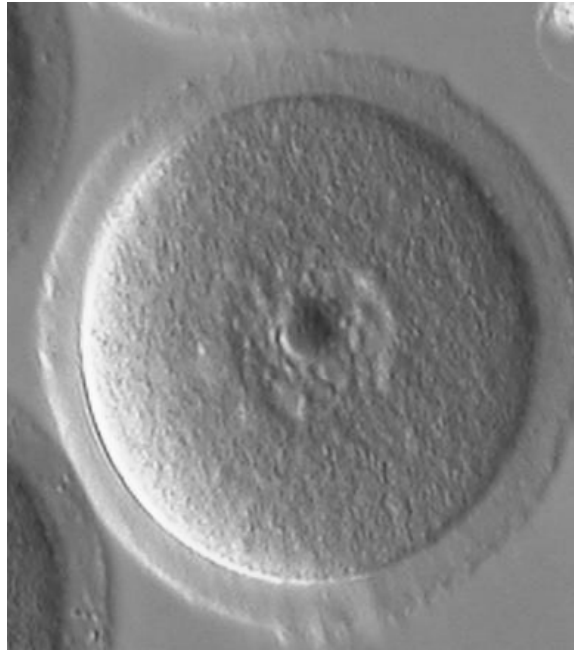


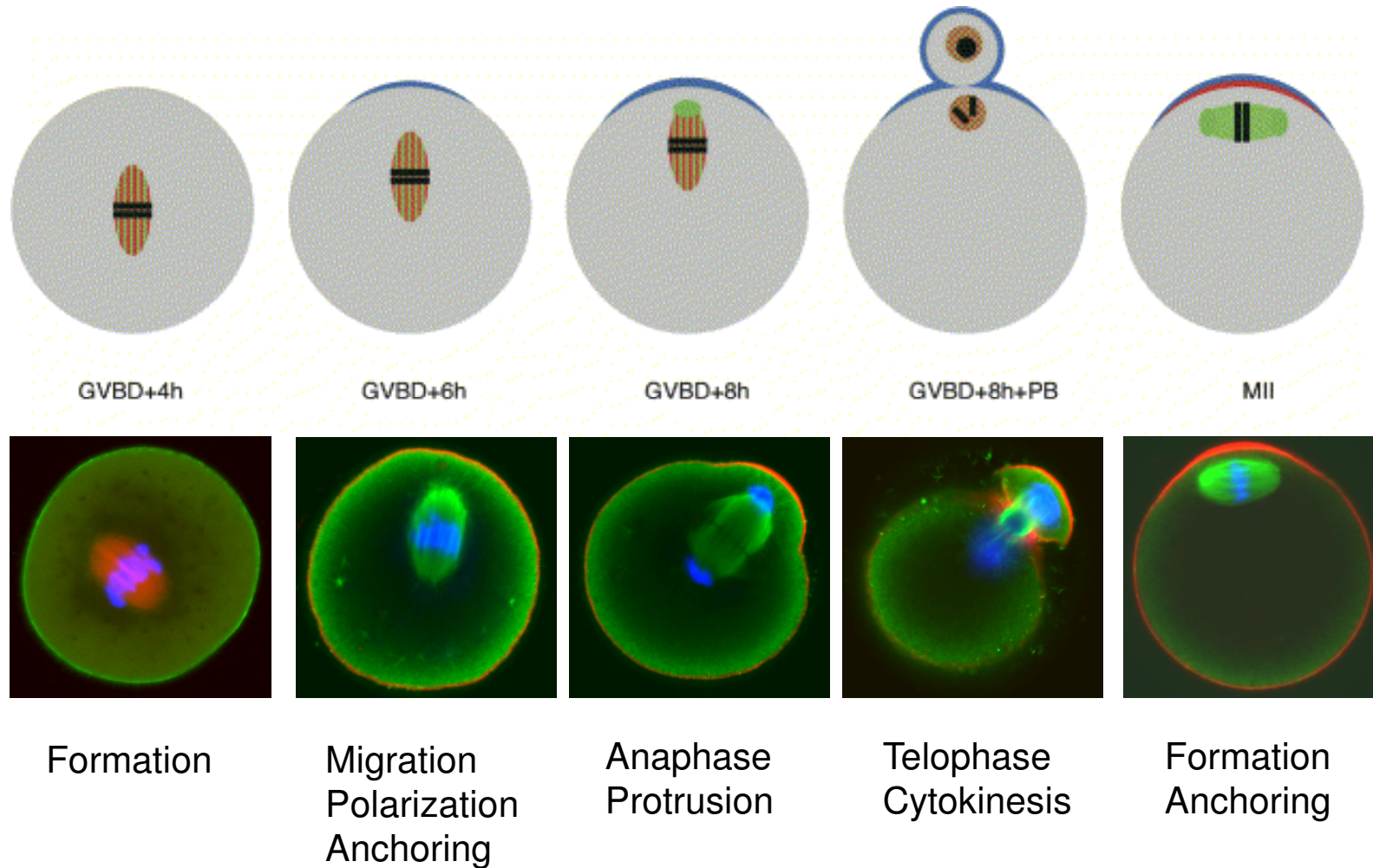


The role of RAC1 and CDC42 in oocyte polarity

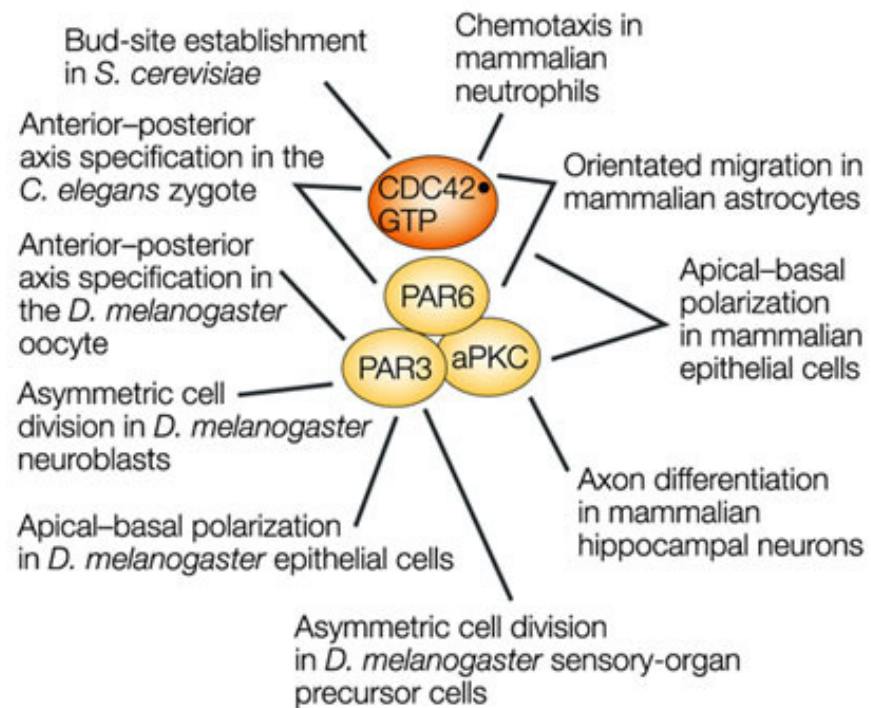
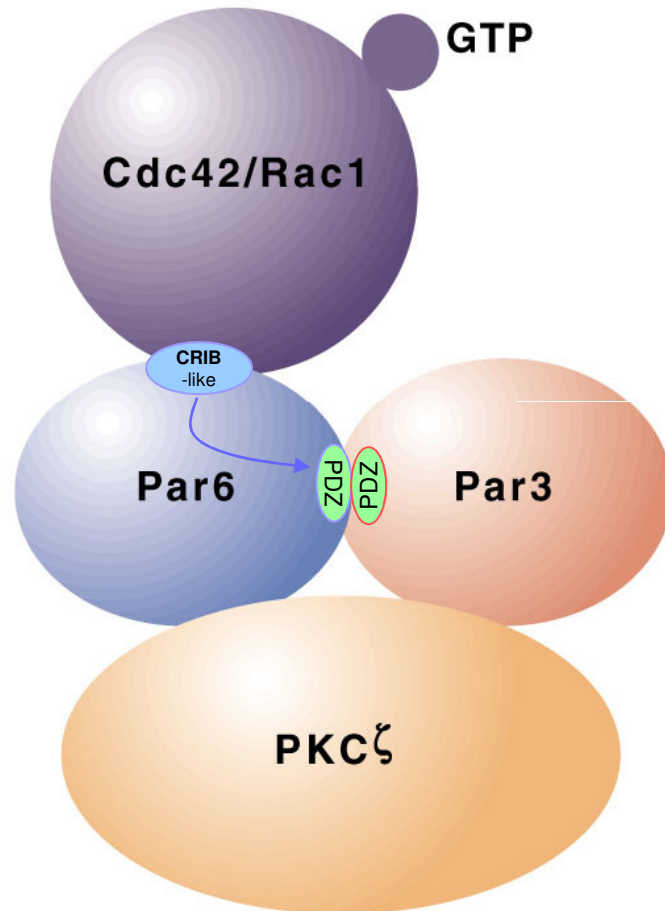


John Carroll
Department of Cell and Developmental Biology
Division of Biosciences
UCL

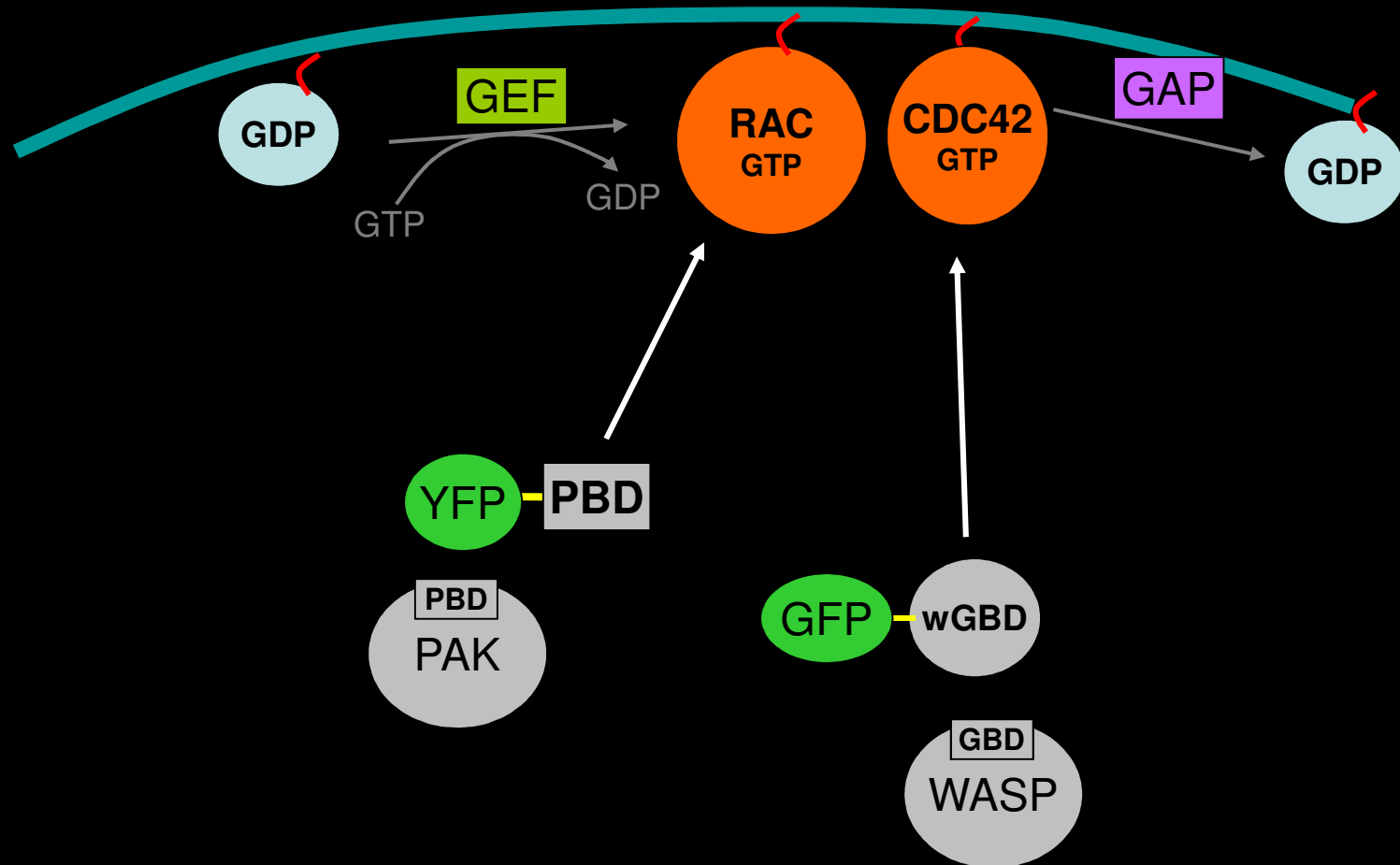
Spindle migration and cortical polarization



