

Dicty 2023 Annual International Dictyostelium Conference

27-31 August 2023, Opatija, Croatia

Book of Abstracts



Welcome from the organizers

The International Dictyostelium meeting 2023 is taking place in the town of Opatija, at the Croatian Adriatic coast. The Dictyostelium conference series is thereby returning to the Mediterranean: it started in 1977 with the meeting in Sardinia.

The meeting is taking place at the Conference Park Amadria, starting with a keynote lecture on the evening of Sunday 27th August, and finishing after breakfast on Thursday 31th.

In keeping with tradition, we have invited two Keynote speakers outside of the Dictyostelium research field and are pleased to announce talks by Iva Tolić and Tomislav Domazet-Lošo from the Ruđer Bošković Institute in Zagreb. All other talks have been selected from early submitted abstracts by the International Scientific Board.

We look forward to a pleasant and productive meeting. See you in Opatija!

Conference co-chairs,
Vedrana Filić and Igor Weber

Local organizing Committee

Igor Weber, Ruđer Bošković Institute, Co-chair
Vedrana Filić, Ruđer Bošković Institute, Co-chair
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Vedrana Filić, Ruđer Bošković Institute

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Dictyostelium discoideum fruiting bodies at various stages of development. False-color fluorescence image, focus stack composite. Photo: Jeff Smith

Conference is organized by:



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TIME SLOTS	SUNDAY	TIME SLOTS	MONDAY	TUESDAY	WEDNESDAY	THURSDAY
7:30 - 9:00	Arrivals	7:30 - 9:00	Breakfast	Breakfast	Breakfast	Breakfast
9:00 - 10:40		9:00 - 10:40	Morning 1 (L1-L4)	Morning 5 (L11-L14)	Morning 9 (L21-L24)	Departures
10:40 - 11:10		10:40 - 11:15	Break	Break	Break	
11:10-12:30		11:15-12:30	Morning 2 (L5-L7)	Morning 6 (L15-L17)	Morning 10 (L25-L27)	
13:00 - 14:00		12:30 - 14:15	Lunch	Lunch	Lunch	
14:00 - 15:20		14:15 - 15:30	Afternoon 3 (L8-L10)	Afternoon 7 (L18-L20)	14:15 - 15:30 Keynote 2	
16:30 - 18:00	Registration	15:30 - 16:00	Break	Break	15:30 - 17:00 Opatija walking tour	
18:00 - 19:15	Opening and Keynote 1	16:00 - 17:00	Afternoon 4 (F1-F7)	Afternoon 8 (F8-F14)	Free time	
		17:00 - 18:00	Dicty Community Resource presentation	Poster Session (P1-P14)		
19:30	Welcome reception	18:00-20:00	Free time	Free time	19:15 Bus departure for Conference Dinner	
		20:00	*Joint dinner		19:30 Conference Dinner	

TALK = 20+5 minutes

FLASH TALK = 5 minutes

*JOINT DINNER = not included in registration fee

Programme

Sunday, August 27

15:30 Registration

18:00 Opening and keynote lecture 1
Iva Tolić (Croatia)
The quest for perfection: spindle assembly and mitotic (in)fidelity in healthy and cancer cells

19:30 Welcome reception

Monday, August 28

Bacterial interactions
Chair: Monica Hagedorn

9:00 **Monica Hagedorn** (Germany)
Recruitment of both the ESCRT and autophagic machineries to ejecting *Mycobacterium marinum*

9:25 **Joseph Oddy** (Switzerland)
Discovering new effectors of bacterial killing in *Dictyostelium*: CPRD

9:50 **Jordi van Gestel** (Germany)
Putative mutational hotspot underlies predation defense in *Bacillus subtilis* against *Dictyostelium discoideum*

10:15 **Davide D'Amico** (Switzerland)
Unraveling function and ligands of *Dictyostelium discoideum* lectins during *Mycobacterium marinum* infection

10:40 Break

Development/collective behavior
Chair: Jordi van Gestel

11:15 **Jan Faix** (Germany)
Cell sorting and development are controlled by the Ras-regulated bundling protein ForC

11:40 **Allyson Sgro** (USA)
Understanding the emergence of microbial collective behaviors

12:05 **Hugh Ford** (UK)
Cells swarm as an active droplet

12:30 Lunch

Motility

Chair: Xuehua Xu

- 14:15 **Jinqiang Yu** (China)
Amoeboid jumping: a novel evolutionary conserved migration mode
- 14:40 **Annette Müller-Taubenberger** (Germany)
Adaptive pathfinding by nucleokinesis in migrating *Dictyostelium* amoebae
- 15:05 **Marko Šoštar** (Croatia)
Oscillatory dynamics of Rac1 in *Dictyostelium* motility: an integrated theoretical and experimental study
- 15:30 Break

Flash Talks

Chair: Huaqing Cai

- 16:00 **Kazutoshi Takebayashi** (Japan)
Field model for multistate lateral diffusion of various transmembrane proteins observed in living cells
- Anja Privara** (Croatia)
The emerging role of *Dictyostelium discoideum* IQGAP-related protein IqgD in large-scale endocytosis
- Matthew Wielgat** (USA)
Harnessing dictyostelids to combat *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm infections
- Antonia Ramos Guzmán** (Chile)
DNA extracellular trap production in response to *Klebsiella pneumoniae* and its lipopolysaccharide by vegetative *D. discoideum* cells
- Lyudmil Raykov** (Switzerland)
A TRAF-like E3 ubiquitin ligase TrafE coordinates ESCRT and autophagy in endolysosomal damage response and cell-autonomous immunity to *Mycobacterium marinum*
- Nina Sey Sey Ng** (Japan)
Chemical communications that exist between dictyostelids and the free-living nematode *C. elegans*
- Kana Hayashi** (Japan)
Nematode repellent active substance derived from *Dictyostelium discoideum*

- 17:00 **Petra Fey & Siddhartha Basu**
Dicty community resource presentation

- 18:00 Free time

- 20:00 Joint dinner

Tuesday, August 29

Cell Biology A

Chair: Davide D'Amico

- 9:00 **Darija Putar** (Croatia)
Small GTPases RasG and Rab5A regulate IqgC localization during macropinocytosis
- 9:25 **Huaqing Cai** (China)
Regulation of macropinocytosis in response to changing nutrient conditions
- 9:50 **Robert Huber** (Canada)
Mechanisms regulating the trafficking of Cln5 and cathepsin D in *Dictyostelium discoideum*
- 10:15 **Tian Jin** (USA)
How *Dictyostelium* amoebae recognize and hunt bacteria as food
- 10:40 Break

Chemotaxis

Chair: Tian Jin

- 11:15 **Richard Gomer** (USA)
An endogenous chemorepellent inhibits Ras signaling to bias movement of *Dictyostelium* cells
- 11:40 **Peter van Haastert** (The Netherlands)
A complete cGMP signaling complex associated with G_{βγ} mediates both local and global information for directed cell movement
- 12:05 **Satomi Matsuoka** (Japan)
Sphingomyelin metabolism underlies Ras excitability for efficient cell migration and chemotaxis
- 12:30 Lunch

Ecology

Chair: Catherine Pears

- 14:15 **Sandrine Adiba** (France)
Single-cell phenotypic plasticity modulates social behavior in *Dictyostelium discoideum*
- 14:40 **Tsuyoshi Araki** (Japan)
Chemical communications between dictyostelids and soil nematodes
- 15:05 **Imen Ayadi** (Switzerland)
Predator-prey relationships between *D. discoideum* and *P. aeruginosa*
- 15:30 Break

Flash Talks

Chair: Marko Šoštar

- 16:00 **Yihong Yang** (China)
GxcM-Fbp17/RacC-WASP signaling regulates polarized cortex assembly in migrating cells via Arp2/3
- Vlatka Antolovic** (UK)
Collective signalling drives rapid jumping between cell states
- Kateryna Maslova** (UK)
Investigating therapeutic mechanisms and targets of non-psychotropic cannabinoids in the model system *Dictyostelium discoideum*
- Masaki Muromoto** (Japan)
 β -arrestin orchestrates the extension of the concentration ranges in eukaryotic chemotaxis
- Tetsuya Muramoto** (Japan)
The bioresource project of cellular slime molds in Japan (NBRP Nenkin)
- Pundrik Jaiswal** (USA)
Metabolic changes during the development of *Dictyostelium*
- Koji Iwamoto** (Japan)
RasGEF B and X break the symmetry of Ras activation to drive spontaneous cell migration

17:00 Poster Session

18:00 Free time

Wednesday, August 30

Cell Biology B

Chair: Robin S.B. Williams

- 9:00 **Ludwig Eichinger** (Germany)
Proteasomes of autophagy-deficient cells exhibit alterations in regulatory proteins and a marked reduction in activity
- 9:25 **Yousef Alhammad** (UK)
Unveiling the identity of *Dictyostelium discoideum* peroxisomes. A walking plant or talking amoeba?
- 9:50 **Hui Tu** (China)
The PripA-TbcrA complex-centered Rab GAP cascade facilitates macropinosome maturation in *Dictyostelium*

10:15 **Xuehua Xu** (USA)
C2GAP2 is a common regulator of Ras signaling for chemotaxis, phagocytosis,
and macropinocytosis

10:40 Break

Signalling and gene expression
Chair: Jonathan Chubb

11:15 **Christian Hammann** (Germany)
Distinct mechanisms keep Skipper-1 under control

11:40 **Robin S.B. Williams** (UK)
Investigating molecular mechanisms of the Chinese herbal remedy, tanshinone
2A, in *Dictyostelium* identifies a new approach to treat brain cancer
(glioblastoma multiforme)

12:05 **Bart Edelbroek** (Sweden)
Evolution of microRNAs in Amoebozoa

12:30 Lunch

14:15 Keynote lecture 2
Tomislav Domazet-Lošo (Croatia)
Evolution of complexity: gene family gain and loss in eukaryotes

