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Welcome and Acknowledgements

Welcome to the 2012 International Dictyostelium conference in Madrid, Spain. This is the 33th conference since the first Sardinia meeting in 1977. Following the 2011 International Dictyostelium conference format in Baltimore, we have shortened the meeting to three and a half days (from the evening of July 29th to mid-day, Thursday August 2nd). We have planned a total of 56 talks and 32 posters, and organized a visit to the historical town of Segovia, where we will have dinner. The scientific program is intense and we hope that you will enjoy it as much as your stay in our country. We deeply appreciate your coming to Madrid at this particular time.

The organizers wish to thank the people from VIBO DP Eventos VLC that have taken care of all the administrative work required for the organization of the Meeting, in particular to Jose Antonio García Montesinos, Marien Clemente and Amanda Gayol.

We are greatly indebted to Dictybase, most particularly to Yogesh Pandit and Siddartha Basu, who have been in charge of the meeting webpage including registrations and abstract submissions. We sincerely appreciate their hard work and dedication. Petra Fey has been a constant and generous supporter and adviser at all times.

We are deeply thankful to Carlos Suarez for his artistic work, designing all logotypes and pictures used at the meeting webpage and in the meeting documentation.

Finally, the organizers wish to thank the following sponsors for supporting the Conference:

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Teresa Suarez, Ricardo Escalante and Leandro Sastre (Organising committee)

Timetable

Sunday, 29th	Monday, 30th	Tuesday, 31st	Wednesday, 1st	Thursday, 2nd	
	Breakfast	Breakfast	Breakfast	Breakfast	7:00-9:00
	Transcriptional Regulation	Membrane Trafficking	Cell-cycle	Dictyostelium-microbial interaction	9:00-11:00
	Coffee	Coffee	Harry MacWilliams' memorial Coffee Break	Coffee	11:00-11:30
	Signalling	Cytoskeleton	Cell Differentiation	Evolution and development	11:30-13:15
	Lunch (SAB Meeting)	Lunch	Lunch	Lunch	13:30-15:00
	Chemotaxis-I	Stress and Disease-I	Chemotaxis-III/DictyBase	Departure	15:30-17:15
	Coffee	Coffee	Visit to Segovia and Dinner		17:15-17:45
Registration	Chemotaxis-II	Stress and Disease-II			17:45-19:30
	Dinner	Dinner			20:00-21:30
Welcome Dinner					
	Posters	Posters			21:30-23:30

Dicty 2012 Meeting Program

Monday, July 30th

9:00-11:00. **Session 1. Transcriptional regulation**
Chair: **Gad Shaulsky**

Jonathan Chubb. Dynamics of signalling to transcription in living cells
Matthew Jones-Rhoades. Diverse microRNAs in dictyostelid social amoebae.
Catherine Pears. Acetylation of histone H3 variants.
Tsuyoshi Araki. Dissection of the Dictyostelium STATc activation pathways.
Thomas Winckler. Deeply conserved gene regulatory functions of the carboxy-terminal domains of dictyostelid C-module-binding factors

11:00-11:30. **Coffee Break**

11:30-13:15. **Session 2. Signalling**
Chair: **Robert Insall**

Louise Fets. A PIP5kinase required for signalling downstream of Ras is crucial for chemotactic gradient sensing.
Doris Heinrich. Probing PI3-Kinase based feedback in alternating chemotactic gradient fields with ongoing cell starvation time.
Jesús Lacal. Regulation of chemotaxis by GSK-3 and a related kinase GlkA.
Satoshi Sawai. Geometries of phosphatidylinositol waves and large-scale membrane deformation in randomly moving Dictysotelium.

13:30-15:00. **Lunch**
(DictyBase Scientific Advisory Board Meeting, 13:20-15:30)

15:30-17:15. **Session 3. Chemotaxis-I**
Chair: **Rick Firtel**

Bill Loomis. Adaptation, amplification and memory of a chemotactic gradient.
Joseph Brzostowski. Phosphorylation of chemoattractant receptors regulates chemotaxis, actin re-organization and signal-relay in Dictyostelium discoideum
Dawid Jowhar. Reversing the polarity of migrating Dictyostelium cells.
Chris Janetopoulos. The influence of polarized morphologies on directed migration.

17:15-17:45. **Coffee Break**

17:45-19:30. **Session 4. Chemotaxis-II**
Chair: **Bill Loomis**

Rick Firtel. Chemotaxing cells implement two distinct modes of adhesion dynamics and lateral and axial contractility to control their migration speed.
John Nichols. The phosphoproteomics of chemotaxis.
Rama Kataria. Ric8 an important regulator of heterotrimeric G-protein signalling.
Gadi Shaulsky. The role of self-recognition in cAMP signalling, differentiation and cheating.

20:00-21:30. **Dinner**

21:30-23:30. **Posters' Visit**

Tuesday, July 31th

9:00-11:00. **Session 5. Membrane trafficking**
Chair: **Gerald Weeks**

Julia von Bülow. AqpB: the first functional aquoporin water channel from Dictyostelium discoideum amoebae is potentially gated.
Thierry Soldati. Rab8a regulates the exocyst-mediated kiss-and-run discharge of the Dictyostelium contractile vacuole.
Khalid Arhzaouy. Heteromeric p97/p97R155C complexes induce dominant negative changes in wild-type and autophagy 9-deficient Dictyostelium cells.
Ricardo Escalante. Autophagy in Dictyostelium: Vmp1 is required for the origin of the autophagosomes.
Pierre Cosson. TM9 proteins: role in intracellular sorting of transmembrane domains.

11:00-11:30. **Coffee Break**

11:30-13:15. **Session 6. Cytoskeleton**
Chair: **Ralph Gräf**

Richard Tyson. Elucidating the mechanisms of blebbing.
Julia Gallinger. Filactin: a primadonna among actin variants in Dictyostelium amoebae.
Annette Müller-Taubenberger. Filamin in Dictyostelium amoebae and leukocytes.
Margaret A. Titus. Expression of the TalinA FERM domain disrupts cell-substrate adhesion.

13:30-15:00. **Lunch**

15:30-17:15. **Session 7. Stress and disease-I**
Chair: **Ludwig Eichinger**

Arjan Kortholt. Dictyostelium Roco proteins to study LRRK2-mediated Parkinson disease.
Robin Williams. A conserved role of Human Presenilin 1 from amoeba to humans.
Liliana Malinovska. Dictyostelium discoideum - A new paradigm to study protein aggregation and proteostasis.
Chris Thompson. Identification of a novel molecular mechanism by which ATP-gated ion channels regulate intracellular processes.

17:15-17:45. **Coffee Break**

17:45-19:30. **Session 8. Stress and disease-II**
Chair: **Robin Williams**

Hellen C. Ishikawa-Ankerhold. Composition and dynamics of nuclear and cytoplasmic actin rods in Dictyostelium.
Salvatore Bozzaro. The Nramp (Natural Resistance Associated Macrophage Protein) protein family and iron homeostasis in Dictyostelium.
Sascha Thewes. About the connection of calcineurin and mitochondria in Dictyostelium discoideum.
James L. Platt. A CHD Chromatin-remodeler regulates nucleosome phasing in a limited gene set in Dictyostelium discoideum.

20:00-21:30**. Dinner**

21:30-23:30. **Posters' Visit**

Wednesday, August 1st

9:00-11:00. **Session 9. Cell cycle**
Chair: **Adrian Tsang**

Adrian Tsang. Transcriptome analysis of cell-cycle mutants: a glimpse into the molecular events underpinning cell-fate bias.
Tatjana Peter. Molecular and microscopic characterization of CP75, a novel Dictyostelium centrosome protein.
Christoph Gallinger. The role of Mo25 for cytokinesis in Dictyostelium discoideum.
Nick Lakin. Regulation of DNA repair in Dictyostelium.
Kimchi Strasser. Regulation of cell proliferation and cell-type specification in Dictyostelium by Cdk1.

11:00-11:30. **Harry MacWilliams' memorial Coffee Break**

11:30-13:15. **Session 10. Cell differentiation**
Chair: **Adriano Ceccarelli**

Zhi-Hui Chen. Dictyostelium discoideum uses the prokaryote second messenger c-di-GMP as an apically secreted signal for stalk cell differentiation.
Hideko Urushihara. Involvement of the plant fertilization protein Hap2/GCS-1 in the gamete interaction of Dictyostelium discoideum.
Koki Nagayama. Generating order from chaos: noise, heterogeneity and robustness during developmental patterning in Dictyostelium.
Suzy Battom. Cell type specific sorting out during Dictyostelium pattern formation.

13:30-15:00. **Lunch**

15:30-17:15. **Session 11. Chemotaxis-III/DictyBase**
Chair: **Rex Chisholm**

Vladimir Zykov. Selection of spiral waves in excitable media with a phase wave at the wave back.
Seido Nagano. A molecular network underlying spontaneous cAMP oscillation and a key role of G-protein dynamics.
Robert Insall. SCAR/WAVE, actin, and how pseudopods are controlled
Petra Fey. dictyBase literature curation and how authors can help.

17:15-24:00. **Visit to Segovia**

Thursday, August 2nd

9:00-11:00. **Session 12. Dictyostelium-microbial interactions**
Chair: **Pierre Cosson**

Hubert Hilbi. Subversion of phosphoinositide lipids and small GTPases by Legionella in Dictyostelium
Monica Hagedorn. Role of actin and actin-binding proteins during mycobacterial infections in a Dictyostelium model system
Matthias Leippe. Saposin-like proteins are implicated in antibacterial defence of Dictyostelium.
Xuezhi Zhang. Exploring the roles of NADPH oxidases in Dictyostelium discoideum.
Adam Kuspa. Initial characterization of the Dictyostelium Microbiome.

11:00-11:30. **Coffee Break**

11:30-13:15. **Session 13. Evolution and development**
Chair: **Adam Kuspa**

David C. Queller. Dictyostelium development shows a novel pattern of evolutionary conservation.
Kurato Mohri. Switching expression of prespore and prestalk marker orthologues during fruiting body development of Acytostelium subglobosum.
Elizabeth Ostrowski. Birth-and-death evolution of the tgr genes in Dictyostelium discoideum.
Joan E. Strassmann. Dictyostelium discoideum as a social organism.

13:30-15:00. **Lunch**

15:00. **Departure**

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4 Tsuyoshi Araki
5 Thomas Winckler

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Talk1

Dynamics of signaling to transcription in living cells

Adam Corrigan, Tetsuya Muramoto, Jonathan Chubb
Department of Cell and Developmental Biology and MRC LMCB, University College London, Gower Street, London, WC1E 6BT

Coordinated transcriptional behavior is generally considered a pre-requisite for developmental and physiology. Yet transcription is an inherently noisy process, occurring in irregular pulses or bursts. To understand how such a noisy process can be channeled into functional development, we observe single transcriptional events in individual living cells, to monitor gene expression decisions in the context of cell choices. Although over short integration times, it is difficult to observe any kind of coordinated behavior for the pulsing of most genes, a more detailed analysis, using high throughput automated image analysis revealed a degree of regularity in the transcriptional behavior for one developmental gene, with correlated transcription in cell neighbourhoods, periodic transcription and persistent bias (memory) in responsiveness to extracellular signals.

Talk 2

Diverse microRNAs in dictyostelid social amoebas

Ya-Lin Lu, Sara F. DeMaria, and Matthew W. Jones-Rhoades
Knox College, 2 E South Street, Galesburg, IL 61601, USA

MicroRNAs (miRNAs) are a class of endogenous, small non-coding RNAs derived from stem-loop precursor RNAs by the action of Dicer-like proteins. MicroRNAs are important regulators of gene expression in both plants and animals, but have been less widely studied in other eukaryotes. We have used next generation sequencing to analyze the sets of small RNAs expressed in Dictyostelium discoideum and Dictyostelium purpureum. We find that each species of social amoeba contains genomic regions that give rise to large of numbers of presumptive small interfering RNAs (siRNAs) that correspond to both strands of the genome; in D. discoideum, these siRNAs primarily derive from known transposable elements. In each species we also find sets of small RNAs with patterns of expression consistent with processing from single stranded stem-loop precursors, suggesting a mechanism of biogenesis similar to those of plant or animal miRNAs. In contrast to the evolutionary conservation observed for some plant and animal miRNAs, we see little evidence of conservation of miRNAs between the two Dictyostelium species. At this point, biological roles for the Dictyostelium miRNAs remains uncertain, although bioinformatic analysis suggests some potential to regulate protein coding genes and to interact with genomic regions that generate phased siRNAs.

Talk 3

Acetylation of histone H3 variants.

Duen-Wei Hsu, Jonathan R Chubb*, Tetsuya Muramoto, Louis Mahadevan, Catherine Pears**
Biochemistry Department, Oxford University, South Park Road, Oxford OX1 3QU United Kingdom.
* MRC Lab for Molecular and Cellular Biology, University College London, Gower Street, London WC1E 6BT, United Kingdom
**Quantitative Biology Cdentre, RIKEN, Osaka, 565-0874 Japan

Dynamic acetylation of all lysine-4-trimethylated histone H3 is a complex phenomenon involved in Immediate-Early gene induction in metazoan eukaryotes. Higher eukaryotes express repeated copies of three closely related H3 variants, inaccessible to genetic analysis. We demonstrate conservation of these phenomena in Dictyostelium which has three single-copy H3 variant genes. Although dynamic acetylation is targeted to two H3 variants which are K4-trimethylated, K9-acetylation is preferentially targeted to one. In cells lacking Set1 methyltransferase and any detectable K4-trimethylation, dynamic acetylation is lost demonstrating a direct link between the two. Gene replacement to express mutated H3 variants reveals a novel interaction between K4-trimethylation on different variants. Cells expressing only one variant show defects in growth and induction of an immediate-early gene, demonstrating the functional importance of variant expression. These studies confirm that dynamic acetylation targeted to H3K4me3 arose early in evolution and reveal a very high level of specificity of histone variant utilization in Dictyostelium.

Talk 4

Dissection of the Dictyostelum STATc activation pathways

Tsuyoshi Araki[1], Linh Vu Hai[2], Norimitsu Sasaki[3], Jianbo Na[2], Takefumi Kawata[3], Ludwig Eichinger[2] and Jeffrey G. Williams[1]

[1]College of Life Sciences, Wellcome Trust Building, University of Dundee, Dow St., Dundee DD1 5EH, United Kingdom; [2]Center for Biochemistry, Medical Faculty, University of Cologne, Joseph-Stelzmann-Str. 52, 50931 Cologne, Germany; [3]Department of Biology, Faculty of Science, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan

SH2 domains are integral to many animal cell signaling pathways. By interacting with specific phosphotyrosine residues, they provide regulatable protein-protein interaction domains. Dictyostelium is the only non-metazoan organism with functionally characterised SH2 domains but the cognate tyrosine kinases are unknown. There are no orthologues of the animal tyrosine kinases but there are very many tyrosine kinase-like kinases (TKLs); a group of kinases which, despite their family name, are mainly classified as serine-threonine kinases. STATc (Signal Transducer and Activator of Transcription-c) is rapidly phosphorylated on Tyr992 when cells are treated with the prestalk inducer DIF-1 or exposed to hyper-osmotic stress. We recently identified one of TKLs, Pyk2, as the STATc kinase that functions in the DIF-1 activation pathway (1). Pyk2 is absolutely essential for DIF-1 inducted activation of STATc. Furthermore, Pyk2 forms a complex with STATc, by an interaction between the STATc SH2 domain and phosphorylated tyrosine residues on Pyk2 that are generated by autophosphorylation. An immuno-precipitation kinase assay shows that Pyk2 functions as a constitutively active STATc kinase; regulation is effected at the level of controlled de-phosphorylation of STATc by the PTP3 tyrosine phosphatase (2). In marked contrast to DIF-1, activation of STATc in a pyk2 null mutant by stress is only marginally delayed. We now find that a null mutant for the most closely related TKL to Pyk2, Pyk3, is more impaired in the STATc stress response. Moreover, the pyk2-/pyk3- double mutant is entirely non-activatable by stress. Further consistent with their both having a direct role in the stress response, the IP-kinase activities of Pyk2 and Pyk3 are elevated by hyper-osmotic stress. Thus a key difference between stress and DIF-1 signaling is that DIF controls only at the level of the tyrosine phosphatase while stress regulates both the phosphatase and the TKL activities. A known intracellular effector in the stress response pathway, 8Br-cGMP, does not induce STATc activation in the pyk2-/pyk3- double mutant. This is consistent with our previous demonstration that the only cGMP binding protein in Dictyostelium, GbpC, is required for 8Br-cGMP activation of STATc (3). Interestingly, we now find that only Pyk3 is activated in the IP kinase assay by 8Br-cGMP treatment. This implies two parallel stress activation pathways, with Pyk2 utilising an effector other than cGMP.

(1) Araki et al. (2012) Proc. Natl. Acad. Sci. USA (in press)
(2) Araki et al. (2008) Development 135 (7): 1347-1353
(3) Araki et al. (2010) J Cell Sci. 12 (123): 837-841

Talk 5

Deeply conserved gene regulatory functions of the carboxy-terminal domains of dictyostelid C-module-binding factors

Anika Schmith, Marco Groth, Josephine Ratka, Sara Gatz, Thomas Spaller, Oliver Siol, Gernot Glöckner, Thomas Winckler

The Dictyostelium discoideum C-module-binding factor (CbfA) belongs to the family of jumonji-type transcription regulators, which often contribute to the deciphering of the histone code by JmjC domain-catalyzed removal of methyl groups from histone tails. CbfA contains a JmjC domain, two characteristic zinc finger (ZF) regions, and a distinct carboxy-terminal domain (CbfA-CTD). Illumina RNA sequencing of a CbfA-depleted mutant has revealed that CbfA regulates more than 1000 genes expressed in growing D. discoideum cells. Thus, CbfA affects fundamental cellular processes such as the organization of the actomyosin cytoskeleton and phagocytic vesicles, cytokinesis, and cell morphogenesis. Intriguingly, complementation studies revealed that most of the CbfA-dependent gene regulation is mediated by CbfA-CTD in the absence of the JmjC/ZF portion of the protein.

