Annual International Dictyostelium Meeting
Dicty 2010

1st August 2010 - 6th August 2010
Cardiff University
Wales, UK

Programme and Abstract Book

Index:

Welcome:

Welcome to the **2010 International Dictyostelium Conference** in Cardiff, UK, the 24th conference in a series that stretches back to the first meeting of its kind in Sardinia in 1977.

New this year, we have invited four world-leading researchers Greg Velicer, Orion Weiner, Darren Gilmour and Eric Brown from outside the field to give Plenary talks that complement our *Dictyostelium* research. It is important to place *Dictyostelium* within the context of the global research effort, and we hope that you will find it valuable to discuss your work with our special guests. This conference also sees the launch of "The Pathostelium Club" for researchers with a common interest in using *Dictyostelium* to address major aspects of human pathogenesis.

We would like to acknowledge the generous support from companies, whose details can be found later in the programme booklet. Not only has their sponsorship supported our plenary speakers, but also provided support for 7 students and junior scientists to attend the meeting. Congratulations to Daniel Calovi, Divya Nair, Sanjanie Fernando, Maja Marinovic, Kelly Dunnning, Marthe H.R. Ludtmann and Daniele Conte.

Finally, we would like to wish you an enjoyable stay in Cardiff and a successive conference.

Adrian Harwood, Cathy Pears and Chris Thompson

(Organising committee)

Maps and Locations:

Talks:

Julian Hodge Lecture Theatre
Cardiff University
Julian Hodge Building
Colum Drive
Cardiff
Wales
CF10 3EU

Posters:

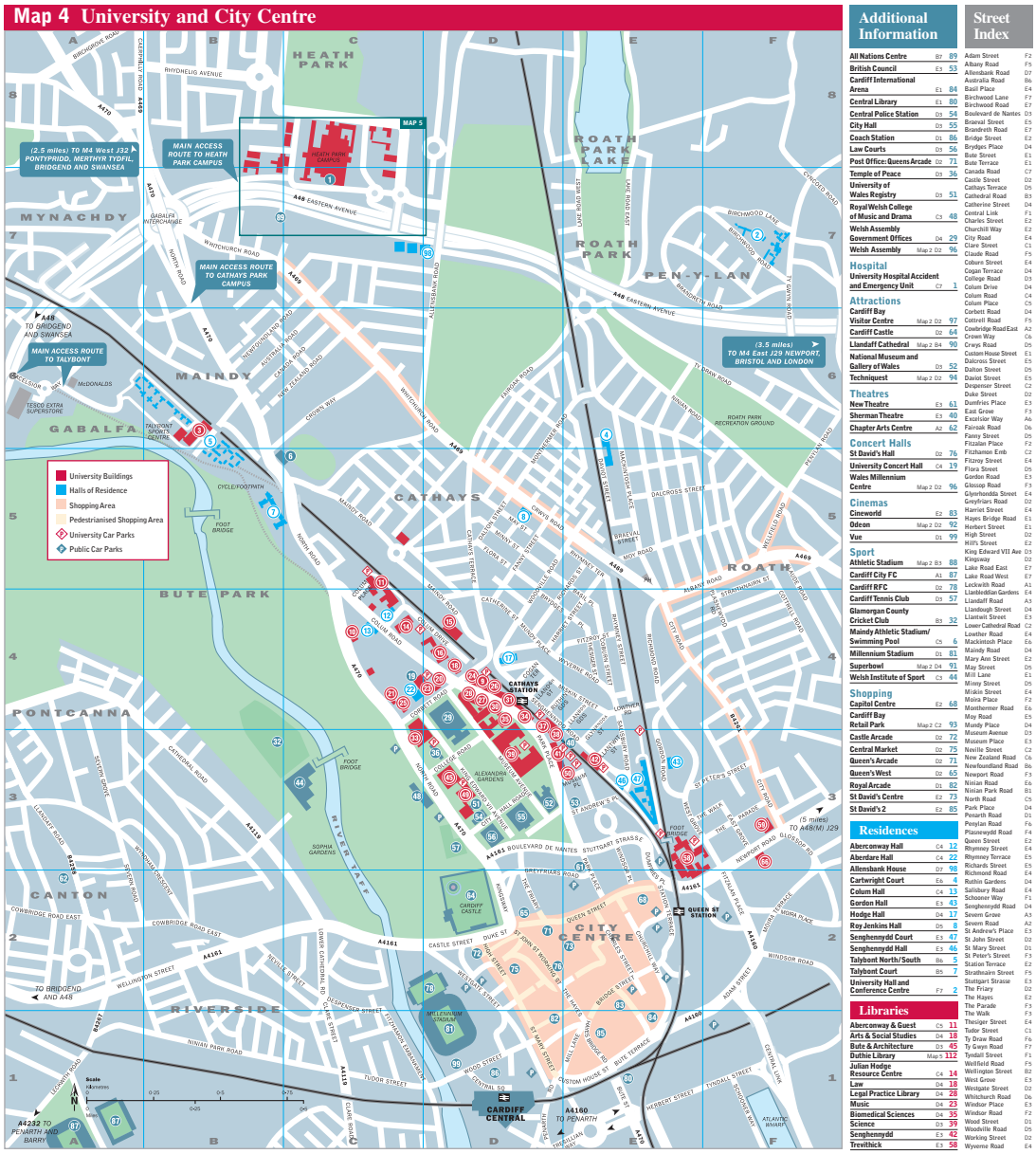
National Museum Cardiff
Cathays Park,
Cardiff
CF10 3NP

Gala Dinner:

Millennium Stadium
Westgate Street

Cardiff
CF10 1NS

Central Cardiff and Cardiff University Buildings:



Additional Information

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Aberdare Hall	C4	22
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Senghennydd Court	E3	47
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Talybont Court	B5	7
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Trevithick	E3	58

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Cathays Park Campus (Map 4)

Tel Switchboard: 029 2087 4000

Aberconway Building	C5	11
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Development and Alumni Relations	D3	41
Earth and Ocean Sciences	D3	39
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English Communication and Philosophy	D4	16
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Finance Division	F3	66
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Health Centre	D4	37
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Humanities Building	D4	16
Human Resources	F3	66
International Development and English Language Services	D3	41
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* Indicates main site

Heath Park Campus (Map 5)

Tel Switchboard: 029 2074 7747

The University shares the Heath Park Campus with the University Hospital of Wales (UHW).

Brecknock House		101
Cardiff Medicentre		102
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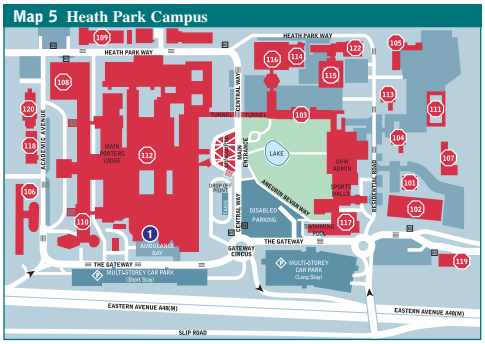
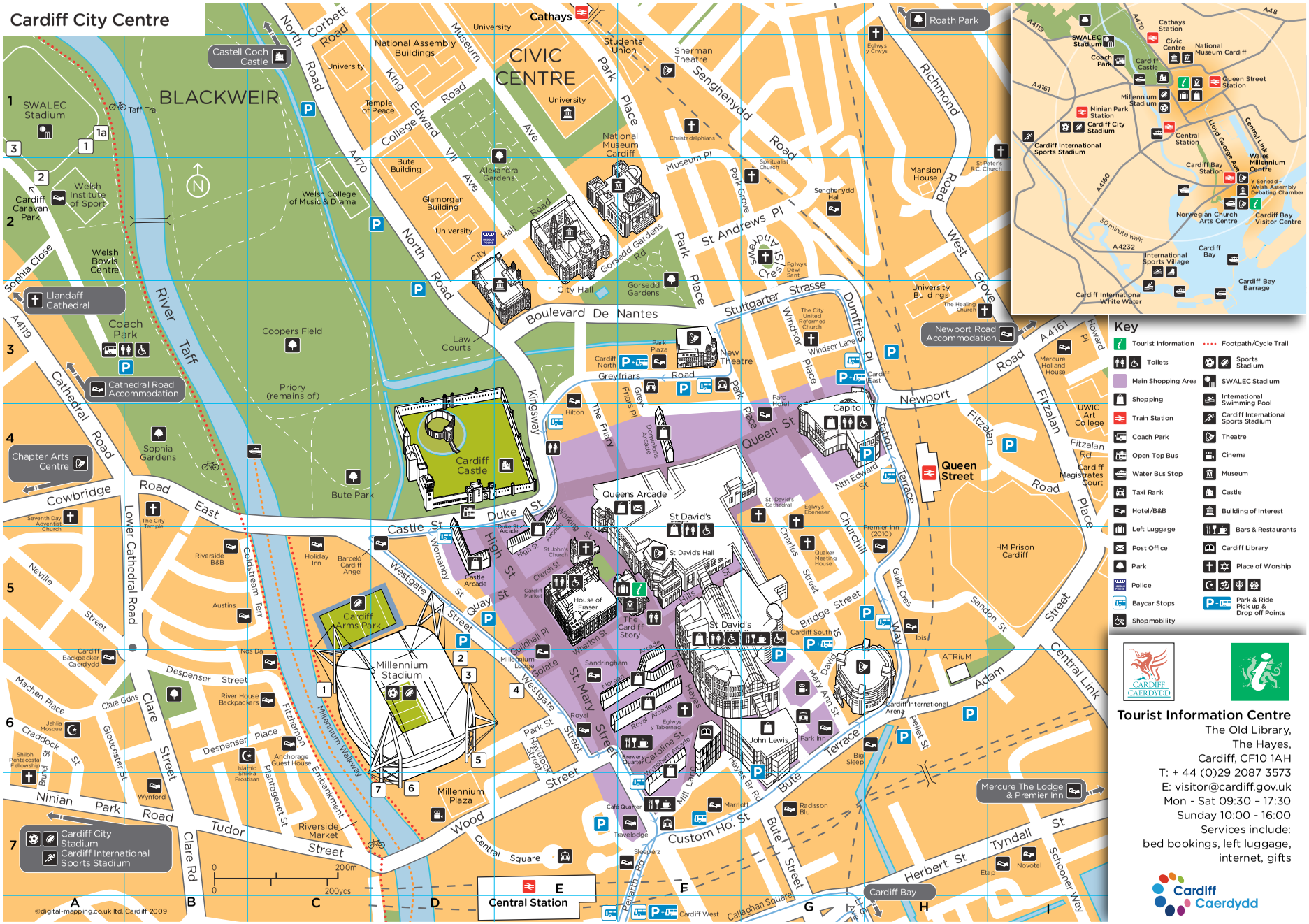


Central Cardiff (Close up):

Cardiff City Centre



Key

- Tourist Information
- Toilets
- Main Shopping Area
- Shopping
- Train Station
- Coach Park
- Open Top Bus
- Water Bus Stop
- Taxi Rank
- Hotel/B&B
- Left Luggage
- Post Office
- Park
- Police
- Baycar Stops
- Shopmobility
- Footpath/Cycle Trail
- Sports Stadium
- SWALEC Stadium
- International Swimming Pool
- Cardiff International Sports Stadium
- Theatre
- Cinema
- Museum
- Castle
- Building of Interest
- Bars & Restaurants
- Cardiff Library
- Place of Worship
- Park & Ride Pick up & Drop off Points

Tourist Information Centre

The Old Library,
The Hayes,
Cardiff, CF10 1AH
T: + 44 (0)29 2087 3573
E: visitor@cardiff.gov.uk
Mon - Sat 09:30 - 17:30
Sunday 10:00 - 16:00
Services include:
bed bookings, left luggage,
internet, gifts

Cardiff Caerdydd


Cardiff Bay
Central Station
Sleeperz
Cardiff West
Callaghan Square
Herbert St
Etap
Novotel
Mercure The Lodge & Premier Inn
Pendyris Street
Merry St
Shree Kutchi Leva Patel Samaj
Sri Swaminarayan Temple
New Hope Centre UPC
RIVERSIDE
Pentre Gardens
Cornwall Street Baptist Church
St Dyfrig & St Samson
Clare Road
Penarth Road
Corporation Road
Paget
A4119
A4160
Tresillian Terrace
Waterbus
Taff Embankment
Taff Trail
River Taff
Cardiff City Stadium
Cardiff International Sports Stadium
Grangetown
St Patricks R.C. Church
Orange Grove
Clive Street
St Pauls Church
St Fagans Street
Ty Rosa Boutique B&B
GRANGETOWN
Ebenezer Church
The Salvation Army
Grangetown Baptist Church
Acondale
Holmesdale Street
IKEA
Channel View Centre & Cardiff Bay Water Activity Centre
Ferry Road
Jim Driscoll Way
Hamadryad Park
River Taff
Cardiff Bay Yacht Club
A4232
CARDIFF BAY
Port of Cardiff
Cardiff Bay Barrage
Penarth Marina
Penarth
Cogan
Windsor Road
Nova Way
Terra Nova Way
Watkiss Way
A4055
International Drive
Olympian Drive
Ice Rink
Cardiff International Pool
International Sports Village
Empire Way
Cardiff Yacht Club
Pont y Werin (Summer 2010)
Cardiff International White Water (Spring 2010)
M4 J33 Cardiff Airport
Cardiff City Stadium
Cardiff International Sports Stadium
St Nicholas Orthodox Church
St Mary The Virgin
New Testament Church of God
Masjid I Noor
Canal Park
Dumballs Road
Bute Street
Lloyd George Avenue
A4232
City Centre
Express by Holiday Inn
ATLANTIC WHARF
Central Link
A4234
County Hall
Cardiff South (Saturday & Sunday only)
Schooner Way
Hemingway Road
Travelodge
Future Inn Cardiff
Red Dragon Centre
Doctor Who Up Close
Butetown Link
Craft in the Bay
Cardiff Bay
BayArt
BUTETOWN
Butetown History & Arts Centre
Coal Exchange
Mount Stuart Sq.
South Loudoun Pl
St Paul's Methodist Church
South Wales Islamic Centre
Bute Place
Wales Millennium Centre
Urdd City Sleepover
National Assembly Offices
Roald Dahl Plass
Jolyon's
Mermaid Quay
Pierhead Building
Y Senedd
Roath Basin
Lightship 2000
Cardiff Bay Visitor Centre (The Tube)
Norwegian Church Arts Centre
Techniquest
St. David's
Taff Trail Start/Finish
Environment Building
Drop Off On Request
Cardiff Harbour Authority Queen Alexandra House
Locks
Clarence Road
James Street
Adelaide Street
West Bute St
New George St
Stuart Street
Harbour Drive
Entrance Quay
Pierhead Street
Key
Tourist Information
Toilets
Changing Places
Road Train
Train Station
Open Top Bus
Water Bus Stop
Taxi Rank
Hotel/B&B
Post Office
Park
Police
Ice Rink
Footpath/Cycle Trail
Sports Stadium
International Swimming Pool
Theatre
Cinema
Museum
Building of Interest
Bars & Restaurants
Place of Worship
Park & Ride Pick up & Drop off
Baycar Stops
Water Sports
0 200m
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N
AA
BB
CC
DD
EE
FF
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Outline Timetable

	Sunday	Monday	Tuesday	Wednesday	Thursday
	Day 1 01-08-2010	**Day 2** 02-08-2010	**Day 3** 03-08-2010	**Day 4** 04-08-2010	**Day 5**05-08-2010
08.45 - 08.55	People coming in prior to this can collect rooms keys - details to follow REGISTRATION from 12pm	**Arrival**	**Arrival**	**Arrival**	**Arrival**
08.55- 09.45		**1 Greg Velicer** **PLENARY**	**21. Orion Weiner** **PLENARY**	**28. Darren Gilmour** **PLENARY**	**45. Eric Brown** **PLENARY**
09.45- 10.05		2. David Queller	22. Regina Teo	29. Magdalena Eder	46. Pierre Cosson
10.05- 10.25		3. Thomas Winckler	23. Louise Fets	30. Chris Sugden	47. Matthias Leippe
10.25-11.00		**Morning Break** **With coffee/tea**	**Morning Break** **With coffee/tea**	**Morning Break** **With coffee/tea**	**Morning Break** **With coffee/tea**
11.00 -11.20		4. Chris Thompson	24. Seiji Ura	31. Alan Kimmel	48. Hubert Hilbi
11.20 -11.40		5. Zhihui Chen	25. Börn Meier	32. Alex Chattwood	49. Thierry Soldati
11.40 -12.00		6. Gad Shaulsky	26. Arjan Kortholt	33. Doris Heinrich	50. Monica Hagedorn
12.00 -12.20		7. Gareth Bloomfield	27. Rick Firtel	34. Cathy Pears	51. Adam Kuspa
12.20 -13.20		**Buffet Lunch**	**Collect Packed Lunch**	**Buffet Lunch**	**Buffet Lunch**
13.20 -13.40		8. Richard Gomer	**Free Afternoon for Trips:** 1.Cardiff Castle and Bay 2.The Big Pit and Vineyard 3.The Brecon Beacons 4. Bath Spa	35. Robin S.B. Williams	52. Bill Loomis
13.40 - 14.00		9. Jason King		36. Sanjanie Fernando	53. Daniel Calovi
14.00 -14.20		10. Annette Müller-Taubenberger		37. Wanessa De Lima	54. Masashi Fukuzawa
14.20 -14.40		11. Ralph Gräf		38. Salvatore Bozzaro	55. Daniele Conte
14.40 - 15.00		12. Nick Lakin		39. Aurelie Gueho	56. Chi-Hung Siu
15.00 - 15.30		**Afternoon Break** **With coffee/tea**		**Afternoon Break** **With coffee/tea**	**Afternoon Break** **With coffee/tea**
15.30 - 15.50	**Buffet Available from 3pm**	13.David Knecht		40. Tian Jin	**57.** Robert Huber
15.50 = 16.10		14. Paul Steimle		41. Wouter-Jan Rappel	**58.** Ricardo Escalante
16.10 - 16.30		15 Alba Hykollari		42. Deborah Wessels	59. Michael Schleicher
16.30 - 16.50	**Welcome**	16 Laura Macro		43. Xiong Liu	60. Angelika Nögel
16.50 - 17.10	**Introductory Lecture By Steve Griffiths**	**Break for walk around**		44. Douwe Veltman	**Closing Comments**
17.10 - 17.25		17. Hideko Urushihara		* * * Pathostelium Workshop 5.30-7pm (optional)	**Free time to get ready?**
17.25 - 17.40		18. Petra Fey			
17.40 - 17.55	**Drinks reception**	19. Pascale Gaudet			
17.55 - 18.10		20. Open Discussion			
18.10 -18.30		Time to set up posters / Dictybase Advisory Board Meeting (until 20.30)		Time to set up posters	Walk down to the Stadium
18.30 - 19.00	Free Evening	**Poster Session I**		**Poster Session II**	Wine Reception at the Millennium Stadium Riverside Suite
19.00 - 20.00	End of The Big Admiral Weekend	& Dinner @7pm At National Museum of Wales, Cardiff		& Dinner @7pm At National Museum of Wales, Cardiff	Tours of the Stadium
20.00 - late		Session ends 10.30pm		Session ends 10.30pm	Gala Dinner, Prize Giving & Disco at the Millennium Stadium, Millennium Suite

Session Breakdown:

SESSION	Day	Talks	SESSION TITLE	CHAIR
Session 1	Monday	1-7	Evolution & Diversity	Pauline Schaap
Session 2		8-12	Cell Division	Harry MacWilliams
Session 3		13-16	Cell Biology I	Rex Chisholm
Session 4		17-20	Genome Resources Workshop	Wolfgang Nellen
Session 5	Tuesday	21-27	Chemotaxis I	Rob Kay
Session 6	Wednesday	28-34	Development I	Jeff Williams
Session 7		35-39	Biomedical	Paul Fisher
Session 8		40-44	Chemotaxis II	Robert Insall
Session 9	Thursday	45-51	Pathostelium	Ludwig Eichinger
Session 10		52-56	Development II	Jonathan Chubb
Session 11		57-60	Cell Biology II	Tomo Abe

Plenary speakers with generous support from specific sponsors:

Session 1: Evolution & Diversity; Talk 1	Greg Velicer	FORMEDIUM™
Session 5: Chemotaxis; Talk 21	Orion Weiner	BSCB BRITISH SOCIETY FOR CELL BIOLOGY
Session 6: Development; Talk 28	Darren Gilmour	Roche
Session 9: Pathostelium; Talk 45	Eric Brown	DMM Disease Models & Mechanisms dmm.biologists.org

= Bell 2 minutes before session starts, or before delegates are leaving Julian Hodge Building for the Museum etc

Day-by-Day Timetable:

Sunday 1st August 2010 – Day 1

Location – Julian Hodge Building (unless otherwise stated):

12.00 -15.00	**Registration from 12pm**
15.00 – 16.30	Buffet Food available on arrival (from 3pm)
16.30 – 16.50	**Welcome: Dicty 2010 Committee**
16.50 – 17.40	**Introductory Lecture by Steve Griffiths**
17.45 – 19.00	Drinks reception in Julian Hodge Lounge
19.00 – late	Free Evening End of The Big Admiral Weekend

Monday 2nd August 2010 – Day 2

Location – Julian Hodge Building (unless otherwise stated)

08.45 – 08.55	**Arrival**
Session 1. Evolution and Diversity **Chair: Pauline Schaap**	
08.55- 09.45	**PLENARY:** **1. Greg Velicer** A small RNA controls Myxococcus development and mediates a major social adaptation.
09.45- 10.05	**2. David Queller** The evolutionary importance of chimerism and cheating
10.05- 10.25	**3. Thomas Winckler** Initial characterization of glorin-mediated gene expression in Polysphondylium pallidum
	Morning Break **With coffee/tea**
11.00 -11.20	**4. Chris Thompson** A simple genetic basis for complex social behaviour

11.20 -11.40	**5. Zhihui Chen** A prokaryote diguanylate cyclase gained a novel role as a secreted signal that induces fruiting body formation in Dictyostelium
11.40 -12.00	**6. Gad Shaulsky** A matching pair of tgrB1 and tgrC1 genes is necessary and sufficient for attractive self-recognition during the establishment of multicellularity in Dictyostelium
12.00 -12.20	**7. Gareth Bloomfield** Three genes specify the three sexes of Dictyostelium discoideum
12.20 -13.20	**Buffet Lunch**
Session 2. Cell Division **Chair: Harry MacWilliams**	
13.20 -13.40	**8. Richard Gomer** A Dictyostelium chalone uses G proteins and RasG to inhibit proliferation
13.40 - 14.00	**9. Jason King** The mitotic activation of SCAR and its role in myosin II-independent cytokinesis
14.00 -14.20	**10. Annette Müller-Taubenberger** Regulation of the kinase NdrC is crucial for cytokinesis
14.20 -14.40	**11. Ralph Gräf** Molecular characterization of NE81, the first lamin-like nucleoskeleton protein in a unicellular organism
14.40 - 15.00	**12. Nick Lakin** Mechanisms of DNA double strand break repair in Dictyostelium
15.00 – 15.30	**Afternoon Break** **With coffee/tea**
Session 3. Cell Biology I **Chair: Rex Chisholm**	
15.30 - 15.50	**13. David Knecht** The organization of the actin cytoskeleton by actin binding proteins.
15.50 - 16.10	**14. Paul Steimle** Cellular and Biochemical Characterization of a Development Specific Myosin II Heavy Chain Kinase
16.10 – 16.30	**15. Alba Hykallori** Elucidation of the N- glycan structures of selected glycosylation mutants, axenic and natural isolates of Dictyostelium discoideum
16.30 – 16.50	**16. Laura Macro** The dynamics of clathrin and AP-2 during endocytosis
16.50 – 17.10	**Break for walk around**

Session 4. Genome Resources Workshop **Chair: Wolfgang Nellen**		
17.10 - 17.25	**17. Hideko Urushihara** Genome structure and gene models of Acytostelium subglobosum	
17.25 - 17.40	**18. Petra Fey** dictyBase 2010: D. purpureum database, gene curation update, and orthologs	
17.40 - 17.55	**19. Pascale Gaudet** dictyBase: Community participation in curation	
17.55 - 18.10	**20. Open Discussion**	
18.10 -18.30	Time to set up posters (Go to Museum)	dictyBase Advisory Board Meeting (until 20.30) (Museum Augustus and Gwen John Suite)
18.30 - 19.00	**Poster Session I** **& Dinner @7pm** **At National Museum of Wales, Cardiff** Session ends 10.30pm	
19.00 - 20.00		
20.00 - late		

Tuesday 3rd August 2010 - Day 3

Location - Julian Hodge Building (unless otherwise stated)

08.45 - 08.55	**Arrival**
Session 5. Chemotaxis I **Chair: Rob Kay**	
08.55- 09.45	**PLENARY** **21. Orion Weiner** Spatial control of signaling during chemotaxis
09.45- 10.05	**22. Regina Teo** Multiple roles of PI(4,5)P2 during Dictyostelium chemotaxis
10.05- 10.25	**23. Louise Fets** PI(4,5)P2 is a Key Regulator of Chemotaxis
10.25-11.00	**Morning Break** **With coffee/tea**
11.00 -11.20	**24. Seiji Ura** Dephosphorylation of SCAR induces its accumulation and promotes pseudopod splitting

11.20 -11.40	**25. Börn Meier** Dynamics of cellular repolarization in spatiotemporally varying gradient fields
11.40 -12.00	**26. Arjan Kortholt** Ras and Rap signaling during cell polarity and chemotaxis
12.00 -12.20	**27. Rick Firtel** Regulation of leading edge function
12.20 -13.00	**Collect Packed Lunch**
13.00 – late	**Free Afternoon** **or one of the Trips** Coaches leave at 12.45-13.00 1.Cardiff Castle and Bay 2.The Big Pit and Vineyard 3.The Brecon Beacons 4. Bath Spa

Wednesday 4th August 2010 – Day 4

Location – Julian Hodge Building (unless otherwise stated)

08.45 – 08.55	**Arrival**
Session 6. Development I **Chair: Jeff Williams**	
08.55- 09.45	**PLENARY** 28. **Darren Gilmour** "A slug inside a fish" - shaping organs through collective migration
09.45- 10.05	**29. Magdalena Eder** Response of Dictyostelium discoideum to micropatterned materials
10.05- 10.25	**30. Chris Sugden** The Dictyostelium SH2 domain protein LrrB is a regulator of prestalk differentiation and slug behaviour
10.25-11.00	**Morning Break** **With coffee/tea**
11.00 -11.20	**31. Alan Kimmel** High Throughput Chemical and Genomic Screens to Identify New Factors Involved in Dictyostelium Differentiation
11.20 -11.40	**32. Alex Chattwood** Antagonistic roles for RasD and RblA in the regulation of cell fate bias and DIF-1 responsiveness
11.40 -12.00	**33. Doris Heinrich** Cell Motility in Surface-Structured Environments
12.00 -12.20	**34. Wolfgang Nellen** Chromatin Organisation in Dictyostelium
12.20 -13.20	**Buffet Lunch**

