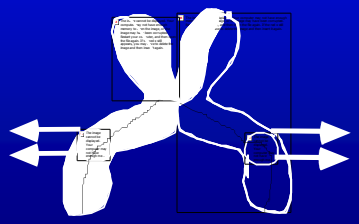
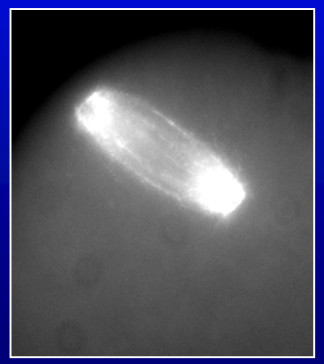
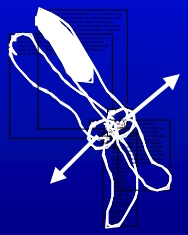
Modelling of age-associated alterations in expression in mammalian oocytes

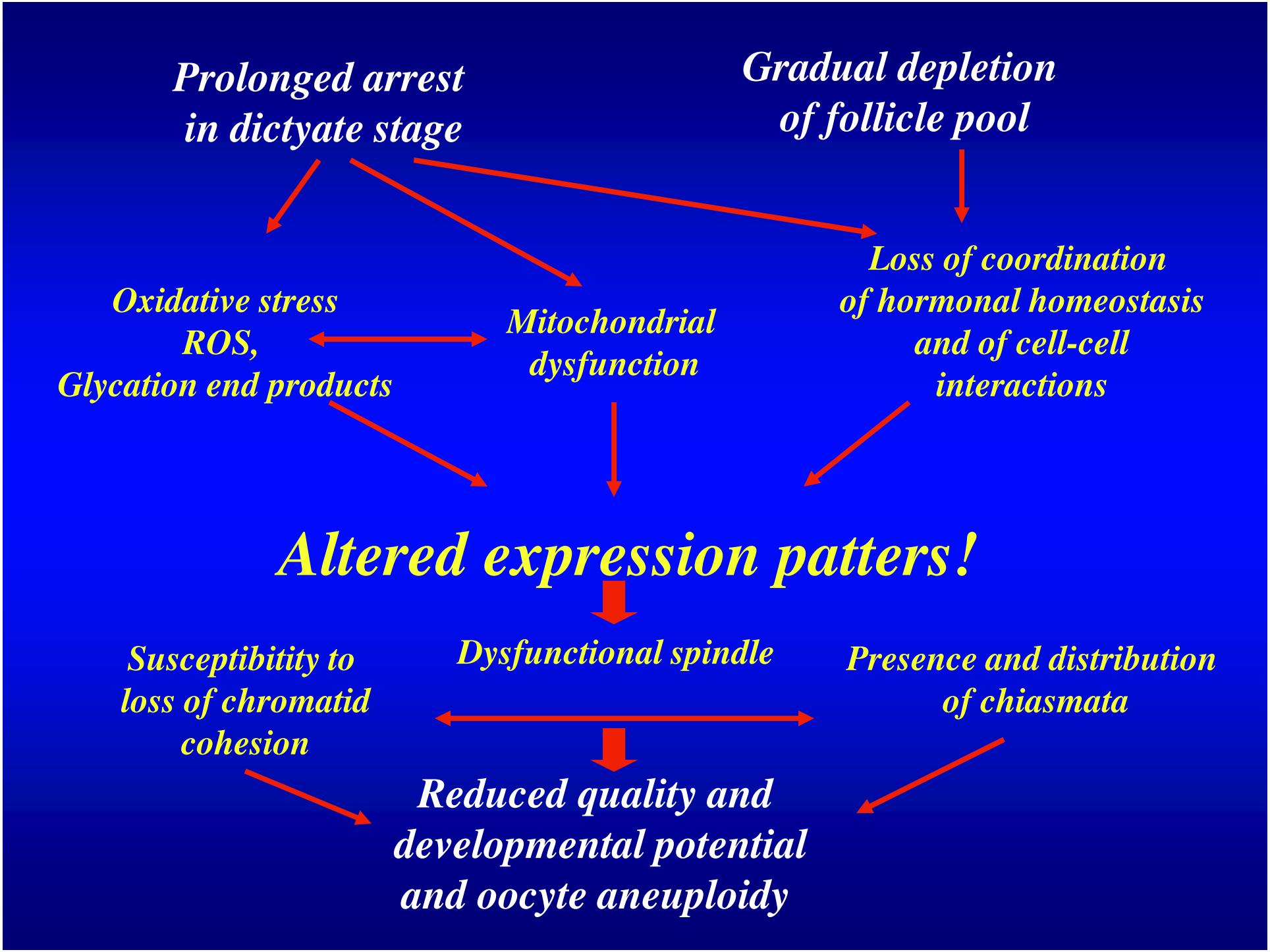
Eichenlaub-Ritter, U.,

Vogt, E., Sanhaji, M., Kipp, A., Betzendahl, I.








Prolonged arrest in dictyate stage
Gradual depletion of follicle pool
Oxidative stress ROS, Glycation end products
Mitochondrial dysfunction
Loss of coordination of hormonal homeostasis and of cell-cell interactions
Altered expression patters!
Susceptibitity to loss of chromatid cohesion
Dysfunctional spindle
Presence and distribution of chiasmata
Reduced quality and developmental potential and oocyte aneuploidy

Gene profiling: Altered expression in aged oocytes (transcript level)

Study in mouse suggests that
from 11 000 genes about 5% exhibit changes in expression in aged GV-stage oocytes and even more (33%) in MII stage (Pan et al., 2008)

Spindle formation
e.g. tubulin
kinesins

Cell cycle regulation
SAC
e.g. Mad2, BubR1, Bub1,
Aurora kinases

Chromatin structure
DNA methylation
Chromosome cohesion
e.g. Smc's

Expression
Metabolism

Mitochondrial
Function; oxydative stress

Maternal
products

Steuerwald et al., RBM Online 2000
Hamatani et al., Hum Mol Genet 2004
Steuerwald et al., Mol Hum Reprod 2005
Pan et al. Biol Reprod 2008

Mad2
Aurora kinase B
MCAK

Modelling loss of functional spindle checkpoint I:

Mad2

Exposure of oocytes to nocodazole show concentration-dependent meiotic arrest, increased spindle aberrations and chromosome congression failure (Shen et al.Mutat Res 2008)

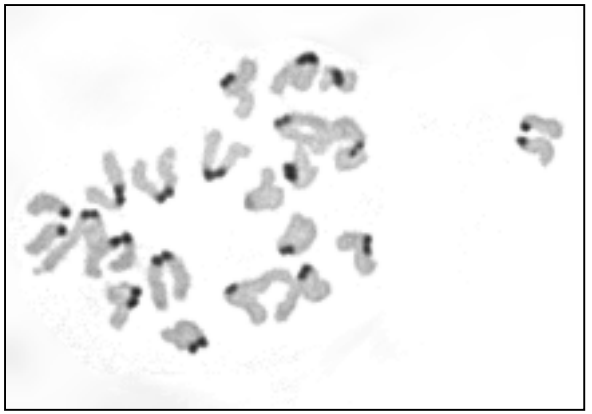
Mad2 overexpression causes meiotic arrest at M I

Knockdown of Mad2 causes faster progression to anaphase I (Homer et al., 2005)