15:30 Opatija walking tour

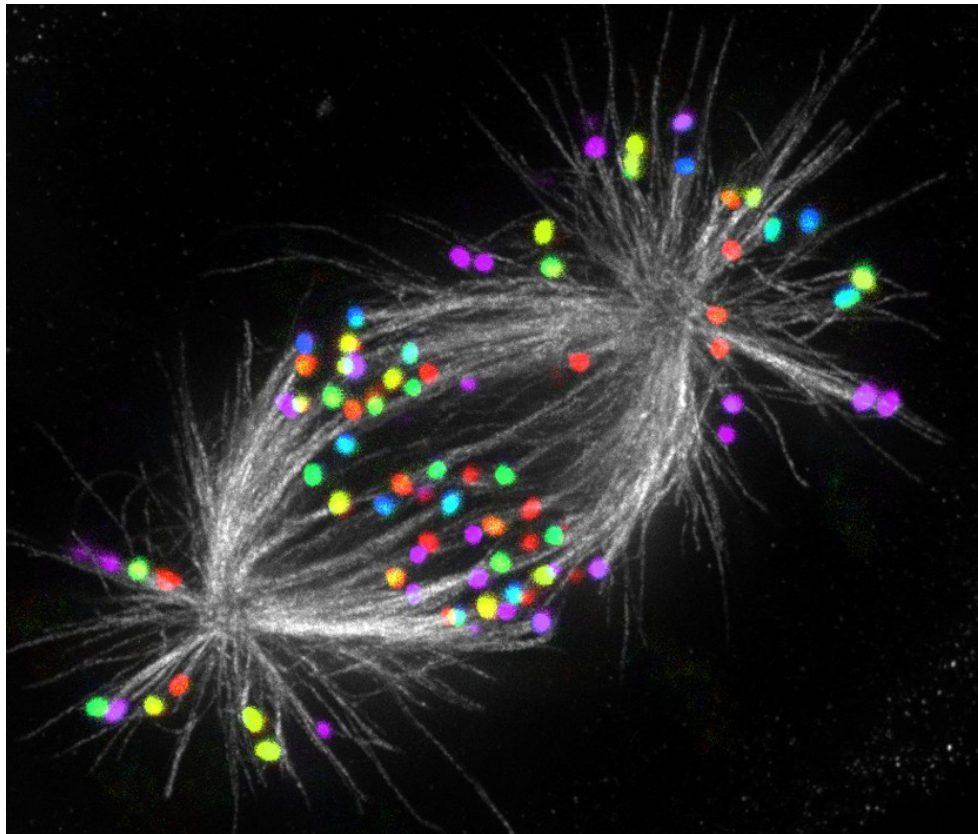
17:00 Free time

19:15 Bus departure for conference dinner

19:30 Conference dinner

K1**The quest for perfection: spindle assembly and mitotic (in)fidelity in healthy and cancer cells**Iva Tolić¹¹*Ruđer Bošković Institute, Zagreb, Croatia*

Segregation of the genome from a mother cell into two daughter cells during cell division is at the heart of life. Physical separation of chromosomes is carried out by the spindle, a fascinating molecular assembly composed of microtubules and numerous other proteins. The main task of the spindle is to accurately segregate the chromosomes, but sometimes errors occur that result in mis-segregation of one or more chromosomes, especially in chromosomally unstable cancer cells. Are all chromosomes at equal risk of mis-segregation? What are the typical types of segregation errors in cancer cells? How can these errors be prevented or corrected? I will discuss our efforts to answer these questions and understand spindle mechanobiology, focusing on microtubule forces that control chromosome movements and their role in ensuring chromosome segregation fidelity.



K2**Evolution of complexity: gene family gain and loss in eukaryotes**

Mirjana Domazet-Lošo¹, Tin Široki¹, Korina Šimičević¹, Tomislav Domazet-Lošo^{2,3}

¹*Department of Applied Computing, Faculty of Electrical Engineering and Computing, University of Zagreb, Unska 3, HR-10000 Zagreb, Croatia,* ²*Laboratory of Evolutionary Genetics, Ruđer Bošković Institute, Bijenička cesta 54, HR-10000 Zagreb, Croatia,* ³*School of Medicine, Catholic University of Croatia, Ilica 242, HR-10000 Zagreb, Croatia*

The evolutionary dynamics of gene gain-and-loss has been recently explored in animals, and to lesser extent in fungi and plants. However, these studies lack the full phylogenetic depth and do not cover some crucial evolutionary periods including the origin of cellular organisms, archaeal divergence and eukaryogenesis. To address this gap, we explored gene family gain-and-loss patterns in animals, plants and fungi by covering the full phylogenetic trajectory from the ancestor of all cellular organism to the four focal species. We reconstructed the gene family content of ancestral genomes and overlaid it with functional data, which surprisingly precisely uncovered macroevolutionary innovations and reductions for thousands of phenotypes.

We found that in our focal lineages gene family diversity shows a common pattern, where the most complex genomes appear on the brink of major evolutionary radiations. This peak in gene family diversity is followed by a strong reduction phase that underpins evolutionary radiation itself. Our profiles suggest that, during the most of Phanerozoic, including the present time, animals, fungi and plants are in the reductive mode of gene family evolution. We proposed a link between this genomic regularity and the increasing ecological specializations that is established via "functional outsourcing".

Our results are the first empirical support for the current theoretical models of genome complexity evolution, which predict that genome complexification precedes a protracted phase of genome simplification. In addition, we suggest that the poor correlation between genome and organismal complexity could be alleviated if biological interactions are considered as a missing parameter.

L1**Recruitment of both the ESCRT and autophagic machineries to ejecting *Mycobacterium marinum***

Monica Hagedorn¹, Christian Hammann¹, Lilli Gerstenmaier², Ombretta Colasanti², Hannah Behrens², Margot Kolonko²

¹Health and Medical University, Potsdam, Germany, ²Bernhard-Noch-Institute, Hamburg, Germany

The human pathogen *Mycobacterium tuberculosis* remains responsible for over 1.5 million deaths each year. To advance our understanding of the basic interactions between pathogenic mycobacteria and their phagocytic host cell, we combine *M. marinum* with the professional phagocyte *Dictyostelium discoideum*. Whilst uptake of mycobacteria by their host cell is well investigated little is known about how bacteria exit and spread the infection. It has been previously described that cytosolic *M. marinum* are ejected from the amoeba *Dictyostelium discoideum* in a non-lytic fashion. During this process the autophagic machinery is recruited to the bacteria and supports host cell integrity during egress. Here, we show that the ESCRT machinery is also recruited to ejecting bacteria, partially dependent on an intact autophagic pathway. As such, the AAA-ATPase Vps4 shows a distinct localization at the ejectosome structure in comparison to fluorescently tagged Vps32, Tsg101 and Alix. Along the bacterium engaged in ejection, ESCRT and the autophagic component Atg8 show partial colocalization. We hypothesize that both, the ESCRT and autophagic machinery localize to the bacterium as part of a membrane damage response, as well as part of a “frustrated autophagosome” that is unable to engulf the ejecting bacterium.

L2

Discovering new effectors of bacterial killing in *Dictyostelium*: CPRD

Joseph Oddy¹

¹*Université de Genève, Switzerland*

The main aim of the Cosson lab is to discover and characterize gene products in *D. discoideum* responsible for the killing of bacteria. We mostly carry out these investigations using biochemical and genetic methods. Many results point to an important role for proteases. Mutants like Kil2 KO, Far1 KO, LrrkA KO, have decreased protease activity in phagosomes and kill less efficiently. These proteins are not proteases, instead, they regulate proteases in phagosomes. There are many proteases in phagosomes, but we don't know which ones are important for killing, or how specific they are for killing each bacteria. We have tested a few proteases and found one, CPRD, to be necessary for efficient killing of *K. pneumoniae* in a Kil2-dependent manner and for efficient protease activity in a Kil2-independent manner. As yet, other proteases have not been found to be necessary for efficient killing of *K. pneumoniae*. This is the first time we have identified a protease involved in the killing of bacteria, and implies protein degradation is important for bacteria killing.

L3**Putative mutational hotspot underlies predation defense in *Bacillus subtilis* against *Dictyostelium discoideum***Jordi van Gestel¹¹EMBL

Protozoan predation forms one of the major selection pressures in natural microbial ecosystems, driving the evolution of both predator and prey. In our lab, we study how protozoan predators and bacterial prey coevolve in the soil and, as model, examine the interaction between the soil-dwelling protozoan *Dictyostelium discoideum* and gram-positive bacterium *Bacillus subtilis*. In this study, we combined transposon screens and experimental evolution to identify a wide range of predation defense mechanisms in *B. subtilis*, which either provide complete or partial resistance against predation by *D. discoideum*. We show that many of these defenses carry strong fitness costs in the absence of predation, resulting in antagonistic selection pressures. Consistently, we identified a putative mutational hotspot underlying the transcriptional regulator, *slrC*, which controls several predation defenses. This hotspot allows for a switch-like activation of defenses (i.e., phase variation), thereby facilitating survival in the presence of predation while simultaneously minimizing the costs in its absence. We anticipate that mutational hotspots might commonly be found in microbial predator-prey coevolution.

L4**Unraveling function and ligands of *Dictyostelium discoideum* lectins during *Mycobacterium marinum* infection**

Davide D'Amico¹, Ana Teresa Lopez-Jiménez¹, Giselle Hevia Hernandez², Stephan von Gunten², Thierry Soldati¹

¹Faculty of Science, Department of Biochemistry, University of Geneva, Switzerland, ²Faculty of Medicine, Institute of Pharmacology, University of Bern; Switzerland

Lectins play a fundamental role in host-pathogen interactions because lectin-glycan binding is often the first molecular event when host and pathogen meet. Mammalian cells express a family of cytosolic and secreted lectins called galectins with affinities for β -galactosides, that participate in both self and non-self-glycoconjugate recognition. The social amoeba *Dictyostelium discoideum* expresses four canonical discoidins (Dsc), lectins that share molecular and biological characteristics with galectins. Upon infection with *Mycobacterium marinum*, Dsc-GFP foci form in the vicinity of the mycobacterium containing vacuole (MCV) in a damage-dependent manner, but it is unclear whether Dsc recognise self-glycans exposed by pathogen-induced membrane rupture, or bacterial surface glycosylations made accessible upon vacuole damage. Strikingly, membrane damage by chemicals or osmotic stress is not sufficient to stimulate the formation of Dsc foci. On the other hand, *in vitro* assays performed by flow-cytometry and validated by immunofluorescence, showed that chemically-labelled Dsc isoforms (DscI and DscII) bind *M. marinum* and other species of bacteria. The specificity of binding of purified Dsc was determined by probing an expanded glycan array. In mammalian cells galectins are fundamental for bacterial restriction by autophagy and their absence promotes intracellular growth. Surprisingly, the knock-out of all Dsc isoforms in *D. discoideum* increased the resistance to *M. marinum* intracellular growth and restriction was not restored by re-expression of any single Dsc isoform. We are currently investigating which glycosylated Dsc ligands are recognised in the mycobacteria cell wall.

