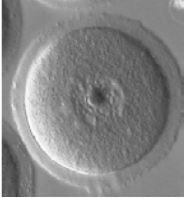



Organizing the cytoplasm during oocyte maturation
 John Carroll
 Laboratory for Oocyte and Embryo Development
 Biosciences
 UCL

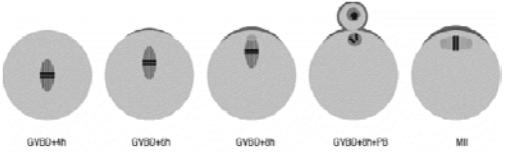
Why investigate oocyte maturation?


→


Prophase I
Metaphase II

- Necessary step in production of mature fertile oocyte.
- Involves cell cycle control and cytoplasmic modifications.
- Model system for studying cell biology and physiology.
- Application in clinic could have a major impact on ART.

Oocyte maturation: *from symmetry to asymmetry*



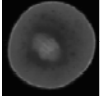

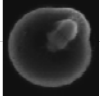


GYSD+M

GYSD+M

GYSD+M

GYSD+M+PB

MII

Formation

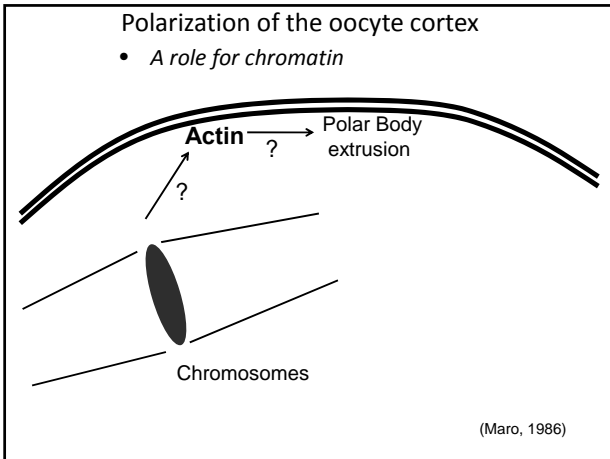
Migration
Polarization
Anchoring

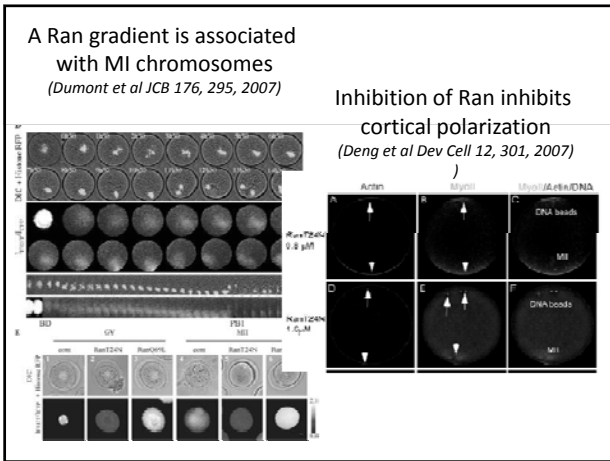
Anaphase
Protrusion

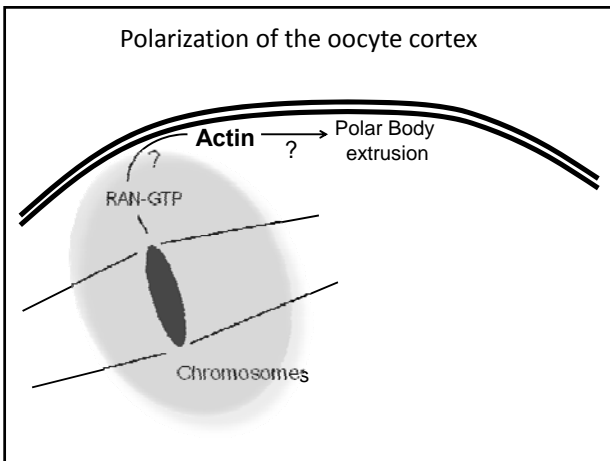
Telophase
Cytokinesis

Formation
Anchoring

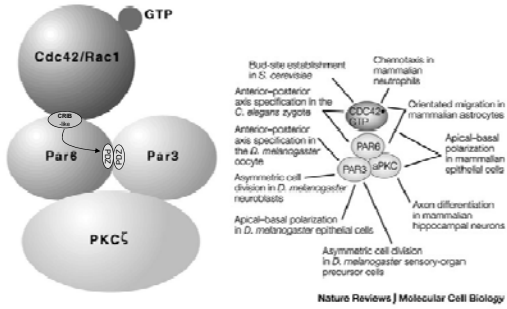
Vinot et al., 2004



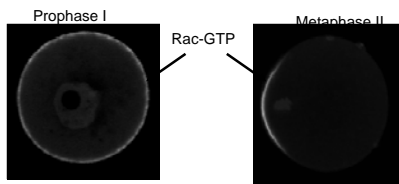




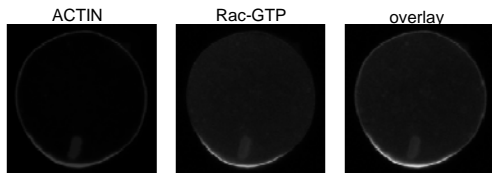
Cellular Polarity: the main players



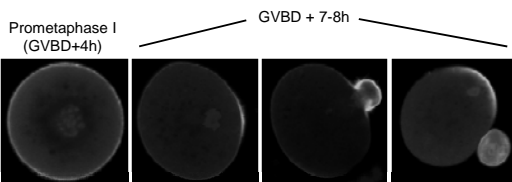
Rac1-GTP is polarised during oocyte maturation



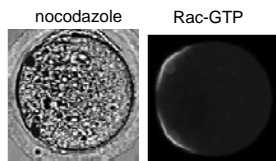
Rac1-GTP co-localises with the actin cap



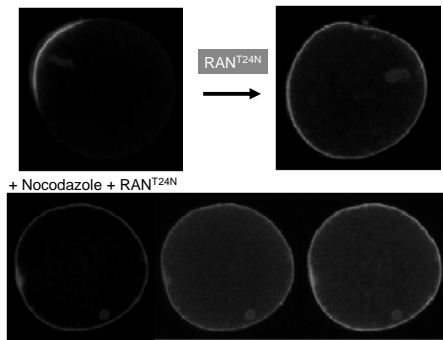
Rac1-GTP polarises as chromatin moves to the cortex