Session 7. Biomedical **Chair: Paul Fisher**	
13.20 -13.40	**35. Robin S.B. Williams** Dictyostelium as a basic biomedical model: investigating targets and new treatment for epilepsy
13.40 – 14.00	**36. Sanjanie Fernando** Alpha-Synuclein cytotoxicity in Dictyostelium.
14.00 -14.20	**37. Wanessa De Lima** Role of mucolipin calcium channel in the endocytic pathway of Dictyostelium discoideum
14.20 -14.40	**38. Salvatore Bozzaro** Another pathogen for Dictyostelium cells: Salmonella thyphimurium and its subversion of the starvation response
14.40 - 15.00	**39. Aurelie Gueho** Characterisation of M. marinum niches : establishment of an isolation procedure.
15.00 – 15.30	**Afternoon Break** **With coffee/tea**
Session 8. Chemotaxis II **Chair: Robert Insall**	
15.30 - 15.50	**40. Tian Jin** The coupling mechanism of a GPCR and heterotrimeric G-proteins that control chemoattractant gradient sensing in Dictyostelium
15.50 - 16.10	**41. Wouter-Jan Rappel** Gradient sensing in defined chemotactic fields
16.10 – 16.30	**42. Deborah Wessels** Dissecting the Calcium Response Pathway
16.30 – 16.50	**43. Xiong Liu** Expression of Actin Tyr53Ala in Dictyostelium Disrupts the Cytoskeleton and Inhibits Intracellular and Intercellular cAMP-Signaling
16.50 – 17.10	**44. Douwe Veltman** Actin nucleation: WASP family proteins divide the labour.
17.10 - 17.25	Free Time For non-Pathostelium Club / Free Time **Pathostelium Workshop 5.30-7pm** **(Optional)**
18.10 -18.30	Time to set up posters (Go to Museum)
18.30 – 19.00	**Poster Session II** **& Dinner @7pm** **At National Museum of Wales, Cardiff**
19.00 – Late	Session ends 10.30pm

Thursday 5th August 2010 – Day 5

Location – Julian Hodge Building (unless otherwise stated)

08.45 – 08.55	**Arrival**
Session 9. Pathostelium **Chair: Ludwig Eichinger**	
08.55- 09.45	**PLENARY** **45. Eric Brown** Mycobacterium marinum infection in macrophages, fish, and mice.
09.45- 10.05	**46. Pierre Cosson** Evolution of Pseudomonas aeruginosa virulence in infected patients revealed in a Dictyostelium discoideum host model
10.05- 10.25	**47. Matthias Leippe** From amoebae to higher organisms: Ancient effector proteins with functions in nutrition and immunity
10.25-11.00	**Morning Break** **With coffee/tea**
11.00 -11.20	**48. Hubert Hilbi** Dictyostelium signaling complexes on Legionella-containing vacuoles
11.20 -11.40	**49. Thierry Soldati** Mechanisms of mycobacteria interference with cell-intrinsic immunity processes
11.40 -12.00	**50. Monica Hagedorn** Differentiation as a cure for infection
12.00 -12.20	**51. Adam Kuspa** The requirement of TirA for bacterial defense during growth, and for Sentinel cell production of 'extracellular traps' during development in D. discoideum.
12.20 -13.20	**Buffet Lunch**
Session 10. Development II **Chair: Jonathan Chubb**	
13.20 -13.40	**52. Bill Loomis** SDF-1 Signaling in Preparation for Terminal Differentiation
13.40 – 14.00	**53. Daniel Calovi** A Green's functions and boids based model of Dictyostelium discoideum
14.00 -14.20	**54. Masashi Fukuzawa** Evidence for two distinct subtypes of pstA-cells and characterization of a transcription factor that mediates pstA cell differentiation

14.20 -14.40	**55. Daniele Conte** A light controlled switch between different fates during Dictyostelium development
14.40 - 15.00	**56. Chi-Hung Siu** cadA Is a Single-Gene Green Beard that Regulate Morphogenesis through Differential Cell Adhesion in Dictyostelium
15.00 – 15.30	**Afternoon Break** **With coffee/tea**
Session 11. Cell Biology II **Chair: Tomo Abe**	
15.30 - 15.50	**57. Robert Huber** DdEGFL1-enhanced cell motility in Dictyostelium functions via calcium signaling independently of the cAMP-mediated signaling pathways
15.50 = 16.10	**58. Ricardo Escalante** Autophagy in Dictyostelium: a model for the identification of new autophagic proteins
16.10 – 16.30	**59. Michael Schleicher** The Ste20-like kinase DstC has a cortical localization and is involved in phagocytosis
16.30 – 16.50	**60. Angelika Nögel** Coronin proteins
16.50 – 17.10	**Closing Comments**
17.10 - 17.25 17.25 - 17.40 17.40 – 17.55	**Free time to get ready?**
17.55 – 18.10 18.10 -18.30	Walk down to the Stadium
18.30 – 19.00	**Wine Reception at the Millennium Stadium - Riverside Suite**
19.00 – 20.00	**Tours of the Stadium**
20.00 – late	**Gala Dinner, Prize Giving & Disco until 12.45am** **At the Millennium Stadium, Millennium Suite**

Summary of Speakers:

Evolution & Diversity
1 Greg Velicer - Plenary
2 David Queller
3 Thomas Winckler
4 Chris Thompson
5 Zhihui Chen
6 Gad Shaulsky
7 Gareth Bloomfield

Cell Division
8 Richard Gomer
9 Jason King
10 Annette Müller-Taubenberger
11 Ralph Gräf
12 Nick Lakin

Cell Biology I
13 David Knecht
14 Paul Steimle
15 Alba Hykollari
16 Laura Macro

Genome Resources Workshop
17 Hideko Urushihara
18 Petra Fey
19 Pascale Gaudet
20 Open Discussion

Chemotaxis I
21 Orion Weiner - Plenary
22 Regina Teo
23 Louise Fets
24 Seiji Ura
25 Börn Meier
26 Arjan Kortholt
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Development I
28 Darren Gilmour - Plenary
29 Magdalena Eder
30 Chris Sugden
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32 Alex Chattwood
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Biomedical
35 Robin SB Williams
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Abstracts:

Talk 1 PLENARY Talk (Evolution and Diversity)

A small RNA controls Myxococcus development and mediates a major social adaptation

Presented by Greg Velicer
Yuen-Tsu Nicco Yu, Xi Yuan and Gregory J. Velicer
Department of Biology, Indiana University, Bloomington, IN 47405

Non-coding small RNA (sRNA) molecules regulate a vast array of processes in biology, but evidence for evolutionary adaptations mediated by mutation of sRNA elements has been indirect. Here we identify the first regulatory sRNA, Pxr, in the fruiting myxobacteria, and demonstrate that Pxr negatively controls fruiting body development in Myxococcus xanthus. We further show that a spontaneous mutation in Pxr abolished its regulatory function and thereby adaptively restored developmental proficiency to a socially defective "cheater" strain of M. xanthus. In wild-type M. xanthus, development is initiated only upon starvation, but deletion of the pxr gene allows development and spore production to proceed at high nutrient levels. Thus, Pxr serves as a major checkpoint controlling the transition from growth to development in the myxobacteria.

Talk 2

The evolutionary importance of chimerism and cheating

Presented by David Queller
David C. Queller, Joan E. Strassmann

Social amoebas can form chimeric fruiting bodies, allowing one clone to cheat another with respect to who forms the sterile stalk. Numerous genes affect cheating, but we want to understand whether chimerism and cheating have been important in the evolutionary history of Dictyostelium. As with most model systems, it can be difficult to understand behavior in the field, but we have used several approaches. First, different clones are found in close proximity to each other in soil, suggesting they could join in the same fruiting body. However, fruiting bodies collected from dung were usually, though not always, clonal. Part of this results from recognition and segregation of clones that initially aggregate together. Though recognition currently reduces chimerism, having recognition makes sense only if it is important to exclude other clones or to treat them differently, suggesting that chimerism has been historically important.

We also conducted a number or indirect tests. 1. The tendency of chimeric slugs to travel less far is consistent with them having evolved to avoid the front pre-stalk region. 2. Unhealthy cells (too little glucose or too much acid) tend to go to stalk, consistent with it being a competitive process. 3. Social genes tend to evolve more rapidly than other genes, consistent with

arms races due to conflict. 4. For a novel kind of test, we conducted a mutation accumulation experiment, putting 90 cell lines through about 1000 generations and 70 single-cell bottlenecks. The effect of the bottlenecks is to reduce the effect of selection and allow mutations to accumulate. Traits that have been selected and maintained by selection should show a decline when random mutations accumulate. We found this to be true for the ability to cheat, consistent with it being a trait maintained by selection. Though most of these findings have possible alternative explanations, In aggregate they suggest that cheating has been important in the evolutionary history of Dictyostelium.

Talk 3

Initial characterization of glorin-mediated gene expression in Polysphondylium pallidum

Presented by Thomas Winckler
Asma Asghar (1), Marco Groth (2), Christoph Enzensperger (2), Friedemann Gaube (1), Oliver Siol (1), Thomas Winckler (1)
(1) School of Biology and Pharmacy, Institute of Pharmacy, Department of Pharmaceutical Biology, University of Jena (Germany
(2) Fritz-Lipmann-Institute for Age Research, Jena (Germany)
(3) School of Biology and Pharmacy, Institute of Pharmacy, Department of Pharmaceutical/Medicinal Chemistry, University of Jena (Germany)

The monophyletic evolution of the social amoebae (Dictyostelia) and their periodic transition from uni- to multicellularity provides a valuable model to study the development and adaptation of intercellular communication systems. Published data of experiments mainly performed on Polysphondylium violaceum has suggested that polysphondylids coordinate aggregation by using an intercellular communication system based on the dipeptide acrasin glorin. However, the recent observation that, within the Dictyostelia clade, the taxon Polysphondylium is polyphyletic has raised the question when the glorin-mediated communication has been invented and how widespread it is among the social amoebae. We used a modified cellophane square chemotaxis assay to survey the response of social amoebae from the phylogenetic groups 1-4 towards glorin and cyclic AMP. As expected, we found that species from the youngest group 4 (Dictyostelium discoideum group) responded to cAMP but not to glorin. In our assay, P. violaceum, which is phylogenetically more related to group 4 species than to other Polysphondylia, responded strongly to glorin, but also weakly to cAMP. Communication with glorin or peptides closely related to glorin seems to be deeply conserved in social amoeba evolution. We confirmed previous reports that P. pallidum (group 2) responds to glorin, but not to cAMP, and found that glorin responsiveness is a property common to polysphondylids, but not acytostelids. We point out that many Dictyostelium species organized in the most ancient group 1 (D. fasciculatum group) responded to glorin in our assay. Taking advantage of the recently completed reference genome sequence of P. pallidum we used Illumina next generation sequencing

technology to show that glorin mediates both induction and repression of a number of genes at the transition from vegetative growth to aggregation.

Talk 4

A simple genetic basis for complex social behaviour
Presented by Chris Thompson
Katie Parkinson1, Neil J. Buttery1, Jason B. Wolf2 and Christopher R.L. Thompson1
1 Faculty of Life Sciences, Michael Smith Building, University of Manchester, Oxford Rd, Manchester, M13 9PT, UK
2 Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath, BA2 7AY, UK

The evolution of cooperative behaviour is counterintuitive because natural selection should favour individuals that cheat by not paying their fair share of any costs. Competition between cheaters and altruists is therefore expected to result in complex social strategies due to an evolutionary arms race akin to the Red Queen process. The social amoeba Dictyostelium discoideum shows such complex strategies, including facultative behaviours that change in interactions with foreign clones. We have generated a mathematical model which shows clonal variation in signal intensity and signal response are sufficient to generate apparent facultative behaviour during chimeric development, without the need for partner recognition. Consistent with this idea, we find that a single gene mutation can lead to complex facultative changes in behaviour. Furthermore, simple measurements of developmental signal production and response during clonal development are sufficient to predict behaviour during partner specific social competition. This is true whether variation is caused by the perturbation of a single gene that acts through a developmental signaling system or the complex variation derived from a natural population.
These studies therefore suggest that complex social strategies can evolve from a simple underlying mechanism governed by modulation of the production of - and response to - behavioural signals.

Talk 5

A prokaryote diguanylate cyclase gained a novel role as a secreted signal that induces fruiting body formation in Dictyostelium
Presented by Zhihui Chen
Zhihui Chen and Pauline Schaap

Starvation triggers the development of Dictyostelium cells. At the late developmental stages the process that prestalk cells differentiate into the stalk tube is essential to the fruiting body formation, which requires coordination of cellulose synthesis by all cells in the centre of the fruiting structure. It is not yet known how this is regulated. Dictyostelium has a cellulose synthase (DcsA) which is more similar to bacterial than plant

cellulose synthases. Cellulose synthases in some bacteria are regulated by c-di-GMP. This compound is produce by diguanylate cyclases with a characteristic GGDEF domain.

Dictyostelid social amoebas are the only eukaryotes with a prokaryote GGDEF protein. To understand its functions we have knocked out the gene coding for the GGDEF protein in D. discoideum. ggdef- mutants develop normally into migrating slugs, but cannot form fruiting bodies and migrate until exhausted. This is somewhat similar to the dcsa- phenotype, but unlike dcsa- mutants, which are only defective in forming spore and stalk cell walls, the ggdef- mutants do not express spore and stalk genes. Overexpression of a bacterial c-di-GMP phosphodiesterase does not phenocopy a ggdef- mutant, suggesting that Dictyostelium GGDEF does not produce c-di-GMP. Dictyostelium GGDEF is expressed in prestalk cells and is secreted or released after cleavage from its N-terminal transmembrane domain. The secreted peptide restores fruiting body formation in ggdef- cells. In conclusion, GGDEF is a novel secreted signal that triggers fruiting body formation. It is possibly an evolutionary relic that originally controlled cell wall construction by cellulose synthase, but gained a novel role in Dictyostelid evolution.

Talk 6

A matching pair of tgrB1 and tgrC1 genes is necessary and sufficient for attractive self-recognition during the establishment of multicellularity in Dictyostelium
Presented by Gad Shaulsky
Shigenori Hirose, Rocio Benabentos, Adam Kuspa and Gad Shaulsky

Natural isolates of D. discoideum segregate from each other, depending on their genetic distances (Ostrowski et al., 2008 PLoS Biol. 6:e287) and this correlates with the degree of polymorphism observed in the tgrB1-tgrC1 gene pair (Benabentos et al., 2009 Curr Biol. 19:567-72). We have now tested the role of tgrB1 and tgrC1 in self-recognition by generating single and double gene-replacements of alleles from wild strains into the AX4 laboratory strain. We found that only pairs of tgrB1 and tgrC1 genes from the same wild strain allow normal development of AX4 and are both necessary and sufficient for self-recognition during aggregation and mound formation. We then examined chimeras of single-gene replacement strains, and tgrB1/tgrC1 merodiploid strains, to test the cellular basis of the self-recognition mechanism. We found evidence for mutual attraction between cells that express complementary sets of alleles, and no evidence for rejection between cells that express non-matching sets.

Talk 7

Three genes specify the three sexes of Dictyostelium discoideum
Presented by Gareth Bloomfield
Gareth Bloomfield and Robert R Kay

Most eukaryotic species possess two sexes, with one gamete of each type needed to enter into every sexual cross. Dictyostelium discoideum has three sexes, each of which is able to mate with each of the others. We recently identified the genetic locus that determines the sex of each amoeba. It is complex, containing one, two or three genes between unvarying flanking sequences. However we find that for each sex only one gene is required to fully specify mating behaviour when introduced into a mat null background. Two of these genes, matC and matS, are homologues, but the other, matA, is completely unrelated in sequence. All encode small, cytoplasmic proteins with no recognisable similarity to genes outside of Dictyostelid mating loci. We are now attempting to discover how these novel sex-determining genes function, and are assessing whether they can be used to provide new tools for Dictyostelium genetics.

Talk 8

A Dictyostelium chalone uses G proteins and RasG to inhibit proliferation.

Presented by Richard Gomer
Deenadayalan Bakthavatsalam1, Parvin Bolourani2, Sarah Herlihy3, Gerry Weeks2, and Richard H. Gomer1,3
1 Department of Biochemistry and Cell Biology, Rice University, Houston, TX USA,
2 Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada, and
3 Department of Biology, Texas A&M University, College Station, TX USA

In mammals, the sizes of tumor metastases and some tissues may be regulated by negative feedback loops in which autocrine secreted factors called chalones inhibit proliferation. Little is known about chalones, in part because attempts at purification have generally failed, and even less is known about how cells sense chalones. Two secreted proteins, AprA and CfaD, function as Dictyostelium chalones. Cells lacking AprA or CfaD proliferate faster than wild type cells, and adding recombinant AprA (rAprA) or rCfaD to cells slows proliferation. We previously found that AprA uses a signal transduction pathway involving Galpha8. We have now found that CfaD uses a signal transduction pathway involving Galpha1 and RasG. Galpha1 and RasG do not affect AprA signaling, indicating that AprA and CfaD use separate signal transduction pathways. AprA and CfaD require each other to inhibit proliferation. The existence of separate signals and pathways suggests that previous attempts to purify chalones may have failed because two separate signals, acting as message authenticators for each other, are needed to function as a chalone.

Talk 9

The mitotic activation of SCAR and its role in myosin II-independent cytokinesis

Presented by Jason King
Jason S. King, Marios Georgiou, Douwe Veltman, Buzz Baum and Robert H. Insall

Cell division requires the tight coordination of multiple cytoskeletal pathways. The best understood of these involves myosin II-dependent constriction around the cell equator but both Dictyostelium and mammalian cells also use a parallel, adhesion-dependent mechanism to generate furrows. We show that the actin nucleation factor SCAR/WAVE is strongly activated during cytokinesis generating large polar protrusions and driving separation of the daughter cells. This continues for 10 minutes after division before the daughter cells revert to normal random motility, indicating that this is a tightly regulated process. We demonstrate that SCAR activity is essential to drive myosin II-independent cytokinesis, and stabilises the furrow, ensuring symmetrical division. SCAR is also responsible for the generation of MiDASes (mitosis-specific actin-rich adhesions). Loss of SCAR in both Dictyostelium and Drosophila leads to a similar mitotic phenotype, with severe mitotic blebbing, indicating conserved functionality. We also find that the microtubule end-binding protein EB1 is required to restrict SCAR localisation and direct migration. EB1-null cells also exhibit decreased adhesion during mitosis. Our data reveal a spindle-directed signalling pathway that regulates SCAR activity, migration and adhesion at mitosis.

Talk 10

Regulation of the kinase NdrC is crucial for cytokinesis
Presented by Annette Müller-Taubenberger
Peter M. Kastner,Parvin Bolourani,Michael Schleicher,Gerald Weeks, Annette Müller-*Taubenberger*

Regulation of cell division has been subject to numerous studies, but the underlying molecular mechanisms are still largely unknown. Recently, we described a SIN (septation initiation network)-homologous pathway which is required for cytokinesis in Dictyostelium discoideum. Upstream components of the SIN-pathway localize to centrosomes like the polo-like kinase Plk, the GAP Bub2, the GTPase Spg1 and the kinase SepA. Since NDR (nuclear Dbf2-related) kinases are very likely to act as potential downstream effectors of SepA, we have characterized three of the four NDR kinases of D. discoideum. Sequence comparisons indicate that NdrA and NdrB correspond to the human kinases NDR1 and NDR2 that were shown to play a role in centrosome duplication, while NdrC and NdrD are related to the large tumor suppressors LATS1 and LATS2.
During the course of our studies of NDR kinases we have identified NdrC (Lats2) to be crucial for the regulation of cell division in D. discoideum. Deletion of NdrC caused impaired cell division and loss of centrosome integrity. Interaction studies identified a Ras binding domain in NdrC. Further in vitro and in vivo assays revealed that both RasB and RasG bind to and regulate NdrC. These Ras proteins have been shown previously to be involved in the regulation of cytokinesis. We now present an updated model how

NdrC/Lats2 and members of the oncogenic Ras family GTPases, RasB and RasG, may be implicated in the regulation of cell division.

Reference:
Müller-Taubenberger, A., Ishikawa-Ankerhold, H. C., Kastner, P. M., Burghardt, E., and G. Gerisch. 2009. The STE group kinase SepA controls cleavage furrow formation in Dictyostelium. Cell Motil. Cytoskel. 66, 929-939.

Talk 11

Molecular characterization of NE81, the first lamin-like nucleoskeleton protein in a unicellular organism
Presented by Ralph Gräf
Anne Krüger, Otto Baumann, Eva Luckert, Irene Meyer, Ralph Gräf

Lamins build the nuclear lamina and are required for mechanical stabilization of cells, chromatin organization, gene expression, cell cycle progression and cell migration. Despite these functions of universal importance, lamins have so far only been found in metazoans. With Dictyostelium NE81 we have now identified a protein, whose properties justify denomination as a lamin-like protein in a lower eukaryote. This based on its primary structure, its localization in live cells and immuno EM preparations, its regulation during mitosis and the requirement of the C-terminal CaaX box as a post translational processing signal for proper localization of the protein. Our knock out and overexpression mutants revealed an important role of NE81 for nuclear integrity, chromatin organization and mechanical stability of cells. All our results are in agreement with a role of NE81 in formation of a nuclear lamina that allow the nucleus to serve as a mechanical support for the cytosolic cytoskeletal elements. The discovery of a lamin-like protein in a unicellular organism is not only very interesting in the light of evolution, it could also provide a simple experimental platform for studies of the molecular basis of laminopathies.

Talk 12

Mechanisms of DNA double strand break repair in Dictyostelium.
Presented by Nick Lakin
Rhian Kiely, Duen-Wei Hsu, Claudia Couto, Jessica Hudson, Catherine Pears and Nicholas Lakin

DNA is under continual assault from a variety of agents that cause DNA damage. As such cells have evolved a DNA damage response (DDR) that detects, signals and repairs DNA damage to restore genome integrity. The importance of the DDR is underscored by the findings that disruption of these pathways leads to gross chromosome instability and a variety of clinical outcomes including increased cancer risk.

DNA double strand breaks (DSBs) are considered the most pathological variety of DNA damage. They can be repaired by two principle pathways, homologous recombination (HR) or non-homologous end joining (NHEJ). Whilst HR requires sequences homologous to the damaged DNA to facilitate repair, NHEJ is achieved by limited processing and direct ligation of DNA termini. Recently, we and others found that components of the human NHEJ pathway absent from commonly used invertebrate DNA repair model organisms are conserved in Dictyostelium. Here we report further characterisation of the Dictyostelium NHEJ pathway. We illustrate that restriction enzyme mediated integration (REMI) of DNA into the Dictyostelium genome is dependent on NHEJ. We use REMI as an assay to establish that Dclre1, a protein with similarity to the human NHEJ protein Artemis, is required for NHEJ. In addition, experiments that asses how a cell determines whether to repair a DSB by NHEJ or HR will be discussed.

Talk 13

The organization of the actin cytoskeleton by actin binding proteins.
Presented by David Knecht
David A. Knecht, Ran-der Hwang, Michael Lemieux

Different actin binding proteins (ABPs) are proposed to organize actin filaments into orthogonal, parallel and anti-parallel arrays in order for the cell to carry out specific mechanical functions. Since all of three types of ABPs are present in cells, it is unclear how different forms of actin can be created if all ABPs bind to new actin filaments. We have shown that the CH-domain actin binding proteins filamin and alpha-actinin have overlapping localization in unpolarized cells, but localize to completely distinct parts of the cytoskeleton in polarized cells. The actin binding domains of these proteins are sufficient to recapitulate these patterns as long as the dimerization status of the proteins is maintained. We have extended these results by examining a variety of different actin binding proteins and show that there is a distinct spatiotemporal order of localization of incorporation into the cytoskeleton. Examining macropinosome cups, phagocytic cups and polarized cells, we find that some proteins bind as new actin filaments are polymerized, while others arrive later. In polarized cells, the different ABPs are generally either in the front or back of the cell, however some proteins have unique distributions. These results suggest that actin filament networks mature over time based upon the composition of actin binding proteins that are associated. One of the challenges of this work is determining the distribution of the F-actin itself. We have found that expression of probes for F-actin alter the amount and localization of F-actin, and as a result alter cell behavior. We have developed a new Lifeact-based fluorescent probe that appears to associate with F-actin while causing minimal changes in the cytoskeleton. Our model of cytoskeletal dynamics proposes that newly polymerized filaments at the front of the cell associate initially with ABPs that are prevalent free in the cytoplasm due to their lower binding affinity. As filaments age and move rearward, the catalog of bound ABPs shift to

higher affinity proteins and this transition alters the functionality of the posterior cytoskeleton.

Talk 14

Cellular and Biochemical Characterization of a Development Specific Myosin II Heavy Chain Kinase

Presented by Paul Steimle
Jonathan Greene, Travis Russell, Paul Steimle
University of North Carolina at Greensboro, Department of Biology, Greensboro, North Carolina, USA.

Contraction-dependent processes such as cytokinesis and cell migration rely on the proper assembly and localization of myosin II bipolar filaments. In Dictyostelium, as well as in mammalian cells, myosin II filament disassembly can be driven by phosphorylation of the myosin II heavy chain (MHC)-tail. MHC phosphorylation in Dictyostelium is catalyzed by at least three kinases (MHCK-A, -B, and -C) that share homologous-kinase catalytic and WD-repeat domains. Another enzyme, MHCK-D, is structurally similar to the other MHCKs, but appears to have little effect on myosin II filament turnover in vegetative cells. In the current study, we examined the cellular and biochemical characteristics of MHCK-D. We found that over-expression of MHCK-D slows suspension growth, with cells becoming large and multinucleated over time. Localization of GFP-tagged MHCK-D is uniform throughout the cytoplasm in both nonmotile and chemotaxing cells, and remains unchanged upon uniform stimulation of cells with cAMP. RT-PCR revealed that MHCK-D expression is, in contrast to the other MHCKs, essentially absent in vegetative cells, but is induced during development with peak expression around 16h after starvation. Moreover, affinity purified MHCK-D phosphorylates MHC to ~3 mol/mol MHC and in manner that leads to myosin II filament disassembly. We conclude that MHCK-D is a bone fide MHCK that is likely to play a central role in myosin II filament turnover during development.

Talk 15

Elucidation of the N- glycan structures of selected glycosylation mutants, axenic and natural isolates of Dictyostelium discoideum

Presented by Alba Hykollari
Alba Hykollari, Katharina Nöbauer, Ebrahim Razzazi-Fazeli, Iain B. H. Wilson

The examination of the N-glycans of Dictyostelium discoideum has been a challenge for over 30 years. During its life cycle different changes in the N-glycome take place; there is a shift of the major N-glycan structures from the unicellular to the multicellular stages of development. For the elucidation of the structures various approaches (reverse and normal phase HPLC, MALDI-TOF mass spectrometry and MS/MS) were employed.

In the work of our and other laboratories, a range of oligomannosidic structures (e.g., Man8GlcNAc3-4Fuc0-1) were found to contain modifications such as core alpha 1,3 fucose or intersecting and/or bisecting N-acetylglucosamine residues.

The fucosyltransferase required for the biosynthesis of the core ± 1,3-fucose modification requires GDP-fucose as donor - this substrate can be synthesized by two pathways, a de novo and a salvage pathway. The HL250 (modC) mutant is defective in the former pathway due to a defect in the GDP-mannose dehydratase (gmd) gene and so cannot incorporate fucose into the glycans of cells grown in liquid medium unless exogenous fucose is added.