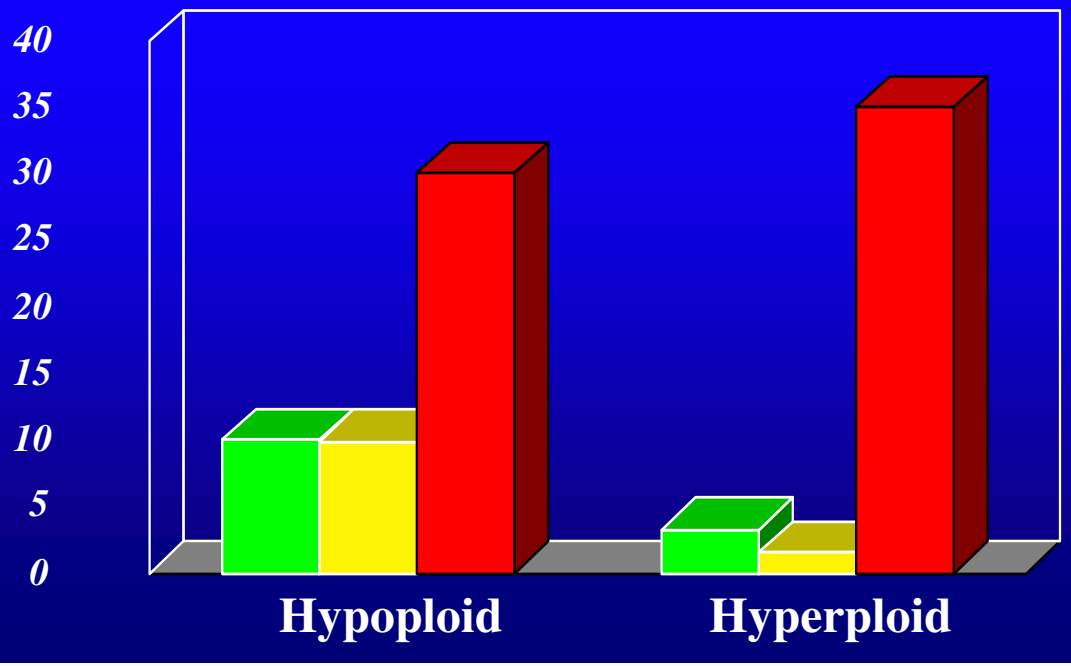
Mad2 knockdown overcomes arrest by nocodazole and induces highly aberrant spindles plus significant increases in aneuploidy

Increase in aneuploidy by RNAi-induced maturation in presence of nocodazole

Control, euploid, 20 dyads



Mad siRNA, hyperploid, 21 dyads



Modelling loss of intact spindle checkpoint part II:

Aurora kinase B

Interference with normal activity of Aurora kinase B (more message of AURKB but less of INCENP in aged MII oocyte, Pan et al., 2008)

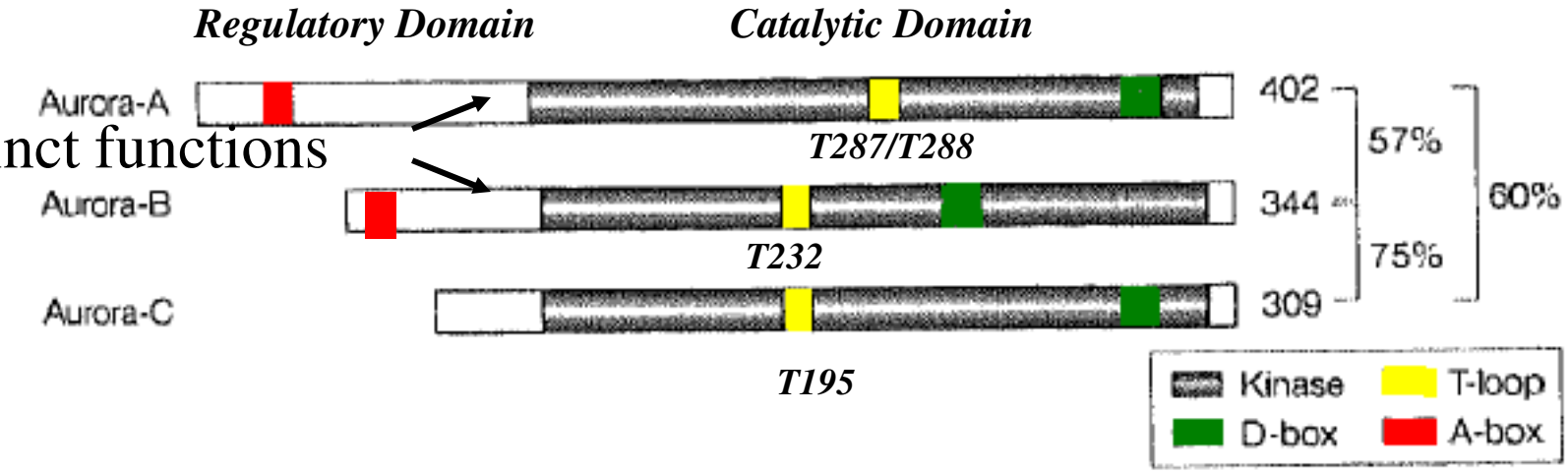
Aurora kinase B is a component of the chromosomal passenger complex (CPC) containing INCENP, survivin, Aurora kinase B and Dasra/borealin (Ruchaud et al., 2007; Vogt et al., RBM Online, Sep. 2009)

Aurora Kinases:

Ubiquiously expressed family of serine/threonine kinases performing multiple functions in mitosis and meiosis/ deregulation involved in tumorigenesis and aneuploidy

Mammals: Aurora kinase A, B, and C.

