the primordial function of the gamma-secretase complex **Robin S.B. Williams**

18:30 - 20:00 Dinner

20:00 - 22:00 Poster Session I

Tuesday, August 5

1st morning session: Chemotaxis and cell migration: Peter van Haastert

9:00 19 A master protein kinase regulating Dictyostelium chemotaxis **Rob Kay**

9:25 20 Structure function relationships of membrane proteins involved in cell migration **Julia von Bülow**

9:50 21 Ate1-mediated posttranslational arginylation in Dictyostelium discoideum **Annette Müller-Taubenberger**

10:15 22 SILAC-based proteomic quantification of chemoattractant-induced cytoskeleton dynamics **Grzegorz J. Sobczyk**

10:40 - 11:10 Coffee break

2nd morning session: Biophysics of cell migration; Chair: Carsten Beta

11:10 23 Cellular memory in eukaryotic chemotaxis **Wouter-Jan Rappel**

11:35 24 Sorting of Dictyostelium cells in vertically constrained aggregates **Albert Bae**

12:00 25 Dynamics of wave patterns in giant Dictyostelium cells **Matthias Gerhardt**

12:25 26 Three-dimensional organization of actin filaments in propagating waves **Marion Jasnin**

13:00 - 14:30 Lunch break

1st afternoon session: Nucleus and chromatin; Chair: Christian Hammann

14:30 27 Knockout zaps are done on Friday mornings **Douwe M. Veltman**

14:55 28 Developmental Regulation of Nucleosome Positioning and Gene Expression by a CHD Type III Chromatin Remodeling Protein **Alan R. Kimmel**

15:20 29 A compendium of chromatin remodelling complex mutants in Dictyostelium discoideum and the nucleosome positioning roles of the CHD family **Mark E. Robinson**

15:45 30 Nuclear envelope-associated Proteins of Dictyostelium **Petros Batsios**

16:10 - 16:40 Coffee break

2nd afternoon session: gene expression and genomics; Chair: Thomas Winckler

16:40 31 Dissecting transcriptional mechanism using live cell imaging **Jonathan R. Chubb**

17:05 32 RNA mediated RNA regulation by Argonautes and dsRBD proteins **Wolfgang Nellen**

17:30 33 Dicer-like proteins in Dictyostelium **Christian Hammann**

17:55 34 Evolutionary conservation of a small set of developmentally expressed genes in social amoebae **Gernot Glöckner**

18:30 - 20:00 Dinner

20:00 - 22:00 Poster Session II

Wednesday, August 6

1st morning session: Phagocytosis; Chair: Markus Maniak

9:00 35 Dictyostelium WASP: roles in endocytosis and cell polarity, but not in pseudopod formation (plus a note about the evolution of phagocytosis) **Robert Insall**

9:25 36 Biochemical and biological properties of cortexillin III, a component of Dictyostelium DGAP1-cortexillin complexes **Shi Shu**

9:50 37 The identification of axeB, the master regulator of macropinocytosis and phagocytosis **Gareth Bloomfield**

10:15 38 Multiple phases and functions of WASH in the phagocytic cycle **Jason S. King**

10:40 - 11:10 Coffee break

2nd morning session: Pathogen interactions I; Chair: Netra Meena

11:10 39 Impact of Lipid Metabolism on the Infection of Dictyostelium with Mycobacterium marinum **Thierry Soldati**

11:35 40. Take the bitter with the sweet: Discoidins and mycobacterial infection **Ana T. López-Jiménez**

12:00 41 The autophagic machinery ensures non-lytic ejection of mycobacteria and efficient cell-to-cell transmission **Rachel Pilla**

12:25 42 Dictyostelium discoideum as a host model to study the interaction with pathogenic and apathogenic yeast **Sascha Thewes**

12:50 - 13:30 Luxury coffee break

1st afternoon session: Pathogen interactions II; Chair: Netra Meena

13:30 43 Autophagy 16 and 9 mutants have similar endocytosis defects but deficiencies in development and proteasomal activity are much more severe in the double mutant **Qiuhong Xiong**

13:55 44 Functional characterization of Dictyostelium iron transporters Nramp1 and Nramp2, involved in resistance to bacterial infection and in iron homeostasis: an update **Salvatore Bozzaro**

14:30 Bus call for **Sanssouci Tour**

15:00 Tour through the castle and park Sanssouci

17:00 Free time to visit the park and downtown area

19:30 **Conference Dinner** at the Mövenpick Restaurant "Zur historischen Mühle" at the historic wind mill.

22:45 Bus call for transfer to the Seminaris hotel

Thursday, August 7

1st morning session: Evolution; Chair: Pauline Schaap

9:00 45 Eastern Japanese Dictyostelia species adapt while populations exhibit neutrality **Shun Adachi**

9:25 46 Functional evolution of adenylate cyclase R and adenylate G in Dictyostelia **Yoshinori Kawabe**

9:50 47 Trade-offs and the illusion of social success in Dictyostelium discoideum **Christopher R.L. Thompson**

10:15 48 Divide and conquer: vegetative conflict in D. discoideum **Balint Stewart**

10:40 - 11:10 Coffee break

2nd morning session: Cell biology / Organelle function; Chairs: Ralph Gräf/Sascha Thewes

11:10 49 CP161, a new component of the centrosome **Salil K. Sukumaran**

11:35 50 Characterization of four novel core components of the Dictyostelium centrosome **Irene Meyer**

12:00 51 Adaptations in the proteostasis network of the social amoebae Dictyostelium discoideum cause an unusual resilience to protein aggregation **Liliana Malinovska**

12:25 52 Peptidyl prolyl cis-trans isomerases (PPIases): a study in Dictyostelium discoideum **Aruna Naorem**

13:00 Lunch

14:30 Departure

Talks (in the order of appearance in the programme)

1

Self-organized wave patterns on the substrate-attached cell surface

Günther Gerisch 1, Mary Ecke 1, Marion Jasnin 1, Britta Schroth-Diez 2

1 Max Planck Institute of Biochemistry, 82152 Martinsried, Germany
2 Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany

On the substrate-attached surface of Dictyostelium cells dynamic wave patterns are formed that resemble the leading edges of chemotaxing cells in phosphoinositide composition, Ras activation and actin organization (1). The distinct feature of the wave patterns is their self-organizing capacity (2). Since these patterns develop on a planar glass surface, they are optimally suited to image state transitions in the plasma membrane and the cortical actin layer. In particular, the spatiotemporal relationship of interacting components during switches from a “front like” to a “tail-like” state and vice versa can be quantitatively analyzed (3). We will explore the patterns of transition in the membrane and the role of the actin system in their generation.

The waves on a substrate-attached cell surface resemble the organization of a phagocytic cup, suggesting that they are involved in recognizing surface geometry. Consequently, we imaged cells that migrate on a structured surface and found that it is primarily the area encircled by an actin wave that responds to curvature of the substrate. This inner territory is known to be enriched in PIP3 and the Arp2/3 complex.

References

1. Gerisch, G., Ecke, M., Wischnewski, D., and Schroth-Diez, B.: Different modes of state transitions determine pattern in the Phosphatidylinositide-Actin system. BMC Cell Biology 12: 42 (2011).

2. Bretschneider, T., Diez, S., Anderson, K., Heuser, J., Clarke, M., Müller-Taubenberger, A., Köhler, J., and Gerisch, G. (2004). Dynamic actin patterns and Arp2/3 assembly at the substrate-attached surface of motile cells. Current Biology 14, 1-10.

3. Gerisch, G., Schroth-Diez, B., Müller-Taubenberger, A., Ecke, M., : PIP3 Waves and PTEN Dynamics in the Emergence of Cell Polarity. Biophys. J. 103 (6), 1170-1178 (2012).

The Dicty World Race

Daniel Irimia, Monica Skoge, Christopher Janetopoulos, Elisabeth Wong, Bashar Hamza, Albert Bae, Joseph M. Martel

Massachusetts General Hospital, Boston, MA,
University of California, San Diego, CA
Vanderbilt University, Nashville, TN

Neutrophils ability to move is critical for our protection against infections but can also facilitate the destruction of normal tissues during chronic inflammation. Thus, any intervention to accelerate responses against microbes or reduce organ damage has to be precise and calibrated. To accelerate the development of such interventions, which are not available today, we are designing novel tools, based on microfluidic technologies that enable the characterization of cell motility phenotype with high precision. For example, by confining moving neutrophils into microfluidic channels we can decouple the measurement of speed from persistence, and by implementing bifurcations in these channels we can quantify the directionality based on series of unique decisions. So far, these tools helped us characterize the defects of neutrophil migration after burns, optimize treatments to improve animal survival after sepsis, and characterize compounds that might reduce chronic inflammation without impairing antimicrobial defenses. However, the goal of finely tuning neutrophil migration in various clinical conditions remains elusive. To better engage the science community into these efforts and increase the awareness for the latest technologies for measuring neutrophil migration, we organized the first ever Dicty World Race, comparing Dictyostelium discoideum (aka Dicty) and neutrophil-like HL60 cells. Where Dicty cells shine in precision, they lag in speed, and where HL60 are good sprinters, they lag in precision. Thus, we asked laboratories around the world to prepare their fastest and smartest cells. 20 teams of scientists worldwide took on the challenge and contributed to the race on May 16 in Boston. The first Race, the winners, and the learning from the first race will be presented.

Cell signaling during development of Dictyostelium

William F. Loomis

Cell and Developmental Biology, University of California San Diego, La Jolla, CA 92093; e-mail: wloomis@ucsd.edu

The order and tempo of events is critical to multicellular development and is carefully monitored by regular checks and balances as development progresses. Later stages depend on earlier stages in a dependent sequence that is rigidly maintained by natural selection. Development in Dictyostelium discoideum is simple enough that it can be approached in a systems manner. Progression through the developmental stages is regulated by intercellular signals that include proteins, peptides, amino acids, nucleic acids, steroids and polyketides. Quorum effectors signal the presence of sufficient cells to warrant transition from growth to differentiation. Some of the products induced by early signaling allow the cells to enter subsequent stages. cAMP, DIF, adhesion proteins and differentiation factors control subsequent timing through positive and negative feedback loops, coherent and incoherent feedforward loops, signal amplification, double negatives and coincidence detectors. Following aggregation the cells diverge into two specialized cell types that continue to signal each other in complex ways to form the spores and stalk cells of well proportioned fruiting bodies.
Mammalian embryogenesis can be considered as probabilistic navagation of an increasingly divided landscape as suggested by C. H. Waddington (1942). In other cases, it might be productive to consider the interplay of kinetic equilibria. Regenerative medicine is based on the hope that we can learn to program pluripotent stem cells through the appropriate stages to reproducibly differentiate into specific cell types that can replace missing or diseased cells. Learning how to program stem cells rapidly and efficiently may benefit from studies on the regulation of differentiation in Dictyostelium.

Allorecognition mediates the transition from unicellularity to multicellularity in D. discoideum

Shigenori Hirose 1, Balaji Santhanam 2,3, Mariko Katoh-Kurosawa 2, Gad Shaulsky 2,3, Adam Kuspa 1,2

1 Verna and Marrs McLean Department of Biochemistry and Molecular Biology; 2 Department of Molecular and Human Genetics; 3 Structural and Computational Biology and Molecular Biophysics Program, Baylor College of Medicine, Houston, TX 77030, USA

In D. discoideum mound formation marks the first instance of cell coalescence into a single tissue, so it is a critical event in the establishment of multicellularity as the cells begin to act as an integrated organism. We have described an allorecognition system that promotes the cooperation of cells with close relatives during development (1-4). The allorecognition proteins TgrB1 and TgrC1 are polymorphic in nature and mediate heterotypic cell-cell adhesion (2, 5-7), providing a possible mechanism for the cooperation observed in this system (1, 8-10). We tested the role of allorecognition in the acquisition of multicellular properties using isogenic strains with divergent tgrB1/tgrC1 allele-pairs and therefore distinct allotypes. When strains with incompatible allotypes are mixed, cells of each type stream together into admixed mounds, but then segregate into distinct organisms and develop separately (3). Allorecognition is not required for the cell streaming behavior that forms the initial mound, but it is required to maintain the mound through the transition to multicellularity. By examining the behavior of cells with incompatible allotypes compared to the interaction of cells with compatible allotypes we now show that TgrB1 and TgrC1 are required for cell polarization, coordinated cell movement, and cell differentiation during mound formation. Our results show that allorecognition controls the integration of individual cells into a unified organism and acts as a gating step for multicellularity in D. discoideum.

1. Ostrowski, et al., PLoS Biol 6, e287(2008).
2. Benabentos, et al., Current biology 19, 567(2009).
3. Hirose, et al., Science 333, 467(2011).
4. Ho, et al., Current biology 23, 1590(2013).
5. Wang, et al., Devel. Biol. 227, 734(2000).
6. Chen, et al., The Biochemical J 452, 259(2013).
7. Chen, et al., The Biochemical J 459, 241(2014).
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9. Mehdiabadi, et al., Nature 442, 881(2006).
10. Gilbert, et al., PNAS 104, 8913(200

Genetic analysis of Dictyostelium cell death control

Yu Song, Marie-Françoise Luciani, Corinne Giusti, Pierre Golstein

Centre d'Immunologie de Marseille-Luminy

Most investigations on cell death have dealt with caspase-dependent apoptosis, which is limited to animal cells. No major systematic effort has been made to analyze cell death in the vast majority of extant species, outside the animal kingdom. We are analyzing molecular bases of developmental cell death in the protist Dictyostelium discoideum. The genome of this model organism encodes no member of the caspase or the bcl-2 families. Also, it is haploid, lending itself well to genetic studies. We described phenomenological steps of this cell death, including ultimate vacuolization. Induction of this cell death proceeds in two stages. In a first stage, starvation both sensitizes the cell to the second stage and triggers autophagy which protects cells from premature starvation-induced death. In a second stage, the polyketide DIF-1 1 or the cyclic dinucleotide c-di-GMP 2 can induce in these starvation-sensitized cells (a) pathway(s) leading to developmental vacuolar cell death. We identified a number of genes required for this cell death and mapped the site of their requirements 3. The results to be reported include a brief description of successive steps of this developmental cell death as mimicked in vitro, and mostly an analysis of the corresponding signaling pathways with markers thereof including required molecules recently identified through insertional and targeted mutagenesis.

1 Morris, H. R., Taylor, G. W., Masento, M. S., Jermyn, K. A. & Kay, R. R. Nature 328, 811-814 (1987).
2 Chen, Z. H. & Schaap, P. Nature 488, 680-683, doi:10.1038/nature11313 (2012).
3 Giusti, C., Tresse, E., Luciani, M.-F. & Golstein, P. Biochimica Biophysica Acta: Molecular Cell Biology 1793, 1422-1431 (2009).

Investigating 'rhomboid' membrane proteases' roles in development and signalling in Dictyostelium

Mehak Rafiq 1, David Traynor 2, Elinor Thompson 1

1 University of Greenwich, Medway Campus, Chatham Maritime
2 MRC-LMB, Cambridge, UK

The rhomboid family of intramembrane serine proteases is almost ubiquitous across all kingdoms of life. The enzymes are poorly conserved at sequence level but are similar in their structures and active-site motifs. They cleave disparate substrates, and it is the role of rhomboids across evolution that seems to be better conserved, since a membrane location means they are ideally placed for signalling and proteolytic activation events. Thus, we are investigating rhomboid function in the development and chemotaxis of Dictyostelium discoideum, the microbial, biomedical model organism that is capable of unicellular vegetative growth and multicellular development. A small group, including four apparently enzymatically active, rhomboids was identified in Dictyostelium. We found that development was unaltered following deletion of rhmC, whereas attempts to knock out the putative mitochondrial rhmD proved lethal. rhmA and rhmB null mutants give rise to changes in development, the lack of RhmA altering the response to chemoattractants and decreasing motility of the multicellular 'slug'. rhmB null cells have lower viability, a smaller spore-sorus and a decreased response to folic acid stimulation. These results correspond with qRTPCR analysis, in which rhmA and rhmB transcript levels are highest during the multicellular growth phase. TEM intriguingly suggests a role in mitochondrion ultrastructure for RhmA, supported by bioinformatic interaction networks. These reveal Dictyostelium RhmA cotranscription with homologues of the Saccharomyces cerevisiae mitochondrial rhomboid's substrates, which also regulate mitochondrial morphology.

The Actions of Chemoattractants Folate and cAMP during Adaptive and Non-Adaptive Signaling Response

Netra Pal Meena, Alan R. Kimmel

National Institutes of Health, Bethesda, MD, USA

In the natural environment, bacteria are one of the crucial food sources for Dictyostelium. Dictyostelium chemotax towards bacterially-derived folate, and eventually engulf the bacteria to fill their nutritional requirements. On the other hand, aggregating Dictyostelium are responsive to endogenously secreted cAMP. Dictyostelium have developed complex mechanisms to enable detection of these chemical gradients and to ensure positive cellular movement during chemotaxis. First, secreted enzymes specifically degrade either folate or cAMP to maintain gradient directionality and to limit concentrations at the cell surfaces for maximal receptor sensitivity. In addition, cells respond in both adaptive and non-adaptive modes, which reinforce and guide signal responses only within positive gradients. The high activities of the folate/cAMP degrading enzymes have made it difficult to directly examine adaptive responses to non-varying concentrations of the chemoattractants. For cAMP, the use of specific analogs and enzyme inhibitors has helped re-define the pathways. But similar analyses have not been applied to folate. Here, we have re-examined the kinetics of folate degradation and developed defined conditions to investigate adaptive and non-adaptive pathways for folate response and the role of G proteins.

A GPCR-controlled imperfectly adaptive Ras signaling guides directional responses to a chemoattractant gradient

Tian Jin

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GPCR-mediated Ras activation is a key signaling step for eukaryotic cells to generate temporal adaptation or to cause spatial amplification in response to spatially uniform stimuli or a chemoattractant gradient. It is not clear how a GPCR/G-protein machinery regulates Ras activation to achieve these cellular responses. Here, using quantitative live cell imaging methods, we measured spatiotemporal dynamics of Ras activation in Latrunculin treated Dictyostelium discoideum cells in response to various cAMP stimuli. A uniform activation of cAR1-GPCR triggers a transient Ras activation, followed by an imperfect adaptation of Ras signaling. In response to a two-step increase in the concentration of uniformly applied cAMP stimuli, while G-protein displays a step-like activation, Ras shows two transient activations with imperfect adaptions, and PIP3 production shows two transient responses followed by perfect adaptions. In cells that lack polarity and functional actin cytoskeleton, a cAMP gradient induces a transient Ras activation that quickly adapts to lower levels around cell membrane. There is no clear spatial amplification at the step of Ras activation between the front and back, which is in clear contrast to the spatially amplified PIP3 response in the front. Our study shows that cAR1-mediated signaling network encodes information in dynamic patterns of signaling steps; and these dynamics provide readout for understanding how these steps are mechanistically linked to produce chemotactic responses.