Two other N-glycosylation mutants were analysed: HL241 and modA. The former strain accumulates Hex5-6GlcNAc2 in both cells grown in liquid medium and in fruiting bodies; the reduced size of the glycans in comparison to those of the AX3 strain is putatively due to a mutation in a mannosyltransferase gene required for synthesis of the dolichol linked oligosaccharide precursor. The modA mutant, which has a mutation in the alpha 1,3-glucosidase II gene, also altered oligomannosidic N-glycans, with compositions such as Hex11GlcNAc2; in this case no fucose is present and the same N-glycans occur in cells and fruiting bodies. Finally, other strains such as the natural isolate NC4, the axenic form of NC4 (NC4A2) and AX4 were also analysed. We found that there are slight differences regarding glycosylation patterns between the axenic and non-axenic strains.

The study of the alteration of the N-glycans present in several glycosylation mutants of D. discoideum, will help us to find out more about the function of the defective enzymes and the biosynthesis of key intermediates in the N-glycosylation pathway.

This work was supported by the FWF P19615-B11.

Talk 16

The dynamics of clathrin and AP-2 during endocytosis

Presented by Laura Macro

Laura Macro & Sanford M. Simon
Laboratory of Cellular Biophysics, The Rockefeller University, 1230 York Ave, New York, USA.

Clathrin-mediated endocytosis (CME) is a conserved process by which eukaryotic cells internalize proteins from the plasma membrane. Two key molecules involved are the coat protein clathrin and the adaptor complex AP-2. In Dictyostelium clathrin is involved in many processes that require membrane trafficking such as osmoregulation, cytokinesis and fluid-phase endocytosis. However, evidence of internalization of specific plasma membrane cargo by CME in Dictyostelium is lacking. In Dictyostelium AP-2 has previously been shown in fixed cells to co-localize with clathrin in puncta which are believed to be sites of coated vesicle formation. We have used live cell imaging to study the dynamics of these puncta. We fluorescently

tagged clathrin or AP-2 and observed their behavior by total internal reflection fluorescence microscopy (TIR-FM). We found that these proteins localize to distinct membrane puncta which are highly dynamic, with all puncta disappearing from the cell surface within six minutes. Simultaneous imaging of clathrin and AP-2 shows that these proteins co-localize and that they disappear together, providing strong evidence that puncta of AP-2 and clathrin are sites of

CME in Dictyostelium. These findings can be extended to study the physiological role of CME during single and multicellular stages of development.

Talk 17

Genome structure and gene models of Acytostelium subglobosum.
Presented by Hideko Urushihara
Hideko Urushihara, Hidekazu Kuwayama, Takehiko Itoh, Atsushi Toyoda, Tateaki Tanigichi, Hideki Noguchi, Hiroshi Kagoshima, Tadasu Shin-I, Kensuke Fukuhara, Yoko Kuroki, Yuji Kohara, Asao Fujiyama

We have been performing genome and cDNA analyses of Acytostelium subglobosum, one of the group 2 species that constructs fruiting bodies with acellular stalks. Since cell-type differentiation does not occur in this species, its genomic differences from Dictyostelium and Polysphondylium species should contain key information related to the history for the system of somatic cell differentiation to have been established. Although our preliminary examination had suggested that A. subglobosum possessed orthologous genes for most of the developmental genes identified in D. discoideum, we were aware that some of the gene family did not have the full set of counterparts for family members. Nor were we confident that orthologous genes carried out the same function in the same pathway. To know exactly what genes were missing in A. subglobosum, we continued genome analysis including employment of SOLEXA sequencing, and now obtained 31.5 Mbp sequence in total extension, longer than roughly estimated genome size. We also determined internal sequences of cDNA, and the deduced natures of A. subglobosum coding sequences, such as codon usages and intron distribution, were used for ab initio gene modeling. Approximately 12,500 genes were predicted, 40% of which did not overlap with Dicty-based gene models we previously constructed. Thus, altogether 14,000 CDS were mapped on the draft genome. Summary of the A. subglobosum gene repertoire and possibility for functional analyses will be discussed.

Talk 18

dictyBase 2010: D. purpureum database, gene curation update, and orthologs
Presented by Petra Fey

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dictyBase recently released the D. purpureum database (http://genomes.dictybase.org/purpureum), the first additional genome in a planned multi-genome environment. The genome consists of 838 unassembled contigs, of which 794 harbor 12,410 genes that can be viewed in the genome browser. If selected, ESTs and D. discoideum orthologs are aligned when available. Entries on each track link to their respective gene- or sequence pages. All D. purpureum sequences are available for download. A redesigned dictyBase website facilitates easy transition from one organism database to another, and a universal BLAST server allows easy access to sequences of all available organisms.

We have revised our gene model curation procedures with the goal of completing a first-pass annotation of D. discoideum genes by the end of 2010. We have categorized uncurated genes into different groups according to their available support (agreement of different gene predictions, ESTs, RNAseq data, D. purpureum ortholog), and prioritized annotation based on this data. We will present gene curation updates, describe our curation practices and show a new tool that helps us to process gene annotations more efficiently.

We have classified and annotated nearly 500 transposable elements. The gene IDs of retrotransposons and putative DNA transposons now carry _RTE and _TE extensions to allow easy identification and querying. This will also enable us to display transposable elements on a separate track in the genome browser in the future.

The gene page now also contains a section of orthologs from 6 different species, Homo sapiens, Mus musculus, Drosophila melanogaster, Caenorhabditis elegans, Saccharomyces cerevisiae, and, Escherichia Coli. The orthologs are displayed in a table that includes the gene product, the source of the orthology assignment and a link to each ortholog's UniProt record.

Talk 19

dictyBase: Community participation in curation
Presented by Pascale Gaudet
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Biological databases such as dictyBase, UniProt, and GenBank, have become common tools for researchers. The data published in papers are read by curators, and converted to standardized formats for integration in databases. Data from annotated papers can thus be accessed from a wide variety of

resources, which increases visibility of the published findings. It is thus to the advantage of researchers to have their papers annotated in a timely fashion.

The limited resources of publically funded databases don't allow all papers to be curated immediately upon publication, and sometimes, not at all. To address this problem, many groups, including dictyBase, have employed mechanisms to integrate annotations provided by researchers. We have implemented simple forms for curation of strains, phenotypes, and Gene Ontology (GO). In a trial several researchers have been asked to do first-pass curation of gene names, products, and GO annotation. We will introduce GO in a short tutorial and present the community annotation forms.

Besides increasing the curation rate in dictyBase, we also hope to increase community feedback in general and user education regarding the use of the GO and other bioinformatics tools.

Talk 20

Open Discussion:

Talk 21 PLENARY Talk (Chemotaxis)

Spatial control of signaling during chemotaxis
Presented by Orion Weiner
Anselm Levskaya, Andrew Houk, Alexandra Jilkine, Jared Toettcher, Delquin Gong, Steven Altschuler, Lani Wu, Chris Voigt, Wendell Lim, Orion Weiner

Our research aims to develop novel tools to perturb and measure the detailed spatiotemporal dynamics of the signaling molecules that orchestrate cell polarity. We aim to move beyond a parts list of components involved in migration to a systems level understanding of cell polarity. We recently developed a genetically encoded system that uses light to control protein localization with micrometer spatial resolution and temporal resolution on the order of seconds. This approach gives us unprecedented control of signaling on a subcellular level. The light-gated protein-protein interaction optimized in these studies should be useful for the design of diverse light-programmable reagents, potentially enabling a new generation of perturbative, quantitative experiments in cell and developmental biology. In our most recent work, we use a combination of experimental and theoretical approaches to define the mechanisms used by leukocytes to establish a unique axis of polarization during cell movement.

Talk 22

Multiple roles of PI(4,5)P2 during Dictyostelium chemotaxis

Presented by Regina Teo
Regina Teo, Jonathan Ryves and Adrian J Harwood

We have previously shown that synthesis of the phospholipid $PI(4,5)P_2$ is required for generation of $PI(3,4,5)P_3$, and important for cAMP-mediated chemotaxis (King et al 2009 DMM 2:306-312). To investigate further, we examined $PI(4,5)P_2$ synthesis following cAMP stimulation of developing *Dictyostelium*. We find that PIP_2 synthesis is stimulated by cAMP, peaking 10-20 seconds after cAMP addition and is sensitive to lithium.

To manipulate PIP_2, we over-expressed phospholipase C (PLC). Interestingly, we found that PLC over-expressing cells chemotax apparently normally. Treatment with the PI3kinase inhibitor LY294002 or lithium, which lowers $PI(3,4,5)P_3$ via depletion of $PI(4,5)P_2$, causing a strong chemotaxis phenotype. This phenotype causes a large drop in chemotactic index (CI) and hence is far stronger than those caused by loss of $PI(3,4,5)P_3$ synthesis, such as in PI3 kinase mutants. These results indicate that in addition to supplying the substrate for PI3 kinases, $PI(4,5)P_2$ is required for other signalling events that mediate chemotaxis.

Talk 23

PI(4,5)P2 is a Key Regulator of Chemotaxis
Presented by Louise Fets
Louise Fets & Rob Kay

Proteins that alter the turnover of cellular phosphoinositides are ubiquitous in chemotactic signal transduction, from Dictyostelium to mammals. Until recently, it was thought that the primary role of this group of proteins was to generate internal gradients of PI(3,4,5)P3, creating an intracellular 'chemotactic compass'. It is now known that in the absence of PI(3,4,5)P3 gradients, cells can chemotax to cAMP with near wild-type efficiency, indicating the potential importance of other members of this lipid family. To test this idea, we disrupted a gene involved in the turnover of PI(4,5)P2.

Loss of this gene generates cells with a severe impairment in gradient sensing and a significantly reduced chemotactic index, despite being able to move at speeds comparable to wild-type cells. The mutant cells express the cAMP receptor apparently normally, but signal transduction downstream of PKB/PKBR1 is almost completely abolished, indicating that PI(4,5)P2 is a key, early regulator of signalling in response to external chemotactic cues.

Talk 24

Dephosphorylation of SCAR induces its accumulation and promotes pseudopod splitting
Presented by Seiji Ura
Seiji Ura, Robert H. Insall

SCAR is a member of WASP family of proteins which promote actin polymerization by activating the Arp2/3 complex. SCAR is involved in multiple cellular processes, especially indispensable in pseudopod extension and split in streaming Dicty cells. However, it is still unclear how SCAR activity is regulated during these processes. We have demonstrated that SCAR is constitutively phosphorylated in its acidic region, which Arp2/3 complex binds to. Although both unphosphorylatable and phosphomimetic mutants of SCAR rescued the growth defect of vegetative SCAR-null cells, neither mutants rescued the ability of developed cells to split pseudopods. Unphosphorylatable SCAR showed hyperaccumulation of SCAR complex in pseudopod tips, and massive polymerization of actin filaments, which resulted in continuous pseudopod extension without splits. In contrast, phosphomimetic SCAR could only form thin pseudopods after splits, and the pseudopod extension was mainly driven by blebbing, similar to SCAR-null cells. These observations indicate that SCAR dephosphorylation amplifies the amount of activated SCAR to the required level to expand the splitting pseudopod to normal size. We also observed that the expression level of unphosphorylatable SCAR rapidly decreased during development, which suggests that dephosphorylated SCAR is mainly inactivated by degradation. Taken together, our data imply a key role of dephosphorylation in SCAR activity regulation during the pseudopod cycle.

Talk 25

Dynamics of cellular repolarization in spatiotemporally varying gradient fields

Presented by Börn Meier
B. Meier, A. Zielinski, C. A. Weber, D. Arcizet, T. Franosch, J. O. Rädler, D. Heinrich

Embryonic cell differentiation, inflammatory response, growth of neurons or food gathering of amoeba are all based on the directed response of eukaryotic cells to an external chemical gradient. We have developed a microfluidic chamber that enables us to manipulate migration properties of Dictyostelium discoideum in a spatio-temporally controlled gradient field. This experimental assay generates bidirectional chemical gradients over a width of more than 100 µm and timescales from seconds to several hours.
For slow alternations of the cAMP gradient direction (<0.01Hz), 70% of the cells show reversal of their migration direction resulting in alternating directed runs. However, for high switching rates (>0.1Hz), we find a ‘chemically trapped’ cell state, characterized by stalled migration. Furthermore, the definition of a fluorescence dipolar moment of the LimE-Gfp protein distribution allows for quantitative time dependent description of cellular repolarization in response to a reversal of gradient direction. The fluorescence dipolar moment of this actin polymerization label exhibits a characteristic down time separating two phases of opposing polarization. In a next step, imaging of chemotactic key players will enable us to resolve intracellular feedback events on a broader scale, identifying time scales and interconnections in chemotactic feedback.

The parallel exposure of entire cell ensembles to tunable chemical gradient fields, in combination with statistical analysis on the single cell level, makes this function generator applicable to the stochastic aspects in feedback schemes of cellular sensing, polarization and motility.

Talk 26

Ras and Rap signaling during cell polarity and chemotaxis
Presented by Arjan Kortholt
Arjan Kortholt, Ineke Keizer-Gunnink, Wouter N. Van Egmond, and Peter J.M. Van Haastert.
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Chemotaxis involves activation of surface receptors, symmetry breaking to generate a front, and amplification to sense very shallow gradients. Recent studies have shown that chemotaxis does not depend on a single molecular mechanism, but rather depends on several interconnecting pathways. So far four major signaling enzymes have been implicated in chemotaxis, PI3K, TorC2, PLA2 and sGC. Surprisingly, small G-proteins of the Ras subfamily appear to play essential roles in all these pathways. Thus far, Ras is the most upstream component of the signalling pathway that shows stronger activation at the leading edge than the steepness of the gradient, suggesting that symmetry breaking and/or amplification of the gradient occurs at or before Ras activation. To understand the molecular mechanisms of chemotaxis we have investigate the Ras and Rap signaling cascade in more detail.

Talk 27

Regulation of leading edge function
Presented by Rick Firtel
Sebastian Kicka, Zhouxin Shen, Sarah J. Annesley, Susan Lee, Paul R. Fisher, Steven Briggs and Richard A. Firtel

We have identified a new kinase signaling pathway that is required for leading edge protrusion. The pathway controls F-actin polymerization and involves GTPases normally thought to regulate vesicular transport. Epistatis studies combined with biochemical analysis and proteomics provide evidence that this new pathway helps control cell motility in Dictyostelium and is most likely conserved in higher cells.

Talk 28 PLENARY Talk (Development)

"A slug inside a fish" - shaping organs through collective migration.
Presented by Darren Gilmour
Darren Gilmour

The collective migration of epithelial cohorts or tissues is a hallmark of organogenesis, wound repair and many metastatic cancers. The zebrafish lateral line primordium is a migrating epithelial tissue that becomes assembled into a series of rosette-like mechanosensory organs en route, a process that is highly reminiscent of Dictyostelium slug migration. We exploit the imaging and experimental strengths of this model to address the cell biology of tissue migration in intact embryos. Previous genetic studies have shown that an extrinsic stripe of the chemokine SDF1 controls the behaviour of 'leader' cells at the tissue edge, whereas internal 'follower' cells are organised into organs through the activity of an internal FGF-signaling circuit. I will present our recent work addressing how these and other signals are integrated to coordinate cell polarity and movement within migrating tissues.

Talk 29

Response of Dictyostelium discoideum to micropatterned materials
Presented by Magdalena Eder
Magdalena Eder, Eduard Arzt, Ingrid M. Weiss

Dictyostelium discoideum was grown on gecko-inspired micropatterned surfaces and multicellular development was studied by time-lapse video microscopy (1). Micropatterned polymer surfaces have adhesive properties, which, in contrast to conventional tape, are reversible and bear the potential for switchability (2). These properties combined with biocompatible materials make gecko adhesives good candidates for various biomedical applications. Previous studies have shown that single cells are very sensitive to the geometry of pillars and other material properties (3). This motivated us to study the influence of micropatterned pillar surfaces on the differentiation and multicellular development of Dictyostelium. Time lapse and environmental scanning electron microscopy showed that Dictyostelium altered its development and differed in terms of shape and size of accumulated cells when grown on micropatterned surfaces. For example, cell differentiation was arrested in particular stages of development and stalks tended to adhere to the micropatterned surface. Developmental variation depended on several effects, amongst them the pillar size and the hydrophobicity of the material. The results show that Dictyostelium discoideum may be used as a convenient model organism for studying multicellular tissue formation on biomedical materials in vivo and that the pattern geometry may be correlated with extracellular matrix composition and particular signaling molecules.

References:
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2. M. Kamperman, E. Kroner, A. d. Campo, R. M. McMeeking, E. Arzt, Advanced Engineering Materials 12, 335 (2010).
3. N. Q. Balaban et al., Nature Cell Biology 3, 466 (2001).

Talk 30

The Dictyostelium SH2 domain protein LrrB is a regulator of prestalk differentiation and slug behaviour

Presented by Chris Sugden

Chris Sugden, Christian Cole, Geoff Barton, Sarah Annersley, Paul Fisher and Jeffrey G. Williams

SH2 domain adapter proteins commonly mediate the interactions of effector molecules but thus far none have been characterised in Dictyostelium signalling pathways. LrrB contains two protein-protein interaction domains, a leucine-rich repeat and an SH2 domain [1]. There is also a potential 14-3-3 binding site near the C terminus and LrrB binds to 14-3-3 in a developmentally regulated manner. While, the significance of this interaction is unknown, the SH2 domain is known to be required for interaction with CldA, another SH2 domain protein. CldA also contains a Clu domain. The first Clu domain protein to be described was Dictyostelium CluA [2], the eukaryotic mitochondrial clustering protein, but the LrrB null has a normal mitochondrial distribution. Three genes, cinB, tatD and abcG10, are, however, greatly under-expressed in both the LrrB and CldA null mutants. Here we describe the late phenotypes of the LrrB null (lrrB-). The pstO region of the slug is aberrant and LrrB- cells are excluded from the prestalk region when co-developed with parental cells. Slug responsiveness, a function of the prestalk zone, is also defective in the mutant. When developed in the dark the mutant cells arrest their development as a mound. When developed in the light migratory slugs are formed but these are highly defective in phototaxis and thermotaxis. Array and transcriptomics analysis reveal several alterations in the level of gene expression in LrrB- slugs. One of the genes under-expressed is the pre-basal disc marker, ecmB. Concordantly, the mutant fruiting bodies lack a basal disc. This is a morphogenetic hallmark of mutants defective in signalling by DIF, the polyketide that induces prestalk cell differentiation, and the null mutant is indeed defective in DIF responsiveness. During early development LrrB interacts physically and functionally with CldA. However, the CldA- mutant does not phenocopy the LrrB null in its multicellular development, suggesting that the early and late functions of LrrB are effected in different ways. The defects in development of the LrrB- mutant are reversed when the parental form of LrrB is expressed but not when a mutant with a point change that inactivates the SH2 doman is expressed. These data suggest that, in addition to its early functions, LrrB is an adaptor protein in one or more SH2 domain signaling pathways necessary for correct prestalk patterning and functionality.

References:

1. Sugden, C. et al., Two novel Src Homology Domain proteins interact to regulate Dictyostelium gene expression during growth and early development. J Biol Chem., in press.
2. Zhu, Q.L., et al., The cluA(-) mutant of Dictyostelium identifies a novel class of proteins required for dispersion of mitochondria. Proc. Natl. Acad. Sci. USA, 1997. 94: p. 7308-7313.

Talk 31

High Throughput Chemical and Genomic Screens to Identify New Factors Involved in Dictyostelium Differentiation

Presented by Alan R. Kimmel
Xin-Hua Liao and Alan R. Kimmel
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The molecular mechanisms that direct cellular differentiation in Dictyostelium are complex. We have utilized unique biological aspects to develop high-throughput chemical and genomic screens to identify novel regulatory pathways. Separately, we miniaturized and optimized chemotactic/aggregation and sporulation assays in 1536-well plate formats, using GFP expression as the initial reporter. Both assays have been validated by counter screening for toxic compounds and by dose-response analyses using known inhibitors in the pathways (e.g. latrunculin B). To date, we have screened ~4,000 compounds from a small molecule library for activators and inhibitors of sporulation and for inhibitors of chemotaxis. Several of the identified compounds (e.g. PDE inhibitors, GABA modulators) are known regulators of Dictyostelium development; other compounds have presumptive targets in mammalian cells, which will assist their biochemical evaluation in Dictyostelium. Finally, we have designed experiments that combine random mutagenesis, FACS, and deep sequencing for whole-genome genetic screenings against a similar background. These large-scale applications will systematically discover new factors/pathways involved in Dictyostelium differentiation and further explore the potential for shared mechanisms among the complex metazoa.

Talk 32

Antagonistic roles for RasD and RblA in the regulation of cell fate bias and DIF-1 responsiveness
Presented by Alex Chattwood

Alex Chattwood 1, Koki Nagayama 1, Parvin Boularani 2, Gerry Weeks 2 & Christopher Thompson 1

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The generation of pattern is one of the most important features of multicellular development. One idea gaining recognition is pattern formation in the absence of positional information. In this, different cell types differentiate within an identical signalling environment before sorting out to form discrete tissues. Current models cannot explain how symmetry is broken under these conditions because the amount of signal received by each individual cell is the same. However, fate choice is possible if the cells interpret the signal they receive differently.
Dictyostelium discoideum provides an excellent model with which to study this patterning mechanism. It is thought that different prestalk cell types arise scattered in response to the diffusible signalling molecule DIF-1 (DIF). Those cells that respond to DIF are thought to be intrinsically more DIF sensitive due to biases

caused by growth history (e.g. nutrition, cell cycle, etc). One clue as to how biases operate at a molecular level has come from studies of the rblA- mutant. From these studies, RblA has been proposed to be a central mediator of cell fate bias. Surprisingly, however, we have found that careful examination of bias effects (using cell type specific markers) caused by nutritional status and RblA mutation actually showed considerable differences. Most notably, although both affect DIF dependent pstB cell differentiation only mutation of RblA affects DIF dependent pstO cell differentiation. These studies therefore suggest that biased decisions exhibit greater molecular complexity and cell type specificity than might have been expected.
We therefore devised a genetic selection to identify novel regulators of cell fate bias in which the interpretation of nutritional bias was affected. One such mutant contained a disruption of GefE, an activator of RasD. Cell type marker expression studies reveal GefE regulation to be sufficient to explain nutritional bias. We also show that GefE and RblA antagonistically regulate DIF dependent pstB cell differentiation in normal development but have distinct roles in other cell types. DIF response assays on rblA- and gefE- mutant strains suggest they antagonistically control the threshold at which a DIF-1 sensitivity switch can be turned on/off. We therefore propose a mechanism whereby DIF signalling drives and maintains differentiation in several cell types based upon the activity of multiple bias switches. In this way, diverse cellular responses can be integrated via a common signal.

Talk 33

Cell Motility in Surface-Structured Environments

Presented by Doris Heinrich
Felix Keber, Carolin Leonhardt and Doris Heinrich

Cellular adhesion and migration are governed by intracellular feedback schemes in combination with external stimuli, which influence the nano-scale reorganisation of the cytoskeleton architecture. Recent advances have been made in examining cell adhesion behaviour on flat surfaces. Still, we lack profound knowledge on cell migration processes in 3D micro- and nano-structured environments, where living cells sense mechanical properties of their environment and especially motile cells react to the surrounding 3D topographical conditions.

Here, we investigate influences of defined 3D mechanical stimuli, like pre-patterned surface topography, on the cytoskeleton reorganisation and the induced actin pattern generation in living cells. We fabricate well-defined micro-structured substrates, consisting of pillar arrays with varying pillar distance, diameter, and density to study the influence of the substrate topography on cell velocity, motion persistence, and branching morphology of the cells. We aim at controlling and predicting cellular migration in this predefined 3D environment.

Our results indicate that the substrate topography significantly influences Dictyostelium discoideum cell motility. To analyze migrational phenomena in obstacle arrays, we developed an algorithm [1] to extract cell motility states, distinguishing directed and non-directed motion. A combined angle,

velocity, and mean square displacement (MSD) analysis reveals that the cells move faster and more directed on a flat substrate, compared to their motion within several pillars. Splitting the cell trajectories into sections classified as directed or non-directed sub-trajectories shows, that the network shape of the pillar array can be identified in the angle distribution of the directed path sections.

Further, we will concentrate on identifying intracellular signaling, which triggers cellular reaction upon contact with 3D topography.

Reference:
[1] D. Arcizet, B. Meier, E. Sackmann, J. Rädler, and D. Heinrich, Temporal analysis of active and passive transport in living cells. Phys. Rev. Lett. 101, 248103 (2008)

Talk 34
Chromatin Organisation in Dictyostelium
Presented by Wolfgang Nellen
Located at the evolutionary branchpoint between plants and animals, Dictyostelium has become a useful model organism to study chromatin organisation and epigenetics. We have established an extensive catalog of histone modifications and find a wide overlap with modifications present in higher organisms. The composition of centromeres and their dynamics during the cell cycle was determined. With the identification of a CenH3 homolog we determined the loading time of centromeres at the G2 - prophase transition which is in contrast to loading in metazoans during telophase - G1. Interestingly, transgenes that are frequently integrated in multicopy tandem arrays tend to form inefficient pseudo-centromeres that share some features with centromeres but fail to function as genuine microtubule attachment sites.

We have further analysed the heterochromatin protein HP1 which is mostly associated with centromeres in Dictyostelium. Preliminary analysis shows that posttranslational modifications of HP1 are essential for its function and presumably affect dimerisation of the protein

Talk 35

Dictyostelium as a basic biomedical model: investigating targets and new treatment for epilepsy
Presented by Robin SB Williams
Lucy M Elphick, Pishan Chang, Manik Dham, Benoit Orabi, Nadine Pawolleck, Leila Chaieb, Frauke Haenel, Irina A Guschina, Rania M. Deranieh, John L Harwood, Miriam L. Greenberg, Markus Maniak, Matthew C. Walker and Robin S.B. Williams

Many biomedical conditions prove problematic to investigate in human or animal studies, due to difficulties in manipulating aspects of molecular cell

biology and biochemistry. We have developed Dictyostelium as a simple model system to investigate the effects of the world's most highly prescribed epilepsy treatment, valproic acid (VPA). Research into the mechanism(s) of this compound span 46 years, but it still remains unclear how the drug works. We have found that, in parallel to animal studies, VPA regulates fatty acid turnover, and we describe our advances in understanding this mechanism. We have also discovered a novel mechanism of action for VPA in regulating phosphoinositide signalling, and this latter effect has lead to the identification of a family of novel compounds that we show gives efficacy in seizure control. These two mechanisms provide distinct modes of action for VPA and this work will thus help to disentangle complex cellular mechanism of VPA in the treatment of epilepsy.