Distinct functions

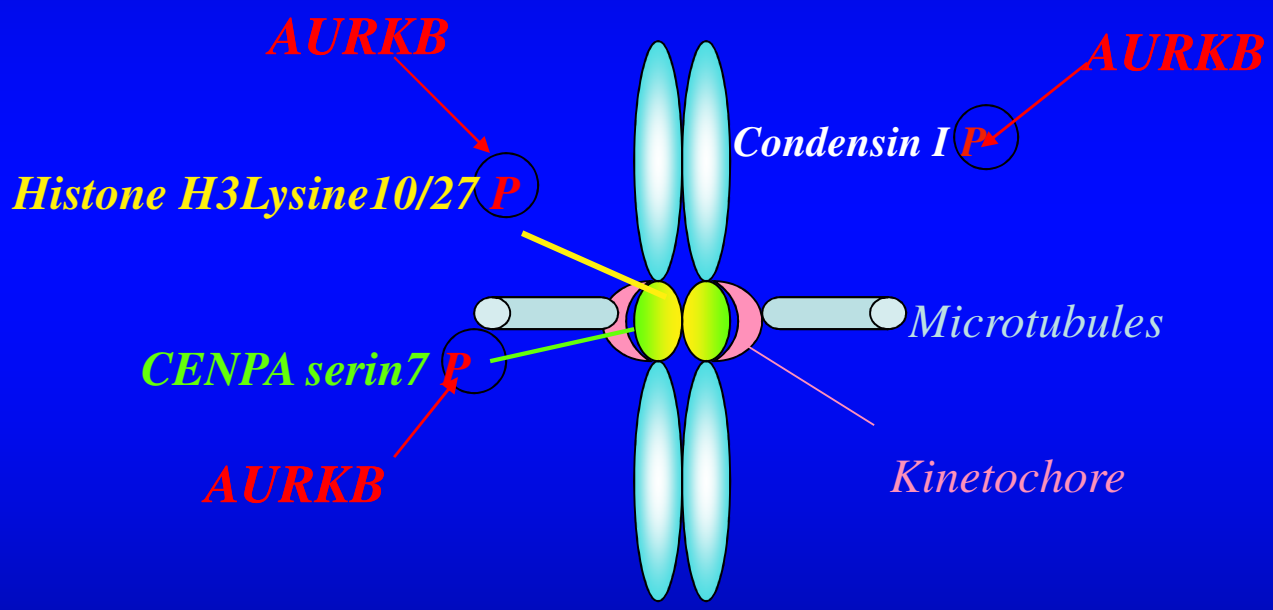


D-Box: destruction box; ***A-box: destruction activating box***

Activation T-loop: Phosphorylated threonine; ATP and substrate binding

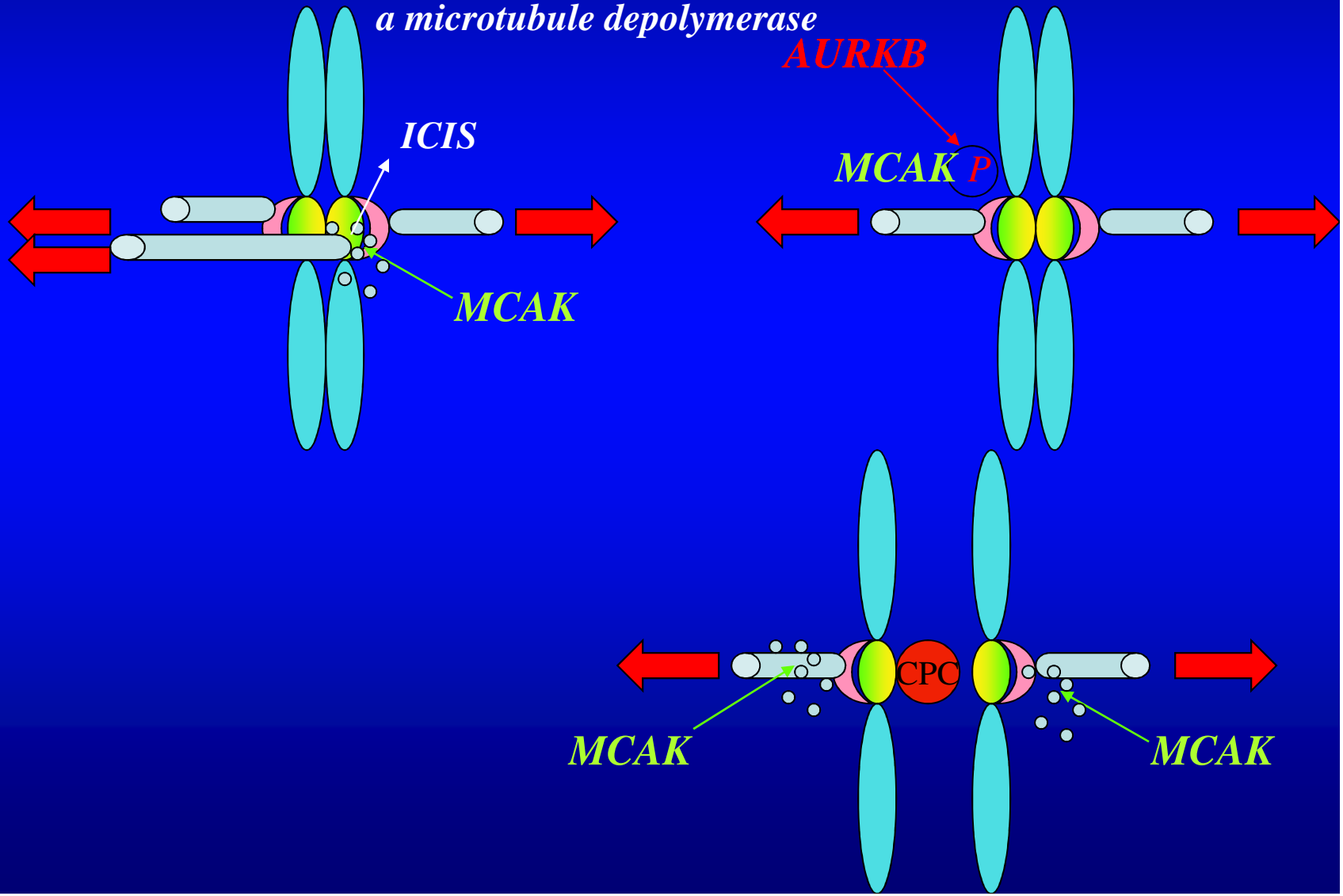
(modified from Keen und Taylor, 2004; Fu et al., 2007)

Aurora Kinase B (AURKB) is involved in chromosome condensation and in organization of a functional centromere

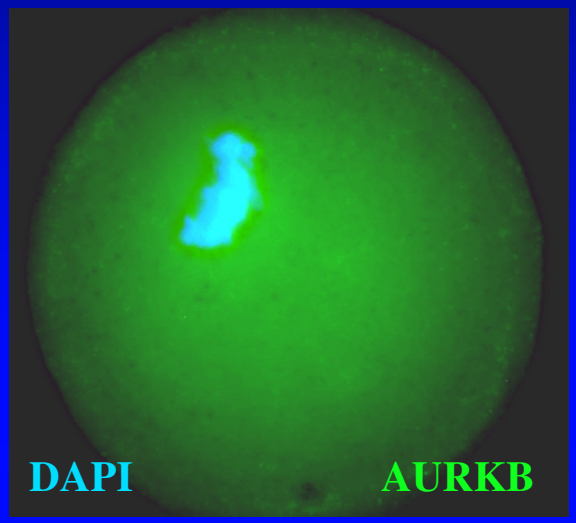


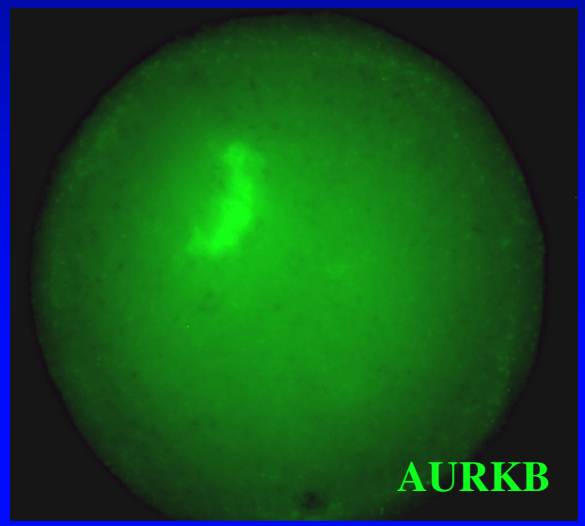
Aurora Kinase B (AURKB) regulates microtubule disassembly of improperly attached tubules

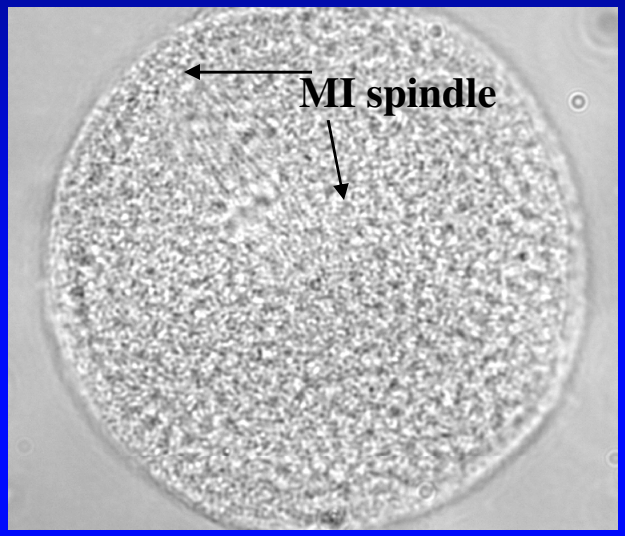
Phosphorylation of the kinesin-like protein MCAK (mitotic centromere-associated kinesin), a microtubule depolymerase

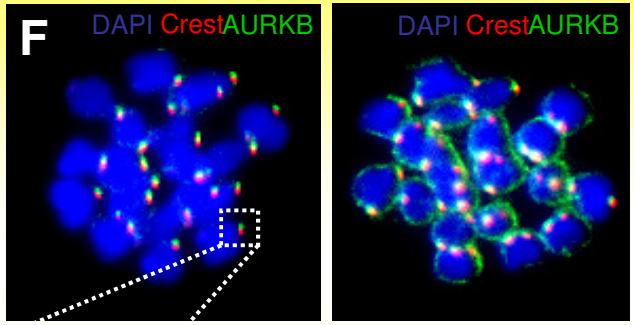


Aurora kinase B associates with chromosomes in meiosis I and is enriched at centromeres of chromosomes