L5**Cell sorting and development are controlled by the Ras-regulated bundling protein ForC**Jan Faix¹¹*Institute for Biophysical Chemistry, Hannover Medical School, Germany*

Development of *D. discoideum* is a multifaceted process in which coordinated cell movement, differentiation, and patterning are regulated by intracellular signals. Starvation initiates a developmental programme, and by about 9 h, the initially indistinguishable amoebae have gathered by chemotaxis towards cAMP into compact mounds in which the two major types of progenitor cells, denoted prespore and prestalk cells, are initially scattered as they form randomly. This differentiation process, followed by cell sorting of the two cell types, is associated with a large-scale rotational flow of the mound (resembling a vortex), with the pre-stalk cells moving into the center. After cell sorting, the collectives develop into migratory slugs that subsequently culminate to form a fruiting body. We have previously characterized the Ras-regulated FHOD1-like formin ForC from *Dictyostelium* cells. ForC is an important regulator of morphogenesis since ForC-null cells are severely impaired in development resulting in the formation of aberrant aggregates with very few spores. ForC is absent during growth, but becomes maximally expressed in prespore cells during the stage of cell sorting in the mounds. Time-lapse imaging revealed that mound rotation in ForC-null mutants is almost entirely abolished strongly suggesting that cell sorting is largely blocked. Notably, unlike most other Diaphanous-related formins, recombinant ForC does not nucleate actin polymerization or promote filament elongation, but crosslinks actin filaments into bundles of mixed polarity. Moreover, it accumulates in the leading edge. According to a long-standing hypothesis, cell sorting is believed to be driven by differential adhesion and selective chemotaxis of the different cell types. However, based on our findings we propose an additional mechanism in which the mechanical properties of prespore cells are selectively changed by expression of an actin-bundling protein in the leading edge. This is expected to specifically reduce cell migration of this cell type and allow sorting within the mound.

L6**Understanding the emergence of microbial collective behaviors**Emily Hager^{1, 2}, Allyson Sgro²¹*Boston University*, ²*HHMI Janelia Research Campus, USA*

Groups of cells of all kinds work together and make decisions to form patterns and generate large-scale behaviors. These behaviors are coordinated via information cells can sense locally, and this information is shaped by the complex and heterogeneous environments the cells exist in. To better understand how population-scale coordination is shaped by these local environments and what properties of single cells allow for robust coordination of large-scale behaviors, we focus the cellular slime mold *Dictyostelium discoideum*, which during starvation uses a biochemical environmental signal to coordinate aggregation into multicellular groups for continued survival. Existing models capture how on flat, homogenous substrates this signal relay produces emergent, population-wide spiral waves that lead cells to aggregate and develop into a fruiting body structure. However, this behavior originally evolved in complex, three dimensional environments like soil. We have designed a naturalistic environmental soil model system where we can quantify how cellular changes such as number, density, and genotype, as well as environmental features such as particle size perturb signaling and collective outcomes. Our current findings suggest that the single-cell and population-wide signaling behaviors that coordinate development are robust in highly complex, three-dimensional environments, but that there are also other important cellular properties we do not yet fully understand required for success in nature.

L7**Cells swarm as an active droplet**

Hugh Ford¹, Jonathan Chubb¹

¹*University College London, UK*

Cells tend to be surrounded by kin and so swarm as they crawl in response to external signals. Although cell swarming is prevalent in nature, it is unclear how coherent collective motion emerges when crawling forces are mostly exerted on neighbouring cells. We address this problem by studying *Dictyostelium* feeding fronts, where large numbers of cells self-organise as a large swarm as they chemotax towards and consume bacteria. Combining light-sheet microscopy, 3D image analysis and mathematical modelling, we developed and experimentally tested an active fluid model of cell swarming. Both data and model reveal that, like a droplet, surface tension maintains swarm structure as it travels via a self-generated gradient and, in response to a flat signal gradient, drives cell deposition in large groups. The motion of active droplets and cell swarms are characterised by a vortex emerging from the local transfer of propulsion forces. The data also reveal that the cell vortex results from a transition in cell transport from advective to diffusive transport across swarm depth, and also drives the redistribution of the external signal, concentrating it at the leading edge of the swarm, thereby sharpening the chemoattractant gradient. Our results identify the active fluid dynamic principles of directed collective cell motion.

L8**Amoeboid jumping: a novel evolutionary conserved migration mode**

Jinqiang Yu¹, Hongyu Wang², Huaqing Cai³, Li Yu¹

¹The State Key Laboratory of Membrane Biology, Tsinghua University-Peking University Joint Center for Life Sciences, School of Life Sciences, Tsinghua University, Beijing 100084, China, ²MOE Key Laboratory of Model Animal for Disease Study and State Key Laboratory of Pharmaceutical Biotechnology, Model Animal Research Center, Nanjing University, Pukou District, Nanjing, China, ³National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

Cell migration plays important roles during many cellular processes, such as predation, immune defense and metastasis. Both the extracellular environment and the cytoskeleton play important roles in cell migration. For the *Dictyostelium discoideum*, which lives in soil and prey on bacteria, the physical properties of the surface they live on may have a profound impact on their physiology. We found that dicty initiated a novel migration mode upon exposure to a positively charged surface. Firstly, PI(4,5)P₂, F-actin and myosin II formed a ring at the edge of the adherent cell and started to contract, which is similar to the contraction process during cytokinesis. *Pik1*⁻, *mhcA*⁻ or *racE*⁻ mutants cannot form this contraction ring. Next, the cells re-polarized on the z-axis, which make them look like “standing up”. Finally, a scission was made at the contraction site when the contraction ring became small enough. After that, the cells were able to migrate forward and the shed membrane became vesicles called migrasomes. We named this novel migration behavior “jumping mode”. Interestingly, neutrophils also have “jumping mode” on fibronectin coated surface. Further studies showed that migrasomes were highly enriched with negatively charged molecules and adhesion receptors. By shedding migrasomes, cells successfully adjusted adhesion force, reset their adhesion properties and finally restored amoeboid migration. Mechanistically, this process is an asymmetry cytokinesis, driven by re-purposing of the cytokinesis machinery for cellular migration and selective membrane shedding. This adaptation strategy is vital for both *Dictyostelium discoideum* and neutrophils: for mutants that cannot form the contraction ring and shed migrasomes, the cells stuck to the ground and therefore failed to prey on bacteria freely. Our study found that in response to a change of surface property, amoeboid cells such as *Dictyostelium discoideum* and neutrophils can mount an evolutionary conserved program to adapt to the new environment.

L9**Adaptive pathfinding by nucleokinesis in migrating *Dictyostelium* amoebae**

Janina Kroll¹, Hellen Ishikawa-Ankerhold², Jörg Renkawitz¹, Annette Müller-Taubenberger³

¹Biomedical Center Munich (BMC), Department of Cardiovascular Physiology and Pathophysiology, Walter-Brendel-Centre of Experimental Medicine, University Hospital, Faculty of Medicine, LMU Munich, 82152 Planegg-Martinsried, Germany, ²Department of Internal Medicine I, University Hospital, and Walter-Brendel-Centre of Experimental Medicine, University Hospital, Faculty of Medicine, LMU Munich, 81377 Munich, Germany, ³Biomedical Center Munich (BMC), Department of Cell Biology (Anatomy III), Faculty of Medicine, LMU Munich, 82152 Planegg-Martinsried, Germany

The ability of cells to efficiently migrate through complex microenvironments plays an important role for many processes in cell biology including development, immune responses, and cancer metastasis. During these processes, cells navigate through environments that are composed of heterogenous mechanochemical signals and encounter diverse mechanochemical cues at the same time, raising the question how cells respond to locally diverse and potentially competing guidance cues.

We investigated how fast migrating cells, mammalian immune cells and *Dictyostelium* amoebae, respond to locally competing guidance cues, namely mechanical pore size and chemokine cues, and discovered that motile cells efficiently adapt their path to the dominating guidance signal. This path adaption requires reorientation of large cytoplasmic parts, including the nucleus, to re-adjust the cytoplasmic content to the selected path along the dominating guidance signal. Our previous investigations of *Dictyostelium* cells as a model for amoeboid movement showed that the centrosome is preferentially located rearward of the nucleus in different 2D and 3D environments used to explore directed migration, while the nucleus is oriented toward the expanding front. It is well accepted that actin polymerization is the driving force at the cell front, while the microtubule network was thought to play at most a regulatory function in coordinating front protrusion and retraction of the rear. We now provide evidence that adaptive pathfinding along heterogenous guidance cues requires rapid and long-range nuclear repositioning, which is driven by myosin-IIA forces and is accompanied by two consecutive cell polarity switches. Motile amoeboid cells actively move and position their nuclei during pathfinding, and this 'amoeboid nucleokinesis' is independent of microtubules but requires forces of the actomyosin cytoskeleton. In summary, our comparative studies revealed that the cellular principles of amoeboid migration and mechanisms of adaptive pathfinding during cellular navigation are conserved from single cell amoebae to mammalian immune cells.

L10**Oscillatory dynamics of Rac1 in *Dictyostelium* motility: an integrated theoretical and experimental study**

Marko Šoštar¹, Vedrana Filić¹, Nenad Pavin², Igor Weber¹

¹*Ruđer Bošković Institute*, ²*University of Zagreb, Croatia*

Rho GTPases play a key role in controlling numerous actin cytoskeleton-driven processes. Our focus lies in exploring their influence on cell motility. *Dictyostelium* amoebas, known for exceptional reorganization speed of their actin network and ability to reverse polarity within a minute, serve as our model organism. These attributes make them an ideal system for examining theoretical models linking Rho signalling to cellular morphodynamics during motility. In our research, we adopt a dual theoretical and experimental approach to probe the intracellular dynamics of the small GTPase Rac1 and its effector DGAP1. Monitoring the proteins that shape and regulate the quickly remodelling cytoskeleton of *Dictyostelium* cells is essential. To achieve this, we utilize fluorescent biosensors, which are adept at tracking these swift dynamics and can withstand extended periods of high-frequency imaging. We have devised a fluorescent probe specifically designed for the activated form of Rac1. It generates minimal cytoplasmic background, allowing us to discern subtle fluctuations in Rac1 activity in the cellular cortex. We used point-scanning confocal microscopy to record spatio-temporal distributions of the fluorescently labelled active Rac1 and DGAP1 in living cells. The data were then processed and subjected to principle component analysis. We identified three predominant patterns in the dynamics of monitored probes: standing waves, travelling waves, and persistently polarized states. Alongside these, we found that Rac1 activity and DGAP1 dynamics mostly displayed anti-correlated behaviour, barring occasional stationary patterns that showed overlapping distributions of the two proteins. We explore various mathematical modelling methods to depict the observed dynamics and juxtapose the outcomes of a reaction-diffusion model with the experimentally derived distributions of Rac1 and DGAP1. Notably, our reaction-diffusion model employing straightforward mass action kinetics was successful in mimicking almost all observed dynamical patterns.