Talk 36

Alpha-Synuclein cytotoxicity in Dictyostelium.
Presented by Sanjanie G. Fernando
Sanjanie G. Fernando, William A. Burrage, Sarah J. Annesley, Paul R. Fisher

Alpha-synuclein is implicated in the pathogenesis of Parkinson's disease (PD) which is a neurodegenerative disorder. Hyperexpression or mutations in the alpha-synuclein gene are believed to be associated with mitochondrial abnormalities and onset of familial PD. To establish a Dictyostelium model for PD and to investigate the role of mitochondrial dysfunction in its cytopathology, we created stable, clonal Dictyostelium transformants hyperexpressing these normal and mutant forms of alpha-synuclein. All three forms were cytotoxic and showed that the protein impairs phagocytosis and growth on bacterial lawns. We have also used immunofluorescence and confocal microscopy to show that alpha-synuclein is concentrated in the membrane particularly at the leading edge of the cell (resembling the presynaptic membrane in neurons) which is the same subcellular location as in human cells. However, it did not colocalize or associate with mitochondria. Further phenotypic characterisation showed that the aberrant phenotypes caused by alpha-synuclein are distinct from those observed in mitochondrial disorders in Dictyostelium. This indicates that there are distinct alpha-synuclein cytotoxicity pathways that differ from those associated with mitochondrial dysfunction.

Talk **37**

Role of mucolipin calcium channel in the endocytic pathway of Dictyostelium discoideum
Presented by Wanessa de Lima
Wanessa de Lima and Pierre Cosson
Dept of Cell Physiology and Metabolism, University of Geneva, Switzerland

Mucolipidosis type IV is a lysosomal storage disorder caused by alterations in the mucolipin protein, a lysosomal integral membrane channel presumably

involved in calcium transport. This lysosomal calcium channel may be a key factor during lysosome exocytosis, a process in which secretory lysosomes fuse to the cell surface. In this study, a mucolipin knockout was generated in Dictyostelium discoideum and its phenotype analyzed, providing new insights on the role of mucolipin in the endo-lysosomal pathway. In mucolipin mutant cells, the general organization of the endosomal pathway is normal when compared to wild type cells, indicating that the composition and structure of endosomes are unaffected. Contrary to the abnormal lysosomal pH observed in knockout mammalian cells, Dictyostelium mutant cells have completely normal values of lysosomal and post-lysosomal pH. However, lysosome exocytosis is markedly increased. In addition, post-lysosomal calcium concentration is significantly lower in the mucolipin mutant when compared to wild type cells. The data altogether suggests that mucolipin may be acting as a negative regulator of fusion events in the endosomal pathway, by controlling calcium fluxes into endocytic compartments.

Talk 38

Another pathogen for Dictyostelium cells: Salmonella thyphimurium and its subversion of the starvation response

Presented by Salvatore Bozzaro

Salvatore Bozzaro, Alessio Sillo, Jan Matthias, Roman Konertz and Ludwig Eichinger**

The list of invasive bacteria pathogenic for both Dictyostelium and macrophages includes sofar L. pneumophila, M. marinum and avium, N. meningitidis and, conditionally, K. aerogenes.

Salmonella typhimurium, the agent of food-borne gastroenteritis and thyphoid fever, is phagocytosed by both macrophages and Dictyostelium cells. In macrophages, S. thyphimurium escapes degradation and grows intracellularly.

By using a mix of cell biological assays and global transcriptional analysis with DNA microarrays, we show that S. typhimurium is pathogenic also for Dictyostelium cells. Depending on the degree of virulence, which in turn depended on bacterial growth conditions, Salmonella could kill Dictyostelium cells or inhibit their growth and development.

In the early phase of infection in non-nutrient buffer, the ingested bacteria escaped degradation, causing a starvation-like transcriptional response. However, expression of genes required for chemotaxis and aggregation, whose expression is normally regulated by starvation and cAMP signaling, was selectively inhibited. Some evidence suggests that this was due to a bacterial factor that interfered with the establishment of the cAMP relay.

By inhibiting development of the aggregation competence, differentiation of the host cells into spore and stalk cells is blocked or delayed, thus favouring establishment of a replicative niche for Salmonella.

Talk 39

Characterisation of M. marinum niches: establishment of an isolation procedure.

Presented by Aurelie Gueho

Aurelie Gueho and Thierry Soldati

Tuberculosis remains a public health issue and still kills 2 million people each year. Mycobacterium tuberculosis, the agent responsible for this disease, is able to manipulate the phagocytes of the innate immune system of its host. After uptake by phagocytosis, it stops the maturation of the phagosome where it resides and establishes a niche where it can proliferate.

Mycobacterium marinum, a fish pathogen and a close cousin to M. tuberculosis, is able to infect the social amoeba Dictyostelium discoideum and arrest phagosome maturation. Dictyostelium normally uses phagocytosis for nutrition and its phagocytic pathway is very similar to the macrophage one. Therefore, we make use of this host-pathogen system to characterise the virulence mechanisms and the manipulation of the phagocytic pathway. In particular, we want to study the impact of M. marinum on the composition of the compartment that evolves into a proliferation niche.

For that, we have established a protocole to isolate pure fractions of phagosomes containing the pathogenic strain M. marinum or the non-pathogenic strain Mycobacterium smegmatis or the avirulent strain M. marinum-L1D.

Our protocole is based on the low density of latex. We have optimised the fixation of latex beads on different mycobacteria strains to form bacteria+beads complexes (BBCs). After verification that Dictytyostelium is able to phagocytose those BBCs, we have observed that the M. marinum present in the BBCs are still infectious and that the fate of the BBC in a Dictyostelium is determined by the mycobacterium strain incorporated in the BBC. After homogeneisation of the infected cells, we recover the BBCs-containing phagosomes by flotation on a sucrose gradient.

The characterisation of isolated niches at 1hpi by IF and WB shows the presence of both early (VatA) and late endosomal markers (p80, LmpA). We will also check the purity of the isolated niches by IF using mitochondrial and ER markers.

Using anti-Mycobacteria antibodies, we could observe by IF that mycobacterial cell wall components are detected on the beads of the BBCs, suggesting that they are shed by the mycobacteria. Those cell wall components at the beads surface allows the BBCs to be recognised as mycobacteria by the Dictyostelium cells. By performing IF on infected cells, we could also observe that those cell wall components are shed by the mycobacteria during infection.

Using isobaric labelling and mass spectrometry, we will study and compare the proteomic composition of those isolated phagosomes in order to identify potential host factors of resistance or of susceptibility. We also want to identify mycobacterial proteins expressed intraphagosomaly and involved in the phagosome manipulation.

Talk 40

The coupling mechanism of a GPCR and heterotrimeric G-proteins that control chemoattractant gradient sensing in Dictyostelium

Presented by Tian Jin

Xuehua Xu1, Tobias Meckel1*, Joseph A. Brzostowski2, Jianshe Yan1, Martin Meier-Schellersheim3, and Tian Jin1*

Coupling of GPCRs with heterotrimeric G-proteins is fundamental for GPCR signaling. However, the mechanism of coupling is still an issue of debate. Moreover, it remains unclear how possible mechanisms affect dynamics of downstream signaling. We investigated the coupling mechanism between cAR1 and the Ga2Gbg heterotrimer by measuring the mobility of receptor and G-ptoeins. We developed computational models to describe the possible coupling mechanisms between a GPCR and G-protein. We computed the expected temporal kinetics of the level of G-protein heterotrimers, and discovered that each coupling mechanism showed a distinct kinetic pattern. We determined the kinetics of cAR1-induced dissociation between Ga2 and Gbg in live cells by FRET imaging. We applied a detailed cAMP sensing model to examine dynamic cell responses by incorporating different coupling mechanisms between cAR1 and G-proteins, and our computational simulations indicate that cAR1 GPCRs use a ligand-induced coupling, rather than pre-coupling, mechanism to control spatiotemporal activation of G-proteins for proper chemosensing.

Talk 41

Gradient sensing in defined chemotactic fields
Presented by Wouter-Jan Rappel
Monica Skoge, Micha Adler, Alex Groisman, Herbert Levine, William F. Loomis, and Wouter-Jan Rappel

Dictyostelium cells are known to be able to sense extremely shallow gradients but the processes underlying their exquisite sensitivity are still largely unknown. Here, we determine the responses of developed Dictyostelium cells to stable linear gradients of cAMP of varying steepness generated in 2 micron deep gradient chambers of microfluidic devices. These low ceiling gradient chambers constrained the cells in the vertical dimension, facilitating confocal imaging, such that subcellular localization of fluorescently tagged proteins could be followed for up to 30 minutes without noticeable phototoxicity. Chemotactic cells enter these low ceiling chambers by flattening and elongating and then move almost as rapidly as unconstrained cells. By following the localization of activated Ras using a Ras Binding Domain fused to Green Fluorescent Protein, we observed the rapid appearance of membrane associated patches at the tips of pseudopods. These patches remained associated with pseudopods while they continued to extend but were rapidly disassembled when pseudopods stalled and the cell moved past them. Correlation of the size and persistence of RasGTP patches with extension of pseudopods may set the rules for understanding how the signal transduction mechanisms convert a weak external signal to a strong directional bias.

Talk 42

Dissecting the Calcium Response Pathway
Presented by Deborah Wessels
Deborah J. Wessels, Amanda Scherer, Daniel F. Lusche, Terra Simon, Spencer Kuhl and David R. Soll

We have shown that Dictyostelium discoideum cells chemotax towards calcium and do so in very steep gradients that can be generated and maintained in a microfluidic device (Scherer et al., submitted). From these and other observations, we hypothesized that a calcium-sensing receptor analogous to the widely studied calcium-sensing receptor in mammalian cells functioned in Dictyostelium discoideum. The affinity of the mammalian calcium-sensing receptor for calcium is very low, approximately 100,000 times lower than the affinity of the Dictyostelium cAR1 receptor for cAMP. Therefore, the steepness of the calcium gradient detected by the Dictyostelium putative calcium-sensing receptor in our microfluidic device would be comparable to the known steepness of the cAMP gradient in a cAMP wave. We further hypothesized that the signal transduction pathway initiation by this putative calcium-sensing receptor in Dictyostelium is responsible for Ca++ facilitation of cell motility (Lusche et al., 2009) and ultimately impinges upon myosin II localization and activation in the posterior cortex of the cell. In order to test this hypothesis, we measured responses of myosin heavy chain phosphorylation mutants and myosin heavy chain kinase mutants to calcium gradients, to combinatorial calcium gradients and cAMP gradients as well as the responses of these cells to rapid shifts in the direction of these gradients. We show that myosin heavy chain kinase C is essential for calcium chemotaxis, but not for cAMP chemotaxis.

Talk 43

Expression of Actin Tyr53Ala in Dictyostelium Disrupts the Cytoskeleton and Inhibits Intracellular and Intercellular cAMP-Signaling
Presented by Xiong Liu
Shi Shu, Xiong Liu, Paul W. Kriebel, Myoung-Soon Hong, Mathew P. Daniels, Carole A. Parent, and Edward D. Korn

We showed previously that phosphorylation of Tyr-53, or its mutation to Ala, inhibits actin polymerization in vitro with formation of aggregates of short filaments, and that expression of Y53A-actin in Dictyostelium blocks differentiation and development at the mound stage (Liu et al. (2006) Proc. Natl. Acad. Sci. U.S.A. 103, 13694-13699; Liu et al. (2010) J. Biol. Chem. 285, 9729-9739). We now show that expression of Y53A-actin, which does not affect cell growth, phagocytosis or pinocytosis, inhibits the formation of head-to-tail cell streams during cAMP-induced aggregation (although individual amoebae chemotax normally) because the cells do not secrete sufficient cAMP to relay the cAMP signal to neighboring cells. Expression of Y53A-actin causes a 50% reduction of cell-surface cAMP-receptors in cAMP-stimulated cells, and inhibits cAMP-induced increases in adenylyl cyclase A

activity, phosphorylation of ERK2 and actin polymerization in differentiated cells. Trafficking of vesicles containing adenylyl cyclase A to the rear of the cell, and secretion of the ACA-vesicles, is also inhibited. The actin cytoskeleton of cells expressing Y53A-actin is characterized by numerous short filaments, and bundled and aggregated filaments similar to the structures formed by copolymerization of purified Y53A-actin and wild-type actin in vitro. This disorganized actin cytoskeleton may be responsible for the inhibition of intracellular and intercellular cAMP signaling in cells expressing F-Y/A-actin.

Talk 44

Actin nucleation: WASP family proteins divide the labour.
Presented by Douwe Veltman
Douwe M. Veltman and Robert H. Insall

Arp2/3 is the major nucleator of new actin filaments in pseudopodia and is known to drive processes such as chemotaxis, endocytosis and vesicle trafficking. Signalling pathways that are activated in response to chemoattractant receptor occupation are relatively well understood in Dictyostelium, but how these signals lead to actin polymerisation is mostly unknown. We have approached this problem bottom-up by investigating the localisation of all known Arp2/3 activators using high resolution TIRF microscopy. We find that WASP co-localises with clathrin during endocytosis, WASH generates actin coats on post-lysosomal vesicles and SCAR is the exclusive driver of actin nucleation in pseudopodia. Surprisingly, when SCAR is deleted, WASP is able to compensate for many aspects of loss, in particular by organising unusual pseudopodia. This raises important questions about the specificity of regulatory pathways upstream of SCAR and WASP.

Talk 45 PLENARY Talk (Pathostelium)

Mycobacterium marinum infection in macrophages, fish, and mice.
Presented by Eric Brown
Eric Brown

Mycobacterium marinum (Mm) is is a pathogen of fish and frogs that is a close relative of Mycobacterium tuberculosis (Mtb). After uptake into macrophages, Mm escapes from phagosomes and can polymerize actin, leading to motility in the cytoplasm and infection of contiguous cells. There is a delay between escape from phagosomes and initiation of actin polymerization, during which some cytosolic Mm become ubiquitinated by host cell enzymes. Ultimately, ubiquitinated Mm become enclosed in a host-membrane derived compartment that expresses LAMP-1 but is not acidic. Cytosolic Mm can shed ubiquitinated components of their cell walls, and these cell wall fragments also are taken up into host vacuoles. Escape from phagosomes requires a genetic locus in Mm homologous to RD1, a locus required for virulence in Mtb. This locus encodes a specialized secretion

system, called Esx-1, or Type VII secretion. Esx-1 deficient Mm are attenuated for disease in both zebrafish, a natural host for Mm, and in mice. In mice, direct analysis of lesions shows that Esx-1 regulates secretion of TNFα, Interferon-γ, and IL-1ß. IL-1ß secretion appears to be regulated by the same membrane lytic property that allows for escape from phagosomes. In conclusion, Mm exhibit a complex interaction with host cells in vitro and in vivo, which, though not observed for other pathogenic Mycobacteria, requires a similar atypical secretion mechanism. Thus Mm phagosome escape may be functionally related to basic processes in the pathogenesis of tuberculosis.

Talk 46

Evolution of Pseudomonas aeruginosa virulence in infected patients revealed in a Dictyostelium discoideum host model

Presented by Pierre Cosson

Emmanuelle Lelong, Anna Marchetti, Marianne Simon, Jane L. Burns, Christian van Delden, Thilo Köhler, Pierre Cosson

Background:
Pseudomonas aeruginosa can cause acute infections as in intubated patients or chronically infect patients with cystic fibrosis (CF). P. aeruginosa has been shown to adapt to these particular lung environments by progressive phenotypic modulation, in particular genetic inactivation of lasR. Whether these adaptive changes affect virulence has not been tested on a large scale.

Methods:
We analyzed a collection of clinical isolates from 16 CF patients and from 10 intubated patients for their virulence in a Dictyostelium discoideum amoebae model. Virulence was assessed by following growth of D. discoideum on a lawn of P. aeruginosa bacteria for 10 days, and scored on a scale from 0 (avirulent) to 8 (virulent).

Results:
With one exception (a CF patient), all initial isolates whether from CF or from intubated patients were virulent (score = 8). For eight CF patients, no decrease in virulence was observed in the two late samples, while for the remaining seven CF patients, at least one of the two late isolates was significantly less virulent. Isolates from 8 out of the 10 intubated patients were virulent at all times analyzed. In two intubated patients, less virulent isolates were seen within a few days (score 4 to 0). Mutations in the quorum sensing regulator LasR were identified both in CF and non-CF isolates; however their presence did not correlate with loss of virulence.

Conclusions:

Loss of virulence is not the main driving force for the adaptation of P. aeruginosa to the human host and does not occur predominantly through mutations in LasR.

Talk 47

From amoebae to higher organisms: Ancient effector proteins with functions in nutrition and immunity

Presented by Matthias Leippe
Matthias Leippe
Zoological Institute, Zoophysiology, University of Kiel

Antimicrobial systems in animals have been characterized at the molecular level primarily for vertebrates and arthropods. A variety of active peptides have been found and they possess highly diverse structures. The majority of them share the common feature of amphipathicity and appear to act by physical disruption of the membranes of their targets. Among the several groups of membrane-permeabilizing peptides classified so far, the one to which the subjects of our studies belong is extraordinary: the members of the saposin-like protein family are relatively large polypeptides and are characterized by a compact alpha-helical and disulfide-bonded fold. Such polypeptides can be found in species of amoeboid protozoa (amoebapores from Entamoeba histolytica/naegleriapores from Naegleria fowleri), organisms which are human pathogens but may be viewed primarily as insatiable phagocytic cells that use bacteria as a nutrient source, and in invertebrates as well as in vertebrates. Porcine and human cytotoxic lymphocytes contain similar peptides, termed NK-lysin and granulysin, respectively, which appear to be important constituents of the internal defence against pathogens, e.g. intracellular bacteria. We compared the tertiary structures of various members of this superfamily of antimicrobial and/or cytotoxic polypeptides and monitored their biological activities to extract the similarities and differences of effector molecules from evolutionarily highly divergent animals. As an intensively studied cellular model system, we are using the free-living and non-pathogenic amoeboid protozoon Dictyostelium discoideum to study the molecular armament which such a primitive phagocyte may use to combat growth of phagocytozed bacteria inside its phagolysosomes. Dictyostelium possesses a variety of genes potentially coding for a large variety of saposin-like proteins which we termed provisionally amoebapore-like peptides (Apls) and for several members of different lysozyme classes. Moreover, with the bacteria-feeding nematode Caenorhabditis elegans we are studying a multicellular model organism in parallel to analyze the antimicrobial system at the molecular and organismal level using recombinant expression, transgenic animals, RNA interference technology and functional knock-out mutants. Data exemplarily demonstrating the antimicrobial activity, mode of action and physiological significance of the aforementioned effector proteins in nutrition and prevention of infection will be presented suggesting an ancient protein fold fulfiling an ancient function.

(Supported by Deutsche Forschungsgemeinschaft – LE 1075/2-4).

Talk 48

Dictyostelium signaling complexes on Legionella-containing vacuoles

Presented by Hubert Hilbi

Hubert Hilbi

Dictyostelium discoideum is a powerful and versatile model to study the virulence of Legionella pneumophila, the causative agent of Legionnaires disease. The environmental bacterium L. pneumophila replicates within a unique membrane-bound host compartment, the Legionella-containing vacuole (LCV). Formation of LCVs is a complex and robust process that requires the bacterial Icm/Dot type IV secretion system and more than 150 different translocated effector proteins, which subvert host cell signal transduction and vesicle trafficking pathways. LCVs acquire markers of the endoplasmic reticulum and are decorated with small GTPases, phosphoinositide (PI) lipids and PI-metabolizing enzymes. A number of L. pneumophila effector proteins anchor via PI lipids to the LCV membrane, where they target small GTPases or PI-metabolizing enzymes.

Recently, we established a simple and fast LCV purification protocol using fluorescently labeled LCVs from infected D. discoideum. Intact LCVs were purified by immuno-magnetic separation with a primary antibody against an Icm/Dot-secreted effector protein localizing exclusively to LCV membranes and a secondary antibody coupled to magnetic beads, followed by density gradient centrifugation. The proteome of purified LCVs was analyzed by LC-MS/MS and revealed more than 560 host cell proteins, including small GTPases and protein or lipid kinases and phosphatases.

Talk 49

Mechanisms of mycobacteria interference with cell-intrinsic immunity processes

Presented by Thierry Soldati

Thierry Soldati, Natascha Sattler, Sonia Arafah, Xuezhi Zhang, and Monica Hagedorn

Pathogenic mycobacteria such as M. tuberculosis, and M. marinum utilise common strategies to invade innate immunity phagocytes, manipulate their bactericidal phagocytic apparatus and ensure cell-to-cell transmission. Using the amoeba Dictyostelium, we have identified and characterized mycobacterial and host factors that modulate uptake, resistance to infection and cell-to-cell spreading. Dictyostelium has three paralogs of the CD36 family of scavenger receptor type B. LmpB is present at the surface and acts as a receptor, while LmpA and LmpC are lysosomal and are implicated in trafficking of hydrolases. Uptake of M. marinum was strongly impaired in lmpA- and lmpB- mutants, but only lmpA- and lmpC- cells showed significantly higher susceptibility to M. marinum infection. The mutants show no attenuation in killing the Gram- K. aerogenes but, lmpB- and lmpA- show

a delay in killing the Gram+ B. subtilis. We also study the response of NADPH-oxidases to bacterial sensing and their role in production of Reactive Oxygen Species and killing. After phagosome maturation arrest, M. marinum and M. tuberculosis escape from their vacuole to the cytosol. We study the importance of autophagy in the genesis, maintenance and breakage of that compartment. Finally, cytosolic mycobacteria are ejected from the cell through an ejectosome. This virulence strategy is conserved and occurs in amoeba and macrophages. Ejection is crucial for the maintenance of an infection and is a concerted process that requires both host and pathogen factors.

Talk 50

Differentiation as a cure for infection

Presented by Monica Hagedorn

Monica Hagedorn and Thierry Soldati

Dictyostelium discoideum is a social amoeba that naturally lives in the soil feeding on environmental bacteria by phagocytosis. In this vegetative state, Dictyostelium lives as single cells, however, under starving conditions, these cells undergo a 24 hour-developmental cycle. After chemotactic aggregation, 100,000 cells form a slug, a true multicellular structure that finally differentiates into a fruiting body with a stalk supporting the spore mass. It is a general understanding, that Dictyostelium spores are sterile, namely free of bacteria. However, nothing is known about the mechanism by which this "cleansing process" occurs during differentiation.

In our laboratory we have established vegetative Dictyostelium amoebae as an efficient model host for Mycobacterium marinum infection, a close cousin to M. tuberculosis. As observed in mammalian cells, in Dictyostelium M. marinum bifurcates from normal phagosomal maturation and establishes itself a niche, in which it replicates. At later stages of infection, this vacuole breaks open and the bacteria are released into the cytosol. Furthermore, we discovered a non-lytic release mechanism of cytosolic bacteria from the host. This ejection process is crucial for intercellular spreading that ensures persistence of the infection.

We observed that a highly infected culture is cured of M. marinum infection when the cells are starved and undergo differentiation. In effect, starting with a population of over 80% infected cells, the cellulose-encased slug and culminants did not contain any visible bacteria. Observation with live fluorescence microscopy showed that this process occurred at a cellular level and, in addition, via a mechanism based on social behaviour.

In principle, infected cells can eliminate the intracellular bacteria in three ways: exocytosis, ejection and autophagy. To test the contributions of these three modi we infected mutants that were strongly affected in the named processes and starved them. To our surprise the three mutant strains were able to clear the infection to various degrees at the cellular level, but all produced sterile spores. However, closer examination at late stages of differentiation showed that remaining infected cells were sorted out of the stream, excluded from the aggregate and left behind in the basal disk.

In conclusion, we find that three cell intrinsic ways allow cell curing from bacteria during starvation induced differentiation: exocytosis, ejection and autophagy. In addition, remaining infected cells are not included in the streams that lead to aggregate formation.

Talk 51

The requirement of TirA for bacterial defense during growth, and for Sentinel cell production of 'extracellular traps' during development in D. discoideum.

Presented by Adam Kuspa
Olga Zhuchenko, Waleed Nasser, Anup Parikh, Roshan Miranda, Chris Dinh, Rui Chen, Gad Shaulsky and Adam Kuspa

We will describe genetic and transcriptional evidence that D. discoideum discriminates between different bacteria and that this enables the amoeba to respond appropriately for optimal feeding and defense against pathogens. For example, mutants in the Toll/interleukin receptor-1 (TIR) domain protein, TirA, grow poorly on Gram(-) bacteria, but they grow normally on Gram(+) bacteria and on heat-killed Gram(-) bacteria. Transcriptional profiles of the mutant amoebae growing on Gram(-) versus Gram(+) bacteria indicate that the response to Gram(-) bacteria depends significantly on the tirA gene, and suggests that TirA is involved in the detection of Gram(-) bacteria during growth. We recently described 'Sentinel' cells (S cells) that appear to provide innate immunity in the slug during development (Chen et al., 2007 Science, 317:678). We have now found that S cells form extracellular nets that appear analogous to 'extracellular traps' (ETs) produced by neutrophils and eosinophils that are known to trap and kill bacteria. Purified S cells are able to elaborate DNA-containing nets upon stimulation with bacterial lipopolysaccharide. Bacteria bind to these nets and appear to be actively killed, similar to what is seen with mammalian ETs.

Finally, we have found that TirA is required for net formation by S cells. Our results suggest that TirA is critically required for D. discoideum's response to gram-negative bacteria in growth and in innate immune function during development. Characterization of the molecular basis of this regulation should shed light on the antibacterial defense mechanisms of the social amoebae and may shed light on innate immune responses in other organisms.

Talk 52

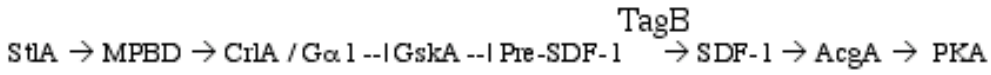
SDF-1 Signaling in Preparation for Terminal Differentiation

Presented by Bill Loomis
Christophe Anjard and William F. Loomis

SDF-1 is a small phosphopeptide that is released from Dictyostelium cells developed as monolayers above the density of 10 e4 cells/ cm2. Adding back

purified SDF-1 to KP cells developing at 2 x 10 e3 cells/cm2 induces them to start sporulating after a period of 45 minutes during which time protein synthesis must occur. The action of SDF-1 can be mimicked by phosphokemptide (LRRASpLG) and is blocked by antibodies to this peptide.

We found that the polyketide MPBD (methyl- pentyl-benzene diol) induces the release of SDF-1 within 2 minutes. MPBD is the product of Steely A (stlA), which is expressed during the late slug stage. By analyzing specific mutants affected in MPBD or SDF-1 production, we delineated a signal transduction cascade through the membrane receptor CrlA coupled to G alpha1, leading to the inhibition of GskA such that the precursor of SDF-1 is released. It is then processed by the extracellular protease of TagB on prestalk cells. SDF-1 apparently acts through the adenylyl cyclase ACG to activate PKA and trigger the production of more SDF-1. This signaling cascade shows similarities with the SDF-2 signalling pathway that acts at a later stage to induce rapid spore encapsulation:



Talk 53

A Green's functions and boids based model of Dictyostelium discoideum
Presented by Daniel S. Calovi
Daniel S. Calovi, Leonardo Gregory Brunnet
Instituto de Física, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, P.B. 15051, 91501-970 Porto Alegre, Brazil

In 1987 Martiel and Goldbeter proposed a set of equations to describe the dynamics of cAMP signaling. Our work proposes solving these equations, with the addition of cAMP diffusion, using Green's functions so that the problem scales with the number of amoebas and not with the discretization of space. Moreover our work will show our model reproduces most of the properties of Dicty, such as synchronization, adaptation, spatial cAMP patterns (spirals, target waves and more complex reaction-diffusion behaviors). The movement in our system comes from a self propelled animoids model first proposed by Vicsek, which takes into account noise, adhesive and chemo attractant forces. It reproduces stream and mound formation and also reproduces internal circular movement inside two dimensional mounds. We will show simulations with large numbers of amoebas in two and three dimensions. In our simulations we use all but one of the biologic inspired parameters used in Martiel and Goldbeter paper, demonstrating a sturdiness of this model that can reproduce many different aspects of Dictyostelium cycle with only one model and one set of parameters.