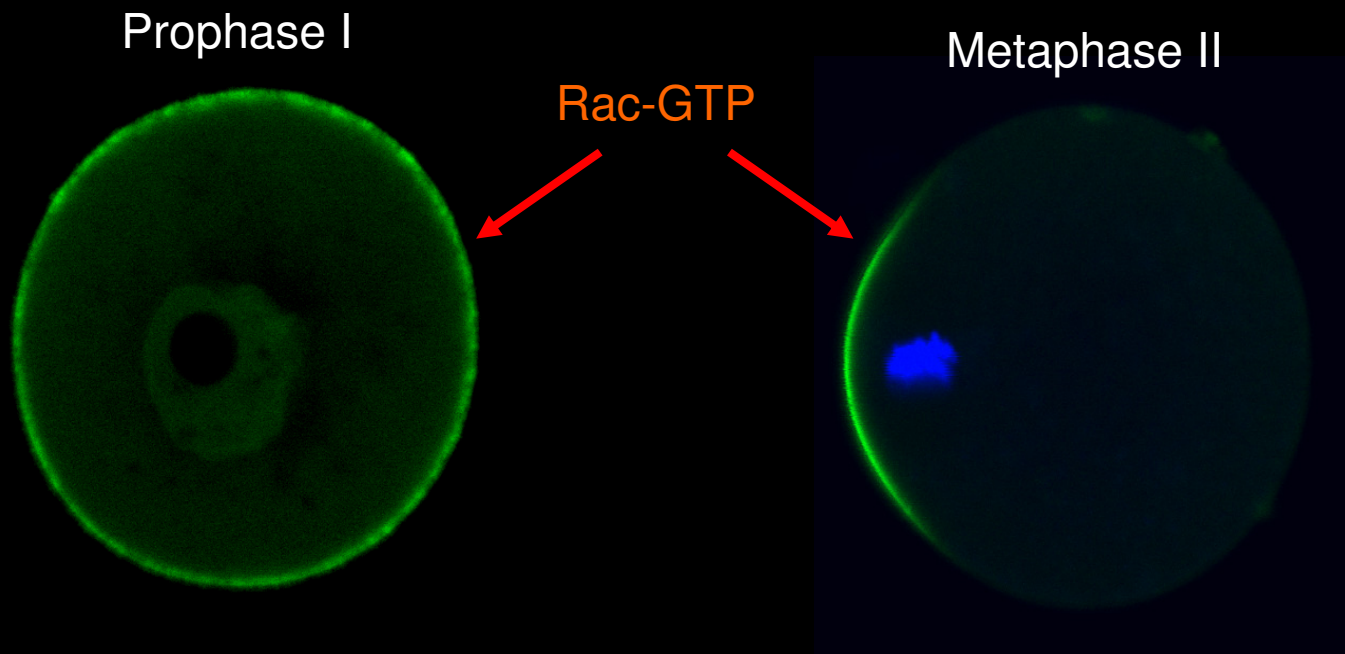
Cellular Polarity: the main players



The role of small GTP-binding proteins in oocytes



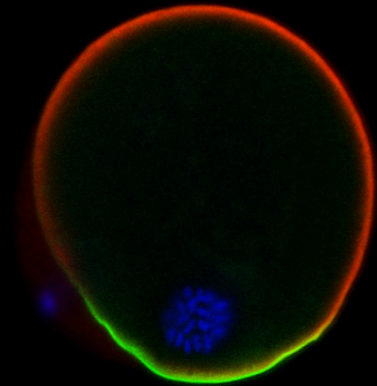
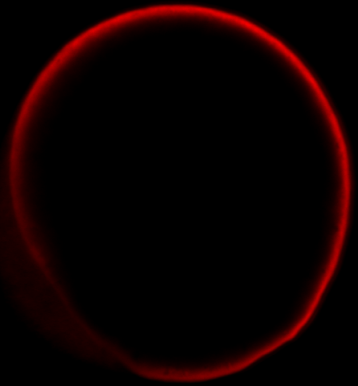
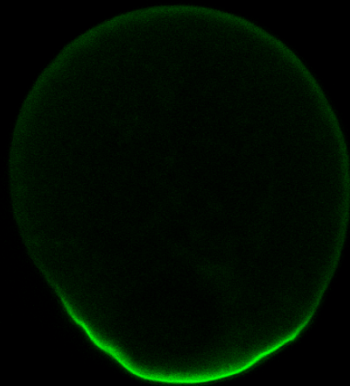
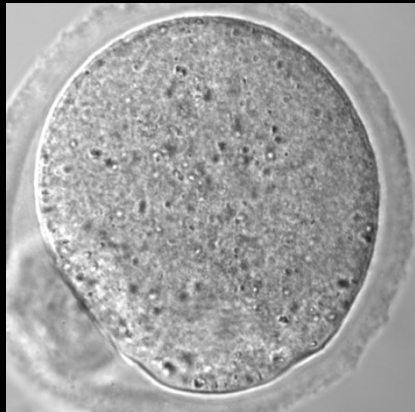
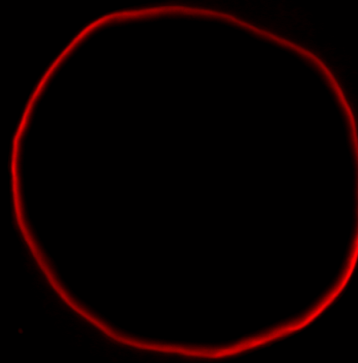
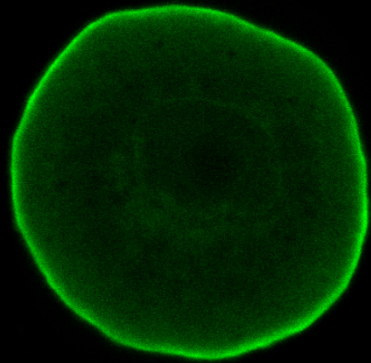
Rac1-GTP is polarised during oocyte maturation



Rac1 is activated in the 'animal pole'