L11**Small GTPases RasG and Rab5A regulate IqgC localization during macropinocytosis**

Darija Putar¹, Anja Privara¹, Xiaoting Chao^{2,3}, Marko Šoštar¹, Tamara Čutić¹, Lucija Mijanović¹, Ana Smolko¹, Hui Tu^{2,3}, Pierre Cosson⁴, Igor Weber¹, Huaqing Cai^{2,3}, Vedrana Filić¹

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IqgC is a *D. discoideum* GTPase-activating protein (GAP) specific for the small GTPase RasG. It negatively regulates both types of macroendocytosis, however, its effect on macropinocytosis is quite modest in axenic background. It is known that macropinocytosis in AX2 cells already operates at a high level due to a deletion of NF1, another RasGAP strongly involved in the regulation of Ras activity during this process. Therefore, we investigated the effect of *iqgC* deletion in the non-axenic DdB strain. We demonstrated a strong negative effect of IqgC on both macropinocytosis and phagocytosis in this genetic background. We also noticed that, although IqgC colocalizes with the active Ras throughout the macropinosome formation, it resides on closed macropinosomes after Ras has dissociated. This prompted us to investigate requirements for the IqgC presence on macropinosomes. By examining the localization of IqgC in *rasG* null cells, and of RasG-nonbinding IqgC mutants in *iqgC* null cells, we showed that RasG is indispensable for the recruitment of IqgC to the forming macropinosomes. To address the retention of IqgC on the Ras-vacated vesicle, we searched for additional IqgC binding partners. IqgC non-selectively binds to all phosphoinositides, with the highest affinity for PI(4,5)P₂. However, the interaction with PIs was abrogated when the interaction between IqgC and RasG was interrupted, thus further underlining the crucial role of RasG in the membrane localization of IqgC. To find protein interactors, we reanalyzed previously published interactome of IqgC. Another small GTPase from the Ras superfamily, Rab5A drew our attention since mammalian Rab5 is a major regulator of early endosome maturation. We showed that IqgC binds the active form of Rab5A directly but does not act as its GAP. The binding is mediated via the RasGAP domain, which also binds RasG, suggesting a competition between the two GTPases. Moreover, Rab5A inhibits IqgC GAP activity toward RasG in a concentration-dependent manner. When co-expressed in the cell, Rab5A and IqgC colocalize on the macropinosome but show opposite recruitment dynamics, i.e. Rab5A accumulates as the IqgC signal declines. In addition, the IqgC lifetime on the macropinosome is significantly shortened when Rab5A is overexpressed. On the other hand, the initial recruitment of Rab5A to early endosomes is delayed in the absence of IqgC. We propose that IqgC and Rab5A mutually affect each other, with IqgC being important for the early accumulation of Rab5A, and Rab5A, in turn, causing dissociation of IqgC from the early macropinosome.

L12**Regulation of macropinocytosis in response to changing nutrient conditions**Huaqing Cai¹¹*Institute of Biophysics, Chinese Academy of Sciences, Beijing, China*

Macropinocytosis is an evolutionarily conserved mechanism for non-specific bulk uptake of extracellular fluid. Recent studies in *Dictyostelium* and cancer cells have uncovered an important metabolic function of macropinocytosis. Axenic *Dictyostelium* cells utilize macropinocytosis to obtain nutrients from liquid medium. Various types of cancer cells exploit macropinocytosis to survive in nutrient-poor environments by ingesting extracellular macromolecules and breaking them down in the lysosome to fuel cell growth. How cells calibrate their macropinocytic activity according to changes in the environmental and cellular nutrient conditions remain largely unknown. By establishing a genetic screening system in *Dictyostelium*, we discovered a number of novel macropinocytosis regulators. Characterization of these regulators, in particular an oligopeptide transporter and several transcription factors, allowed us to reveal how nutrient stress conditions are transduced to modulate macropinocytic activity of the cell.

L13**Mechanisms regulating the trafficking of Cln5 and cathepsin D in *Dictyostelium discoideum***

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In humans, homozygous mutations in ceroid lipofuscinosis neuronal 5 (*CLN5*) and cathepsin D (*CTSD*) cause CLN5 disease and CLN10 disease, respectively, which are two subtypes of neuronal ceroid lipofuscinosis (NCL, commonly known as Batten disease). *CLN5* and *CTSD* encode soluble lysosomal enzymes that also localize extracellularly. However, the mechanisms regulating the intracellular trafficking of CLN5 and CTSD, and their release from cells, are not well understood. In addition, while CLN5 and CTSD are both detected extracellularly, it is not clear whether they function outside cells. Thus, understanding the pathways regulating the intracellular/extracellular trafficking of CLN5 and CTSD and how mutations affect this trafficking are of particular importance. In this study, we used the social amoeba *Dictyostelium discoideum* as a model system to examine the pathways and processes that regulate the release of CLN5 and CTSD from cells. *D. discoideum* encodes homologs of both proteins (Cln5 and CtsD) and accumulated evidence indicates that the localization and function of CLN5 and CTSD are conserved from *D. discoideum* to human. Here, we show that both Cln5 and CtsD contain signal peptides for secretion that facilitate their release from cells. Furthermore, we demonstrate that extracellular CtsD, like Cln5, is glycosylated. Cln5 and CtsD are secreted upon the induction of autophagy and require the autophagy genes, *atg1* and *atg5*, with Cln5 additionally requiring *atg9* and autophagosomal-lysosomal fusion. The release of Cln5 and CtsD from cells also requires microfilaments and the *D. discoideum* homologs of human AP-3 complex subunit mu-1, LYST, and mucopolin-1, which all regulate lysosomal exocytosis. In addition, the intracellular/extracellular distribution of Cln5 is regulated by ABC transporters, microtubules, and the *D. discoideum* homologs of sortilin and cation-independent mannose-6-phosphate receptor. ABC transporters and microtubules also regulate the intracellular/extracellular distribution of CtsD. Finally, we show that osmotic stress inhibits the release of Cln5 from cells but increases the release of CtsD. Collectively, our data indicate that there are two pools of Cln5 and CtsD in cells; one that localizes to lysosomes and the other extracellularly. In addition, our findings suggest that the trafficking and secretion of Cln5 and CtsD may be regulated by slightly different mechanisms. In total, this study identifies mechanisms regulating the release of Cln5 and CtsD from *D. discoideum* cells and lays the foundation for follow up work in mammalian models to better understand how altered trafficking of CLN5 and CTSD causes disease in humans.

L14**How *Dictyostelium* amoebae recognize and hunt bacteria as food**Tian Jin¹¹*Chemotaxis signal section, LIG, NIAID, NIH, USA*

The social amoebae *Dictyostelium discoideum* are prototypical phagocytes that chase various bacteria via chemotaxis and consume them as food via phagocytosis. We discovered that *D. discoideum* amoeba use fAR1, a class C GPCR, to detect folic acid released from bacteria, chase bacteria via chemotaxis, and recognize LPS on the surface of Gram-negative bacteria for ingesting them via phagocytosis. Class C GPCRs have a common structure consisting of an extracellular Venus-flytrap (VFT) domain for ligand binding. The *D. discoideum* genome encodes 14 class C GPCRs, including fAR1. We propose that these Class C GPCRs consist of a new class of major microbe-associated molecular pattern (MAMP) receptors that allow *Dictyostelium* amoeba to chase and engulf not only Gram-negative but also Gram-positive bacteria as food.

L15**An endogenous chemorepellent inhibits Ras signaling to bias movement of *Dictyostelium* cells**

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The ability of eukaryotic cells to detect chemical gradients is crucial for development, morphogenesis, and immune responses. While much has been learned about chemoattraction, the movement of cells towards a signal, relatively little is known about chemorepulsion, the movement away from a signal. Proliferating *Dictyostelium* cells secrete the protein AprA, which acts as a chemorepellent, causing cells at the edge of a colony to move away from the colony, potentially in search of new food sources. In *Dictyostelium*, AprA-induced chemorepulsion is mediated by the G protein-coupled receptor GrhH, the G proteins Gα8 and Gβ, the enzyme phosphatidylinositol phosphate kinase A (PIPkinA), the GTPases RasC, RasG, and RacC, components of the target of rapamycin complex 2 (mTORC2), protein kinase C (PKC), and extracellular signal-regulated kinase Erks of the MAPK pathway. Localized Ras activation at one side of a cell induces actin filament formation, pseudopod formation, and subsequent cell movement in the direction of the pseudopod. In an AprA gradient, Ras activation and pseudopod formation do not occur at the side of the cell closest to the source of AprA, causing cells to move randomly in any direction other than towards the source of AprA, resulting in chemorepulsion. Cells lacking GrhH, Gβ, Gα8, PakD, Erk1, RasG, and PKB showed defective inhibition of Ras cortical activation by AprA. Cells lacking GrhH, RasC, and Erk1 showed a reduced number of pseudopods, and cells lacking RasC, RasG, and AprA showed a reduced number of filopods. These findings suggest that these proteins are either in, or are necessary for, the pathway between the AprA receptor GrhH and the inhibition of Ras activation. A partially overlapping pathway mediates an AprA-induced inhibition of cell proliferation. AprA causes decreased phosphorylation of 49 proteins involved in the Ras signaling pathway and cytoskeletal organization and increased phosphorylation of 13 proteins associated with the cell cycle and differentiation. Together, these findings suggest that 1) AprA regulates Ras regulatory proteins to locally inhibit Ras to induce cells to move away from a colony in search of new food sources, and 2) regulates proteins to inhibit proliferation of cells at the center of a colony in anticipation of starvation.

L16**A complete cGMP signaling complex associated with $G_{\beta\gamma}$ mediates both local and global information for directed cell movement**

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Cells in a complex environment often integrate local and global information to induce a specific response. During directed movement cells make local actin-filled protrusions in the cell front and global myosin filaments in the rest of the cell to inhibit protrusions. The mechanism how cells receive and integrate global and local information is poorly understood. Here we show that in *Dictyostelium* a complete cGMP signaling pathway, including a guanylyl cyclase, a cGMP target protein and a phosphodiesterase, is associated in a complex with $G_{\beta\gamma}$. Chemotactic activation of a guanylyl cyclase protein leads to the production of 200 cGMP molecules in 10 seconds. Combining estimates on the structure of the proteins in the complex with observed kinetics of all cGMP signaling proteins and diffusion of cGMP we calculated the fate of these 200 cGMP molecules. With more than 95% probability the cGMP-binding protein of the complex becomes activated by one of these 200 cGMP molecules, thereby transducing local information to pseudopod formation in the front of the cell. In addition, two molecules are degraded by phosphodiesterase in the complex and 197 cGMP molecules escape the complex, diffuse into the cell and mix with other cGMP molecules to provide global information for myosin filament formation. Detailed analysis reveals that during *Dictyostelium* chemotaxis the signaling pathway is organized in such a way that the cell receives optimal local and global information at a very wide range of chemoattractant concentrations. The results uncover how cells can use a fast diffusing signaling molecule to transmit both local and global information for very sensitive directional movement.