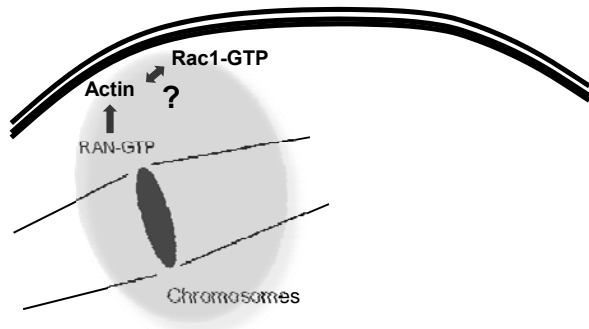
Rac1-GTP accumulates close to chromatin



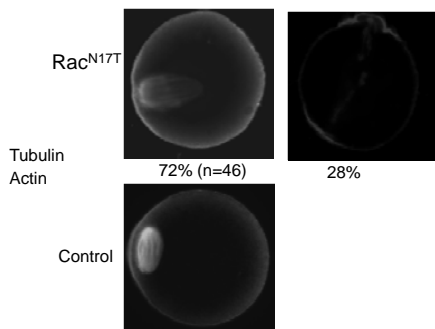
Ran activity is necessary for polarised Rac-GTP



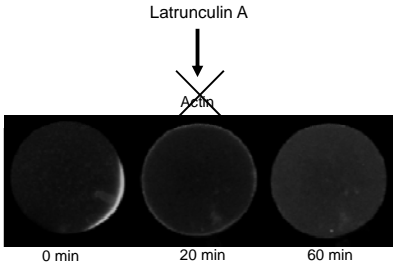
Polarisation of the oocyte cortex



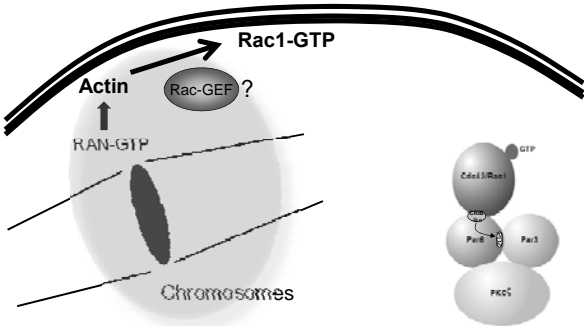
Inhibition of Rac1-GTP has no effect on formation of polarised actin.
But does inhibit Pb extrusion and causes spindle defects.



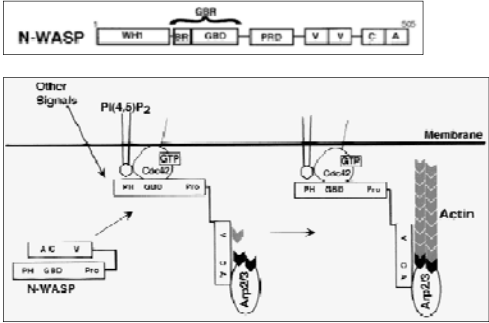
Cortical actin is necessary for polarised Rac



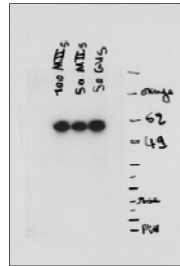
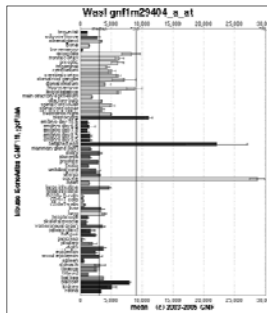
Polarisation of the oocyte cortex



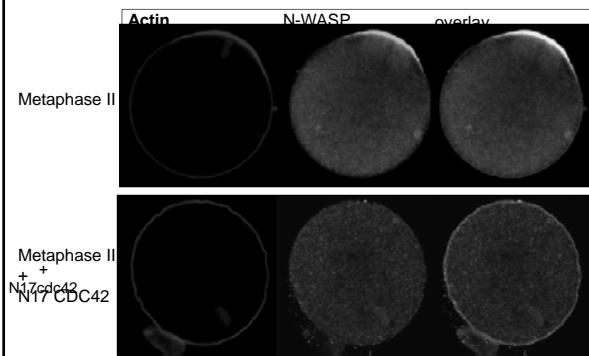
Cdc42 induces actin filaments through N-WASP



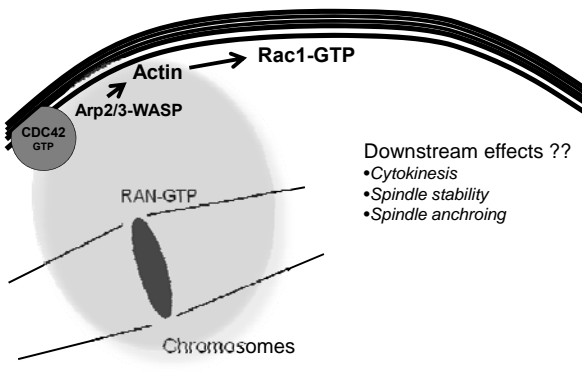
N-WASP is highly expressed in oocytes



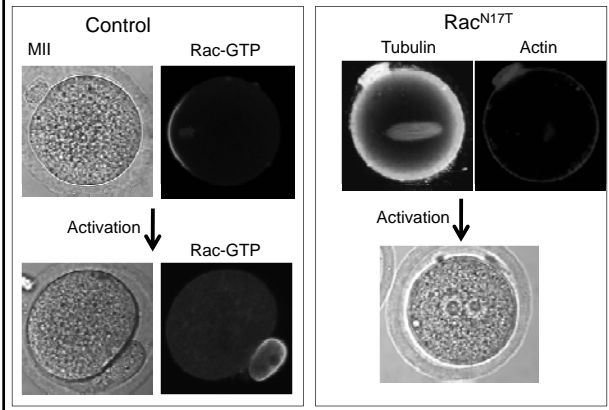
CDC42 is necessary for polarised N-WASP and actin.



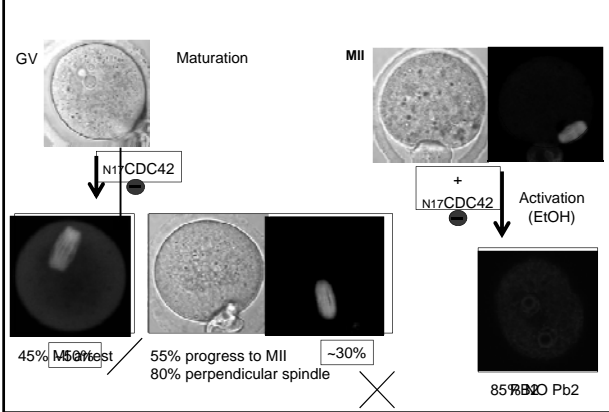
Polarization of the oocyte cortex



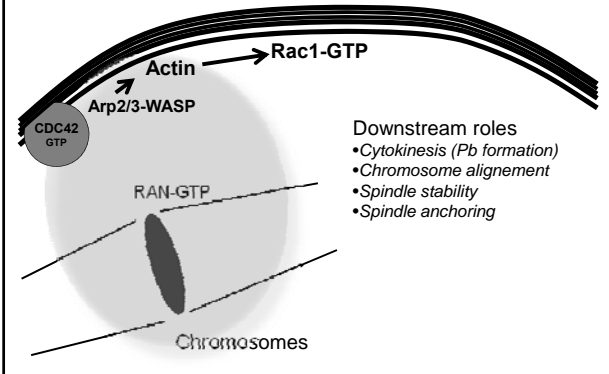
Rac is necessary for spindle anchoring and cytokinesis