Talk 54

Evidence for two distinct subtypes of pstA-cells and characterization of a transcription factor that mediates pstA cell differentiation

Presented by Masashi Fukuzawa

Hiroshi Senoo, Satoshi Kuwana, Hong Yu Wang, Jeff Williams, Masashi Fukuzawa

The prestalk region of the slug is comprised of two major subtypes, pstA and pstO cells,

that are defined by their differential utilization of sub-fragments of the ecmA promoter. The anterior-like cell (ALC) population is a mixture of cells the pstO-ALC, that share many characteristics with pstO cells, and a recently discovered population, the pstU cells. There are also ALC that express the ecmB gene at a high level, the pstB cells. Here we demonstrate that there is a hitherto undetected cell types within the pstA region. We propose naming the novel subtype as pstA1, and the

authentic one as pstA2. We labeled pstA1 and pstA2 cells with promoter-GFP/RFP constructs and examined their behavior through

development. The two cell types are mutually exclusive within the tip of a slug, with a small number of cells co-expressing both markers. Analysis of the initial differentiation of pstA1 cells suggests a novel mode of prestalk cell differentiation.

Finally, we present evidence that the known transcription factor RcdK may be involved in both pstA2 differentiation and in pstB cell differentiation (see poster of Senoo *et al*. for further details).

Talk 55

A light controlled switch between different fates during Dictyostelium development

Presented by Daniele Conte

Daniele Conte and Adriano Ceccarelli

During Dictyostelium development light regulates slug phototaxis and onset of culmination. Very little is known about the molecular mechanisms that mediate its action, and light has always been regarded as a physiological stimulus triggering or accelerating events without changing the differentiation pathways of cells involved in the response. We show here that light can activate a switch of fate between different cell types.

We have observed previously that Btg controls ALC cell differentiation and that rblA lies on the same pathway downstream of btg. DIF negatively regulates btg expression and rblA controls cell responsiveness to DIF. Our

present work on the mechanism by which btg induces ALC fate shows that btg overexpression controls cell sorting to positions in the slug and in the culminant that are compatible with ALC or pst fates, depending on light/dark conditions respectively. While btg regulates ALC differentiation in an rblA dependent manner, the dark-operated btg-dependent pathway controlling the sorting of cells to the tip is independent from the presence of rblA. However dimB, a transcription factor regulated by DIF, is downstream of btg and is necessary to the dark-activated pathway, as shown by the inability to sort of dimBnull btg overexpressors. The lack of dimB in the recipient strain of a chimera also inhibits sorting, suggesting an additional non cell-autonomous role of dimB in controlling the dark-dependent phenotype. GSK, a kinase involved in the control of pst differentiation, is on the same pathway with btg and dimB, and the GSKnull mutation abolishes the effects of btg overexpression on sorting and differentiation in the dark.

A western blot analysis of the dimB protein shows that it is upregulated in btg overexpressing strains and downregulated in rblAKO and GSKnull, suggesting a trancriptional control on dimB exerted by rblA and GSK. Btg overexpression can restore expression of dimB.

A possible interpretation of our observations suggests that btg overexpression may result in the inactivation of dimB in the light, and a consequent overexpression of inactive of dimB protein. Development in the dark might restore dimB activity resulting in sorting to the pst territory. GSK regulates fate preference by regulating dimB activity in the same cells.

Talk 56

cadA Is a Single-Gene Green Beard that Regulate Morphogenesis through Differential Cell Adhesion in Dictyostelium

Presented by Chi-Hung Siu
Shrivani Sriskanthadevan, Yingyue Zhu, Kumararaaj Manoharan and Chi-Hung Siu
From the Banting and Best Department of Medical Research and Department of Biochemistry, University of Toronto, Toronto, Ontario M5S 1A8, Canada

During development of the social amoeba Dictyostelium discoideum, multiple cell types are formed and undergo a coordinated series of morphogenetic cell movements to generate the fruiting body. Here we describe how the cadA gene which codes for a homophilic cell adhesion protein, acts as a single-gene green beard. In chimera experiments, the cells expressing DdCAD-1 on the cell surface were more likely recruited into aggregates and gave rise to mature fruiting bodies than cadA-null cells when development was carried out on soil plates. Here cadA behaved as a single gene green beard. However, cadA exhibited anti-green beard behaviour when development was carried out on non-nutrient agar. Wild-type cells differentiated mostly into prestalk cells and eventually died for the sake of the whole organism whereas the cadA-null survived as spores. It became evident that cells expressing DdCAD-1 had the tendency of sorting to the anterior region of the

slug. Ectopic expression of the DdCAD-1 in the cadA-null strain rescued the sorting defects in slugs in a Ca2+-dependent manner. To investigate how DdCAD-1 was involved in cell sorting, ectopic expression of DdCAD-1-GFP driven by its endogenous promoter revealed spatial and temporal changes in the subcellular distribution of DdCAD-1 during development. In the anterior zone, DdCAD-1 was enriched in cell-cell contact regions while DdCAD-1 was mostly localized in the cytoplasm of prespore cells in the posterior zone. Using recombinant proteins, we found that externally applied DdCAD-1 bound tightly to the cell membrane of cadA-null cells and restored the wild-type pattern of cell sorting in slugs. Whereas DdCAD-1 remained on the cell surface of anterior cells, it was internalized in the most of the posterior cells. Taken together, these results show that DdCAD-1 regulates the social behaviour of Dictyostelium through its differential expression in prespore and prestalk cells.

(Supported by the Canadian Institutes of Health Research.)

Talk 57

DdEGFL1-enhanced cell motility in Dictyostelium functions via calcium signaling independently of the cAMP-mediated signaling pathways

Presented by Robert Huber
Robert Huber 1 and Danton H. O'Day 1,2
1. Department of Cell and Systems Biology, University of Toronto, Canada
2. Department of Biology, University of Toronto Mississauga, Canada

The Epidermal Growth Factor (EGF) is a polypeptide that binds to the EGF receptor (EGFR) to regulate a number of cellular processes such as cell movement, division, differentiation, and morphogenesis. EGF-like (EGFL) repeats are domains within proteins that possess strong sequence similarity to EGF. Ten14, an EGFL repeat from the extracellular matrix protein tenascin C, has been shown to enhance cell motility by binding to the EGFR. Our research investigates the function of a synthetic EGFL peptide (DdEGFL1), whose sequence was obtained from the first EGFL repeat of cyrA, a matricellular, calmodulin-binding protein in the model eukaryote Dictyostelium discoideum. DdEGFL1 has previously been shown to enhance cell motility and cAMP-mediated chemotaxis in Dictyostelium (Huber and O,ÄôDay, 2009). The extracellular localization of DdEGFL1 suggests the peptide binds to the cell surface to enhance cell movement. To identify the proteins that bind to DdEGFL1, we generated a DdEGFL1-coupled agarose resin and pulled-down four phospho-proteins. Using mutants of cAMP signaling (carA-, carC-, gpaB-, gpbA-), the endogenous calcium release inhibitor TMB-8, the calmodulin antagonist W-7, and a radial motility bioassay, we have shown that DdEGFL1 functions independently of the cAMP-mediated signaling pathways through a mechanism involving calcium signaling and calmodulin. Finally we have shown that DdEGFL1 increases the amounts of polymeric myosin II heavy chain and actin in the cytoskeleton. Together, our research provides insight into the functionality of EGFL peptides in Dictyostelium. It also identifies several mechanistic components

of EGFL peptide-enhanced cell movement in this model organism, which may ultimately provide a model system for understanding EGFL peptide function in higher organisms.

Talk 58

Autophagy in Dictyostelium: a model for the identification of new autophagic proteins

Presented by Ricardo Escalante
*Javier Calvo-Garrido; Sergio Carilla-Latorre; Natalia Santos-Rodrigo; Ana Mesquita; Olivier Vincent and Ricardo Escalante**
* Instituto de Investigaciones Biomedicas 'Alberto Sols'. CSIC-UAM.

Macroautophagy (referred to as autophagy hereafter) is a degradative pathway characterized by the formation of double-membrane vesicles called autophagosomes that engulf part of the cytoplasm or even organelles for lysosomal degradation and recycling. Autophagy is essential for cell survival under starvation conditions but is also induced in other circumstances such as for the elimination of protein aggregates, defective organelles or in response to invasive pathogens, and it is therefore very relevant in diverse pathological processes and aging (1). Dictyostelium genome codes for most proteins that have been described so far to regulate autophagy in other organisms. Our current studies make use of yeast two-hybrid techniques to characterize the map of interactions among the identified proteins and to discover potential new components of the pathway.

Dictyostelium autophagy proteins show a strong level of similarity with animals. Moreover, the presence of certain proteins conserved in Dictyostelium and human that seems to be absent in yeast emphasize the high level of conservation of the basic autophagy machinery between this simple social amoeba and higher eukaryotes. One of these proteins is Vmp1, an ER-resident protein conserved between Dictyostelium and human but absent in yeast. Cells deficient in Vmp1 show defects in autophagy and aberrant formation of poly-ubiquitinated protein aggregates (2). These aggregates have also been detected in other Dictyostelium autophagic mutants and show similarities with those associated with human degenerative diseases.

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Talk 59

The Ste20-like kinase DstC has a cortical localization and is involved in phagocytosis
Presented by Michael Schleicher
Meino Rohlfs, Gergana Gateva and Michael Schleicher

Ste20-like kinases are ubiquitous and highly conserved S/T kinases with distinct activities. We reported earlier that the Dictyostelium discoideum kinase Krs1 is important for chemotaxis (Arasada et al. (2006) Eur. J. Cell Biol., 85:1059-1068), whereas the severin kinase SvkA regulates late stages of cytokinesis (Rohlfs et al. (2007) J. Cell Sci., 120:4345-4354). Here we describe the Ste20-like kinase DstC as a major regulator of the actin driven phagocytosis. The catalytic domain of DstC shows a 50% identity to the mammalian kinases Mst1/Krs2 and Mst2/Krs1, which were shown to modulate cell growth and apoptosis. The overall identity of DstC when compared to Polysphondylium pallidum is as high as 83%. In D. discoideum the gene knockout leads to a reduced growth and a severe phagocytosis defect. GFP constructs of DstC localize to phagocytic cups, the leading edge and to a subset of acidic vesicles. This localization indicates a connection to the actin-cortex which was supported by the presence of DstC in the Triton-insoluble cytoskeleton fraction. We could map the signal that localizes DstC to actin-rich structures down to 66 amino acids in the C-terminal half of the protein.

Talk 60.
Coronin proteins
Presented by Angelika Nögel
Maria C. Shina, Annette Müller-Taubenberger, Michael Schleicher, Ludwig Eichinger, Angelika A. Nögel

Dictyostelium discoideum harbors a short (CRN12) and a long coronin (CRN7) composed of one and two beta-propellers, respectively. They are primarily present in the cell cortex and cells lacking CRN12 (corA-) or CRN7 (corB-) have defects in actin driven processes. We characterised and compared mutant cell lines lacking CRN12 (corA-), CRN7 (corB-) and both proteins (corA-/corB-) focusing on cytokinesis, phagocytosis, chemotaxis and development. Cytokinesis, uptake of small particles, and developmental defects were not enhanced in the corA-/corB- strain as compared to the single mutants, whereas motility and phagocytosis of yeast particles were more severely impaired. It appears that although both proteins affect the same processes they do not act in a redundant manner. Rather, they often act antagonistically which is in accordance with their proposed roles in the actin cytoskeleton where CRN12 acts in actin disassembly whereas CRN7 stabilizes actin filaments and protects them from disassembly.

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4 Santosh Sathe
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39 Judith Langenick
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Abstracts:

Poster 1

Impact of WASP inhibition on cytoskeletal dynamics in Dictyostelium cells

Presented by Eva Pfannes
Eva Pfannes and Carsten Beta

N-WASP (Neural-Wiskott-Aldrich syndrome protein) is a key regulator of cell migration and actin polymerization in humans, linking upstream signals to the activation of the Arp2/3 complex. Dictyostelium WASP was identified by Myers and co-workers and reduced expression of WASP culminated in less F-actin levels and inability to establish polarity during chemotaxis [1]. Here, we tested the drug Wiskostatin, a 14-aa cyclic peptide identified in a high-throughput screen, known to bind and inhibit N-WASP [2], to selectively disrupt the Dictyostelium WASP analog. We determined the LD50 (lethal dose) of Wiskostatin in Dictyostelium to be 6μM. Drug treatment induces prominent morphological changes, with cells rounding up and showing no or very few pseudopods. Developed cells show less persistent directional movement towards a gradient of cAMP, but are still able to chemotax. Using a GFP-labeled fluorescent marker for F-actin (DdLimEΔ), we observed that drug treated cells still maintain an actin cortex indicated by the characteristic DdLimEΔ cortical localization. Compared to non-treated cell, they exhibit recurrent spots of localized actin polymerization. Also other cell lines with fluorescently labeled actin-binding proteins were used, indicating that the cortical localization may be impaired in some cases after drug exposure. Since N-WASP down-regulation was found in human breast cancer cells and N-WASP over-expression reduced tumour growth in vivo [3], we hope to elucidate changes in cytoskeletal dynamics that are important for the progression of invasive metastatic tumours.

[1] Myers S A, Han J W, Lee Y, Firtel R A and Chung C (2005) Molecular Biology of the Cell, 16: 2191-2206
[2] Peterson J R, Lokey R S, Mitchison T J, and Kirschner M W (2001) PNAS 98: 10624-10629
[3] Martin T A, Pereira G, Watkins G, Mansel R E and Jiang W G (2008) Clin Exp Metastasis 25:97-108

Poster 2

Modification of histone H3 variants

Presented by Cathy Pears
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Division of Cell and Developmental Biology, College of Life Sciences, University of Dundee, Dundee DD1 5EH, United Kingdom

Histone modifications play a vital role in regulation of gene expression. Combinations of modifications on individual histone molecules generate

binding interfaces for proteins that regulate DNA function including the transcription machinery. We have focussed on interactions between modifications at the N-terminus of histone H3 looking at the interplay between trimethylation of lysine K4 and acetylation of lysine residues on the same tail. Higher eukaryotes express variants of histone genes which differ by only a small number of amino acids. Mammalian cells express three variants of histone H3, one of which is incorporated into DNA in a replication independent manner and is associated with active genes. Genetic analysis of the role of these variants is complicated by the large number of genes encoding them. Dictyostelium also contains three genes encoding H3 and each is present as a single copy. One variant contains and extra three amino acids facilitating analysis of modification patterns on individual variants. Analysis of strains in which genes encoding two of the three have been deleted suggests that these histone variants offer a selective advantage, for example, in growth on bacteria and induction of gene expression of some genes.

Poster 3

Targets of adenosine and caffeine in Dictyostelium and their role in endocytosis and pattern formation.

Presented by Ramamurthy Baskar

Jaiswal Pundrik 1, Sascha Thewes 2, Thierry Soldati 3 and Ramamurthy Baskar 1.

1 Department of Biotechnology, Indian Institute of Technology-Madras, Chennai-600036. India.

2 Institute for Biology,Microbiology; Department of Biology, Chemistry, Pharmacy; Freie Universität Berlin; Germany.

3 Department de Biochimie, Faculté des Sciences, Université de Genéve, CH-1211-Genéve-4, Switzerland.

The potent morphogenetic regulator adenosine induces large aggregate formation in Dictyostelium while its antagonist caffeine favours the formation of small aggregates and promotes multiple tip formation in distantly related slime molds. Little is known about the targets of adenosine and caffeine in cellular slime molds. In the presence of caffeine or adenosine, we verified the aggregate morphology in mutants that have a genetic lesion in the putative caffeine target genes such as those involved in TORC1 pathway and calcineurin signalling. By a candidate gene approach, we have identified mutants that are hypersensitive or resistant to caffeine and adenosine treatments, which surprisingly play also an important role in endocytosis. We examined the role of lst-8, a component of TORC1 complex, in detail and found that this gene product seems to be one of the caffeine targets. The aggregate size of lst-8 mutants remains unaltered in the presence of caffeine suggesting insensitivity of aggregation process to the presence of caffeine. However the insensitivity is aggregate specific, as endocytosis seems to be hypersensitive to the presence of caffeine. We will further discuss the various targets of caffeine action in slime molds.

Poster 4

Exploitation and co-existence in strains of Dictyostelium giganteum

Presented by Santosh Sathe
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This work was carried out with genetically distinguishable strains of Dictyostelium giganteum that were isolated from undisturbed forest soil in the Mudumalai reserve, southern India. Laboratory experiments using these strains show that they can develop together. In other words, they can form chimaeric aggregates and fruiting bodies. When they do so, one strain often exploits the other during sporulation - that is, it forms a disproportionate number of spores relative to the other. Despite this, the strains co-exist in close proximity. We have begun to address some issues that are raised by this observation.
1) What might account for the co-existence of different strains of same species even when, in a mixture, one is more efficient at sporulating than the other?
2) How do group-level traits such as aggregation territory size, overall spore forming efficiency and strain productivity (the spore forming efficiency of a given strain when part of a mixture) compare between clonal and chimaeric groups?
On the basis of the cases studied so far, our preliminary findings are as follows: (a) There is a trade-off between different life cycle components of fitness, and this may help two strains to co-exist; (b) With respect to territory size and spore forming efficiency, but not strain productivity, clonal groups and chimaeras are comparable. We discuss these results in the light of evolutionary models for cooperative behaviour in the cellular slime moulds.

Reference:

Kaushik S, Katoch B and Nanjundiah V (2006) Social behaviour in genetically heterogeneous groups of Dictyostelium giganteum. Behav Ecol Sociobiol 59 521-530.

Poster 5

Efficient generation of gene knock out vectors for Dictyostelium using combinatorial cloning

Presented by Christian Hammann
Stephan Wiegand, Janis Kruse, Sina Gronemann, Uwe Carl & Christian Hammann

We present the use of combinatorial cloning for the fast and efficient generation of deletion vectors, that are produced in a one-step reaction by inserting two PCR products in an organism-specific, generic acceptor system. This worked efficiently for all 16 tested constructs directed against genes in Dictyostelium discoideum. Using appropriate selection markers, similar

systems should be useful in all organisms, where genes can be knocked out by homologous recombination.

Poster 6

Preliminary characterization of peptide based communication in social amoebae

Presented by Asma Ashgar

Asma Asghar, Marco Groth, Christoph Enzensperger, Friedemann Gaube, Oliver Siol, Thomas Winckler*

Cell aggregation by chemotaxis in response to a specific acrasin marks the transition from the myxamoebal growth phase to multicellular development. In contrast to Dictyostelium discoideum whose acrasin is cyclic AMP, Polysphondylium violaceum uses the unusual dipeptide N-propionyl-gamma-L-glutamyl-L-ornithine-delta-lactam ethyl ester (glorin) as acrasin. The fact that P. pallidum is also responsive to glorin suggested that glorin communication during early development may be a common property of polysphondylids.

The recently established dictyostelid phylogeny (Science 314:661-663, 2006) indicated that polysphondylids are polyphyletic and P. violaceum and P. pallidum are only distantly related. The data suggested that communication with glorin or glorin-related peptides is an ancient property of social amoebae that predates the use of cAMP to coordinate aggregation.

Using chemically synthesized glorin, we studied the responsiveness of a collection of species across the phylogenetic tree of social amoebae. Our preliminary data suggest that peptide based aggregation/signaling is conserved in the most ancient groups 1 and 2, i.e. in Polysphondylium and Dictyostelium species.

Poster 7

Phenotypic analysis of single-gene knock-out mutants for antimicrobial protein genes of Dictyostelium discoideum

Presented by Ranjani Dhakshinamoorthy

Ranjani Dhakshinamoorthy, Moritz Bitzhenner*, Thierry Soldati**, Matthias Leippe**

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In Dictyostelium, two major groups of genes may contribute to the antimicrobial armament, namely genes coding for lysozymes and Apls. D. discoideum possesses a variety of genes putatively coding for members of different classes of lysozymes, amoeba lysozymes (alys), chicken-type lysozymes, T4-phage-type lysozymes and Entamoeba histolytica-type

lysozymes. The multifarious apl family comprises genes putatively coding for a range of amoebapore-like peptides. Amoebapores are pore-forming peptides isolated from Entamoeba histolytica that display antibacterial activity and are cytotoxic to human cells. Structurally, both amoebapores and Apls belong to the protein superfamily of saposin-like proteins (SAPLIPs), characterized by the presence of a conserved motif of six cysteine residues involved in three disulfide bonds. Apart from antibacterial and cytolytic activities, some SAPLIPs are also involved in degradation of complex lipid structures internalized by endocytosis. The presence of the wide variety of lysozyme and Apl genes in D. discoideum raises questions concerning the physiological significance and specificity of a particular gene product of these groups. Accordingly, we generated Dictyostelium cell lines with single-gene knock-out mutations for selected members of each group and phenotypically analyzed them with respect to growth and phagocytosis. Currently, we are in the process of testing the ability of these knock-out mutants to grow on different bacterial strains. We hope this approach will shed some light on the arsenal that Dictyostelium uses to kill internalized microbes and to combat potential pathogens.

(Supported by Deutsche Forschungsgemeinschaft – LE 1075/2-4)

Poster 8

Variation, sex and social cooperation: Molecular population genetics of the social amoeba Dictyostelium discoideum

Presented by Si Li

Jonathan M. Flowers, Si Li, Angela Stathos, Gerda Saxer, Elizabeth A. Ostrowski, David C. Queller, Joan E. Strassmann, and Michael D. Purugganan

Dictyostelium discoideum is a eukaryotic microbial model system for multicellular development, cell-cell signaling and social behavior. Key models of social evolution require an understanding of genetic relationships between individuals across the genome or possibly at specific genes, but the nature of variation within D. discoideum is largely unknown. We re-sequenced 137 gene fragments in wild North American strains of D. discoideum, and examined the levels and patterns of nucleotide variation in this social microbial species. We observe surprisingly low levels of nucleotide variation in D. discoideum across these strains, with a mean nucleotide diversity of 0.08%, and no strong population stratification among North American strains. We also do not find any clear relationship between nucleotide divergence between strains and levels of social dominance and kin discrimination. Kin discrimination experiments, however, show that strains collected from the same location show greater ability to distinguish self from non-self than do strains from different geographic areas. This suggests that a greater ability to recognize self versus non-self may arise among strains that are more likely to encounter each other in nature, which would lead to preferential formation of fruiting bodies with clonemates and may prevent the evolution of cheating behaviors within D. discoideum populations. Finally, despite the fact that sex has rarely been observed in this species, we document a rapid decay of linkage disequilibrium between SNPs, the

presence of recombinant genotypes among natural strains, and high estimates of the population recombination parameter ρ. The SNP data indicate that recombination is widespread within D. discoideum, and that sex as a form of social interaction is likely to be an important aspect of the life cycle.

Poster 9

Deep conservation of Adenylate cyclase B and its role in encapsulation in social and solitary amoebae

Presented by Christina Schilde

Christina Schilde, John Sinclair, Pauline Schaap

Many free-living protists possess the ability to form durable cysts upon unfavourable environmental conditions. This ability to encyst has been conserved in the form of microcysts in the more basal groups of Dictyostelids like Polysphondylium pallidum, but has been lost in group 4 species like Dictyostelium discoideum. We have previously shown that the process of encystation in Polysphondylium pallidum is mediated by a rise in intracellular cAMP which utilises a conserved cAMP signalling pathway. Like in P. pallidum, encystation in Acanthamoeba is also accompanied by a rise in intracellular cAMP. Furthermore, analysis of the preliminary genome sequence of Acanthamoeba showed that many of the cAMP-signalling genes with roles in Dictyostelids are conserved. Surprisingly we could only identify a single adenylate-cyclase B-like (ACB-like) protein in Acanthamoeba that could be responsible for the measured production of cAMP. We have cloned and sequenced the full-length AcACB cDNA and have recombinantly expressed its cyclase domain in E. coli. Biochemical characterisation of the purified protein showed that it has a Mn2+- rather than Mg2+-stimulated cyclase activity and is also stimulated by high concentrations of Ca2+.

We set out to search for inhibitors of encystation of Acanthamoeba, which is an important opportunistic pathogen of humans and where encystation during antibiotic treatment poses a problem rendering the pathogen insensitive to antibiotic treatment. We believe that AcACB is a good target for inhibition of encystation because (i) it is the only adenylate cyclase present in the Acanthamoeba genome that could be responsible for cAMP production during encystation and (ii) adenylate cyclases of this type are not present in the host metazoans, so possible side effects can be avoided.

Poster 10

The CHD Chromatin Remodeling Family in Dictyostelium discoideum.

Presented by James Platt

James L. Platt, Ben Rogers, Alan R. Kimmel and Adrian J. Harwood

DNA within the nucleus of a eukaryotic cell is packaged into a nucleoprotein complex, chromatin, of which the basic subunit is the nucleosome. In the context of transcription, chromatin structure can control the access of

transcription factors and the transcription machinery to the DNA sequence. Changing the access to the DNA in either a repressive or activating manner is termed chromatin remodeling.

The CHD proteins are one of the four major ATP-dependent chromatin remodeling families, CHD being one of these, originally characterised by the presence of a double Chromodomain, Helicase/ATPase domain and a DNA-binding domain. Humans have nine CHD proteins that are linked to various disorders including dermatomyositis, Hodgkin's lymphoma, neuroblastoma and a syndrome termed CHARGE, which leads to multiple defects of the eye, ear, heart and renal anomalies as well as growth and developmental retardation. The social amoeba Dictyostelium discoideum possesses three CHD proteins with high homology to human counterparts it offers a simple yet powerful system in which to study chromatin remodeling. We have knocked out each Dictyostelium CHD protein, and each has a different developmental phenotype. Discussed will be our current analysis of the different phenotypes.

Poster 11

FbiA, a potential target of ubiquitin-mediated degradation, regulates cell-type proportioning in Dictyostelium discoideum

Presented by Margaret Nelson

H. B. Heeter, R. J. Beichner, K. E. Miller, R. M. Farmer, S.E. Stein, A. M. Stas, M. D. Richter, and M. K. Nelson

FbiA is an evolutionarily-conserved protein identified via its interaction with the WD-40 repeat region of FbxA, a component of an SCF E3 ubiquitin ligase. This interaction, as well as the phenotypes of null mutants, suggests that FbxA-mediated ubiquitination of FbiA plays a role in cell-type proportioning. The C-terminal region of FbiA is homologous to proteins in humans, mice, Drosophila, C. elegans, Arabidopsis, S. pombe, S. cerevisiae, N. crassa, and P. falciparum. The function of these FbiA homologues is, however, unknown. Hence, an understanding of FbiA's role in Dictyostelium development may shed light on the function of this evolutionarily conserved protein family. Here we focus on functional characterization of FbiA and analysis of a putative fbiA promoter region. To complement our prior qualitative data on the proportioning defect of fbiA- mutants (decreased prespore region in pspA-lacZ transformants; culminants with very long stalks and small sporeheads), we present quantitative data on the prespore and spore populations. In addition, since in situ hybridization analysis indicates a strong up-regulation of fbiA expression in the prespore region as culmination begins, we assess the consequences of FbiA absence on the detergent-resistance and germination rate of spores. Finally we present further analysis of the 800 bp region that we previously identified as a putative promoter region, based on its ability to mimic the complex, developmentally-regulated expression of fbiA when cloned upstream of lacZ.