L17**Sphingomyelin metabolism underlies Ras excitability for efficient cell migration and chemotaxis**

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In eukaryotic motile cells, the active Ras (Ras-GTP)-enriched domain is generated in an asymmetric manner on the cell membrane through the excitable dynamics of intracellular signaling network. This asymmetric Ras signaling regulates pseudopod formation for both spontaneous random migration and chemoattractant-induced directional migration. It has been revealed that membrane lipids such as sphingomyelin and phosphatidylserine contribute to Ras signaling in various cell types, but it remains to be unsolved whether such lipids are involved in the Ras excitability for cell motility. Here we show that functional Ras excitability requires normal metabolisms of sphingomyelin for efficient cell motility and chemotaxis. Pharmacological blockades of the sphingomyelin metabolism by using an acid-sphingomyelinase inhibitor, fendiline, and various other inhibitors suppressed excitable generation of stable Ras-GTP-enriched domain. The suppressed excitability by fendiline treatment failed to invoke enough basal motility to achieve directed migration under shallow chemoattractant gradients. Ras expression was also suppressed slightly. These fendiline-induced defects in Ras excitability, motility and stimulation-elicited directionality were due to an accumulation of sphingomyelin on the membrane, which could be recovered by externally added sphingomyelinase or phosphatidylserine without recovery of Ras expression level. These results indicate a novel regulatory mechanism of the excitable system by membrane lipids, in which sphingomyelin metabolism provides a membrane environment to ensure Ras excitation for efficient cellular motility and chemotaxis.

L18**Single-cell phenotypic plasticity modulates social behavior in *Dictyostelium discoideum***Mathieu Forget^{1, 2}, Sandrine Adiba^{1, 3}, Silvia De Monte^{1, 2, 3}

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Within multicellular chimeras, the spread of strains that reap more than their share of benefits from the group - the so-called cheaters - is predicted to undermine collective functions whenever social behaviors are genetically determined. Indeed, a crucial condition for cheaters to be selected, is that their social behavior is genetically determined, so that advantages are heritable across successive social cycles. However, an increasing number of studies suggests that social behavior also reflects single-cell properties (that can also vary in isogenic populations) and social context (that depends on cell's neighborhood composition), thus weakening the role of direct genetic control.

In this study, we explore the relative role of genetic and plastic phenotypic differences in *Dictyostelium discoideum* strain evolutionary success. We have designed an experimental setup, that we call 'chrono-chimeras', to study within a common framework how plastic variation influences social behavior in binary mixtures of cells with the same or different genetic background. The phenotypic state of cells is modulated by changing the growth phase of cultures when starvation-induced aggregation begins. The social behavior was evidenced as usually by comparing, for one of the co-aggregating types, the fraction of cells that are passed on to the following generation -the spores- to the fraction of cells before aggregation (spore bias). First, we show that, for two isogenic populations, spore bias depends both on growth phase and on population composition, and is negatively correlated to the fraction of 'loners' (cells that do not join aggregates). Secondly, when examining single-cell properties that are expected to affect aggregation efficiency, we found that variations in the fraction of slowly moving cells with growth phase may explain why earlier cultures appear to be under-represented in the spore pool. We confirm the expected ubiquity of growth-phase induced spore bias variation by showing that it is not negligible in genetic chimeras, and can even reverse the classification of a strain's social behavior. These results suggest that biases emerging during aggregation can provide an efficient 'lottery' system to harness the evolutionary spread of cheaters and may contribute to understand how aggregative multicellular life cycles persist on evolutionary times despite unavoidable genetic conflicts.

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L19**Chemical communications between dictyostelids and soil nematodes**

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Dictyostelids and two major groups in nematodes, free-living nematodes and plant-parasitic nematodes, coexist in soil. Free-living nematodes, such as *Caenorhabditis elegans*, are considered to be the competitors for food and have a relationship of prey-predator with dictyostelids. They may also function as spreaders of spores (1). We have reported that fruiting bodies of dictyostelids and their released chemicals repel two plant-parasitic nematodes; root-knot nematodes (RKNs), *Meloidogyne* spp. (2), and root-lesion nematodes, *Pratylenchus* spp. (3). In this study, we analysed *C. elegans* behaviours in the presence of amoeba and fruiting bodies of two closely related species in Group 4: *D. discoideum* (*Dd*) and *D. purpureum* (*Dp*). In a chemotaxis behaviour assay with fruiting bodies, *C. elegans* were attracted to *Dd*, as shown previously (1), and *Dp* as well. Contrary, in an assay with amoeba, *C. elegans* exhibited different behaviours towards *Dd* and *Dp*; attraction towards *Dd* and repulsion towards *Dp*. These results may suggest that closely related *Dd* and *Dp* have different coexisting strategies with *C. elegans* in soil. Also, we have been elucidating the chemorepellent(s) in fruiting bodies of dictyostelids against RKNs and found that the conditioned medium of starved *Dd* cells also have the repellent activity against RKNs. In contrast, *C. elegans* appeared to be attracted to conditioned medium of *Dd*. These show that starved *Dd* cells produce and secrete both repellent(s) for RKNs and attractant(s) for *C. elegans*. We will discuss the nature of chemotactic activities towards plant-parasitic and free-living nematodes in dictyostelids. Although further analysis is needed for understanding the underlying mechanism, these results indicate the presence of complex chemical communications between dictyostelids and soil nematodes.

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References

1. Kessin RH *et al.* (1996) How cellular slime molds evade nematodes. *Proc Natl Acad Sci U S A* 93 (10) 4857-4861 <https://doi.org/10.1073/pnas.93.10.4857>
2. Saito YF *et al.* (2018) Chemical compounds from *Dictyostelium discoideum* repel a plant-parasitic nematode and can protect roots. *PLOS ONE* 13(9): e0204671 <https://doi.org/10.1371/journal.pone.0204671>
3. Araki T *et al.* International *Dictyostelium* Conference 2022 (Stirling, UK)

L20**Predator-prey relationships between *D. discoideum* and *P. aeruginosa***Imen Ayadi¹¹*University of Geneva, Switzerland*

The general aim of our laboratory is to understand how phagocytic cells kill microorganisms. For this, I used the professional phagocyte *D. discoideum* and *P. aeruginosa*, a bacterium responsible for life-threatening infections in patients. When *D. discoideum* encounters a *P. aeruginosa* bacterium, it can either ingest it or not. Remarkably, a large proportion of bacteria that are not ingested die a few minutes after being in contact with *D. discoideum*. These observations indicate that *D. discoideum* can kill *P. aeruginosa* intracellularly and extracellularly. Using a large collection of *D. discoideum* mutants we determined that molecular mechanisms necessary for extracellular and intracellular destruction of *P. aeruginosa* are different. On the other hand, *P. aeruginosa* developed an arsenal of mechanisms to resist intracellular and extracellular killing. The aim of my current experiments is to identify and dissect these virulence mechanisms, using a panel of *P. aeruginosa* mutants. My results notably stress the critical role of quorum sensing and LPS in the resistance of *P. aeruginosa* to *D. discoideum* predation.

L21**Proteasomes of autophagy-deficient cells exhibit alterations in regulatory proteins and a marked reduction in activity**

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Autophagy and the ubiquitin proteasome system are the two major processes for the clearance and recycling of proteins and organelles in eukaryotic cells. Evidence is accumulating that there is extensive crosstalk between the two pathways, but the underlying mechanisms are still unclear. We previously found that autophagy 9 (ATG9) and 16 (ATG16) proteins are crucial for full proteasomal activity in the unicellular amoeba *Dictyostelium discoideum*. In comparison to AX2 wild-type cells, ATG9⁻ and ATG16⁻ cells displayed a 60%, and ATG9⁻/16⁻ cells a 90%, decrease in proteasomal activity. Mutant cells also showed a significant increase in poly-ubiquitinated proteins and contained large ubiquitin-positive protein aggregates [1]. Here, we focus on possible reasons for these results. Reanalysis of published tandem mass tag-based quantitative proteomic results of AX2, ATG9⁻, ATG16⁻, and ATG9⁻/16⁻ cells [2] revealed no change in the abundance of proteasomal subunits. To identify possible differences in proteasome-associated proteins, we generated AX2 wild-type and ATG16⁻ cells expressing the 20S proteasomal subunit PSMA4 as GFP-tagged fusion protein, and performed co-immunoprecipitation experiments followed by mass spectrometric analysis. The results revealed no significant differences in the abundance of proteasomes between the two strains. However, we found enrichment as well as depletion of proteasomal regulators and differences in the ubiquitination of associated proteins for ATG16⁻, as compared to AX2 cells. Recently, proteaphagy has been described as a means to replace non-functional proteasomes. We propose that autophagy-deficient *D. discoideum* mutants suffer from inefficient proteaphagy, which results in the accumulation of modified, less-active, and also of inactive, proteasomes. As a consequence, these cells exhibit a dramatic decrease in proteasomal activity and deranged protein homeostasis.

[1] Xiong et al., The phenotypes of *atg9*, *atg16* and *atg9/16* knock-out mutants imply autophagy-dependent and -independent functions. *Open Biol.* **2015**, *5*, 150008.

[2] Xiong et al., RNAseq and quantitative proteomic analysis of *Dictyostelium* knock-out cells lacking the core autophagy proteins ATG9 and/or ATG16. *BMC Genom.* **2021**, *22*, 444.

L22**Unveiling the identity of *Dictyostelium discoideum* peroxisomes. A walking plant or talking amoeba?**

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Peroxisomes are organelles found in most eukaryotic cells. They are typically round in shape, measuring about 1 µm, and play significant roles in various cellular processes, such as the beta (β) oxidation of fatty acids and detoxification of hydrogen peroxide (H₂O₂). These organelles contain important enzymes, including oxidases and catalase, and exhibit diverse metabolic and non-metabolic functions depending on the environmental conditions and organisms. Despite their importance, peroxisomes have received limited attention in studies of *Dictyostelium discoideum*.