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

Rama Kataria, Ineke Keizer-Gunnink, Peter J.M. van Haastert, Arjan Kortholt

Department of Cell Biochemistry, University of Groningen, The Netherlands

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

A novel heterotrimeric G-protein interacting protein (Gip1) and its function in chemotaxis

Yoichiro Kamimura, Yukihiro Miyanaga, Masahiro Ueda

Laboratory for Cell Signaling Dynamics, QBiC, RIKEN
Laboratory of Single Molecule Biology, Osaka University

Chemotaxis is a fascinating cellular behavior that combines motility with the perception of a chemical gradient. Dictyostelium discoideum displays a typical chemotactic response to cAMP during the early stages of multicellular development. Chemoattractants are recognized by a sensor module which sends signals via several signaling modules to a cellular motility module. The trimeric G protein, Gα2 and Gβγ, is an essential component of the sensor module and we are interested in how it processes chemical information. Recently, new interacting proteins of trimeric G proteins including ElmoE and Ric8 have been found but the precise mechanism of regulation is still unknown. We sought new regulators of G-protein by the tandem affinity purification (TAP) assay of Gα2, Gβ, and Gγ subunits. The elution fraction of Gβ-TAP contained a novel protein in addition to Gα, Gγ, and ElmoE, designated Gip1 (trimeric G prtotein interacting protein 1). Although cells lacking this novel factor can complete development to form fruiting bodies, the early aggregation pattern was abnormal. Closer examination showed that chemotaxis was abrogated, especially at the higher concentrations of cAMP. Biochemical analysis has revealed that complex formation between Gα and Gβγ is reduced in gip1- cells. These data suggest that Gip1 may regulate the stability of the G protein complex which is important for biological outputs including chemotaxis.

A putative protein kinase C- like protein affects signaling, cell adhesion, mound morphogenesis and cell type differentiation in Dictyostelium

Wasima Mohamed, Baskar Ramamurthy

Indian Institute of Technology- Madras, Chennai, Tamil Nadu, India

Protein kinase C (PKC) is a major phosphorylating enzyme involved in diacyl glycerol (DAG) mediated signal transduction and influences numerous processes from transcriptional regulation to differentiation, apoptosis and cell fusion. PKCs are animal specific and are absent in plants. A gene encoding PKC domain containing protein has been identified in the Dictyostelium genome (DDB_G0288147) and we generated the corresponding mutant by homologous recombination. pkc- forms loose aggregate with a prominent hollow in the center which progresses to form a flat mound. A prominent phenotype of pkc- is in the formation of multiple tips that arise precociously from the flat mounds and progresses to form migrating slugs and fruiting bodies which however resemble the wild type. The phenotype of pkc- resembles the already known mutants lagC- (late aggregate C) and gbf- (G-box binding factor) which fail to establish a dominant signaling wave center during mound morphogenesis. However, pkc- completes development unlike lagC- and gbf-, possibly due to increased cAMP levels. pkc- are defective in the expression of cell adhesion proteins such as CadA and CsaA. Reconstitution with wild type cells, cell type specific marker expression and stalk cell induction studies reveal that PKC plays role in cell fate determination. When Dictyostelium cells were treated with specific inhibitors of PKC, mounds with multiple tips arose in a dose dependent manner strikingly resembling the pkc-. Transcriptional regulation of post aggregative genes and substrate identification would help in understanding key steps of transition during mound morphogenesis.

The Dictyostelium discoideum RACK1 orthologue has roles in growth and development

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2 Anatomy III – Cell Biology, Ludwig Maximilian University of Munich, Schillerstr. 42, 80336 Munich, Germany

The receptor for activated C-kinase 1 (RACK1) is a conserved protein belonging to the WD40 repeat family of proteins. It folds into a beta propeller with seven blades which allow interactions with many proteins. Thus it can serve as a scaffolding protein and have roles in several cellular processes. We identified the product of the Dictyostelium discoideum gpbB gene as the Dictyostelium RACK1 homolog. The protein is mainly cytosolic but can also associate with cellular membranes. DdRACK1 binds to phosphoinositides (PIPs) in protein-lipid overlay and liposome-binding assays. The basis of this activity resides in a basic region located in the extended loop between blades 6 and 7 as revealed by mutational analysis. Similar to RACK1 proteins from other organisms DdRACK1 interacts with G protein subunits alpha, beta and gamma as shown by yeast two-hybrid, pulldown, and immunoprecipitation assays. Unlike the Saccharomyces cerevisiae and Cryptococcus neoformans RACK1 proteins it does not appear to take over Gbeta function in D. discoideum as developmental and other defects were not rescued in Gbeta null mutants overexpressing GFP-DdRACK1. Overexpression of GFP-tagged DdRACK1 and a mutant version (DdRACK1mut) which carried a charge-reversal mutation in the basic region in wild type cells led to changes during growth and development. DdRACK1 interacts with heterotrimeric G proteins and can through these interactions impact on processes specifically regulated by these proteins.

The inverse BAR-domain protein IBARa drives membrane remodelling to control osmoregulation, phagocytosis and cytokinesis

Joern Linkner[1], Gregor Witte[2,], Hongxia Zhao[3], Alexander Junemann[1], Benjamin Nordholz[1], Petra Runge-Wollmann[2], Pekka Lappalainen[3] and Jan Faix[1]

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As opposed to the comparatively well characterized BAR and F-BAR domain proteins that promote the formation of plasma membrane invaginations, the cellular functions of I-BAR domains that induce membrane protrusions are not well understood. Here, we analyzed the single I-BAR family member IBARa from *Dictyostelium*. The X-ray structure of the N-terminal I-BAR domain solved at 2.2 Å resolution revealed an all-a helical structure that self-associates into a zeppelin-shaped antiparallel dimer and are consistent with its shape in solution obtained by small-angle X-ray-scattering. Cosedimentation, fluorescence-anisotropy as well as fluorescence and electron microscopy revealed the I-BAR domain to bind preferentially to phosphoinositide-containing vesicles and drive the formation of negatively curved tubules. Immunofluorescence labelling further showed accumulation of endogenous IBARa at the tips of filopodia, the rim of constricting phagocytic cups, in foci connecting dividing cells during the final stage of cytokinesis, but most prominently at the osmoregulatory contractile vacuole (CV). Consistently, IBARa-null mutants displayed defects in CV formation and discharge, growth, phagocytosis and mitotic cell division, whereas filopodia formation was not compromised. Of note, IBARa-null mutants were also strongly impaired in cell spreading. Together, these data suggest IBARa to constitute an important regulator of numerous cellular processes intimately linked with the dynamic rearrangement of cellular membranes.

Of lean and fat Dictyostelium cells: Going through a genetically forced yo-yo diet

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If grown axenically, Dictyostelium amoebae completely fail to build one cellular organelle, the lipid droplet. As soon as free fatty acids are added to the growth medium, or amoebae are fed with bacteria, fat synthesis begins and lipid droplets are formed.
As a prerequisite for fat synthesis, fatty acids are activated by Coenzyme A, mainly by the activity of FcsA. The corresponding KO mutant shows reduced fat accumulation, which is even further decreased in cells that also lack the Dictyostelium bubblegum homologue. FcsC may also contribute to the consumption of bacterial lipids, as suggested by a reduced colony diameter on a bacterial lawn.
In the first step of fat synthesis, one Coenzyme A-activated fatty acid is linked to Glycerol-3-Phosphate, mainly through the activity of GPAT3, because the KO mutant has a strikingly “lean” phenotype. Two candidate enzymes for conducting the addition of the second fatty acid, AGPAT3 and PLSC1, failed to affect fat synthesis if knocked out singly or in combination. The third fatty acid is added by enzymes called DGATs. Whereas DGAT2 provides the main activity in mammals, DGAT1 clearly is the dominant enzyme activity in Dictyostelium. Although no defects were observed in a DGAT2 KO, this protein can fully restore the defects seen in a DGAT1 KO, if overexpressed.
Most of the enzymes required for fat synthesis reside in the Endoplasmic Reticulum, when fatty acids are scarce, but move to the surface of lipid droplets only when fatty acids are provided in excess and these fat stores are formed. We saw this dual localization also for many other lipid droplets constituents, among these a number of proteins with as yet unknown functions. This step of protein trafficking is remarkable, because the ER is delimited by a normal membrane bilayer, whereas the hydrophobic core of fat in the lipid droplet is surrounded by just one membrane leaflet. We are currently investigating the basis of this protein redistribution in vivo and in vitro.

Dictyostelium a model system 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 human Leucine-Rich-Repeat Kinase 2 (LRRK2) have been found to be thus far the most frequent cause of late-onset PD. LRRK2 belongs to the Roco family of proteins, which are characterized by the presence of a Ras-like G-domain, called Roc and a kinase domain. Pathogenic mutations in LRRK2 result in decreased GTPase activity and enhanced kinase activity, suggesting a possible PD-related gain of abnormal function.
We use Dictyostelium Roco proteins as model to study the function and activation mechanism of LRRK2. Mutant studies reveal a link between LRRK2/Roco dysfunction and mitochondrial disease. The structure of Dictyostelium Roco4 kinase was obtained for wild-type and PD mutants, and explains the G2019S PD-related increased LRRK2 kinase activity. Importantly, the strong inhibition phenotype in vivo and Roco4 kinase structures in complex with inhibitors provide excellent tools to optimize the current and identify new LRRK2 inhibitors.

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The Role of Mitochondrial Dysfunction in Dictyostelium Parkinson's Disease Models

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Impaired mitochondrial function is believed to play a central role in the cytopathology of Parkinson's Disease (PD). We have studied the phenotypic outcomes and mitochondrial dysfunction associated with 3 different PD proteins - alpha-synuclein (a-syn) , DJ-1 and HtrA2.
Dictyostelium does not have a native homologue of a-syn, but ectopic expression of human wild type, A53T point mutant or C-terminally truncated forms of human a-syn was cytotoxic to Dictyostelium cells. The a-syn was not associated with the mitochondria, but was localized in the cell cortex where it caused defects in phagocytosis and growth on bacteria, as well as slightly deranged fruiting body morphogenesis and a mild impairment of phototaxis. No mitochondrial respiratory dysfunction was detectable. Although alpha-synuclein is toxic to Dictyostelium cells, it does not exert this toxicity by impairing mitochondrial function.
Dictyostelium DJ-1 was overexpressed or knocked down and the phenotypic outcomes studied in the presence or absence of H_2O_2 (oxidative stress). Under oxidative stress, DJ-1 translocated to the mitochondria where it exerted a protective effect, ameliorating defects in phototaxis, morphogenesis (thickened stalks), pinocytosis, phagocytosis and growth. The knockdown phenotypes were rescued partially or fully by AMPK antisense inhibition in the same cells. The results suggest a protective mitochondrial role for DJ-1 in oxidatively stressed cells.
HtrA2 is a mitochondrial serine protease. Under conditions of oxidative stress, knockdown of Dictyostelium HtrA2 dramatically impaired pinocytosis, phagocytosis, and growth in liquid. Phototaxis remained unaffected. Overexpression of catalytically inactive HtrA2 showed that the protein translocates from the mitochondria to the cytosol in response to oxidative stress and that it caused similar phenotypes to HtrA2 knockdown. The results suggest a protective, catalytic role for HtrA2 in the cytoplasm of oxidatively stressed cells.

Tip genes, autophagy and human diseases

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Deficient autophagy causes a distinct phenotype in Dictyostelium discoideum, characterized by the formation of multitips at the mound stage. This lead us to analyze autophagy in a number of multitipped mutants described previously at William Loomis laboratory (tipA–, tipB– , tipC– and tipD–). We found a clear autophagic dysfunction in tipC – and tipD– while the others showed no defects. tipD codes for a homologue of Atg16, which confirm the role of this protein in Dictyoselium autophagy and validates our approach. tipC, on the other hand, codes for a protein whose role in autophagy had not been described previously. It shows high similarity to human VPS13A (also known as Chorein), whose mutations cause the disease Chorea-acanthocytosis. Here we show that Dictyostelium cells lacking tipC show a reduced number of autophagosomes in starvation as determined by the markers GFP-Atg18 and GFP-Atg8. A proteolytic cleavage assay also showed that autophagy degradation was impaired in these cells. Of interest, the expression of a C-terminus fragment of TipC containing a region of similarity to Atg2 largely complement the mutant phenotype with the formation of differentiated stalk and spore cells. We have downregulated VPS13A in human HeLa cells by RNA interference and confirmed the role of the human protein in autophagy.

Dissecting the primordial function of the gamma-secretase complex

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The γ-secretase complex plays a key role in the progression of Alzheimer's disease. Research into the function of the complex in mammalian systems has been difficult, since deletion of components of the complex cause lethality at an early embryonic stage. The complex is comprised of four proteins: Anterior pharynx-defective-1 (Aph-1), Nicastrin, Presenilin enhancer 2, and one of two Presenilins (Psen1/Psen2). The complex has been suggested to have both proteolytic and structural functions, but little is known regarding the latter function.

We have set out to develop *Dictyostelium* as a model system to understand the primordial function of the γ-secretase complex. *Dictyostelium* contains the core components of the complex including two presenilin homologues (PsenA and PsenB). In our previous work[1,2] we have shown that deletion of both presenilin genes leads to a severe developmental block that is rescued by the human Psen1 protein. This developmental role is independent of the proteolytic function since deletion of key catalytic residues within the human or *Dictyostelium* presenilins does not compromise this developmental rescue. We also showed that both human and *Dictyostelium* presenilin proteins localise to the ER and nuclear envelope - in agreement with their localisation in mammalian cells. We now show that Aph1 also localises to the ER, suggesting that the complex forms in *Dictyostelium*. We also show that expression of the human Psen2 protein, unlike Psen1, only partially rescues development. We are continuing this project to identify binding partners for presenilins (human and *Dictyostelium*), and to develop assays for catalytic activity based upon Notch cleavage.

This study will therefore develop our understanding of the ancient function of the g-secretase complex in *Dictyostelium*, and will enable us to dissect the proteolytic and structural functions of the complex in basic cell function which has not been possible in animal models.

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A master protein kinase regulating Dictyostelium chemotaxis

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Every year new chemotaxis genes are discovered, suggesting that we are still well short of having a complete parts list for this process. Genetic screens have revealed many of the key chemotactic genes discovered to date, but struggle where there is redundancy, or the gene is essential for viability or early development. Over the last 5 years we have adopted an alternative approach based on phosphoproteomics and the assumption that many proteins involved in chemotaxis will be regulated by phosphorylation in response to chemotactic stimulation.
Using SILAC and phosphopeptide enrichment, we are able to measure changes in phosphorylation of a good proportion of the proteome in a single, albeit extended, mass-spectroscopic experiment. In this way we have identified more than 100 proteins whose phosphorylation increases by at least 3-fold after cAMP stimulation. These fall into distinct kinetic classes, with one group phosphorylated rapidly, another with a delay of around 10 seconds and a third whose phosphorylation does not adapt, being maintained after 6 minutes. However, the cellular response to cAMP is complex, and includes effects on gene expression and cAMP relay, as well as chemotaxis and it is not clear into which realm many of the phosphorylated proteins fall.
We therefore also examined protein phosphorylation in response to folic acid, which is chemotactic for growing cells, and found that a surprisingly short list of about 30 proteins are phosphorylated in response to both chemoattractants. This list of candidate ‘core’ chemotactic proteins is remarkable in that more than half of them are phosphorylated at a single peptide consensus site, suggesting that a single master protein kinase may be responsible. We have now identified this protein kinase.

Structure function relationships of membrane proteins involved in cell migration

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Cell migration is central in various human processes, such as the immune response and the pathology of cancer [1]. There is evidence that aquaporin (AQP) water channels and tetraspanin (Tsp) membrane proteins are involved in the formation of lamellipodia, i.e. a prerequisite of cell motility. We are studying structure function relationships of AQPs and Tsps with the aim to evaluate their potential as new drug targets using Dictyostelium discoideum amoeba as a model. We found two aquaporins, AqpB and AqpC, to be present in the amoeba state. AqpB turned out to be a water-selective channel that is gated by an extraordinary long intracellular loop [2]. We identified a single amino acid exchange to open the channel, suggesting that tyrosine phosphorylation may initiate gating. Overexpression of permanently active AqpB impaired cell volume regulation of the amoeba upon hypoosmotic stress. Growth and development of an AqpB– strain appeared normal. However, random motility of the amoeba was markedly reduced. Currently we are investigating effects on chemotaxis and the basic biochemical properties of the concurrently expressed AqpC. Besides we detected expression at the cDNA level of TspA, TspC, and TspD, i.e. three of the five genomically encoded Dictyostelium Tsps. We produced antisera against parts of the large extracellular loops. However, the resulting antibodies did not recognize all of the native proteins. We localized the Tsps as GFP fusion proteins in amoeba to internal, vesicular structures and to the plasma membrane. The generation of single, double and triple gene knockout strains is underway with the aim to assess the cellular role of Tsps. We envision to use Dictyostelium amoeba as a model to study human Tsps. A secondary focus is the production of functional recombinant proteins for crystallization and the study of protein-protein interactions.

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Ate1-mediated posttranslational arginylation in Dictyostelium discoideum

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The highly conserved enzyme arginyl-tRNA-protein transferase (Ate1) mediates arginylation, a posttranslational modification that is not well understood at its molecular level. In fibroblasts, arginylation of ß-actin was described to modulate actin filament properties, ß-actin localization, and lamella formation and thus regulates motility. More recent studies have provided evidence that posttranslational arginylation is involved in a number of important processes such as heart development, angiogenesis and tissue morphogenesis in mammals.
In order to test whether arginylation plays a role in actin-dependent and other processes in Dictyostelium discoideum, we knocked out the gene encoding Ate1 and investigated the phenotype of ate1-null cells. Similar to mouse fibroblasts deficient for ate1, the Dictyostelium ate1-null cells were smaller in size compared to wild type. Visualization of actin cytoskeleton dynamics by live cell microscopy using markers for filamentous actin indicated significant changes in comparison to wild-type cells. Ate1-null cells lacked almost completely the actin adhesion sites at the substrate-attached surface and were only weakly adhesive. In chemotaxis assays towards folate, the motility of ate1-null cells was increased. We could also show that the ate1-null phenotype can be mimicked by the small-molecule inhibitors tannic acid and merbromin.
Interaction studies identified several potential proteins including actin that interact either directly or indirectly with Ate1. We currently test whether actin itself is arginylated in Dictyostelium and whether the differences observed in the organization of filamentous actin structures are directly or indirectly linked to arginylation.