Poster 12

Identifying downstream targets of DIF-1 signalling

Presented by Suzy Battom
Suzy Battom, Koki Nagayama, Chris Thompson
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A key feature of multicellular development is cell fate choice and patterning. During Dictyostelium discoideum development, identical cells differentiate into intermingled prestalk and prespore cells that subsequently sort out into distinct tissues to form a multicellular slug. Differentiation-inducing factor 1 (DIF-1) is a signalling molecule that drives the prestalk-prespore cell fate decision, whilst differential adhesion and motility are thought to drive sorting. To further understand how DIF-1 regulates patterning, it will be crucial to reveal how DIF-1 regulates these cell-type specific behaviours.

1.Identification of potential targets of pstO specific HECT ligase HfnA
We have identified a DIF-1 induced HECT ligase, HfnA. HfnA is specifically expressed in prestalk O cells (pstO) and in an hfnA- mutant, pstO cells differentiate but fail to sort to the collar. HfnA contains a filamin domain at its N-terminus, suggesting that filamin, an F-actin binding protein, is a potential target. Genetic interaction in which filamin complex function is elevated or reduced supports this idea. However filamin is not the direct target of HfnA, as filamin levels remain unchanged during development in the hfnA- mutant compared to wild type AX4. Therefore, to identify other targets of HfnA, we have employed a second-site suppressor screen. This is based on the finding that disruption of hfnA- also results in slugs extending out from plaques when developed on bacteria. This defect (like the pstO sorting defect) is rescued when filamin is knocked out in the hfnA- background. Using this approach, we have identified several novel genes, which when disrupted suppress the hfnA- mutant phenotype.

2. Identification of novel DIF-1 targets by RNA-seq and ChIP-seq
Understanding the identity of DIF-1 target genes, as well as how they are co-ordinately regulated in response to a signal will be important to understanding patterning of DIF dependent cell types. To address these questions, we are utilising next-generation sequencing techniques. Firstly, we have developed novel methodology to compare prestalk gene expression patterns in wild type and DIF signalling mutants using flow cytometry. Secondly, we have generated knock-in strains in which DIF responsive transcription factors (DimA, DimB, MybE, GATAc, STATc) are replaced by FLAG-tagged versions, and thus under the control of their endogenous regulatory elements. These FLAG-tagged transcription factors will be used to identify direct downstream target sequences, by ChIP-seq. Combined RNA-seq and ChIP-seq data will provide a rich data set illuminating the principles of DIF-1 dependent gene regulation and pattern formation.

Poster 13

Protein dynamics of the cytoskeleton components during chemotaxis.

Presented by Grzegorz Sobczyk
Grzegorz Sobczyk and Kees Weijer

Stimulation of cells with cAMP results in two phases of actin polymerization. The first rapid phase peaks at 4-8 s after stimulation and does not depend on PI3K activity. The second phase peaks at about 80 s and depends on PI3K. Both of those phases are dependent on Arp2/3 complex, which is a major actin nucleator. The exact mechanisms regulating actin polymerization during the first phase are still unknown.

We are investigating differences between mechanisms regulating the two phases of actin polymerization after cAMP stimulation, using functional proteomics analysis. This is based on the stable isotope labelling by amino acids in cell culture (SILAC) and cytoskeleton preparations from various times after cAMP stimulation corresponding to different phases of actin response.

Results from these experiments revealed very interesting patterns of protein dynamics in those two phases of actin polymerization. There is a whole range of actin binding proteins that show very rapid dynamics and significant changes in the incorporation profiles to the cytoskeleton between the two phases. There are also novel proteins that were not identified previously as components of the cytoskeleton.

All these results will help to understand the signalling pathways leading from the cAMP receptor to the regulation of the cytoskeleton.

Poster 14

The induction of autophagy by mechanical stress

Presented by Jason King

Jason S. King and Robert H. Insall

Autophagy is a multi-functional catabolic process, by which intracellular components can be degraded. This is important both as a general house-keeping process, as well as allowing the cell to respond to a number of stresses such as starvation and cytotoxic challenge. Here, we show that, the formation of autophagosomes is rapidly induced in response to mechanical compression. Importantly, this effect is conserved in mammalian cells and happens under relatively low, and potentially physiological pressures. We therefore propose that autophagy may provide a protective mechanism by which cells minimise and repair mechanically-induced intracellular damage.

Poster 15

The phosphoproteomics of cAMP signal transduction

Presented by John Nichols

John Nichols1, Sew-Yeu Peak-Chew2, Farida Begum2, Rob Kay1 and Elaine Stephens2

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2 Mass Spectrometry Facility, MRC-LMB, Cambridge.

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

We are taking a global phosphoproteomic approach to identify components of this network and to establish their sites of phosphorylation. We have combined the SILAC (Stable Isotope Labelling with Amino Acids in Cell Culture) mass spectrometry technique with phosphopeptide enrichment to quantify changes in protein phosphorylation in response to cAMP. By this method we have identified over 2750 unique phosphorylation sites, including many where the degree of phosphorylation responds to cAMP treatment. Proteins acutely phosphorylated in response to cAMP include several known from earlier work, such as the RasGEF GefS, the scaffold protein Sca1 and the BAR-domain containing protein SHAPS, and also a number not previously implicated in cAMP signal transduction. This approach therefore promises to be a powerful means of discovering new players in the chemotactic response.

Poster 16

Is this the DIF-1 receptor? ~ Approaches to identify novel components of the DIF-1 signaling pathway ~

Presented by Koki Nagayama
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DIF-1 is a morphogen that induces differentiation of Dictyostelium stalk cells. Over recent years, great strides have been made in defining the DIF signaling pathway, most notably through the identification of transcriptional factors required for DIF responsive gene expression (e.g. DimA, DimB, GataC, MybE, StatC). DIF-1 induces nuclear localization of most of these transcriptional factors. However, our understanding of the signaling pathway that leads to transcriptional factor activation is far from complete. Most notably (1) The DIF-1 receptor(s) is unknown. (2) Few components linking receptor activation and transcriptional factor activation/nuclear localization have been identified. In order to address these questions, we have devised two novel approaches:

1) Yeast three hybrid screen for DIF-1 binding proteins:
Whilst the yeast-two-hybrid (Y2H) system is routinely used to detect protein-protein interactions, the Yeast-three-hybrid (Y3H) system is a recently established alternative methodology to study interactions between a small molecule and proteins. This system is based on the fact that a DBD-DHFR fusion will bind to a methotrexate (MTX) conjugated version of your small molecule of interest (e.g. DIF-1-MTX), but not activate transcription. However, this can be used as a bait, to screen for activation domain fused

cDNAs that bind to your compound, and thus activate transcription. In a preliminary screen of a Dictyostelium cDNA library, we identified a colony that could activate reporter gene activity only in the presence of DIF-1-MTX. Importantly, the biologically inactive DIF analogue DIF-3-MTX is not sufficient to stimulate reporter activity, suggesting the binding is DIF-1 specific. Cloning of the cDNA revealed it to encode a novel gene, which is expressed specifically in the DIF-1 dependent pstO cells population. We are currently knocking out this gene to establish a role in DIF-1 signaling.

2) Genetic selection of REMI mutants that show aberrant ecmA expression:
To date, genetic selections for DIF signaling mutants have relied upon stalk cell and spore cell differentiation to provide the readout. We reasoned that a more direct alternative approach would be to use the immediate readout of DIF-1 dependent gene activation. For this, we chose ecmA as its expression is known to be strongly induced by DIF-1. We successfully generated a strain in which the endogenous ecmA gene is replaced by GFP, so that the natural expression behavior of ecmA can be monitored by green fluorescence and flow cytometry. A REMI mutant pool was created in this strain. After DIF-1 treatment, flow cytometry was used to sort out the cells into the different subgroups including: no expression of GFP, weak expression, strong expression and expression without DIF-1 induction. These subgroups were grown up and the process was repeated through several rounds. Using this approach, we have identified novel classes of DIF signaling mutants (e.g. partially DIF insensitive, aberrant ecmA expression during development). The disrupted genes are currently being identified by inverse PCR and the phenotypes defined.

Poster 17

MidA is a putative mitochondrial methyltransferase required for mitochondrial complex I function

Presented by Sergio Carilla-Latorre

Sergio Carilla-Latorre; M. Esther Gallardo; Sarah J. Annesley; Javier Calvo-Garrido; Osvaldo Graʃ±a; Sandra L. Accari, Paige K. Smith, Alfonso Valencia; Rafael Garesse; Paul R. Fisher and Ricardo Escalante*

* Instituto de Investigaciones Biomédicas 'Alberto Sols'. CSIC-UAM.

Dictyostelium and human MidA (DdMidA and hMidA) are homologous proteins that belong to a family of proteins of unknown function called DUF185. We have found by a yeast-two hybrid screening and subsequent validation by pull-down that both proteins interact with the mitochondrial complex I subunit NDUFS2. Consistent with this, Dictyostelium cells lacking MidA showed a specific defect in complex I activity, and knock down of hMidA in HEK293T cells resulted in reduced levels of assembled complex I. These results indicate a role for MidA in complex I assembly or stability. A structural bioinformatics analysis suggested the presence of a methyltransferase domain that was further supported by site-directed mutagenesis of specific residues from the putative catalytic site. Interestingly, this complex I deficiency in Dictyostelium midA- mutant causes a complex phenotypic outcome including phototaxis and thermotaxis defects.

We have found that these aspects of the phenotype are mediated by a chronic activation of AMPK revealing a possible role of AMPK signaling in complex I cytopathology (1).

Reference:

1. Sergio Carilla-Latorre; M. Esther Gallardo; Sarah J. Annesley; Javier Calvo-Garrido; Osvaldo Graña; Sandra L. Accari, Paige K. Smith, Alfonso Valencia; Rafael Garesse; Paul R. Fisher and Ricardo Escalante. (2010). MidA is a putative mitochondrial methyltransferase required for mitochondrial complex I function. J. Cell Sci. 123:1674-83.

Poster 18

Phenotypic screen for components of the WASH-dependent endosomal trafficking system

Presented by Peter Thomason

Peter Thomason and Robert Insall

The WASH proteins are a recently discovered class of Arp2/3 activators that regulate the trafficking and maturation of endosomes. Depletion of WASH in mammalian cells causes endosome elongation and distension and results in defective sorting and recycling of internalized materials. Likewise, in Dictyostelium WASH-mutants, endosomes fail to properly mature into neutral post-lysosomal compartments. One consequence of this is that indigestible material (such as high molecular weight dextran) cannot be properly exocytosed and therefore accumulates inside WASH-mutant cells.

WASH forms part of a multi-protein complex analogous (or even homologous) to that regulating SCAR/WAVE activity during pseudopod extension. Dictyostelium mutants in other members of the WASH complex (Fam21, Strumpellin, SWIP) share features of the WASH-null phenotype, though some differ in important details. We are using the accumulation of fluorescently labelled dextran in WASH-mutants as the basis of a phenotypic screen to look for regulators of WASH-complex activity in Dictyostelium. A successful screen should identify not only the core components of the complex, but also other potential upstream regulators and downstream effectors. We are in the process of screening REMI libraries for appropriate mutants and will present our current data.

Poster 19

Comparing transcriptional firing of housekeeping and developmental genes

Presented by Tetsuya Muramoto

Tetsuya Muramoto and Jonathan R. Chubb

Common methods for studying transcription such as Northerns and microarrays measure cell population averages at fixed time points. However, these approaches cannot address how the dynamic nature of transcription varies between genes and between individual cells, or how these properties are affected by developmental time. To gain insight into how transcriptional

pulsing can be modulated for different genes and by developmental timing, we have used a system which allows us to visualise nascent transcription in individual living cells using fluorescence microscopy. We are studying the in vivo transcription of both housekeeping and developmentally-induced genes. We have selected 17 genes for this comparative study, including housekeeping genes such as an actin and a ribosomal protein and developmentally-induced genes including cell adhesion molecules and membrane receptors. We have addressed how the pulsatile behaviour (frequency, duration, amplitude) vary between genes of different expression requirements.

Poster 20

Determining the Role of FtsZ Proteins in Mitochondrial Division in D. discoideum

Presented by Kelly Dunning

Kelly Dunning

The powerhouses of the cell, mitochondria, are extremely dynamic. Imaging of these organelles in yeast and mammalian cells demonstrates that the steady state tubular structure undergoes numerous fission and fusion events. The balance of these events ensures that the mitochondria can exchange vital components, as well as remain distributed throughout the cell.

The endosymbiont theory states that mitochondria arose from engulfed primitive prokaryotes. Since prokaryotes undergo cell division by forming a z-ring made primarily of the tubulin homolog FtsZ, one would assume that mitochondrial fission would use a similar mechanism. Yet, in an interesting twist, the well studied yeast and mammalian systems instead use dynamin related proteins to mediate mitochondrial fission. Another surprising discovery was that several lower eukaryotes such as Dictyostelium discoideum encode for two mitochondrial FtsZ proteins, FszA and FszB rather than a dynamin related protein.

To gain understanding of this potential alternative fission mechanism, our lab is currently characterizing mitochondrial dynamics in D. discoideum wild-type cells using time-lapse microscopy, and creating FszA and FszB knockout strains by homologous recombination.
While most mitochondria in D. discoideum cells are spherical, our preliminary data suggests that fission and fusion do occur but these events are rare. By analyzing these events in live cells we will further our understanding of the mechanism regulating fission in D. discoideum. We will also gain insight into mitochondrial dynamics of all eukaryotes, as well as increase our understanding of mitochondrial evolution.

Poster 21

Transcriptional dynamics and polymerase distribution

Presented by Michelle Stevense

Michelle Stevense and Jonathan R. Chubb

The relationship between nuclear organisation and gene regulation remains unclear, largely because subtle perturbations of nuclear structure and single cell transcriptional dynamics have been difficult. The ability to examine gene regulation in living cells combined with the ability to perturb nuclear structure using genetics is an attractive route to understanding links between structure and function. We therefore observe single gene transcriptional responses in the genetic model Dictyostelium. We will present data relating single gene transcriptional activity and RNA polymerase distribution. We also present evidence for strong cooperativity in the immediate early transcriptional response.

Poster 22

The evolution of morphology in the social amoebas

Presented by Anna Skiba

Anna Skiba and Pauline Schaap

Social amoebas (Dictyostelia) interchange between uni- and multicellular morphology in response to environmental changes.
100 species of Dictyosteloids have been described thus far, which considerably vary s in the morphology and behaviour of their multicellular structures, the morphology of differentiating cells and the presence of alternative life cycles. A recently constructed molecular phylogeny subdivides all known species into 4 major taxon groups (Baldauf, Roger et al. 2000).
To understand in what order these features evolved we measured a broad range of morphological and behavioural traits and mapped these traits to the molecular phylogey of the Dictyostelids.

Our results show that many of the morphological traits display a clear tendency across the evolutionary tree of Dictystelium species and are more or less randomly distributed across the entire phylogeny.
However we found that all slugs and early coulminants of the most derived, group four species, display a clear anterior- posterior pattern of prestalk and prespore cells, whereas, in contrast, none of the three more basal group species show clear patterning.
Moreover in all four group species cAMP seems to play a key role during aggregation and fruiting body formation. In comparison more basal species use different chemoattractants for aggregation. Furthermore the group four species display the most robust development under a range of culture conditions.
Eventually we are hoping to be able to correlate the evolution of phenotype with the evolution of genotype as evident from genome sequencing of group-representative species. Combined with genetic manipulation of gene of interest this can inform us how genetic changes gave rise to evolution of novel form.

Reference:

Baldauf, S. L., A. J. Roger, et al. (2000). "A kingdom-level phylogeny of eukaryotes based on combined protein data." Science 290(5493): 972-977.

Poster 23

Towards a proteomic analysis of the antimicrobial arsenal of Dictyostelium discoideum

Presented by Moritz Bitzhenner

Moritz Bitzhenner, Ranjani Dhakshinamoorthy*, Christoph Gelhaus*, Matthias Leippe**

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Dictyostelium discoideum is a professional phagocyte and is capable of internalising and degrading several hundred bacteria per hour. Besides the abundant knowledge concerning phagocytosis, little is known about the antimicrobial processes inside the Dictyostelium phagolysosome. Genome analyses revealed that D. discoideum possesses an immense arsenal of members of a structurally defined, so-called saposin-like protein (SAPLIP) superfamily. These proteins are functionally characterised as lipid-binding and membrane-interacting proteins found in lysosome-like organelles from amoebae to humans and are involved in various biological processes such as lipid degradation and immunity. Among the most prominent SAPLIPs are the amoebapores, ~ 8 kDa proteins involved in the pathogenicity of Entamoeba histolytica. According to the Dictyostelium database, the D. discoideum genome harbours 17 genes that potentially code for proteins we termed amoebapore-like peptides (Apls). Several Apls appear to be contained in larger prepromultipeptide precursors which simultaneously may give rise to a variety of mature peptides upon proteolytic cleavage. In total, 33 SAPLIP-domain containing peptides may be released.

Moreover, in the Dictyostelium genome at least 12 lysozyme genes representing four classes of lysozymes can be identified. Gene analysis reveals members of c-type (chicken type), phage-type (T4-phage type), Eh-type (Entamoeba-histolytica type) and Aly-lysozymes (amoeba-lysozyme type). AlyA is the only antimicrobial protein isolated and molecularly characterized from Dictyostelium to date and has been the founding member of a unique class of lysozymes (Müller et al., 2005).

We present a proteomic approach to identify and characterise members of the aforementioned families by a combination of various approaches including enrichment of phagosomes, liquid chromatography, 2D-PAGE and mass spectrometry. Heterologous recombinant expression of selected members of the Apl family is another approach of us to characterize antimicrobial effector proteins involved in the process of intracellular killing and degradation of microbes.

(Supported by Deutsche Forschungsgemeinschaft – LE 1075/2-4)

Poster 24

mAM4, a new protein involved in phagocytosis and cell spreading in Dictyostelium

Presented by Marco Dias
Marco Dias, Anna Marchetti and Pierre Cosson

The amoeba Dictyostelium is a simple genetic system for analyzing phagocytosis and substrate adhesion. A new mutant named mAM4 was isolated in this system. Mutant mAM4 cells phagocytose two times more than wild type cells. The expression of adhesion protein as SibA, Talin and Phg1 is similar in the mutant mAM4 and in wild type, as well as the strenght of adhesion to a substrate. Mutant mAM4 cells have no significant defects in the organization of actin. Remarkably, mutant cells spread two times faster than wild type cells on a substrate, as assessed by RICM. At equilibrium, mAM4 mutant cells are two times more spread on the substrate than wild-type cells. These results suggest that the mAM4 protein functions as a regulator of cell spreading on a substrate.

Poster 25

Understanding presenilin signalling using Dictyostelium discoideum

Presented by Marthe H.R. Ludtmann
Marthe H.R. Ludtmann, P.W. Beesley, P. Alifragis, R. Killick, Robin S.B. Williams

Background:
The inherited form of Alzheimer's disease (AD) is caused by numerous mutations in two presenilin (PS) genes. However, the cell signalling changes caused by these mutations remain unclear. Dictyostelium is the simplest biomedical model possessing two PS homologues, both of which contain the active aspartic acid endopeptidase residues suggesting a common cellular function to that of human PS proteins. The Dictyostelium proteins contain conserved amino acids which, upon mutation in humans, give rise to AD. This project will create Dictyostelium mutants lacking one or both PS genes and mutants expressing only AD-inducing Dictyostelium PS proteins (C263R, P264L, L286V, and M233V) in the absence of wild type background activity. These mutants will be analysed for alterations in biochemical pathways for example phospholipid turnover, calcium signalling and GSK3 signalling.

Material & Methods:
Phylogenetic analysis was employed to establish the homology between Dictyostelium PS proteins and PS proteins of other species. Gene knock-outs were generated by using the Cre-loxP technology. Phosphoinositide turnover was assessed by in vivo [32 alpha] ATP phospholipid labelling.

Results:
Phylogenetic analysis established that the Dictyostelium PS homologues derived from a single common ancestral enzyme. Both genes are differentially expressed, at either high (PSb) or low (PSa) levels throughout

development. Knock-out mutants for both PSa and PSb did not show gross morphological changes during development. Our data suggest ablation of PS genes elevates phosphoinositide turnover, proposing a new mechanism for presenilin signalling.

Conclusions:

Dictyostelium is a useful model to characterise presenilin-controlled signalling. Ongoing work will identify AD-mutation induced signalling changes.

Poster 26

Characterization of the Roco family of proteins

Presented by Bernd Gilsbach

Bernd K. Gilsbach, Wouter N. van Egmond, Arjan Kortholt, Peter J. M. van Haastert

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Roco proteins constitute a novel family of Ras-like GTP-proteins, characterized by the presence of a Ras-like Guanine nucleotide binding domain, called Roc (Ras of complex proteins), followed by a COR domain (C-terminal of Roc). Human leucine-rich-repeat kinase 2 (LRRK2) belongs to the Roco family of proteins and has been found to be thus far the most frequent cause of late-onset and idiopathic Parkinson,Äôs disease (PD). Although, the Roco proteins have been studied intensely since the discovery of missense mutations in LRRK2 segregating with PD, many intriguing questions remain open. By means of biochemical and structural characterization of the prokaryotic Roco protein it was shown that COR is a constitutive dimerisation device and that the Roc G-domain proteins seem to belong to the GAD (G proteins activated by nucleotide-dependent dimerization) class of molecular switches whose GTPase switch-off reaction is regulated by transient dimer formation (Gotthardt et al., 2008, Gasper et al., 2009).

The genome of the cellular slime mold Dictyostelium discoideum encodes 11 Roco proteins. In the current study we are aiming for a detailed biochemical and biophysical analysis of different LRRs from D. discoideum Roco proteins to elucidate their biological functions and binding partners. In this way we hope to get more insights into signaltransduction pathways and how specificity is mediated by Roco-LRRs.

References:

Gotthardt, K., Weyand, M., Kortholt, A., van Haastert, P. J. and Wittinghofer, A.(2008). Structure of the Roc-COR domain tandem of C. tepidum, a prokaryotic homologue of the human LRRK2 Parkinson kinase. EMBO J. 27, 2239-2249.

Raphael Gasper, Simon Meyer, Katja Gotthardt, Minhajuddin Sirajuddin and Alfred Wittinghofer (2009). It takes two to tango: regulation of G proteins by dimerization. Nature Reviews Molecular Cell Biology 10, 423-429 (June 2009)

Poster 27

Physical aspects of Dictyostelium slug locomotion

Presented by Konstantin Doubrovinski

Konstantin Doubrovinski and Edward Cox

In response to starvation solitary Dictyostelium discoideum amoebae aggregate into an amoeba pile. Under appropriate environmental conditions the pile develops into a migratory multicellular organism known as the slug. Slugs may migrate for weeks, during which time the speed of migration remains roughly constant.

In the course of migration, the slug maintains its characteristic bullet-like shape, although the tip may occasionally branch, and the slug splits in two. The surface of the slug is covered in a thin extracellular matrix of protein and cellulose termed the slime sheath.

Shape maintenance and locomotion of the slug are known to principally involve cell-cell adhesion, cell-ECM interactions and coordinated cell migration within the slug tissue. How the interplay of these developmentally vital processes determines the dynamics of the slug remains largely unknown.

We propose to tackle this problem by systematically examining the dynamics of the slug under various external mechanical constraints. To this end we developed an assay that confines a crawling slug in a microfabricated channel of a given shape. We show that this mechanical manipulation can induce a range of behaviors that are absent in or atypical of unconstrained slug locomotion.

Based on our observation we propose a mechanical model for slug locomotion capable of accounting for all of the dynamical behaviors observed in a confined locomoting slug. Our preliminary data suggest that shape and dynamics of this living sausage is primarily a consequence of mechanical interactions between the cell mass and its surrounding slime sheath.

Poster 28

UNDERSTANDING FUNCTIONAL ROLE OF POLYKETIDE SYNTHASES AND ASSOCIATED PROTEINS IN DICTYOSTELIUM DISCOIDEUM

Presented by Divya R Nair

Divya R. Nair, Mauld Lamarque, Ratna Ghosh, Debasisa Mohanty, Shweta Saran, Rajesh S. Gokhale

Dictyostelium genome analysis has revealed the presence of an unprecedented number of Polyketide Synthases (PKSs). PKSs are multi-functional enzymes, capable of synthesizing diverse metabolites with varied biological functions. Our lab is interested in dissecting out how these forty functional PKSs (DiPKSs) could play a role during the morphogenesis of this organism. Recently, we showed that one of the developmental regulating factors, MPBD, is a biosynthetic product of DiPKS1. By combining genetic and

biochemical studies, we are presently investigating the relevance of DiPKS2. Transcriptional profile reported earlier suggests that this gene shows differential temporal expression (4-10 h and 22 h of development). Our studies further indicate cell-type specific expression of dipks2. We are also attempting to identify metabolites produced by this PKS. However, in order to obtain functional form of PKSs, a key step involves their post-translational modification by phosphopantetheinyl transferases (PPTases). We have identified 2 classes of PPTases from Dictyostelium and shown their distinct functions and relevance in its biology.

Poster 29

The transcriptional correpresor PadA regulates aggregation in D. discoideum

Presented by A. Garciandia
Garciandia, A.; Suarez, T. (CIB, Madrid, Spain)

PadA is an NmrA-like protein that belongs to a new class of transcriptional regulators that use NAD(P)+ as cofactors and may function as direct sensors of the metabolic state of the cell. We had previously described padA- as a thermosensitive mutant. We showed that it is defective in PstA and PstAB cell differentiation and unable to complete development at the restrictive temperature (27°C). The padA- mutant carries an allele that codes for a truncated protein lacking 25 aminoacids at the carboxi-terminus (out of 301). Here we show that padA- is probably a lack of function mutation because no PadA protein could be detected in the padA- strain. During development at 22°C, padA- is impaired in aggregation and takes 14-16 h to reach the mound stage. In submerged aggregation experiments, padA- forms 50 times more aggregation centres than the wild type, with very short streams. Our results show that this defect is not rescued in chimaeras with 5-10% AX2 cells but, padA- cells were homogenously distributed in long streams in chimaeras with more than 50% AX2 cells. We looked at the expression of genes involved in cAMP signaling and found that carA, acaA and pdsA were downregulated at the onset of development in padA-. Accordingly, PdsA protein levels were slightly lower in the mutant, which supported the finding that extracellular cAMP levels were higher in padA-. Our results suggest that PadA is required to generate long streams and normal size aggregation territories probably modulating cAMP signaling genes.