We compared CbfA sequences of representative species from all four major groups of dictyostelids and found a striking conservation of the CbfA domain structure even in most ancient species. Heterologous expression of a CbfA-CTD from Polysphondylium pallidum in the D. discoideum CbfA mutant restored 97% of CbfA-CTD-dependent gene expression, indicating a deep conservation of this domain to facilitate important gene regulatory functions in dictyostelid evolution.

Searching for proteins with similarity to CbfA revealed a new family of ""CbfA-like proteins"" in dictyostelids (CbfB) and in filamentous fungi, which all share the CbfA-archetypical JmjC/ZF design but contain completely diverged carboxy-terminal domains. We hypothesize that two independent gene regulatory functions may have been combined in CbfA-like proteins that were either conserved to facilitate general functions (JmjC/ZF) or were diverged (CTD) to ensure adaptation processes in gene regulatory networks that may be critically required either in response to the evolution or to drive the evolution of dictyostelid genomes.

Talk 6

A PIP5kinase required for signalling downstream of Ras is crucial for chemotactic gradient sensing

Louise Fets and Rob Kay
MRC Laboratory of Molecular Biology, Hills Road, Cambridge

PIP5Kinases, the enzymes responsible for the production of the signalling lipid PI(4,5)P2, have been suggested to play a role in chemotaxis in mammalian systems both at the uropod, and at focal adhesions. So far however, these enzymes have not been well studied with regard to leading edge signalling in gradient sensing, despite high turnover of PI(4,5)P2 at this site. We have found that PikI, a Dictyostelium PIP5K that is phosphorylated in response to cAMP stimulation, is vital for gradient sensing. PikI- cells have a severe chemotactic phenotype yet remarkably, cell speed is unaffected. Despite normal activation of Ras in response to chemoattractant, Ras-dependent cAMP responses are significantly diminished in the mutants. Creation of a phospho-mimic mutant suggests that phosphorylation of PikI in response to chemoattractant increases its activity and may form the basis of a positive-feedback loop, enabling signal maintenance in the face of high PI(4,5)P2 hydrolysis.

Talk 7

Probing PI3-Kinase based feedback in alternating chemotactic gradient fields with ongoing cell starvation time

Boern Meier, Doris Heinrich
Faculty of Physics and Center for Nanoscience, Ludwig-Maximilians-Universitat Munich, Geschwister-Scholl-Platz 1, 80539 Munich, Germany

It is essential to understand intracellular signalling pathways involved in the sensing of a chemical gradient by a single cell and its migratory response.

We developed a microfluidic chamber to manipulate cell migration in spatio-temporally controlled gradient fields. Bidirectional chemical gradients over a width of more than 300 um and timescales from seconds to several hours allow for parallel exposure of entire cell ensembles. This setup greatly facilitates statistical analysis of cellular migration properties in response to changing gradient directions and for genetic or pharmacological perturbation of the underlying regulatory network.

We analyse the cytoskeletal response dynamics by fluorescently labelling relevant signalling proteins and the subsequent actin polymerization process to determine involved molecular mechanisms during cell reorientation.

In the amoeboid model system Dictyostelium discoideum, we found time scales for chemotactic cell trapping, when cell migration is stalled due to the fact that the internal signalling reorients on longer time-scales than the external cues. Further, we identified two different types of cell reorientation by analyzing the spatiotemporal actin dynamics during cell repolarisation in the alternaiting gradient fields.

We found that PI3-kinase based formation of new pseudopods is promoted by steep chemctic gradients and reduced cellular starvation times, while reduction of the gradient steepness and ongoing starvation enhances persistent cell migration.

B. Meier, A. Zielinski, C. Weber, D. Arcizet, S. Youssef, T. Franosch, J. O. Rädler, and D. Heinrich, "Chemical cell trapping in controlled alternating gradient fields" Proc. Natl. Acad. Sci. 108(28):11417-11422 (2011)

Talk 8

Regulation of chemotaxis by GSK-3 and a related kinase GlkA

Jesus Lacal Romero, Verena Kölsch[2], Kimberly Baumgardner, Zhouxin Shen, Susan Lee, Pouya Lotfi, Pascale G. Charest[1], Steven P. Briggs and Richard A. Firtel
Section of Cell and Developmental Biology, Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093-0380
[1,2]Present addresses: [1],Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ: [2], ZMBE, Institut für Zellbiologie, Von-Esmarch-Str. 56, 48149 Münster, Germany

Recently studies have demonstrated that glycogen synthase kinase-3 (GSK-3) is essential for proper chemotaxis in Dictyostelium (Teo et al., 2010, Kim et al., 2011). Coincident with this work, we identified a new Ras/Rap1 effector, Daydreamer, whose functions are partially regulated by direct phosphorylation by GSK-3. To understand how the GSK-3 network controls chemotaxis, we used a phosphoproteomic screen to identify potential substrates and are in the process of confirming these candidates and understanding how GSK-3 mediates their function.

Interestingly, Dictyostelium contains a gskA homolog, glkA. We have analysed the glkA and gskA/glkA null strain and find that both kinases have distinct and overlapping functions in controlling chemotaxis. Our analysis of the GSK-3 substrates and GlkA functions will be presented.

Teo R, Lewis KJ, Forde JE, Ryves WJ, Reddy JV, Rogers BJ, Harwood AJ. (2010) Glycogen synthase kinase-3 is required for efficient Dictyostelium chemotaxis. Mol Biol Cell. 21:2788-96.
Kim L, Brzostowski J, Majithia A, Lee NS, McMains V, Kimmel AR. (2011) Combinatorial cell-specific regulation of GSK3 directs cell differentiation and polarity in Dictyostelium. Development. 138:421-30.

Talk 9

Geometries of phosphatidylinositol waves and large-scale membrane deformation in randomly moving Dictyostelium

Daisuke Taniguchi, Shuji Ishihara, Takehiko Oonuki, Mai Honda, Kunihiko Kaneko and Satoshi Sawai
Graduate School of Arts and Sciences, University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan

By employing phase map analysis, we show that geometry of phosphatidylinositol (3,4,5)- trisphosphate (PIP3) and F-actin waves at the basal membrane in randomly moving growth-stage Dictyostelium cells can be characterized by the number, charge and position of spatial phase singularities; i.e. points which represent organizing centers of rotating waves. A single isolated singularity near the cellular edge induced a rotational protrusion, whereas a pair of singularities supported translational extension. These singularities appeared by strong phase resetting due to de-novo nucleation at the back of pre-existing waves. Analysis of a theoretical model indicates excitability of the system that is governed by a positive feedback from PIP3 to PI3K activation, and we show experimentally that this requires polymerization of actin. By comparing experimental data with the model, we demonstrate that outwardly propagating spiral and concentric waves and those that are reflected back from the cell edge compete for dominance and dictate the complex patterns of temporal and spatial changes in the global cell morphology.

Talk 10

Adaptation, Amplification and Memory of a Chemotactic Gradient

Monica Skoge, Haicen Yue, Herbert Levine, Wouter-Jan Rappel, Michael Erickstad, Alex Groisman, and William F. Loomis
Departments of Biology and Physics, University of California San Diego, La Jolla, CA

We have followed membrane-localization of Ras-GTP in developed cells exposed to various regimes of extracellular cAMP in microfluidic devices. When cells are aburptly exposed to spatially uniform cAMP concentrations ranging from 1 nM to 1 mM, Ras-GTP becomes membrane localized within a few seconds and returns to the cytoplasm within 35 seconds even in the presence of latrunculin (Takeda et al., 2012). Such perfect adaptation is mediated by an incoherent feedforward loop in which the trimeric G-protein activated by CAR1 receptor stimulates both Ras-GEF and Ras-GAP with slightly different kinetics.

In a spatial gradient where the concentration of cAMP is only 5% higher at the front of a cell than at the back, membrane localized Ras-GTP is more than 10 fold higher at the front. This amplification may occur when GEF activity is slightly higher than GAP activity at the front and lower elsewhere. If GEF is membrane localized and GAP is freely diffusing in the cytoplasm, ultrasensitivity in the incoherent feedforward loop can result in the observed switch-like response. Using a microfluidic device that limits the height of cells to 2 microns and allows rapid switching of gradients (< 10 sec), we found that the anterior patch of Ras-GTP disappears immediately after withdrawing cAMP or reversing the gradient. However, if the concentration of cAMP is maintained, the anterior patch persist for at least 2 minutes even in the absence of a gradient.
When a cell that had been at the mid-point of a 0 - 100 nM gradient (ie. at 50 nM cAMP varying at 2 nM/mm) is rapidly exposed to uniform 20 nM cAMP, there is a period of about 25 seconds when no Ras-GTP patch is seen. After this quiescent period, a patch reappears at the same position where it had been found previously. This memory also occurs in cells treated with latrunculin or LY294002.
When the direction of the gradient is reversed, the Ras-GTP patches switch from one end of the cells to the other within10 seconds. However, the probability of a patch forming on the opposite side within 2 minutes is dependent of the steepness of the reversed gradient.
The incoherent feedforward loop could account for the kinetics of adaptation and amplification but requires an additional feedback loop to account for the spatial memory. We have simulated a circuit with a second membrane localized GEF that is activated by Ras-GTP or its downstream effectors. If GEF2 activity shows bistability, it can account for the persistence of basal motility as well as directional motility in cAMP gradients.

This work was supported by the National Institutes of Health (PO1 GM078586).

Talk 11

Phosphorylation of Chemoattractant Receptors Regulates Chemotaxis, Actin Re-organization, and Signal-Relay in Dictyostelium discoideum

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Eukaryotic G protein coupled receptors (GPCRs) control physiological processes, as diverse as, homeostasis, vision, and chemotactic movement. Migratory cells, like mammalian leukocytes and Dictyostelium discoideum, utilize GPCR signaling to regulate MAPK/ERK, PI3K, TORC2/AKT, adenylyl cyclase, and actin polymerization, which collectively direct chemotaxis. Upon ligand binding, these GPCRs are often phosphorylated at cytoplasmic residues, uncoupling G protein pathways, but activating others. In developing Dictyostelium, secreted cAMP serves as a chemoattractant, with extracellular cAMP propagated as oscillating waves to ensure directional migratory signals. The cAMP oscillations derive from a transient excitatory response of adenylyl cyclase, which then rapidly adapts. Most chemotactic responses are similarly transient when the cAMP chemoattractant is presented persistently. We have studied chemotactic signaling in Dictyostelium that express non-phosphorylatable cAMP receptors and provide evidence that receptor phosphorylation is required for effective polarization and chemotaxis of Dictyostelium to cAMP. We also show that these cells are unable to regulate adaptation of adenylyl cyclase, which disrupts long-range oscillatory cAMP wave production and cytoskeletal actin response. These data indicate that chemoattractant receptor phosphorylation is required to co-regulate essential pathways for Dictyostelium and perhaps other migratory cells.

Talk 12

Reversing the polarity of migrating Dictyostelium cells

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Cells migrating in a chemical gradient typically have a polarized morphology, where they display a distinct front and back. Cells can also have different localized sensitivities to chemoattractants depending on their degree and type of polarity. To better understand these phenomena, we used the social amoeba Dictyostelium discoideum that expressed GFP-tagged proteins known to localize to distinct regions of the cell during cell migration. Dictyostelium cells were lured into a specialized microfluidic device developed in our laboratory which confined the cells in narrow 3-dimensional PDMS channels. The chemoattractant gradient in this experimental platform could be reversed so that the rear of the cell was exposed to a higher concentration of chemoattractant than the front of the cell. Cells in the channels would freeze, become unpolarized, and were then capable of forming a new front at the former rear of the cell. We have observed the temporal and spatial loss and gain of localization of various signaling and cytoskeletal molecules at both the rear and front of the cell as polarity is broken down and re-established. We monitored the localization of PHcrac (a biosensor for PI(3,4)P2 and PI(3,4,5)P3), RBD (a biosensor for Ras activity), PTEN-GFP (a phosphatase which elevates levels of PI(4,5)P2), Tubulin-GFP (a biomarker for microtubules) and redistribution during polarity re-establishment. Further experiments were performed in the absence of an actin cytoskeleton by treating cells with Latruncllin A. These experiments have helped elucidate the molecular mechanisms that contribute to the establishment and maintenance of cell polarity.

Talk 13

The influence of polarized morphologies on directed migration

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Chemotaxis plays a vital role during the life cycle of the social amoeba Dictyostelium discoideum. Cells use directed migration to find food during vegetative growth and also use chemotaxis during aggregation in the developmental phase of the life cycle. My laboratory is using the distinct morphologies of these two different states to elucidate the mechanisms underlying this process. During vegetative growth, unpolarized cells are grown in the presence of bacteria and become competent for folic acid chemotaxis and do not require the developmental program. During aggregation, cAMP-mediated chemotaxis occurs after starvation and the onset of development. These cells can become highly polarized and have a distinct leading and trailing edge. Presumably, the same directional sensing module guides a cell during cAMP and folic acid-mediated chemotaxis. We have used a variety of approaches, including genetic analysis and various chemotaxis assays to tease out the underlying molecules regulating chemotaxis in these two cell morphologies. Recent studies using microfluidic devices have given us insight into the role polarity plays in cell migration. In addition, we have used these devices to characterize the temporal and spatial regulation of signaling and cytoskeletal proteins that are critical for both polarity and migration. Cells responding to folic acid or cAMP appear to use a local excitation, global inhibition based mechanism to sense the chemical gradients. The unpolarized cells responding to folic acid have an added component that contributes to directed migration. This additional module appears to lower their chemotactic efficiency when crawling on a smooth coverslip lacking any features or obstructions.

Talk 14

Chemotaxing Cells Implement Two Distinct Modes of Adhesion Dynamics and Lateral and Axial Contractility to Control Their Migration Speed

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Dictyostelium cells adjust their speed depending on the stiffness and adhesive properties of the substrate and other factors. However, the mechanism by which the speed of motility is largely unknown. We used Fourier Traction Force Cytometry (FTFC) to measure the spatiotemporal evolution of shape and traction stresses and show that cells control their speed by switching between two motility modes with distinct adhesion and contractility dynamics. In the "slow" mode, the adhesion sites are stationary while the body translocates forward by periodic axial contractions. The back adhesions break after new frontal adhesions are created. In the "fast" mode, the cell reduces the magnitude of the traction stresses, increases the frequency of axial contractions, and maintains the frontal adhesion stationary while continuously dragging the back adhesion forward. Cells with cytoskeletal crosslinking defects exhibit ineffective axial contractility impeding the implementation of these modes and rely on periodic lateral contractions for their motion.

Talk 15

The phosphoproteomics of chemotaxis

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The responses of Dictyostelium amoebae to chemoattractants are mediated rapidly by robust signalling networks. In such signalling networks, protein phosphorylation is a key and common regulator of protein activity and signal transduction.

We have undertaken a global phosphoproteomic study to identify signalling components involved in chemotaxis. We have used the SILAC mass spectrometry technique to quantify changes in protein phosphorylation in response to the chemoattractants cAMP and folate. By this method we have identified over 6000 unique phosphorylation sites, many of which show a change in phosphorylation in response to chemoattractant treatment. We have identified chemoattractant-sensitive phosphorylation sites on known players in chemotactic signal transduction, such as PI3Ks and the RasGEF gefR. We have also found a number of proteins not previously implicated in chemotactic signal transduction that have chemoattractant-sensitive phosphorylation sites. Such proteins represent putative new players in signalling.

Additionally, we have used quantitative phosphoproteomics to investigate signal transduction in known chemotaxis mutants. Two key kinases involved in Dictyostelium chemotaxis are the Akt orthologue PKBA and the related kinase PKBR1. By adapting our SILAC approach to study the double knockout mutant of PKBA and PKBR1, we have identified putative new substrates of these two kinases.

Talk 16

Ric8 an important regulator of heterotrimeric G-protein signaling

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Previously we have used Dictyostelium mutants to investigate the minimal requirements for chemotaxis, and identified a basal signaling module consisting of heterotrimeric and monomeric G-proteins, which is sufficient for chemotaxis (Kortholt et al., 2011). To identify further components of this basal pathway we performed a pull-down screen with Ga□proteins as bait. Mass-spectrometry revealed Ric8 as one of the interacting partners of Ga2 and Ga4. Our results show that Ric8 is a non-receptor GEF for Ga protein, essential for the regulation of development and chemotaxis.

Talk 17

The role of self-recognition in cAMP signaling, differentiation and cheating

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Self-recognition during the establishment of multicellularity in Dictyostelium is mediated by tgrB1 and tgrC1, two polymorphic genes that encode developmentally-regulated cell-cell adhesion proteins (1). Strains that carry incompatible pairs of matching tgrB1-tgrC1 alleles segregate from one another during aggregation and develop as separate multicellular organisms (2). We will show that self-recognition can protect Dictyostelium cells against cheaters, suggesting that cheaters may be one of the evolutionary pressures that increase tgrB1-tgrC1 sequence diversity. We will also show that when cells which carry one pair of matching tgrB1-tgrC1 alleles are present at low frequency within fields containing a majority of cells carrying a different pair of alleles, the rare cells fail to participate in cooperative cAMP signaling, streaming, or subsequent differentiation despite the fact that they are exposed to the same cAMP signals as the majority cells. These findings suggest that models of cAMP signaling and chemotaxis should consider recognition through tgrB1-tgrC1 as an essential feature of morphogenesis and cell differentiation. These findings also establish Dictyostelium as a model system for the role of self-recognition in social evolution.

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2. Hirose, Benabentos, et al. (2011). Self-recognition in social amoebae is mediated by allelic pairs of tiger genes. Science 333, 467-470.