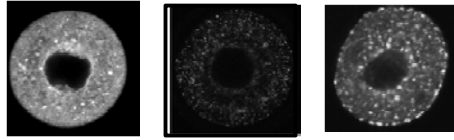
CDC42 is needed for Pb extrusion and spindle attachment



Polarisation of the oocyte cortex allows highly asymmetric cell division in meiosis



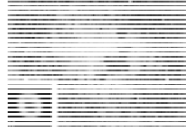
Reorganization of organelles during oocyte maturation



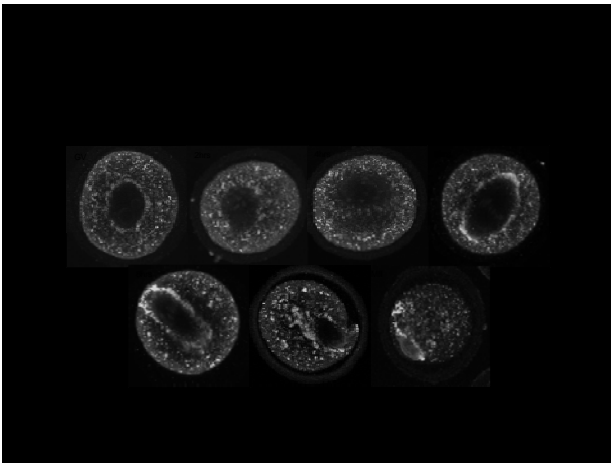
ER

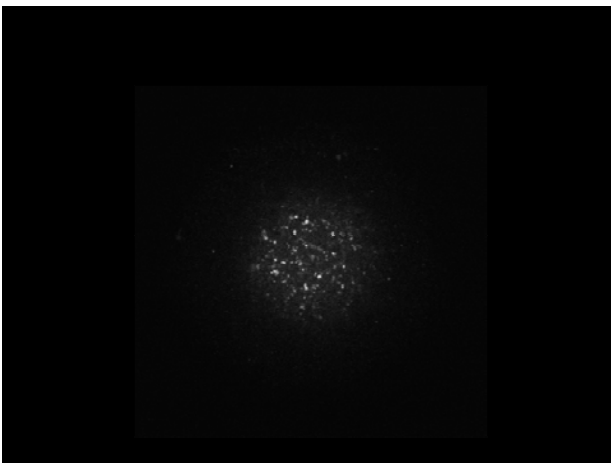
Mitochondria

Golgi

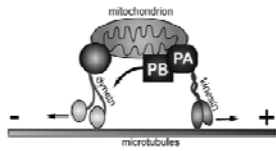
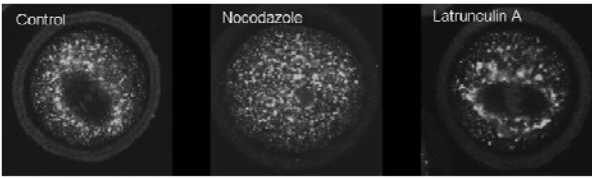


Van Blerkom and Runner, The American Journal of Anatomy, 1984



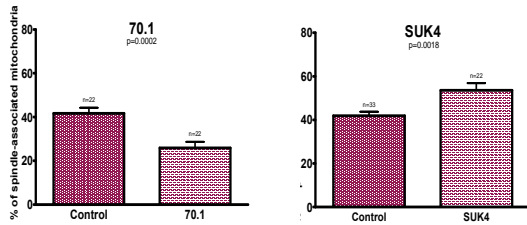
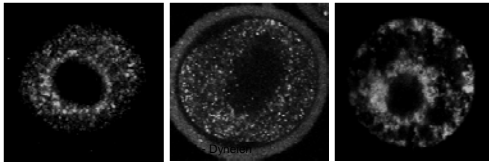


Mitochondrial aggregation requires microtubules not microfilaments

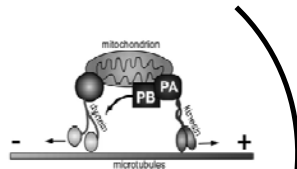
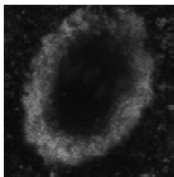


Milton controls the early acquisition of mitochondria by *Drosophila* oocytes.
Cox and Spradling, *Development*, 2006

Motor proteins actively distribute mitochondria

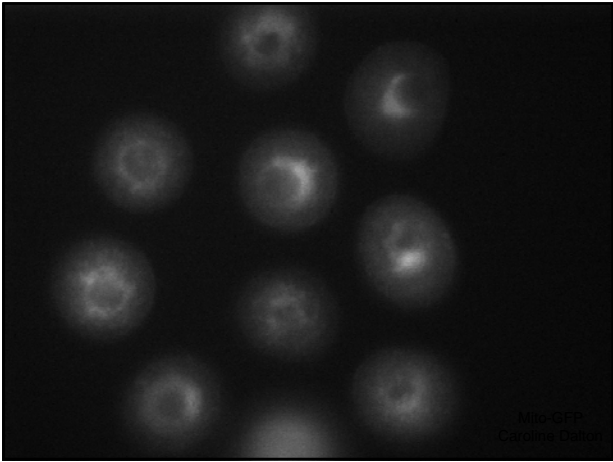


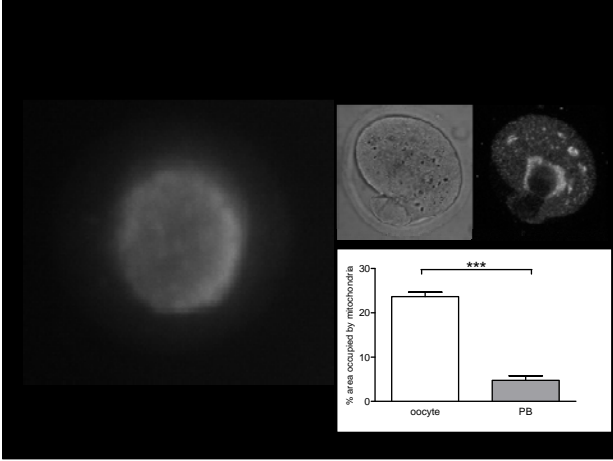
Mitochondrial dynamics during oocyte maturation

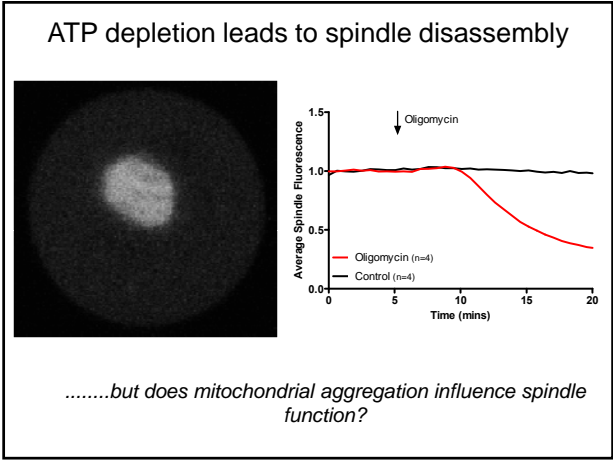


During maturation the balance favours dynein mediated trafficking.