Poster 30

Dual role of a Rac1A GTPase in the regulation of cell motility

Presented by Maja Marinovic
Maja Marinovic1, Vedrana Filifá1, Jan Faix2, Igor Weber1
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Rac proteins are members of a broad family of monomeric Rho GTPases that act as key regulators of the actin cytoskeleton. Previously it was shown that Rac1A binds to the IQGAP-related protein DGAP1, which forms a cortical complex with cortexillin heterodimer . This complex localizes to the rear end of moving cells and to the cleavage furrow of dividing cells, where it supports efficient cytokinesis .To gain further insight into the role of Rac1A in Dictyostelium cells, we created a fluorescent probe that specifically binds its GTP-bound form. A screen based on yeast two-hybrid and GST pull-down assays resulted in the selection of an interaction partner specific for the active form of Rac1A, a GBD (GTPase-binding domain) from rat PAK1 kinase. PAK1_GBD was fused N-terminally to YFP and expressed in Dictyostelium cells. In non-motile cells, our probe was strongly enriched throughout the cortex, while in motile cells it always localized to the leading edge. During phagocytosis and macropinocytosis, the probe localized to endocytotic cups. During cytokinesis and chemotaxis the probe didn,ÄôÈt show any prominent localization. In order to demonstrate an interaction between PAK1_GBD and GTP-Rac1A in living cells, we employed fluorescence resonance energy transfer (FRET) approach. A unimolecular probe was constructed, where PAK1_GBD and Rac1A were sandwiched between florescent proteins YFP and mRFP. This FRET probe has a prominent cortical localization and measurements by sensitized emission of the acceptor indicate that PAK1_GBD and GTP-Rac1A interact in the cortex of living cells. Altogether, our results demonstrate that localization of active Rac1A, as reported by our probe PAK1_GBD-YFP, does not correspond to localization of GFP-DGAP1 and GFP-cortexillin, the other components of the aforementioned cortical complex that contains Rac1A. Based on these results, we propose that Rac1A has a dual role in regulation of the actin cytoskeleton. In addition to its established role in recruitment of the DGAP1-cortexillin complex to the rear parts of a polarized cell, it also participates in signaling pathways that control de novo actin polymerization at the protruding regions of the cell.

Poster 31

Using Dictyostelium discoideum as a model organism to explore the cellular role of dopamine receptor interacting proteins

Presented by Nicholl Pakes

Nicholl Pakes 1, Jamal Nasir 2, Robin Williams 1

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The dopamine signalling pathway has been implicated in the pathophysiology of neuropsychiatric conditions including bipolar disorder and schizophrenia. A detailed analysis of this pathway is essential for understanding these conditions. Previous work identified eleven novel human dopamine receptor interacting proteins (DRIPs), but their role in cell signalling remains unclear.

In this project, we have employed a simple biomedical model, Dictyostelium discoideum, to elucidate the role of these DRIPs in cell signalling. This model

organism has previously been used to identify cell signalling pathways targeted by bipolar disorder drugs. BLAST analysis of these proteins in Dictyostelium revealed five potential homologues. From these DRIPs, two have been chosen for further analysis (DRIP2 and DRIP9), based on sequence comparison and paucity of current literature. The DRIP2 homologue in Dictyostelium shares 50% similarity and 30% identity with the human DRIP2 protein. These proteins are dedicator of cytokinesis (DOCK) family proteins which have guanine exchange factor (GEF) activity and are involved in the regulation of small GTPases. The Dictyostelium and human DRIP9 homologues share 49% similarity and 29% identity. These are serine/threonine kinase proteins of the microtubule affinity regulating kinase (MARK) family, which are involved in the regulation of microtubule dynamics.

To investigate the function of these proteins, gene knockout mutants have been created in isogenic strains. These mutant cells did not show gross developmental changes in response to bipolar disorder treatments. We are currently creating cell lines expressing tagged proteins, for cellular localisation and proteomic studies. This approach will enable us to identify the cellular pathways and down stream targets of these two DRIPs using the Tandem Affinity Purification (TAP) method. Finally, analysis of pathway specific regulation and novel potential bipolar disorder treatments will be pursued.

This project therefore aims to characterise the molecular function of two DRIPs in Dictyostelium, to better understand their role in cell signalling and their potential involvement in the aetiology of bipolar disorder and schizophrenia.

Poster 32

An RcdK transcription factor regulates DIF signaling, pstA differentiation and pstB differentiation

Presented by Hiroshi Senoo

Hiroshi Senoo, Hong Yu Wang, Jeff Williams and Masashi Fukuzawa

In order to identify the signaling pathway directing pstA cell differentiation, we have been studying the ecmAO promoter sub-region that directs pstA expression. We found two linked CA-rich sequences (R3 and R4) within the ecmA promoter sub-region that are necessary for pstA expression. When R3-R4 was multimerized 4 times and cloned in A15deltaBamGal (4xR3R4-lacZ), it was expressed in pstA cells and in scattered pstO cells. Using R3 and R4 as baits, we purified proteins by DNA affinity chromatography. Several transcription factors including MybE and DimB were identified but here we focus on another transcription factor, RcdK. RcdK shares homology with Saccharomyces cerevisiae Ndt80/PhoG. This binds conserved CA-rich sequences and activates expression of more than 200 genes at mid-meiosis, including those required for meiotic divisions and spore formation. Although Dictyostelium rcdK- cells grow normally, there is a marked delay (~24h) in aggregation. When aggregates are formed, the null exhibits a multi-tipped phenotype and the slugs tend to break up, resulting in small fruiting bodies.

Although ecmA expression is normal in the null slugs the 4xR3R4-lacZ construct is completely inactive. Also, the rcdK null has a defect in basal disc formation, a phenotypic characteristic of DIF-signaling mutants, and it shows an aberrant ecmB expression pattern in the fruiting body; all staining in the upper/lower cup is lost. Furthermore, ecmB-lacZ is not DIF-inducible and monolayer differentiation of stalk cells is much reduced (<10% of WT). These results suggest that RcdK has a role in pstA-cell differentiation as well as in the DIF-1 signaling pathway controlling ecmB expression. Consistent with this, we separately isolated RcdK as a transcription factor that binds to an activator element in the stalk-tube (ST) region of the ecmB promoter.

Poster 34

Dictyostelium discoideum as a Potential Model for Identifying Emetic Liability

Presented by Steven Robery

Stven Robery, Jana Mukanowa, Dr Robin Williams, Prof Paul Andrews

Nausea and emesis (also known as vomiting) are common potentially serious side effects associated with over 33% of currently marketed therapeutic drugs. Identification of emetic liability is only possible during later stages in drug discovery and development employing a range of experiments on various sentient animals. Dictyostelium discoideum is a social amoeba used as a simple biomedical model since it contains a number of genes with therapeutically-relevant human homologues that are absent in more simple models such as yeast.

In this project, we have examined the possibility of using Dictyostelium for use in a preliminary screen for emetic liability to reduce or replace animal use. Preliminary results suggest that a number of emetic compounds display an acute effect on cell movement. Using this approach, we have shown that three known emetic compounds- copper salts, rolipram, capsaicin had a rapid and significant effect on Dictyostelium chemotaxis.

These initial results suggest that Dictyostelium has the potential to identify the increased risk of emeticity of novel therapeutic drugs.

(This project has been funded by Universities Federation for Animal Welfare UFAW).

Poster 35

Functional analysis and subcellular localization of SunB, a novel type of SUN protein

Presented by Nao Shimada

Nao Shimada, Takefumi Kawata and Satoshi Sawai

The conventional SUN-1 proteins are inner-nuclear membrane proteins containing at least one transmembrane domain and a C-terminal Sad1/UNC-84(SUN)-domain. These proteins together with KASH-domain containing proteins are thought to act as adaptors that connect the nuclear outermembrane to cytoskeletons. We have recently shown that a there is a new family of SUN protein that possesses a SUN-domain in the middle of the amino acid sequence and that its member SunB in Dictyostelium is essential for development. Here we show that during the interphase, SunB localizes to the nucleus, cell cortex and microtubles. During mitosis, SunB protein is enriched in the centrosome. A knockdown strain of sunB cells are hypersensitive to nocodazole treatment and harbor multiple MTOCs and centrosomes. The centrosomes were enlarged and appeared to be detached from the nucleus. Moreover, based on BrdU-incorporation the ratio of S-phase cells increases from 7% in the wildtype to 14% in the sunB mutant strain. Flow-cytometric analysis also indicates that DNA content of nucleus is doubled in the mutant strain. To summarize, SunB is required for proper size and numbering of microtubules and centrosomes during the interphase. In the S-phase, SunB may be involved in regulation of DNA replication and its loss results in extension of the S-phase and increase in nuclear DNA-content. The correct positioning of the centrosome via SunB during mitosis may thus be responsible for proper timing of nuclear division and DNA replication.

Poster 36

Well-defined chemoattractant patterns generated by the flow photolysis technique modulate the spatio-temporal protein recruitment to the cell cortex of D. discoideum

Presented by Hellen C. Ishikawa-Ankerhold

Hellen C. Ishikawa-Ankerhold, Till Bretschneider, Günther Gerisch, Annette Müller-Taubenberger, Eberhard Bodenschatz and Carsten Beta

Dictyostelium discoideum respond to cAMP stimulation with a distinct chemotactic response by rearranging their actin network. Here, single- and double-mutants lacking one or two proteins of interest were analyzed by confocal live-cell microscopy, employing the flow photolysis stimulation technique. This approach uses a microfluidic device in combination with photo-induced uncaging of the cAMP chemoattractant (Beta et al., 2007). This uncaging technique offers a precise temporal control of the chemoattractant delivered to the cells and allows to perform multiple single-cell experiments in one setup.

Using the uncaging technique the patterns of cAMP stimulation were modulated and consecutive pulses of chemoattractant at different intervals were applied to the cells. Under these conditions, the actin response was studied in the presence or in the absence of two actin-binding proteins, coronin (CorA) and/or actin-interacting protein (Aip1).

Wild-type cells exhibited sharp and distinct actin response peaks upon a series of three cAMP pulses at intervals of 15, 20, 25, 30, 35 and 40 seconds,

and irregular and ineffective responses upon intervals of less than 10 seconds.

In the single-mutant cells of CorA or Aip1 the recovery of the actin response after repetitive pulses of cAMP could be observed only at intervals longer than 15 seconds. In the double-mutant cells the recovery of actin was further delayed. These data suggest the participation of both proteins in de-adaptation of the actin response.

Poster 37

Possible functions of the stalk-cell vacuole

Presented by Toru Uchikawa

Toru Uchikawa, Kei Inouye

Most plant and fungal cells develop large vacuoles. Although vacuoles in these organisms are known to have various functions, such as degradation, turgor pressure formation, cell volume expansion and storage, the mechanism of their de novo formation is not well understood. We are studying the mechanism of de novo vacuole formation and the function of stalk-cell vacuoles in Dictyostelium.

We have previously shown that stalk-cell vacuoles are formed by repeated fusion of autophagosomes and lysosomes, which suggested that stalk-cell vacuoles have a function of degradation. As fully differentiated stalk cells are non-viable, vacuoles are not likely to have a function of storage. To examine the possibility that stalk-cell vacuoles generate turgor pressure, we quantitatively analyzed the vacuole and cell volumes and cell wall formation during stalk cell differentiation in vitro and in vivo. Live imaging of differentiating stalk cells revealed that the cell volume increases in parallel with vacuole enlargement, suggesting that vacuolization contributes to cell expansion. It was also shown, using Calcofluor as a cell wall marker, that the rates of increase in vacuole and cell volumes drop upon initiation of cell wall formation. This suggests that expansion of the vacuole and the resistive force by the cell wall generate turgor pressure. Observation of culminating fruiting bodies revealed that a large vacuole formation and cell expansion occur in the upper part of the stalk where stalk cells are confined within the small space in the stalk tube, a tough structure composed of cellulose fibrils. Expansion of stalk cells and the resistive force by the stalk tube would generate large pressure, like turgor pressure, which would also contribute to the rigidity of the stalk needed to hold the spores aloft.

Poster 38

Single cell imaging of cytoplasmic Ca2+ fluxes using the FRET reporter Cameleon

Presented by David Traynor

David Traynor and Robert R. Kay

Ca2+ is one of the most important messenger molecules in biology and consequently there has been a substantial effort to develop tools to image Ca2+ dynamics in living cells. In Dictyostelium Ca2+ sensitive small molecule reporters including Fura-2, Fluo-3 and Calcium Green have been utilised but these can be subject to compartmentalisation and leakage. In addition they are often expensive and are introduced into the cell by harsh treatments, such as scrape-loading or electroporation, that can have adverse physiological effects. Genetically encoded Ca2+ indicators (GECIs) such as aequorin offer an alternative but this indicator has proven to be more suited to population measurements of Ca2+ dynamics in Dictyostelium. Cameleon is a GFP based GECI that links a conformational change upon Ca2+ binding to changes in FRET between the donor CFP and acceptor YFP fluorophores present in this protein. We have constitutively expressed Cameleon in the cytoplasm of amoebae and measured changes in cytoplasmic Ca2+ ([Ca2+]c) in single amoebae. The addition of micromolar concentrations of extracellular ATP produces rapid and transient changes in [Ca2+]c whereas similar concentrations of cAMP elicit a delayed [Ca2+]c response. These results show that [Ca2+]c can be reliably monitored in single cells using FRET.

Poster 39

The Dictyostelium nuclease Xpf is required for G2/M checkpoint activation after cisplatin treatment

Presented by Judith Langenick

Judith Langenick, Tetsuya Muramoto, David Traynor, Rob R. Kay, Jonathan R. Chubb and Ketan J. Patel

Fanconi anaemia (FA) is a rare genetic disorder characterised by progressive bone marrow failure, congenital abnormalities, cancer predisposition and cellular hypersensitivity to DNA interstrand crosslinks (ICLs) ‚Äì very toxic DNA lesions blocking DNA replication and transcription. Vertebrates use four main DNA repair pathways to resolve ICLs; translesion synthesis, the Fanconi anaemia pathway, homologous recombination and excision repair nucleases. Proteins from all these repair groups are believed to work in a coordinated manner to repair crosslinks, however much work still needs to be done to understand how precisely this repair is achieved.

All gene groups required for ICL repair are present in the Dictyostelium genome. We recently determined that Dictyostelium Fanconi and Translesion synthesis null mutants are mildly sensitive to the DNA crosslinking agent cisplatin ‚Äì while disruption of the Xpf nuclease results in profound hypersensitivity*1. As Xpf is essential for the extreme tolerance to ICLs in Dictyostelium we further characterised the xpf-null mutant. By overexpressing the S-phase marker GFP-PCNA*2 in xpf-null and wild type cells we were able to monitor the cell cycle response to cisplatin. Wild type cells respond to cisplatin with an activation of the G2/M checkpoint and an arrest of cells in the G2 phase of the cell cycle. xpf-null cells in contrast are unable to activate the G2/M checkpoint, fail to arrest in G2 but instead continue

undergoing mitosis. In response to bleomycin, a DNA double strand break inducing agent, however xpf-null cells are able to activate the G2/M checkpoint and arrest. Taken together these results indicate that incision at the crosslink by Xpf and the resulting creation of a DNA double strand break are requirements for cell cycle arrest and thereby successful repair. However once the double strand break has occurred the checkpoint can be activated in the absence of Xpf.

References:

*1 Zhang, X., Langenick, J., Traynor, D., Babu, M. M., Kay, R. R. and Patel K. J. (2009). Xpf and not the Fanconi anaemia proteins or Rev3 accounts for the extreme resistance to cisplatin in Dictyostelium discoideum. PLoS Genetics, 5(9):e1000645.

*2 Muramoto, T., Chubb, J. R. (2008). Live imaging of the Dictyostelium cell cycle reveals widespread S phase during development, a G2 bias in spore differentiation and a premitotic checkpoint. Development 135: 1647-1

Poster 40

Putative signaling role of *agpA*, in the regulation of macropinocytosis, motility and cell division

Presented by David Knecht

Charito Romeo, Deneene Doyker and David A. Knecht

Macropinocytosis is a major endocytic pathway in living organisms characterized by non-selective, clathrin-independent and receptor-independent bulk-phase uptake of extracellular fluid. This process is important for many biological processes including nutrient uptake, membrane recycling, antigen processing and pathogen invasion, however many aspects of its spatiotemporal regulation are unknown. We have used REMI mutagenesis and flow cytometry to screen for cells conditionally defective in macropinocytosis, and have isolated two genes that were disrupted in some of the mutants. We have named the first mutated gene agpA since it contains conserved domains similar to human 1-acylglycerol phosphate O-acyltransferase [AGPAT8] and mouse lysocardiolipin acyltransferase isoform 1 [ALCAT]. These enzymes convert lysophosphosphatidic acid to phosphatidic acid, which are key signaling molecules and intermediates in phospholipid biosynthesis. Preliminary phenotype characterization of agpA null cells shows a reduced rate in fluid uptake, absence of growth in HL5, a dramatic increase in random motility, impairment of cell division and a decrease in cell size. Our current goal is focused on recapitulation of the REMI mutation, construction of fluorescent fusion probes for agpA and determination of alterations in lipid profiles in order to identify the metabolic and signaling roles of agpA in macropinocytosis.

Poster 41

The role of MEGAP2 in the regulation of the SCAR/WAVE complex during chemotaxis.
Presented by Andrew Davidson
Andrew Davidson, Robert J. W. Heath & Robert H. Insall

Dictyostelium MEGAP2 (srGAP3/WRP/ArhGAP14) potentially couples membrane curvature events to intracellular signaling and subsequent cytoskeleton reorganization. Its homologues have been implicated in regulation of Rac GTPases and the SCAR/WAVE complex, mental retardation, and neuronal migration during development. MEGAP2 possesses a N- terminal F-BAR domain, which induces membrane curvature (Itoh et al., 2005), and a C-terminal RhoGAP domain. Mammalian MEGAP1 has also been shown to interact with the Arp2/3 activator SCAR1 through its C-terminal SH3 domain and selectively stimulate the GTPase activity of Rac1 (Soderling et al, 2002). Since Rac is a known upstream activator of the SCAR/WAVE complex, these findings suggest MEGAP2 may connect membrane curvature to subsequent inactivation of the SCAR/WAVE complex.

We have found that MEGAP null Dictyostelium cells have a range of interesting phenotypes involved in cytoskeleton reorganization. GFP-MEGAP1 has been shown to induce tubulation of the contractile vacuole network and it has been proposed that it drives the expulsion of excess water from the cell under hypo-osmotic conditions (Heath and Insall, 2008). We find that MEGAP2 is involved in macropinocytosis and also localizes to the leading edge of cells. Mgp2- null cells present with morphological, adhesion and chemotactic defects which are being investigated. The spatial/temporal relationship between MEGAP2 and the SCAR/WAVE complex will also be demonstrated and how this is perturbed in mgp2 nulls.

Poster 42

Expression profile of Polyketide Synthase genes in Dictyostelium discoideum
Presented by Kazuki Akabane
Kazuki Akabane & Tamao Saito

Polyketides are a ubiquitous class of natural products produced by bacteria, plants and fungi.They are made by polyketide synthases (PKSs), which are currently classified into three groups: type I, type II, and type III-the chalcone synthase-PKS.

The genome project has revealed that Dictyostelium discoideum has 45 polyketide synthase (PKS) genes in its genome. It seems that Dictyostelium has more PKS genes than any other organisms, though the function of the most PKSs has not been analysed. As a first step of the functional analysis of these PKSs, we analysed the expression profile of them by RT-PCR with different feeding conditions (i.e. HL-5, gram positive and negative bacteria). Interestingly, most of the PKS genes seemed to be expressed in the later stage of the development, suggesting that they are involved in the cell type

differentiation. According to the expression profile, we categorized them into 4 groups. The different expression patterns of PKS genes with the different feeding conditions will also be discussed.

Poster 43

Extracellular calmodulin in Dictyostelium discoideum: A new way to study an old protein

Presented by Robert Huber
Andres Suarez 2, Robert Huber 1 and Danton H. O,ÄôDay 1,2
1. Department of Cell and Systems Biology, University of Toronto, Canada
2. Department of Biology, University of Toronto Mississauga, Canada

Calmodulin (CaM) is a ubiquitous calcium binding protein that has been found in every eukaryote studied to date. It has been demonstrated to be involved in a wide variety of processes such as growth, proliferation and movement. CaM exerts its effects by regulating the activity of proteins collectively known as CaM-binding proteins (CaMBP). Most of the research done to date on CaM and its binding partners has focused on their function in the intracellular environment. However, CaM has also been detected extracellularly in several organisms, including plants, frog, rat, rabbit and human. Furthermore, CaMBPs have also been detected extracellularly, providing evidence that the established importance of CaM extends to the outside of the cell. Here we show that Dictyostelium CaM is constitutively expressed and secreted throughout asexual development. In order to gain a better understanding into the role that extracellular CaM plays in Dictyostelium we treated cells with exogenous CaM and assayed its effect on cAMP-mediated chemotaxis and cell proliferation. Treatment with exogenous CaM increased the rate of chemotaxis and decreased the rate of cell proliferation. In addition, we have identified a novel matricellular CaMBP, cyrA, which is secreted throughout development. Interestingly, treatment with the CaM antagonist W-7 suggests that CaM regulates the events leading to proteolytic cleavage of cyrA. Our research provides the first direct evidence for the existence of extracellular CaM in the Dictyostelium and provides insight into its functions in this model organism.

Poster 44

Four-dimensional chemotaxis of Dictyostelium cells using optical coherence tomography

Presented by Sara Rey
Sara Rey, Boris Považay, Bernd Hofer, Wolfgang Drexler and Adrian Harwood

Cell chemotaxis is typically performed on 2D glass substrates, which are very different from their natural environment. Cell substrates, for example biomaterials, have been shown to affect cell function and behaviour. However, these materials are often non-transparent. Consequently, due to current limitations in optical imaging technology, probing the effects of

different environments on cells generally requires the use of labelling modalilties which can perturb the cell.

The non-invasive imaging technique of frequency domain optical coherence tomography (OCT) is used to image chemotaxing label-free Dictyostelium discoideum cells in timelapse, during development and on exposure to a cAMP gradient. Cells are visualized in 2D on opaque substrates and suspended within a 3D agarose gel. Preliminary findings indicate that movement of Ax2 cells on a nitrocellulose filter and on agar are comparable in terms of speed and directionality. Ax2 cells are able to move within agarose and form streams. However, myosin heavy chain knockout cells, which are able to move in 2D, are unable to move within the more challenging environment of agarose.

Migratory and chemotactic changes in mutant cells within complex environments can be non-invasively visualized using OCT. Therefore, OCT is demonstrated as a useful method for imaging Dictyostelium cell migration within 3D constructs and on non-transparent 2D surfaces.

Poster 45

Dictyostelium discoideum Amoebae Chemotax to Relatively Steep Gradients of Ca++: Further Characterization of the Ca++ Response

Presented by Amanda Scherer
Amanda Scherer, Spencer Kuhl, Deborah Wessels, Daniel F. Lusche, Brent Raisley and David R. Soll
The W. M. Keck Dynamic Image Analysis Facility, Department of Biology, The University of Iowa, Iowa City, IA 52242

By generating stable Ca++ gradients in a microfluidic chamber, we have demonstrated that Ca++ is a highly effective chemoattractant for aggregation-competent D. discoideum cells and that parallel spatial gradients of cAMP and Ca++ in vitro are more effective than either alone. The Ca++ gradient we found to be optimal for chemotaxis was estimated to be 100,000 times steeper than the optimal cAMP gradient. This seemingly large difference is, however, consistent with the approximately 100,000-fold difference between the binding constants of the known Ca++-sensing receptors in animal cells and the cAMP receptor in D. discoideum. To further characterize Ca++ chemotaxis in D. discoideum, we measured the dose-response to Ca++, tested the effects of pertussis toxin on Ca++ chemotaxis, and assessed the capacity of various mutant strains to chemotax to Ca++. Based on our results and the extensive number of examples of Ca++ chemotaxis in cell types of higher eukaryotes, we consider the hypothesis that combinatorial signals consisting of both a cell type-specific attractant and Ca++ may be involved not only in D. discoideum aggregation but also in the chemotaxis of some animal cells. Ca++ chemotaxis may have been conserved as an enhancing system for cell type-specific chemotaxis in the evolution of the eukaryotes. However, although very high soluble concentrations of Ca++ have been demonstrated in the environment of free

living cells and cells in multicellular organisms, steep soluble Ca++ gradients have not been physically demonstrated in vivo. A putative receptor for Ca++ chemotaxis, IplA, has been identified (see Lusche et al. presentation).

Poster 46

How a Cell Crawls during Chemotaxis: 3D Analysis of Dictyostelium Pseudopodial Dynamics in a cAMP Gradient

Presented by Spencer Kuhl

Spencer Kuhl and David R. Soll

Lateral pseudopod suppression is a hallmark characteristic of efficiently chemotaxing Dictyostelium amoebae. Our efforts to understand how this key event is regulated began in 1987 when Varnum-Finney et al. showed that Dictyostelium amoebae orient in a cAMP spatial gradient by extending approximately equal numbers of pseudopods towards and away from the cAMP source. However, pseudopods extended towards the source were more likely to produce a turn. We subsequently developed and applied the 3D Dynamic Image Analysis System (3D-DIAS) to derive an integrated, 3D view of cell crawling that incorporated the dynamics of pseudopod extension, retraction and uropod behavior (Soll et al., 2009) during random cell motility. We have now similarly analyzed cell crawling and pseudopod dynamics in spatial cAMP gradients, using our conditions for chemotaxis as well as that of other laboratories. Using cells expressing the F-actin marker ABD-GFP to more accurately identify pseudopods, confocal microscopy and high resolution DIC imaging, we will present 3D data related to the origin, volume, shape, expansion and retraction rates of pseudopods generated by cells crawling up a cAMP gradient. In addition, we have assessed the process referred to as splitting, in which cells extend a new anterior pseudopod from the previous anterior pseudopod through pseudopod bifurcation.

Poster 47

Cations regulate cellular behavior through multiple receptors in Dictyostelium discoideum.

Presented by Daniel F. Lusche

Daniel F. Lusche, Deborah Wessels, Amanda Scherer, Spencer Kuhl and David R. Soll.

We have previously shown that high Ca++ (10-20mM) and high K+ (40mM) facilitate cell motility (Lusche et al. 2009, Soll et al. 2009, Soll et al. 2002) in the absence and presence of a cAMP gradient. We have also recently demonstrated that aggregation-competent amoebae undergo chemotaxis up a Ca++, but not a K+ gradient (see Scherer et al. presentation). Here we present evidence that IplA, a mechanoreceptor (Fache et al. 2004), functions as the putative receptor for Ca++ chemotaxis. Its role in Ca++ facilitation, however, is ambiguous. We also present evidence that Nhe1, a monovalent cation-hydrogen exchanger functions as the putative receptor for K+ facilitation, but not Ca++ facilitation. The receptor for Ca++ facilitation has

not as yet been identified. A model is presented that includes the K+ facilitation receptor, the unidentified Ca++ facilitation receptor, the identified Ca++ chemotaxis receptor and the cAMP receptor. Finally, which functions in both facilitation and chemotaxis.

Poster 48

Defining cationic facilitation by a comparison of the major buffer systems used to study Dictyostelium disocoideum chemotaxis, and cationic facilitation solutions.