Talk 18

AqpB: the first functional aquaporin water channel from Dictyostelium discoideum amoebae is potentially gated

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Aquaporins (AQP) are integral membrane proteins of about 30 kDa and have been found in all kingdoms of life. They facilitate the selective passage of water and uncharged solutes, such as urea and glycerol[1]. AQP water permeability has been linked to various physiological and pathophysiological processes, e.g. cell volume sensing, osmotic stress regulation, or cell migration[2]. However, the underlying molecular mechanisms remain largely unknown. D. discoideum is a well established model organism to address questions touching basic cell biology. Therefore, we analyzed AQP expression and functionality in D. discoideum. Until then, two AQPs had been found, which are expressed exclusively in multicellular developmental stages, yet neither protein has been shown to conduct water. We identified and functionally characterized the first functional aquaporin, AqpB, in D. discoideum amoebae [3]. Unexpectedly, we found wild-type AqpB to be impermeable to water, glycerol and urea when expressed in Xenopus laevis oocytes. Neither acidification of the assay buffer, truncation of the N-terminus nor introduction of selected point mutations activated the channel. Eventually, truncation of 12 aa from an intracellular connecting loop, loop D, increased water-permeability 6-fold above background using Xenopous oocytes. This result was confirmed by reconstitution of truncated AqpB into proteoliposomes and osmotic light scattering experiments. We did not detect urea or glycerol permeability. These results hint at a water-selective AQP with a novel channel-gating mechanism located in loop D. The active mutant was further inhibitable by mercuric chloride confirming the presence of a cysteine residue in the selectivity filter region. Furthermore, AqpB was found to be constitutively expressed in all developmental stages and appeared to be glycosylated as shown by Western blot with a specific antibody. N- and C-terminal GFP fusion proteins with AqpB were localized intracellularly to vacuolar structures, in a punctuate pattern throughout the plasma membrane, and in lamellipodia-like protrusions of D. discoideum amoebae. This localization is consistent with cellular functions of AqpB in osmoregulation and cell motility.

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[2] Saadoun, S., Papadopoulos, M.C., Hara-Chikuma, M., Verkman, A.S. Nature 2005
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Talk 19

Rab8a regulates the exocyst-mediated kiss-and-run discharge of the Dictyostelium contractile vacuole

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Water expulsion by the contractile vacuole (CV) in Dictyostelium is carried out by a giant kiss-and-run focal exocytic event during which the two membranes are only transiently connected but do not completely merge. We present a molecular dissection of the GTPase Rab8a and the exocyst complex in tethering of the contractile vacuole to the plasma membrane, fusion, and final detachment. Right before discharge, the contractile vacuole bladder sequentially recruits Drainin, a Rab11a effector, Rab8a, the exocyst complex, and LvsA, a protein of the Chédiak–Higashi family. Rab8a recruitment precedes the nucleotide-dependent arrival of the exocyst to the bladder by a few seconds. A dominant-negative mutant of Rab8a strongly binds to the exocyst and prevents recruitment to the bladder, suggesting that a Rab8a guanine nucleotide exchange factor activity is associated with the complex. Absence of Drainin leads to overtethering and blocks fusion, whereas expression of constitutively active Rab8a allows fusion but blocks vacuole detachment from the plasma membrane, inducing complete fragmentation of tethered vacuoles. An indistinguishable phenotype is generated in cells lacking LvsA, implicating this protein in postfusion detethering. Of interest, overexpression of a constitutively active Rab8a mutant reverses the lvsA-null CV phenotype.

Talk 20

Heteromeric p97/p97R155C complexes induce dominant negative changes in wild-type and autophagy 9-deficient Dictyostelium cells

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p97 (VCP or valosin containing protein in mammals and Cdc48p in yeast) is a ubiquitously expressed and evolutionarily highly conserved hexameric member of the magnesium-dependent Walker P-loop AAA-ATPases. p97 has been associated with various essential cellular processes including ubiquitin-proteasome protein degradation and autophagy. Point mutations in the human p97 gene can cause autosomal-dominant IBMPFD (Inclusion Body Myopathy with early-onset Paget disease and Frontotemporal Dementia), ALS14 (Amyotrophic Lateral Sclerosis), or HSP (Hereditary Spastic Paraplegia). The most prevalent mutation in p97 that causes IBMPFD is the R155C mutation.

We studied the function of p97 in Dictyostelium discoideum and have generated strains that ectopically express p97-RFP or p97R155C-RFP in AX2 wild-type and autophagy 9 (ATG9) knock-out cells. Co-immunoprecipitation studies using an anti-RFP antibody showed that endogenous p97 and p97R155C-RFP form heteromers. The mutant strains displayed changes in cell growth, phagocytosis and phototaxis indicating misregulation of multiple essential cellular processes. Immunofluorescence analysis revealed an increase of protein aggregates in ATG9KO/p97R155C-RFP and ATG9KO cells. They were positive for ubiquitin in both strains, however, immunoreactive for p97 only in the ATG9KO mutant. Immunoblotting showed an increase of ubiquitinated proteins and of the autophagy marker ATG8(LC3). Proteasomal activity was slightly reduced in AX2/p97R155C-RFP cells, but nearly completely inhibited in the ATG9KO mutant and partially rescued in the ATG9KO/p97R155C-RFP double mutant. The observed R155C specific cellular alterations in the single versus the double mutants suggest a novel mode of p97 regulation based on the interaction and mutual inhibition of wild-type p97 and core autophagy proteins.

Talk 21

Autophagy in Dictyostelium: Vmp1 is required for the origin of autophagosomes

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Autophagy is a lysosomal degradation pathway of cell´s own material, which is highly conserved in all eukaryotes. It is triggered by starvation but it is also induced in other circumstances such as for the elimination of protein aggregates, defective organelles, in response to bacterial pathogens and even to mechanical stress. During the last years our laboratory has been developing the use of Dictyostelium as model to discover new autophagic proteins. We have optimized the tools to monitor autophagy and have begun to analyze the different protein complexes involved in the autophagic machinery. A number of autophagic proteins are strongly conserved between Dictyostelium and human but are absent in yeast, the best characterized model for autophagy, and thus, Dictyostelium can be a suitable simple model to address the function of these new proteins. This is the case of Vmp1, an endoplasmic reticulum (ER) transmembrane protein that we believe is required for the initial steps of autophagosome formation. Mechanistically, autophagy begins with the formation of double membrane vesicles called autophagosomes, which engulf parts of the cytoplasm and later fuse with lysosomes for degradation. The autophagosome membrane can be originated from other organelles including the ER, but the mechanisms involved in the specialization of this membrane are unknown. Cells deficient in Vmp1 show an abnormal pattern of the signaling mediated by the class-III PI3K VPS34. This kinase is responsible for a transient accumulation of PI(3)P (Phosphatidylinositol 3-phosphate) phospholipid at the membrane of the ER and this is believed to be an important step in the specialization of the autophagosome membrane. The use of GFP-Atg18 and FIVE markers that recognize PI(3)P-rich regions have shown an increasing and persistent PI(3)P signaling in the mutant. Interestingly, patches of this abnormal PI(3)P-rich membrane regions surround the ubiquitinated protein aggregates typically found in this mutant. These regions also colocalize partially with ER-markers suggesting that Vmp1 is required for the correct formation of a competent autophagosome membrane from the ER by regulating PI(3)P signaling.

Talk 22

TM9 proteins: role in intracellular sorting of transmembrane domains

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Previous studies have shown that Phg1/TM9 proteins can control cell adhesion of Dictyostelium discoideum by determining the amount of SibA adhesion molecules present at the cell surface. Our current experiments indicate that Phg1 may directly interact with the transmembrane domain of SibA, and that this interaction is necessary for efficient cell surface expression of SibA. Phg1 may be generally involved in the recognition and intracellular sorting of transmembrane domains. This observation may account for the variety of phenotypes reported in phg1 KO cells.

Talk 23

Elucidating the mechanisms of blebbing

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Cell motility plays an important role throughout biology, the polymerisation of actin being fundamental in producing protrusive force. However, it is increasingly apparent that intracellular pressure, arising from myosin-II contraction, is a co-driver of motility. In its extreme form, pressure manifests itself as hemispherical protrusions, referred to as blebs, where membrane is torn from the underlying cortex. Although many components and signalling pathways have been identified, we lack a complete model of motility, particularly of the regulation and mechanics of blebbing.

We develop the next generation of the successful QuimP software designed for automated analysis of motile cells, producing quantitative measures protein distribution and cell morphology. Key to QuimP's new functionality, we present the Electrostatic Contour Migration Method (ECMM) that provides rapid, high resolution tracking of local deformation with better uniformity and efficiency than rival methods. We employ ECMM to build an automated protrusion tracking method (ECMM-APT) sensitive not only to pseudopodia, but also the complex characteristics of high-speed blebs in Dictyostelium. Our high-throughput approach has yielded new hypotheses into the mechanics of blebbing.

Talk 24

Filactin: a Primadonna Among Actin Variants in Dictyostelium Amoebae

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Actin is one of the most abundant proteins in eukaryotic cells. The conventional actin is crucial for a wide range of cellular functions including cell shape, cell motility, cell division and membrane dynamics. Besides the conventional actins many higher organisms harbor actin variants with unknown function. The model organism Dictyostelium discoideum comprises an actinome of a total of 41 actins, actin isoforms and actin-related proteins (Joseph, J.M. et al., PLoS ONE 3: e2654. 2008).

Among them is 'filactin', a highly conserved actin with an elongated N-terminus. The 105 kDa protein has a distinct domain organization with an Ist-1 domain at the very N-terminus followed by filamin-like Ig repeats. A homolog of this protein is present in other Dictyosteliidae and in Entamoeba histolytica which is responsible for amoebic colitis.

We study the functions of filactin in vivo and in vitro. Immunofluorescence studies in D. discoideum localize endogenous and GFP-filactin in the cytoplasm at vesicle-like structures and in cortical regions of the cell. A most peculiar behavior is the stress-induced appearance of full length filactin in nuclear actin rods.

To perform in vitro analyses we expressed recombinant filactin in Sf9 cells. Fluorescence studies with the filactin actin-domain suggest that it interferes with actin polymerization by sequestering G-actin or even capping filaments. Gelfiltration assays propose a tetrameric structure of full length filactin. We assume that filactin is involved in the ESCRT (endosomal sorting complexes required for transport) pathway which is responsible for multivesicular body formation via its N-terminal domains. Protein-interaction studies show that vta1, a component of the ESCRT machinery, associates with the filamin-like Ig repeats of filactin.

The data on filactin suggest that only the conventional actins are the backbone for the microfilamentous system whereas less related actin isoforms have highly specific and perhaps cytoskeleton-independent subcellular functions.

Talk 25

Filamin in Dictyostelium amoebae and leukocytes

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Filamins are large F-actin crosslinking proteins that organize actin filaments into orthogonal networks and contribute to the regulation of cell signaling cascades. Structurally, filamins form homodimers that are characterized by conserved N-terminal actin-binding domains which are followed by a flexible rod segment consisting of 6 (Dictyostelium discoideum filamin, ddFLN) to up to 24 (human filamins) homologous repeats that adopt an immunoglobulin (Ig)-like fold. Dimerization is mediated by the C-terminal domain. DdFLN plays a crucial role in motility, chemotaxis and phototaxis, and mediates responses to mechanical stress. In mammals, three filamin isoforms, FLNA, B and C, are known which share about 70% sequence identity. Filamins have been described to bind to about 70 different cellular proteins including migfilin, caveolin, small GTPases, ß-integrins and vimentin, and most interactions seem to be mediated by the C-terminal repeats. However, detailed interaction cascades have not been described and the functional relationship between individual cytoskeletal systems is a very important but poorly understood area of research.

We want to elucidate the role of human and Dictyostelium filamin as cytoskeletal organizers of motile activities in Dictyostelium discoideum and leukocytes. As a model for leukocytes we use HL-60 cells, a promyelocytic leukemia cell line. We have generated HL-60 cell clones that express full-length or truncated filamin or vimentin in order to analyze the distribution of the cytoskeletal elements during migration, adhesion and spreading. In parallel, we use Dictyostelium amoebae as a highly efficient expression system for full-length or truncated filamin and vimentin, and employ the constructs for the identification of interacting proteins in pull-down assays. Furthermore, by using different chemotaxis devices and microfluidics we analyze the migratory behavior of Dictyostelium and HL-60 cells expressing different filamin constructs. The long-term objective of our project is to determine the molecular basis of filamin/vimentin and other interactions.

Talk 26

Expression of the TalinA FERM domain disrupts cell-substrate adhesion.

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TalinA (TalA) plays a critical role in Dictyostelium cell-substrate adhesion. The N-terminal region of talins contains a FERM domain (band 4.1, ezrin, radixin, moesin) that binds to the cytoplasmic tails (CT) of adhesion receptors as well as actin, phosphoinositides and regulatory proteins. A GFP-TalA FERM fusion was expressed in wild type (wt) cells and distributed diffusely throughout the cell, with a small fraction present in the cytoskeleton. The FERM cells exhibit a significant defect in adhesion, as assayed by measuring adhesion to plates, only ~20% of wt cells are detached by shaking whereas over 40% of the FERM cells are, comparable to that observed for the talA null mutant. FERM cells also exhibit a significant reduction in the uptake of both 1 µm beads and B/r bacteria and have a mild cytokinesis defect, consistent with the FERM domain causing defects in substrate adhesion. The strong similarity of the FERM phenotype to that of the talA null suggested that expression of this domain causes a significant loss of TalA and/or TalB expression. Quantitative immunoblotting showed that the TalA FERM cells have reduced overall levels of endogenous TalA (~70% of wild type levels) while the TalB levels are unchanged. The relatively small reduction in TalA levels is not sufficient to account for the strong adhesion phenotype. Interestingly, expression of a mutant TalA FERM that is predicted to be unable to bind to CTs also causes the same adhesion defects, suggesting that the FERM domain is not simply acting by occupying TalA binding sits on receptor tails. Together, these data suggest that the TalA FERM domain interferes directly with the function of endogenous talins, by potentially disrupting intramolecular interactions necessary for activating talin function or by blocking the activity of a key talin regulator.

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Talk 27

Dictyostelium Roco proteins to study LRRK2-mediated Parkinson disease

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Parkinson Disease (PD) is a neurodegenerative disorder affecting more than five million people worldwide. Recently a number of genetic factors causing PD have been discovered. Mutations in human leucine-rich-repeat kinase 2 (LRRK2) have been found to be thus far the most frequent cause of late-onset PD. LRRK2 belongs to the Roco family of proteins, which are characterized by the presence of a Ras-like G-domain, called Roc and a kinase domain. Importantly, pathogenic mutations in LRRK2 result in decreased GTPase activity and enhanced kinase activity, suggesting a possible PD-related gain of abnormal function. Here we show that Dictyostelium discoideum Roco4 is an excellent model to study the structural and biochemical characteristics of the LRRK2 kinase and can be used for optimization of the current and identification of new LRRK2 inhibitors. We have solved the structure of Roco4 kinase wild-type, PD related mutants G1179S and L1180T (G2019S and I2020T in LRRK2) and the structure of Roco4 kinase in complex with the LRRK2 inhibitor H1152. Together our data give important new insight in the LRRK2 activation mechanism and most importantly it explains the G2019S related increased LRRK2 kinase activity.

Talk 28

A conserved role of Human Presenilin 1 from amoeba to humans

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Inherited forms of Alzheimer's disease are often caused by mutated forms of one of two highly related Presenilin proteins, PS1 or PS2. These proteins control a wide variety of signalling pathways and molecular effectors, making our understanding of the pathological mechanisms of inherited Alzheimer's disease complex. In order to unravel these effects, a model is required in which all wild-type Psen activity is deleted to enable the investigation of disease-related signal transduction catalysed by mutant isoforms in the absence of background activity. Deletion of both mammalian PS-encoding genes is highly problematic, as gene deletion causes lethality at an early embryonic stage. We have thus set out to develop a better model system for understanding PS1 cellular function using a simple model system.

Dictyostelium, a social amoeba, is the simplest biomedical model possessing two psen homologues (psenA and B). Deletion of either Dictyostelium psen gene did not cause a lethal phenotype, and did not affect the process of development in this model. However, deletion of both psen genes in a single cell line lead to a severe developmental block suggesting a redundant activity of these proteins, in a similar manner to that seen in mammalian systems. This phenotype was rescued by reintroducing a GFP-tagged copy of the Dictyostelium psenB gene, and the resulting protein was found to localise to the endoplasmic reticulum (ER) and nuclear envelop.

To show the functionality of this model in the analysis of the human PS1 (hPS1), we expressed the gene encoding hPS1 tagged with GFP in the Dictyostelium psen null background. Isogenic cell line expressing the human gene showed restored wild-type development, suggesting the human protein is functional in Dictyostelium and can completely complement the endogenous protein in development. The hPS1 protein also showed ER and nuclear envelop localisation - in agreement with its localisation in mammalian systems.

We then confirmed the conserved function of the Dictyostelium protein in mammalian presenilin (Notch cleavage) assays. Expression of the Dictyostelium psen genes in mouse blastocysts lacking all copies of presenilin showed comparable Notch-cleavage activity to that of cells transformed with the human psen1 encoded gene. This confirms that the Dictyostelium and human Psen proteins show functional conservation in both models.

This study is the first to describe a conserved function of human PS1 from the simple biomedical model Dictyostelium to mammalian systems. This model therefore provides an excellent system to further investigate cellular roles of human presenilin in basic cell function and disease-related signalling.

Talk 29

Dictyostelium discoideum - A new paradigm to study protein aggregation and proteostasis

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Protein misfolding and aggregation can dramatically interfere with cellular integrity. Aberrant protein structures can occur under normal growth conditions, but are particularly enhanced during stress or aging. As a defense against misfolded proteins, cells induce a system for protein quality control, which consists of molecular chaperones and protein degradation machines. However, protein aggregation is not always detrimental and can even have important biological functions. S. cerevisiae for example employs heritable protein aggregates or prions to generate phenotypic diversity in clonal populations of yeast cells. Importantly, the prion properties of these prions reside in characteristic domains (prion domains, PrDs), which are highly enriched for asparagines (N) and/or glutamines (Q).