SILAC-based proteomic quantification of chemoattractant-induced cytoskeleton dynamics

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Cytoskeletal dynamics during cell behaviours ranging from endocytosis, exocytosis to cell division and movement is controlled by a complex network of signalling pathways, the full details of which are as yet unresolved. We have shown that SILAC based methods can be used to characterise the rapid chemo-attractant induced dynamic changes in the actin-myosin cytoskeleton and regulatory elements on a proteome wide scale with a second time scale resolution. This novel unbiased proteomic approach to quantify changes in the composition of the cortical cytoskeleton, required establishment of the SILAC procedure for use in Dictyostelium. This approach provides novel insights in the ensemble kinetics of key cytoskeletal constituents and association of known and novel identified binding proteins. The proteomic incorporation data were confirmed by detailed microscopy based analysis of in-vivo translocation dynamics for key signalling factors.
There are several important general conclusions about cytoskeletal dynamics that can be drawn from the SILAC dataset. Firstly it is very clear, based on protein abundance and incorporation dynamics, that the Arp2/3 complex is the main nucleator of actin filaments in both phases of cAMP dependent actin polymerisation. We can also conclude that the Cap32/34 complex is the main actin filament capping factor in chemotaxing cells. We detected three major temporal patterns of incorporation of the cytoskeletal proteins. The most common pattern is an actin-like biphasic profile. The other two patterns are formed by proteins that show enrichment mainly during either the first or the second phase of actin polymerisation. These patterns are less frequent but very informative since they may suggest a specific role of the relevant proteins in one of the two phases. DockA and DockB Rac GEF's were among the signalling factors that showed the strongest incorporation during the first phase of actin polymerisation.

Cellular memory in eukaryotic chemotaxis

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We use microfluidic devices to examine the presence of cellular memory in chemotaxing Dictyostelium cells. In one set of experiments, we quantify cell motion and localization of a directional-sensing marker after rapid gradient switches. In a second microfluidic device, we expose cells to traveling waves of chemoattractant with varying periods. These experiments reveal that cells exhibit cellular memory and that they maintain direction towards the wave source in the back of the wave for the natural period of 6 minutes. Furthermore, we probe the role of the cAMP and the cGMP pathways in this cellular memory. The experimental results can be explained using a model that couples directional sensing to bistable memory.

Sorting of Dictyostelium cells in vertically constrained aggregates

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We revisited the problem of how cells sort out in disc-like aggregates 2-3 cells thick using a microfluidic device which provides fine control of aggregate thickness. The microfluidic setup is compatible with traction force microscopy, enabling us to examine the forces as an aggregate sorts out into regions of prestalk and prespore cells. Cells were imaged either with a cytosolic label, ecmA::GFP (prestalk), or with a novel double nuclear label, ecmA::HST-GFP (prestalk) ; cotB::HST-RFP (prespore). When vertically constrained, the cells swirl around in aggregates before sorting out. We found that the forces exerted on the substrate are directed radially outward, indicating that the cells are pushing inward. Swirling was observed even in populations of cells lacking adenylyl cyclase ($acaA^-$ $pkaC^{O/E}$) suggesting that chemotaxis to cAMP is not involved.
We also found that the cellulose in the slime sheath obscures the forces cells are exerting on the substrate to gain traction. Using cellulose synthase null ($dcsA^-$) cells we were able to properly observe these forces as the cells vortexed and eventually sorted out.

Dynamics of wave patterns in giant Dictyostelium cells

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Dynamic actin and PIP3 waves spontaneously emerge on the substrate-attached membrane of Dictyostelium cells. The typical wavelength is on the order of the cell size, so that waves in normal sized cells evolve under strongly confined conditions. Are the dimensions of these wave patterns enforced by the cell size, or do they exhibit an intrinsic self-organized wavelength? To resolve this question, we studied actin and PIP3 waves on the substrate-attached membrane of giant Dictyostelium cells obtained either by electric pulse induced fusion or by myosin II knockout. In the giant cells, wave patterns can freely evolve, independent of the effect of the cell border. We observed that waves are composite structures that consists of a PIP3-rich region that is circumscribed by an actin-rich band. As the PIP3-rich domain travels across the cell membrane, the enclosing actin band consists of two segments: a leading actin segment that moves towards the PIP2 rich area outside of the wave and the trailing actin segment that moves towards the PIP3 rich region in the center of the composite wave. As a key feature in both electro-fused cells and myosin II-null cells, we found that the waves indeed display a self-organized characteristic wavelength that is independent of the cell size and on the order of the size of normal cells. Expanding waves maintain their finite wavelength by forming arc-shaped segments or circular ring structures (doughnuts). They show many signatures of waves in an excitable medium. They can move at a constant speed over large distances, they may annihilate upon mutual collision or upon collision with the cell border, and they can curl into spirals waves. However, they may also be reflected at the cell border and can reverse their direction of motion.

Three-dimensional organization of actin filaments in propagating waves

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The actin system of Dictyostelium discoideum is able to self-organize into waves that propagate on the planar, substrate-attached membrane of the cell (1). Actin waves are formed in live cells from molecular motors and proteins controlling actin polymerization and depolymerization, including the Arp2/3 complex, myosin IB and coronin (1, 2). Wave propagation is based on an actin treadmilling mechanism, indicating a program that couples actin assembly to disassembly in a three dimensional pattern (2). We aim to decipher the mechanism of actin polymerization at the front of the waves. Using cryo-electron tomography, we explored the 3D architecture of the actin filaments inside the waves, in correlation with the localization of fluorescent actin-associated proteins in the cell cortex and PIP3 in the membrane. Actin waves were identified using cryo-light microscopy of vitrified cells grown on EM grids. Thin (200-500 nm) wedges suitable for cryo-ET were prepared using focused-ion-beam milling (3). Actin filaments were traced automatically from the 3D EM-reconstructions (4). In this talk, we will present the first results on the architecture of actin waves, either running freely at the surface of Dictyostelium or reaching a cell border.

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Knockout zaps are done on Friday mornings

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The ability to genetically manipulate Dictyostelium cells has been essential for numerous molecular biological discoveries and has established it as one of the core model organisms. However, genetic manipulation is currently only practical in a limited set of axenised strains. Moreover, we have recently shown that the high levels of macropinocytosis in these strains interfere with chemotaxis, a widely studied process in Dictyostelium. To overcome the current limitations, we developed methods for gene expression and gene disruption in non-axenic Dictyostelium discoideum cells. All methods were optimised with robustness and simplicity in mind. After 3 years of iterative refinements, final protocols are now taking shape. The optimised transfection efficiency is about 1:50 using 5x10e6 cells and 1 µg of vector DNA. In combination with a 4-hour doubling time, this means that sufficient transfected cells for experiments such as fluorescence microscopy can be routinely obtained within 24 hours. Colonies for experiments that require clonal selection are typically obtained after 2-3 days. Due to the 5-hour recovery time before selective pressure is applied, this makes Friday mornings the most ideal time for doing knockout electroporations. After letting go of old routines and getting used to the new timing of experiments, we think that molecular biology in non-axenic strains will be a competitive alternative to the conventional methods.

Developmental Regulation of Nucleosome Positioning and Gene Expression by a CHD Type III Chromatin Remodeling Protein

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The placement and positioning of nucleosomes within chromatin can direct transcriptional effects in individual genes; however, the precise interactions of these events are complex and largely unresolved at the whole genome level. The Chromodomain-Helicase-DNA binding (CHD) Type III proteins are members of a family of factors that can remodel chromatin, are required for metazoan and Dictyostelium development, and are identified as haploinsufficient in several severe, complex congenital human disorders. Although Type III CHDs are able to remodel nucleosome positions during in vitro assays, their in vivo actions in chromatin organization have not been established. To investigate the relationship between nucleosome re-positioning and gene expression, we have applied deep sequence analyses of genome-wide chromatin structure and transcription profiles during growth and development of wild type (WT) Dictyostelium and cells lacking ChdC, a Type III CHD protein.
We demonstrate that chromatin of a developmentally regulated gene subset is specifically remodeled during WT multicellular development, causing a dramatic phase change in nucleosome positioning, when compared to growing cells. Loss of ChdC results in the mis-regulation of chromatin structure by increasing DNA linker-length nucleosome spacing, but not through changes in nucleosomal phasing. The altered nucleosomal spacing pattern in chdC-null cells is highly restricted to a gene subset that encompasses approximately half of the genes that are normally remodeled during WT development and that also exhibit aberrant developmentally regulated gene expression in chdC-nulls in comparison to WT. Our data provide new insight into the in vivo function of CHD Type III chromatin remodeling proteins, their role in developmentally regulated nucleosomal positioning, and their requirement for WT gene expression.

A compendium of chromatin remodelling complex mutants in Dictyostelium discoideum and the nucleosome positioning roles of the CHD family

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ATP-dependent chromatin remodelling complexes are essential regulators of nucleosome dynamics, important for all aspects of DNA regulation including replication, repair and transcription. Disruption of nucleosome positioning perturbs transcriptional regulation and can generate spurious cryptic transcripts, yet how positions are established and their precise relationship with the transcriptional machinery remains unclear. We have established knockout strains of the catalytic subunits of each of the major chromatin remodelling complexes (SWI2/SNF2, INO80, SWR, ISWI and CHD families) and observed varied roles in growth, development, chemotaxis and transcription. The most severe phenotypes are observed in chdC-, as observed previously, and swr1- which display unique defects to cytokinesis and form large multi-nucleated cells. Generation of double knockouts further allowed us to screen for genetic interaction between these chromatin remodellers.
Nucleosome maps were generated for null strains of the remaining CHD family members: chdA and chdB, to complement existing chdC-null maps. Both chdA and chdB regulate large portions of the genome with opposing effects on genic and inter-genic nucleosome occupancy; chdA further regulates positioning of nucleosomes within gene bodies to maintain nucleosomal arrays. In contrast to the specific regulatory roles of chdC, chdA and chdB appear to play more widespread genomic roles with no direct correlation to transcription. Cross-analysis of these complementary data sets reveals a common subset of genes susceptible to disruption. The level of dependency upon remodellers to maintain nucleosome organisation may relate to both gene structure and transcriptional activity.

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Nuclear envelope-associated Proteins of Dictyostelium

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The nuclear envelope (NE) includes proteins of the nuclear membrane and the nuclear lamina. Recently, we identified NE81, a protein that forms the nuclear lamina in Dictyostelium discoideum. Our analyses revealed that NE81 is not only evolutionary related to lamins, but that it also functionally behaves like a bona fide lamin. Interestingly, several inner nuclear membrane proteins of animal cells are also conserved in the amoeba.
Src1 was in silico identified as a putative MAN1 homolog in Dictyostelium. GFP-Src1 localizes to the inner NE. Like NE81 it exhibits no detectable mobility at the NE during interphase in FRAP experiments. When expressing truncated Src1 as mRFP fusion its C-terminus co-localizes with mutant GFP-NE81, which is intentionally mis-expressed in cytosolic clusters and the outer NE. In BioID close proximity assays NE81 is biotinylated by BirA-Src1.
Another transmembrane protein, Erg24, shows similarity to the Lamin B Receptor (LBR), a protein of the inner NE that anchors the lamina and the heterochromatin to the membrane. Like the LBR, the Erg24 sequence carries a delta(14)-sterol reductase signature, however it lacks the N-terminal nucleoplasmic domain of LBR, which is required for binding to lamin B and chromatin. Yet, unlike the other known sterol reductases of this protein family, GFP-Erg24 localizes not only to the ER, but is concentrated at the NE. Its function at the NE and possible relationship to nuclear lamina proteins is subject of further investigations.
Our results show that Dictyostelium contains a surprisingly large set of proteins with a function in the nuclear lamina of higher eukaryotes. This suggests that the lamin-based nuclear lamina of metazoans with its interacting proteins was invented much earlier in evolution as previously thought. This is interesting not only from an evolutionary point of view, it also makes the amoeba an interesting experimental platform to study laminopathies originating from mutations in these proteins.

Dissecting transcriptional mechanism using live cell imaging

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With a developing appreciation of how noisy gene expression can be, and difficulties in deciphering conventional gene expression data into cell control mechanisms, it has become clear that single cell techniques for measuring transcription are necessary to illuminate basic cell regulation strategies. The resultant use of live cell RNA visualisation approaches in single cells revealed transcription is not adequately reflected by the smooth, seamless process we tend to infer from standard measures of RNA level. When RNA production is measured in single cells, the process of transcription has been shown to occur in bursts, or pulses. The nature of these pulses is a product of basic transcriptional mechanism, and is an instantaneous dynamic readout of the gene expression decisions of a single cell. I will describe our approaches to understand the mechanisms underlying discontinuous transcription, using a combination of live cell imaging, mathematical modelling and molecular genetics.

RNA mediated RNA regulation by Argonautes and dsRBD proteins

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siRNAs and miRNAs contribute substantially to the regulation of gene expression in eukaryotes. In Dictyostelium, a number of genuine miRNAs has been identified and siRNAs are mostly derived from the retroelement DIRS-1.
We have analysed the function of the argonaute proteins agnA and agnB in DIRS-1 regulation and find that AgnA is required for accumulation of siRNAs. A knock-out of agnA results in accumulation of DIRS-1 transcripts, the expression of DIRS-1 encoded proteins and the appearance of a DIRS-1 cDNA in the cytoplasm. In contrast, AgnB appears to be a positive regulator of DIRS-1 and its knock-out inhibits the accumulation of the cDNA in an agnA-/agnB- strain.
dsRBD proteins are known to participate in siRNA and miRNA generation. The dsRBD-B protein is required for the production of all miRNAs we have investigated since in a knock-out, no mature miRNAs can be detected, however there are indications for the accumulation of miRNA precursors. dsRBD-B co-localises and associates with Dicer B and is apparently required for a functional micro-processor complex. Surprisingly, the isolated dsRBD domain contained in Dicer B can rescue a dsRBD-B KO.
By an in-vivo reporter system we have shown that miRNAs can regulate mRNA expression on a posttranscriptional level.
Though many miRNAs are tightly regulated in development, their complete loss by disruption of the dsRBD-B gene does not result in an obvious mutant phenotype in growth or in development. We assume that miRNAs in Dictyostelium play a minor role under laboratory conditions but may be essential for survival in the natural environment.

Dicer-like proteins in Dictyostelium

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Dicer proteins are conserved endoribonucleases of the RNase III family that cleave double stranded RNA into small regulatory RNAs with functions in posttranscriptional gene silencing or chromatin modifications. The genome of *Dictyostelium* encodes for two similarly structured but non-redundant Dicer-like proteins, referred to as DrnA and DrnB that were shown to localize in cytoplasmic and nucleolar associated foci, respectively. The identity of those structures is still unknown in *Dictyostelium*, but their appearance is reminiscent of animal P-bodies for DrnA, and of plant Dicing- or Cajal-bodies for DrnB. We show that the known function of DrnB in miRNA biogenesis (1) is essentially tied to its nucleolar localization and is independent of the internal double-stranded RNA binding domain dsRBD of DrnB. DrnB co-localizes with dsRBP-B, a protein featuring a dsRBD as the only annotated domain, and the lack of this protein exhibits the same phenotype in miRNA maturation as a knock out of the *drnB* gene. The assembly close to or within nucleoli suggests furthermore a role in rRNA maturation, for which the observed accumulation of 17S derived small RNAs in the absence of DrnB is an initial indication. This phenomenon has not been described for Dicer proteins before, however, prototypical RNase III proteins in bacteria and yeast act in rRNA maturation. *In vitro* assays employing the recombinant RNase III domains of the Dicer proteins reveal that they preferentially cleave dsRNA molecules at junctions of double- and single-stranded, without specificity for perfectly base paired dsRNA or ssRNA, offering new insights into the catalytic properties of Dicer proteins which appear to be distinct in *Dictyostelium.*

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Evolutionary conservation of a small set of developmentally expressed genes in social amoebae

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Background
The developmental cycle of social Amoebae fascinates biologist since several decades. Mutant studies revealed that it integrates components of signal transduction chains, cell adhesion, fruiting body formation, and tissue determining factors. Genes involved in the basic cellular machinery seem to play also a role since their mutation can lead to a developmental phenotype. It is not clear, how such a complicated cycle was initially established and how many genes are required for its proper functioning.
Results
We compared the global gene expression profiles of four social amoebae species during the full developmental cycle. More than 2000 genes with a 3x higher expression in development than in the vegetative state are present in each species. However, genes with orthologs in all species add up to only ~700 genes. Furthermore, 101 genes are developmentally expressed in 3 species and have no ortholog in the fourth species. This core set of developmentally expressed genes is enriched for signal transduction components, developmental genes, and cellulose metabolism genes. A large number of the core set genes is not yet characterized.
Conclusion
Evolution of a complex developmental cycle can be traced back to constituting components, if later additions and modifications can be discerned from a core set of indispensable functions. By comparing distantly related species we were able to remove species specific additions and spurious expression patterns. Our study opens up the possibility to investigate the core components of a developmental cycle in detail.

Dictyostelium WASP: roles in endocytosis and cell polarity, but not in pseudopod formation (plus a note about the evolution of phagocytosis)

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WASPs are conserved proteins that regulate local actin polymerisation by triggering the Arp2/3 complex. The literature on WASPs (both in Dictyostelium and other organisms) is confusing - a number of papers associate WASP activity with chemotaxis and the generation of new pseudopods, but others are contradictory. In Dictyostelium mutants lacking the pseudopod activator SCAR, WASP relocalises to the pseudopod edges and takes over SCAR's function.
We have generated a number of new WASP knockout strains, including an inducible knockout to guard against secondary phenotypes, and clean knockouts to control for residual WASP function. The results are unequivocal - WASP is not important for pseudopod function, but is essential for the formation of actin at the sites of clathrin-mediated endocytosis, and greatly contributes to the efficiency of coated pit uptake. Perhaps surprisingly, SCAR cannot replace WASP's roles.
WASP mutants make normal pseudopods, but have significantly slowed chemotaxis and cell polarity. We have analysed why this occurs; it is connected with the efficiency of recycling Rac proteins and their regulators rather than new actin polymerisation at the front.
We will also discuss some fascinating new modelling data concerning the evolutionary origins of actin in migration, phagocytosis, and chemotaxis.

Biochemical and biological properties of cortexillin III, a component of Dictyostelium DGAP1-cortexillin complexes

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Cortexillins I, II and III are members of the α-actinin/spectrin sub-family of Dictyostelium calponin homology proteins. Unlike recombinant cortexillins I and II, which form homodimers in vitro, we find that recombinant cortexillin III is an unstable monomer but forms more stable heterodimers when co-expressed in E. coli with cortexillin I or II. Expressed cortexillin III also forms heterodimers with both cortexillin I and II in vivo and the heterodimers complex in vivo with DGAP1, a Dictyostelium GAP protein. Binding of cortexillin III to DGAP1 requires the presence of either cortexillin I or II, i.e. cortexillin III binds to DGAP1 only as a heterodimer, and heterodimers form in vivo in the absence of DGAP1. Expressed cortexillin III co-localizes with cortexillins I and II in the cortex of vegetative amoebae, the leading edge of motile cells and, if DGAP1 is present, the cleavage furrow of dividing cells. Functionally, cortexillin III may be a negative regulator of cell growth, cytokinesis, pinocytosis and phagocytosis as all are enhanced in cortexillin III-null cells.