In our study, a proximity labelling approach (BioID) was employed to identify the components of peroxisomal import and contents. By comparing putative biotinylated peroxisomal proteins among different organisms, it was observed that there exists a substantial quantity of peroxisomal proteins, comparable to those observed in plants and mammalian cells, particularly human cells. These findings suggest possible similarities in cellular processes and metabolic pathways.

L23**The PripA-TbcrA complex-centered Rab GAP cascade facilitates macropinosome maturation in *Dictyostelium***

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Macropinocytosis, an evolutionarily conserved mechanism mediating nonspecific bulk uptake of extracellular fluid, has been ascribed diverse functions. How nascent macropinosomes mature after internalization remains largely unknown. By searching for proteins that localize on macropinosomes during the Rab5-to-Rab7 transition stage in *Dictyostelium*, we uncover a complex composed of two proteins, which we name PripA and TbcrA. We show that the Rab5-to-Rab7 conversion involves fusion of Rab5-marked early macropinosomes with Rab7-marked late macropinosomes. PripA links the two membrane compartments by interacting with PI(3,4)P₂ and Rab7. In addition, PripA recruits TbcrA, which acts as a GAP, to turn off Rab5. Thus, the conversion to Rab7 is linked to inactivation of the upstream Rab5. Consistently, disruption of either *pripA* or *tbcra* impairs Rab5 inactivation and macropinocytic cargo processing. Therefore, the PripA-TbcrA complex is the central component of a Rab GAP cascade that facilitates programmed Rab switch and efficient cargo trafficking during macropinosome maturation.

L24**C2GAP2 is a common regulator of Ras signaling for chemotaxis, phagocytosis, and macropinocytosis**Xuehua Xu¹¹*National institute of allergy and infectious diseases, USA*

Phagocytosis, macropinocytosis, and G protein coupled receptor-mediated chemotaxis are Ras-regulated and actin-driven processes. The common regulator for Ras activity in these three processes remains unknown. Here, we show that C2GAP2, a Ras GTPase activating protein, highly expressed in the vegetative growth state in model organism *Dictyostelium*. C2GAP2 localizes at the leading edge of chemotaxing cells, phagosomes during phagocytosis, and macropinosomes during micropinocytosis. *c2gapB*⁻ cells lacking C2GAP2 displayed increased Ras activation upon folic acid stimulation and subsequent impaired chemotaxis in the folic acid gradient. In addition, *c2gapB*⁻ cells have elevated phagocytosis and macropinocytosis, which subsequently results in faster cell growth. C2GAP2 binds multiple phospholipids on the plasma membrane and the membrane recruitment of C2GAP2 requires calcium. Taken together, we show a shared negative regulator of Ras signaling that mediates Ras signaling for chemotaxis, phagocytosis, and macropinocytosis.

L25**Distinct mechanisms keep Skipper-1 under control**

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Skipper-1 is a chromovirus-type retrotransposon that accumulated in (peri-)centromeric regions of the *Dictyostelium discoideum* genome. We determined that Skipper-1 expression and mobility are controlled by two independent RNA interference pathways. In the cytoplasm, the Argonaute protein AgnA and the RNA-dependent RNA Polymerase (RdRP) RrpC inhibit Skipper-1 by small RNAs. Dysregulation of either protein disrupts this posttranscriptional gene silencing (PTGS) and leads to Skipper-1 mobilization, similar to what has been observed for the retrotransposon DIRS-1. Distinct from the latter is an additional nuclear silencing of Skipper-1 that is mediated by the RdRP RrpA, the Dicer-like protein DrnB and the Argonaute protein AgnB. Skipper-1 transcripts accumulated in mutants lacking any of these proteins, but retrotransposition was still blocked by cytoplasmatic PTGS. DrnB and RrpA co-localized in the nucleus with Skipper-1 genomic DNA and the heterochromatin marker H3K9me3. We suggest that Skipper-1 transcription might be reduced via small RNA-induced heterochromatization of its genomic copies, whereas Skipper-1 amplification is suppressed in the cytoplasm by a small RNA-induced silencing complex.

L26**Investigating molecular mechanisms of the Chinese herbal remedy, tanshinone 2A, in *Dictyostelium* identifies a new approach to treat brain cancer (glioblastoma multiforme)**

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Glioblastomas are a highly aggressive cancer type which respond poorly to current pharmaceutical treatments, thus novel therapeutic approaches need to be investigated. One such approach involves the use of the bioactive natural product tanshinone IIA (T2A) derived from the Chinese herb Danshen, where mechanistic insight for this anti-cancer agent is needed to validate its use. Here, we employ a tractable model system, *Dictyostelium discoideum*, to provide this insight. T2A potently inhibits cellular proliferation of *Dictyostelium*, suggesting molecular targets in this model. We show that T2A rapidly reduces phosphoinositide 3 kinase (PI3K) and protein kinase B (PKB) activity, but surprisingly, the downstream complex mechanistic target of rapamycin complex 1 (mTORC1) is only inhibited following chronic treatment. Investigating regulators of mTORC1, including PKB, tuberous sclerosis complex (TSC), and AMP-activated protein kinase (AMPK), suggests these enzymes were not responsible for this effect, implicating an additional molecular mechanism of T2A. We identify this mechanism as the increased expression of *sestrin*, a negative regulator of mTORC1. We further show that combinatory treatment using a PI3K inhibitor and T2A gives rise to a synergistic inhibition of cell proliferation. We then translate our findings to human and mouse-derived glioblastoma cell lines, where both a PI3K inhibitor (Paxalisib) and T2A reduce glioblastoma proliferation in monolayer cultures and in spheroid expansion, with combinatory treatment significantly enhancing this effect. Thus, we propose a new approach for cancer treatment, including glioblastomas, through combinatory treatment with PI3K inhibitors and T2A.

L27**Evolution of microRNAs in Amoebozoa**

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MicroRNAs (miRNAs) are important regulators of gene expression in both plants and animals. They are thought to have evolved convergently in these lineages and hypothesized to have played a role in the evolution of multicellularity. In line with this hypothesis, miRNAs have so far only been described in a handful of unicellular eukaryotes. To investigate the evolution of miRNAs and their potential role in multicellularity, we studied the presence and evolution of miRNAs in Amoebozoa. The amoebas analysed ranged from truly unicellular species to amoebas where cells go through aggregative multicellularity. Through small RNA sequencing, we identified miRNAs which adhere to both the stringent plant and animal miRNA criteria in all examined amoebae, greatly expanding the total number of protists featuring miRNAs. We found miRNAs conserved between closely related species, but the majority of species feature only unique miRNAs. To further resolve the evolution of miRNAs, we investigated the conservation of miRNA between the Group 4 dictyostelids *D. discoideum* and *D. firmibasis*. For this, we de-novo sequenced the genome of the latter, greatly improving its quality and allowing for a side-by-side comparison of the two species. In conclusion, our results show that the miRNAs in Amoebozoa varies greatly in numbers and that conservation between species is rare. This suggests that miRNAs evolved independently and rapidly in different amoebozoan lineages where they fulfil specific roles, but they do not seem to play a role in the evolution of multicellularity in this supergroup.

P1**Field model for multistate lateral diffusion of various transmembrane proteins observed in living cells**Kazutoshi Takebayashi¹, Yoichiro Kamimura², Masahiro Ueda^{1, 3}¹Center for Biosystems Dynamics Research (BDR), RIKEN, ²Nara Medical University, ³Graduate School of Frontier Biosciences, Osaka University, Japan

The lateral diffusion of transmembrane proteins on plasma membranes is a fundamental process for many cellular functions. Various membrane proteins display distinct modes of diffusion, largely due to membrane structure complexity and protein species-specific interactions. Although diffusion properties specific to individual protein species have been extensively studied, the common features among protein species are poorly understood. To develop a universally applicable model, we must analyze two aspects of diffusion: one tied to the protein's intrinsic properties, and another associated with the surrounding membrane environment. In this study, we systematically studied the lateral diffusion of various transmembrane proteins, with one to ten transmembrane regions and molecular weights ranging from 27 to 163 kDa, in *Dictyostelium discoideum* cells using a hidden Markov model for single-molecule trajectories obtained experimentally. Despite structural differences, all membrane proteins consistently adopted three free diffusion states with similar coefficients, a feature common to all. The fact that all proteins exhibited free diffusion is in contrast to mammalian cells, where a variety of diffusion modes have been observed. Moreover, all protein species reduced their mobility similarly upon the inhibition of microtubules, actin cytoskeleton, or myosin II. The correlation between protein size and diffusion coefficient aligned with the Saffman-Delbrück model, suggesting that membrane viscosity, not protein size, significantly influences lateral diffusion. Hence, membrane environment plays a pivotal role in lateral mobility, rather than proteins' intrinsic properties like molecular size. To account for the multistate free diffusion commonly observed in many transmembrane proteins, we proposed a simple membrane field model. We assumed the membrane field to have three regions—fast, middle, and slow simple diffusion areas—with fast and slow squares randomly positioned in the middle region. By creating a field with only four parameters, including the size of the fast and slow regions and the occupied area relative to the whole field, and allowing molecules to diffuse freely over that field, the model successfully reproduces multi-state diffusion under control and drug treatment conditions. Our model suggests that *Dictyostelium* cells have a relatively simple membrane structure capable of producing multistate free diffusion. This study reveals that the lateral diffusion of membrane proteins in *Dictyostelium* is broadly determined by the surrounding plasma membrane environment. The protein species-independent nature of multi-state free diffusion was simply and quantitatively explained by free diffusion over three membrane regions of different viscosity, in sharp contrast to the complex diffusion behavior of transmembrane proteins in higher eukaryotes.

P2**The emerging role of *Dictyostelium discoideum* IQGAP-related protein lqgD in large-scale endocytosis**

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IQGAPs are multidomain proteins conserved from amoeba to humans. They play a vital role as scaffold proteins enabling the harmonious integration of diverse signalling pathways and precise regulation of cellular processes that require the reorganization of the actin cytoskeleton such as migration, adhesion, cytokinesis, and endocytosis. The N-terminal calponin homology domain (CHD) enables them to interact directly with actin filaments. Due to the oligomerization, IQGAPs cross-link actin filaments into bundles. The oligomerization is facilitated by the binding of Rho family GTPases Rac1 and Cdc42 via the GAP-related domain (GRD). Additionally, IQGAPs have a regulatory role in the actin cytoskeleton dynamics by interacting with actin nucleation-promoting (N-WASP) and assembly factors (Arp2/3, Dia1), thus facilitating the generation of protrusive structures at the cell leading edge. Among the four IQGAP-related proteins found in *D. discoideum*, lqgD is the sole protein possessing a CHD, alongside a coiled-coil region, a GRD, and a RasGAP_C-terminal (RGCT) extension, and it has not been characterized yet. Here we investigated the localization of lqgD in live AX2 cells using confocal microscopy. In interphase cells, lqgD localizes over the entire cell cortex and is notably enriched at the macroendocytic cups. It exhibits no prominent localization during cytokinesis. lqgD's almost complete colocalization with active Ras/PI(3,4,5)P₃ patches and active Rac1 and F-actin further corroborates its involvement in large-scale endocytosis. Using yeast two-hybrid assay, we identified the Rac1 GTPases as direct binding partners of lqgD. In addition, co-immunoprecipitation was employed to demonstrate the binding of lqgD and actin. Further experiments to confirm these interactions are underway. We have also generated *lqgD* knock-out cells to examine the physiological role of lqgD. For now, our findings suggest that lqgD functions as an IQGAP-related protein regulated by small GTPases from the Rho family, with a mechanistic role in remodelling of the actin cytoskeleton during both macropinocytosis and phagocytosis.