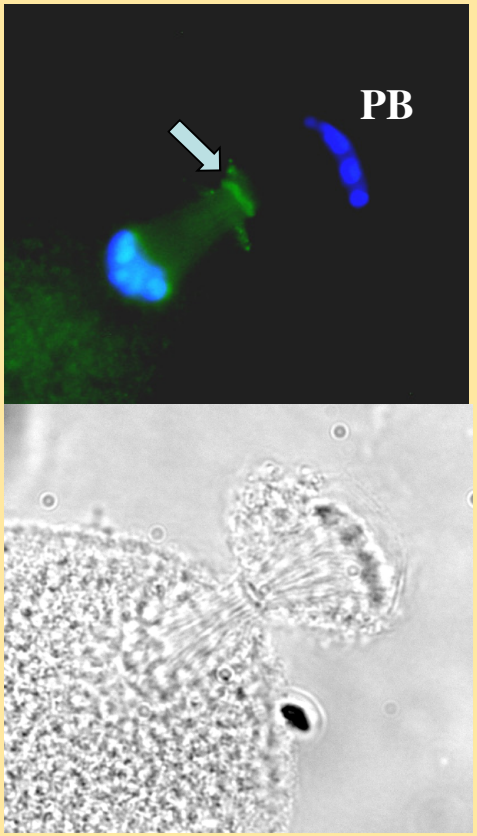


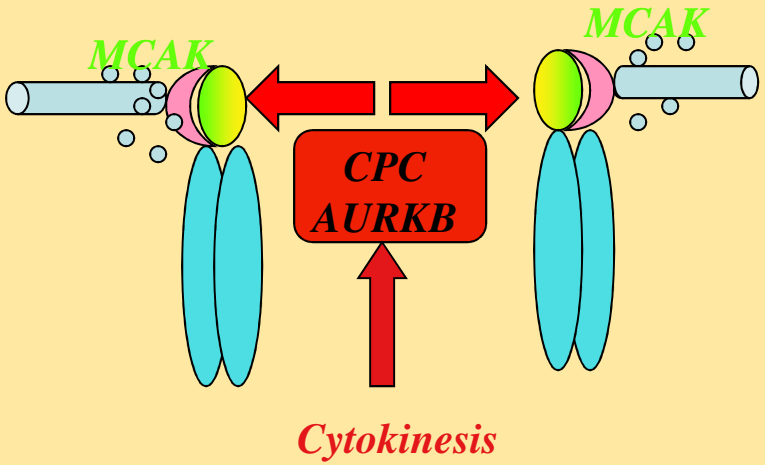


DAPI: Chromosomes
CREST:Centromere protein A/C
Anti-AURKB

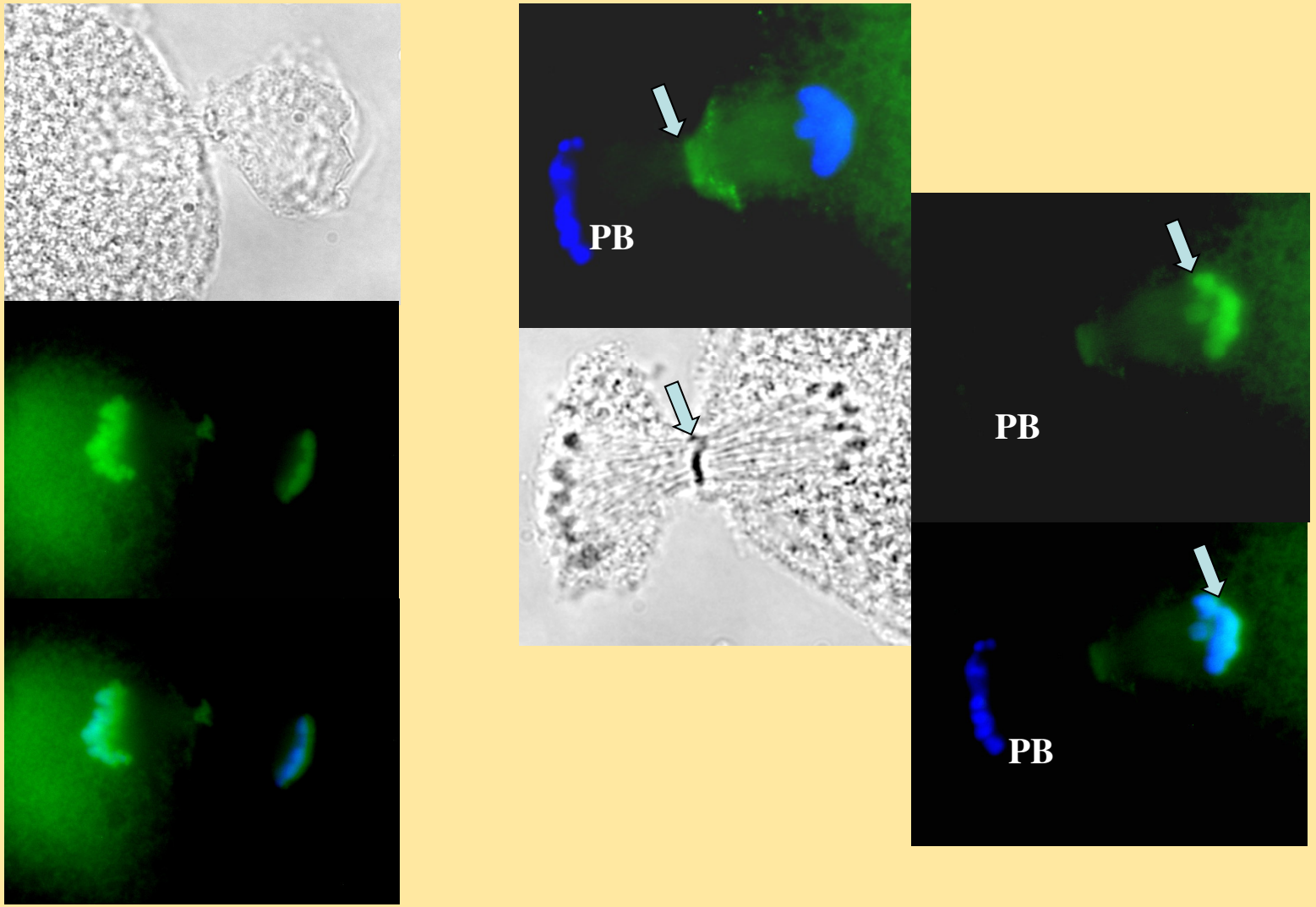
Vogt et al., 2009,
RMB Online
19,352-368; 2009

AURKB is associated with the spindle midzone at anaphase I and telophase I

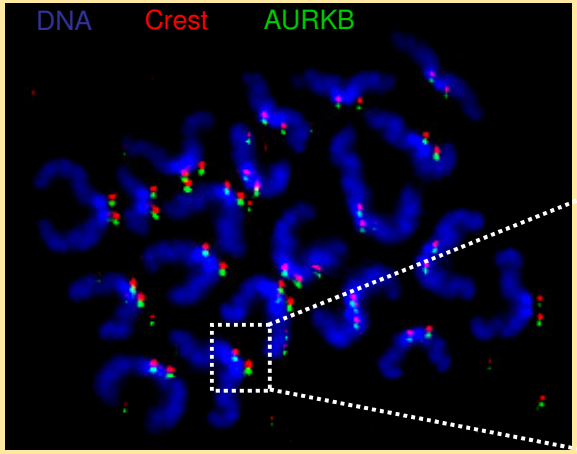


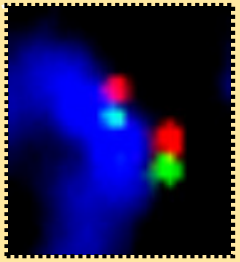


AURKB relocates to metaphase II chromosomes immediately after chromosome separation at the transition to meiosis II

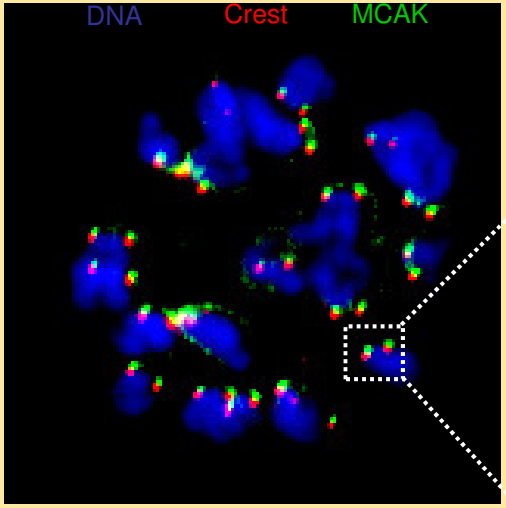


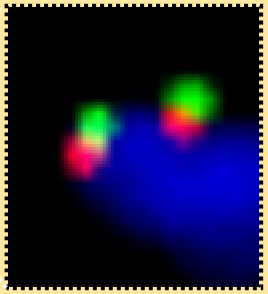
AURKB is enriched at centromeres of metaphase II chromosomes in proximity to MCAK