Using an algorithm for the detection of Q/N-rich PrDs, we uncovered an extraordinarily high number of prion-like proteins in the slime mold D. discoideum. To investigate how the presence of these Q/N-rich proteins affects the biology of Dictyostelium, we expressed several well-defined heterologous Q/N-rich proteins in Dictyostelium and compared their aggregation behavior to the yeast and mammalian system. We found that the PrD of the yeast protein Sup35 formed aggregates in Dictyostelium, which were reminiscent of prion aggregates formed in yeast. A poly-glutamine-rich protein derived from human huntingtin, however, did not form aggregates, in contrast to what has been observed in yeast or mammals. In addition, we analyzed several Dictyostelium chaperones in S. cerevisiae. We found that D. discoideum Hsp101 can functionally replace the yeast disaggregase Hsp104 and can support the inheritance of yeast prions. In summary, our data suggest that Dictyostelium is able to promote the formation and propagation of prion aggregates. Thus, Dictyostelium promises to be a valuable model organism for the study of protein aggregation and we expect that it will provide important insight into the strategies that organisms employ to deal with an aggregation-prone proteome.

Talk 30

Identification of a novel molecular mechanism by which ATP-gated ion channels regulate intracellular processes

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Ion channels represent one of the major therapeutic drug targets for wide ranging diseases. For example P2X receptors are ion channels that drive cellular responses to ATP and regulate diverse physiological processes in mammals, including inflammation and pain. P2X receptors therefore represent a novel target for disease including pain and inflammation, with many pharmaceutical companies actively pursuing this potential. Understanding how P2X receptors are regulated will therefore be important for the further development of such drugs.

Although P2X receptors were discovered as cell surface proteins that respond to extracellular ATP, we recently found that in the social amoeba Dictyostelium they also function on internal membranes, where they respond to intracellular ATP (Nature 2007). These findings were field-changing as they suggest hitherto unknown intracellular roles in mammals that may have been overlooked whilst also providing a great advance in our understanding of the molecular function and structure of this poorly understood biomedically important ion channel family.

Currently, we are using a unique combination of genetics (mutageneis), biochemistry (proteomic), live cell imaging and electrophysiology to determine the role and regulation of intracellular P2X receptor function. Through these studies we have uncovered a novel mode of action of P2X receptors, and have elucidated the novel molecular mechanism by which P2X receptors function to regulate intracellular processes. We suggest that this mechanism represents an evolutionarily conserved mode of action of this biomedically important class of ion channels.

Talk 31

Composition and dynamics of nuclear and cytoplasmic actin rods in Dictyostelium

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Actin rods are bundles formed either in the nucleus or in the cytoplasm under certain chemical or mechanical stress conditions. The formation of actin rods in the nucleus of skeletal muscle cells can lead to distinct myopathies. In stressed neurons, cytoplasmic rods lead to synaptic dysfunction, impaired vesicle transport and may mediate cognitive deficits in dementias. Rods consist mainly of actin and cofilin, but contain also other cytoskeletal proteins. Essentially, it is unknown what exactly triggers rod formation, which proteins are recruited or excluded, what the underlying mechanisms of rod formation are and whether rods exhibit a dynamic instability.

We use D. discoideum as a model to study appearance, composition and stability of nuclear and cytoplasmic rods. Intranuclear rod formation was induced by incubation of the cells with 5% DMSO for 15 min at 28°C. Cytoplasmic rod formation was induced by treatment of the cells with 10 mM sodium azide for 20 min at 28°C. Rods were visualized by using cells expressing RFP-actin and GFP-cofilin, or by immunofluorescence with a selection of antibodies. Both types of rods contain in addition to actin and cofilin only a distinct set of other proteins like filactin, actin-interacting protein 1 (Aip1) and coronin. We found a finely tuned spatio-temporal pattern of protein recruitment during rod formation. To analyze rod stability, we performed FRAP (fluorescence recovery after photobleaching) experiments and analyzed the kinetics of rod disassembly. The FRAP assays at different stages of rod formation show the high stability of these structures. Cytochalasin D and latrunculin A do not block rod formation. However, when the stress stimulus is removed the rods disassemble. We show that nuclear rods differ from the cytoplasmic rods in several aspects including internal stability, temporal protein recruitment and properties during different stress conditions."

Talk 32

The Nramp (Natural Resistance Associated Macrophage Protein) protein family and iron homeostasis in Dictyostelium

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D.discoideum genome harbours two genes encoding members of the Nramp superfamily, which is conserved from bacteria (MntH proteins) to humans (Slc11 proteins). Nramps are proton-driven metal ion transporters with a preference for iron and manganese. Acquisition of these metal cations is vital for all cells, as they act as redox cofactors and regulate key cellular processes, such as DNA synthesis, electron transport, energy metabolism and oxidative stress. In mammals, Nramp1 is specifically expressed in macrophage phagogomes, and polymorphisms are associated with increased susceptibility to bacterial infections. Nramp2 isoforms are expressed ubiquitously in recycling endosomes or specifically at the apical membrane of epithelial cells in gut and kidney. Nramp2 mutations cause microcytic hypochromic anemia.

Dictyostelium Nramp1, like its mammalian ortholog, is expressed in phagosomes, mediates iron transport to the cytosol and resistance to infection by invasive bacteria. We have extended the analysis to the nramp2 gene, by generating single and double knockout mutants. In contrast to Nramp1, the Nramp2 protein is expressed exclusively at the membrane of the contractile vacuole. Both proteins co-localize with the V-H+ ATPase, which provide the electrogenic force for vectorial iron transport. Like nramp1, nramp2 gene disruption affects resistance to Legionella pneumophila. Disrupting both genes additionally leads to defects in development, with strong delay in aggregation and formation of large streams and multi-tipped aggregates. Single and double mutants display differential sensitivity to cell growth under conditions of iron overload or depletion. All mutants are more sensitive than the parental strain to iron depletion, whereas the double mutant is more resistant to iron overload. The results favour the hypothesis that Nramp1 and Nramp2 synergistically regulate iron homeostasis, with the contractile vacuole acting as a store for metal cations. Altering iron homeostasis affects development, innate immunity and, under conditions of iron overload or depletion, cell growth.

Talk 33

About the connection of calcineurin and mitochondria in Dictyostelium discoideum.

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The Ca2+/calmodulin dependent protein phosphatase calcineurin, which is composed of a catalytic (calcineurin A) and a regulatory (calcineurin B) subunit, is a major mediator of Ca2+-signalling. We could previously show that calcineurin plays a role in the stress response and development in D. discoideum. However, in contrast to other organisms little is known about the targets of calcineurin in D. discoideum. For example, in mammalian cells calcineurin regulates the translocation of the profission dynamin related protein 1 (Drp1) to the mitochondria. When the dephosphorylation of Drp1 by calcineurin is disturbed this leads to a loss of mitochondrial integrity. In the filamentous fungus Aspergillus fumigatus calcineurin mutants show decreased mitochondrial metabolic activity. We therefore investigated the potential connection between calcineurin and mitochondria in Dictyostelium. Using RNAi-silenced calcineurin A and B mutants we find that the mutant cells are smaller but have more copies of mitochondrial DNA compared to the wild type. However, no differences in respiration between WT and mutants could be observed. Measuring the expression of genes, which are involved in respiration, we show that only the alternative oxidase (aoxA) is differentially expressed in the calcineurin mutants. During development of the mutants the alternative oxidase seems to be especially important as they show severe developmental defects under treatment with benzohydroxamic acid (BHAM), an inhibitor of the alternative oxidase, but are more resistant towards KCN. In summary, this is the first time that a link between calcineurin and mitochondria is described in D. discoideum.

Talk 34

A CHD Chromatin-Remodeler Regulates Nucleosome Phasing in a Limited Gene Set in Dictyostelium discoideum

James L. Platt, Nick A. Kent, Adrian J. Harwood and Alan R. Kimmel

Regulated changes in chromatin organization are critical for multicellular development and epigenetic control of cell differentiation. The CHD (Chromodomain-Helicase-DNA binding) protein family is one of the major ATP-dependent, chromatin remodeling factors that regulate nucleosome positioning and gene expression in eukaryotes. Mammalian CHD proteins group into three subfamilies and several human diseases are associated with impaired CHD function. Here, we identify three CHDs (ChdA, ChdB, and ChdC) in Dictyostelium discoideum that are expressed with unique developmental patterns. Null mutants for each have distinct, non-redundant phenotypes, indicating functional specificity. ChdC clusters with members of CHD subfamily 3 that includes CHD7. Mutations in CHD7 are associated with the human CHARGE syndrome, characterized by multiple congenital developmental defects, and in Dictyostelium, chdC-nulls have severe and diverse developmental phenotypes. To understand the mechanistic function of ChdC and related CHDs, we compared genome-wide transcriptional profiles and nucleosomal maps in Wild-Type and chdC-null cells. We show that nucleosome linker-spacing is altered in a subset of genes in chdC-nulls and that gene-specific transcriptional profiling changes correlate with these nucleosomal patterning differences. This study provides novel mechanistic insight into the action of a class of CHDs in chromatin organization and may serve as a basis to better understand certain human genetic defects.

Talk 35

Transcriptome analysis of cell-cycle mutants: a glimpse into the molecular events underpinning cell-fate bias

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At the time of starvation, newly divided cells preferentially develop into stalk cells whereas cells residing in mid and late G2 tend to become spores. We posited that perturbing the key cell-cycle control proteins would help to expose the molecular mechanisms underlying cell-fate bias. We constructed mutations in two major cell-cycle control proteins of Dictyostelium: the retinoblastoma-like protein RblA and the cyclin-dependent protein kinase Cdk1. In plant and mammalian cells, the retinoblastoma protein plays a pivotal role in G1/S transition and is involved in cell differentiation. The Cdk1 protein, on the other hand, is critically involved in G2/M transition. The loss of RblA activity (rblA-null) results in reduced cell size, premature growth-development transition, and accelerated development. The expression of the unphosphorylatable T14A/Y15F allele of Cdk1 causes arrest at mitosis. Transcriptome analysis revealed that both RblA and Cdk1 mutants express growth-specific and early development genes abnormally. In addition, they alter the expression of several genes encoding putative transcription factors. Some of these affected transcription factors, including CudA and SrfA, have previously been shown to regulate the expression of mid-to-late developmental genes. Moreover, the expression of these transcription factor genes fluctuates during the cell cycle. Using SrfA as an example, we show that, in addition to altering the expression of genes of the stalk and spore pathways, the srfA-null mutant down-regulates early developmental genes and up-regulates growth-specific genes during growth. These results suggest that some regulators of developmental genes play a role in growth-development transition and that the periodic expression of these gene regulators during the cell cycle may impart cell-fate bias.

Talk 36

Molecular and microscopic characterization of CP75, a novel Dictyostelium centrosome protein

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Centrosomes of Dictyostelium amoebae consist of a core structure with three major layers surrounded by a corona containing microtubule-nucleation complexes. Centrosome duplication starts in prophase with growth of the core structure, disappearance of its central layer and loss of the corona. Centrosome duplication proceeds with splitting of the core structure. The two former outer layers then form two spindle poles maturating to two new, complete centrosomes in telophase. CP75 is one of nine novel proteins identified in a centrosomal proteome analysis (Schulz et al. 2009, Cell Motil. Cytoskeleton 66, pp. 915). CP75 localizes to the centrosomal core structure in a cell cycle-dependent manner. GFP-CP75 dissociates from the core structure in early prometaphase and reappears at spindle poles in telophase. Since this pattern fits to the disappearance and reappearance of the central layer, this indicates that CP75 is a constituent of this layer. This behavior is mimicked by another putative constituent of the central layer, CP39, which showed two-hybrid interaction with CP75. FRAP experiments reveal that GFP-CP75 exhibits no mobility at the centrosome during interphase. This may indicate a role in structural maintenance of the centrosomal core. Both the N- and C-terminal halves of the protein contain a centrosomal targeting domain, since both fragments localized at the centrosome when expressed as GFP fusion proteins. Upon overexpression, GFP-CP75 colocalized also with F-actin at the cell cortex. This association with actin was even more prominent in the GFP-CP75ΔN strain. In contrast, GFP-CP75ΔC was not associated with actin, but overexpression caused a dominant negative effect. GFP-CP75ΔC overexpressors frequently contained supernumerary centrosomes, more or less absent interphase microtubules and enlarged, deformed nuclei. A very similar phenotype was observed upon knockdown of CP75 expression by RNAi. Thus, CP75 appears to be required for microtubule organization and centrosome biogenesis.

Talk 37

The role of Mo25 for cytokinesis in Dictyostelium discoideum

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Mo25 (morula protein 25) is a highly conserved 40 kDa scaffolding protein with 60% identity from amoeba to man. It facilitates and activates a complex of a Ste20-like kinase and other downstream kinases. In human Mo25 forms a triple complex together with STRAD (STE20-related adaptor protein) and the kinase LKB1. Disruption of this complex results in Peutz-Jeghers-syndrome (Boudeau et al., 2003, EMBO J., Oct 1;22(19):5102-14), an inherited disease with high disposition to develop cancer (Jansen et al., 2009 Physiol. Rev., Jul;89(3):777-98). Here we generated a Dictyostelium discoideum Mo25-minus cell line. The knockout of the mo25 gene in D. discoideum results in very large, multinucleated cells unable to complete cytokinesis. Also growth and development of the Mo25-minus strain are massively disturbed. Furthermore, in phototaxis assays the Mo25-minus strain is unable to move towards the light source. These findings imply that Mo25 plays an important role in cytokinesis, growth and cell polarity. By using GFP-Trap resin we identified a binding partner of Mo25, the Ste 20-like kinase SvkA (Eichinger et al., J Biol Chem. 273: 12952, 1998), a homolog of the human MST3, MST4 and YSK1 kinases. To further elucidate the interaction of Mo25 with SvkA and other possible interactors we generated a series of Mo25 rescue constructs with distinct point mutations in the protein/protein interaction sites. With these mutations in Mo25 we hope to reduce the complex multilayer phenotype to its basic modules and identify the different downstream signaling pathways that branch off from the Mo25/SvkA kinase complex.

Talk 38

Regulation of DNA repair in Dictyostelium

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DNA is under continual assault from a variety of agents that cause DNA damage. As such cells have evolved multiple pathways to repair DNA damage and restore genome integrity. The molecular basis of these pathways is becoming increasingly well defined. However, a remaining challenge is to understand how these pathways integrate to maintain genome integrity and cell viability should one pathway fail.

DNA double strand breaks (DSBs) are considered to be one of the most pathological varieties of DNA damage. They can be repaired by two pathways, homologous recombination (HR) or non-homologous end joining (NHEJ). Whilst HR requires sequences homologous to the damaged DNA to facilitate repair, NHEJ is achieved by limited processing and direct ligation of DNA termini. We and others have found that human DSB repair factors absent from commonly used model organisms to study DNA repair are conserved in Dictyostelium. Recently, we exploited this observation to uncover a novel role for ADP-ribosylation in regulating non-homologous end-joining (NHEJ). Similar to humans, Dictyostelium contain multiple ADP-ribosyltransferases (ARTs) and two of these (Adprt1b and Adprt2) are required for resistance to DNA single strand breaks (SSBs). In contrast, whereas Adprt1b and to a lesser extent Adprt2 are dispensable for ADP-ribosylation at DSBs, a novel ART (Adprt1a) is required for this event and to promote NHEJ by promoting accumulation of Ku at sites of damage. Here we extend these studies and assess redundancy between different ARTs and DNA repair pathways following induction of DNA SSBs. The implications of these data with regards to how ADP-ribosylation regulates SSB and DSB repair will be discussed.

Talk 39

Regulation of cell proliferation and cell-type specification in Dictyostelium by Cdk1

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A Dictyostelium cell chooses its fate based largely on the time of its last division prior to the initiation of development. At the onset of development, freshly divided cells are more likely to form the stalk of the fruiting body while late G2 cells tend to become the reproductive spores. Precisely how the phases of the cell cycle are linked to cell-type differentiation is unknown. To address this issue, we targeted for analysis the cell-cycle regulator Cdk1.

Using RNA-blot analysis and reporter-gene studies, we showed that cdk1 is expressed shortly before mitosis and then again during development, but purely in cells that form spores. To determine the role of Cdk1 in the Dictyostelium cell cycle, we generated a series of conditional cdk1 mutants. Cells expressing cdk1Y15F, where the tyrosine residue at position 15 was replaced with a non-phosphorylatable phenylalanine, bypassed a control point in late G2. These cells were blocked in mitosis and displayed defects in spindle assembly. We observed the same phenotype in cells expressing the double mutant, cdk1T14AY15F, in which the neighbouring threonine phosphoacceptor residue was also mutated. This suggests that, like animal cells, Cdk1 dephosphorylation on tyrosine 15 is a pivotal step in the G2/M transition of Dictyostelium. We found that cells expressing the cdk1T14A single mutation were viable and developed normally. However cell size was reduced in comparison to the control strain, suggesting that Cdk1T14 phosphorylation is one mechanism by which Dictyostelium cells coordinate cell growth with cell division. When given the choice between becoming spores or stalk cells, Cdk1T14A cells opted for the stalk fate. Importantly we found that the developmental phenotype was rescued when cdk1T14A was expressed in a strain lacking the Retinoblastoma orthologue, RblA.

Talk 40

Dictyostelium discoideum uses the prokaryote second messenger c-di-GMP as an apically secreted signal for stalk cell differentiation

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Cyclic-di-GMP is a major prokaryote signalling intermediate, which is synthesized by diguanylate cyclases and triggers sessility and biofilm formation. We detected the first eukaryote diguanylate cyclases (DgcAs) in all major groups of Dictyostelia. Upon food depletion, Dictyostelium discoideum amoebas collect into aggregates, which first transform into migrating slugs and next into sessile fruiting structures. These structures consist of a spherical spore mass that is supported by a column of stalk cells and a basal disk. A polyketide, DIF-1, was isolated earlier, which induces stalk-like cells in vitro. However, its role in vivo proved recently to be restricted to basal disk formation. Here we show that Dictyostelium DgcA produces cyclic-di-GMP as the morphogen responsible for stalk cell differentiation. D.discoideum DgcA synthesized cyclic-di-GMP in a GTP-dependent manner and was expressed at the slug tip, the site of stalk cell differentiation. Disruption of the DgcA gene blocked the transition from slug migration to fructification and the expression of stalk genes. Fructification and stalk formation were restored by exposing dgca- slugs to wild-type secretion products or to cyclic-di-GMP. Moreover, cyclic-di-GMP, but not cyclic-di-AMP, induced stalk gene expression in dilute cell monolayers. Apart from identifying the long elusive stalk-inducing morphogen, our work also identifies the first role for c-di-GMP in eukaryotes.