P3**Harnessing dictyostelids to combat *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm infections**

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Wound infections account for approximately 34% of nosocomial infections. The formation of biofilms, a major bacterial virulence factor, accounts for 65% of all microbial infections and 80% of chronic infections. *Staphylococcus aureus* and *Pseudomonas aeruginosa* represent two of the most common pathogens in wound infections and are notorious for forming biofilms. These biofilms provide antibiotic tolerance, immune evasion, and facilitated acquisition of antibiotic drug resistance, making these infections difficult to treat. Consequently, biofilm-related wound infections have emerged as a substantial public health concern, warranting novel treatment strategies that target pre-established biofilms in wounds.

We previously discovered that compounds capable of degrading biofilms are produced when dictyostelids prey on bacteria, such as *Staphylococcus epidermidis*. Here, we determine whether such compounds produced by the dictyostelid species, *Polysphondylium pallidum*, are effective at degrading biofilms of the wound pathogens, *S. aureus* and *P. aeruginosa*, using *in vitro* biofilm infection models and an *ex vivo* porcine skin wound infection model. Our work demonstrates that *P. pallidum*-associated compounds eradicate up to 90% of pre-established *S. aureus* and *P. aeruginosa* mono- and polymicrobial biofilms. Scanning electron microscopy indicates that *P. pallidum* selectively targets the extracellular polymeric substances found within *S. aureus* and *P. aeruginosa* biofilms, resulting in the complete elimination of the biofilm matrix. Furthermore, we demonstrate that *P. pallidum*-associated compounds also prevent the formation of these biofilms. In conclusion, *P. pallidum*-associated compounds effectively prevent biofilm formation and eradicate pre-established biofilms of *S. aureus* and *P. aeruginosa*, suggesting their potential application as therapeutic agents for combating biofilms in wound infections.

P4**DNA extracellular trap production in response to *Klebsiella pneumoniae* and its lipopolysaccharide by vegetative *D. discoideum* cells**

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The formation of extracellular traps (ETs) has been previously described in neutrophils and other immune cells of mammals. These structures, composed of DNA and surrounded by antimicrobial granules, play a crucial role in innate immune defense by capturing and eliminating intracellular pathogens. Recent studies on *Dictyostelium discoideum* have revealed the vital role of ET formation in phagocytic S cells during social development, where they trap and eradicate bacteria, contributing to the formation of sterile fruiting bodies. However, the involvement of ETs in the host-defense mechanisms against pathogens in vegetative *D. discoideum* cells remains poorly understood. In this study, employing live-cell fluorescence imaging in conjunction with global proteomics analysis, we have discovered that vegetative cells of *D. discoideum* are capable of producing extracellular traps in the presence of both virulent and avirulent strains of *Klebsiella pneumoniae*, as well as in the presence of the lipopolysaccharide (LPS) from this strain. Furthermore, we have observed changes in the proteomic profile of the ETs when induced by LPS from *Klebsiella pneumoniae*, and we have also determined that treatment with DNase impairs the efficient clearance of bacteria. These findings provide evidence that *D. discoideum* serves as a valuable model organism for investigating the evolution and conservation of traits involved in the dynamic formation of ETs. Additionally, our results support the idea of *D. discoideum* as a model for studying cell-autonomous defenses.

P5**A TRAF-like E3 ubiquitin ligase TrafE coordinates ESCRT and autophagy in endolysosomal damage response and cell-autonomous immunity to *Mycobacterium marinum***

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Cells are perpetually challenged by pathogens, protein aggregates or chemicals, that induce plasma membrane or endolysosomal compartments damage, recognised as severe stress and controlled by the endosomal sorting complex required for transport (ESCRT) and the autophagy machineries that are recruited to damaged membranes to either repair or to remove membrane remnants. Yet, the insight is limited on how damage is sensed and which effectors lead to extensive tagging of the damaged organelles with signals, such as K63-polyubiquitin, required for the recruitment of membrane repair or removal machineries. To explore the key factors responsible for detection and marking of damaged compartments we use the professional phagocyte *Dictyostelium discoideum*. We found an evolutionary conserved E3-ligase, TrafE, that is robustly recruited to intracellular compartments disrupted after infection with *Mycobacterium marinum* or after sterile damage caused by chemical compounds. TrafE acts at the intersection of ESCRT and autophagy pathways and plays a key role in functional recruitment of the ESCRT subunits ALIX, Vps32 and Vps4 to damage sites. Importantly, we show that the absence of TrafE severely compromises the xenophagy restriction of bacteria as well as ESCRT-mediated and autophagy-mediated endolysosomal membrane damage repair, resulting in early cell death.

P6**Chemical communications that exist between dictyostelids and the free-living nematode *C. elegans***

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Our research focuses on investigating the chemical communication between *Dictyostelia* and the free-living nematode *Caenorhabditis elegans* in the soil environment. In previous studies, these two organisms exhibit a predator-prey relationship, with nematodes preying on the *Dictyostelium discoideum* amoeba. During multicellular development, the slime molds create a slime shield to protect their spores and when ingested by nematodes, spores remain viable and are subsequently excreted back in the soil. This intriguing phenomenon is believed to be a survival strategy of the cellular slime molds, potentially indicating that *C. elegans* is attracted to facilitate the dispersal of spores for these organisms. In contrast, *Dictyostelium purpureum*, a closely related species to *D. discoideum*, repels *C. elegans* of the *Rhabditidae* family during the amoeba stage. (1)

In this study, we conducted an analysis of *C. elegans* behaviours in response to the presence of amoeba and fruiting bodies of two closely related species in Group 4: *D. discoideum* (*Dd*) and *D. purpureum* (*Dp*). In a chemotaxis behaviour assay with fruiting bodies, *C. elegans* were attracted to both *Dd*, as shown previously (1), and *Dp* as well. Interestingly, when subjected to an assay with amoeba, *C. elegans* exhibited distinct behaviours towards *Dd* and *Dp*; showing attraction towards *Dd* and repulsion towards *Dp*. These findings suggest that closely related *Dd* and *Dp* employ different coexistence strategies with *C. elegans* in soil. Additional chemotaxis assays were performed on different groups of *Dictyostelia*, revealing a strong attraction of *C. elegans* towards all groups of dictyostelid's fruiting bodies. This led us to hypothesise that chemoattractant(s) may be secreted from individual cells during the multicellular development. To further elucidate the impact of slime molds on *C. elegans*, we conducted chemotaxis behaviour assay at various development stages of *D.d* and *D.p*. Surprisingly, we observed an increase in attractant activity as development progressed.

In conclusion, our findings shed light on the complex chemical communications that exist between dictyostelids and soil nematodes, serving as a significant stride towards comprehending the intricate interactions within the soil community.

P7**Nematode repellent active substance derived from *Dictyostelium discoideum***

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We are studying the relationship between *Dictyostelia* and plant parasitic nematodes. Plant parasitic nematodes inhibit growth of plants through parasitizing plants, causing significant damage to agriculture. Previous studies have shown that *D. discoideum* repel root-knot nematodes⁽¹⁾ and root-lesion nematodes. At last year's conference, we reported that Ethyl-a-D-glucoside (aEG) was one of the active substances which is purified TLC and HPLC analysis. In this study, we attempted to further identify nematode-repellent substances possessed by *D. discoideum*. Metabolomics analysis was performed to know the substances contained in *D. discoideum*. As a result, it was found that *D. discoideum* contained 126 kinds of substances. We performed bioassays on all of these substances and identified eight substances with repellent activity. Four of these are amino acids, and the others have antioxidant properties. This work will be useful for the development of new methods to control nematodes, which are causing serious damage around the world.

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Reference

(1) Saito YF *et al.* (2018) PLOS ONE 13(9): e0204671

P8**GxcM-Fbp17/RacC-WASP signaling regulates polarized cortex assembly in migrating cells via Arp2/3**Dong Li¹, Yihong Yang¹, Huaqing Cai¹¹*Institute of Biophysics, Chinese Academy of Sciences, China*

The actin-rich cortex plays a fundamental role in many cellular processes. Its architecture and molecular composition vary across cell types and physiological states. The full complement of actin assembly factors driving cortex formation and how their activities are spatiotemporally regulated remain to be fully elucidated. Using *Dictyostelium* as a model for polarized and rapidly migrating cells, we show that GxcM, a RhoGEF localized specifically in the rear of migrating cells, functions together with F-BAR protein Fbp17, a small GTPase RacC, and the actin nucleation-promoting factor WASP to coordinately promote Arp2/3 complex-mediated cortical actin assembly. Overactivation of this signaling cascade leads to excessive actin polymerization in the rear cortex, whereas its disruption causes defects in cortical integrity and function. Therefore, apart from its well-defined role in the formation of the protrusions at the cell front, the Arp2/3 complex-based actin carries out a previously unappreciated function in building the rear cortical subcompartment in rapidly migrating cells.