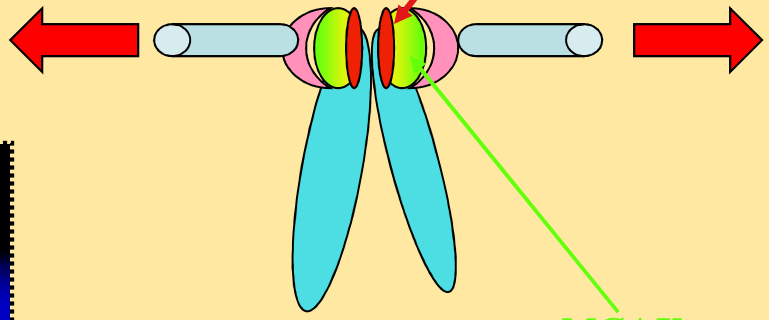


Anti-AURKB
DAPI: Chromosomes
CREST:Centromere protein A/C

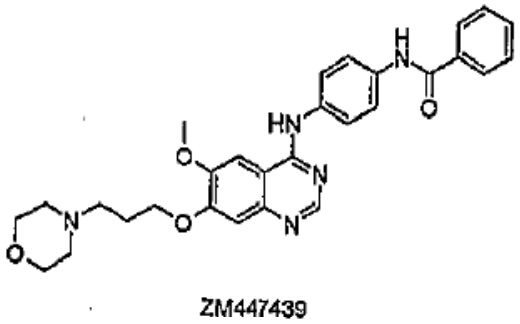




Anti-MCAK

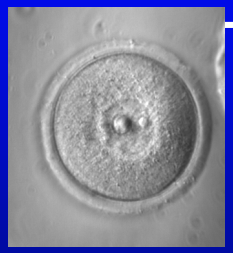


Consequences of Deregulation of AURKB: Effects of Inhibition



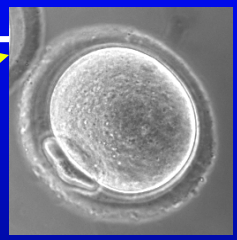
Selective inhibitor of AURKB
(Ditchfield et al., 2003, Keen and Taylor, 2004, Girdler et al., 2006)