The identification of axeB, the master regulator of macropinocytosis and phagocytosis

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Almost all Dictyostelium research during the past forty years has made use of axenic mutant strains. These mutants are able to grow in the absence of bacteria by gaining nutrients through macropinocytosis, but the genetic basis of this behaviour has remained mysterious. By selecting new axenic mutants and sequencing their genomes, we have identified the Dictyostelium axeB gene. Inactivating mutations in axeB, which encodes the orthologue of the Ras GTPase-activating protein Neurofibromin (NF1), lead to increased macropinocytosis and also enable phagocytosis of larger-than-normal particles. The NF1 protein localises transiently to membrane ruffles and nascent macropinosomes, indicating that it functions there directly to restrain Ras signalling. We suggest that NF1 evolved to limit feeding activity in the remote phagotrophic common ancestors of amoebae and animals, and highlight practical issues associated with the use of axenic mutants.

Multiple phases and functions of WASH in the phagocytic cycle

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As both phagocytic and macropinocytic vesicles transit through the cell, they undergo a complex series of maturation steps. This involves the sequential delivery and retrieval of specific proteins and is crucial to ensure efficient killing and digestion of bacterial food, as well as preventing the accumulation of indigestible material and maintaining cellular homeostasis.
The WASH complex is an important regulator of vesicle trafficking. By generating actin on the surface of vesicles, WASH can regulate the sorting and retrieval of proteins. Here we show that WASH is active at distinct phases of Dictyostelium phagosome and macropinosome maturation. Furthermore we show that WASH interacts with the Retromer sorting compex, but is able to use both Retromer-dependent and independent mechanisms to retrieve phagocytic receptors, lysosomal hydrolases and the vacuolar ATPase at the appropriate times. We therefore present a new model of Dictyostelium phagocytic trafficking with WASH functioning at multiple key phases.

Impact of Lipid Metabolism on the Infection of Dictyostelium with Mycobacterium marinum

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Induction of foamy macrophages filled with lipid droplet (LD), is a response to mycobacterial infection. During the establishment of infection, mycobacteria switch metabolism to use almost exclusively host-derived lipids, fatty acids and sterols. At the same time, intracellular mycobacteria have been seen in close apposition to LDs, but the mechanism by which they get access to the cytosolic lipids is still unknown.
We use the Dictyostelium/Mycobacterium marinum model to monitor the impact of lipid metabolism on the course of infection. To mimic the foamy characteristics of macrophages we induce LDs prior to infection by adding fatty acids. Infection with mycobacteria expressing bacterial luciferase allows to quantitate the impact of lipid metabolism on bacterial growth. Interactions between intracellular bacteria and LDs are monitored by EM and by using fluorescent lipid probes in confocal microscopy. When Dictyostelium cells were fed a high fat diet prior to infection, LDs are recruited to intracellular bacteria already at 4 hpi. At later time points, massive accumulation of neutral lipids was observed in the bacterium-containing compartment. As a consequence, intracellular bacteria harbored many more lipid inclusions under these conditions. Interestingly, feeding Dictyostelium with fatty acids before the infection had no effect on intracellular bacterial growth, but addition of fatty acids at 18 hpi completely inhibited growth.
The Long-Chain Fatty Acid CoA Synthase (LC-FACS) 1, an enzyme that activates fatty acids for triglyceride synthesis, is recruited to the mycobacterium-containing compartment. Strikingly, the Dictyostelium homologue of Perilipin, the most prominent LD protein, closely surrounds the cytosolic bacteria as soon as they escape their compartment. The functional role of LC-FACS1, Perilipin and DGAT 1 and 2 during infection is studied by monitoring the growth of fluorescent or luminescent bacteria infecting the various knockout mutants.

Take the bitter with the sweet: Discoidins and mycobacterial infection

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Tuberculosis is a life-threatening infectious disease caused by Mycobacterium tuberculosis, which mainly infects alveolar macrophages. In order to study host-pathogen interactions, we use Dictyostelium discoideum as a macrophage surrogate and Mycobacterium marinum, a species closely related to M. tuberculosis. After uptake by phagocytosis, M. marinum is able to subvert the acidification and maturation of the phagosome in which it resides. This creates a friendly niche where it can survive and replicate. Around 24 hours post-infection, M. marinum escapes from its compartment to the host cytosol.
Since the 80's, a family of D. discoideum lectins, the discoidins, have been studied in relation to cell aggregation during multicellular development. However, our recent MS analysis revealed that discoidins are enriched in compartments containing M. marinum, compared to non pathogenic mycobacteria. This suggests that the four cytosolic discoidins may play a role in innate immunity similar to the one of the mammalian galectins, which share many molecular and biological similarities with discoidins.
Using specific antibodies and GFP-tagged discoidins we have studied their localization. They are found in the cytosol, but also secreted to the extracellular medium. When cells are infected with M. marinum, we observed the appearance of discoidin foci, often located close to the bacterial poles. Discoidins also extensively labelled the damaged M. marinum compartment during bacteria escape.
We want to study if the discoidins, as lectins, recognize the glycans from M. marinum or the ones from the host membranes that are exposed to the cytosol when the compartment breaks. To dissect this process, we perform bacteria binding assays in vitro and induce sterile damages to phagosomes.
Finally, we want to investigate discoidin downstream signalling and crosstalks with other pathways such as autophagy, using immunofluorescence, pull downs and blot overlays.

The autophagic machinery ensures non-lytic ejection of mycobacteria and efficient cell-to-cell transmission

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Mycobacterium tuberculosis infections still represent a global burden, and no effective new vaccines or treatment have been developed in the last decades. In contrast to mechanisms mediating uptake of bacterial pathogens, egress and cell-to-cell transmission of intracellular pathogens are still poorly understood. In the Dictyostelium-Mycobacterium marinum model, a F-actin based structure, the ejectosome, allows non-lytic egress and transmission of mycobacteria. However, how the host cell maintains integrity of its plasma membrane during the ejection process was so far unknown. Using a correlative light/electron microscopy approach, we were able to observe that the intracellular pole of the ejecting bacteria was tightly enclosed by a vacuolar structure. With immunofluorescence staining, we were able to demonstrate that members of the autophagic machinery, as well as SQSTM1 and ubiquitin localized specifically to the intracellular pole of the bacteria. To further dissect the role of autophagy in this process, we used an atg1- mutant to evaluate the structure and function of ejectosomes in a cell without the autophagic pathway. While ejectosomes were still formed when autophagy is impaired, cell-to-cell transmission is reduced. In addition, the host plasma membrane becomes compromised, leading to leakage of intracellular material, and eventually, cell death. These findings highlight a new, highly ordered interaction between bacteria and the autophagic pathway and we hypothesize it represents an ancient membrane sealing mechanism.

Dictyostelium discoideum as a host model to study the interaction with pathogenic and apathogenic yeast

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Dictyostelium discoideum is well known for its ability to phagocytose bacteria and other microorganisms or small particles. Furthermore D. discoideum has been established as a host model for different pathogenic bacteria like Legionella or Mycobacterium. However, the interaction of the amoebae with yeast has only been rarely investigated. Heat killed yeast are used to study phagocytic events but experiments with living yeast cells have not been conducted or failed so far. Here we describe for the first time the interaction between D. discoideum with living pathogenic and apathogenic yeast. We modified a protocol originally established to study the interaction between Acanthamoeba and Mycobacterium to investigate the killing efficiency of Dictyostelium using different yeast strains. It turns out that different yeast strains show differences in their resistance towards killing by D. discoideum. Interestingly, S. cerevisiae strains isolated from human blood turned out to be more resistant compared with laboratory strains. Using different D. discoideum knock-out strains, which have been shown to play a role in phagocytosis and/or intracellular growth of pathogenic bacteria, we show that autophagy partially contributes to killing of yeast cells. Finally we investigated the interaction of Dictyostelium with the human fungal pathogens Candida albicans and C. glabrata. Whereas the non-filamentous yeast C. glabrata behaves similar like S. cerevisiae blood isolates, C. albicans is more resistant and produces hyphae upon interaction with D. discoideum. Using a transwell-assay we show that hyphae-formation is not only induced upon contact or phagocytosis but also with secreted substances and lysate of Dictyostelium cells. This points to an ancient mechanism of C. albicans to escape from phagocytic cells.

Autophagy 16 and 9 mutants have similar endocytosis defects but deficiencies in development and proteasomal activity are much more severe in the double mutant

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Macroautophagy is an intracellular bulk degradation system that is highly conserved in all eukaryotic cells. It is governed by a large number of core and accessory autophagy protein (ATGs) and is involved in many physiological and pathological processes. Macroautophagy also constitutes an important mechanism in cell autonomous immunity and we previously found that infection of *Dictyostelium discoideum* with medically relevant pathogens caused differential regulation of the core autophagy genes ATG8a, ATG8b, ATG9, ATG12 and ATG16. Here, we describe the phenotypes of ATG16^{-} and ATG9^{-}/16^{-} cells, of the previously reported ATG9^{-} mutant, and of cells that express ATG16-GFP in the knock-out mutants. ATG16 deficiency caused an increase in the expression of ATG8a, ATG8b and ATG9, suggesting a sensing system for autophagosome completion. ATG16, ATG9 as well as ATG9/16 deficient cells have complex phenotypes and display severe defects in development, pinocytosis and phagocytosis. Uptake of *L. pneumophila* was reduced. Moreover, proteasomal activity of the single mutants was strongly reduced and the proteasomal activity of the double mutant was even more compromised. The decrease in proteasomal activity correlated with an increase in the overall level of poly-ubiquitinated proteins. Mutant cells contained large protein aggregates which stained positive for ubiquitin and partially colocalised with ATG16. Our results show that ATG16 and ATG9 are required for normal development, efficient pinocytosis and phagocytosis and, opposite to the expectation, normal proteasomal activity. The phenotype of the double mutant suggests that ATG9 and ATG16 likely have additional functions which are independent of each other and of autophagy.

Functional characterization of Dictyostelium iron transporters Nramp1 and Nramp2, involved in resistance to bacterial infection and in iron homeostasis: an update

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The Dictyostelium genome harbours two genes of the Nramp family, Nramp1 and Nramp2. Nramp1 is recruited to phagosomes or macropinosomes and confers resistance against legionella and mycobacteria infection. Nramp2 is localized in the contractile vacuole membrane, and synergistically with Nramp1 regulates iron homeostasis. During L. pneumohila infection, the pathogen hinders recruitment of V-H+ ATPase, but not Nramp1, to the Legionella-containing macropinosome, thus generating a non-acidic, iron-rich proliferation vacuole. We suggested that legionella avoids fusion with acidic vesicles by secreting a PI3-phosphatase that hydrolyses PI3P sites on the membrane of the proliferation vacuole (Peracino et al., 2010). We now show that legionella mutants defective in PI3P and PI3,4P2 phosphatases display reduced pathogenicity.
Evidence that Nramp1 transports iron was inferred from in vitro studies with isolated phagosomes. To show that in-vivo Nramp1 mediates iron traffic, we exploited the iron-sensitive fluorochrome calcein. By incubating cells with Fe-quenched calcein, we assessed calcein fluorescence de-quencing in macropinosomes by confocal microscopy and flow cytometry. De-quenching occurred in wild type or Nramp2- but not Nramp1-KO cells, suggesting that Nramp1 is essential for iron export from macropinosomes.
Iron transport was further characterized by functional expression of Nramp1 or Nramp2 in Xenopus oocytes. For expression on the oocyte surface, the Nramp N- and C-termini were replaced by the corresponding regions of murine DMT1 (formerly Nramp2). Following cRNA injection, the oocytes were subjected to electrophysiological and radiochemical uptake assays. In parallel, they were also injected with calcein, incubated with divalent metals and calcein-quenching assessed in confocal microscopy. These studies confirmed that Dictyostelium Nramp1 and Nramp2 are electrogenic proton-dependent iron transporters with cation selectivity comparable to mammalian DMT1.

Eastern Japanese Dictyostelia species adapt while populations exhibit neutrality

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The dynamics of biological hierarchies are the result of social activities among constituents that occur across multiple scales. Furthermore, the differences between population dynamics and species dynamics are poorly understood, which has created a level of ambiguity associated with the concepts of both population and species. In the present study, Dictyostelia was utilized as a model community in order to clarify these concepts. Distributions of logarithmically ranked populations could be explained by unified neutral theory and a zero-sum patch game, while distributions of ranked species could not be explained by unified neutral theory. Furthermore, principal component analysis demonstrated that species had distinct niches, which supported the idea that species-level organization was explained by adaptive structure, while population-level organization was explained by unified neutral theory. Genetic structures of both populations and species, and differences in characteristic time scales of these hierarchies based on actual and deduced point mutation rates and speciation rate were also examined. These results indicated that species of Eastern Japanese Dictyostelia may have behaved adaptively along a longer time scale, while populations may have behaved in accordance with unified neutral theory along a shorter time scale.

Functional evolution of adenylate cyclase R and adenylate G in Dictyostelia

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Spore forming Dictyostelid social amoebas evolved from cyst forming solitary amoebas by inventing a unique form of multicellularity where amoebas assemble into fruiting structures. Molecular phylogeny subdivides Dictyostelia into four major groups. In the group 4 species Dictyostelium discoideum, spore formation and maintenance of spore dormancy are triggered by intracellular cAMP, requiring activation of the adenylate cyclases, ACR and ACG, respectively. Spores are kept dormant in the fruiting body by high osmolarity and this effect is mediated by ACG. Many species in groups 1, 2 and 3 have retained the ability to encysts from single cells and we showed earlier that encystation is requires PKA. ACR and ACG are conserved in all four taxon groups. We here studied the roles of ACR and ACG in sporulation and encystation in the group 2 species Polysphondylium pallidum.
Despite its critical roles in D. discoideum development, single or double ACR and ACG null mutants in the P.pallidum showed normal development and spore differentiation. However, inhibition of spore germination by high osmolarity was less effective in the acr- cells than in wild type and was lost completely in acr-acg- cells, indicating that ACR and ACG are have overlapping roles in spore dormancy. High osmolarity induces encystation in P. pallidum. This response was normal in acg- cells and only slightly delayed in acr- cells. However, encystation was lost in the acr-acg- double mutant, indicating that ACR and ACG play overlapping roles in encystation. High osmolality also maintains cyst dormancy and this still occurred in acg-, but not in acr-, indicating that ACR mediates the inhibition of cyst germination by high osmolarity. These findings show that the specific roles of ACR and ACG in spore formation and germination differ between D.discoideum and P.pallidum. However, their roles in both processes are likely to be evolutionary derived from an original role in encystation and cyst germination.

Trade-offs and the illusion of social success in Dictyostelium discoideum

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Despite the constant force of natural selection favouring the fittest individuals, many traits with clear fitness advantages show a surprisingly high amount of genetic variation. One particularly striking example of such variation is seen in traits involved in social interactions, where it is expected that the genotypes that confer the highest social success, and hence fitness, should be fixed in a population. For example, in the social amoeba, Dictyostelium discoideum, social success is clearly linked to fitness because it is manifested as the relative number of spores produced in different genotypes in chimaeric fruiting bodies. However, natural populations harbour enormous genetic diversity, including genotypes with apparently very low social success. To understand the persistence of variation in social success in populations, we test the hypothesis that trade offs exist between social and non-social traits. We find that genotypes that appear to be the most socially successful gain a numerical advantage by producing smaller spores, but this advantage comes at a significant cost because these smaller spores have reduced viability. Because of this trade-off, higher apparent social success does not correspond to higher fitness in social interactions. Consequently, genotypes with very different apparent social success have similar total fitness, leading to very weak selection on social traits that allows for diversity in social traits to persist. This result helps explain the observed diversity in social strategies that coexist in natural populations.

Divide and conquer: vegetative conflict in D. discoideum

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Understanding the maintenance of natural variation in the face of Darwinian selection remains a central problem in evolutionary biology. Key to its solution is the idea of fitness trade-offs, where organisms cannot maximize the fitness of all traits simultaneously either because a trait that is beneficial in one scenario is harmful in another, or because of constraint in trait evolution through pleiotropy, where one gene affects multiple traits. The social amoeba D. discoideum is a compelling model organism for studying trade-offs because its simple life history facilitates quantification of an entire suite of traits across the organism's entire life-cycle. In this work, I look at growth across a set of 24 natural isolates of D. discoideum from North Carolina with well-characterized social phenotypes. Highly significant variation was observed in growth rates across the strains when fed K. aeroginosa, the standard laboratory food source for wild-type strains. This variation was only weakly negatively correlated with social success, and could not explain the maintenance of such variation in the face of strong selective pressure for fast growth. In the wild, natural isolates of D. discoideum consume a variety of bacterial species thus presenting the possibility that growth on one species may trade-off with growth on another. When fed on different strains of gram negative and positive bacteria, faster growth on KA across 8 strains from the top, middle and bottom of the growth hierarchy did not necessarily correlate with faster growth on other food sources, with switches in the hierarchy observed across different food sources. Furthermore, 2 mutants identified in a genetic screen for faster growth on KA lost their advantages when grown on different food sources. Taken together, these data indicate that strains specialize in predation of certain bacterial strains over others helping to explain natural variation in a trait expected to be under strong selective pressure.

CP161, a new component of the centrosome

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The Dictyostelium centrosome is a nucleus-associated body consisting of an amorphous matrix functionally equivalent to the pericentriolar material of animal centrosomes that is responsible for the nucleation and anchoring of microtubules. Here we describe CP161 as a novel component of the D. discoideum centrosome, which also localises to the Golgi complex. We identified CP161 in a search for interaction partners of CP250, a previously described component of the pericentriolar matrix (Blau-Wasser et al., 2009, Mol Biol Cell. 20:4348-61). CP161 is present at the centrosome throughout interphase and all phases of mitosis. We find that amino acids 1-763 of the 1381 amino acids are sufficient for centrosomal targeting and centrosome association. AX2 cells over-expressing truncated and full length CP161 proteins have defects in growth and development. We further studied the interaction with CP250 and carried out a search for new binding partners.

Characterization of four novel core components of the Dictyostelium centrosome

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A proteomic approach for new components of Dictyostelium centrosome led to the identification of 34 candidates proteins. Eight of these could already be confirmed using GFP-fusion proteins. Here we present the characterization of the four new components of the centrosomal core region. While CP55 localizes to the centrosome throughout the cell cycle, while CP39, CP75 and CP91 show a cell cycle-dependent localization. CP39 and CP75 can be observed at the core region of the centrosome in interphase cells, but are absent from the spindle poles of metaphase cells. CP39 leaves the centrosome already in prophase, CP75 persist until prometaphase. CP91 can always be detected at the centrosome, but its level is dramatically reduced during mitosis. All three proteins re-accumulate at the centrosome during telophase. This cell cycle-specific behavior of the four core components is informative regarding their subcentrosomal localization. EM studies had shown that the central layer of the core complex is disappearing in prometaphase. The two remaining layers separate to form the two poles of the mitotic spindle. Since CP39 and CP75 disappear during mitosis and CP91 level is reduced, these three proteins are likely to be part of the central layer. CP55, which persists throughout mitosis, is supposed to be located at the two outer layers. Our functional analyses revealed that CP55 plays a role in the stabilization of the corona. In null mutants additional MTOCs can be observed, which do not contain a core structure, as shown by electron microscopy. Live cell studies revealed, that these MTOCs arise in telophase. A knock out of the three other proteins appears to be lethal. Overexpression of GFP-CP39 or CP91 fusion proteins, respectively, causes supernumerary centrosomes. Therefore these proteins appear to be involved in mitosis and centrosome duplication. Overexpression of GFP-CP75 causes colocalization with F-actin, indicating a functional link to the actin cytoskeleton.