.....But what happens at Pb formation.....



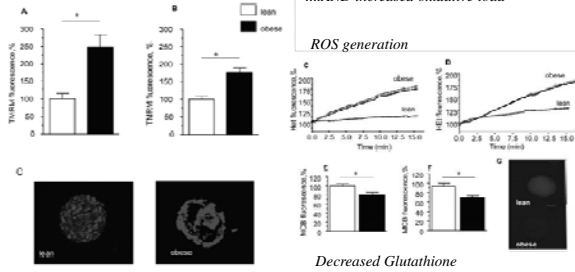


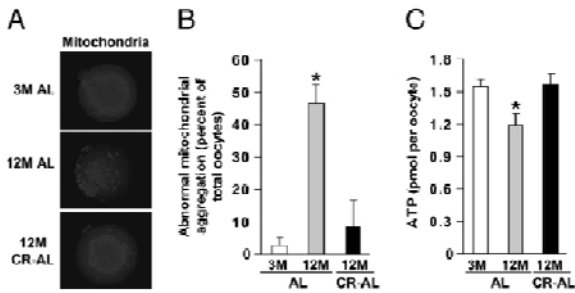


Maternal diet impacts on oocyte mitochondria

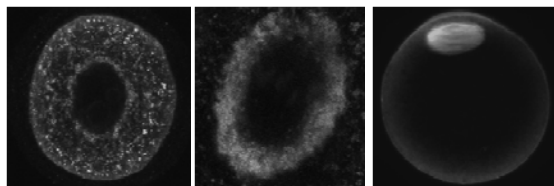
Igosheva et al., PLOS One, 2010.

Obese mice have increased Mitochondrial activity.





Spatial reorganization of cytoplasm during oocyte maturation



Symmetrical distribution of organelles

Redistribution of Organelles

- Microtubules
- Cytoplasmic dynein

Polarization of cortex

- CDC42 / WASP
- Actin
- RAC-GTP

Polar Body formation

Acknowledgements



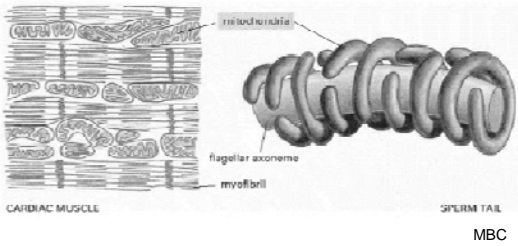
MRC and Wellcome Trust

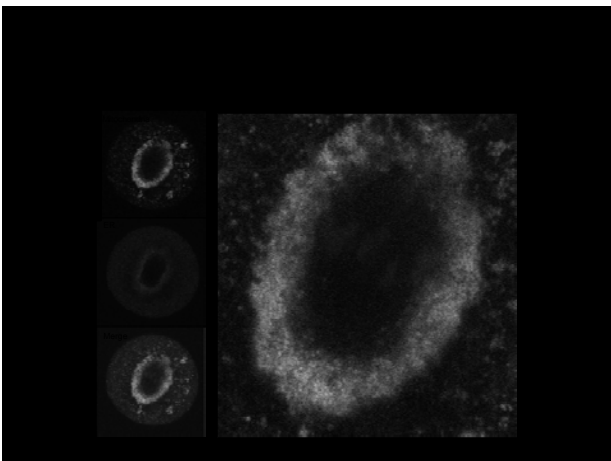
Laboratory for
Oocyte and Embryo
Development

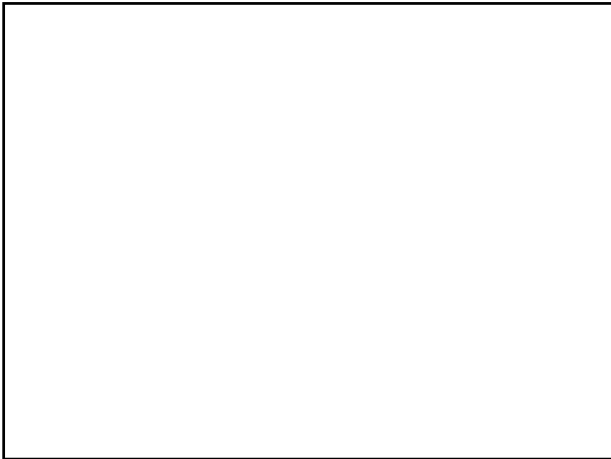
Guillaume Halet
Petros Marangos
Caroline Dalton
Biba Nabti
Roberta Dale

Collaborators
Greg FitzHarris
Hayden Homer
Michael Duchen
Gyuri Szabadkai

*Mitochondria are located at sites of
high energy demand*







Acknowledgements



Laboratory for
oocyte and embryo
development

Greg FitzHarris
Guillaume Halet
Petros Marangos
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Roberta Dale

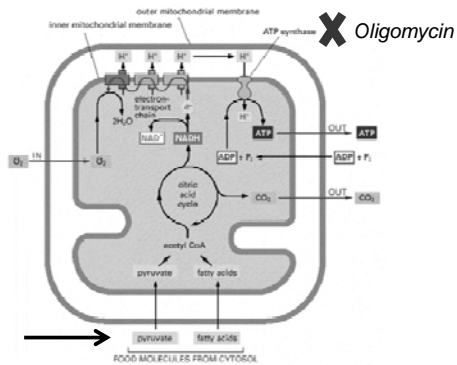
MRC and Wellcome Trust

Featuring the work of.....