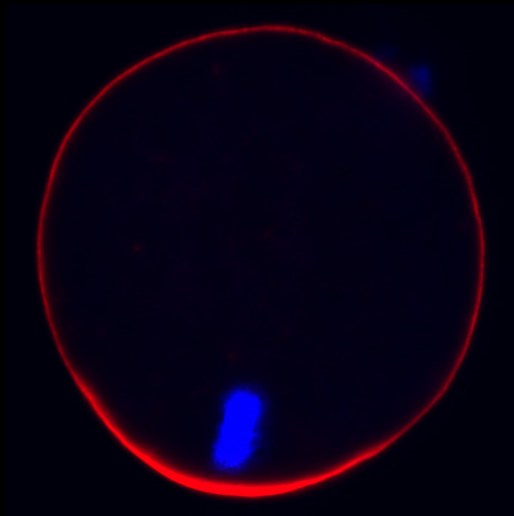
Rac-GTP

Rac1 Aby

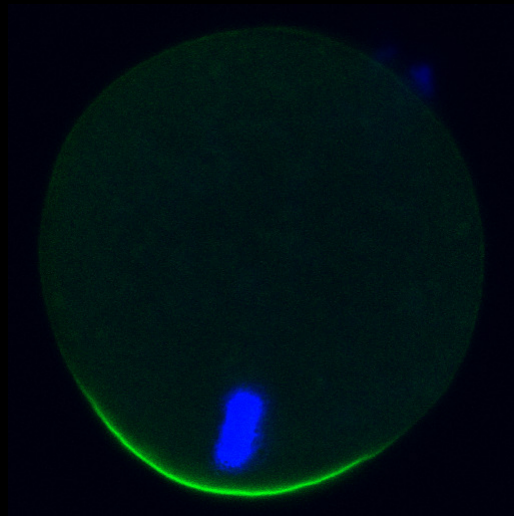


Rac1-GTP co-localises with the actin cap

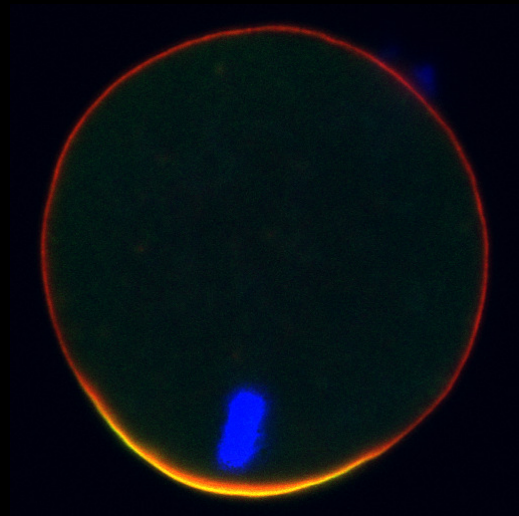
ACTIN



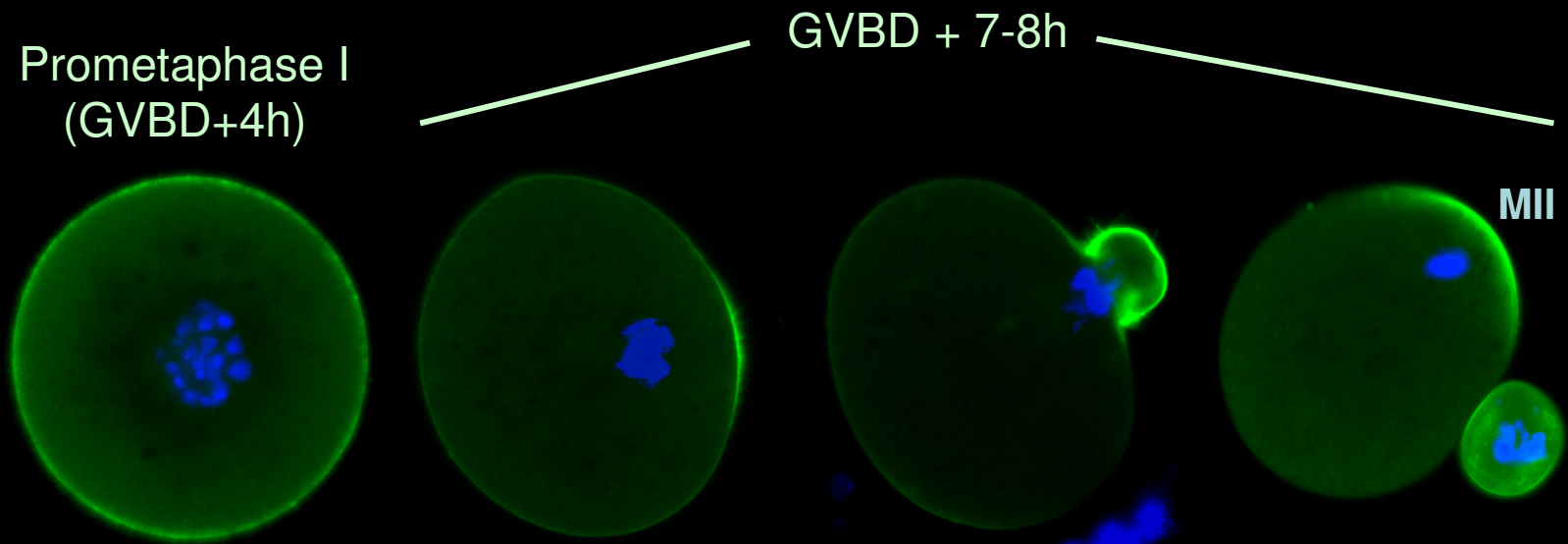
Rac-GTP



overlay



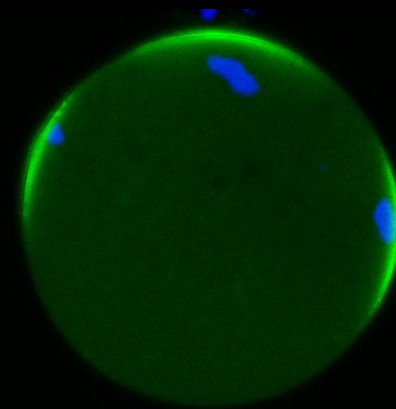
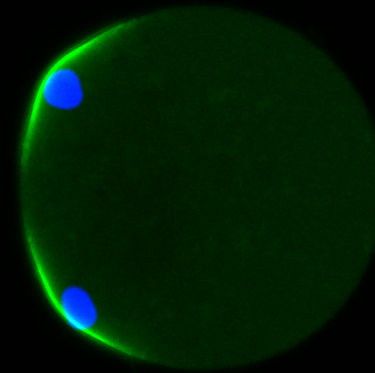
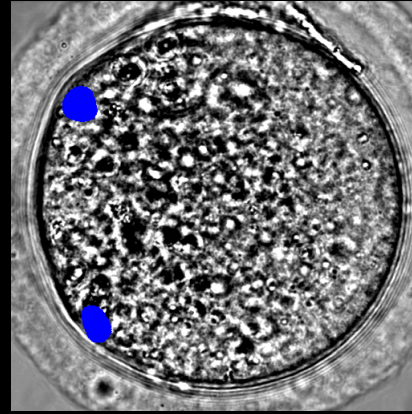
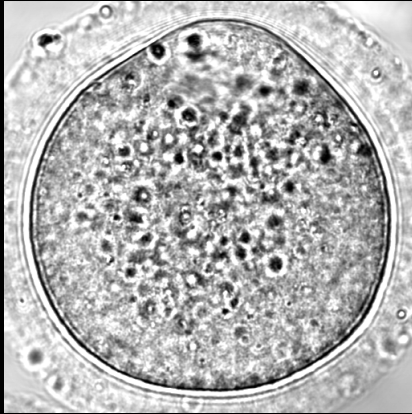
Rac1-GTP polarises as chromatin moves to the cortex



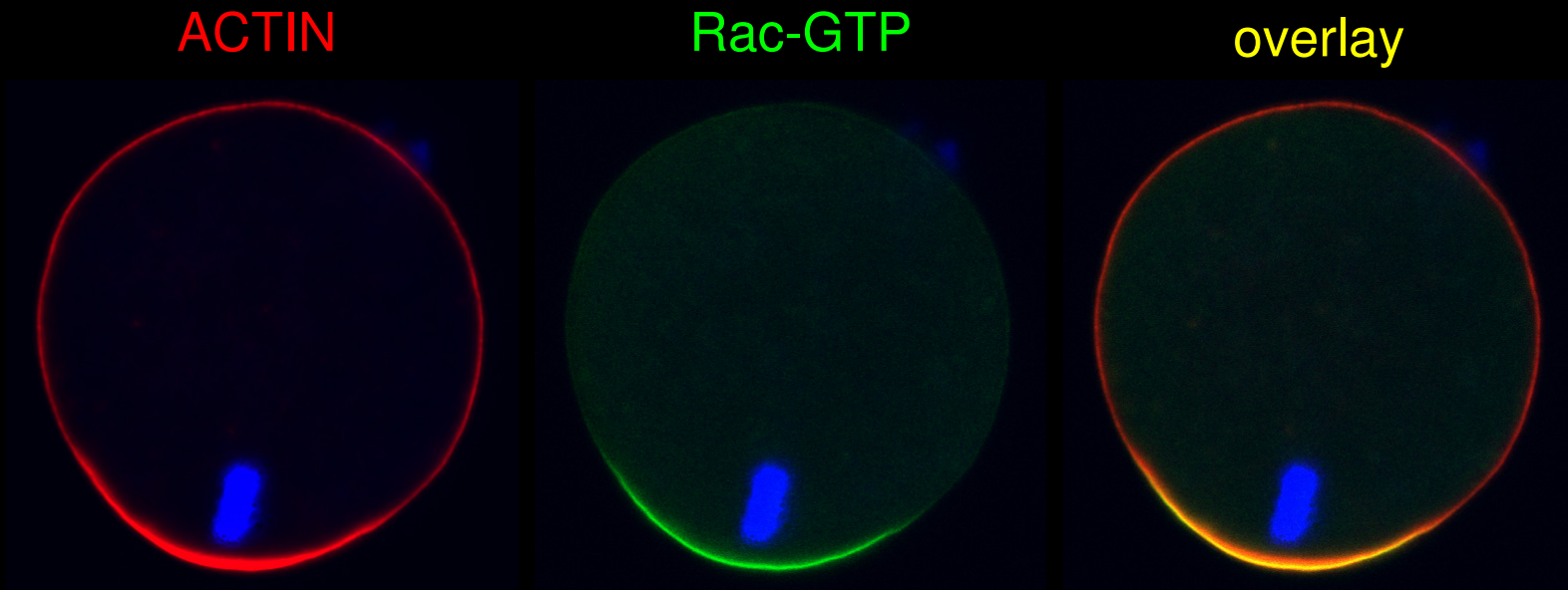
Rac1-GTP accumulates close to chromatin

nocodazole

Rac-GTP



How does chromatin remodel the cortex?



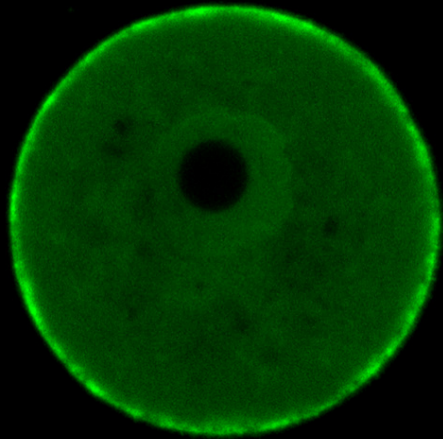
Is Rac-GTP necessary for actin cap formation?

Rac1-GTP is inhibited by RacN17T

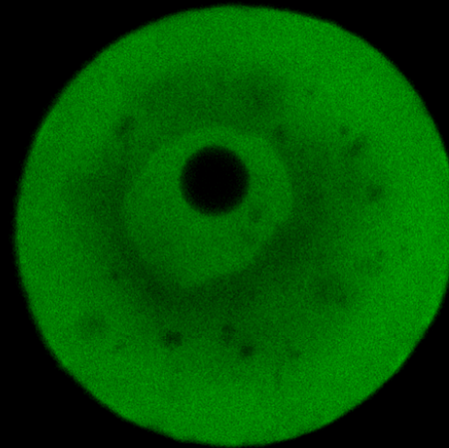
PBD

YFP

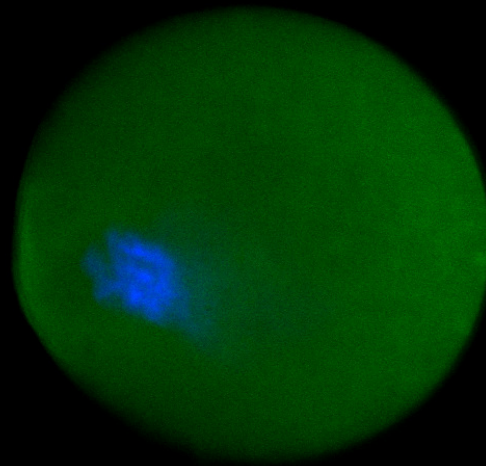
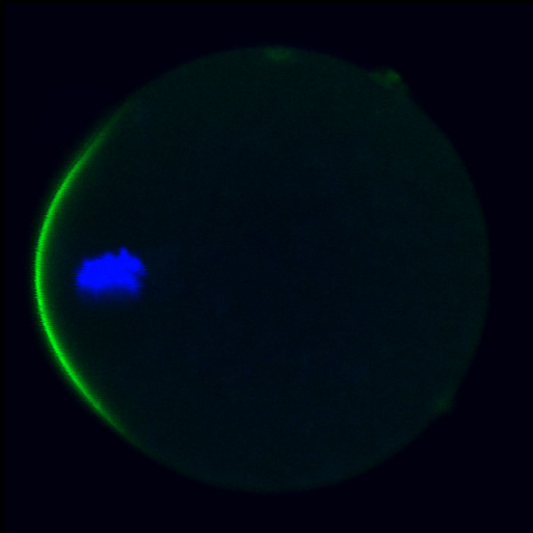
GV
Rac-GTP



Rac^{N17T}



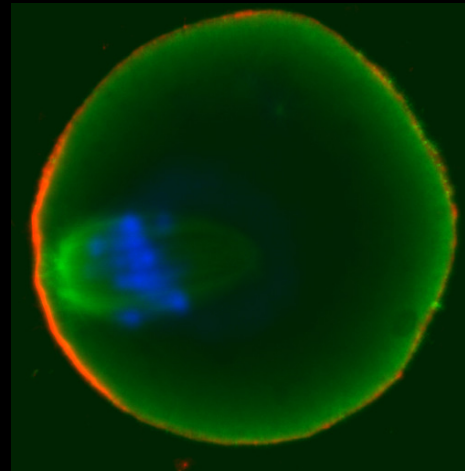
MII
Rac-GTP



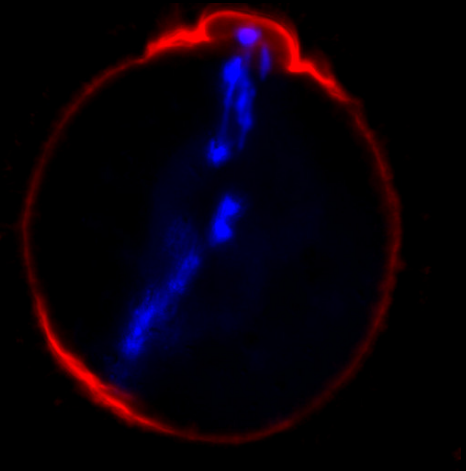
In MI Rac-N17T inhibits:

Chromosome congression, spindle stability, Pb extrusion
But NOT cortical polarization.

Rac^{N17T}
Tubulin
Actin

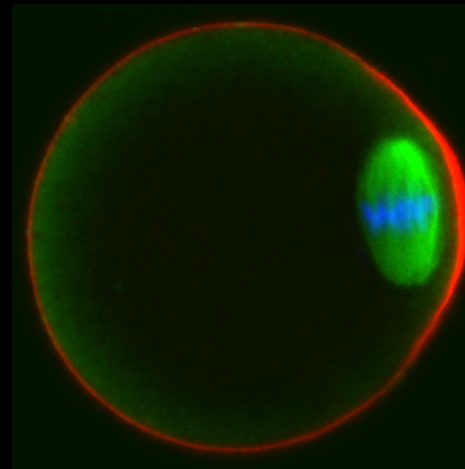


72% (n=46)



28% (n=46)

Control

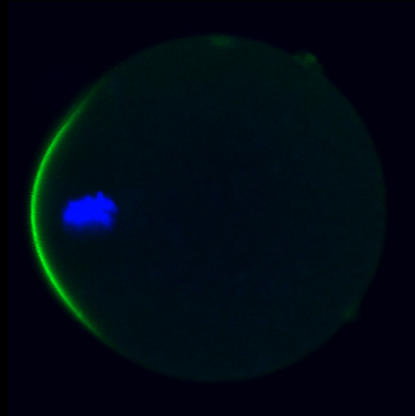
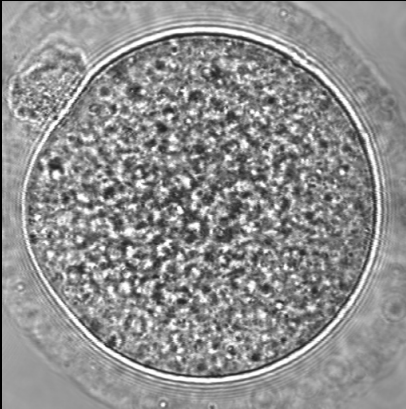


At MII, Rac1-GTP maintains the spindle at the cortex.