Adaptations in the proteostasis network of the social amoebae Dictyostelium discoideum cause an unusual resilience to protein aggregation

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A key prerequisite for cellular and organismal health is a functional proteome. To maintain its integrity, cells have evolved an elaborate protein quality control system (PQC) system. However, under stress condition protein misfolding increases and overwhelms the PQC system, leading to the formation of protein aggregates. The aggregation properties of a protein are determined by specific sequence stretches such as poly-asparagine(N) and poly-glutamine(Q)-rich regions or low sequence complexity regions, also known as 'prion-like' due to their sequence similarity to yeast prions. Recent studies investigating the abundance of N/Q-rich stretches in the proteomes of several species, identified *D. discoideum* as the organism with the highest N/Q content. Using bioinformatics tools we searched for aggregation-prone proteins in the proteome of *Dictyostelium* and found a remarkably high number. To identify mechanisms that allow *Dictyostelium* to tolerate such a highly aggregation-prone proteome, we studied the behavior of several characterized aggregation-prone marker proteins. Our findings showed that, in contrast to observations made in other organisms, these marker proteins remain soluble and innocuous. However, we showed that conditions compromising the PQC system induce formation of cytotoxic aggregates in the cytosol, thus suggesting a role for the PQC system in the regulation of protein aggregation. We identified the disaggregase Hsp101 as key player in the response to and recovery from stress-induced protein aggregation. Furthermore, we suggest that the nucleus serves as place of proteostasis, where aggregation-prone proteins might be degraded. Taken together, we suggest that the PQC system of *D. discoideum* has been subjected to specific adaptations that increase the proteostatic capacity of the organism and allow for an efficient regulation of protein aggregation

Peptidyl prolyl cis-trans isomerases (PPIases): a study in Dictyostelium discoideum

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Peptidyl prolyl cis-trans isomerases (PPIases) (EC 5.2.1.8) catalyze the otherwise a slow process of cis-trans isomerisation of peptide bond preceding proline residue. PPIases function primarily in protein folding until they are found to have profound effect on target protein functions by regulating phosphorylation status, stability, subcellular localization and as targets of immunosuppressive drugs. Based on their structure and sensitivity towards immunosuppressive drugs, PPIases are of four types: Parvulins, Cyclophilins, FKBPs and PTPA. Parvulins specifically recognized phosphorylated serine/threonine (pSer/Thr-Pro) preceeding proline bonds by their N-terminal WW domain while cis-trans isomerisation of target peptide or protein is catalysed by C-terminal isomerase domain. Parvulin-type isomerases, Ess1 in Saccharomyces cerevisiae and Pin1 in Homo sapiens, are important in cell cycle progression and transcription regulation. Our objective is to characterize the function of Ess1/Pin1 type of PPIase in Dictyostelium discoideum. Based on sequence homology of Ess1 protein (39% identity), a single gene DDB_G0268618, pinA, coding for 268 amino acids, in D. discoideum genome was amplified using gene-specific primers. Unlike S. cerevisiae and H. sapiens homologs, PinA is predicted to have FHA domain at N-terminal to PPIases domain thus studying the homologue of Pin1-type PPIases in D. discoideum may open a new function of this PPIase family. D. discoideum PinA can complement the lethality associated with S. cerevisiae ess1H164R mutant at the restrictive temperature (37°C) showing that the function of these proteins is conserved. Expression profiling showed that pinA transcript is expressed throughout D. discoideum life cycle. Confocal analyses showed that PinA-YFP is localized in both nucleus and cytoplasm of growing cells. Towards elucidating the function of PinA, knockout and overexpression cell lines were generated. Analyses of overexpressor cells showed that PinA activity is important during development. Results of these studies carried out to understand the function of *D. discoideum* PinA during growth and starvation-induced development will be presented.

Posters (in alphabetical order according to the presenter's name)

1

Characterization of regulatory elements in promoters of glorin-induced genes in Polysphondylium pallidum

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The dipeptide glorin is an extracellular signaling molecule that probably coordinates aggregation of cells from the earliest diverged species of social amoebae. We have shown in previous experiments that glorin induces gene expression during early development of Polysphondylium pallidum. Elevation of transcripts from glorin-induced genes is detectable after a few minutes of exposure of P. pallidum cells to glorin, suggesting that a latent transcription factor may be activated in response to glorin receptor-stimulated intracellular signaling pathways. By comparing promoter sequences of glorin-induced genes we identified a degenerate C-rich element as a putative "glorin response element" in glorin-induced genes. Preliminary data from reporter gene-based promoter deletion and mutagenesis analyses seem to support this hypothesis.

Identification and characterization of tetraspanins from Dictyostelium amoeba: role in cell migration?

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Cell migration is key in human health and disease e.g. the immune response and cancer spreading. Although there is evidence that tetraspanins (Tsps) are involved in these processes, little is known about the structure and function of these evolutionary highly conserved, four transmembrane spans containing proteins. The complexity of 33 human Tsp isoforms prompted us employ Dictyostelium discoideum as a simple and manipulatable model to study the role of Tsps in cell migration. In silico, we identified five Tsps in the D. discoideum genome (TspA-E). Carrying out cDNA synthesis with oligo(dT)primers from D. discoideum total RNA and PCR-amplification with specific primers we found tspA, C, and D to be expressed in the amoebae stage. We produced and affinity purified antibodies against peptides of the large extracellular loops (EC2) of TspA, C, and D. However, the antibodies failed to recognize the native Tsps. In this context, we are currently investigating whether glycosylation may mask the epitopes by mutation of candidate glycosylation sites and enzyme-mediated deglycosylation. Initial results hint at N-glycosylation of the EC2 of TspC. For intracellular localization of Tsps, we generated fusion proteins with an N- or C-terminal GFP and obtained signals mainly at cytosolic organellar structures, presumable representing contractile vacuoles. We deleted tspC by homologues recombination yielding a strain with normal growth and development of which we are now analyzing random motility and chemotaxis. Single/double/triple knockouts of tspA, tspC, and tspD are in the works to clarify the contribution of Tsps in Dictyostelium cell migration. We are further aiming at using such tsp knockout strains to heterologuously express human Tsps in D. discoideum for investigating migration phenotypes, cell adhesion, protein-protein-interactions etc. on a molecular level.

G-quadruplex and Dictyostelium

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Guanine-rich DNA and RNA sequences can form unusual secondary structures named G-quadruplex (G4). These tetrahelical structures result from the stacking of several quartets, each quartet being composed of four guanines linked together by Hoogsteen hydrogen bonding, with additional stabilization provided by a monovalent cation. These motifs are found in the promoter region of important genes, near the transcription initiation site. G-quadruplex structures are associated with a number of important aspects of genome function such as the control of gene expression, replication or splicing and represent potential therapeutic targets. Quadruplex ligands are small molecules able to recognize G4. The identification of specific ligands for these G4 structures is a novel approach for finding new treatments against pathologies such as cancer. Our unpublished bioinformatic studies suggest that there are even more G4-prone motifs in the human genome than expected (over 106). The deconvolution of these potential "G4 pathways" is therefore extremely difficult. On the other hand, Dictyostelium, with its small, extremely AT-rich genome, offers a limited number of well-defined potential G4 sites.
We performed a bioinformatic analysis of the genome of Dictyostelium to predict putative G4 sequences with 3 analysis tools: QGRS Mapper, Quadparser and one developed in house. We validated our predictions by different spectroscopy techniques such as UV experiments, circular dichroism and NMR.
To study the impact of G4 structures on the transcription of genes under their control we are mutating 4 interesting profiles identify by NMR in Dictyostelium. To investigate the mechanism of action of G4 ligands and describe the various molecular processes involved, we are developing a toxicity assay with quadruplex ligands known.

Cluster analysis of the cell migration in Dictyostelium

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Research of eukaryotic cell motility had been based on the persistent random walk model before careful analysis of locomotion paths revealed additional features of the cell migration that lead to modifications and extensions of this standard model. The initial aim of our research was to characterize motility of cortexillin double-null cells of D. discoideum within the framework of a generalized Langevin model extended with a memory term. In the course of the analysis of wild-type and mutant cells, we noticed that cells belonging to a single strain are highly heterogeneous with respect to their mode of migration. This prompted us to perform unsupervised classification of all measured cell trajectories according to a set of attributes that quantitatively describe cell locomotion. The motile behavior of cells from two different strains, wild-type (AX2) and the strain lacking cortexillins I and II (CI-/CII-), was monitored by dark-field microscopy. For both strains, we measured the trajectories of randomly migrating vegetative and aggregation-competent cells. Migration of individual cells was characterized by a set of parameters derived from the speed distribution, the velocity autocorrelogram and the velocity periodogram.
We determined that each of the four analyzed populations, i.e. AX2 and CI-/CII- cells in vegetative and aggregation-competent stages, can be divided into several homogenous subpopulations specified by the average speed and the low-frequency segment of the periodogram. CI-/CII- cells showed a larger dispersion of the average speed values relative to AX2 in both phases of their life cycle, whereas the aggregation-competent CI-/CII- cells displayed a loss of low-frequency oscillations. Finally, the motility data for each of the identified subpopulations were fitted to the generalized Langevin equation.

Two Dictyostelium Tyrosine Kinase-Like kinases function in parallel, stress-induced STAT activation pathways

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When Dictyostelium cells are hyper-osmotically stressed STATc is activated by tyrosine phosphorylation. Unusually, activation is regulated by serine phosphorylation and consequent inhibition of a tyrosine phosphatase: PTP3. The identity of the cognate tyrosine kinase is unknown and we show that two Tyrosine Kinase-Like (TKL) enzymes, Pyk2 and Pyk3, share this function; thus for stress-induced STATc activation, single null mutants are only marginally impaired but the double mutant is non-activatable. When cells are stressed both Pyk2 and Pyk3 undergo increased auto-catalytic tyrosine phosphorylation. The site(s) that are generated bind the SH2 domain of STATc and then STATc becomes the target of further kinase action. The signalling pathways that activate Pyk2 and Pyk3 are only partially overlapping and there may be a structural basis for this difference because Pyk3 contains both a TKL domain and a pseudokinase domain. The latter functions, like the JH2 domain of metazoan JAKs, as a negative regulator of the kinase domain. The fact that two differently regulated kinases catalyse the same phosphorylation event may facilitate specific targeting because under stress Pyk3 and Pyk2 accumulate in different parts of the cell; Pyk3 moves from the cytosol to the cortex while Pyk2 accumulates in cytosolic granules that co-localize with PTP3.

REMI-seq: generation of a genome wide mutant resource for Dictyostelium functional genomics

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Dictyostelium offers a powerful model system for discovery genetics and biomedical research, with strength in many areas including chemotaxis, cell signalling and developmental biology. Existing resources include the complete genome sequence and transcriptional profiles. However, full exploitation of these data is hampered by lack of a systematic, genome-wide collection of Dictyostelium mutants; there are null mutants available for just 4% of the total gene complement.
To rectify this a novel technique, REMI-seq, is being employed. REMI-seq combines Restriction Enzyme Mediated Insertional (REMI) mutagenesis with Next Generation Sequencing (NGS) to generate large numbers of mutants and then identify the disrupted gene by NGS. Each mutation position will be mapped onto the genome sequence, and be searchable online via the existing Dictyostelium genomic resource, dictyBase. Individual, groups and even large pools of mutants will be available to the research community via the linked Dicty Stock Centre.
The proposed resource will thus revolutionize Dictyostelium research by providing genome-wide coverage of mutants available to the global Dictyostelium research community, improving access to non-Dictyostelium groups and enabling new systems level functional genomics approaches.
Technical development is currently underway and mutant generation will commence shortly. At this early stage, we are interested to hear how you would like to use this resource, and how it will benefit your research.

Using Dictyostelium to dissect host-pathogen interactions in Francisella infection

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The genus of Francisella comprises of Gram-negative, facultative intracellular bacteria that infect various species of both vertebrates and invertebrates. Francisella species can be divided into two lineages represented by F. tularensis causing potentially fatal tularemia in humans and F. noatunensis infecting aquatic animals. We established a novel infection model using Dictyostelium discoideum as a surrogate macrophage and the fish pathogen F. noatunensis subsp. noatunensis (F.n.n.). The Dictyostelium-F.n.n. system shared high similarities with the mammalian system demonstrated by comparable phagosomal maturation and bacterial growth in the cytosol. Using this new model system we want to identify and study bacterial virulence factors as well as host defence mechanisms.
To investigate Francisella virulence mechanisms we characterized the infection of Dictyostelium with a F.n.n. ΔiglC mutant. The gene locus iglC encodes a virulence factor, which has been shown to be essential for bacterial escape into the cytosol. Mimicking the situation in macrophages F.n.n. ΔiglC was not able to block phagosomal maturation and was exocytosed after 2-4 hours post infection.
First screenings of Dictyostelium knock-out mutants revealed the importance of host factors in our infection model regarding autophagy, iron homeostasis and the PI3K pathway. In F. tularensis infection the role of autophagy is disputed. In Dictyostelium Δatg1 cells lacking an intact autophagic pathway F.n.n. showed increased growth after 24 hpi suggesting interaction with this pathway during infection. Overall, usage of the Dictyostelium-F.n.n. system will contribute to our understanding of host-pathogen interactions during Francisella infection.

Role for Coronin A in the Induction of development of Dictyostelium discoideum

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Upon starvation Dictyostelium discoideum secrete conditioned medium factors that initiate cAMP signal transduction by inducing expression of genes such as cAMP receptors and adenylate cyclase. The mechanisms involved in the activation of the first pulses of cAMP release are only partially described. Our recent results implicate coronin A in the initiation of the cAMP response. Upon starvation, coronin A-deficient cells fail to up-regulate the expression of cAMP-regulated genes, thereby failing to initiate development. Of importance, external addition of cAMP to coronin A-deficient cells results in normal chemotaxis and aggregate formation, thereby restoring the developmental program and implicating a functional cAMP relay in the absence of coronin A. These results suggest that coronin A is dispensable for cAMP sensing per se but is part of a signal transduction cascade essential for sensing factors that initiate the multicellular development in Dictyostelium. Our current aim is to identify the coronin A-dependent factor(s) in conditioned medium from starving Dictyostelium by using an unbiased biochemical approach. This work may contribute to a better understanding of the molecules and signaling pathways involved in the early starvation response of Dictyostelium.

LKB1-mediated signalling in the Dictyostelium mitochondrial disease model

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Mitochondrial diseases are a poorly understood group of degenerative diseases characterized by a complex array of symptoms affecting major organ systems in unpredictable combinations. *Dictyostelium discoideum* provides a tractable, established model for mitochondrial disease which revealed previously that diverse mitochondrial disease phenotypes are caused by chronic activation of an energy-sensing alarm protein AMP-activated protein kinase (AMPK). Chronic AMPK hyperactivity impairs growth, cell cycle progression, multicellular morphogenesis and photo/thermosensory signal transduction. In other organisms AMPK is mainly activated in response to various cellular stresses by the upstream protein kinase LKB1-STRADα-MO25 complex. LKB1 is a serine/threonine kinase, originally identified as a tumour suppressor. Loss of function mutations in the human LKB1 gene can lead to Peutz–Jeghers syndrome, an autosomal dominant disease characterized by benign gastrointestinal tumors (hamartomas).pTo determine the potential role of LKB1 in AMPK-mediated mitochondrial disease phenotypes in *Dictyostelium*, we overexpressed LKB1. This caused impairments in growth and morphogenesis similar to those observed in mitochondrially diseased strains. Unexpectedly, LKB1-overexpression also increased the resistance to DIF-induced cell death. Cotransformation with overexpression constructs for LKB1 and STRADα (one of its binding partners in the LKB1-STRADα-MO25 complex) produced more severe defects than overexpression of LKB1 alone. Whereas AMPK activity was reported not to influence the rates of phagocytosis and pinocytosis, LKB1 overexpression inhibited both. This could result from LKB1 phosphorylation of additional downstream target kinases other than AMPK. Together the results suggest that LKB1 phosphorylates both AMPK and other targets, and that its activation of AMPK is responsible for AMPK-mediated mitochondrial disease phenotypes.

dictyBase Help and Discussion Desk

Petra Fey, David Jiménez-Morales

Northwestern University, Chicago, IL, USA

We will have a table and chairs to dicuss any questions you might have about dictyBase. You can also just hear what's currently going on or give us your suggestions. Bring your computer or use ours. Open at both poster sessions.

The Argonaute protein AgnB interacts with chromatin components and participates in regulation of developmental genes

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The classical RNA-interference (RNAi) process uses small RNA species to regulate gene expression, transposable elements as well as the defence against viral RNA. Argonaute proteins are well known key players for RNAi mechanisms in the cytoplasm.
More recently, many studies linked Argonaute proteins to function in the nucleus. Depending on (different) interaction partners the Argonautes are able to control various aspects of gene regulation such as the chromatin status.
We have identified three novel interaction partners of the Argonaute B protein (AgnB) in Dictyostelium discoideum: De-etiolated A (DetA), DNA repair protein E (RepE) and Cullin D (CulD). DetA is a well-characterized chromatin remodeller that suppresses gene expression whereas CulD together with RepE is involved in the transfer of ubiquitin to target proteins.
Interestingly these proteins form a stable complex and localize exclusively in the nucleus, indicating nuclear functions of AgnB.
We used homologous recombination to generate a knock out strain of agnB for analysis.
Real time qRT-PCR data of genes that are associated with the repressor DetA revealed three target genes (cotA, cotB, psvA) which are regulated by AgnB. These genes showed a strong reduction of transcript level during the development of the amoeba in agnB- strains demonstrating a potential antagonistic effect of DetA and AgnB.
While common phosphorylation patterns of AgnB seem to be absent in D. discoideum we use specific antibodies to determine its ubiquitin level. Quantification of the ubiquitin signals demonstrate a clear reduction of ubiquitinylated AgnB in the development relative to the vegetative phase, which is consistent with expression level and modified functions. We will present a model where the DetA/RepE/CulD complex in association with AgnB regulates the expression of developmental genes.