Petros Marangos - Calcium signalling and GFP-cyclin
Greg FitzHarris - ER organization and mitosis
Guillaume Halet - PH-GFP, PKC-GFP
Caroline Dalton - ATP and mitochondria
Remi Dumollard - Mitochondrial physiology
Tasos Siskoglou - Nuclear PI signalling
Sophie Brind - InsP3 R downregulation
Rachel Webb - cAMP in meiosis

Collaborators: mark larman
karl swann
tony lai
keith jones
michael duchen
tomo kono

ATP production by mitochondria

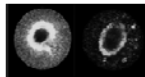


ATP Consuming Processes During Oocyte Maturation

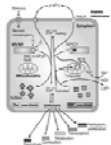
Spindle formation and migration



Organelle movement



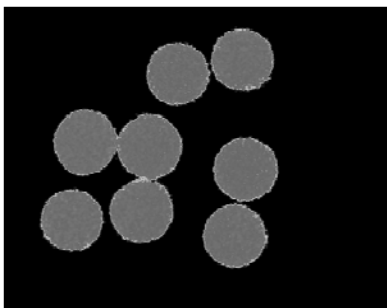
Cellular homeostasis mechanisms

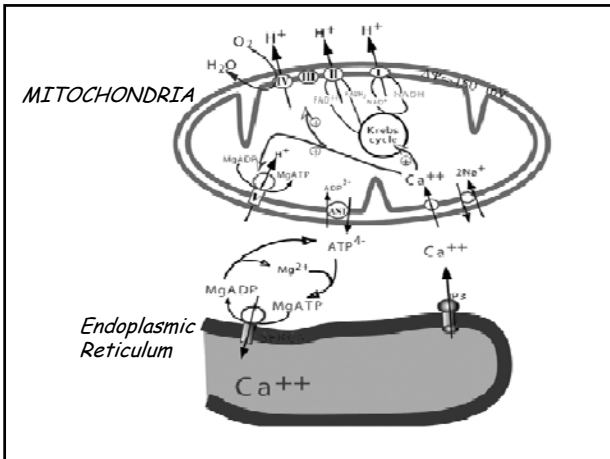


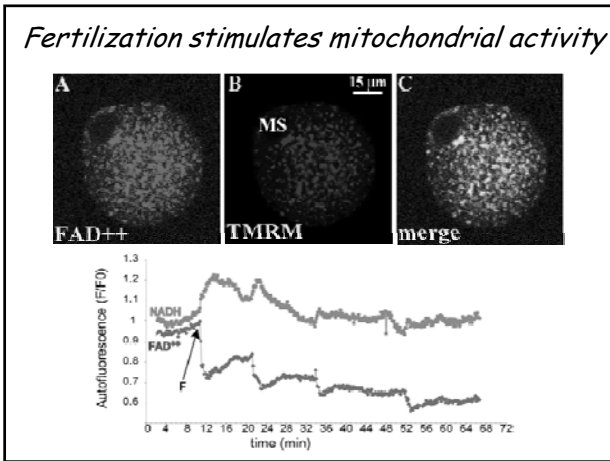
Phosphorylation events

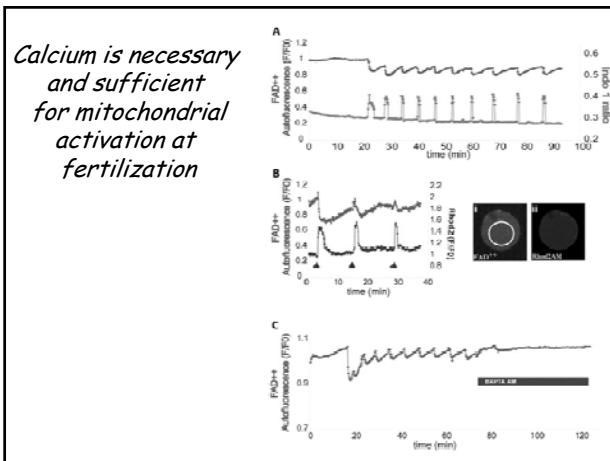


Fertilization stimulates Ca²⁺ oscillations

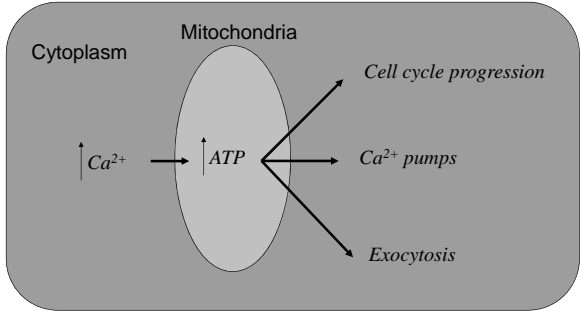




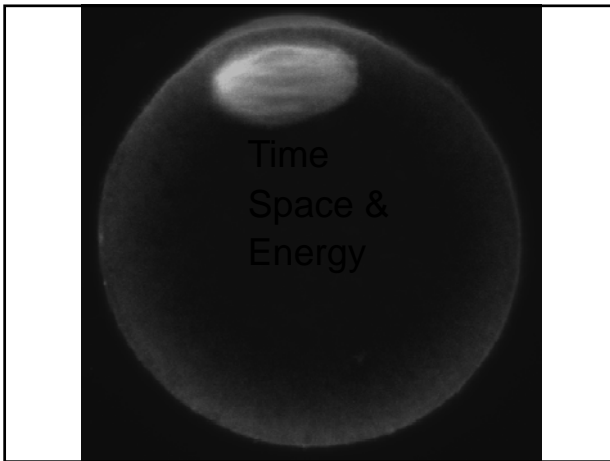




ATP - Ca^{2+} may ensure a match in supply and demand

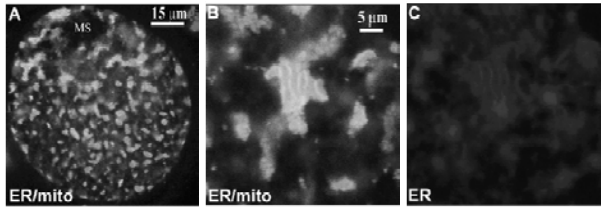


Allows mitochondrial respiration to be set at a minimum level thereby minimizing risk of free radical production to which mitochondria are susceptible.

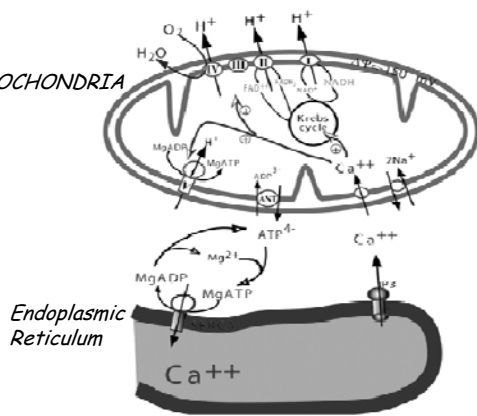




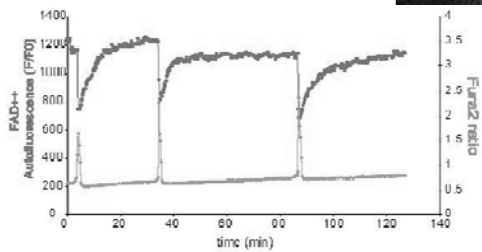
Endoplasmic reticulum and mitochondria are in close proximity