Rac^{N17T}

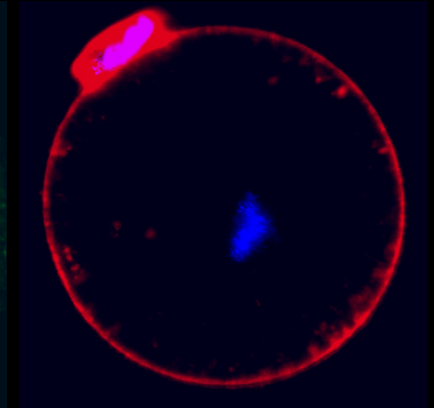
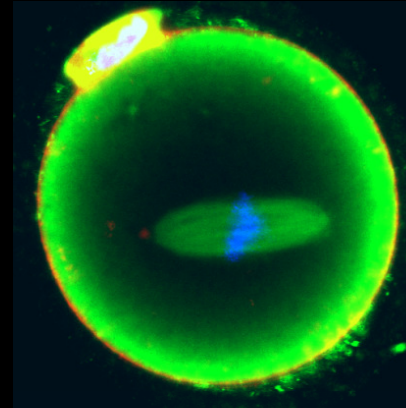
MI I

Rac-GTP



Tubulin

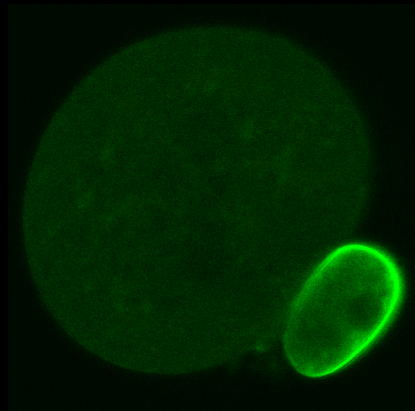
Actin



Activation



Rac-GTP

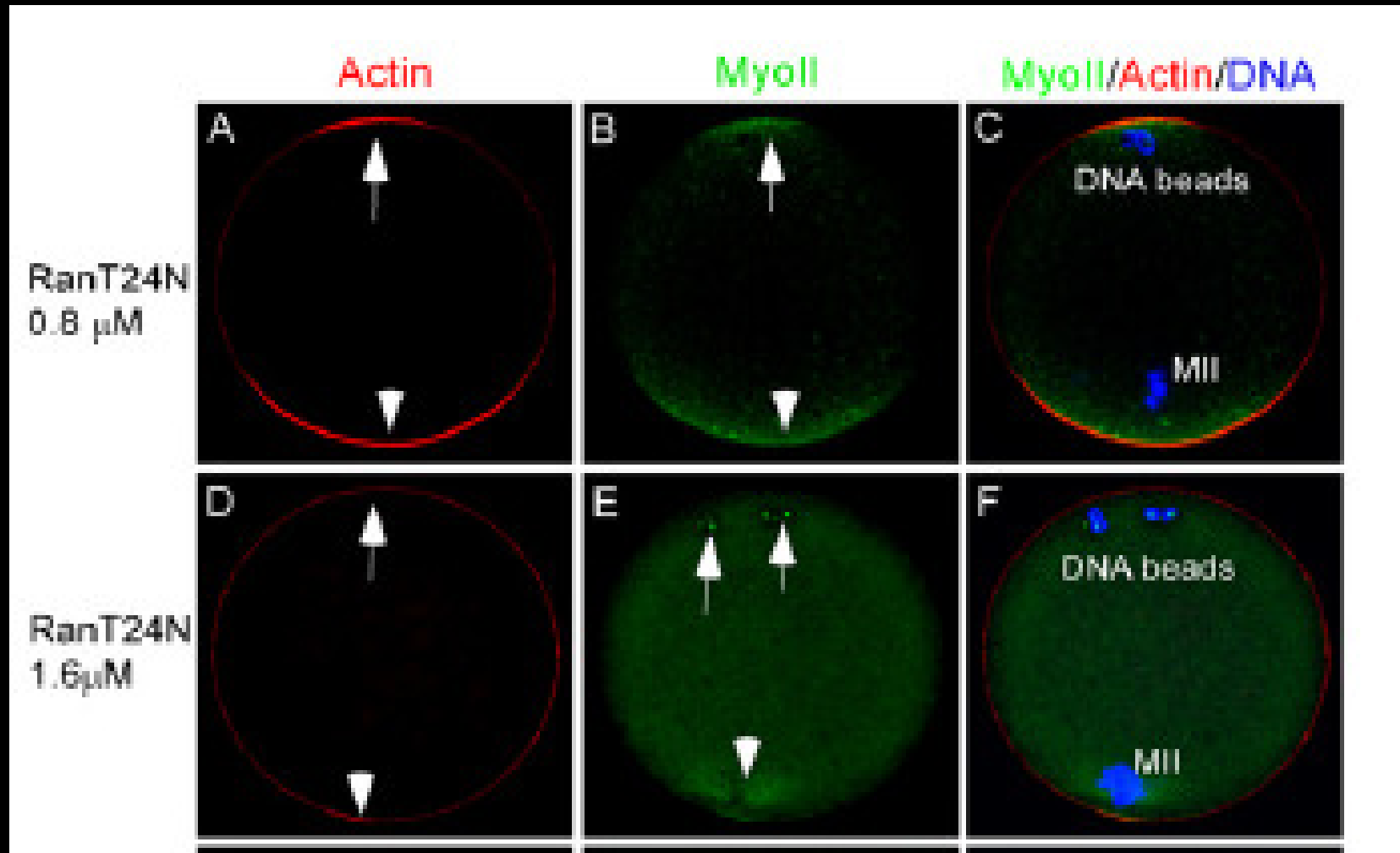


Activation

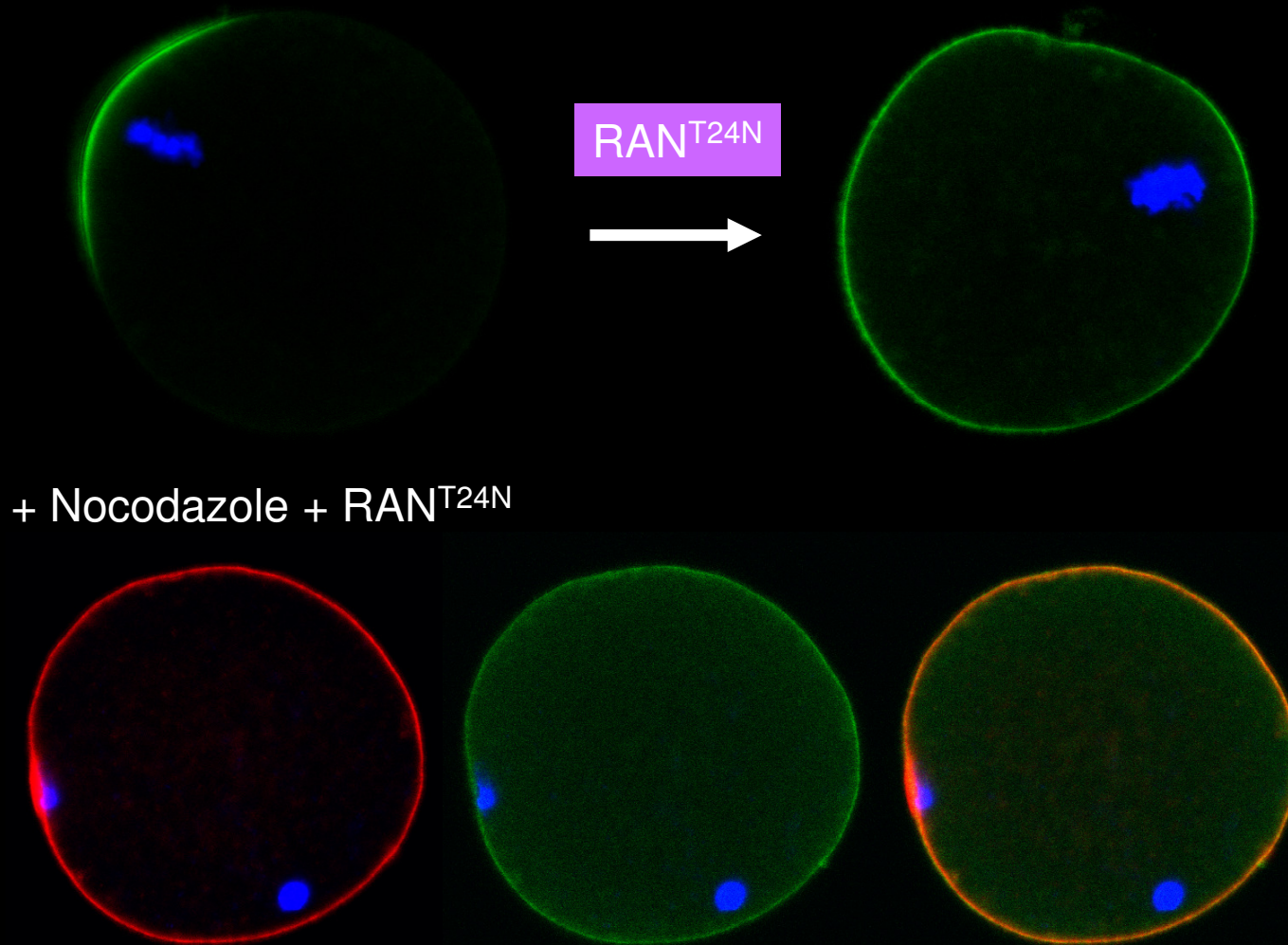


A Ran gradient contributes to cortical polarity

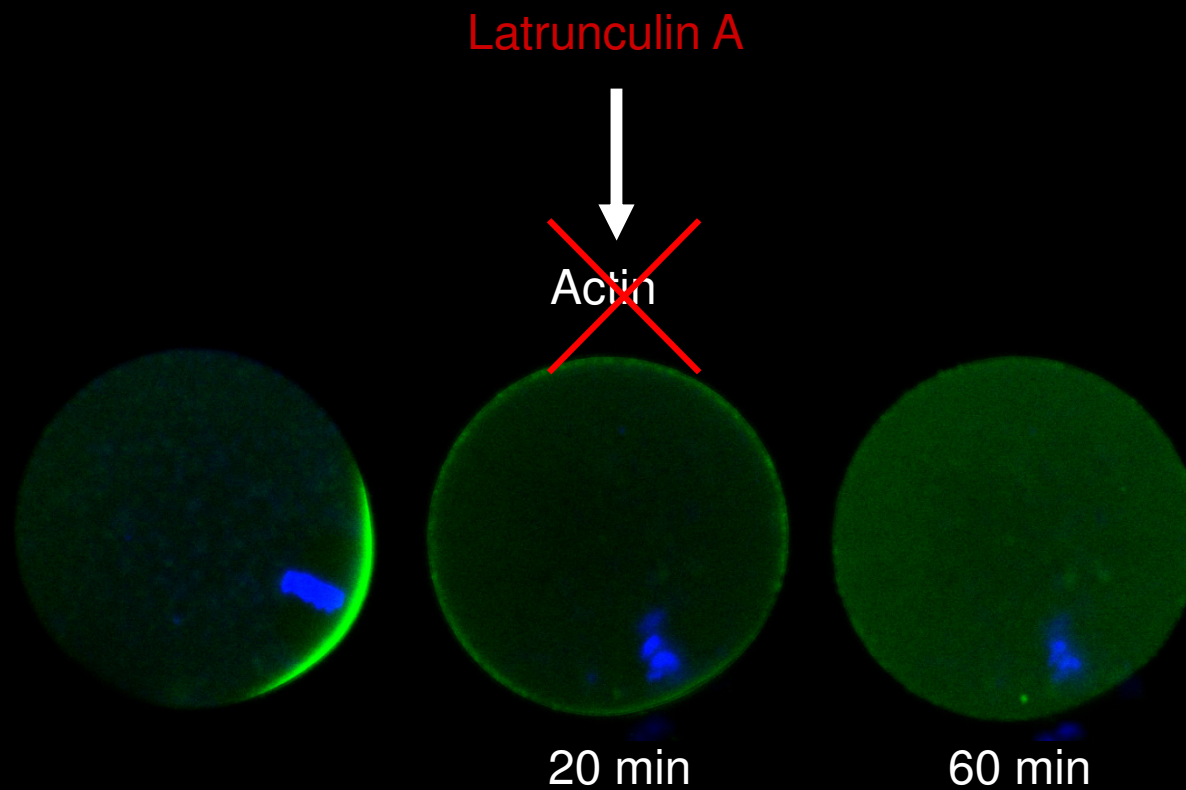
Deng et al., (2007) Dev Cell 12, 301-308