Molecular Mechanisms of bacterial egress studied in the Mycobacteria - Dictyostelium Model system

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The global burden of the infection with Mycobacteria tuberculosis remains enormous with nearly two million people dying from TB every year (WHO). Despite intense research no effective vaccines or treatment targets have been developed in the last decades. Because working with M. tuberculosis in mammalian systems is difficult and time-consuming M. marinum in combination with the amoeba Dictyostelium discoideum was established as a model system to study mycobacterial pathogenicity and cellular host defense mechanisms.
While host cell entry and the establishment of a replication compartment are well understood, little is known about the egress of bacteria from their host cells and how infection is spread between cells. In Dictyostelium a F-actin based structure, the ejectosome, allows non-lytic egress of mycobacteria (Hagedorn M et al., 2009). Applying different microscopy techniques we are investigating the molecular mechanisms underlying its formation and function. The “Vasodilator-stimulated phosphoprotein“ (VASP), a regulator of polymerization and bundling of actin, strongly accumulates in the ejectosome. If VASP is knocked out the cells form less ejectosomes and a strong enrichment of ArpC4 and WASP in the ejectosome is observed. Most importantly, VASP knockout cells show a significant reduction in transmission of mycobacteria and presumably in the ejectosome function.
This data indicates that the protein VASP is directly involved in the maintenance of the ejectosome function and therefore for successful cell-to-cell transmission of mycobacteria. We hypothesize that WASP and ArpC4 are partially able to complement a lack of VASP and suppose a redundancy between actin nucleation machineries. While its precise function needs to be determined we suggest that VASP gives the ejectosome structure rigidity for efficient ejection of mycobacteria.

Segregation – does it protect against cheaters?

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Previous studies have shown that D. discoideum strains exhibit kin discrimination. The decision whether two strains segregate is dependant upon the highly polymorphic genes tgrB1 and tgrC1. These genes are under positive selection but they were also shown to co-evolve and encode for transmembrane proteins that bind each other. At present, two hypotheses about what drives the evolution of tgrB1 and tgrC1 have been proposed. Firstly, as amoebae are vulnerable to exploitation by cheaters, segregation could be a protection mechanism. Secondly, correlation between genetic distance and segregation suggests that, as genomes drift apart and become distinct, they slowly start to become developmentally incompatible.
We chose to investigate segregation and cheating patterns as well as genome diversity within 24 co-occurring D. discoideum strains. Conducting all pairwise comparisons, we found that most strains segregate from each other in varying degrees and that there is a nearly linear cheating hierarchy. By comparing chimaeric behaviour on soil, where strains can segregate, and on agar, where segregation does not occur, we then tested whether cheating behaviour changes when strains are able to segregate.
In order to determine the genome diversity of the strains, we conducted a SNP analysis using NGS, which revealed that they show surprisingly high divergence in several hundred genes. Both tgr genes were among the genes with the highest number of SNPs. Using the extracted tgr sequences, a binding assay showed that the strength of binding is dependant upon the differences in the whole protein not only within the binding domains.
Taken together, we were able to show that segregation is a complex mechanism and our data are consistent with diversification driven by cheating.

Structural analysis of acidic N-glycans of wild type and mutant Dictyostelium strains

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The Dictyostelium N-glycome has been of scientific interest for over three decades. Although it is a simple eukaryotic organism, its N-glycosylation is more complicated and unusual compared to other model organisms. Using modern techniques as HPLC, MALDI TOF MS and MS/MS, the neutral and acidic N-glycan structures were determined. In contrast to the neutral structures (oligomannosidic Man8A isomer forms modified with core a 1,3-fucose and terminal bi- and intersecting GlcNAc), the analysis of the acidic residues was more challenging. A new analytical workflow was established to separate the neutral from the acidic N-glycans, prior to detailed analysis. The major modifications found attached to mannose residues were sulphate, phosphate and methylphosphate. In the acidic N-glycome of the wild type axenic strain Ax3, up to a maximum of five of these residues, were found on a single N-glycan structure (Man8GlcNAc2[PMe]2S3). The analysis of diagnostic fragments generated by MALDI TOF MS/MS in positive and negative ion mode combined with exoglycosidase and chemical treatments enabled the fine analysis of single purified structures. Furthermore, the alteration of the acidic N-glycome was also studied in two N-glycosylation axenic mutant strains. Based on the structural determination of the analysed N-glycans, the potential biosynthetic routes of the N-glycan processing pathways in Dictyostelium can be deduced.

Silencing of calcineurin leads to abnormal mitochondria: the cause of oxidative stress?

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Calcineurin is a Ser/Thr-phosphatase, which has been shown to play a role during stress and development of Dictyostelium discoideum. However, compared to other organisms, only one substrate of calcineurin, the transcription factor TacA, has been identified so far in D. discoideum. Here we describe the first results concerning a putative connection of calcineurin with the mitochondria. We show that in vegetative cells the amount of mtDNA is approximately doubled in the calcineurin A- and B-RNAi mutants (CNA-RNAi and CNB-RNAi, resp.). Using transmission electron microscopy we observe differences in the mitochondrial morphology of the mutants, and the size of the mitochondria is increased compared to the wild-type. During starvation, the amount of mtDNA is reduced to normal levels. To get first insights if the differences in mtDNA content and morphology have an impact on mitochondrial function in vegetative cells we measured the respiration of the cells and analysed the expression of mitochondrial and nuclear-encoded genes. Respiration of wild-type and mutant cells was comparable but in contrast expression of most of the mitochondrial genes was downregulated. One of the upregulated genes was the alternative oxidase (aoxA) and we therefore investigated also other genes involved in oxidative stress and found that those were also upregulated in the RNAi-mutants during vegetative growth. In starving cells the expression levels of genes involved in oxidative stress were similar in wild-type and mutant cells. Therefore we speculate that silencing of calcineurin might induce intracellular oxidative stress specifically during vegetative growth of D. discoideum.

A proteomic approach to identify molecular mechanisms of symmetry breaking and chemotaxis

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Central to chemotaxis is the molecular mechanism by which an external shallow gradient of chemo-attractant induces an intracellular steep gradient of activated signaling molecules. This process, called symmetry breaking, is highly complex and involves numerous signaling molecules and interacting signaling pathways. We previously used Dictyostelium mutants to investigate the minimal requirements for chemotaxis, and identified a basal signaling module providing symmetry breaking at the level of Ras activation [1]. This symmetry breaking absolutely requires Ga2 and Gβγ, but not the cytoskeleton nor the four cAMP-induced signaling pathways, PIP3, cGMP, TorC2, PLA2 [2].
Here we used a highly sensitive proteomic approach to identify additional components of this basal pathway and to determine the mechanism by which heterotrimeric G-proteins regulate Ras activation. In the first step recombinant G-proteins were expressed and purified from bacteria as glutathione-S-transferase (GST)-fusion proteins. To identify both regulators and downstream effectors, the G-proteins were loaded either with non-hydrolysable GTP (GppNHp) or with GDP, and then used as bait in mass-pull-down experiments from Dictyostelium cell lysate. The resulting interactome was characterized using SDS-PAGE and in-gel tryptic digestion followed by LC-MS/MS and bioinformatic analysis. Subsequently we confirmed the interactions of a subset of proteins and characterized their function by biochemical and genetic analysis.
This approach together with the advantage of Dictyostelium as model system will give new insights in the molecular mechanisms of symmetry breaking and chemotaxis.

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2. Kortholt A, Keizer-Gunnink I, Kataria R, Van Haastert PJ (2013). J Cell Sci. 26: 4502-13.

Dicer-like proteins in Dictyostelium

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Dicer proteins are endoribonucleases of the RNase III family that cleave double stranded RNA into small regulatory RNAs with functions in posttranscriptional gene silencing or chromatin modifications. Well studied types of such Dicer products are siRNAs and miRNAs, which were also identified in Dictyostelium, however less is known about their biogenesis. The genome of Dictyostelium encodes for two similarly structured but non-redundant Dicer-like proteins, referred to as DrnA and DrnB that were shown to localize in cytoplasmic and nucleolar associated foci, respectively. The identity of those structures is still unknown in Dictyostelium, but their appearance is reminiscent of animal P-bodies and plant Dicing- or Cajal-bodies. Several attempts to delete the drnA gene were unsuccessful, indicating that the gene might be essential. We show that the known function of DrnB in miRNA biogenesis (Hinas et al., 2007) is obviously essentially tied to its subnuclear, or yet nucleolar localization and is independent of its internal dsRBD. The assembly close to or within nucleoli suggests furthermore a role in rRNA maturation for which an accumulation of 17S derived small RNAs in the absence of DrnB is an initial indication. This observation lacks comprehension and was not yet evidently described for Dicer proteins, however, prototypical RNase III proteins in bacteria and yeast are well known for their role in rRNA maturation. In another part of this study recombinant RNase III domains were shown to cleave dsRNA molecules at junctions of dsRNA and ssRNA without specificity for perfectly base paired dsRNA or ssRNA, offering new insights into the catalytic properties of Dicer proteins which appear to be divergent in Dictyostelium, compared to other species.

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Suppressors of the tgrC1– phenotype: signal transduction and evolvability

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The cell-cell adhesion molecules, TgrB1 and TgrC1, mediate allorecognition and cell differentiation during development in Dictyostelium. They are essential for development as single-gene deletions (tgrB1-null or tgrC1-null) lead to a developmental arrest at the loose mound stage and lack of cell-type differentiation. Moreover, TgrB1-TgrC1 functions as a receptor-ligand pair for kin recognition and are highly polymorphic in wild populations. Cells carrying a divergent allelic pair of tgrB1/tgrC1 genes segregate from one another during streaming.
The TgrB1/TgrC1 system provides a model to study the molecular basis of rapid evolution of adhesion receptors. It is intriguing how adhesion receptors can diverge rapidly despite the fact that they have essential functions in development. To elucidate the evolvability of this system, we are dissecting the signal transduction pathways that mediate TgrB1/TgrC1 signaling. We have devised screens for suppressors that rescue the developmental defects in tgrC1-null cells. We discovered several candidate suppressor genes, including alg9 (glycosyltransferase) and stcA (suppressor of tgrC1–), that suppress some of the tgrC1-null phenotypes. Inhibition of N-glycosylation by insertional mutation in alg9 or by Tunicamycin treatment allow tgrC1-null cells to sporulate. The mutation stcA(ins) also increases the sporulation efficiency of tgrC1-null. Co-development of the suppressor mutants and tgrC1-null cells rescued the development of the latter, suggesting that suppression acts non-cell-autonomously.
The selection for maintaining the essential function in development and the compatibility between TgrB1 and TgrC1 likely restricts the diversification of the tgrB1/tgrC1 genes. However, genetic suppressors, such as alg9 and stcA, may play a role in buffering the effects of novel tgrB1/tgrC1 mutations and allow the organism to tolerate the associated fitness disadvantages while promoting expansion of the allelic repertoire of tgrB1/tgrC1.

Target search for DrnB dependent microRNAs in Dictyostelium discoideum

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We have previously reported the presence of miRNAs in Dictyostelium discoideum and demonstrated that the biogenesis of the tested ones are strictly dependent on DrnB, one of the two dicer-like proteins in D. discoideum. Dicer proteins play key roles in the RNA silencing pathway in eukaryotes commonly by cleaving long double stranded RNAs into small RNA duplex. These small dsRNAs are incorporated into the RNA Induced Silencing Complex (RISC). Subsequently, one strand is discarded and the remaining RNA strand guides RISC to the target RNA which is recognized by base-pairing. Upon binding of RISC to the target RNA, mRNA cleavage and/or translational inhibition is induced, leading to reduced protein synthesis.
To study the regulatory role of DrnB, we performed high throughput sequencing of mRNA and small RNAs from growing as well as developing cells of drnB knock-out and wild-type strains. Our analysis shows that more than 400 genes are up-regulated as an effect of DrnB depletion in either growing or developing cells. Among them, 65 genes are up-regulated at both time points. These genes are involved in several different molecular functions, for example oxidoreductase activity and RNA binding. As expected, the analysis of the sequenced small RNAs showed that mature miRNAs were absent in cells depleted of DrnB, underscoring its essential role in miRNA production. One of the most abundant miRNAs, ddi-mir-1177, was studied in more detail. The absence of mature miRNAs was confirmed by northern blot while its corresponding precursor accumulates in drnB knock-out cells. In order to pin-point the function of ddi-mir-1177, we also performed mRNA analysis on a strain over expressing ddi-mir-1177. Furthermore, comparison of mRNA expression in cells over expressing ddi-mir-1177 and in drnB knock-out cells (lacking miRNAs) identified 44 genes with opposite expression pattern. Hence, these genes are putative targets for ddi-mir-1177.

GflB mediated Rap activation regulates cytoskeletal rearrangement in Dictyostelium

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With a pull-down screen using Dictyostelium Gα proteins as bait we identified a RasGEF and RhoGAP containing protein, GEF-Like Protein B (GflB), as one of the interacting parters of Gα2. GflB-null cells have delayed development, severely defected chemotaxis and decreased myosin II assembly. Further characterization showed that the N-terminal part of GflB interacts with active Gα2, the GEF domain of GflB specifically activates the small G-protein RapA and the GAP domain of GflB specifically interacts with RacB and RacL. In Dictyostelium, RapA is important for cytoskeletal regulation during cell migration and phagocytosis [1]. Together our data suggest that GflB links heterotrimeric and monomeric G-protein signaling to cytoskeletal rearrangement and is therefore important for Dictyostelium development and chemotaxis.

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Skipper retrotransposon mobilization in Dictyostelium discoideum

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Skipper is a 7 kb Long Terminal Repeat (LTR) retrotransposon that occurs in about 60 mostly fragmented copies in the genome of Dictyostelium discoideum. The two identical LTRs, directed in the same orientation, flank two or three open reading frames that code for proteins with typical LTR retrotransposon domains, namely a nucleocapsid protein Gag, a protease Pro, and polyprotein Pol. We investigated the Skipper transcript levels in deletion strains of the three RNA-dependent RNA Polymerases (RdRPs) genes rrpA, rrpB and rrpC of Dictyostelium, which we had generated in all possible combinations. Northern blots and qRT-PCR reveal that elevated Skipper levels in the rrpA and rrpC deletion strains, indicating that RrpA and RrpC are involved in Skipper silencing. Moreover, we observed cumulative effects in the accumulation of Skipper transcripts in the rrpA:rrpC double knock-out, but, surprisingly, not in the triple knock-out of all three rrp genes. Illumina deep sequencing revealed only few Skipper siRNAs in the AX2 wild type. In the rrpC deletion strain, however, two “hot spots” with specific skipper siRNAs were observed, that accumulate compared to AX2. We currently analyze if deletion of the rrp genes leads not only to accumulation of skipper transcripts, but also to genomic mobilization of the retroelement. For this purpose we generated synthetic Skipper retrotransposons (skippermbsrI), carrying a blasticidin reporter cassette in antisense orientation. This cassette is disrupted by an intron positioned in sense orientation with respect to Skipper. The resistance cassette is only functional after a full retrotransposition cycle of Skipper. We will report on the results of this retrotransposition assay that we perform in all rrp gene knock-out variants.

The Argonaut protein agnA affects siRNA levels and accumulation of a novel extrachromosomal DNA from the Dictyostelium retrotransposon DIRS-1

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Non-coding RNAs are essential for gene regulation in eukaryotes and important players in many different biological processes, e.g. in transposon control.
The retrotransposon DIRS-1 has important structural features in genome stability by constituting the centromeres of the six chromosomes in Dictyostelium discoideum. The vast majority of cellular siRNAs is derived from DIRS-1, suggesting that the element is controlled by RNAi related mechanisms. Here we investigated the role of two of the five Argonaute proteins of D. discoideum, AgnA and AgnB, in DIRS-1 silencing. Deletion of agnA resulted in the accumulation of DIRS-1 transcripts and loss of most DIRS-1 derived secondary siRNAs. Hence, DIRS-1 encoded proteins accumulated in the mutant background and extrachromosomal single stranded DIRS-1 DNA was detectable in the cytoplasm. These DNA molecules appear to be products of reverse transcription, associate with the DIRS-1 encoded Gag protein and thus could represent intermediate structures before transposition.
The deletion of agnB alone had no strong effect on DIRS-1 transposon regulation. However, milder phenotypes were observed in agnA−/agnB− double mutant strains compared with the agnA- single mutant. Especially the accumulation of extrachromosomal DNA was strongly reduced compared to the single agnA− strains. This indicated that AgnB has a positive effect on transposon amplification, while AgnA leads to DIRS-1 silencing.

Robustness of tip cell differentiation

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How genetically identical cells develop into distinct cell types is one of the fundamental questions in biology. We have previously shown that gefE null cells are biased to become tip and prespore cells when they are developed in chimera with wild type cells (Chattwood et al. eLIFE 2013). To elucidate the downstream effects of a gefE knock-out, we performed transcriptome analysis to identify mis-regulated genes in the mutant. Among dozens of abnormally expressed genes, the most differentially expressed gene was spb, with 100-fold expression in gefE- compare to wild type. Knocking out spb rescued the tippy bias of gefE- cells but not the sporey one, suggesting spb might be predominantly responsible for tip cell differentiation. Consistent with this idea, a single knock-out of spb results in cells that are unable to form tip cells in chimera, but only under certain conditions.
Cells grown in glucose rich media (G+) are also biased toward tip and prespore cell fate in chimeric development with cells grown in glucose poor media (G-). We found spb- cells are more sensitive to this nutritional bias, although they don't show any bias when they are grown in G+ media. This raises the possibility that there are at least 2 individual pathways to induce tip cell formation; glucose dependent and glucose independent, both of which are able to compensate for the effects of the other. We propose spb is a component of the glucose independent pathway since mutant cells cannot compensate for glucose effect, thus, they directly reflect the outcome of glucose dependent pathway. We will discuss how cells maintain robustness on cell fate decisions when they are grown in various environments.

How confinement induces polarity in motile amoeba

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Motile eukaryotic cells, such as leukocytes, cancer cells, and amoeba, typically move inside the narrow interstitial spacings of tissue or soil. While most of our knowledge of actin-driven eukaryotic motility was obtained from cells that move on planar open surfaces, recent work has demonstrated that confinement can lead to strongly altered motile behavior. Here, we report experimental evidence that motile amoeboid cells undergo a spontaneous symmetry breaking in confined interstitial spaces. Inside narrow channels, the cells switch to a highly persistent, unidirectional mode of motion, moving at a constant speed along the channel. Their actin cytoskeleton exhibits a characteristic arrangement that is dominated by dense, stationary actin foci at the side walls, together with less dense dynamic regions at the leading edge. Our experimental findings can be explained based on an excitable network model that accounts for the confinement-induced symmetry breaking and correctly recovers the spatio-temporal pattern of protrusions at the leading edge. Since motile cells typically live in the narrow interstitial spacings of tissue or soil, we expect that the geometry-driven polarity we report here plays an important role for movement of cells in their natural environment.

SteelyA is required for cAMP signalling during early Dictyostelium development

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The hybrid-type polyketide synthase SteelyA synthesizes 4-methyl-5-pentylbenzene-1, 3-diol (MPBD) and this compound acts as a spore maturation factor during late Dictyostelium development. However, the expression of stlA shows larger peak in the pre-aggregation stage than the late stage involved in spore maturation, suggesting that SteelyA has an additional function other than spore maturation. In this study, we focused on the function of SteelyA enzyme in the early stage of development.
We found that a stlA null mutant showed aggregation delay and abnormally small aggregation territories. The mutant has a defect in cAMP chemotaxis but no defect in folate chemotaxis. MPBD addition during starvation rescued these defects in the mutant cells. Assay for cAMP relay response revealed that during aggregation, the stlA null mutant had lower cAMP accumulation than that of wild-type Ax2, suggesting defective cAMP production in the mutant. Exogenous cAMP pulses rescued the aggregation defect in the mutant in the absence of MPBD. Expression analysis of cAMP signalling genes revealed that their expressions in the mutant were down regulated during early development, especially at 3 hours from starvation which stlA were maximally expressed. These results indicated that SteelyA regulates cAMP signalling gene expressions required for aggregation and makes starving cells aggregation competent during early development.