P9**Collective signalling drives rapid jumping between cell states**Elizabeth Westbrook¹, Tchern Lenn¹, Jonathan Chubb¹, Vlatka Antolovic¹¹*University College London, UK*

Development can proceed in “fits and starts”, with rapid transitions between cell states involving concerted transcriptome-wide changes in gene expression. However, it is not clear how these transitions are regulated in complex cell populations, in which cells receive multiple inputs. It is also not clear to what extent these rapid transitions represent developmental commitment. Here we address these issues using *Dictyostelium* cells undergoing development in their physiological niche. A continuous single cell transcriptomics time series reveals a sharp “jump” in global gene expression marking functionally different cell states. By simultaneously live imaging the physiological dynamics of transcription and signalling over millimetre length scales, we show that the jump coincides with the onset of collective oscillations of cAMP, the positive feedback signal for multicellular development. Different jump genes respond to distinct dynamic features of signalling. The late gene expression changes of the jump are almost completely dependent on cAMP. In contrast, transcript changes at the onset of the jump require additional input. The spatial boundary marking the jump divides cells separated by only a few minutes of developmental time, with cells missing a jump then waiting several hours for the onset of the next wave of cAMP oscillations. This timing variability contrasts the prevailing developmental paradigm of a timed synchronous process and is associated with substantial pre-jump transcriptome variability. The coupling of collective signalling with gene expression is a potentially powerful strategy to drive robust cell state transitions in heterogeneous signalling environments. Based on the context of the jump, we also conclude that sharp gene expression transitions may not be sufficient for commitment.

P10**Investigating therapeutic mechanisms and targets of non-psychotropic cannabinoids in the model system *Dictyostelium discoideum***

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Plant derived cannabinoids, termed phytocannabinoids, are naturally occurring compounds synthesised by *Cannabis sativa* as part of the plant's natural defence system. In humans, specific non-psychotropic cannabinoids have demonstrated an array of medicinal properties, yet the molecular mechanisms for these effects are largely uncharacterised. Initial analysis of these cannabinoids in *Dictyostelium discoideum* identified molecular mechanisms and potential targets associated with FOCC (folate one carbon cycle) signalling, and mTORC1 (mechanistic target of rapamycin complex 1) signalling. This project aims to further investigate these molecular targets for specific cannabinoids in *D. discoideum*. We show a concentration-dependent inhibition of cell proliferation in *D. discoideum* following cannabinoid treatment. Computational analysis of a key enzyme in FOCC, cobalamin-dependent methionine synthase, predicts the binding of these cannabinoids. We are currently employing LCMS (Liquid Chromatography Mass Spectrometry) to analyse FOCC metabolites in response to cannabinoid treatment. In addition, we are analysing effects of these cannabinoids on mTORC1 signalling measured through phosphorylation of the substrate 4EBP1 (Eukaryotic translation initiation factor 4E-binding protein 1). Thus, this project is developing new mechanistic insights regarding the medicinal properties of specific non-psychotropic cannabinoids.

P11 **β -arrestin orchestrates the extension of the concentration ranges in eukaryotic chemotaxis**

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Chemotaxis is the directional migration of cells in response to chemical gradients. *Dictyostelium discoideum*, a model organism for chemotaxis, receives 3',5'-cyclic adenosine monophosphate (cAMP) via G-protein-coupled receptor (GPCR) and exhibits chemotaxis. Chemotaxis signaling pathways include G-protein-dependent and G-protein-independent pathways. But the role of G protein-independent pathways in chemotaxis is not well understood. To reveal the role of this pathway, we focused on β -arrestin, the upstream molecule of the G protein-independent pathway. *Dictyostelium discoideum* has six β -arrestin homologs (Arrestin domain containing protein A-F, AdcA-F). We created knockout mutants for these genes and measured the chemotaxis efficiency. Then, we found that the chemotaxis efficiency was reduced in all mutants. These results suggest that β -arrestin acts to extend the concentration range of chemotaxis.

P12**The bioresource project of cellular slime molds in Japan (NBRP Nenkin)**

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The National Bioresource Project of Cellular Slime Molds in Japan (NBRP Nenkin) has been serving as a bioresource center since 2007. Our main objective is to collect and distribute bioresources, specifically cellular slime molds cells and related plasmids, to the global *Dictyostelium* research community. This initiative aims to facilitate research, particularly focused on the fascinating model organism *Dictyostelium discoideum* cells. To date, we have collected and stored approximately 2,000 strains and around 960 plasmids, primarily sourced from Japanese laboratories involved in diverse research fields. Since 2022, the management of NBRP has transitioned to the University of Tsukuba, taking over the project from RIKEN. Over the past several years, we have made significant contribution to the field. One notable accomplishment is the deposition of all-in-one vectors for CRISPR/Cas9-mediated gene modification, which includes knockouts, inducible knockouts, knockdowns, knock-ins, point mutations and deletions. These plasmids have been ordered by researchers worldwide. Additionally, we have collected and made available a highly bright and sensitive cAMP biosensor called Flamindo2. Flamindo2 enables the detection of changes in cytosolic cAMP concentration ranging from approximately 0.3 to 12 μ M during the early aggregation and mound stages. We believe that these resources will prove valuable for your research endeavors. Furthermore, our stocked strains include field-isolates that are currently aiding in the discovery of pharmacological lead compounds for drug development. All resources within the NBRP are accessible to anyone through our website (<http://nenkin.nbrp.jp>). Moreover, we welcome the submission of bioresources from your studies, regardless of your location in the world. If you have resources to contribute, please contact us at nbrpnengin@shigen.info. Your contribution to this project would be greatly appreciated, and we welcome any comments or feedback you may have.

P13**Metabolic changes during the development of *Dictyostelium***

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The TOR kinase functions in two main protein complexes, mTORC1 and mTORC2. mTORC1 is a key regulator for cell growth, sensing signals from nutrients and energy levels. While mTORC1 activity is inhibited by nutrient removal, it is also inactivated by the immunosuppressive drug rapamycin. In contrast, mTORC2 does not regulate cell growth, is insensitive to the direct action of rapamycin, but is crucial for developmental pathways. Collectively, the mTOR kinase complexes are essential for the life cycle of *Dictyostelium*, where the growth-to-development transition (GDT) is initiated upon nutrient depletion. Thus, withdrawal of nutrients from or addition of rapamycin to growing cells leads to a rapid (<5 min) de-phosphorylation of the mTORC1 mediated growth regulators pS6K and p4EBP1. Even in the presence of nutrients, rapamycin suppression of mTORC1 promotes mRNA unloading from polysomes and inhibition of protein synthesis, similar to that of nutrient-depleted cells. In parallel, following inactivation of mTORC1 by nutrient-starvation or rapamycin-treatment, AMP Kinase (AMPK), an energy status regulator, becomes phosphorylated/activated. As active mTORC1 may inhibit AMPK, AMPK similarly appears to reciprocally suppress mTORC1. Activation of AMPK in growth media by phospho-AICAR, an AMP mimic, (or 2-deoxy glucose) will inhibit mTORC1, while the AMPK-inhibitor dorsomorphin blocks suppression of mTORC1, under conditions of starvation.

We have shown that direct treatment of vegetative cells in growth media with rapamycin alone (in absence of nutrient depletion) is sufficient to drive GDT and developmentally regulated gene expression. Thus, de-activated mTORC1 but activated AMPK are placed at the junction of GDT induction. Significantly, many 1000s of mRNA that are up/down-regulated during starvation-induced development are not similarly up/down-regulated by rapamycin-induced development. RNA sequencing analysis of rapamycin-GDT showed up-regulation of genes previously associated with development, and down-regulation of growth-essential genes, as well as classes of uncharacterized novel gene sets. We wish to identify metabolic pathways required for the result of GDT, and hypothesize that rapamycin-induced development may identify a more limited set than by nutrient withdrawal. We have compared cells in growth media, cells starved for nutrients, and cells in growth media treated with rapamycin, using quantitative mass spectrometry and biochemical assays of small molecules, paralleled by RNA sequencing analyses. We observe alterations in metabolic pathways, including glycolysis, lipolysis, and protein synthesis and degradation, upon mTORC1 inactivation and relate these to the energy deficit state in control of GDT.

P14**RasGEF B and X break the symmetry of Ras activation to drive spontaneous cell migration**Koji Iwamoto¹, Satomi Matsuoka¹, Masahiro Ueda¹¹*Osaka University, Japan*

Ras is a core of an excitable system that generates a signal for cell polarity and spontaneous cell migration without environmental cues. The Ras-GTP enriched region that is self-organized by the excitable system determines the anterior side. However, the activation mechanism by RasGEFs which are activators of Ras has not been elucidated. Here, RasGEFs responsible for the spontaneous excitability were screened comprehensively from 22 RasGEFs coded on the genome in *Dictyostelium discoideum* by live-cell imaging of activated Ras. Systematic characterization of their over-expression effects, followed by a hierarchical clustering, revealed 5 clusters of RasGEFs which positively or negatively regulate Ras to different degrees. Among the 5 clusters, cluster 1 including GEFB, M, U, and X is the most potent positive regulator. In addition, from Ras-GTP dynamics of the knockouts of GEFs in cluster 1, GEFX KO showed reduced Ras activation and low efficient cell migration. Furthermore, among GEFs in cluster 1, GEFB, U and X localized on the membrane. Particularly, GEFB and X colocalizes with Ras-GTP and showed traveling waves, indicating that Ras-GTP, GEFB and X have the same excitable dynamics. And we also found that GEFB and GEFX spatially and temporally regulate the dynamics of Ras-GTP, respectively. Finally, our study suggests that a combination of GEFX and GEFB was essential for the spatiotemporal regulation of Ras activation and for driving cell migration.

Addendum

Extracellular adenosine deamination primes tip organizer development in *Dictyostelium*

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Volatile ammonia plays a major role in *Dictyostelium* morphogenesis and there are several routes by which ammonia can be generated. We show that extracellular adenosine deamination plays a significant role in ammonia formation driving early morphogenesis in *Dictyostelium*. A block in this deamination pathway (insertion in the gene encoding for adenosine deaminase related growth factor-*adgf*) or treating wild type cells with a highly specific extracellular ADA inhibitor results in arrested development with no organizer tip formation. The tip formation in *adgf*- cells can be restored by adding adenosine deaminase (ADA) enzyme, adding 8-Br-cAMP, a cell permeable cAMP analog and interchanging the conditioned medium with wild type cells. Wild type cells physically separated from the mutants on one side of the compartmentalized dish and culture supernatant of *adgf* supplemented with ADA enzyme independently rescued the *adgf*- phenotype suggesting that volatile ammonia generated from the wild type cells/adenosine deamination are responsible for rescuing the defect. Thus, contrary to the long-held view that ammonia generated from proteolytic activity driving tip development, this work demonstrate that extracellular adenosine deamination also contributes to tip development in *Dictyostelium*. Our work suggests that the successive transformation events of cAMP to adenosine and adenosine to ammonia are sequentially integrated in the consecutive steps of *Dictyostelium* development. We tried rescuing mutants with similar phenotypes with ADA and found that *dhkD* mutant getting rescued suggesting that ADGF acts downstream of *dhkD* driving tip formation. The expression of candidate genes involved in cAMP relay were examined and further, we the influence of the substrate, adenosine and the product ammonia were examined on *adgf* expression.

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