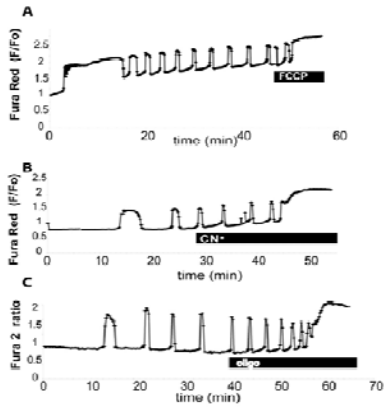
MITOCHONDRIA



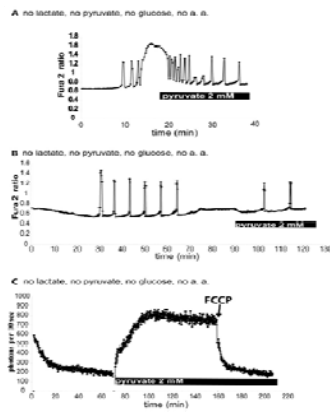
Mitotic Ca²⁺ transients stimulate mitochondrial activity



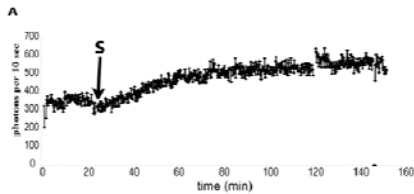
ATP production at fertilization is necessary to maintain calcium oscillations



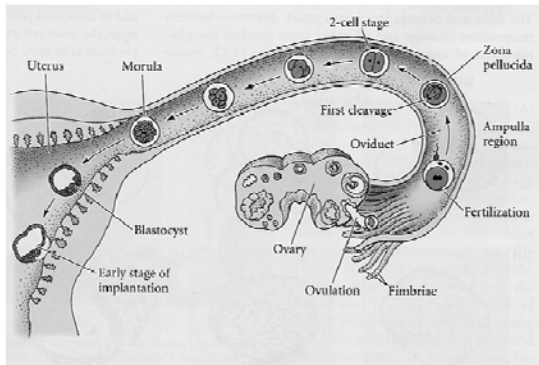
Pyruvate is essential metabolic substrate for maintaining calcium oscillations and ATP.



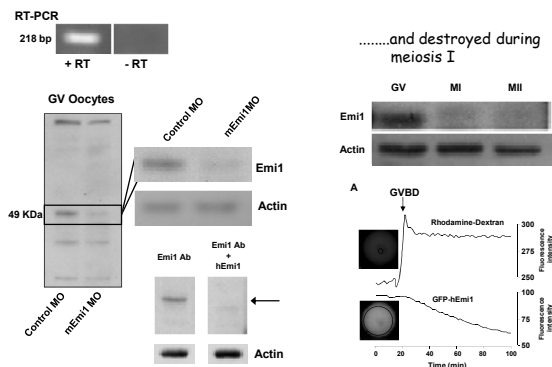
ATP levels are stimulated at fertilization



The first week of life.



Emi1 is expressed in prophase I-arrested oocytes



Featuring the work of.....

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 Greg FitzHarris - ER organization and mitosis
 Guillaume Halet - PH-GFP, PKC-GFP
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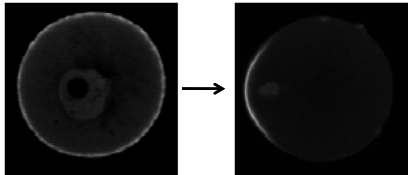
A fertile egg underpins embryo development

*From
De Generatione Animalium
by
William Harvey, 1651*

Summary and Perspectives

1. The oocyte cytoplasm is dramatically reorganised during oocyte maturation.
2. Aggregation of ER and mitochondria create a microenvironment in and around the spindle.
 - ER may charge mitochondria
 - ATP is necessary for spindle dynamics
 - Does this structure promote spindle function?
3. ATP consumption changes during oocyte maturation.
 - highest during meiosis I
 - what is impact of cumulus cells
 - is consumption related to specific events of meiosis

Polarity develops during oocyte maturation



Polarization is essential for asymmetric cell division

Acknowledgements



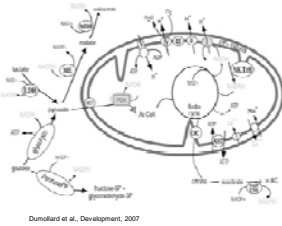
UCL Laboratory for
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Guillaume Halet
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Caroline Dalton
Biba Nabti
Roberta Dale
Jenny Bormann

Greg FitzHarris

MRC and Wellcome Trust

Mitochondria, ATP and the oocyte.

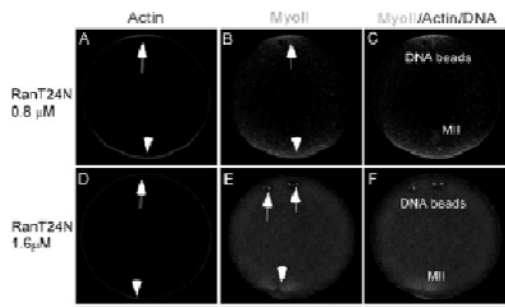


Dunthorn et al., Development, 2007

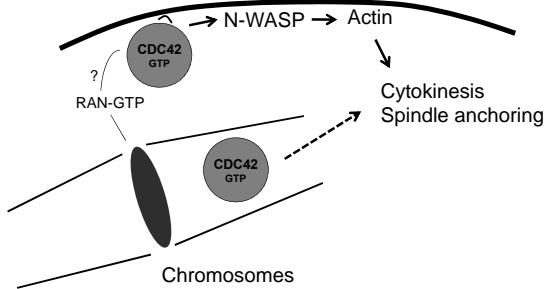
- Mitochondria provide the primary source of ATP for the oocyte
- Oocyte mitochondria expand from 10-100 in primordial follicles to hundreds of thousands in fully grown oocytes
- Mitochondrial dysfunction in the oocyte leads to developmental arrest
- Developmental competence appears to be related to mitochondrial function and ATP levels in the oocyte

A Ran gradient contributes to cortical polarity

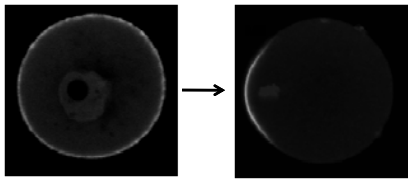
Deng et al., (2007) Dev Cell 12, 301-308



The role of CDC42-GTP in oocytes

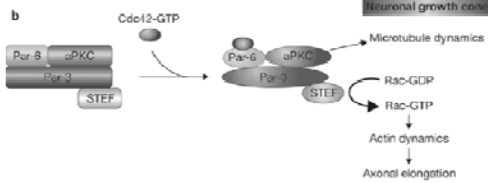


Polarity develops during oocyte maturation

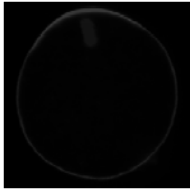


Polarization is essential for asymmetric cell division

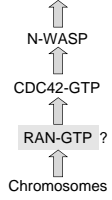
Role and regulation of Rac1 activity during meiosis