Gp64 gene of Polysphondylium pallidum may have a Role in a Light Regulation of Lateral Branching

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Polysphondylium pallidum (WS320) is a cellular slime mold with regularly spaced whorls of lateral branches. A WS320 strain, obtained from Slime Mold Collection, was noticed that the extent of branching of its sorocarps is low and variable. We speculated that an unfavorable mutation occurred in the population overwhelmed, and so we have separated fully branching strain (FBS) from the poorly branching strains (PBS). Previously we showed that gp64 is a GPI-anchored, membrane protein of Polysphondylium pallidum (Manabe et al., 1994; Saito et al., 1994), and has some role in branching of the species. We, therefore, compared sequences of the gp64 genes of fully (FBS) and poorly branching strains (PBS) isolated. It was found that the gp64 gene of PBS has lost thymidine at the 11th base pairs downstream from the initial ATG codon in the gp64 gene of FBS. Both the strains were selected based on differences in branching of sorocarps grown on LP agar plates in the light, and their extents were reproduced consistently on the same plates. Unexpectedly, however, when both strains were allowed to develop on Millipore filters in the light, they both achieved fully branching on the filter, but in the dark, while the FBS showed extensive branching, the PBS developed only elongated, lodging sorophores; there is no sign of branching on their sorophores even after 72 hrs, importantly after short-term light exposure PBS begins to branch. Our findings reveal a critical role for gp64 in light regulation and thereby monitoring for environment around the slime mold.

References:
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Ribosomal RNA processing in Dictyostelium discoideum

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Transcription of rRNA genes is highly regulated to respond to both general metabolism and stress conditions. In the cellular slime mold Dictyostelium discoideum, unicellular amoebae aggregate when starved, leading to formation of multicellular fruiting bodies containing spores. The ribosomal RNA genes in Dictyostelium are located on an extrachromosomal palindrome that exists in~100 copies in the cell. To characterize the ribosomal RNA (rRNA), we have ligated the rRNA ends and performed RT-PCR on these circular RNAs(1). Sequencing revealed that the mature 26 S, 17 S, 5.8 S, and 5 S rRNAs have sizes of 3741, 1871, 162, and 112 nucleotides, respectively. The calculated full length ~ 7.5 Kb precursor rRNA transcript was seen by Northern blotting, and the major processing sites in ITS1 and ITS2 were mapped by primer extension. Northern blots and RT-PCR reveal that from the primary transcript two precursor molecules of the17 S and two precursors of the 26 S rRNA are generated. We have also determined the sequences of these precursor molecules, and based on these data, we have proposed a model for the maturation of the rRNAs in Dictyostelium discoideum (1). Further we have observed that Pre r-RNA level goes up during the development of spores at 16hr and again went down in mature spores. This preliminary data indicates that regulation of ribosomal RNA transcription may have a major role in development of spores in Dictyostelium discoideum.

(1) Boesler et al. (2011) J. Biol. Chem. 286, 17693-17703

The small noncoding RNA response during mycobacterial infection of Dictyostelium discoideum

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Mycobacterium tuberculosis remains a major threat to health worldwide. Despite extensive research several aspects of mycobacterial pathogenesis are unknown. Small noncoding (nc)RNAs such as micro (mi)RNAs are important regulators of gene expression and can mediate RNA silencing at the transcriptional or translational levels by guiding effector protein complexes to target mRNAs. Recent reports indicate that small RNAs play important roles in host-pathogen interactions. Here, D. discoideum was infected with M. marinum, a close relative of M. tuberculosis, to study the small ncRNA response during infection. Total RNA was extracted and mRNA and small RNA libraries were prepared and sequenced using Illumina technology.
Around 1100 D. discoideum genes were differentially expressed upon infection. GO-term analysis identified upregulation of genes involved in e.g. endosome formation and downregulation of cellular respiration and cation transport genes. Interestingly, the Argonaute B gene was upregulated 4fold. Argonautes are effector proteins in gene silencing by small RNAs. The putative ribonuclease eriA that is homologous to a negative regulator of RNA interference in C. elegans was also upregulated. We have verified the differential expression of a set of genes by qRT-PCR.
In the small RNA library from infected samples we observed a marked enrichment of 32-nt reads. Specifically, stable 5'-end fragments of tRNA-Cys were detected and confirmed by northern blot. In addition, a subset of the dictyostelid specific Class I ncRNAs was upregulated, whereas miRNA-expression was downregulated.
In conclusion, M. marinum infection in D. discoideum results in downregulation of known miRNAs, upregulation of Argonaute B and several small ncRNAs and leads to enrichment of stable tRNA fragments. tRNA-fragments have been indicated in regulatory processes upon stress in several organisms. These results strongly indicate that small RNAs are part of the host response to infection.

Characterization of actin variants in D.discoideum

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The actin cytoskeleton in eukaryotic cells plays an important role in cell shape, structure, migration, and intracellular transport. As D. discoideum harbors a relatively large actinome composed of 33 actin genes and eight genes coding for actin related proteins, this social amoebae serves as an excellent model organism to study the actin system. Conventional actin is encoded by 17 distinct genes (Act8 group), whereas the protein sequences of the other 16 actin variants are nearly identical (e.g. 97% identity in Act3) or differ strongly like in Act18 or Act31 (88% or 37% identity, respectively). Selected actin variants with different expression levels and identity to conventional actin have been compared, to understand the functions of actin isoforms. GFP-fusion constructs have been analyzed in D. discoideum. GFP-Act3 colocalizes with conventional actin in the F-actin network, whereas GFP-Act18 and GFP-Act31 are soluble proteins in the cytoplasm. All mutants show defects during growth, germination and phagocytosis. Another interesting observation is the presence of GFP-Act3 in stress-induced nuclear actin rods. GFP-Act18 and GFP-Act31 overexpressors are able to form nuclear rods, but these actin variants are not part of the rods. In vitro studies were performed by amplifying Flag-tagged fusion proteins via the baculoviral protein expression system. Flag-Act3 as a part of the F-actin network could act as a nucleator and favors elongation in actin cables, whereas actin patches remain unaffected. Flag-Act18 and Flag-Act31 are assumed to be either regulators of the cytoskeleton or just subunits of larger protein complexes.

Chemical ecology of Dictyostelium-How does Dictyostelum repel the nematodes?

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Dictyostelium and nematodes seem to have a close relationship with each other. They live in the soil and sometimes compete for foods. Since the relationship between Dictyostelium and non- parasitic nematode C. eleganse has been reported before, we focused on the relationship between Dictyostelium and parasitic nematode, Root knot nematode (RKN), that damages 5% of the total crops in the world.
We found that RKNs avoided Dictyostelium in the laboratory condition. This avoidance reaction is weaker in Polysphondylium and Actyostelium than in Dictyostelium. To try and identify the chemical compound(s) that repel RKN, we analysed the secreted chemical compounds from Dictyostelium cells and found the repulsion activity to the parasitic nematodes. This indicates that the chemical compounds released from Dictyostelium have repulsion activity. For further analysis, cell released materials from the several polyketide synthase knockout mutants were analysed and one mutant strain showed low repulsion activity. This result suggests that Dictyostelium polyketides are involved in this repulsion activity . Further research is needed to determine the contributor of this effect.

Is ‘noisy’ gene expression responsible for scattered differentiation?

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The process of random or scattered differentiation is one that can be observed from metazoans to social amoeba. How this 'salt and pepper' patterning can be achieved in a population of genetically identical cells which are all exposed to uniform differentiation signals though is still not well understood. We postulate that the heterogeneity seen in populations of differentiating cells is caused by variable, or 'noisy' patterns in gene expression of certain lineage priming genes within individuals. This then allows for only a certain proportion of a population to be primed towards a given cell fate at any one time. The creation of this variable gene expression has recently been linked to the presence or absence of various histone modifications, including methylation created by the enzyme set1. To study this idea that 'noise' is responsible for heterogeneity we have created a set1 mutant cell line in D. discoideum. By making use of chimeric developments we have been able to show that modulating variable gene expression through set1 deletion can lead to a bias in mutant cells towards a stalk-cell fate. Using this knowledge we hope to identify genes which may be responsible for lineage priming and study their expression patterns to further understand how scattered differentiation patterns are generated. Beyond this we recognise that methylation by set1 as well as other chromatin marks can often be produced as a result of antisense transcription. These non-coding transcripts have also been shown to have heterogeneous patterns of expression within populations as well as being linked to cell fate decisions and as such we believe they may be involved in the control of variable gene expression. We have been able to detect the presence of antisense transcription in D. discoideum and are interested to observe what affect these transcripts have on lineage priming gene expression.

Microtubule function in directed motility in neutrophils and Dictyostelium

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Chemotactic movement of many well-studied eukaryotic cell types such as leukocytes, metastatic tumor cells or Dictyostelium cells requires sensing of a gradient, the establishment of polarity and coordinated shape changes that involve cytoskeletal rearrangements. Directed migration towards a chemotactic source is largely dependent on coordinated actin cytoskeleton functions that provide the driving forces at the cell front and enables contractility at the cell rear. In contrast to the force-generating properties of the actin cytoskeleton, the microtubule network assumes a more regulatory function in balancing front-to-back polarity in some cell types including neutrophil granulocytes. However, it is not clear whether this also applies for microtubules in migrating Dictyostelium cells. Previous reports together with our studies of the microtubule system of Dictyostelium and neutrophil-like HL-60 cells during migration in 2D- and 3D-environments revealed that their modes of reorganization follow similar patterns.
In migrating neutrophils, microtubules are mostly concentrated at the cell rear, where they are stabilized and kept in place by a Cdc42-, WASP-, EB1- and CD11b-dependent mechanism. EB1, as a +TIP protein, is an ideal candidate to bridge the gap between microtubule- and actomyosin-dynamics. Although knockdown of EB1 did not alter the overall microtubule organization in migrating HL-60 cells, directionality of migration and straightness of EB1 mutant cells moving through 3D collagen gels were significantly reduced, indicating that EB1 is involved in directed migration. Accordingly, an increased number of lateral protrusions in EB1 knockdown cells is observed, possibly pinpointing at alterations in their ability to balance cell polarity. Finally, in EB1 mutants, we found substrate adhesion after spreading or during haptokinetic movement on fibrinogen-coated surfaces to be impaired.

Polyketides induce prestalkA cells in Dictyostelium discoideum.

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We wanted to know the functions of polyketides in the prestalk and stalk cell differentiation. When Dictyostelium cells were treated with high concentration of cerulenin, the expression of pstA, a prestalk marker gene, was completely inhibited. This result indicates that polyketides are indispensable for the expression of prestalk marker genes. Interestingly, even without the prestalk gene expression, the cerulenin treated cells made fruiting bodies, though they looked very fragile. Their spores were not encapsulated and remained as amoebae like cells. These cells looked quite similar with these of stlA null mutant. The stalk was made of single layered cell and so very fragile. Most of the fruiting bodies laid down on the agar surface like stlB null mutant. Microscopic analysis showed that the stalk cells were normally vacuolated. We created stlA and stlB double knockout mutant and confirmed that this mutant showed the very faint expression of pstA marker and made fragile stalk. Both cerulenin treated cells and the double knockout mutant cells made fragile but normally vacuolated stalk. These results indicate that prestalk cells are induced by polyketides and the stalk cell differentiation is controlled by different chemical compound that is most likely c-di-GMP. The normal expression of dgcA was confirmed both in cerulenin treated cell and in the double knockout mutant cells.

The social amoeba Polysphondylium pallidum loses encystation and sporulation, but can still erect fruiting bodies in the absence of cellulose

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Amoebas and other freely moving protists differentiate into walled cysts when exposed to stress. As cysts, amoeba pathogens are resistant to biocides, preventing treatment and eradication. Lack of gene modification procedures has left the mechanisms of encystation largely unexplored. Genetically tractable Dictyostelium discoideum amoebas require cellulose synthase for formation of multicellular fructifications with cellulose-rich stalk and spore cells. Amoebas of its distant relative Polysphondylium pallidum (Ppal), can additionally encyst individually in response to stress. Ppal has two cellulose synthase genes, DcsA and DcsB, which we deleted individually and in combination. Dcsa- mutants formed fruiting bodies with normal stalks, but their spores and cyst walls lacked cellulose, which obliterated stress-resistance of spores and rendered cysts entirely non-viable. A dcsa-/dcsb- mutant made no walled spores, stalk cells or cysts, although simple fruiting structures were formed with a droplet of amoeboid cells resting on an sheathed column of decaying cells. DcsB is expressed in prestalk and stalk cells, while DcsA is additionally expressed in spores and cysts. We conclude that cellulose is essential for encystation and that cellulose synthase may be a suitable target for drugs to prevent encystation and render amoeba pathogens susceptible to conventional antibiotics.

The Elongator complex in Dictyostelium discoideum

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The highly conserved eukaryotic Elongator complex is known to be involved in numerous cellular mechanisms like transcriptional elongation, histone acetylation as well as tRNA modification at the wobble base of certain tRNAs. The unstable holo-complex consists of 6 proteins and can be divided into two subcomplexes; the core-complex consisting of the Elongator Proteins Elp1-3 and the HAP complex consisting of Elp4-6. Applying pulldown experiments of GFP-tagged Elp3 and Elp4 homologues using a GFP-nanotrap, a multiprotein complex was identified, indicating the existence of a holo-complex (Elp1-Elp6) in Dictyostelium discoideum. Further, analyses of tRNA wobble base U34 modifications mcm5 and ncm5 in the AX2 wildtype and Elp3 knockout strain suggest that these tRNA modifications are potentially executed by the Elongator complex. We will investigate the role of the wobble base tRNA modification by the Elongator complex in gene expression, for which we will be using computational data on codon usage combined with expression analysis of certain genes.

Evolutionary reconstruction of pattern formation in 98 Dictyostelium species reveals that cell-type specialization by lateral inhibition is a derived trait

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Multicellularity provides organisms with opportunities for cell-type specialization but also requires novel mechanisms to position correct proportions of different cell types throughout the organism. Dictyostelid social amoebas form multicellular fruiting bodies in which, depending on the species, cells differentiate solely into spores, the asexual gametes propagation units of Dictyostelia, or additionally into up four different 'somatic' cell types that support the spore head. Molecular phylogenetic inference subdivides Dictyostelia into four major and some minor groups. Using a cell-type specific antibodies antibody and promoter-reporter fusion constructs, we investigated the order in which cell type specialization evolved in 98 species that represent all groupings. Our results indicate that except for group 4 and clade 2A, most species differentiate into maximally two cell types, with pattern formation being dominated by position-dependent transdifferentiation of prespore cells into prestalk cells. In Clade 2A, the prespore cells also construct an acellular stalk. Group 4 species set aside correct approximate proportions of prestalk and prespore cells early in development, and differentiate into up to three more cell types, that which form specialized support structures. In the group 4 species D.discoideum, these cells and the prestalk cells have been shown to differentiate intermixed with prespore cells by lateral inhibition-type mechanisms and then to sort to their final location. Only the transition of prestalk into stalk cells is still under positional control in group 4. Group 4 spore masses are significantly larger than those of other groups and it is likely that the formation of different support structures by lateral inhibition is an innovation that facilitated this increase in size.

Retrotransposition of TRE5-A in the Dictyostelium discoideum genome is sustained by suppression of RNA interference by a host-encoded factor

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Mobile elements are major drivers of genome evolution; however, excessive transposition activity is a permanent threat to genome stability and can be disastrous for the host. Dictyostelium discoideum is an intriguing model to study transposable element-host interactions because its genome is haploid, highly packed with genes, and presents limited space in intergenic regions as potential transposon integration sites. In such a gene-dense genome there is limited space for transposons to replicate without being harmful (in terms of reduced fitness) to the host. Even though D. discoideum has a potent RNAi machinery, the retrotransposon TRE5-A has established a fairly high amplification rate in growing D. discoideum cells despite the constitutive production of minus strand (antisense) RNA from an element-internal promoter, the C-module. The C-module binding factor (CbfA) is a transcriptional regulator involved in the regulation of TRE5-A retrotransposition. In a CbfA-underexpressing mutant both plus-strand and minus-strand RNA of TRE5-A are reduced by more than 90 %, and this reduction of transcript levels is accompanied by a strong reduction of TRE5-A's in vivo retrotransposition activity. Preliminary data suggest that CbfA supports TRE5-A amplification indirectly by down-regulating putative components of the RNAi machinery. Transcriptome analysis of the CbfA-depleted mutant revealed an approximately 200-fold and 3-fold overexpression of the genes encoding D. discoideum Argonaute-like proteins AgnC and AgnE, respectively. In this study, we show that TRE5-A is overexpressed in knock-out mutants of either agnC or agnE. When the D. discoideum genome was engineered to overexpress the agnC gene, TRE5-A expression was drastically silenced. Thus, our results indicate that TRE5-A amplification in D. discoideum cells is under surveillance by putative components of the cellular RNAi machinery, but this control is partially overcome by suppression of RNAi by the host factor CbfA.

Intracellular, intercellular and cell-substrate dynamics

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One essential feature of cell migration is pseudopod formation, where the cell membrane is locally pushed out by actin polymerization. Here we study the dynamics and pattern formation in the cortex of D. d.. Besides signaling pathways of directional sensing and motility, chemotaxis includes polarity, a breaking of the symmetry that gives the cell a leading an retracting edge. In a theoretical analysis we have studied the pattern formation of cell polarity.
Furthermore, D. d. synchronizes itself during the cell signaling phase with waves of cAMP. We have quantified these collective phenomena on the intercellular level by applying for the first time electric impedance measurements (ECIS). We showed that cells seeded on electrodes provoke impedance oscillations. The results provide a quantitative understanding of the overall cell morphology during early starvation. In another study focused on cell migration of D.d., as opposed all migration experiments done on flat surfaces, we use curved substrates. To our surprise we found that D. d. shows what we call curvotaxis. Our experimental observations show that cells prefer to migrate along the curved axis.
Finally, as cell-substrate adhesion of eukaryotes still remains to be fully understood, we study adhesion of D.d., which lacks integrins; it is ideally suited to study alternative adhesion properties reflective of eukaryotic evolution. In Single Cell Force Spectroscopy (SCFS), we attach single cells to a cantilever and probe quantitatively cell-substrate adhesion. We find that substratum adhesion is dramatically reduced during the first 6 hrs of starvation induced development and can be considered developmentally regulated. In collaboration with the UCSD, we compare null mutants of adhesion transmembrane proteins, suggesting that, as opposed to common literature notion, neither of them play a significant role in the adhesion of during early development.