Talk 41

Involvement of the plant fertilization protein Hap2/GCS-1 in the gamete interaction of Dictyostelium discoideum

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Macrocyst formation of Dictyostelium discoideum is a sexual process and represents a prototype of sexual cell interactions. There are three mating types, type-I, -II, and -III, for heterothallic strains. Although the mating-type genes for each of them, matA, matB and C, and matS, respectively, and a membrane-fusion protein macA have recently been reported, molecular mechanisms for gamete interaction are still elusive. In the present study, we examined the possible existence of other proteins involved in the mating of D discoideum. We first analyzed the structure of matA and macA in the previously isolated cell fusion-defective type-I mutants, XMC7 and XMC17, because the responsible genes had not been identified yet. The nucleotide sequences of both genes were completely same as the parental strain in both mutants. Their expression levels as revealed by RT-PCR were also indistinguishable, indicating the existence of the third genes for gamete interactions. In parallel, we performed the proteome analyses of gamete membranes and found that an orthologue for the plant fertilization protein Hap2/GCS-1 was expressed in the type-I gamete. In addition, the expression of a weekly homologous protein, MrhA (motif related to hap2), was also detected at differential levels. Gene disruption of either hapA or mrhA in KAX3 resulted in the complete loss of mating ability. Thus, D. discoideum appeared to share the molecular mechanisms for the gamete interaction with plants. Understanding how those proteins interact in D. discoideum will provide useful information for broader phyla.

Talk 42

Generating order from chaos: Noise, heterogeneity and robustness during developmental patterning in Dictyostelium

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Embryonic development is a remarkable feat of biological reproducibility. It is generally assumed this is because the underlying mechanisms are highly stereotypical or deterministic. However, recent observations of cell fate choice in simple microbial systems challenge this idea, and even suggest heterogeneity (also termed noise and stochasticity) in cell signalling and responses can be evolutionarily advantageous. Furthermore, heterogeneity in responses to differentiation signals within stem cell cultures (lineage priming) is now widely documented and represents a major challenge to the therapeutic value of this approach. Together, these findings have huge implications, because they raise the question of whether heterogeneity is normally regulated to play a role in cell fate choice and developmental patterning in multicellular organisms.

I will describe our progress in understanding this question through the identification of lineage priming genes that regulate an evolutionarily conserved developmental patterning mechanism exhibited during early mouse development and best known in Dictyostelium: "salt and pepper" differentiation and sorting out. I will focus on one such gene, gefE, which we find specifically primes growing cells to differentiate as pstB cells, partly through regulation of rasD activity. We find that this affects the cellular threshold or sensitivity of responsiveness to the pstB cell inducer DIF-1 within gefE expressing cells. These findings have led us to propose a model in which heterogeneous expression of priming genes during growth affects sensitivity of responses to developmental signals.

Talk 43

Cell type specific sorting out during Dictyostelium pattern formation

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Differential cell motility plays a key role in many developmental processes. This is perhaps most evident in examples of pattern formation in which different cell types arise intermingled before sorting out into discrete tissues. This is thought to require heterogeneities in responsiveness to differentiation-inducing signals that result in the activation of cell type-specific genes and 'salt and pepper' patterning. How differential gene expression results in cell sorting is poorly defined. I will describe a novel gene, hfnA that provides the first mechanistic link between cell signalling, differential gene expression and cell type specific sorting in Dictyostelium.

HfnA defines a novel group of evolutionarily conserved HECT ubiquitin ligases with a Filamin domain towards the N-terminus (HFNs). HfnA expression is induced by differentiation inducing factor DIF-1 and is restricted to a subset of prestalk cells (pstO). In an HfnA- mutant, pstO cells differentiate but their sorting out is delayed. Genetic interactions suggest this is due to misregulation of filamin complex activity. Disruption of Filamin in an HfnA- background effectively rescues pstO cell localization. We therefore propose that HfnA regulates filamin complex activity and cell type specific motility, through the breakdown of filamin complexes. These findings provide a novel mechanism for filamin regulation, and demonstrate that filamin is a crucial mechanistic link between responses to differentiation signals and cell movement in patterning based on "salt and pepper" differentiation and sorting out.

To explore further the link between DIF-1 signalling and cell sorting we have performed a second-site suppressor screen to identify other mutants involved in the HfnA pathway. This screen has identified a putative RNA-binding protein (RBP), which when disrupted in the HfnA- background, also effectively rescues pstO cell sorting to the collar of the developing slug.

Talk 44

Selection of spiral waves in excitable media with a phase wave at the wave back

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Universal relationships between the medium excitability and the angular velocity and the core radius of rigidly rotating spiral waves in excitable media are derived for situations where the wave front is a trigger wave and the wave back is a phase wave [1]. Two universal limits restricting the region of existence of spiral waves in the parameter space are demonstrated. The predictions of the free-boundary approach are in good quantitative agreement with results from numerical reaction-diffusion simulations performed on the Kessler-Levine model, which was originally aimed to simulate spiral wave dynamics during the cell aggregation in Dictyostelium discoideum [2].

[1] V.S. Zykov, N. Oikawa and E. Bodenschatz, Phys. Rev. Lett. 107, 254101 (2011).
[2] D.A. Kessler and H. Levine, Phys. Rev. E 48, 4801 (1993)."

Talk 45

A molecular network underlying spontaneous cAMP oscillation and a key role of G-protein dynamics

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Rhythms of many kinds are involved in the self-regulation of biological systems, with oscillation periods varying widely from milliseconds to years. In the case of Dictyostelium discoideum, the periodic production of cAMP pulses is well studied. Dictyostelium cells secrete cAMP when starved. Secreted cAMP binds to cAR1, then ACA and ERK2 are activated by ligand-bound cAR1, and intracellular cAMP activates cAMP-dependent protein kinase A (PKA) such that it inhibits ERK2 and ACA activation, while the cAMP phosphodiesterase RegA reduces internal cAMP to its resting level. This whole process is responsible to the spontaneous production of cAMP pulses with a periodicity of 5-10 minutes.

Recently, we have proposed a molecular network, incorporating both adaptation and phosphorylation, to account for the spontaneous cAMP oscillation of Dictyostelium[1]. We have modified the scheme previously proposed Maeda et al.[2] to include adaptation by the cAMP receptor and such that extracellular cAMP is formed by the secreted intracellular cAMP instead of being directly produced by adenylyl cyclase. Our scheme provides better robustness than that of Maeda et.al.[2] However, adaptation was introduced only phenomenologically, and the corresponding molecular basis could not be clarified. Thus, we have investigated the role of G-protein dynamics in adaptation and spontaneous cAMP oscillation. We plan to report its progress.

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[2] Maeda, M., Lu, S., Shaulsky, G., Miyazaki, Y., Kuwayama, H., Tanaka, Y., Kuspa, A., and Loomis, W.F., 2004. Science 304, 875-8.

Talk 46

SCAR/WAVE, actin, and how pseudopods are controlled

Douwe Veltman, Seiji Ura, Peter Thomason, Andrew Davidson, Jose Batista, and Robert Insall

Chemotaxis is primarily driven by pseudopods, which protrude from the fronts of cells and move the leading edges forwards. Pseudopods, in turn, are powered by the polymerization of new actin filaments immediately next to the membrane. We are interested in what causes pseudopods to form and grow, and how this process can be steered.

It is usual to portray pseudopods as being initiated at the end of complex signalling pathways. In this world view, pseudopods are rather dull structures, and the control and steering of cell migration are achieved through signal processing in the upstream pathways. We hold a different, "pseudopod-centred" view, in which pseudopods can be generated and grow without direct control from signaling pathways, and in which anything that affects the evolution of pseudopods can contribute to cell steering.

We will support this idea with several different lines of data. We have assessed the phosphorylation of SCAR, the catalyst of actin & Arp2/3 complex assembly, and found a complex control circuit. We have also measured real pseudopod behavior under several different conditions, in targeted SCAR and WASP mutants, and conclude that real-life regulation of pseudopod formation is very different from most textbook models. Finally, generated computational models of cell migration in which simple pseudopod-centred rules can explain much of the complexity of directed cell migration.

Talk 47

dictyBase Literature Curation and how Authors can Help

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Literature curation is a key task in a biocurator's daily work; it entails the extraction of all experimental information from a manuscript that can be stored in a database. Annotations we currently store in dictyBase are:

- Literature topics (broad categories of biological entities)
- Mutant strains
- Phenotypes
- Molecular functions (GO)
- Biological processes (GO)
- Cellular components (GO)
- Gene/Protein names
- Free-text descriptions, notes, summaries
- Gene structure

The most time consuming aspects of literature curation at dictyBase are the gene ontology (GO) and the strain annotations. GO annotations consist of a controlled vocabulary and are amenable to text mining, which we began utilizing in collaboration with Wormbase (Müller et al., 2004). However, challenges remain and we will discuss the bottlenecks and how authors can help improve accuracy for these semi-automatic annotations. Strain annotations, which in turn are needed to curate phenotypes, are categorized into strain descriptors, genotypes, genetic modifications, mutagenesis types, strain characteristics, and more. However, strain information in publications is often not well organized or clearly described; certain aspects may be found in different sections of the paper or missing altogether. Authors might help by listing strains in one place, and by ensuring that all key information is provided.

Literature curation aids individual researchers by providing all annotations related to a gene on one page. Furthermore, systematic annotations enable the mining of databases for functional data and the electronic transfer of experimental annotations to related species for which manual curation is not available. Here we suggest a few simple ways in which authors can facilitate the annotation of their publications.

dictyBase is supported by NIH grants GM64426 and HG00022.

Müller, H.-M., Kenny, E.E., Sternberg, P.W., 2004. Textpresso: an ontology-based information retrieval and extraction system for biological literature. PLoS Biol. 2, e309.

Talk 48

Subversion of phosphoinositide lipids and small GTPases by Legionella in Dictyostelium

Stephen Weber, Eva Rothmeier, Christine Hoffmann, Maria Wagner & Hubert Hilbi
Max von Pettenkofer Institute, Ludwig-Maximilians University, Munich, Germany

Dictyostelium discoideum is a versatile model organism to study interactions with the facultative intracellular bacterium Legionella pneumophila. The "accidental" pathogen L. pneumophila employs a conserved mechanism to replicate in protozoa and macrophages within a unique compartment, the "Legionella-containing vacuole" (LCV) (1). Formation of LCVs is a complex and robust process that requires the bacterial Icm/Dot type IV secretion system and involves more than 250 different translocated "effector" proteins. The effector proteins likely interfere with host vesicle trafficking and signal transduction pathways, yet the cellular targets and functions of most of these bacterial proteins are unknown (2).
Intact LCVs can be purified from L. pneumophila-infected D. discoideum amoebae by "immuno-magnetic separation", using an antibody against an Icm/Dot-secreted effector exclusively localizing to the LCV membrane. Proteomics and fluorescence microscopy of LCVs revealed the presence of small GTPases including Arf1, RanA and members of the Rab family (3), phosphoinositide (PI)-modifying enzymes such as OCRL1, as well as the PI lipid phosphatidylinositol-4-phosphate (4). Current studies focus on the dynamics of different PIs on LCVs and on the characterization of several L. pneumophila effector proteins that subvert PI lipids and small GTPases, or promote microtubule-dependent LCV dynamics.

(1) Hilbi, H., Hoffmann, C. & Harrison, C. (2011) Legionella spp. outdoors: colonization, communication and persistence. Environ. Microbiol. Rep. 3: 286-296.

(2) Hilbi, H. & Haas, A. (2012) Secretive bacterial pathogens and the secretory pathway. Traffic. doi: 10.1111/j.1600-0854.2012.01344.x. Epub ahead of print.

(3) Urwyler, S., Nyfeler, Y., Ragaz, C., Lee, H., Mueller, L. N., Aebersold, R. & Hilbi, H. (2009) Proteome analysis of Legionella vacuoles purified by magnetic immuno-separation reveals secretory and endosomal GTPases. Traffic 10: 76-87.

(4) Weber, S. S., Ragaz, C. & Hilbi, H. (2009) Pathogen trafficking pathways and host phosphoinositide metabolism. Mol Microbiol 71, 1341-1352.

Talk 49

Role of actin and actin-binding proteins during mycobacterial infection in a Dictyostelium-model system

Margot Kolonko, Monica Hagedorn
Department of Parasitology, Bernhard Nocht Institute for Tropical Medicine. Bernhard-Nocht-Strasse 74, 20359 Hamburg, Germany

Tuberculosis is an infectious disease that accounts for nearly two million deaths each year. It is caused by Mycobacterium tuberculosis, which is taken up by human alveolar macrophages and establishes its replication niche by circumventing the phagosomal maturation processes. To study the dynamics of the host-pathogen interaction on the cellular level, we use the Dictyostelium discoideum-Mycobacterium marinum model system that has previously been established (Hagedorn et al., Science, 2009). While host cell entry has been studied extensively, the mechanisms of establishment and maintenance of the mycobacterial replication niche remain largely unknown. The host cytoskeletal protein actin is important at many steps during cell entry and egress.

Applying confocal microscopy, we showed that the M. marinum containing vacuole is partially decorated with actin. After depolymerization of actin at the compartment in three independent ways, we observed that the vacuole is severely remodeled and lacks typical compartment membrane markers. Moreover, this remodeling process is restricted to the early phase of the infection and depends on the bacterial pathogenicity factors, such as the RD1 locus. We could show that this process does not involve autophagy, but rather promotes the fusion of lysosomes with the mycobacterial compartment, which subsequently acidifies. Using FACS-based approaches, we investigated the long-term effects of the remodeling and measured the bacterial proliferation. Currently, we are monitoring our key observations in a macrophage model.

We propose that actin controls the fusion events at the mycobacterial compartment and may therefore be a target of the pathogen virulence factors. Shedding light on this host-pathogen cross-talk should help to understand mycobacterial persistence and virulence.

Talk 50

Saposin-like proteins are implicated in antibacterial defence of Dictyostelium

Ranjani Dhakshinamoorthy, Moritz Bitzhenner, Matthias Leippe
Department of Zoophysiology, Zoological Institute, University of Kiel, Olshausenstr. 40, 24098, Kiel, Germany

Although Dictyostelium discoideum has become an interesting model for the study of host-pathogen interactions and the beginning of innate immunity in recent years, the knowledge about the arsenal that Dictyostelium uses to kill internalized microbes and to combat potential pathogens is limited to very few examples - at least at the protein level. Comparable to the free-living nematode and model organism C. elegans (1-2), Dictyostelium possesses a gene family potentially coding for a enormous number of antimicrobial proteins that structurally belong to the saposin-like protein (SAPLIP) family. SAPLIPs are also known from mammals and can fulfil a variety of biological functions in so far as lipid interaction is concerned (3). In amoebae, it is known from Entamoeba histolytica that a SAPLIP termed amoebapore is pore-forming, antimicrobial and cytotoxic to human cells and is considered a virulence factor, the tertiary structure of which has been solved (5). Here, we show for selected members of the SAPLIPs of Dictyostelium provisionally named Apls (amoebapore-like peptides) that they are membrane-permeabilizing proteins that can kill bacteria in vitro and that the ablation of the gene when phenotypically analyzed may result in defects in growth on and intracellular killing of particular bacteria such as Klebsiella aeroginosa. Accordingly, this multifarious protein family presumably constitutes an important part of the amoebic armamentarium that act complementarily and synergistically to allow Dictyostelium to survive in its microbial world.

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J. Biol. Chem. 280, 41125-41128.
4. Andrä, J., Herbst, R. and Leippe, M. (2003) Amoebapores, archaic effector peptides of protozoan origin, are discharged into phagosomes and kill bacteria by permeabilizing their membranes. Dev. Comp. Immunol. 27, 291-304.
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Talk 51

Exploring the roles of NADPH oxidases in Dictyostelium discoideum

Xuezhi Zhang, Thierry Soldati
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Reactive oxygen species (ROS) are short-lived and highly reactive molecules that serve a variety of purposes, such as intracellular or extracellular signaling in development and symbiotic relations, to microbicidal activity of immune phagocytes. The membrane-bound NADPH oxidases (NOX) are the major enzyme complexes devoted to ROS generation. Classically, in neutrophils, NOX2-generated ROS are essential to produce the microbe-killing oxidative burst, while in dendritic cells and macrophages, the exact function(s) of ROS is still under intense study.

Dictyostelium and human phagocytes share a surprisingly high level of conservation in molecular machineries responsible for bacteria sensing, engulfment and killing. By using the most sensitive ROS-detection methods now available, Amplex UltraRed (AUR), Dihydroethidium (DHE), and L-012, we were able to detect unambiguously the extracellular hydrogen peroxide and intracellular superoxide production in Dictyostelium. Our preliminary data indicated that various bacteria culture supernatants have significantly different stimulatory activities on ROS production. Further ongoing work indicates that structurally different lipopolysaccharide (LPS) can stimulate ROS production to extremely different levels, which indicate for the first time that the recognition of LPS and possibly other PAMPs is involved in bacteria sensing and ROS generation in Dictyostelium. With the help of Oxyburst-coated silica beads, we were able to visualize the intra-phagosomal ROS generation in real-time, further quantification of fluorescence emission from Oxyburst revealed that the generation of ROS occured within 1 min after ingestion of the particle. The use of a catalase (CatA) knockout Dictyostelium strain considerably increased the measurable level of extracellular H2O2, whereas inhibitors of the superoxide dismutase increased the measurable level of intracellular superoxide. In addition, the single knockouts of NoxA, B, C and p22phox showed impaired ROS production under stimulation, indicating that NOXs are important for ROS generation in Dictyostelium. Due to the possible compensation of expression and redundancy of functions of NOXs, we are generating multiple knockouts in Dictyostelium using cre-lox system.