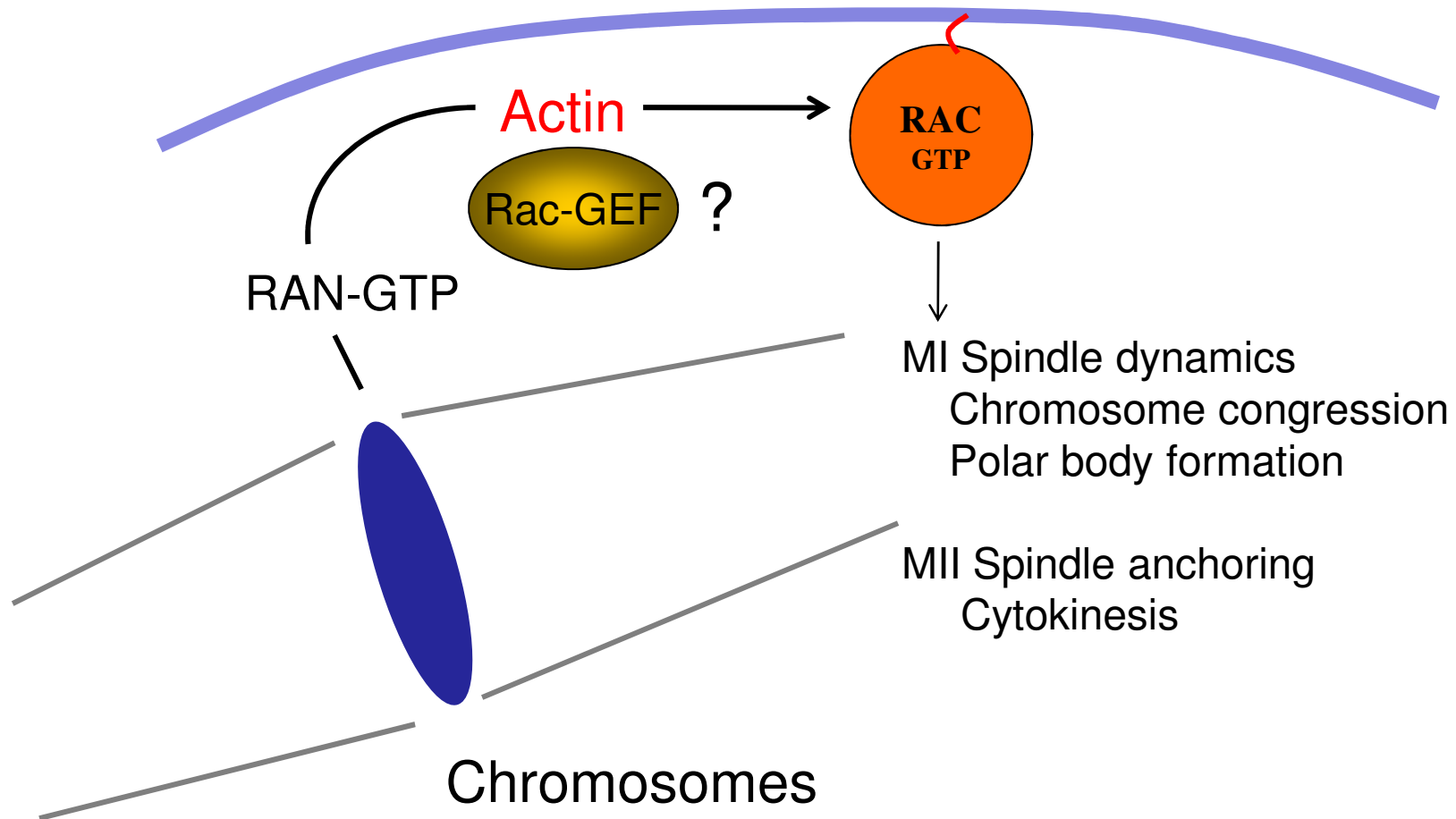
Ran activity is necessary for polarised Rac-GTP



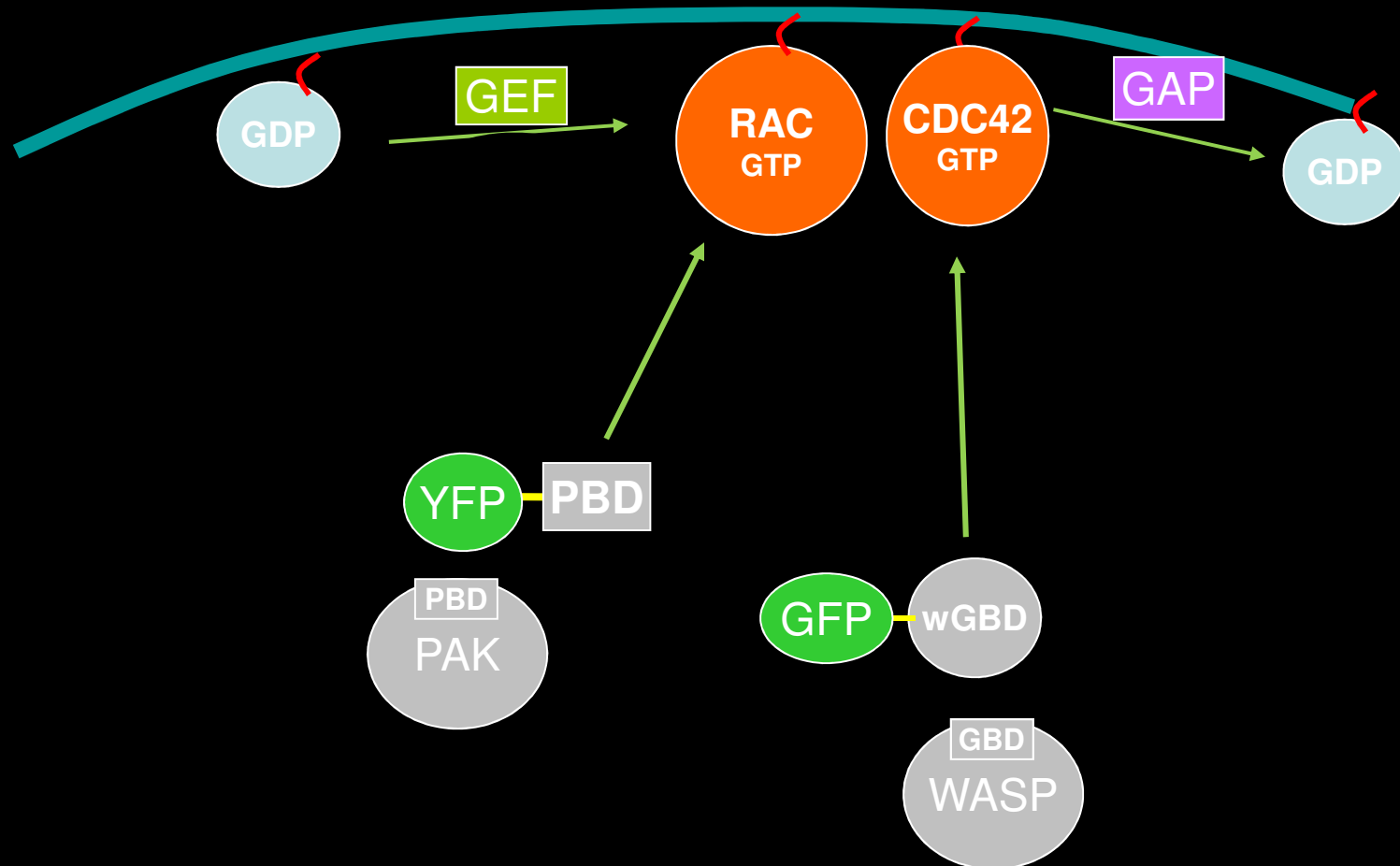
Polarized Rac activity is maintained by cortical actin



The role of Rac-GTP in oocytes



The role of small GTP-binding proteins in oocytes

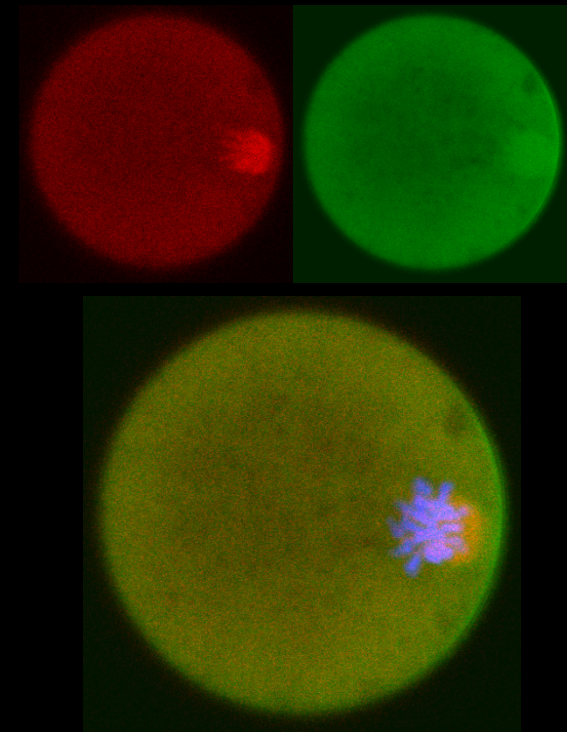
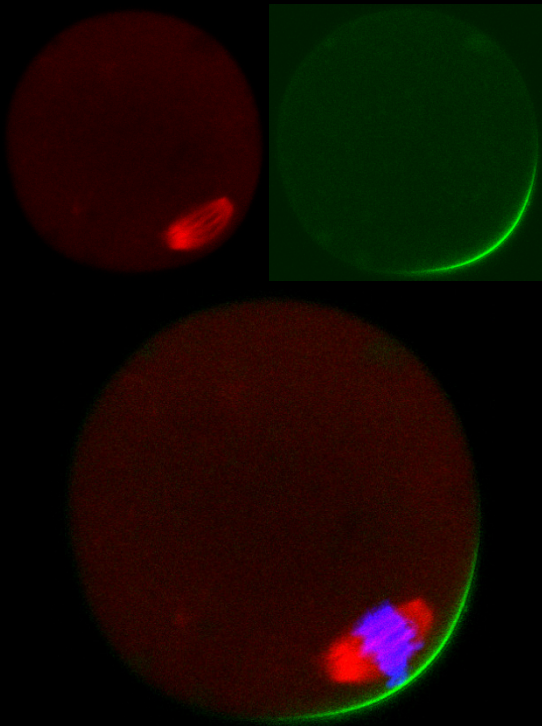