GV GVBD MI/AnaI PB/MII

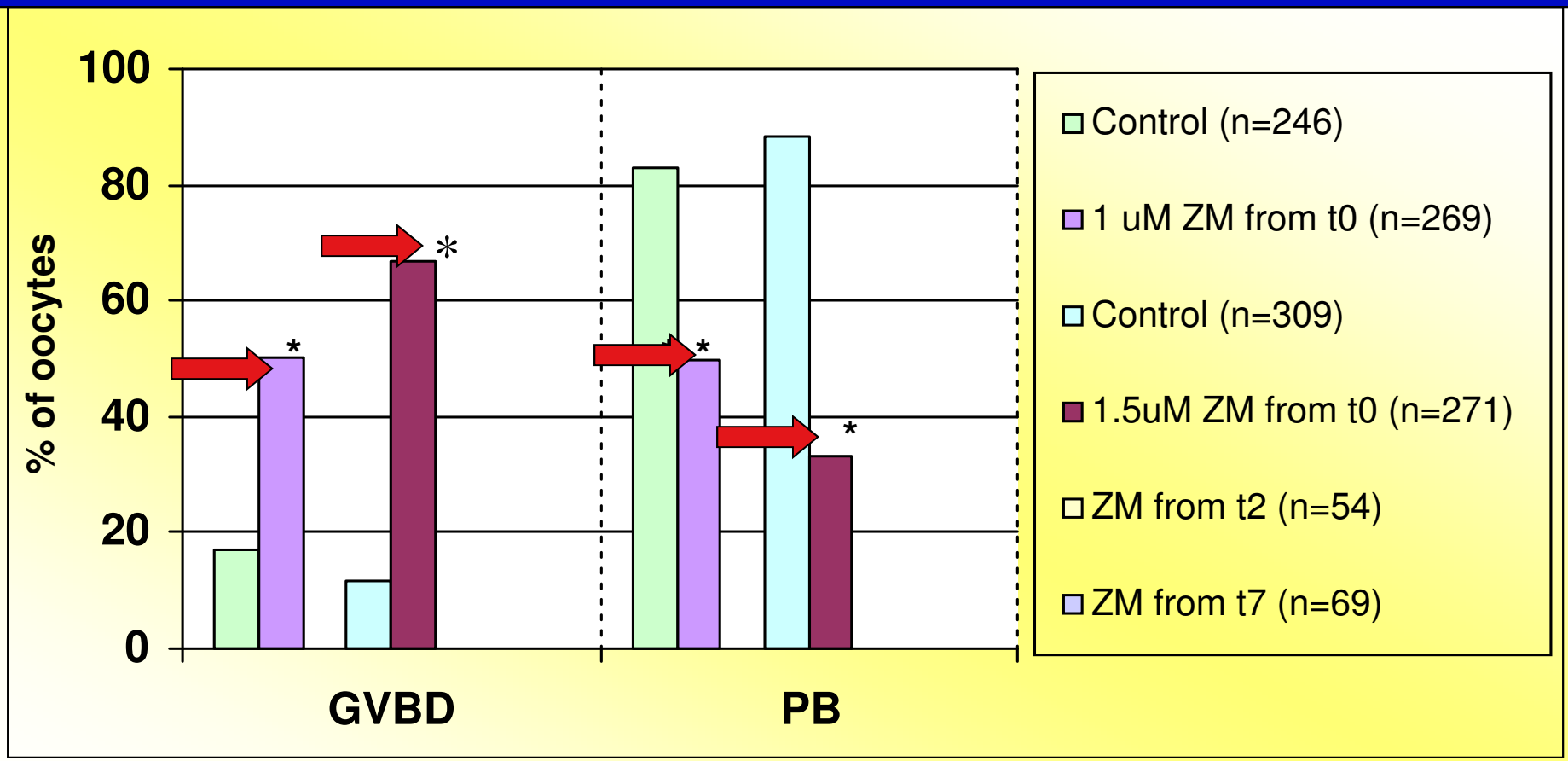


16h

1μM ZM

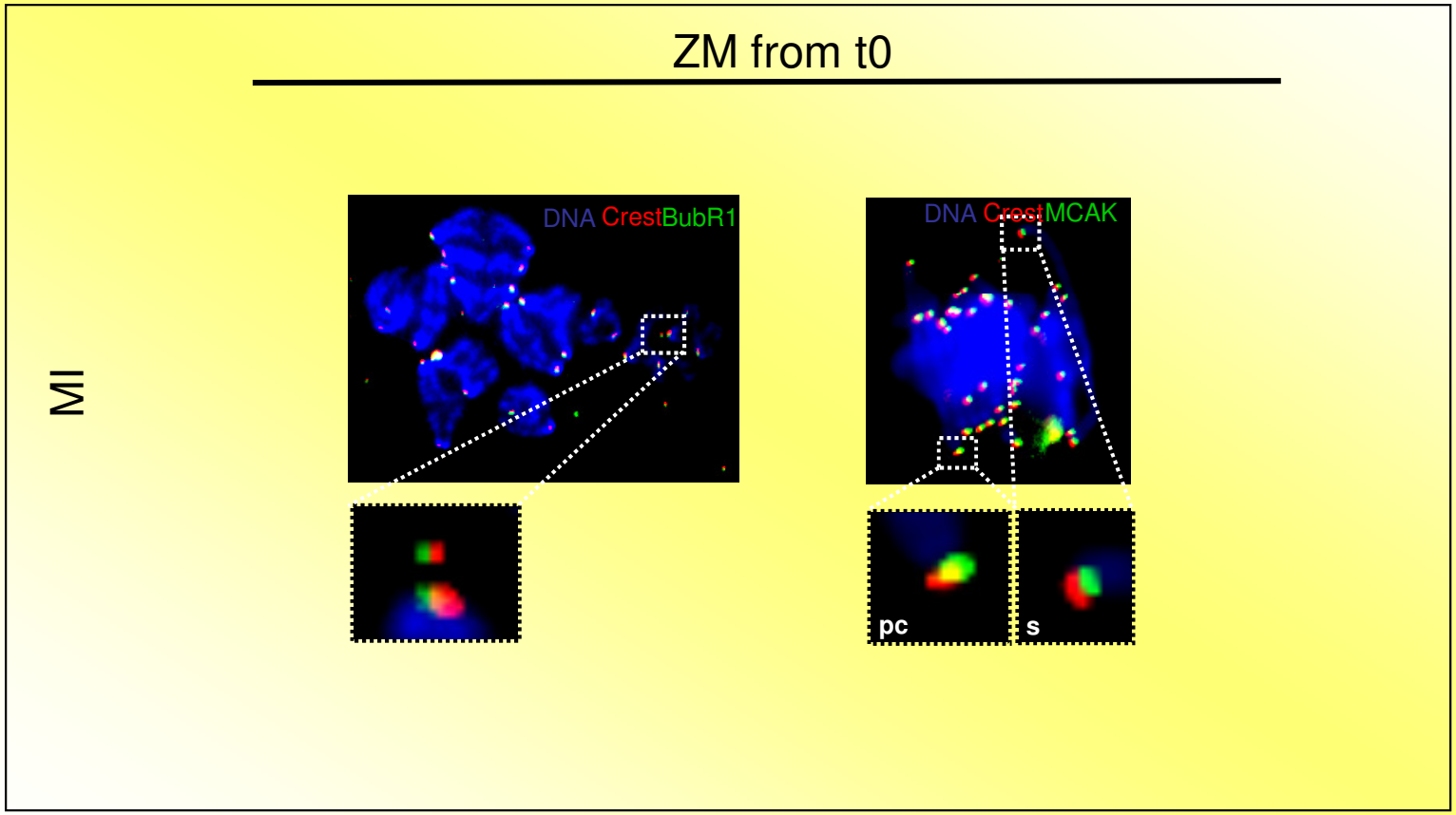


Inhibition of AURKB by ZM causes dose-dependent block in cytokinesis



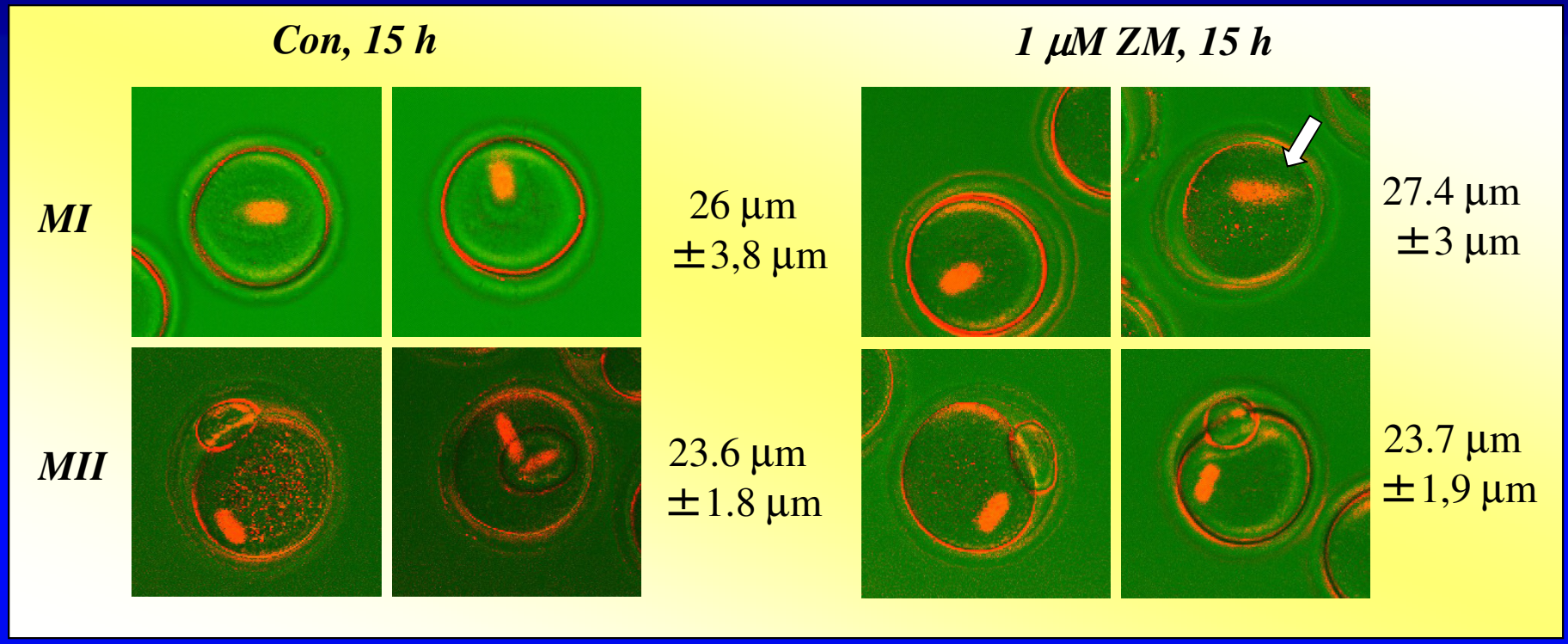
Significant difference to control; *p<0.001

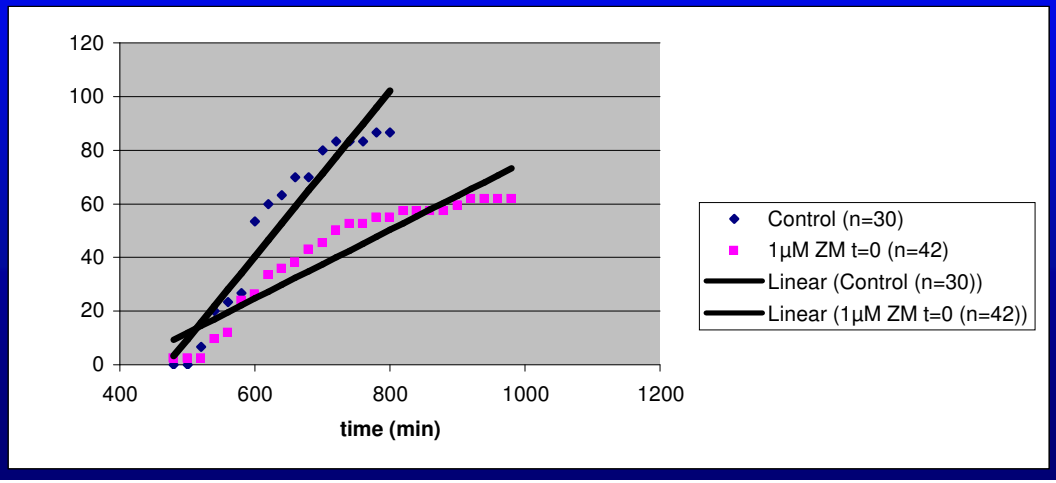
Inhibition of AURKB appears to prolong also the spindle assembly checkpoint (SAC)



Vogt et al., 2009, RMB Online 19,352-368; 2009

Octax polarisation microscopy confirms blocked meiotic progression and presence of aberrant spindles in MI of ZM exposed oocytes





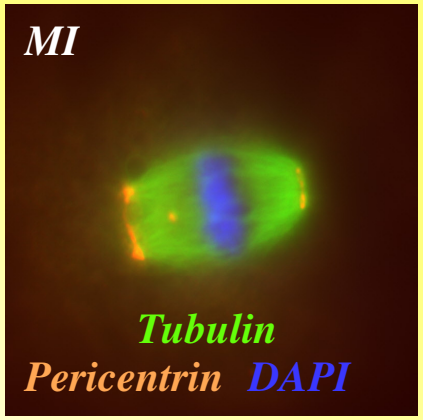
At 12h (720min)

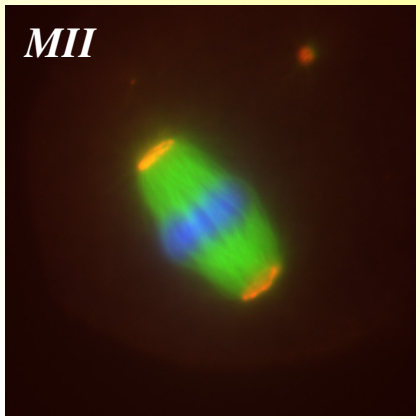
Contr.: 83% PB
ZM:50% PB

Vogt et al., 2009,
RMB Online
19,352-368; 2009

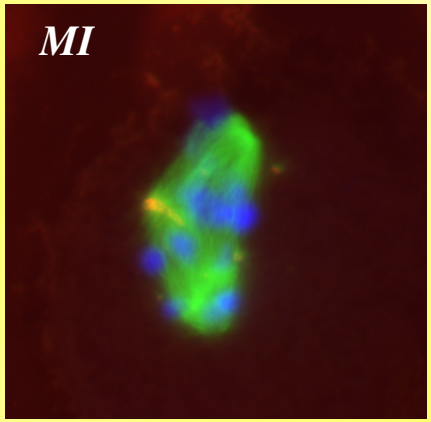
Immunofluorescence detects aberrant spindles and failures in chromosome congression in ZM-exposed oocytes