Talk 52

Initial Characterization of the Dictyostelium Microbiome

Olga Zhuchenko, Chris Dinh, Elizabeth Ostrowski, and Adam Kuspa
Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston TX 77030

Dictyostelium has an innate immune system consisting of Sentinel (S) cells that can clear bacteria from the migrating slug (1). Certain wild strains of Dictyostelium, called farmers, carry bacteria during development for later use as a renewable food supply (2). The ability to carry bacteria while maintaining an innate immune system leads to the intriguing possibility that Dictyostelium interacts with its microbiome during development in ways that influence its physiology.

To explore this we have begun to characterize the variation in immune system function among natural isolates of Dictyostelium. In efficient carrier strains, we find that the bacterial population is maintained over prolonged periods of slug migration. The bacterial population also increases with time during the slug migration of carriers, and there may be variation in this property amongst strains. We also quantified the number of S cells in wild strains and found an inverse relationship between the number of S cells they have and their ability to carry bacteria. In addition, we have been able to convert a non-carrier strain into a carrier by genetic manipulation. The tirA gene is required for efficient feeding on bacteria by amoebae during growth and for S cell function during development (1). The laboratory strain AX4 is a non-carrier, but mutating the tirA gene in AX4 results in a strain that is able to carry bacteria. Thus, a lower number of S cells, or S cells with diminished functionality, is associated with an increased frequency of Dictyostelium slugs that carry bacteria during development. These observations lead to the intriguing possibility that the tendency to carry bacteria is controlled, at least in part, by the Dictyostelium immune system and are suggestive of a complex interaction between the multicellular Dictyostelium host and the bacteria they carry. In further studies we hope to define the interactions between the bacteria and the host that determine the establishment and maintenance of the Dictyostelium microbiome.

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Talk 53

Dictyostelium development shows a novel pattern of evolutionary conservation

David C. Queller, Xiangjun Tian, Joan E. Strassmann
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Von Baers law states that early stages of animal development are the most conserved though more recent evidence supports an "hourglass" pattern in which a somewhat later stage is most conserved. Both patterns have been explained by the relative complexity of either temporal or spatial interactions; the greatest conservation and lowest evolvability occur at the time of the most complex interactions, because these cause larger effects that are harder for selection to alter. This general kind of explanation might apply universally across independent multicellular systems. We use RNA-seq expression data from the development of Dictyostelium to demonstrate that it does not. Instead, most analyses of Dictyostelium show the strongest conservation and weakest evolvability late in development. Either Dictyostelium follows a novel developmental timecourse of complexity, or relative complexity does not always determine relative conservation.

Talk 54

Switching expression of prespore and prestalk marker orthologues during fruiting body development of Acytostelium subglobosum

Kurato Mohri, Yu Kiyota, Hidekazu Kuwayama and Hideko Urushihara
Faculty of Life and Environmental Sciences, University of Tsukuba

Somatic cell differentiation is a crucial event for the development of the multicellular organisms. While fruiting body development of Dictyostelium discoideum represents its superb model with separation of mortal stalk cells from the spore lineage, that of Acytostelium subglobosum is not accompanied with cell differentiation. It produces acellular stalks and seemingly all aggregated amoebae become spores. We have been performing comparative genomic analyses between these two species and found that their gene repertoires were very similar, almost all D. discoideum stalk-lineage genes having their counterparts in A. subglobosum. In this study, we analyzed the spatio-temporal expression patterns of A. subglobosum orthologues for D. discoideum stalk- or spore-lineage marker genes to clarify the developmental process of A. subglobosum. Expression of a stalk-lineage gene orthologue, As-ecmA, started at the mound stage and was prominent in the protruding sorogens. On the other hand, although a spore-lineage gene orthologue, As-cotD, was likewise detected shortly after the cell aggregation and increased in intensity until tip-formation, it diminished in the protruding sorogens. The double-fluorescence staining revealed that the expressions of those two genes were mostly exclusive and that switching from As-cotD to As-ecmA expression occurred in the early tips. The prespore vesicles started to accumulate in the tip regions and were observed in the entire sorogens throughout later development. Our results indicate that A. subglobosum cells become committed to spores first and then, while keeping this commitment intact, participate in stalk formation. The discrete spatial pattern shown by differential gene expression reflects the temporal change of cellular activity and all the cells devote their energy serially to spore and stalk formation.

Talk 55

Birth-and-death evolution of the tgr genes in Dictyostelium discoideum

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Department of Biology and Biochemistry, University of Houston, Houston, TX 77204

Many genes that exhibit extreme polymorphism are members of large gene families that evolve by a "birth-and-death" process. Under this scenario, new genes are born through a process of gene duplication, but are lost through gene conversion, unequal crossing over, or mutational inactivation. The hallmarks of birth-and-death evolution are large numbers of physically clustered genes, rapid expansion and contraction of the gene family, and numerous "dead" pseudogenes. In addition to the possibility that duplicated genes might diverge in sequence and function, close proximity and sequence similarity among the members is thought to facilitate rearrangements that can drive the acquisition of proteins with novel functions.

As part of a larger genome sequencing project, we have been examining the evolution of the tgr gene family in D. discoideum. Two members of this gene family, tgrB1 and tgrC1, show extraordinary levels of sequence polymorphism among different natural isolates, and sequence variation at these loci has been shown to underlie allorecognition during development [1,2]. However, tgrB1 and tgrC1 are members of a much larger family, comprising nearly 60 genes and pseudogenes, many of which occur in clusters in close proximity to the centromeres. Molecular evolution analysis of the tgr gene family indicates that other members aside from tgrB1 and tgrC1 also show extraordinary polymorphism, although there are also many tgr genes that are well conserved. We performed computational analyses to detect genome-wide structural variation, which identified several candidate regions as hotspots for rearrangements, including the region corresponding to the tgrB1-tgrC1 cluster. Our results suggest that gene duplication and rearrangements may be important for generating novel allelic variants at these loci, helping to explain their function as rapidly evolving, highly sensitive indicators of close kinship among strains.

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Talk 56

Dictyostelium discoideum as a social organism

Joan E. Strassmann, David C. Queller
Department of Biology, Washington University in St. Louis, One Brookings Drive, Campus Box 1137, St. Louis MO 63130 USA

How we view a biological problem influences the kinds of questions we ask. For example, a developmental approach inspires questions about morphogenesis. One of the advantages of working on Dictyostelium is that we can take multiple perspectives and answer different kinds of questions. Here we take a social perspective. This means we look at Dictyostelium as a group of individuals of the same species. An important difference from a developmental perspective is that amoebae, or cells, not the multicellular organism, are the focus. There are some standard questions about social organisms that can then be asked. Are group members related? Is there conflict within the group? Are individual roles changeable? What biological problems does grouping solve? How do the answers to the above questions vary with group size? In this talk we will cover the social problems of Dictyostelium that have been answered. We will identify and explore some of the questions that remain.

Posters

Summary of presenters:

1. Ramamurthy Baskar
2. Yoshinori Kawabe
3. Azam Gholami
4. Wei-Chi Lin
5. Marco Tarantola
6. Hsin-Fang Hsu
7. Margot Kolonko
8. José Negrete Jr
9. Christoph Blum
10. Mari Gorelashvili
11. Ineke Keizer-Gunnink
12. Morgan N. Thompson
13. Yukie G. Sato
14. Takaaki B. Narita
15. Linh Vu Hai
16. Tetsuya Muramoto
17. María Galardi-Castilla
18. Teresa Suarez
19. Nicole Gruenheit
20. Thomas Spaller/Jana Schiefner
21. Robert Dodson/Petra Fey
22. Sarah Annesley/Paul Fisher
23. Małgorzata Wojtkowska
24. Ana Mesquita
25. Jan Matthias
26. Jasmina Ilievska
27. Clèmence Habourdin
28. Matthieu Delincé
29. Lauren Kolski
30. Frauke Bach
31. Christina Schilde
32. Balint Stewart

Poster 1

Evaluation of caffeine and adenosine action on growth and development in Dictyostelium discoideum mediated via TOR complexes.

Pundrik Jaiswal, Rakhil Akkali, Jan Matthias, Linh Vu Hai, Thierry Soldati, Michael Gromiha, A. Mary Thangakani, Ludwig Eichinger and Ramamurthy Baskar

The xanthine alkaloid, caffeine, has pleotropic effects affecting growth and development in Dictyostelium discoideum. The caffeine antagonist, adenosine is a morphogen in Dictyostelium. In S. cerevisiae, caffeine is known to act on the TOR1 of target of rapamycin complex-1(TORC1) and increases their life span. In Dictyostelium, it is not clear if growth and development in the presence of caffeine and adenosine are being regulated by TORC1 or TORC2. FRB (FKBP12 rapamycin binding domain) and PI3Kc kinase (Phosphatidyl inositol 3-kinases) domains of the yeast TOR1 complex show strong similarity with the Dictyostelium orthologues suggesting that TOR is likely to be a caffeine target in Dictyostelium as well. This assumption is supported by our finding that rapamycin and caffeine treatment caused a similar effect, a decrease in aggregate size. Furthermore, the knockout mutants in genes encoding components of the TOR complex (RIP3- and lst8- cells) showed insensitive or hypersensitive responses respectively to caffeine treatment. Based on sequence similarity of the TOR kinase domain to other kinases like PikD (phosphatidylinositol kinase-D), PikE (phosphatidylinositol kinase-E), PIkF (phosphatidylinositol kinase-F), smg1 (serine/threonine-protein kinase-1), ATR (ataxia telangiectasia and Rad3 related) and dnapkcs (DNA-dependent protein kinase catalytic subunit), adenosine may act on PikD, PikE, PIkF, upstream of the TOR complex or on downstream TOR target genes involved in DNA repair mechanism. To identify the spectrum of genes whose expression is modulated by the presence of caffeine and adenosine, we undertook a large scale gene expression analysis using DNA microarrays. This will help us uncover the targets regulating growth and development. Upon caffeine treatment, 49 genes were differentially expressed at 3 h and a total of 344 at 6h of development in comparison to control cells, developed for 3 and 6 h, respectively. The same comparison in the presence of adenosine resulted in approximately three-fold less differentially regulated genes, 17 and 98 respectively. Classification of the differentially regulated genes based on their biological function showed an overrepresentation of genes involved in signal transduction and multicellular organization. Among the differentially regulated genes, we selected RIP3, cnbA (calcineurin B), ctnA (countin), PdiA (cAMP phosphodiesterase inhibitor-A) and lagC (tgrC1; loose aggregate C) and monitored the respective knock-out mutants in comparison to control cells for their timing, differentiation and aggregation in response to caffeine and adenosine treatment."

Poster 2

Functional Conservation and Evolution of Extracellular cAMP Signaling in Dictyostelids.

Yoshinori Kawabe, Pauline Schaap
College of Life Sciences, University of Dundee, MSI/WTB/JBC complex, Dundee DD1 5EH, UK

Cyclic AMP signaling plays many roles in Dictyostelium discoideum. Extracellular cAMP acts as chemoattractant and also induces the expression of aggregation genes and prespore genes.

Most Dictyostelium species can be subdivided into four major groups with D.discoideum residing in group 4, together with all other species that use cAMP as the chemoattractant for aggregation Although species in the other three groups do not use cAMP to aggregate, cAMP receptors which are most diagnostic for use of extracellular cAMP are present throughout the Dictyostelia and have functions after aggregation. To gain further insight into the evolution of extracellular cAMP signaling, we also analysed the role of adenyl cyclase A (ACA) in non-group 4 species. Genomic DNA sequencing projects in several basal species showed that ACA genes are conserved throughout Dictyostelid evolution. Expression of an ACA gene from the group 3 species D. minutam partially restore aggregation of a D. discoideum ACA knockout mutant. The group 2 species P. pallidum has 3 ACA genes, which are expressed at the tip region of multicellular structures. Single null mutants in these genes show normal morphology, while double knockout mutants shows changes in fruiting body morphology, These finding suggests that ACA also produces extracellular cAMP in lower group species and has functions for fruiting body morphogenesis.

Poster 3

Influence of an external flow on cAMP wave formation during aggregation of Dictyostelium discoideum

Azam Gholami, Mahboobeh Naghipoor, Eberhard Bodenschatz
Max Planck Institute for Dynamics and Self-Organization, Am Fassberg 17, 37077 Goettingen, Germany

We study the effect of an external flow on pulse waves of cAMP in aggregating Dictyostelium, both experimentally and by means of mathematical modelling. External parabolic flow profile is generated using mili fluidic channels to investigate spiral wave formation in an excitable system with axial anisotropy. External flow effects propagation velocity of circular and spiral pulses, and the annihilation of pulses propagating against the flow in a spatially two-dimensional system. We use Martiel-Goldbeter kinetic model [1] to describe our experimental observations.

[1] J. L. Martiel and A. Goldbeter, Biophy. J. 52, 807 (1987)

Poster 4

Identification of Rmo as a novel regulator of cell motility in Dictyostelium

Wei-Chi Lin, Te-Ling Pang and Mei-Yu Chen.

Directional cell migration is an important process involved in various physiological and pathogenic events. Here, through analyses of a mutant obtained from a genetic screen for Dictyostelium discoideum defective in chemotactic responses, we have identified a previously uncharacterized gene involved in the regulation of cell motility and named it rmoA. Dictyostelium mutants with disrupted rmoA could not develop into fruiting bodies as wild-type cells did on bacteria lawns, and could only form smaller-than-wild-type fruiting bodies on non-nutrient agar plates. Under the cAMP gradient, rmoA- cells displayed normal directional persistence, yet migrated significantly slowlier than the wild-type cells did. Expressing rmoA in rmoA- cells restored their defect in cell motility. Western results demonstrated that a significant portion of cellular Rmo protein could be found in a detergent insoluble fraction rich in polymerized cytoskeletal components. The fluorescence signals of mRFP-tagged Rmo accumulated at the leading edge of migrating cells and colocalized with signals of F-actin. Results from domain mapping experiments using different truncation forms of Rmo suggested that the aa401-600 region of Rmo is essential for the accumulation of Rmo to the cell periphery or the leading edge. In vitro assays demonstrated that the aa401-600 Rmo fragment was sufficient to associate with the detergent insoluble cytoskeletal fraction and to directly bind to polymerized actin. In addition, compared to wild-type cells, rmoA- cells showed increased levels of filamentous actin and decreased cell-substratum adhesion. Taken together, our data suggest that Rmo is a novel F-actin interacting protein involved in the regulation of Dictyostelium cell motility.

Poster 5

Chemotaxis of Dictyostelium Discoideum monitored by impedance analysis: collective oscillations of 2d aggregates and individual cell-substrate contacts

Edith Schaefer, Marco Tarantola, Christian Westendorf, Noriko Oikawa, Carsten Beta, Burkhard Geil, Eberhard Bodenschatz, Andreas Janshoff
Max Planck Institute for Dynamics and Self-Organization, Fassberg 17, 37077 Goettingen, Germany

So far, most of our knowledge on collective shape oscillations of Dictyostelium Discoideum is based on population-level optical density observations or isotope dilution assays of cAMP. Individual cells or small ensembles are rarely investigated with respect to shape oscillations, while the migratory behavior of single cells is abundantly studied using optical microscopy. An entirely different approach to assess the shape changes of individual amoebae as well as small ensembles during starvation is to employing electric cell-substrate impedance sensing (ECIS). ECIS provides a means to follow subtle shape changes of living cells as well as the dynamics of cell-substrate and cell-cell contacts with a time-resolution in the millisecond range.

Here, we report on direct spatio-temporal correlation of video microscopy (bright field and TIRF) with time-resolved impedance recordings from corresponding areas supported by acoustic resonator measurements to unequivocally show what morphological changes occur on the electrode once the cells start to oscillate in response to starvation conditions.

We found that the main contribution to the impedance oscillations in response to cAMP waves could be attributed to changes in the cell-substrate distance. TIRF measurements clearly reveal that a smaller cell-substrate distance leads to an increase in impedance giving rise to oscillations. These findings are in good accordance with acoustic resonator measurements using a QCM-D setup.

More subtle contributions originate from oscillations in circularity of the amoebae that although reproducing the oscillation frequency they fail to display the same signal shape. In contrast, periodic changes in the 2-D aggregation state reproducing both signal shape and oscillation frequency. We show that this periodic formation of small cell clusters is responsible also for impedance shift in which larger clusters produce larger impedance signals preserving the overall electrode coverage.

Poster 6

Dynamics of actin network in Dictyostelium

Hsin-Fang Hsu, Eberhard Bodenschatz
Am Fassberg 17, 37077 Goettingen, Germany

Cells can response to chemicals in the environment. The cellular motility is driven by the assembly and disassembly of actin filaments. In addition to the dynamic of actin, the contraction of Myosin II in the back of cells also play important role in effective movement. Without Myosin II, cells move much slower and have difficulty in dividing. Here we observed Myosin II mutant cells showed frequent actin dynamic even without external stimulus. We thus investigate how external stimulus perturbs the self-sustained system. We use microfludic device accompanied with caged cAMP to precisely control the external stimulus applied to Dictyostelium cells. Our results show that external stimulus can elongate the oscillation period of actin dynamic.

Poster 7

Role of actin and actin-binding proteins during mycobacterial infection in a Dictyostelium-model system

Margot Kolonko and Monica Hagedorn
Bernhard-Nocht-Institute for Tropical Medicine, Department of Cellular Biology and Parasitology, Bernhard-Nocht-Str. 74 ,20359 Hamburg

Tuberculosis is an infectious disease that accounts for nearly two million deaths each year. It is caused by Mycobacterium tuberculosis, which is taken up by human alveolar macrophages and establishes its replication niche by circumventing the phagosomal maturation processes. To study the dynamics of the host-pathogen interaction on the cellular level, we use the Dictyostelium discoideum-Mycobacterium marinum model system that has previously been established (Hagedorn et al., Science, 2009). While host cell entry has been studied extensively, the mechanisms of establishment and maintenance of the mycobacterial replication niche remain largely unknown. The host cytoskeletal protein actin is important at many steps during cell entry and egress.