CDC42 localizes to the spindle and overlying cortex

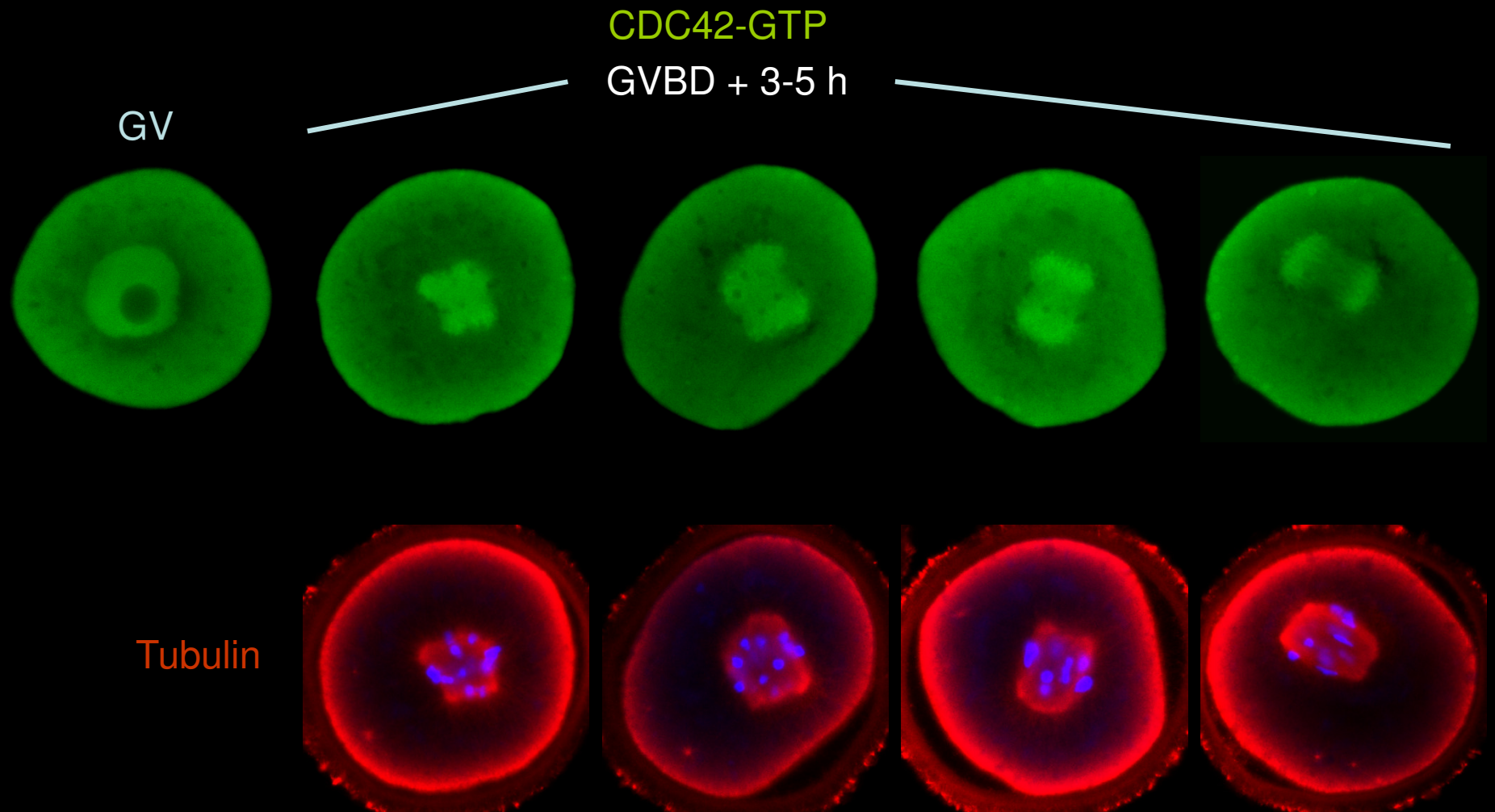
Rac-GTP

Metaphase II oocytes

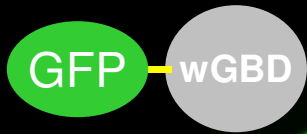
CDC42-GTP



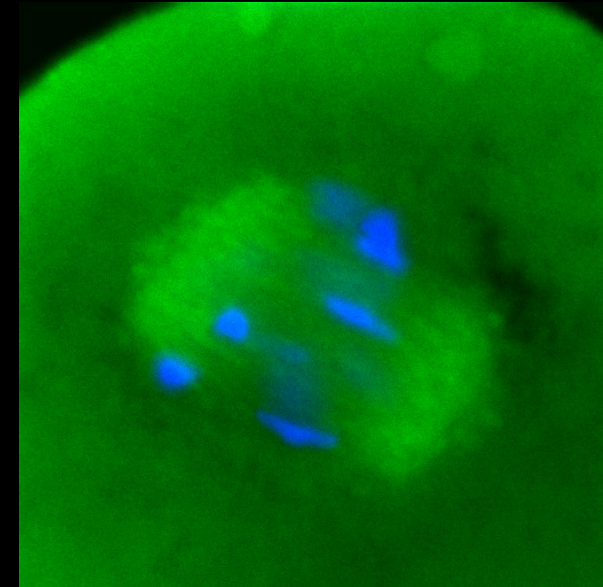
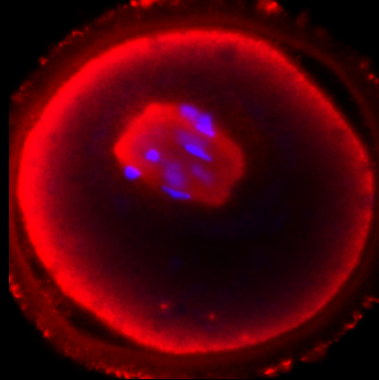
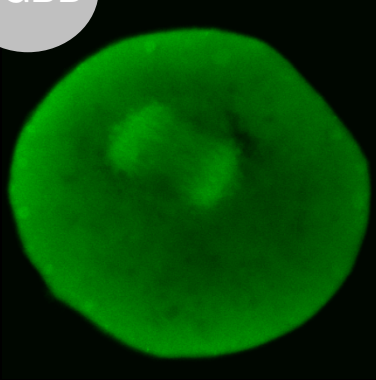
CDC42-GTP localizes to the developing spindle



CDC42-GTP associates with the developing spindle

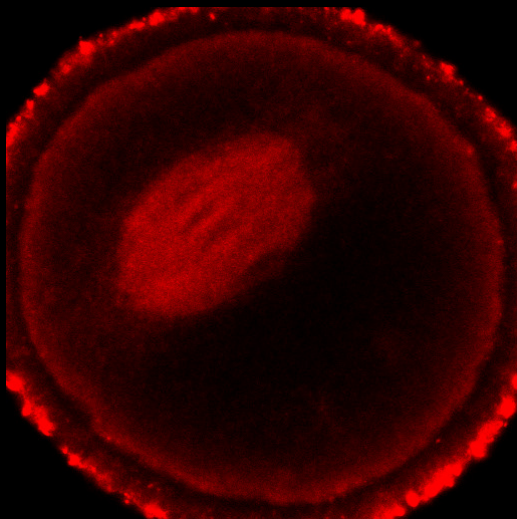


Tubulin

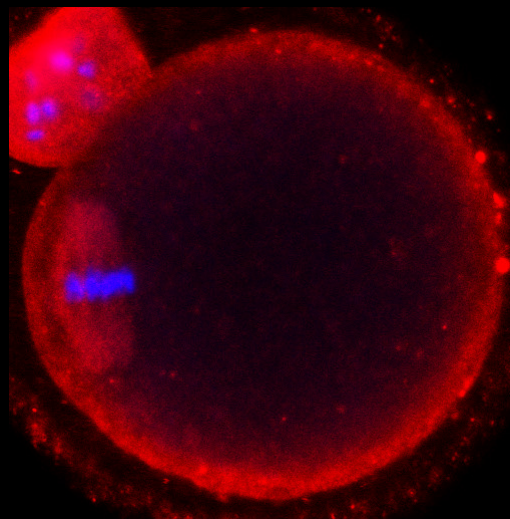


CDC42 Immunofluorescence

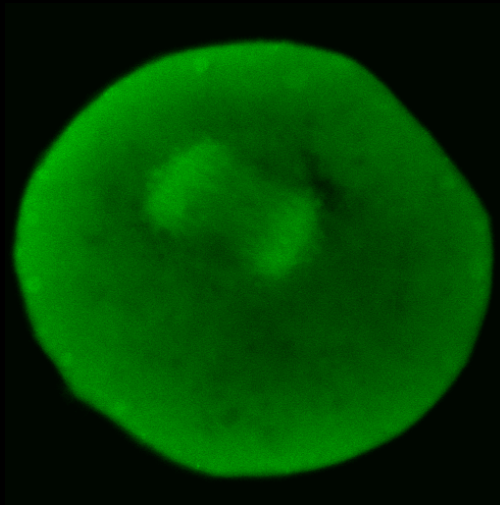
GVBD + 4 h



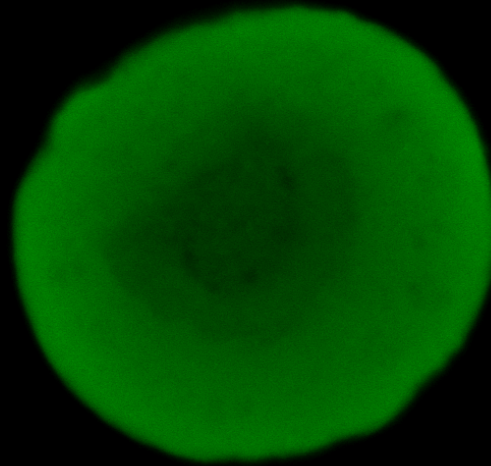
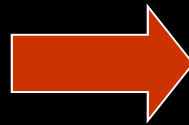
Metaphase II