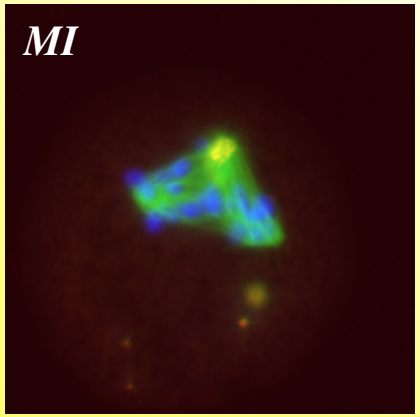
Control

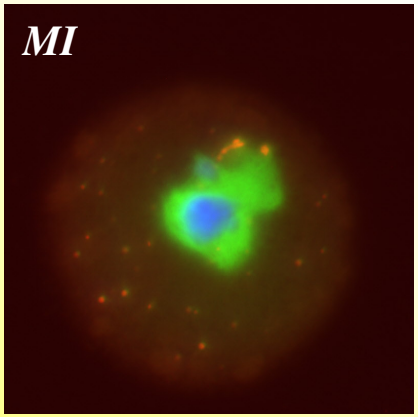


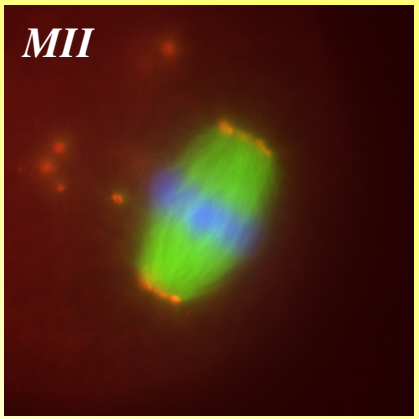


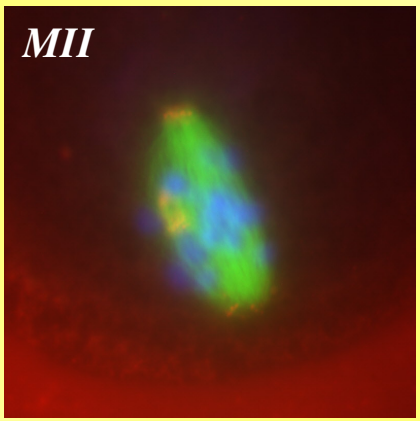
ZM-exposed



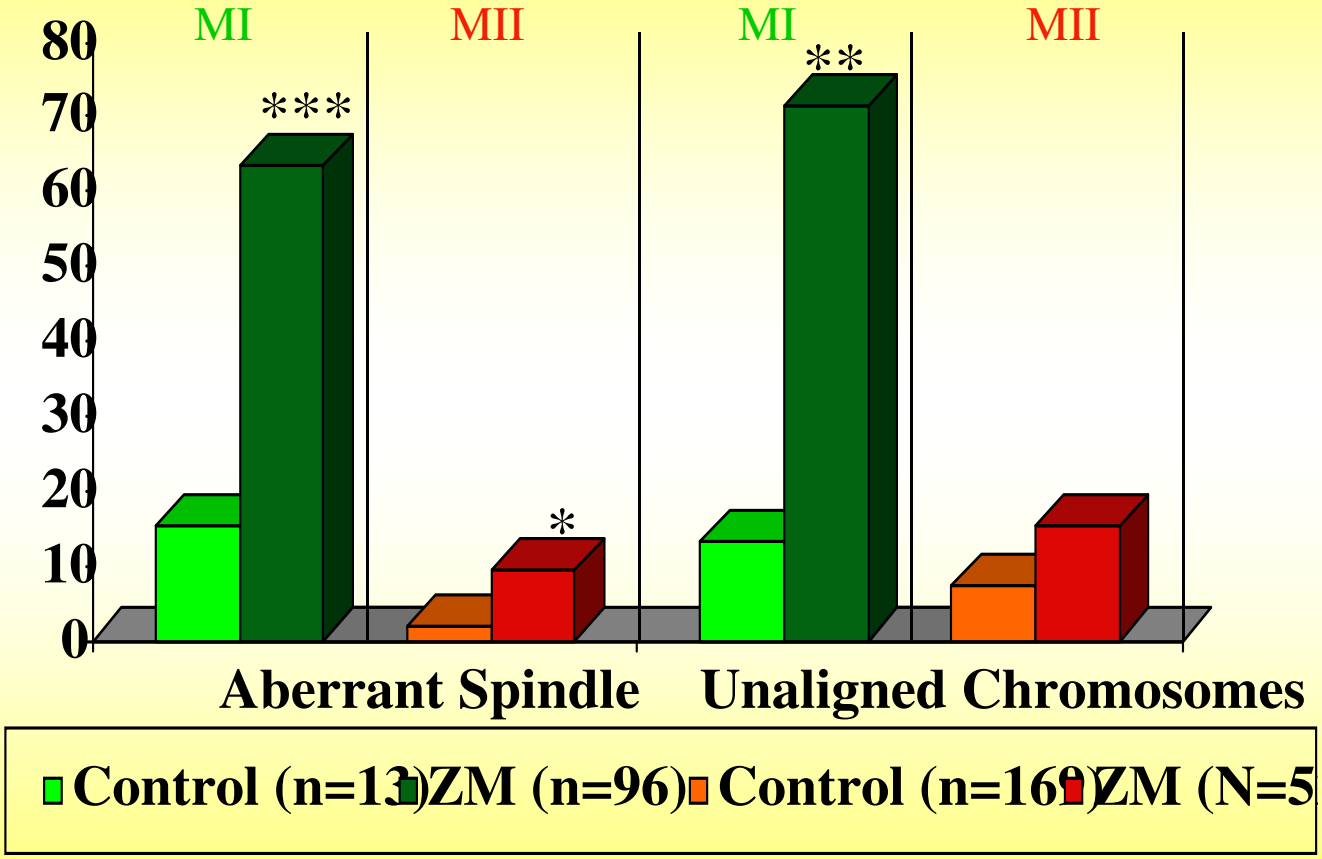








Spindles of ZM exposed MI and MII oocytes are aberrant and chromosomes fail to align at spindle equator



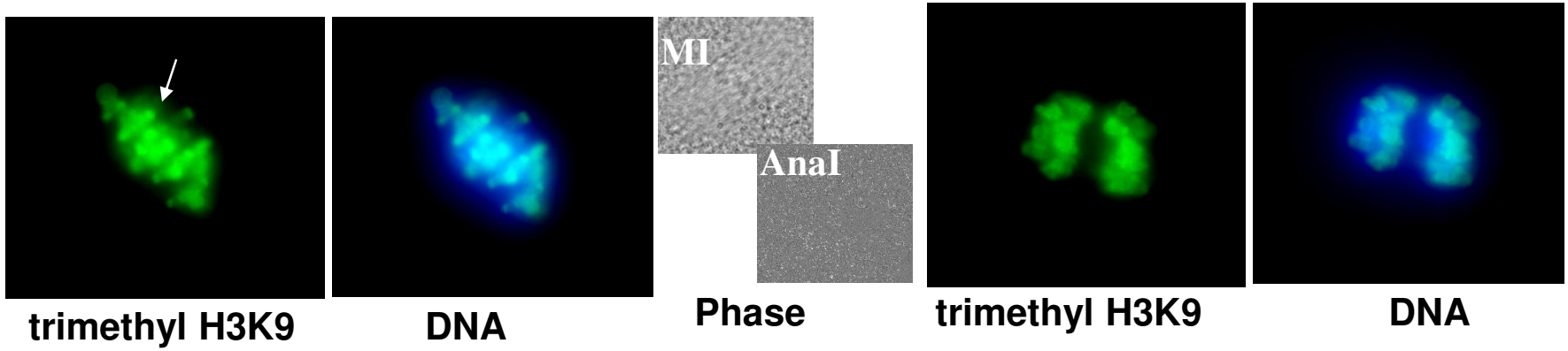
Significant difference to control, *p<0,05; **p<0.005; ***p<0.001