TRE5-A retrotransposition in Dictyostelium reveals RNA polymerase III transcription units on the extrachromosomal ribosomal DNA element

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The retrotransposon TRE5-A shows a remarkable amplification rate in the Dictyostelium discoideum genome. TRE5-A integrates in the close vicinity of tRNA genes, suggesting that this preference protects the host cell from damage caused by insertional mutagenesis. We have previously shown that the selection of tRNA genes as integration sites by TRE5-A requires an active B box, which is part of the tRNA gene-internal promoter region and binding site for the RNA polymerase III transcription factor TFIIIC. TRE5-A integration upstream of tRNA genes most likely involves direct protein interactions of a retrotransposon protein with TFIIIB, which is recruited by TFIIIC in a B box-dependent manner. Analysis of the D. discoideum genome revealed no evidence for "off-target" integrations of TRE5-A elements, yet it remained unclear whether gene disruption by TRE5-A is not observed because affected cells are rapidly lost from the cell population. To address this question, we designed tagged TRE5-A retrotransposons that added blasticidin resistance to cells in which a productive retrotransposition had occurred. As expected, genome-wide analysis revealed new integrations of the tagged TRE5-A element upstream of tRNA genes. However, we also noticed integrations into the extrachromosomal DNA element that carries the rRNA genes of D. discoideum. We detected 22 previously unnoticed B boxes on each palindrome arm that exactly match the tRNA gene consensus. Since TRE5-A targets the TFIIIC/TFIIIB complex that binds RNA polymerase III to initiate transcription, we assume that targets of TRE5-A integration are active transcription units of RNA polymerase III. We provide evidence that TRE5-A integration targets on the extrachromosomal rDNA palindrome are in fact transcribed, pointing to a possible indirect role of active RNA polymerase III transcription units in the nuclear organization and/or transcription regulation of rRNA genes.

Dissection of Francisella virulence in Dictyostelium; a genetically tractable host system

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Francisella is one of the most infectious bacterial agents known where less than 10 CFU can cause severe invasive disease in both vertebrates and invertebrates. The genus is broadly divided into two lineages; F. tularensis subsp that cause tularemia and F. noatunensis subsp that cause francisellosis in aquatic organisms. Upon infection Francisella invades and replicate in a number of cell types where macrophages are an important target. Francisella has also been identified in protists. The professional phagocyte Dictyostelium discodeum could therefore be an attractive model system to dissect host-pathogen interactions. Here the establishment of a Dictyostelium–Francisella infection model is described using the fish pathogen F. noatunensis subsp noatunensis (F.n.n) which has the same optimal growth temperature as Dictyostelium. Within mammalian macrophages Francisella will after cell entry escape from the Francisella-containing phagosomes into the host cell cytosol where it replicates thereby avoiding phagolysosomal fusion and degradation. Mutations in the iglC gene, located within the Francisella pathogenicity island, impair Francisella in phagosomal escape and intracellular growth. Within Dictyostelium, wild-type F.n.n is initially associated with the endosome marker p80 and vATPase, a proton pump for phagosomal acidification. This colocalization is disrupted at 6 hrs post infection illustrating that F.n.n is able to escape from phagosomal maturation and into the cytosol where it is able to persist. This behavior is in contrast to the ΔiglC mutant which after entry most bacteria is efficiently exocytosed within 2 hrs post infection. The mutants that are still present within Dictyostelium fail to translocate into the host cell cytosol and follows the normal phagosomal maturation pathway. Taken together these results mimicking the situation reported for Francisella in other systems and illustrate the power of the Francisella-Dictyostelium infection model.

C2RasGAP1 controlled Ras signaling reveals a novel molecular mechanism of inhibition process in G protein coupled receptor mediated gradient sensing.

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Optimal polarization and continuous chemotaxis in a chemoattractant gradient requires inhibition processes. No molecular mechanism of inhibition process has ever been identified although various models had been proposed to explain how inhibition balances activation to achieve gradient sensing. We have previously demonstrated that a locally regulated inhibition process is essential for gradient sensing. Here, we demonstrate that cells control an appropriate Ras activation for an optimal polarization through an activation-dependent recruitment of a Ras GAP protein. Our finding reveals a novel molecular mechanism of inhibition process at the layer of Ras signaling through C2RasGAP1.

Lineage priming and cell fate choice

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Several cellular events are known to prime cells towards a certain cell fate. For instance, metabolic activity or position in the cell cycle at the start of development are known to bias the outcome of differentiation. Likewise, cytoplasmic calcium levels have been shown to strongly bias cell fate decisions.
Although these factors are known to bias cell fate decisions, it is completely unknown which pathways lie downstream and how such components actually control priming of cells. Consequently, it is also not clear whether these biasing factors may even work through similar pathways.
We therefore aim to better understand how lineage priming is controlled. To do this, we are using forward genetic screens to find mutants that could overcome a stalk-cell bias and whole genome RNA sequencing approaches to identify genes associated with lineage priming. I will report on the progress of these studies.

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Nightlife

just a few suggestions without ranking. The whole area around Friedrich-Ebert-Strasse, Brandenburger Strasse and Holländerviertel is full of Bars and Restaurants. Prices are usually moderate.

Bar

- 11-Line Charlottenstraße 119, 14467 Potsdam, 0331 87908899
- B-West, Zeppelinstraße 146, 14471 Potsdam. 0331/9792013
- Cuhibar, Luisenplatz 7, 14471 Potsdam, 0331/236 9744, *smoking allowed*
- Quo Vadis? Friedrich-Ebert-Straße 20, 14467 Potsdam, 0331/2712232
- Hafthorn, Friedrich-Ebert-Straße 90, 14467 Potsdam, 0331 2800820
- Rossini, Friedrich-Ebert-Str. 88 14469 Potsdam, 0331/6012476
- unscheinBAR, Friedrich-Ebert-Str. 118 14469 Potsdam, 0331/2700642, *smoking allowed*
- Café Labendig, Kurfürstenstrasse 70, 14467 Potsdam, 0176 23906194 *smoking allowed*
- Daily Coffee (Bar/Bistro), Friedrich-Ebert-Straße 31, 14467 Potsdam, 0331/2011979 *smoking allowed in separate room*

Restaurants

German

- Hohle Birne, Mittelstr. 19, 14467 Potsdam, 0331/2800715; Among the best selections of beers in the city center
- Der Butt, Gutenbergstraße 25, Potsdam, 0331 2006066; fish restaurant

Italian

- Villa Apostoli, Sellostr. 19, 14471 Potsdam, 0331/9678574
- Ristorante Da Vinci, Dortustr. 4, 14467 Potsdam 0331/2805189
- Osteria La Maiella, Gutenbergstraße 90, 14467 Potsdam, 0331/6264893
- Pfeffer und Salz Brandenburger Straße 47, Potsdam, 0331/2002777

Spanish

- Mea Culpa, Dortustr. 1, 14467 Potsdam, 0331 2011780
- El Puerto, Lange Brücke 6, 14467 Potsdam, 0331/2759225

Greek

- Athos, Zeppelinstraße 152, Potsdam, 0331/974524

Croatian

- Adriatic, Dortustraße 71c, Potsdam, 0331 8773360

Asian *(China, Thai, Japan, Vietnam, India):*

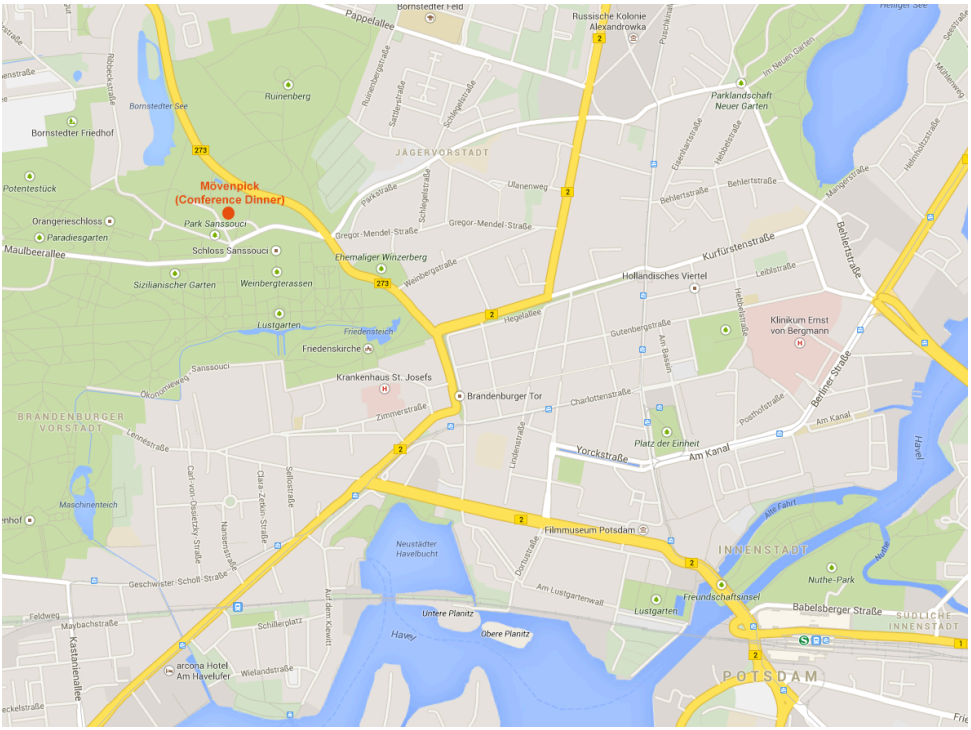
- My Keng, Brandenburger Straße 20, 14467 Potsdam, 0331/9793044, Famous for sushi
- Sala Thai, Dortustr. 71 c, im Innenhof, 14467 Potsdam, 0331/2803670
- China Restaurant Mandarin, Lindenstraße 44, 14467 Potsdam 0331/2804445
- India Haus, Lindenstraße 65, 14467 Potsdam 0331/2804813

Clubs/Discos

find links to the individual clubs here: http://www.cojito.de/potsdam_clubs_22587.htm

- Club 18, Pietschkerstr. 50, Potsdam
- Club Laguna, Friedrich-Ebert-Str.34, Potsdam, 0331/20056500
- Lindenpark, Stahnsdorfer Str. 76-78, Potsdam, 0331/747970
- Princehall, Zeppelinstraße 136, Potsdam, 0331-8673743
- Waschhaus, Schiffbauergasse 4c, Potsdam, 0331/271560

City center



Time schedule "Wassertaxi"

(by boat to the city center or main station)

you start at "Seminaris Hotel Pirschheide"

A	B	A	B	A	B	A		B	A	B	A	B	A	B
	9:45	**11:15**	13:30	**15:00**	17:15	**18:45**	Park Glienicke - Krughorn	9:45	**11:15**	13:30	**15:00**	17:15	**18:45**	
	9:55	**11:25**	13:40	**15:10**	17:25	**18:55**	Sacrow - Heilandskirche	9:35	**11:05**	13:20	**14:50**	17:05	**18:35**	
	10:05	**11:35**	13:50	**15:20**	17:35	**19:05**	Cecilienhof - Meierei	9:25	**10:55**	13:10	**14:40**	16:55	**18:25**	
	10:15	**11:45**	14:00	**15:30**	17:45	**19:15**	Glienicker Brücke - Potsdam	9:15	**10:45**	13:00	**14:30**	16:45	**18:15**	
	10:25	**11:55**	14:10	**15:40**	17:55	**19:25**	Schloß und Park Babelsberg	9:05	**10:35**	12:50	**14:20**	16:35	**18:05**	
	10:33	**12:03**	14:18	**15:48**	18:03	**19:33**	Schiffbauergasse - Hans Otto Theater	8:57	**10:27**	12:42	**14:12**	16:27	**17:57**	
8:40	10:45	**12:15**	14:30	**16:00**	18:15	**19:45**	Potsdam Hauptbahnhof - Hafen	8:45	**10:15**	12:30	**14:00**	16:15	**17:45**	19:50
8:50	10:55	**12:25**	14:40	**16:10**	18:25		Neustädter Havelbucht - Sanssouci		**10:05**	12:20	**13:50**	16:05	**17:35**	19:40
8:58	11:03	**12:33**	14:48	**16:18**	18:33		Potsdam West - Arcona Hotel		**9:57**	12:12	**13:42**	15:57	**17:27**	19:32
9:03	11:08	**12:38**	14:53	**16:23**	18:38		Inselhotel Potsdam - Hermannswerder		**9:52**	12:07	**13:37**	15:52	**17:22**	19:27
9:12	11:17	**12:47**	15:02	**16:32**	18:47		Kongresshotel am Templiner See		**9:43**	11:58	**13:28**	15:43	**17:13**	19:18
9:18	11:23	**12:53**	15:08	**16:38**	18:53		Seminaris Seehotel - Pirschheide		**9:37**	11:52	**13:22**	15:37	**17:07**	19:12
9:30	11:35	**13:05**	15:20	**16:50**	19:05		Forsthaus / Strandbad Templin		**9:30**	11:45	**13:15**	15:30	**17:00**	19:05

Tram connections from Pirschheide to the city center and back

Tram 91 Bhf Rehbrücke ▸ Bhf Pirschheide

ViP

Tram 91

Tram 91 **Montag - Samstag**

		A	B	A	B			20			20		C	20	C
Bhf Rehbrücke	ab	.	.	.	.	5.07		19.47	20.02			0.22	0.29		1.29
Am Moosfenn		.	.	.	.	5.08		19.48	20.03			0.23	0.30		1.30
Friedrich-Wolf-Str.		.	.	.	.	5.09		19.49	20.03			0.23	0.30		1.30
Zum Kahleberg		.	.	.	.	5.10		19.50	20.04			0.24	0.31		1.31
E.-Claud.-Str./H.-Mann-Allee		.	.	.	.	5.11		19.51	20.05			0.25	0.32		1.32
Waldstr./Horstweg		4.27	4.32	4.47	4.52	5.12		19.52	20.06			0.26	.		.
Kunersdorfer Str.		4.28	4.33	4.48	4.53	5.13		19.53	20.07			0.27	.		.
Sporthalle		4.29	4.34	4.49	4.54	5.14		19.54	20.07			0.27	.		.
Friedhöfe		4.30	4.35	4.50	4.55	5.15		19.55	20.08			0.28	.		.
S Hauptbahnhof	an	4.33	4.38	4.53	4.58	5.18		19.58	20.11			0.31	.		.
S Hauptbahnhof	ab	4.33	4.38	4.53	4.58	5.18		19.58	20.12			0.32	.		.
Lange Brücke		4.34	4.39	4.54	4.59	5.19		19.59	20.13			0.33	.		.
Alter Markt/Landtag		4.36	4.41	4.56	5.01	5.21		20.01	20.15			0.35	.		.
Platz der Einheit/West		.	4.43	.	5.03	5.23		20.03	20.17			0.37	.		.
Dortustr.		.	4.45	.	5.05	5.25		20.05	20.18			0.38	.		.
Luisenplatz-Süd/Park Sanssouci		.	4.47	.	5.07	5.27		20.07	20.19			0.39	.		.
Feuerbachstr.		.	4.48	.	5.08	5.28		20.08	20.20			0.40	.		.
Auf dem Kiewitt		.	4.49	.	5.09	5.29		20.09	20.21			0.41	.		.
Bhf Charlottenhof		.	4.50	.	5.10	5.30		20.10	20.22			0.42	.		.
Schloss Charlottenhof		.	4.51	.	5.11	5.31		20.11	20.23			0.43	.		.
Kastanienallee/Zeppelinstr.		.	4.53	.	5.13	5.33		20.13	20.25			0.45	.		.
Im Bogen/Zeppelinstr.		.	4.55	.	5.15	5.35		20.15	20.27			0.47	.		.
Luftschiffhafen		.	4.56	.	5.16	5.36		20.16	20.28			0.48	.		.
Bhf Pirschheide	an	.	4.58	.	5.18	5.38		20.18	20.30			0.50	.		.

A bereits ab Bisamkiez und ab Alter Markt/Landtag weiter nach Platz der Einheit/Nord
B bereits ab Bisamkiez
C ab E.-Claud.-Str./H.-Mann-Allee weiter nach Bisamkiez

Tram 91 Bhf Pirschheide ▸ Bhf Rehbrücke

ViP

Tram 91

Tram 91 **Montag - Samstag**

Verkehrshinweise						98 A			20			20		
Bhf Pirschheide	ab	.	.	5.10	8.10	.	8.50			19.50	20.00			1.00
Luftschiffhafen		.	.	5.11	8.11	.	8.51			19.51	20.01			1.01
Im Bogen/Zeppelinstr.		.	.	5.12	8.12	.	8.52			19.52	20.02			1.02
Kastanienallee/Zeppelinstr.		.	.	5.14	8.14	.	8.54			19.54	20.04			1.04
Schillerplatz/Schafgraben		.	.	5.15	8.15	.	8.55			19.55	20.05			1.05
Bhf Charlottenhof		.	.	5.16	8.16	.	8.56			19.56	20.06			1.06
Auf dem Kiewitt		.	.	5.17	8.17	.	8.57			19.57	20.06			1.06
Feuerbachstr.		.	.	5.18	8.18	.	8.58			19.58	20.07			1.07
Luisenplatz-Süd/Park Sanssouci		.	.	5.20	8.20	.	9.00			20.00	20.08			1.08
Dortustr.		.	.	5.21	8.21	8.41	9.01			20.01	20.09			1.09
Platz der Einheit/West		4.43	5.03	5.23	8.23	8.43	9.03			20.03	20.11			1.11
Alter Markt/Landtag		4.44	5.04	5.24	8.24	8.44	9.04			20.04	20.13			1.13
Lange Brücke		4.46	5.06	5.26	8.26	8.46	9.06			20.06	20.14			1.14
S Hauptbahnhof	an	4.48	5.08	5.28	8.28	8.48	9.08			20.08	20.16			1.16
S Hauptbahnhof	ab	4.48	5.08	5.28	8.28	8.48	9.08			20.08	20.17			1.17
Friedhöfe		4.50	5.10	5.30	8.30	8.50	9.10			20.10	20.19			1.19
Sporthalle		4.51	5.11	5.31	8.31	8.51	9.11			20.11	20.19			1.19
Kunersdorfer Str.		4.52	5.12	5.32	8.32	8.52	9.12			20.12	20.20			1.20
Waldstr./Horstweg		4.53	5.13	5.33	8.33	8.53	9.13			20.13	20.21			1.21
E.-Claud.-Str./H.-Mann-Allee		4.54	5.14	5.34	8.34	8.54	9.14			20.14	20.22			1.22
Zum Kahleberg		4.55	5.15	5.35	8.35	8.55	9.15			20.15	20.23			1.23
Friedrich-Wolf-Str.		4.56	5.16	5.36	8.36	8.56	9.16			20.16	20.24			1.24
Am Moosfenn		4.57	5.17	5.37	8.37	8.57	9.17			20.17	20.25			1.25
Bhf Rehbrücke	an	4.59	5.19	5.39	8.39	8.59	9.19			20.19	20.26			1.26

98 nur in den Ferien
A bereits als Linie 99 ab Fontanestr.