Applying confocal microscopy, we showed that the M. marinum containing vacuole is partially decorated with actin. After depolymerization of actin at the compartment in three independent ways, we observed that the vacuole is severely remodeled and lacks typical compartment membrane markers. Moreover, this remodeling process is restricted to the early phase of the infection and depends on the bacterial pathogenicity factors, such as the RD1 locus. We could show that this process does not involve autophagy, but rather promotes the fusion of lysosomes with the mycobacterial compartment, which subsequently acidifies. Using FACS-based approaches, we investigated the long-term effects of the remodeling and measured the bacterial proliferation. Currently, we are monitoring our key observations in a macrophage model.

We propose that actin controls the fusion events at the mycobacterial compartment and may therefore be a target of the pathogen virulence factors. Shedding light on this host-pathogen cross-talk should help to understand mycobacterial persistence and virulence."

Poster 8

The Dynamics of Actin Polymerization under Local External Pulses of cAMP

José Negrete Jr., Christian Westendorf, Carsten Beta and Eberhard Bodenschatz.
Max Planck Institute for Dynamics and Self-Organization, Laboratory for Fluid Dynamics, Pattern Formation and Biocomplexity, Am Fassberg 17, D-37077 Goettingen, Germany.

We have studied the translocation of filamentous actin marker LimE-GFP in Dictyostelium discoideum under external stimulation with cAMP. The experiment consists of single cells stimulated with pulses of caged cAMP placed in a microfluidic device under constant flow. A particular feature of this protein is that a single short stimulus forces the marker to translocate globally from cytosol to cortex and relaxes back to equilibrium translocating back and forth in a repetitive a manner. Since the translocation may occur to cortical areas outside the focal plane, the average cytosolic fluorescence intensity is used as a measure for the temporal evolution of actin polymerization.

In this work we have analyzed the dynamics of LimE under different temporal stimulation patterns as also cells co-expressing LimE-RFP with Myosin II-GFP or Coronin-GFP. Diverse signal processing methods have revealed several features of this dynamical system, its relationship to other variables and suggest mechanisms for adaptation.

Poster 9

Correlation of Ras with pseudopod formation in D. d.

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Actin cytoskeletal dynamics provide the fundamental basis of eukaryotic cell motility. The cross-linked actin network at the front of a cell pushes the leading edge of the membrane towards the source of attractant. It is our aim to provide a quantitative understanding of the spatio-temporal dynamics of the actin cytoskeleton within the actin cortex.

We have developed experimental methods to address single cells with well-controlled mechanical and chemical stimuli [1]. Our experimental techniques are based on microfluidic devices, such as flattening device and micromixer, and fluorescence microscopy (Confocal, TIRF).

Here we present the correlation of Ras-GTP localization with the formation of pseudopods and their dynamics. The localization is visualized by the Ras binding domain probe (RBD-GFP). The pseudopod formation is analyzed by curvature maps.

Poster 10

Cell Migration in Quasi-3D Environments

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Different types of external stimuli can influence cell migration. Chemotaxis, which is the cellular ability to direct the movement according to the gradient of a chemical attractant, is one well-known example [1]. But surface topography of the environment can also impact the direction and the velocity of cell migration.

Here, we study the influence of micro-scale environment topography and cytoskeleton states on amoeboid migration of Dictyostelium discoideum cells in absence of chemoattractant gradients. We investigate amoeboid migration of wild type cells and of cells lacking cytoskeletal elements like microtubules and myosin II motors in well-defined micro-pillar arrays. A time-resolved migration analysis is done by a local mean-squared displacement algorithm [2].

We find that the amoeboid migration consists of alternating phases of directed (dir) runs and random (rm) migration modes. In quasi-3D pillar arrays dir-runs are exhibited less frequent and at higher velocities. The network geometry is reflected by the angular distribution of dir-runs for wild type cells, whereas cells lacking microtubules do not present this characteristic [3].

These results reveal that the environment topography strongly influences cell migration and that the cellular interaction with 3D structures is enhanced by microtubules.

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Poster 11

G-protein interacting components in GPCR-mediated chemotaxis pathways, revealed by mass spectrometry analysis.

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Central to chemotaxis is the molecular mechanism by which cells exhibit directed movement in shallow gradients of chemoattractant. In previous studies we have identified a basal signaling module of chemotaxis in Dictyostelium, which consists of heterotrimeric and monomeric G-proteins (Kortholt et al, 2011). This basal module provides activation of Ras and actin polymerisation at the leading edge, which is sufficient for chemotaxis. The next challenge will be to discover additional components of this basal pathway and to determine the mechanism by which heterotrimeric G-proteins induce and regulate Ras activation. To isolate these effectors and regulators, recombinant glutathione S-transferase (GST)-fused G-alpha and Ras-proteins were used as a bait in pull-down studies from Dictyostelium cell lysate. Potential interacting proteins were identified by mass spectrometry. Their role in chemotaxis was further confirmed by direct binding assays and characterized in more detail by in vivo studies.

Poster 12

Huntingtin is necessary for EDTA-resistant homotypic cell adhesion in Dictyostelium

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Huntington's disease (HD) results from a single causative mutation in the HTT gene, consisting of an expanded version of a polymorphic polyglutamine tract in the huntingtin protein. The ancient eukaryote, Dictyostelium discoideum, possess a HTT homolog (htt), providing a powerful system to study conserved, normal function of huntingtin. I have demonstrated that huntingtin null (htt-) Dictyostelium cells wholly fail to acquire EDTA-resistant cell adhesion in shaking starvation culture. Contact site A (CsA) is the molecular player considered to be primarily responsible for EDTA-resistant adhesion in Dictyostelium. Protein, but not mRNA, levels of CsA are dramatically reduced in htt- cells, yet introduction of CsA via an overexpression construct fails to rescue the adhesion defect. Interestingly, supplementation of exogenous calcium is capable of restoring EDTA-resistant adhesion in htt- cells in a normal developmental timeframe and results in normal levels of CsA. In an effort to explain why CsA overexpression failed to restore EDTA-resistant adhesion in htt- cells, I investigated factors known to affect the stability of the protein, including localization and glycosylation. However, no defects were found, suggesting that despite proper csA mRNA levels, CsA protein synthesis is defective in htt- cells, although cells possess all of the necessary machinery to produce CsA normally upon addition of calcium. Furthermore, these results spurred an examination of the effects of calcium on EDTA-resistant adhesion in csA- null cells. Interestingly, exogenous calcium is able to rescue EDTA-resistant adhesion in the absence of CsA. Taken together my data suggest that, contrary to our prior understanding, calcium alone is capable of bypassing the requirement for CsA, indicating that CsA is necessary but not sufficient for EDTA-resistant adhesion. The precise molecular role of huntingtin in facilitating CsA translation and the mechanism of action of calcium in EDTA-resistant adhesion are currently under investigation.

Poster 13

The prestalk cell inducing factor hunting in Dictyostelium discoideum

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The genome data of several Dictyosteloid species showed that they have a massive potential for the production of polyketides that are known as useful organic compounds such as antibiotics, antitumor agents and/or antifungal agents. Though Dictyostelium has more polyketide synthase (PKS) genes than any other organisms, only a few PKS genes form orthologus groups between the species. They are two hybrid type PKSs (Steely A and SteelyB), fatty acid synthases and unknown PKS genes (pks2 in D. discoideum) . This indicates that the most of the PKS genes showed the species specific expansion probably reflecting the species specific ecological challenge.

On the other hand, stalk differentiation is the essential morphological step and is conserved in Dictyosteloid species. This developmental step is reported to be regulated by polyketides. In order to find the inducer of the major prestalk cell type, prestalk A cells, we decided to focus on conserved PKS genes that are stlA, stlB and pks2.

Steely A is involved in the production of spore maturation factor MPBD and Steely B produces the backbone of the (pre) stalk inducing factor DIF-1. Unlike the single knock out mutants of stlA or stlB, the double knock out strain of stlA and stlB showed the defect in ecmA::LacZ marker gene expression. The defect of ecmA expression was not restored by adding MPBD and/or DIF-1, the products of SteelyA and SteelyB PKSs. Our results suggest that SteelyA or B seemed to be involved in the production of novel polyketide(s) that induces prestalk A cells.

Poster 14

MPBD, the polyketide of a spore maturation factor, regulates the chemotaxis in Dictyostelium discoideum

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4-methyl-5-pentylbenzene-1,3-diol (MPBD) was first identified as stalk cell inducing factor by combination of biochemical and cell biological analyses from dmtA- strain conditioned medium. This compound was shown to have not only stalk cell inducing activity but also spore cell inducing activity. In fact, we recently found that MPBD was the spore maturation factor and was the major product of SteelyA, a hybrid type polyketide synthase. The expression of the stlA gene was peaked in the vegetative stage and declined as the development proceeds. It showed a small peak in the final stage of the development when the spore maturation occurs. Polyketides have been thought to play very important roles in Dictyostelium development and so it is possible that some polyketides are responsible for the regulation of the chemotaxis.

Therefore, we focused on the function of SteelyA in the early stage of the development and found the defect. The stlA null mutant was delayed initiation of aggregation and formed small fruiting bodies compared to these of wild-type cells, Ax2. We then examined the chemotaxis of the mutant and found that this strain showed reduced chemotaxis toward cyclic adenosine monophosphate (cAMP). This defect was restored by adding MPBD during starvation for 6 h, indicating that MPBD was not only the spore maturation factor but also the regulatory factor of chemotaxis. The stlA null mutant restored the defect of chemotaxis by adding MPBD during starvation for 6 h but this 6 h treatment of MPBD did not restore the defect of spore maturation. To restore the spore maturation of stlA null mutant, it was necessary to add MPBD in the late stage of the development. These results indicate that MPBD regulates chemotaxis in the early stage and spore maturation in the late stage of the development, independently.

Poster 15

Identification of two tyrosine kinase-like (TKL) proteins that act upstream of STATc in response to hyper-osmotic stress

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Dictyostelium possesses effective response mechanisms to changes in the osmotic environment that allows it to adapt. Previously, we found that STATc (Signal Transducer and Activator of Transcription c), one of four STAT-Proteins encoded in the Dictyostelium genome, is a key regulator of the transcriptional response to high osmolarity. Under hyperosmotic conditions STATc is activated by tyrosine phosphorylation, dimerises and translocates to the nucleus where it regulates the expression of a large number of target genes (Araki et al., 2003, J Cell Sci.116, 2907; Na et al., 2007, BMC Genomics, 8, 123). In vertebrates, STAT proteins are in most cases activated by an upstream Janus kinase (JAK) upon receipt of extracellular stimuli. In Dictyostelium, however, there are no recognisable JAK orthologues. Recently, Pyk2 a tyrosine kinase-like (TKL) protein that directly phosphorylates STATc in response to DIF-1 has been identified (Araki et al., 2012, Proc. Natl. Acad. Sci. USA, in press). In the corresponding knock-out cells sorbitol induced STATc activation was still possible albeit slightly retarded. This result suggested one or more additional TKL proteins that activate STATc in response to hyper-osmotic stress.

Based on microarray results and sequence analysis we identified four tyrosine kinase-like (TKL) proteins whose differential expression depends on the presence of STATc: Pyk3 (PkyA), MORN, Pyk1 (SplA) and Phg2. We confirmed their STATc-dependent differential regulation in response to sorbitol by quantitative real-time PCR and generated KO strains for Pyk3, Phg2 and the corresponding double KO strain. Here we focus on the analysis in response to hyperosmotic conditions. Real-time PCR experiments showed that absence of pyk3 or phg2 resulted in reduced transcriptional activation of STATc and STATc-regulated genes. Using a phospho-specific STATc antibody we found significantly reduced STATc phosphorylation in the two single KO strains and a further decrease in the double KO strain. Additionally, we obtained a delayed nuclear translocation of GFP-STATc in the absence of Pyk3. Overexpression of myc-tagged Pyk3 in the AX2 wild-type strain resulted in semi-constitutive STATc activation. Furthermore, myc-tagged Pyk3 was activated by sorbitol and 8-bromo-cGMP treatment and could directly phosphorylate bacterially expressed STATc in an in vitro kinase assay. Our results show that Pyk3 is a further STATc kinase and that Pyk3 and Phg2 are both required for optimal stress-induced STATc phosphorylation. Whether Phg2 also directly activates STATc is currently under investigation.

Poster 16

Live imaging of transcription reveals distinct types of transcriptional pulse regulation

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Recent progress in imaging transcription in individual living cells has revealed gene transcription occurs in pulses or bursts. These features of transcription are conserved from bacteria to mammalian cells, but it is unclear how transcriptional pulsing is modulated for genes with different functions and expression profiles. We compared the transcriptional pulsing of different housekeeping and developmentally-induced genes, at different stages in pre-aggregative development. Different genes showed distinct transcriptional behaviour, which revealed different types of transcriptional regulation during development, and highlighted how regulatory inputs to gene regulation can reflect the nature of the gene to be transcribed.

Poster 17

The transcription factor Mef2A (SrfC) regulates cell differentiation in D. discoideum.

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Four transcription factors of the MADS-box family are encoded by D. discoideum genome, two of them are more similar to animal SRF (Serum Response Factor) factors, and the other two more similar to animal Mef2 (myocyte enhancer factor 2) factors. The participation of the two SRF-homologous proteins, SrfA and SrfB, in D. discoideum development has been studied previously in our laboratory. The structure and function of the gene coding for one of the two Mef2 homologous factors, presently named SrfC and that we propose to name Mef2A, is presented in this communication. Mef2A is expressed in vegetative cells and in the prespore region of developing structures from two alternative promoters. Developmental expression is markedly induced from 4 hours of development. The biological function of the gene has been studied through the generation of mutant strains where the gene has been partially deleted. Mutant strains showed reduced growth feeding on bacteria, but not in axenic media. These strains also showed impaired formation of slug structures and produced a reduced number of spores. Analysis of the expression of cell-type-specific markers indicated that mef2A mutant structures expressed lower levels of prespore markers and increased levels of pretalk markers. In addition, most of the spores formed by chimeric structures between AX4 and mef2A mutant cells derived from AX4 cells. These experiments also indicated that mef2A-mutant cells do not participate in the formation of the distal tip region either. Mef2A-mutant cells were not able to form spores in *in vitro* differentiation assays. Finally, mRNA-sequencing experiments showed that mef2A-mutant structures expressed increased levels of several pretalk-specific genes and lower levels of several prespore-specific genes. In summary, the results obtained indicate that Mef2A participates in the determination or differentiation of presopore cells, and a population of prestalk cells, although it is not absolutely required for these processes.

Poster 18

The NMRA/NMRAL1 homologue PadA modulates the expression of extracellular cAMP relay genes during aggregation in Dictyostelium discoideum.

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NMRA-like proteins belong to a class of transcriptional regulators that function as direct sensors of the metabolic state of the cell and link basic metabolism to changes in gene expression. PadA was the first NMRA-like protein described in Dictyostelium discoideum and was shown to have a pleiotropic phenotype and to be necessary for prestalk cell differentiation. The initial step in Dictyostelium development is the aggregation of starving cells directed by relayed pulses of extracellular cAMP to form aggregation territories. We describe and characterise padA- deficiency during the onset of development which results in the formation of abnormally small territories. Phenotypic analysis suggests that cAMP signalling is diminished in padA- and correct aggregation is restored at high cell density and in chimeras with wild type cells. Transcriptional analysis shows that cAMP-induced gene expression is downregulated in padA-, particularly the essential genes that establish the extracellular cAMP relay. The mutant phenotype can be rescued with the constitutive expression of one of these genes, carA, encoding the cAMP receptor. Analysis of the transformed strain showed that PadA is required to reach full expression of the carA aggregation promoter. Further analysis demonstrate an orthologous relationship between PadA and NMRA. Our results show a regulatory role of PadA on the carA aggregation promoter and support that PadA is the first NMRA-like protein involved in development.

Poster 19

Differential expression, antisense regulation and annotation of new genes using RNA-seq data

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Identifying genes and conditions that influence the differentiation of cells towards prestalk or prespore cells is a mayor part of Dictyostelium research. With the availability of second generation sequencing technologies large scale analyses of differentially expressed genes between, for example, prespore and prestalk as well as developed and vegetative cells has become feasible.

We isolated RNA using both the Ribo$^-$ and the polyA kit from developed wildtype and mutant cells as well as vegetative cells grown under different conditions. Sequencing was done on an ABI SOLiD platform, which produced 50 bp strand specific reads.

By mapping the reads of each sample to the Dictyostelium genome we obtained several thousand differentially expressed genes. More than 300 genes showed cell specific antisense regulation in at least one of the samples with an antisense to sense ratio of at least 70%. Assembly and mapping of transcripts enabled us to annotate so far undetected genes and non-coding RNAs in the genome.

Poster 20

How to watch retrotransposons jumping around in the Dictyostelium genome

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Transposable elements are genomic parasites, or "selfish DNA" elements, that invade cells and try to increase in copy number to ensure their own "survival" over evolutionary time. Integration of transposable elements into genomes poses a serious challenge to host fitness due to insertional mutagenesis of genes and induction of genome rearrangements. Transposable elements with unrestricted activity may be particularly harmful to cells with gene-dense genomes like that of Dictyostelium discoideum.

Intergenomic spreading of selfish DNA elements and fixation of such elements in a population occurs most efficiently in diploid, sexually reproducing organisms. In haploid organisms molecular parasites are mostly restricted to intragenomic amplification and spread less efficiently in a population. We hypothesize that in a gene-dense genome a predominantly haploid state and rare sexual reproduction could provide defense against the spread of mobile elements, thus enabling the maintenance of a "healthy" population at the cost of the loss of individual cells that were affected by insertion mutagenesis. Otherwise the amplification of transposable elements in diploid populations may lead to the rapid accumulation of heterozygous, potentially lethal mutations and an unduly genome instability that would compromise the entire population. The genome of D. discoideum accomodates retrotransposons that target the vicinity of tRNA genes, which could be interpreted as a means to avoid integrative gene disruptions. The observed "tRNA gene preference" of old integration events of retrotransposons like D. discoideum TRE5-A or DGLT-A could be a misinterpretation of purifying selection that removed cells affected by unfavorable transpositions from the population. We have recently developed genetically traceable retrotransposons that allow us to evaluate de novo integrations in the D. discoideum genome. Using this retrotransposition assay we challenge our earlier hypothesis that retrotransposon TRE5-A effectively prevents mutagenesis of host genes by displaying a strict integration preference at tRNA gene loci.