N17CDC42 disrupts localization of CDC42-GTP



+ N17CDC42

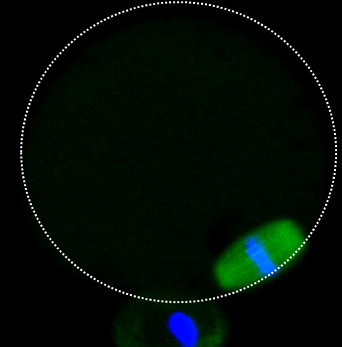


CDC42 is needed for Pb extrusion and spindle attachment

GV



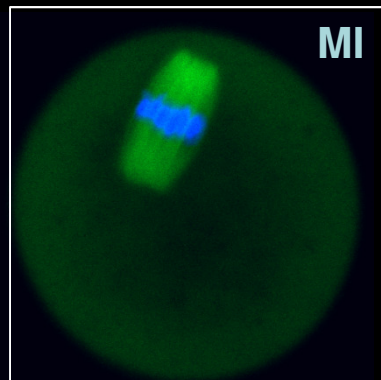
MII



+
N17CDC42
⊖

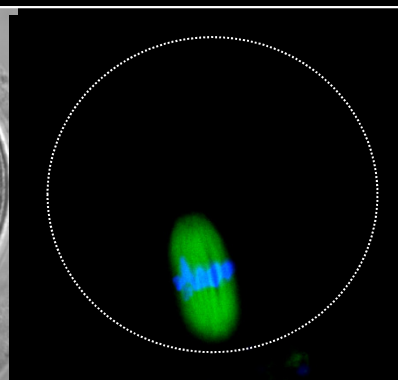


Maturation



MI

45% MI arrest

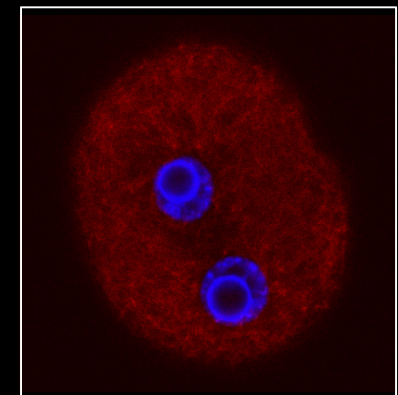


55% progress to MII
80% perpendicular spindle

+
N17CDC42
⊖

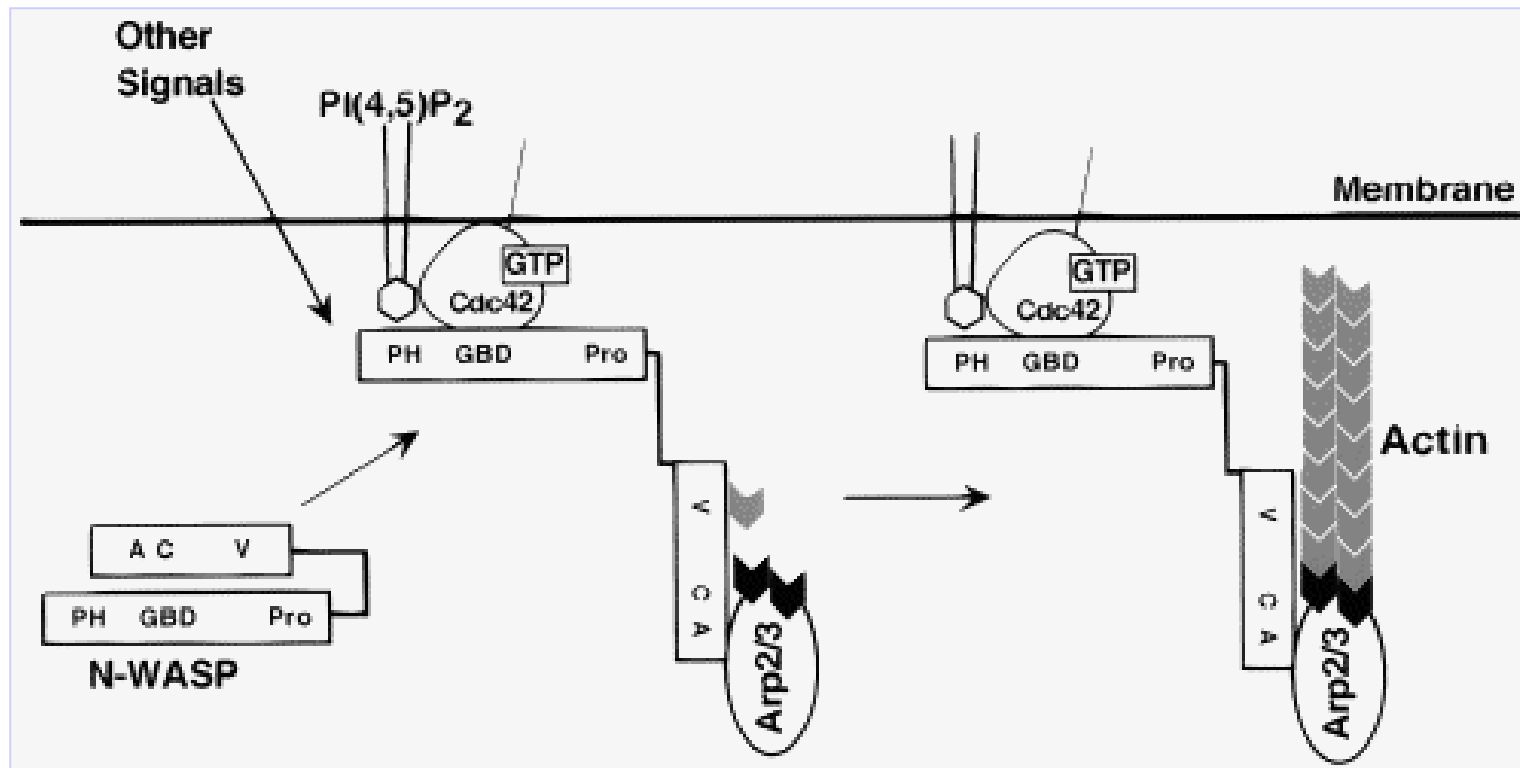
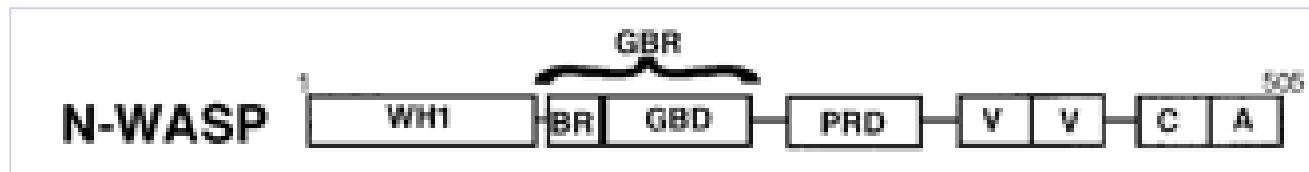


Activation
(EtOH)

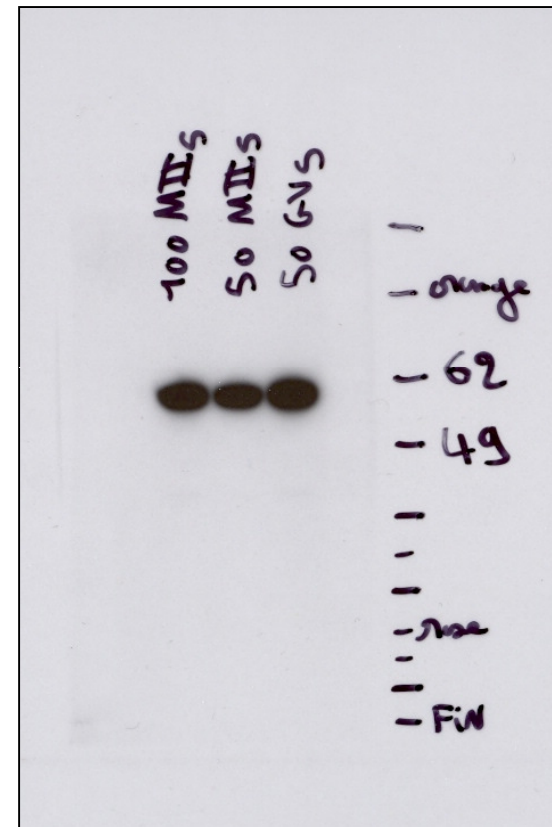
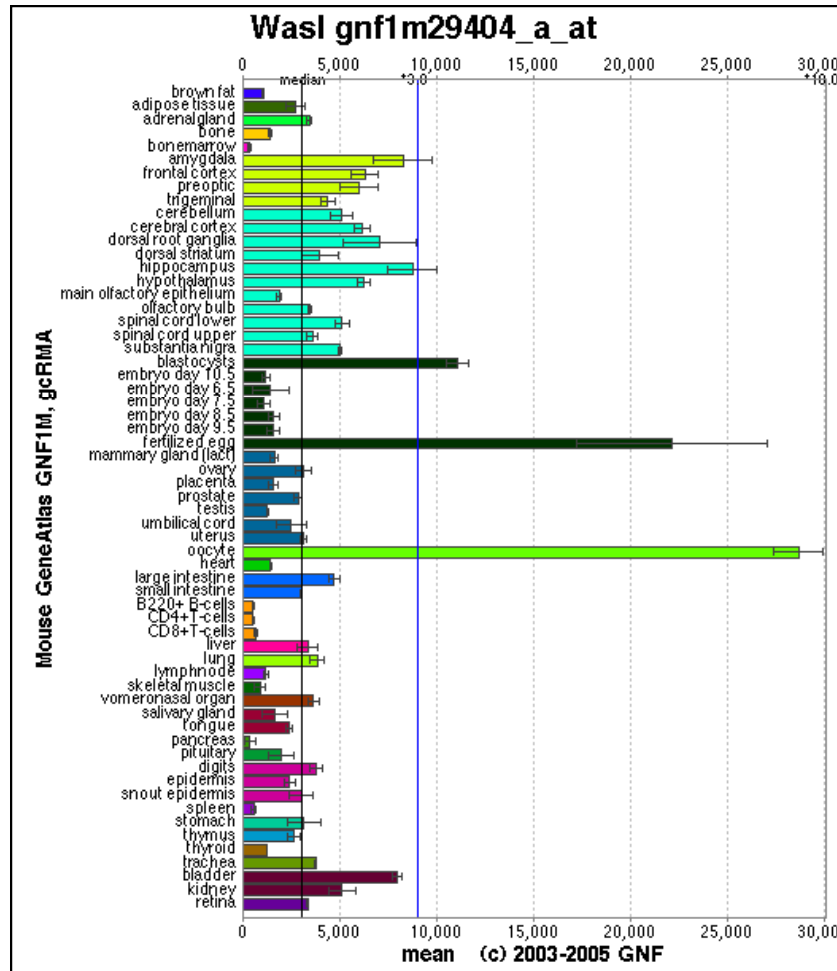