Presented by Daniel F Lusche

Daniel F. Lusche, Amanda Scherer, Deborah J Wessels, Kristin Wood and David R. Soll

Here we show that the great majority of buffer solutions used to study chemotaxis do not facilitate maximum velocity in the absence of chemoattractant (basic cell motility) and in a spatial gradient of cAMP.

They do however, support efficient chemotactic orientation in gradients. All but one of the tested 9 buffer solutions resulted in cells crawling at half the velocity of those crawling in facilitating concentrations of Ca++ (10mM) and K+ (40mM). These results provide a clear distinction between what we define a cationic facilitation and chemotactic orientation.

Poster 49

New insights into DIF-1 3(5) dechlorinase enzyme
Presented by Francisco Velazquez-Duarte
Francisco Velazquez-Duarte and Rob Kay

Differentiation-inducing factor 1 DIF-1 [1-(3,5-dichloro-2,6-dihydroxy-4-methoxyphenyl)-hexan-1- one] was identified as a stalk inducing molecule using in vitro monolayer assays. The continuous work of many laboratories has improved our knowledge of how DIF signalling works during Dictyostelium development although we are still far from getting a comprehensive picture. Recently, using a combined approach of in silico searches and Mass Spec, we reported the identification of the gene encoding DIF-1 3(5)- dechlorinase, otherwise named DIFase, responsible for the first step of DIF-1 metabolism. Here we report further characterization of DIFase both at the biochemical and cellular level. We will discuss insights into a putative enzymatic mechanism of DIF-1 dechlorination as well as the spatial regulation of DIFase expression upon slug cutting.

Poster 50

Open Access Microfluidic Device for the Study of Cell Migration during Chemotaxis
Presented by Dawit Jowhar
Dawit Jowhar, Gus Wright, Philip C. Samson, John P. Wikswo, Christopher Janetopoulos

Cells sense and interpret chemical gradients, and respond by localized responses that lead to directed migration. An open microfluidic device (OMD) was developed to provide quantitative information on both the gradient and morphological changes that occurred as cells crawled through various microfabricated channels. This device overcame problems that many current devices have been plagued with, such as complicated cell loading, media evaporation and channel blockage by air bubbles. We used a micropipette to set up stable gradients formed by passive diffusion and thus avoided confounding cellular responses produced by shear forces. Two versions of the OMD are reported here: one device that has channels with widths of 6, 8, 10 and 12 microns, while the other has two large 100 micron channels to minimize cellular interaction with lateral walls. These experiments compared the migration rates and qualitative behavior of Dictyostelium discoideum cells responding to measurable cAMP and folic acid gradients in small and large channels. We report on the influence that polarity has on a cell's ability to migrate when confined in a channel. Polarized cells that migrated to cAMP were significantly faster than the unpolarized cells that crawled toward folic acid. Unpolarized cells in wide channels often strayed off course, yet migrated faster than unpolarized cells in confined channels. Lastly, it was found that the polarized cells could easily change migration direction even when only the leading edge of the cell was exposed to a lateral gradient.

Poster 51

The dynamic localization of actin binding proteins to the cytoskeleton
Presented by David Knecht
Mike Lemieux, Ran-der Hwang and David Knecht

Actin binding proteins are proposed to organize actin filaments into orthogonal, parallel and anti-parallel arrays in order for the cell to carry out specific mechanical functions. Since all of these ABPs are present in cells, it is unclear how different forms of actin can be created if all ABPs bind to new actin filaments. While there is data using knockout cell lines suggesting that each of these proteins plays some function in cells, it is unclear how all of these different actin cross-linkers act in concert to regulate the dynamic behavior of a living cell. To answer this question, we have created cell lines expressing multiple actin binding proteins fused to fluorescent probes to examine their relative dynamic distribution. Fimbrin and alpha-actinin are present in the distal tips of new phagocytic and macropinocytic cups. Coronin, dynacortin, filamin, and enlazin associate with the proximal portion of the cups. In polarized cells, fimbrin and alpha actinin are localized to the front of the cell and diffuse throughout the cytoplasm, while coronin,

dynacortin, filamin, and enlazin are mostly bound to the cortex in the rear of the cell with little free cytoplasmic protein evident. It is likely that the cytoplasmic protein concentration is indicative of the affinity of the ABP for F-actin. There is a general correlation that proteins that appear to have a high binding affinity are distributed in the rear cortex of polarized cells due to their association with rearward moving actin filaments. When new filaments are formed in the front, there is a low concentration of high affinity ABPs available to bind. New filaments at the front are therefore primarily associated with weak binding proteins that have a high free concentration.

One of the challenges of this work is determining the distribution of F-actin relative to that of the ABPs. We have found that expression of probes for F-actin alters the amount and localization of F-actin, and as a result alters cell behavior. We have developed a new GFP-Lifeact fluorescent probe and compared its distribution in live cells to that of filamin-ABD and LimE alpha coil. The Lifeact probe appears to dynamically associate with F-actin while causing minimal changes to the cytoskeleton.

Poster 52

Cyclic AMP controlled oscillation assessed by acoustic and impedimetric whole cell biosensors

Presented by Edith Schaefer

Edith Schaefer, Andreas Janshoff

Dictyostelium is a well established model system to analyse cell migration and cell-cell communication during aggregation, because starvation induces periodic pulses of cyclic AMP (cAMP) secreted by cells which trigger a cyclic migration of hundreds of amoebae to the aggregation centre to form a multicellular structure (Dormann and Weijer, 2006).

We monitor these cell movements at the early phase of cell aggregation in real time by electric cell-substrate impedance sensing (ECIS) and quartz crystal microbalance (QCM) measurements, two non-optical, label-free and non-invasive methods (Tarantola, Janshoff et al. 2010). In ECIS measurements the time dependent impedance signal is detected, which is sensitive to shape and cell-substrate distance alterations. The QCM technique measures the shift in resonance frequency of an acoustic resonator over time caused by cell attachment or changes of cell stiffness close to the quartz surface. Therefore, both methods provide a means to detect cell motility by analysing the signal fluctuation. Four to six hours after starvation a cyclic, simultaneous movement of cells on the substrate is detectable by periodic spikes in the measuring signal. This oscillation can by influenced by injection of cAMP in the similar way as found in spectrophotometric experiments (Gerisch and Hess, 1973).

Poster 53

The Dictyostelium retinoblastoma ortholog is a major regulator of developmental gene expression
Presented by Harry MacWilliams
Kimchi Strasser, Adrian Tsang, Harry MacWilliams

Using data from DictyExpress, we have examined the expression of the genes which are regulated (repressed) by the retinoblastoma ortholog rblA. Many of these genes have known functions in cell cycle progression. The overwhelming majority of such genes show low expression at T0 and T4, then a prolonged peak between T8 and T16, followed by low expression again at T20 and T24. These include both S-phase and mitotic genes. There is some tendency for mitotic genes to show later expression peaks. One can also see differences among genes whose promoters contain differerent putative cell-cycle elements. In particular, putative targets of the histone acetyl transferase smyd3 show late expression peaks, which correlate well with the late expression peak of symd3 itself.

A number of putative cell cycle genes which are NOT regulated by rblA, prominently the cyclins, nonetheless show expression peaks between T8 and T16. The peaks of cyclin D and cyclin A occur before that of cyclin B. This is the same expression order as is observed in the mammalian cell cycle.

Of the two rblA-regulated transposons, one shows the typical ‚Äúcell cycle‚Äù developmental trajectory. This transposon has a putative E2F binding site in its promoter.

There are old reports suggesting that Dicty cells go through a cell cycle during mid-development, but also several newer papers which dispute this. The observed expression patterns strongly suggest cell cycle activity in Dicty development, if not in present-day laboratory strains, then at least in recent ancestors.

A number of DNA-repair genes are regulated by rblA. For the most part, these are genes with putative functions in replication-coupled repair. Such genes show a somewhat different developmental trajectory: the peak between T8 and T16 is present, but there is an additional expression at T0. The ratio of the T0 to the T8-16 peak heights is very variable. I suggest that this might reflect the relative roles of these genes in replication-coupled and replication-independent repair pathways.

Finally, a number of genes are not repressed but activated by rblA. The vast majority of such genes show activity confined to late development, typically with a peak at T24. These genes show both spore- and stalk-specific activity. This observation is reminiscent of the well-known role of Rb in terminal differentiation in mammalian cells, and suggests that the involvement of Rb in differentiation began in unicellular organisms.

Poster 54

Phosphatidylinositols in Dictyostelium: A procedure for radiolabelling, extraction and analysis by TLC chromatography.
Presented by Jonathan Ryves

W. Jonathan Ryves, Regina Teo and Adrian Harwood.*
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The turnover of Phoshatidyl inositol phospholipids (PIPs) are known to play a critical signalling role in a diverse range of processes. It is therefore crucial to understand how altering the availiability of PIPs can orchestrate cellular events within the cell.

PIPs undergo rapid and transient turnover in developing Dictyostelium (1). In response to cAMP pulses the appearance of PIPs such as Phosphatidylinositol (3,4,5) P3 (PIP3) coincide with activation of signalling proteins, such as PKB, on cell membranes.

We have exposed Dictyostelium cells to radiolabelled ATP and extracted PIPs during cAMP signalling to analyse the changes to PIP species using TLC (2). This approach has provided evidence that, while Lithium treatment limits the entry of Inositol into the PIP network (presumably though inhibition of enzyme in the inositol cycle) it also causes membrane PIP3 levels to be maintained at a higher level. We suggest that it is the de-novo production of higher PIPs rather than levels of those PIPs that are crucial in transmitting the cAMP signal to initiate cellular processes beyond the membrane.

References:
(1) Yi Elaine Huang et al, (2003) Receptor-mediated Regulation of PI3Ks Confines PI(3,4,5)P3 to the Leading Edge of Chemotaxing Cells. Molecular Biology of the Cell , Vol. 14, 1913-1922
(2) Cyber Lipid Centre: http://www.cyberlipid.org/cyberlip/home0001.htm

Poster 55

DlaA, a protein containing La-like RNA-binding domain, is involved in cell growth and differentiation of cellular slime mould Dictyostelium discoideum
Presented by Toshinori Usui
Toshinori Usui and Tomoaki Abe

We identified a novel protein DlaA, that contains an RNA-binding domain highly homologous to metazoan La, with the analysis of REMI mutant isolates. DlaA is a 68 kDa protein of 621 amino acids. DlaA null (DlaA-) cells grow substantially slower than wild-type Ax-2 cells. DlaA- cells also require longer to form multicellular bodies. The basal discs of fruiting bodies of DlaA- cells are deformed. The ratio of prestalk cells to prespore cells in the slugs of DlaA- cells is significantly higher than the normal ratio observed with Ax-2 slugs. We performed a monolayer culture assay on DlaA- to examine the cellular sensitivity to cAMP and DIF-1, and found that the number of prestalk cells in fact decreased under the ordinary conditions favourable for inducing them. However, when DlaA- cells were pretreated with cAMP in a very short time, larger number of prestalk cells were induced. This result suggests that

DlaA- may acquire cAMP-sensitivity unusually prematurely. As supporting evidence, we found that expression timing and the amount of cAMP receptor 4(cAR4)-mRNA was considerably both earlier and greater than that of wild-type cells, respectively. These findings suggest that DlaA is involved in the regulation of the expression of cAR4, which is known to repress prestalk specific genes. We also produced anti-DlaA rabbit polyclonal antibodies and identified a single band with Western blotting. When DlaA localization was observed with immuno-histochemical staining, DlaA was found to be localized mainly in the cytoplasm of vegetative and developing cells. However, a significantly high percentage of nuclei of the cells just hatched from spores was labelled with anti-DlaA antibodies. Considering the fact that the cells right after germination from spores are arrested at the G1 phase of the cell cycle, it is suggested that DlaA may translocate from the cytoplasm to the nuclei just around the G1 phase.

Poster 56

The adenylyl cyclase A (acaA) gene is transcribed from three alternative promoters.

Presented by Leandro Sastre
María Galardi-Castilla1, Ane Garciandía2, Teresa Suarez2 and Leandro Sastre1.
1 Instiuto de Investigaciones Biomedicas
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Adenylyl cyclase A is responsible for cAMP synthesis in a reaction that is activated by extracellular cAMP, forming part of a feed-forward loop that plays a central role during Dictyostelium discoideum development. The first developmental process where this pathway is required is cell aggregation, and, as expected, acaA mutants do not form aggregates. Extracellular cAMP signaling also regulates several post-aggregative developmental processes, including cell sorting, cell-type specific gene expression, culmination and spore terminal differentiation. The importance of Adenylyl cyclase A in these processes is not completely understood. A functional study of the acaA promoter region has been initiated to get further insight in the function of this gene during development. Transcription initiation sites and the sequence or the 5,Äô-untranslated region were determined by rapid amplification of cDNA ends (RACE). The data obtained indicated that acaA is expressed from three different promoters located in the 4 kb-long intergenic region that precedes the acaA gene. Transcription from each promoter originates a specific first exon that is joined, by mRNA splicing, to a common second exon where the translation initiation codon is located. Therefore, the three mRNAs that are synthesized code for the same protein. Reporter vector analysis indicates that the three promoter regions drive transcription in different regions of the developing structures and at distinct developmental stages. In particular, the more distal promoter is specifically active during the aggregation process, the intermediate promoter drives expression in prespore cells, and the one closest to the coding region is active at the tip-organizer. RT-PCR experiments using Exon 1-specific oligonucleotides

corroborate the three mRNAs are expressed at different times during development. In situ hybridization experiments are also in agreement with the patterns of reporter-gene expression observed in the analysis of the promoter regions. In summary, our data show that the acaA gene is expressed in different regions throughout Dictyostelium development, indicating that the encoded protein could be important for cAMP synthesis at several steps of the process, in addition to its well-characterized role in aggregation.

Poster 57

Transcription of the Dictyostelium discoideum mitochondrial genome occurs from a single initiation site

Presented by Phuong Le

Phuong Le, Paul Robert Fisher and Christian Barth

Transcription from single uni- or bidirectional promoters seems to be common to the mitochondria of all vertebrates. In mammalian mitochondria, for example, transcription of the rather small and compact genome (16 kb) is initiated at a single promoter on each strand of the mitochondrial DNA, generating a large polycistronic transcript that is co-transcriptionally cleaved to produce individual RNA molecules (Clayton, 1991; Jaehning, 1993).

Less derived organisms, such as fungi and plants, tend to have larger mitochondrial genomes, which, due to their size, require multiple, if not many promoters for the expression of the more complex genomes (Brennicke et al., 1999; Constanzo and Fox, 1990). In contrast to this, we show here that in the simple protist Dictyostelium discoideum, the relatively large mitochondrial genome of 56 kb in size is transcribed from only a single, unidirectional promoter. We provide evidence for the existence of a large primary transcript in Reverse Transcriptase Polymerase Chain reactions (RT-PCR) and in capping experiments, and we also demonstrate that the sequences upstream of the unique transcription initiation site can be bound by the Dictyostelium mitochondrial RNA polymerase to specifically initiate transcription. This is the first report on a protist mitochondrial DNA that is, although much larger in size than its metazoan counterparts, transcribed from a single transcription initiation site.

Poster 58

The biochemical basis of chlorination in DIF-1 biosynthesis

Presented by Christopher S. Neumann

Christopher S. Neumann, Christopher T. Walsh, Robert R. Kay

Differentiation Inducing Factor I (DIF-1) is a chlorinated polyketide molecule that helps drive differentiation into the prestalk-O cell lineage. A three step biosynthetic pathway has been proposed previously, and the biochemistry of the first (polyketide synthesis) and last (O-methylation) steps has been elucidated; however, the biochemical basis of chlorination has remained

unclear. In this work, we describe the identification of an oxygen- and flavin-dependent halogenase ChlA that catalyzes this key step in DIF-1 maturation. Through a combination of biochemical and genetic experiments, we show that ChlA catalyzes the dichlorintaion of the nascent polyketide trihydroxy-phenylhexanone (THPH) in vitro and is required for the production of DIF-1 in vivo. These data provide the first biochemical characterization of a eukaryotic flavin-dependent halogenase and complete the molecular-level characterization of the DIF-1 biosynthetic pathway.

Poster 59

Ecological context can suppress cheating behaviour

Presented by Neil Buttery

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The environment plays an important role in adaptive evolution because genotypes can interact with a variable environment in non-parallel ways. These are known as genotype-environment (G-E) interactions. G-E interactions have the potential to maintain genetic variation and provide a mechanism by which genotypes can become adapted to different niches. Cheating behaviour is a paradox because selfish individuals that don't cooperate within the social group should be selected for. The social amoeba Dictyostelium discoideum displays both fixed and facultative cheating strategies and strains form a linear cheating hierarchy when competed pair-wise, suggesting that there is one overall 'winner'. We therefore investigated the effect of a variable environment on the cheating behaviour displayed by the social amoeba Dictyostelium discoideum. We find that the amount of cheating is dependent upon ecological context, i.e. their partner as well as the abiotic environment that they both experience. Although there were no significant shifts in the order of the linear hierarchy, facultative cheating could be suppressed in two ways: (1) by complete or partial segregation of genotypes when grown on a natural substrate and (2) by a measurable decrease in the facultative strategy. The study highlights the importance of factoring in the effects the environments when considering the ecology and evolution of social traits.

Poster 60

Cells Expressing the Predicted Caspase I Cleavage Fragments of Human T-Plastin Have Altered Actin Morphology

Presented by Andrew Maselli

Denise Patrick and Andrew G. Maselli

Atypical protein inclusions are a hallmark of neurodegenerative disease (ND). Inclusions composed of actin filaments and actin binding proteins (ABP) have

been reported in association with ND and are called Hirano bodies. Expression of truncated forms of Dictyostelium ABPs have been shown to result in actin inclusions. The cascade of events that leads to inclusion formation is not completely understood. Protease cleavage is one possible mechanism for the production of truncated ABPs in the brain. We hypothesize that caspase I activity in the brain can lead to ABP fragments with altered actin binding. To begin to test this hypothesis, we identified the putative caspase I cleavage sites of T-plastin, the human homologue of the ABP Fimbrin. We expressed the predicted T-plastin fragments in Dictyostelium as GFP fusions. The full length protein had a localization pattern consistent with reports on fimbrin. The amino terminal fragment (aa 1-422) contains the first actin binding domain and part of the second and showed a cortical localization. The fragment extending from aa 422-630 contains most of the second actin binding domain and formed inclusions in the cytoplasm that are labeled with GFP and phalloidin, suggesting that they are actin aggregates. These cells contained areas that have an ultrastructure consistent with Hirano bodies. A third fragment extending from aa 295-630 also generated GFP labeled inclusions in cells. Further investigation is needed to link caspase I cleavage of ABPs to Hirano body formation.

Poster 61

Stress and development in Dictyostelium discoideum: the involvement of the calcineurin signalling pathway.

Presented by Sascha Thewes
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Calcium is one of the most important signalling molecules in eukaryotic cells regulating a plethora of cellular processes. In Dictyostelium discoideum calcium signalling is important for many different cellular processes including chemotaxis, pseudopod and uropod formation, the cortical localisation of myosin, differentiation, and development. One major target in the calcium signalling pathway is the Ca2+/calmodulin dependent protein phosphatase calcineurin, which is composed of a catalytic (calcineurin A) and a regulatory (calcineurin B) subunit. The role of calcineurin for growth and development of D. discoideum has been partially studied. It has been shown for example that pharmacological inhibition of calcineurin leads to slower growth and developmental defects and that RNAi-silencing of the regulatory subunit calcineurin B results in shorter stalks and ectopic tip formation of developing fruiting bodies. Here we show that RNAi-mediated silencing of the catalytic subunit calcineurin A leads to comparable defects during development as

could be observed for RNAi-silenced calcineurin B mutants and that calcineurin is involved in the stress response of D. discoideum. Additionally, we investigated the role of the putative calcineurin-activated transcription factor TacA during growth and development. We show that the localisation of TacA shifts from the cytosol to the nucleus upon Ca2+ treatment. Further, RNAi-silencing of tacA leads to developmental abnormalities and defects during growth under certain stress conditions.

Poster 62

Plasticity of Dictyostelium discoideum mitochondrial phosphoproteome: The role of AMP-dependent protein kinase.

Presented by Malgorzata Czarna
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Recent comparative proteomics studies of Dictyostelium discoideum mitochondria exhibited significant changes in the expression level of numerous mitochondrial proteins during vegetative growth and starvation-induced development and suggested that the phosphorylation status of mitochondrial proteome is likely to be regulated in an energy-dependent manner.
The AMP-dependent protein kinase (AMPK) is a component of a highly conserved signalling pathway that senses the energy status of the cell and nutrient availability. Dictyostelium cells overexpressing the AMPK alpha subunit show increased number of mitochondria and increased intracellular level of ATP whereas antisense inhibition of AMPK alpha expression resulted in decreased number of mitochondria and intracellular ATP level.
With the use of two-dimensional gel electrophoresis combined with phosphospecific in-gel staining we detected a number of phosphorylated mitochondrial proteins of Dictyostelium wild type, AMPKalpha overexpressing and AMPK alpha antisense-inhibited cells, which show possible regulation of expression and/or phosphorylation status during vegetative growth, starvation and starvation-induced development.
This is a first view of the D. discoideum mitochondrial phosphoproteome which unravels possible molecular links between mitochondrial protein phosphorylation and the AMP-dependent signalling pathway controlling the energy status of the cell during vegetative growth and starvation-induced development.

Poster 64

Response of the actin cytoskeleton to rapidly applied periodic stimuli

Presented by Christian Westendorf
Christian Westendorf, Rabea Sandmann, Albert J. Bae, Eberhard Bodenschatz and Carsten Beta

We characterized the response of the actin cytoskeleton in starvation-developed Dictyostelium discoideum AX2 LimE-GFP cells to rapidly applied periodic pulses of cAMP. Cells were distributed in a microfluidic channel and a continuous flow of caged cAMP was applied. Pulses of cAMP, with a rise time of less than one second and a total duration of less than two seconds, were created by photoactivation with a short wavelength laser beam inside a line shaped uncaging region upstream of the cell [1]. The periods of pulse stimulation ranged from 4 to 60 seconds. The filamentous actin response was monitored via LimE-GFP, using laser scanning confocal microscopy. The recorded cells were digitally subdivided into a cortical and a cytosolic region. We observed the characteristic response signal for each period of stimulation. Properties and time scales were investigated by a frequency analysis. This analysis was complemented by automated celltracking, revealing a chemotactic response of the cells towards the periodic source of cAMP.

Reference:
[1] Analytical Chemistry 2007 79(10) p3940-44.

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Poster 65

Transition of the signaling pattern in Dictyostelium discoideum with changing diffusion coefficients

Presented by Noriko Oikawa

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In the absence of nutrients, Dictyostelium discoideum cells enter a developmental cycle--they signal each other, aggregate, and ultimately form fruiting bodies. During the signaling stage, the cells relay waves of cyclic adenosine 3',5' monophosphate (cAMP) via diffusion in the substrate. We experimentally investigated the signaling process with changing diffusion coefficient, using spectral entropy method. It has been revealed that the onset of synchronization shifts to later times and the signaling pattern becomes disordered as the diffusion coefficient decreases. In this presentation, we will show a correlation between the onset of the synchronization and the number of the phase singularity observed in the signaling pattern.

Poster 66

Regulation of autophagy by lithium in Dictyostelium

Presented by Jon Reddy

Jonathan V Reddy and Adrian J Harwood

Previous research established a novel role for the mood stabiliser lithium in the treatment of neurodegenerative disorders. The application of lithium to mammalian cells induced a process known as autophagy, resulting in the enhanced clearance of aggregate-prone proteins linked to neurodegenerative disease. The well characterised protein kinase mTor, a negative regulator of autophagy, remained active following lithium treatment suggesting that lithium induced autophagy via a mTor independent pathway. Lowered inositol (1,4,5)-trisphosphate (IP3) levels were also observed indicating that this regulatory pathway may involve IP3.

However, a recent study by King et al., 2009 has revealed an alternative mechanism of lithium action - the suppression of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) signalling.

In the current study, lithium treatment was found to induce autophagy in Dictyostelium leading to the clearance of huntingtin protein aggregates. New results will be presented describing the mechanisms involved in lithium induced autophagy.

Poster 67

Dictyostelium Myosin IB is involved in membrane recycling from phagosomes during the reneutralisation step.

Presented by Navin Gopaldass

Navin Gopaldass, Robert Insall and Thierry Soldati

We have previously shown that Dictyostelium myosin IB (MyoB) is involved in membrane recycling from endosomes. We now show that MyoB is involved in a recycling step during the transition from the acidic lysosome to the neutral post lysosomal stage. Indeed, phagosomal reneutralisation kinetics measured with FITC/TRITC labelled beads was delayed in myoB-null cells. Consistently, myoB-null cells have an increase number of strongly labelled p80 compartments while the number of vacuolin compartments was similar to wt cells. Western blot analysis of purified phagosomes isolated at different maturation stages revealed a strong delay in LmpB recycling as well as an accumulation of Abp1 and DynaminA on phagosomes isolated from myoB-null cells compared to wt cells. Moreover, knock out of DynaminA resulted in massive recruitement of MyoB to the phagosome at late time points suggesting that MyoB participates in a membrane scission process. Live cell imaging allowed us to visualise the recruitment of GFP-MyoB and GFP-DynaminA to sites of membrane recycling on phagosomes. Our findings suggests that a machinery similar to that involved in endocytosis in yeast is involved in membrane budding from phagosomes in Dictyostelium during the reneutralisation phase. Preliminary results suggest that Myosin IB could act in concert with the WASH machinery for V-ATPase recycling.

Poster 68

Repression of Dd-STATa activity by Dd-PIAS, a Dictyostelium PIAS homologue

Presented by Takefumi Kawata

Takefumi Kawata, Tatsunori Hirano, Ayako Yachi, Shun Ogasawara and Ryota Aoshima

Several mammalian protein families are known to inhibit the activity of signal transducer and activator of transcription (STAT) proteins. The PIAS (protein inhibitor of activated STAT) was initially identified by a two-hybrid screen through its ability to interact with STAT proteins in human. We isolated a gene encoding a Dictyostelium homologue of PIAS, Dd-PIAS, which consists of 843 amino acids with a predicted molecular weight of 94.5 kDa. A Dd-PIAS null mutant strain displayed a normal morphology but accelerated development once aggregated. In contrast, Dd-PIAS overexpressor strains demonstrated slightly weak phototaxis, delayed aggregation, and a prolonged slug migration period. This strain was a near phenocopy of the Dd-STATa null mutant, though it eventually formed a fruiting body. The expression of several Dd-STATa-regulated genes was upregulated in the Dd-PIAS null mutant and downregulated in strains overexpressing Dd-PIAS. A PIAS-GFP fusion protein expressed under its own promoter localised predominantly in the prestalk O (pstO) cells, and partially co-localised with Dd-STATa. The amount of PIAS-GFP was greatest in the pstO cells and gradually decreased with proximity to the tip cells of the slug and culminant, showing a gradated localisation with a pattern opposite that observed for Dd-STATa. Our results indicate the existence of genetic interactions between Dd-PIAS and Dd-STATa that influence gene expression in Dictyostelium.

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