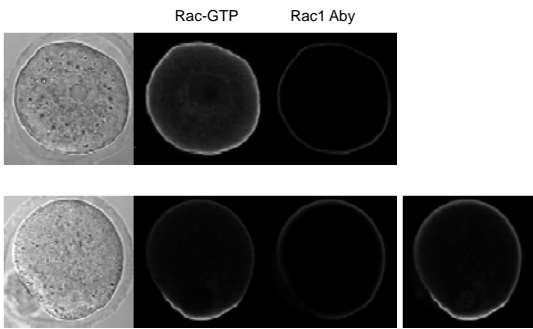
Nishimura et al., 2005



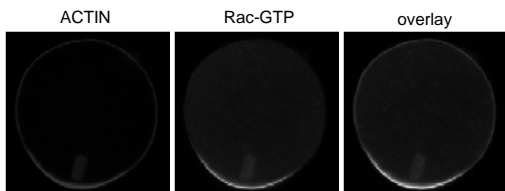
oocyte polarisation, spindle anchoring, PB emission



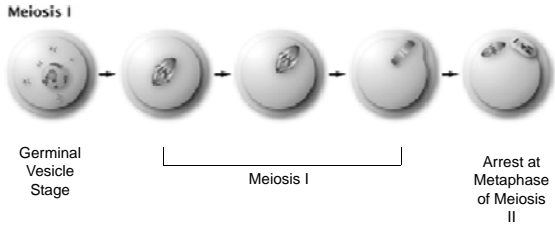
Rac1 is activated in the 'animal pole'



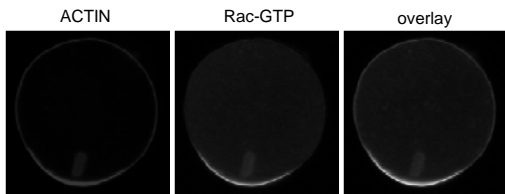
Rac1-GTP co-localises with the actin cap



Oocyte Maturation

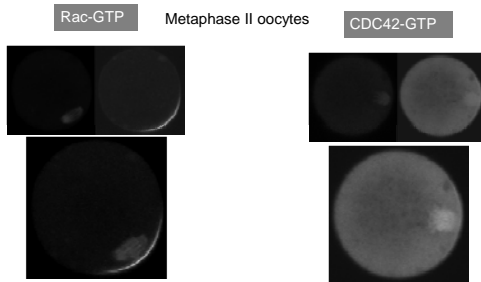


Rac1-GTP co-localises with the actin cap
How does chromatin remodel the cortex?

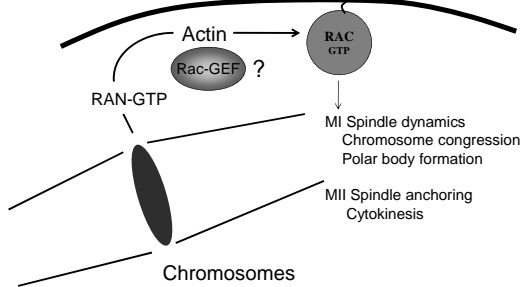


Is Rac-GTP necessary for actin cap formation?

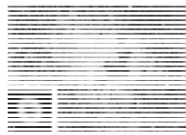
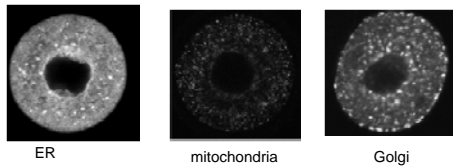
MII: CDC42 localizes to the spindle and overlying cortex



The role of Rac-GTP in oocytes

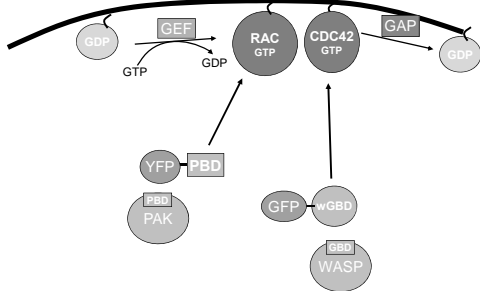


Spatial organisation of the oocyte cytoplasm during maturation

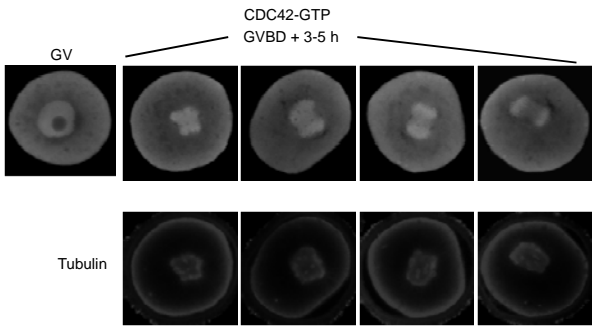


Van Blerkom and Runner, The American Journal of Anatomy, 1984

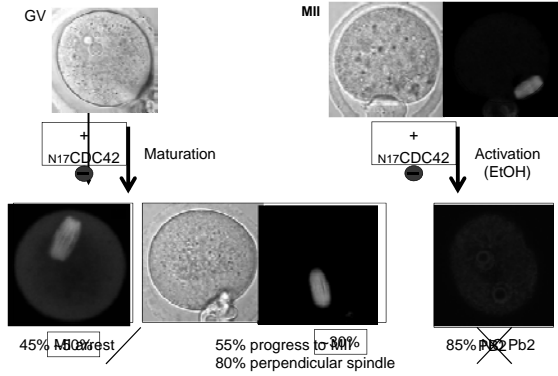
The role of small GTP-binding proteins in oocytes