85% NO Pb2

CDC42 induces actin filaments through N-WASP

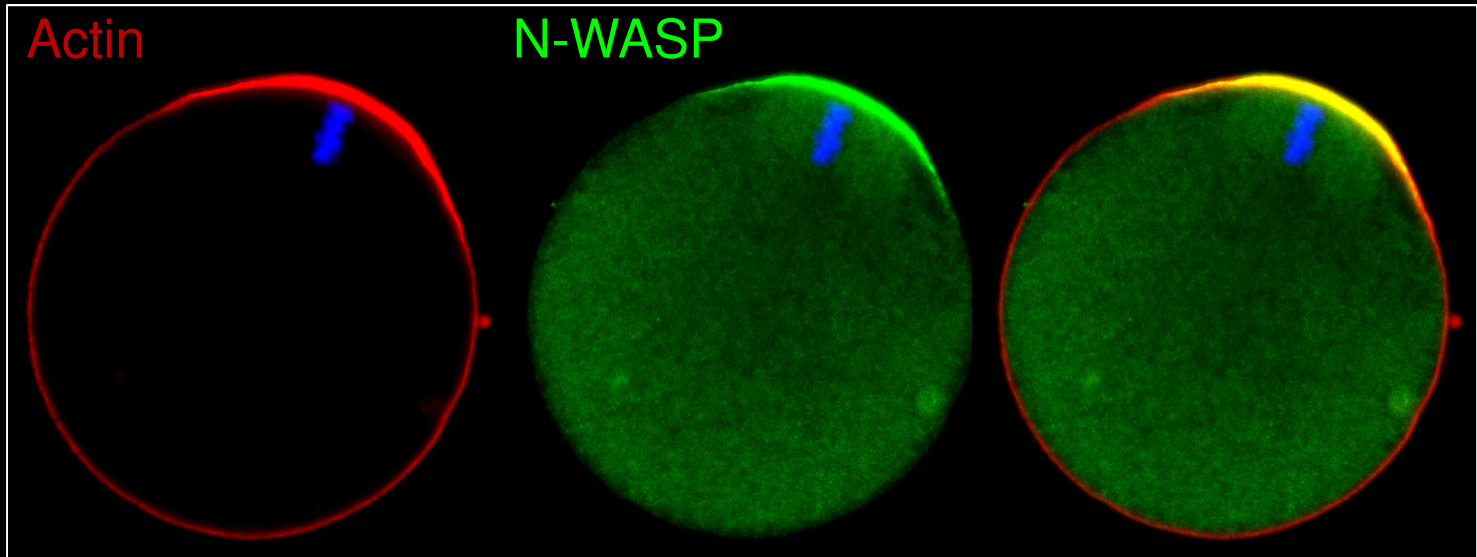


N-WASP is highly expressed in oocytes

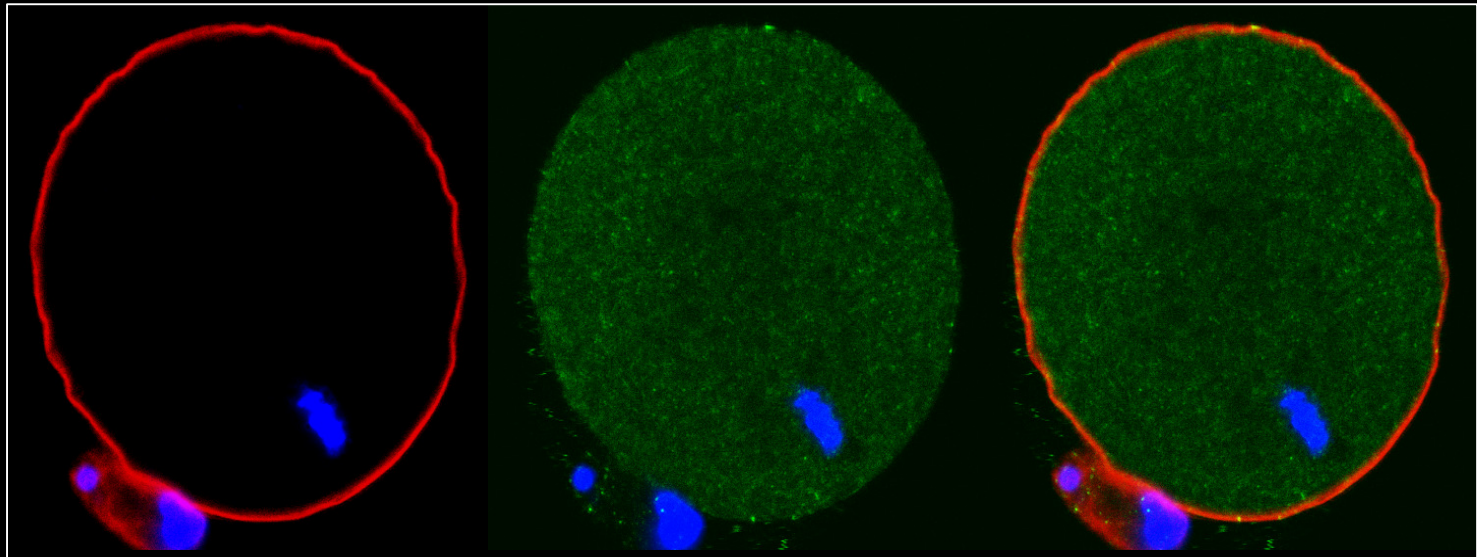


N17CDC42 causes loss of polarised N-WASP and actin.

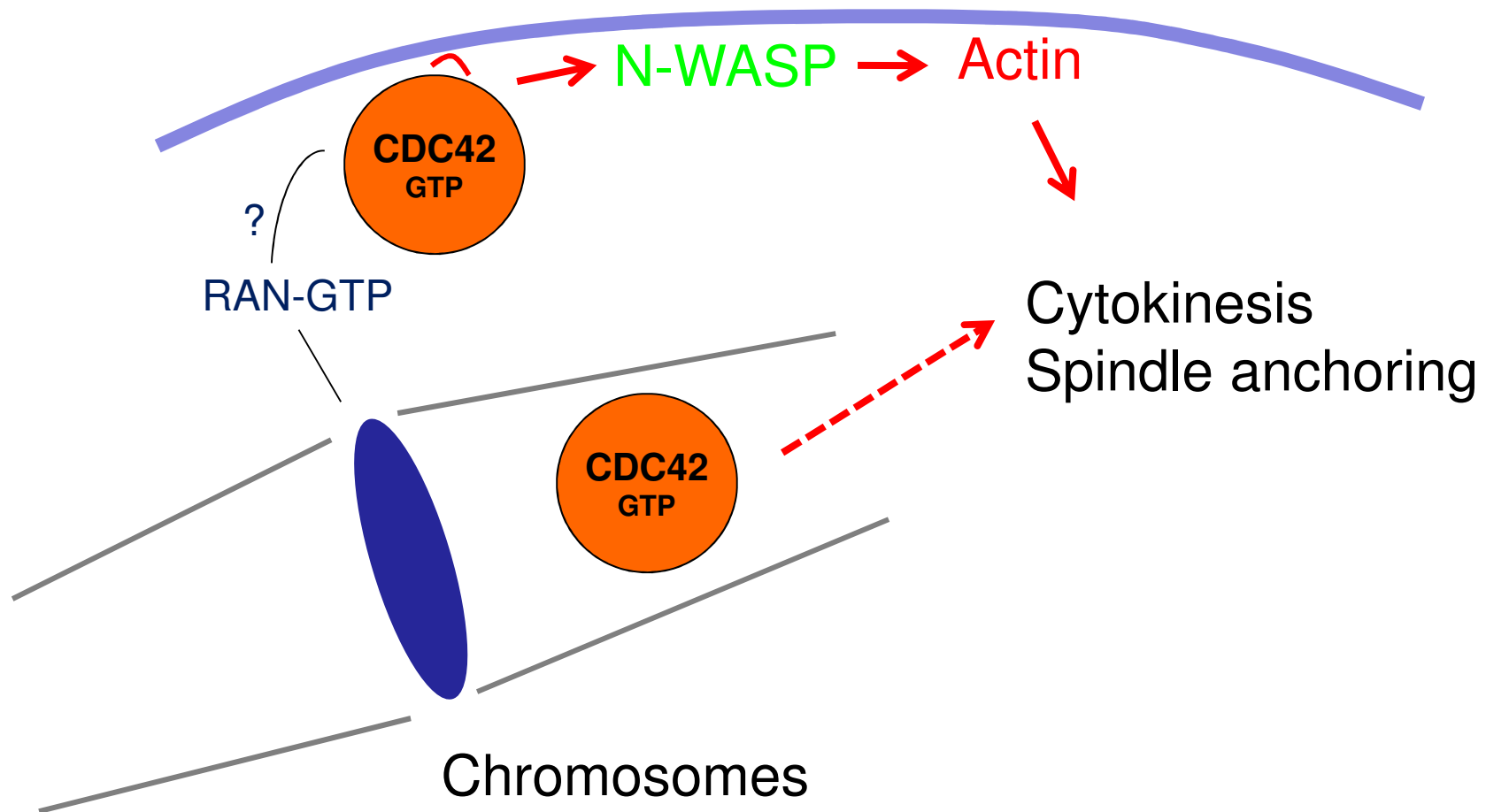
Metaphase II

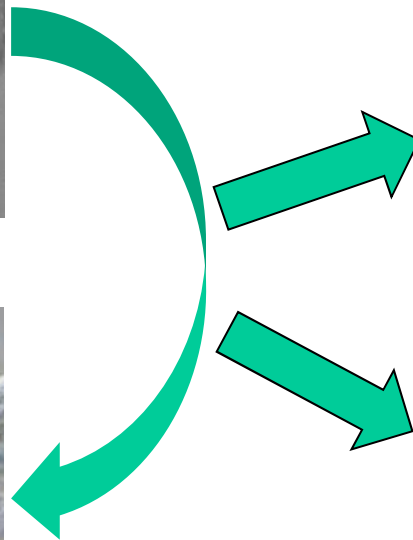
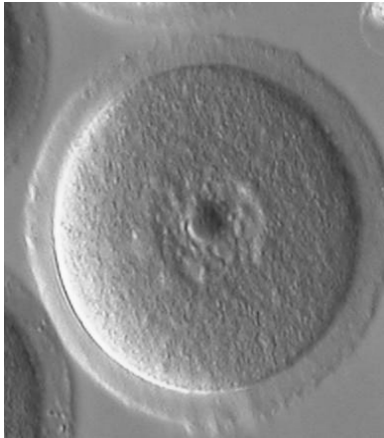


Metaphase II
+
N17 CDC42



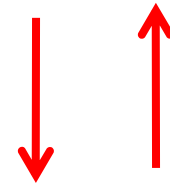
The role of CDC42-GTP in oocytes





Successful oocyte
maturation

Nuclear
maturation



Cytoplasmic
maturation

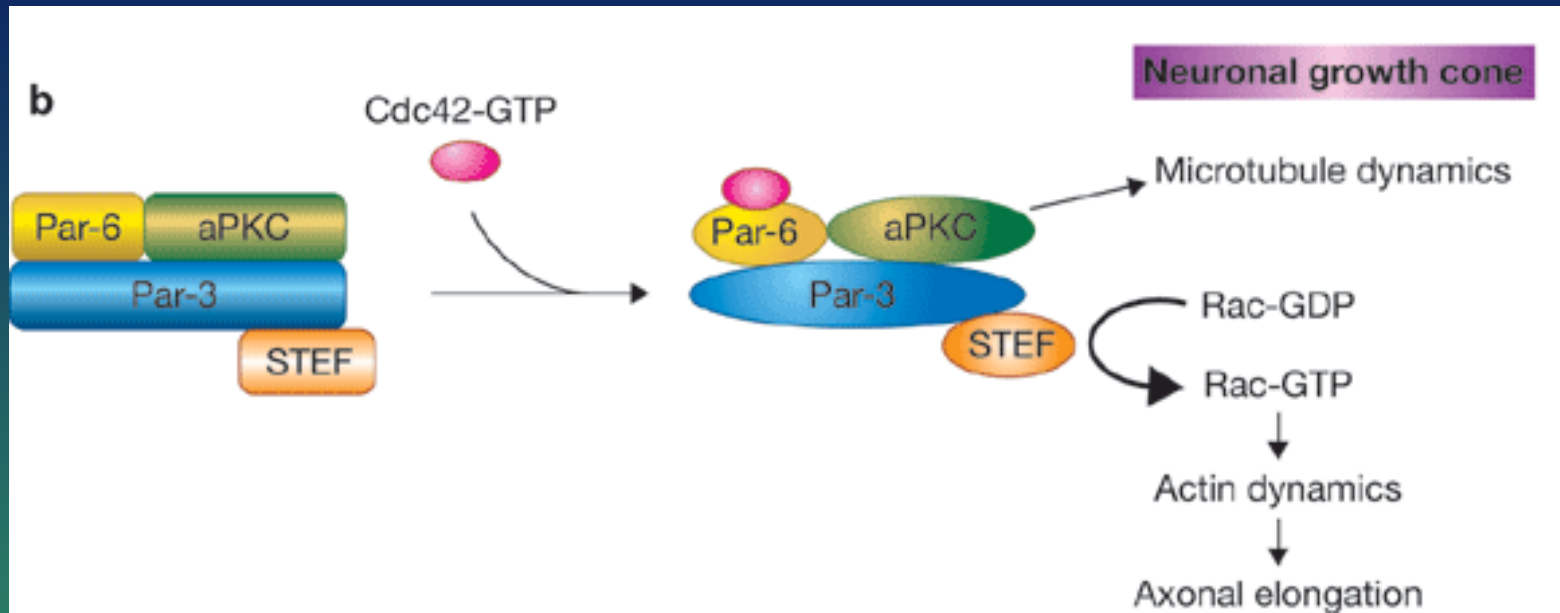
Acknowledgements

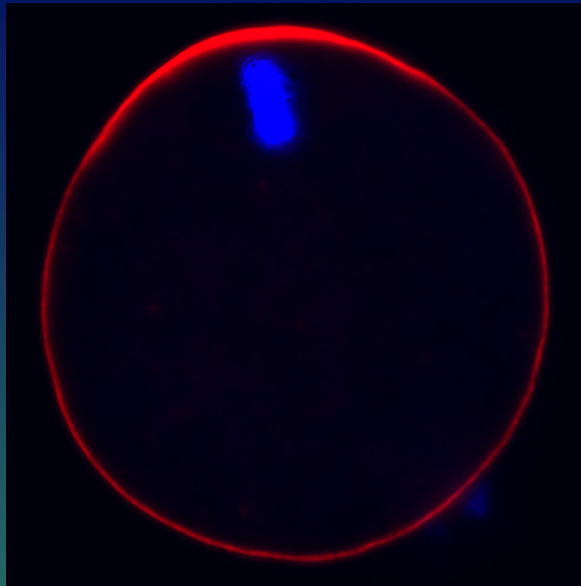


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Development

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Caroline Dalton
Biba Nabti
Roberta Dale
Jenny Bormann

MRC and Wellcome Trust





oocyte polarisation, spindle anchoring, PB emission