Poster 21

Tools to explore new genomes at dictyBase

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At dictyBase we continually seek to introduce new data and tools to better serve our community. We have recently launched our new multi-genome database, adding genomes for Dictyostelium fasciculatum and Polysphondylium pallidum (Heidel et al., 2011), and updating the database of Dictyostelium purpureum (Sucgang et al., 2011). Each of these genome databases contains a gene page for each predicted gene, a gene search function and a download page. In addition, two central tools, BLAST and GBrowse, have been updated to facilitate comparisons between multiple genomes.

We have added a brand new version of our genome browser (GBrowse version 2.4), in which all four genomes in dictyBase are directly accessible via a drop-down. GBrowse is versatile and customizable, allowing the user to select desired chromosomal features to display - these include: the genomic position of genes and gene models based on their chromosomal coordinates, ESTs, GenBank records, interspecies BLAST hits, and RNA sequence data. GBrowse also contains multiple search functions, navigation tools, and sequence downloads, as well as other features described in more detail in this poster.

A unified BLAST server containing all 4 Dictyostelid species available at dictyBase provides an opportunity to compare sequences between the various genomes and links out to the sequence pages of the different databases. The BLAST Server offers the choice of different BLAST programs, several datasets, and configurable parameters.

We will offer online tutorials at the poster session -bring your computer!

dictyBase is funded by NIH GM64426.

Heidel, A.J., et al. (2011) Phylogeny-wide analysis of social amoeba genomes highlights ancient origins for complex intercellular communication. Genome Res. 21, 1882â€“1891.

Sucgang, R., et al. (2011) Comparative genomics of the social amoebae Dictyostelium discoideum and Dictyostelium purpureum. Genome Biol.12, R20.

Poster 22

Mitochondrial Disease and AMP-activated Protein Kinase

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Mitochondrial disease is a disorder that arises from impaired mitochondrial function, resulting in an inability to produce sufficient ATP to meet normal cellular energy demands. Mitochondrial dysfunction is frequently caused by mutations within the nuclear genome (nDNA) and/or mitochondrial genome (mtDNA). In general nDNA abnormalities present in early childhood while mtDNA abnormalities present in late childhood or adult life. Clinical features of mitochondrial disease are diverse and the relationship between genotype and phenotype is complicated and poorly understood. Furthermore, the same genetic defect can result in different symptoms amongst various individuals whereas different mutations can lead to the same phenotype. Such diversity therefore prevents predictions of mitochondrial disease phenotypes based on genetic defects.

It has been generally accepted that the diverse cytopathologies of mitochondrial disease are caused by the depletion of ATP. To elucidate the nature of this relationship, mitochondrial disease was created in the model organism Dictyostelium discoideum via knock down of chaperon 60 (hsp60). These strains showed gene dose dependent defects in phototaxis, growth, and multicellular morphogenesis. AMPK, a highly conserved cellular energy sensor, is known to be activated under ATP depleting stressors. Under these conditions it down regulates energy consuming processes and up regulates energy producing pathways to restore normal ATP levels. Its knock down in the disease background resulted in complete rescue of the (hsp60) mutant phenotype, while overexpression of AMPK in AX2 (wt) cells phenocopied mitochondrial disease. These results indicate that the diverse cytopathologies of mitochondrial disease in D. discoideum are not caused by insufficient ATP but by chronic AMPK signalling. Microarray technology was then used to compare AX2 (wt) to the AMPK mutant strains to identify differentially expressed genes that are regulated by AMPK. These could represent potential drug targets for the treatment and cure of mitochondrial disease.

Poster 23

Studies on the mitochondrial TOM complex of the Dictyostelium

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The majority of proteins found in mitochondria are encoded in the nucleus and are synthesized in the cytosol. The efficiency of their import into mitochondria depends on the wide variety of proteins forming translocases localized in both mitochondrial membrane. One of these translocases is the TOM complex (translocase of the outer membrane). The complex is regarded as the gate into mitochondria for imported proteins as is responsible for decoding of targeting signals, translocation of imported proteins across or into the outer membrane, and their subsequent sorting. Thus, undoubtedly the TOM complex is fundamental for mitochondrial functioning.

The subunit organization of the TOM complex has been shown to be characteristic for a given phylogenetic lineage. Beside common subunits the complex may contain subunit(s) that is(are) not present in representatives of other phylogenetic lineages. Till now the complex has been described for representatives of fungi and animals (termed Opisthokonta in the recent eukaryotic classification system involving six supergroups) as well as for plants (Archaeplastida). However, for representatives of Amoebozoa, Chromalveolata, Excavata and Rhizaria (former Protista) the amount of data concerning the complex is indeed small. Therefore we decided to study the TOM complex of the slime mold Dictytiostelium discoideum classified as a representative of Amoebozoa and applied as an important model for human cell biology and disease.

By expression of his-tagged form of D.discoideum Tom7 protein and using affinity and ion-exchange chromatography we isolated a protein complex of MW about 450 kD that displays channel activity characteristic for the TOM complex. The complex was then analyzed by protein electrophoresis and mass spectrometry that allowed for identification of Tom40, a channel forming subunit of the TOM complex. Thus, we proved that the isolated complex is the TOM complex and therefore it can be used for identification of its other subunits.

Poster 24

The autophagy network in Dictyostelium: analyses of Atg1 complex

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The landmark of autophagy is the formation of double membrane vesicles called autophagosomes that engulf parts of the cytoplasm, organelles or protein aggregates. This is followed by fusion of these vesicles with lysosomes for degradation of cargo. The first proteins involved in this process were identified in yeast (named Atg for ""Autophagosome proteins"") only a decade ago and although a long way has been covered, there are still many questions awaiting further studies. We performed yeast-two-hybrid (Y2H) screening using Dictyostelium Atg proteins as baits and our results confirm some essential interactions between Atg proteins that have been described in other systems, which further validates Dictyostelium as a suitable system in autophagy. We have also identified putative interactions with proteins not previously described to have a role in autophagy that need to be confirmed by immunoprecipitation. We have focused our studies in the Atg1 complex, an essential kinase complex involved in the first stages of autophagy induction. These studies identified the putative autophagic proteins Atg13 and Atg101 in Dictyostelium. We found interactions between Atg1 and Atg13 and between Atg13 and Atg101. In order to confirm the role of these proteins in autophagy we disrupted both proteins and the resulting strains showed the characteristic phenotypes usually associated with autophagic dysfunction. The defects in autophagy are being analyzed using the markers GFP-Atg18, GFP-Atg8 and PgkA-GFP and preliminary results confirm their implication in autophagy. Atg101 is especially interesting as it is present in mammalian cells but absent in the yeast model and its function in autophagy is still poorly characterized. We will also describe the isolation of mutants for other putative Atg1 complex proteins (Atg17 and FIP200).

Poster 25

Functional Analysis of the autophagy proteins ATG8 and ATG8-like

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Macroautophagy (hereafter autophagy) is an ancient cellular pathway that is conserved from yeast to man. Autophagy contributes to many physiological and pathological processes and plays a major role in the degradation of proteins and/or organelles in response to starvation and stress. In this process cytosolic material is captured into double membrane-bound vesicles, the autophagosomes. After fusion with lysosomes, they become autophagolysosomes, the cargo is degraded and then recycled for further use.

Autophagy 8 (ATG8 in mammals LC3), undergoes a unique ubiquitin-like conjugation to phosphatidylethanolamine on the autophagic membrane, a process essential for autophagosome formation. Whereas there are at least seven ATG8 genes in human, yeast, Acanthamoeba and Neurospora each have only a single Atg8 gene. In contrast Arabidopsis, Drosophila and Dictyostelium encode two closely related paralogues. The two Dictyostelium genes encode proteins of a similar size (ATG8: 122aa, ATG8-like: 126aa) that are 54% identical. ATG9 is so far the only known integral membrane protein of the core autophagy machinery and is thought to deliver membrane lipids to the site of autophagosome formation.

To learn more about their function we investigated the Dictyostelium ATG8 and ATG8-like proteins by generating strains that ectopically express mRFP fusion proteins in the AX2 wild-type, the ATG9- strain and a strain that expresses ATG9-GFP in the ATG9- background (Tung et al., 2010, Cell. Microbiol. 12, 765). Co-immunoprecipitation studies using antibodies against ATG8, ATG8-like or RFP revealed similar interaction partners for both proteins. Immunofluorescence studies of fixed AX2 cells expressing mRFP-ATG8 or mRFP-ATG8-like showed dot-like structures of similar size and number for both proteins. Both proteins also co-localize with ubiquitin. In the ATG9- , the ubiquitin-positive dot-like structures were generally bigger, more abundant and the number of ATG8-like positive structures was higher. Live-cell studies with strains expressing ATG9-GFP as well as mRFP-ATG8 or mRFP-ATG8-like showed a very dynamic behaviour of ATG9-containing vesicles and very brief and transient co-localization with mRFP-ATG8 or mRFP-ATG8-like. Taken together our results suggest that ATG8 and ATG8-like are functional isoforms that appear to have largely overlapping functions in Dictyostelium autophagy.

Poster 26

Roles of ESCRT proteins in Dictyostelium discoideum

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The ESCRT (endosomal sorting complexes required for transport) machinery is comprised of four distinct complexes (0, I, II & III) that play a crucial role in the endosomal and autophagic pathway. Functional ESCRT's are required for efficient fusion of autophagic vesicles with the endocytic pathway and for degradation of both endosomal and autophagic vesicle cargoes. In ESCRT-deficient cells misfolded proteins are inefficiently degraded via the autophagic pathway and consequently can accumulate as aggregates within the cell. ESCRT mutations are associated with Alzheimer's, Huntington's and Parkinson's disease. These three neurodegenerative disorders are characterized by an accumulation of intracellular protein aggregates and defective autophagic compartments, raising the possibility that failure to remove damaged organelles and/or misfolded proteins is causative of the disease.

In this research we altered the expression of the ESCRT-II proteins (Vps22, 25 & 36) in order to elucidate their biological roles in Dictyostelium and to create a model for studying the cytopathological pathways resulting from ESCRT deficiency in neurodegenerative disease. We found that antisense inhibition of Vps22, 25 or 36 causes defects in growth, endocytosis and morphogenesis.

Poster 27

Response to osmotic stress: a role for the endosomal arrestin-like protein AdcA?

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The arrestin clan has recently expanded with the addition of arrestin-fold harbouring proteins - arrestin-related proteins and Vps26s - to the well characterized family of beta- and visual arrestins. Recent studies have revealed that these adaptor proteins have a convergent role in membrane protein trafficking despite differences in their mode of function and regulatory partners, providing additional levels of specificity. However, the exact roles for these novel proteins remain largely unknown. Except for the VPS26 counterparts that are involved in endosome to Golgi retrograde transport, no arrestin-like orthologs have been described in plants so far. The arrestin module is however present in the early diverging organism Dictyostelium as part of 6 adaptor proteins AdcA-F. AdcA is highly enriched on endosomes through a FYVE domain that recognizes endosomal PI3P. Our recent data indicate that AdcA phosphorylation is part of the cell response to osmotic stress.

Poster 28

Long-Term Single Cell Resolution Timelapse Microscopy of Dictyostelium co-cultured with Mycobacteria

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The Dictyostelium discoideum-Mycobacterium marinum duo has been shown to be a relevant model to study host-pathogen interactions with recent discoveries of new host defense mechanisms during infection (Hagedorn et al, Science 2009). Microscopic studies of these interactions have currently been limited to short term events due to the difficulty to follow the same cells for an extended period of time because of the rapid movements of Dictyostelium. There was therefore a need for a platform able to trap cells while allowing high-resolution imaging and culturing media perfusion. We solved this problem by designing a microfluidic device made of thousands of micro-scale chemostat. This system has been successfully developed and used to image the very same cells for several days at high resolution, enabling to follow the amoebae cell divisions and motility. The imaging time was only limited to the filling of the cultivation chamber with cells. We have also performed timelapse studies on long-term interactions between M. marinum and Dictyostelium. Using a motorized stage microscope the device allows to multiplex the number of chemostat imaged and therefore to capture the population heterogeneity in a single experiment. Furthermore the simplicity of fabrication and its use will enable its spreading to other laboratories for use with any cell type. This imaging platform is currently being used to study the pathogen´s cell cycle during infection. One of the most basic processes concerns cell division, which is tricky to study in mycobacteria as the septum constriction is not visible due to their thick cell wall. We have therefore tagged the septum with a GFP. Analysis of this strain alone at the single cell level already shows the population heterogeneity of interdivision times and cells length at birth and division.

Poster 29

Micropatterned Surfaces for the Control of Localization of Dictyostelium discoideum

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Cell-substrate dependant polarization, directional movement, and adhesion are crucial for many physiological processes, including embryonic development, wound healing, and functional immune and neural systems. The aim of this study is to gain a better understanding of these complex processes of cellular adhesion and directional movement through the engineering of defined local adhesion characteristics at a micrometric scale. The social amoeba Dictyostelium discoideum was used as the model organism. Since Dictyostelium are capable of adhering to various surfaces regardless of the presence of extracellular matrix proteins, the selective adhesion patterns were engineered utilizing the amoeba´s inability to spread on inert surfaces, specifically the block copolymer of polyethylene glycol and polypropylene oxide (Pluronic). Using appropriate surface chemistry, micropatterned surfaces of glass and Pluronic F-127 were created. The patterned glass surfaces were then used to observe the interaction of Dictyostelium with the inert Pluronic surface. In an assay of randomly moving Dictyostelium, the amoebas fail to move onto the Pluronic surface. Most notably, we found that cells chemotaxing up a gradient of the chemoattractant cAMP develop a head-to-tail streaming morphology that is lost when they encounter the nonadhesive Pluronic surface. Ultimately, we conclude a presence of a cellular mechanism by which Dictyostelium sense the adhesive or nonadhesive nature of their surroundings and direct their movements accordingly. These findings overall show how our novel use of micropatterned surfaces in the study of chemotaxis in Dictyostelium can help shed further light on the machinery powering this vital process.

Poster 30

Subtype specific function of flotillin-like vacuolins during Mycobacterium marinum infection

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Tuberculosis is an infectious disease caused by the strictly human pathogen Mycobacterium tuberculosis. The bacteria are transmitted via aerosols and taken up by alveolar macrophages. Instead of being killed by phagosomal maturation pathogenic mycobacteria circumvent their digestion and remodel their phagosome into a replication vacuole.

Flotillin-like proteins, namely vacuolinB, have been shown to be essential for mycobacteria-induced phagosomal maturation arrest (Hagedorn et al, 2007). Very little is known about the underlying molecular mechanisms and we propose that mycobacteria target host cell flotllins. Raft associated flotillins are well conserved throughout all species, including Dictyostelium discoideum where there are three isoforms, vacuolinA, B and C.

To dissect the role of vacuolins at the mycobacterial compartment in detail we simulated the tuberculosis infection using D. discoideum as a surrogate macrophage combined with M. marinum, a close relative of M. tuberculosis.

Via quantitative real-time PCR analyzes we showed that the mycobacteria infection leads to a transient, pathogen and subtype specific increase in vacuolin mRNA abundance. Strikingly, even though the vacuolin isoforms share a high amino acid identity their recruitment to the mycobacterial compartment occurs in a distinct manner. At the early stage of the infection (3hpi) vacuolinC strongly accumulates at the mycobacterial compartment. In contrast, we find that vacuolinA barely decorates the compartment at any time while vacuolinB is found at the vacuole in a diverse distribution ranging from a strong accumulation to none. The recruitment of vacuolin to the mycobacterial replication niche indicates a conserved function in the infection. Further investigation of the isoform-specific role of vacuolin will improve our comprehension of the biogenesis of the mycobacterial replication compartment.

Poster 31

Detailed Mapping of Phenotypic Evolution in Social Amoebas Highlights Developmental Plasticity as a Consequence of Colonial Multicellularity

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During 600 Myr of evolution, the Dictyostelids have evolved many different aggregate shapes and fruiting body architectures, which are used as species diagnostics. In order to elucidate the evolution of multicellular structures in the Dictyostelids, we have performed analysis of 30 phenotypic and behavioural characters in 99 species. We resolved conflicts between existing single gene phylogenies with a novel 18 gene core phylogeny, and are now able to resolve the root of the Dictyostelids. The novel phylogeny shows subdivision of all Dictyostelia into two branches, each containing two major groups. Using phylogeny-based statistical methods, we show that the last common ancestor (LCA) of all Dictyostelia formed comparatively small fruiting structures. It used secreted cAMP to coordinate fruiting body morphogenesis and a different compound to mediate aggregation and was capable of forming microcysts. This phenotype persisted up to the LCAs of three of the four major groups. The group 4 LCA additionally employed cAMP to mediate aggregation and formed much larger fruiting structures. However, it lost encystation, the survival strategy of solitary Amoebozoa that is retained by many species in groups 1-3. Large structures, phototropism and light-oriented migration of sorogens co-evolved within most groups. Overall, Dictyostelids show considerable plasticity in the size and shape of their multicellular structures, both within and between species. This likely reflects constraints placed by colonial life on developmental control mechanisms, which, depending on local cell density, need to direct from ten up to a million cells into forming a functional fruiting structure.

Poster 32

Pathways to social success in D. discoideum

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I use a combination of mutagenesis and directed evolution to elucidate different developmental pathways important for mediating social outcomes across a set of natural isolates of D. discoideum. Understanding the genetic networks underlying these pathways could indicate mechanisms that limit the evolution of 'cheater' strategies whose emergence would lead to the collapse of cooperative systems.

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