CDC42-GTP localizes to the developing spindle

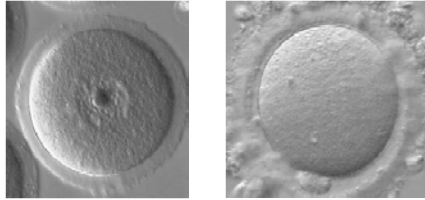


CDC42 is needed for Pb extrusion and spindle attachment



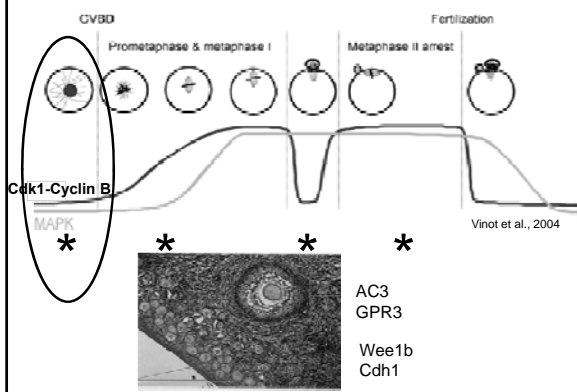


Organizing the cytoplasm during oocyte maturation

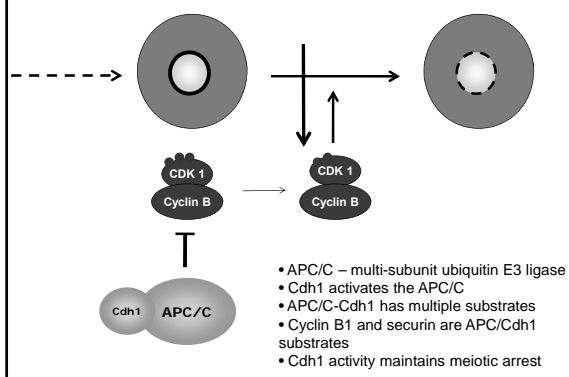


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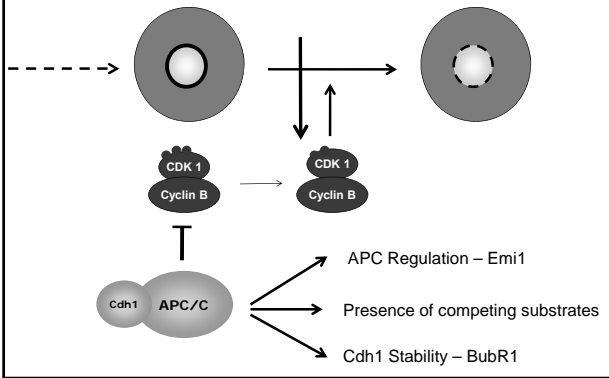
Timing of meiosis in mouse oocytes



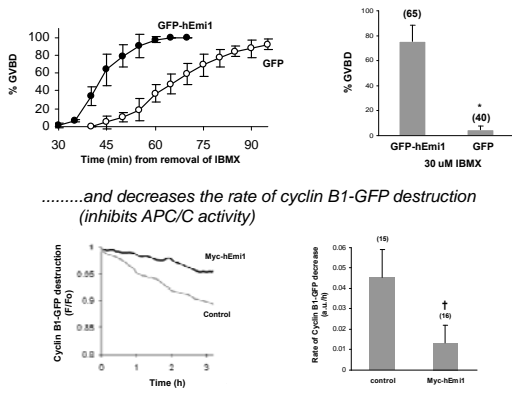
Maintaining meiotic arrest in prophase I



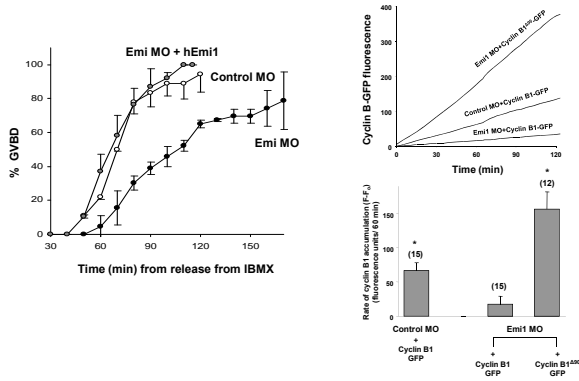
Maintaining meiotic arrest in prophase I

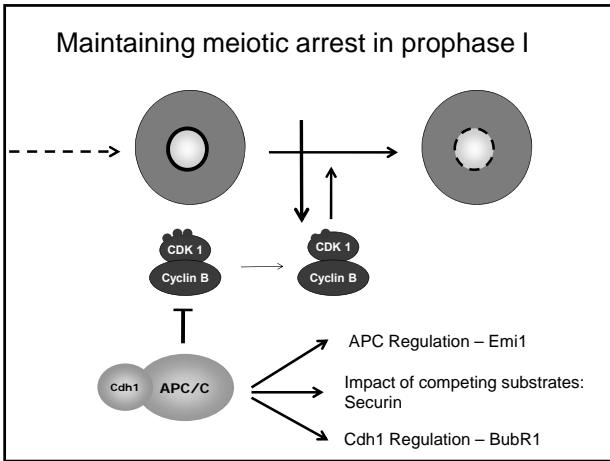


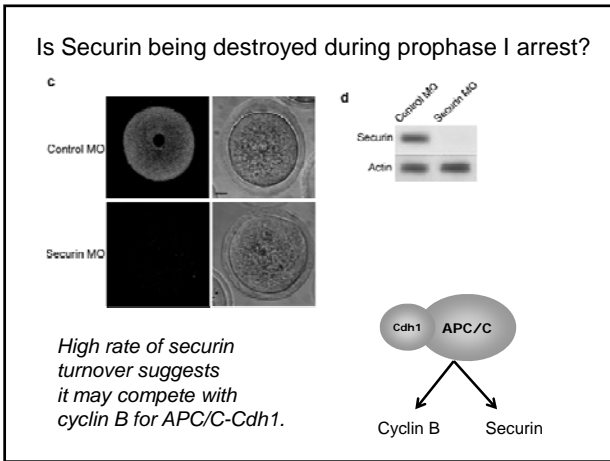
Exogenous Emi1 accelerates entry into M-phase

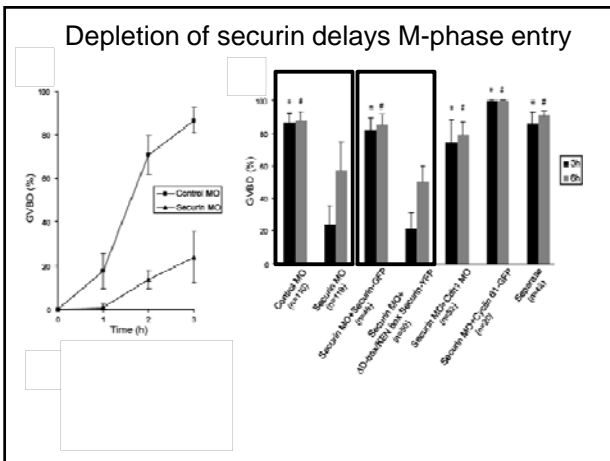


Depletion of Emi1 delays entry into M-phase

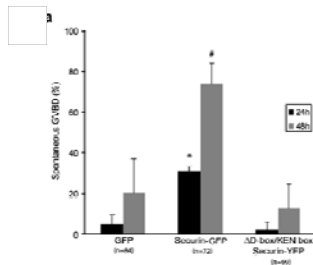




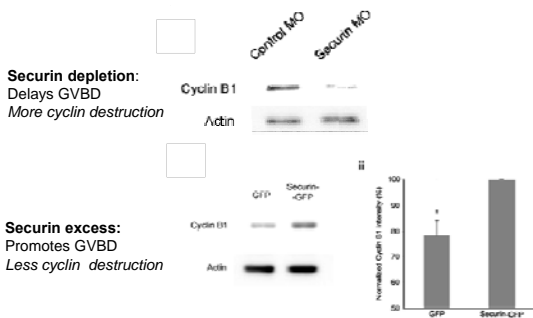




Excess securin promotes M-phase entry



Securin influences the levels of cyclin B1



Maintaining meiotic arrest in prophase I