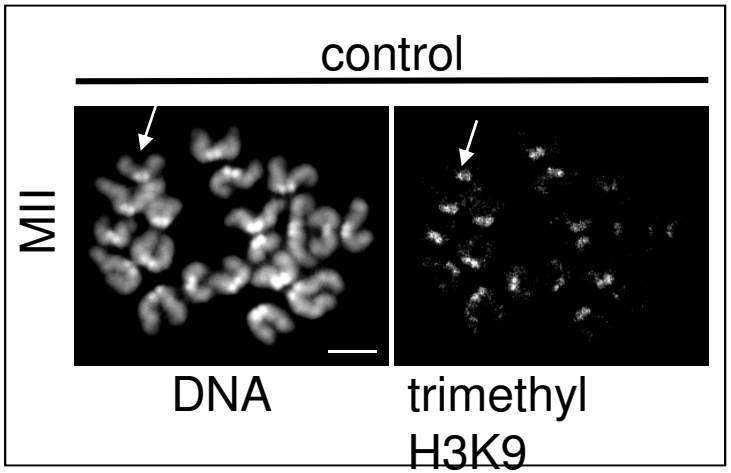
Congression failure by ZM inhibitor is overcome by Aurora kinase B overexpression (not A or C) and it appears therefore to be the primary kinase in regulating chromosome dynamics (congression) during meiosis in oocytes (Shuda et al., 2009)

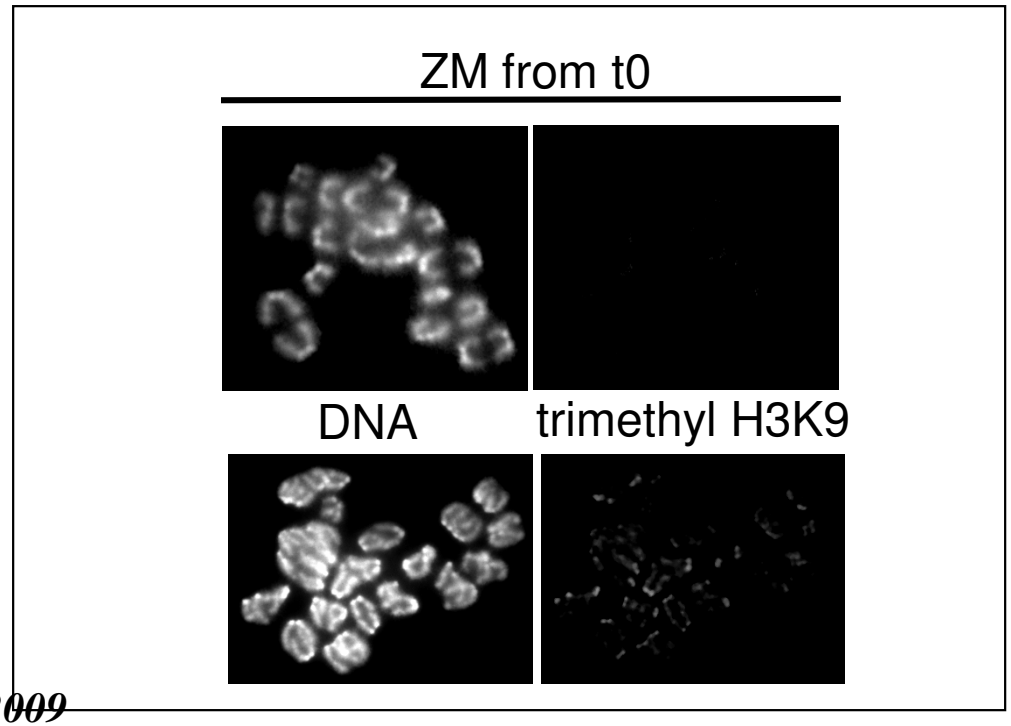
Aurora kinase B phosphorylates histones e.g. serine 10 in histone H3 (Swain et al., 2008):
Other posttranslational modifications may also be affected:
Methylation status H3 lysine 9 tri-methylation (Vogt et al., 2009)

Changes in epigenetic state of centromeric heterochromatin!

Altered histone modifications at centromeric heterochromatin of meiotic chromosomes apart from phosphorylation: reduced/no lysine 9 tri- methylation of histone H3 by ZM4474439 inhibition of AURKB!

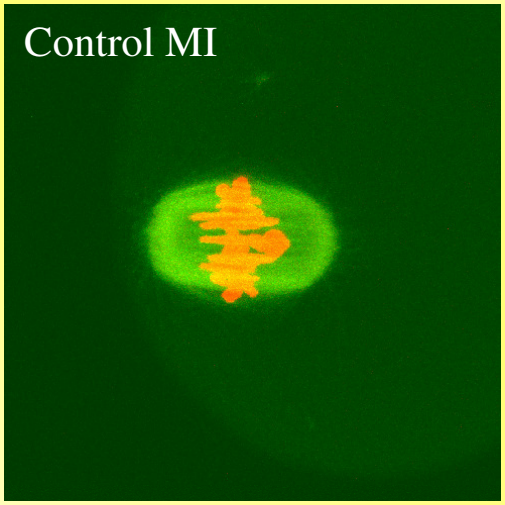


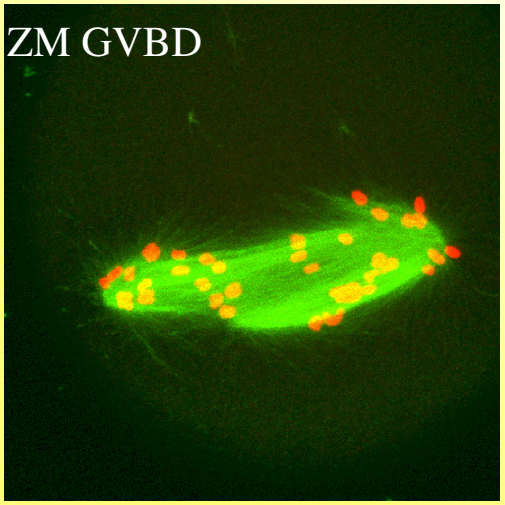


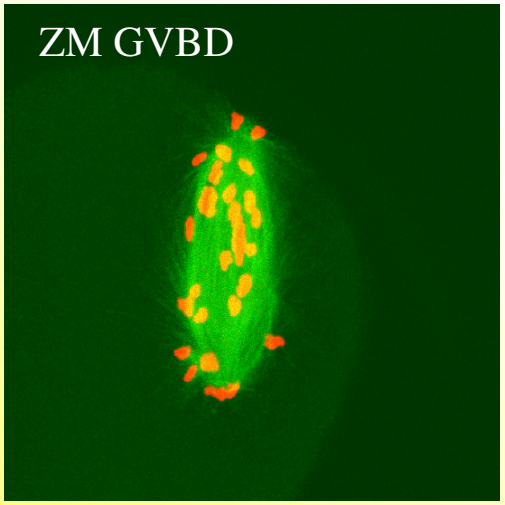


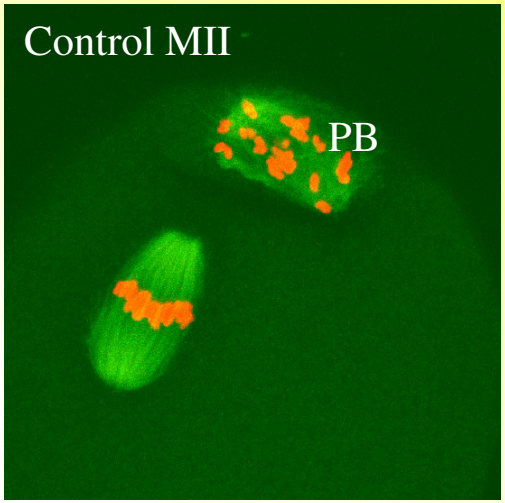
Vogt et al., 2009, RMB Online 19,352-368; 2009

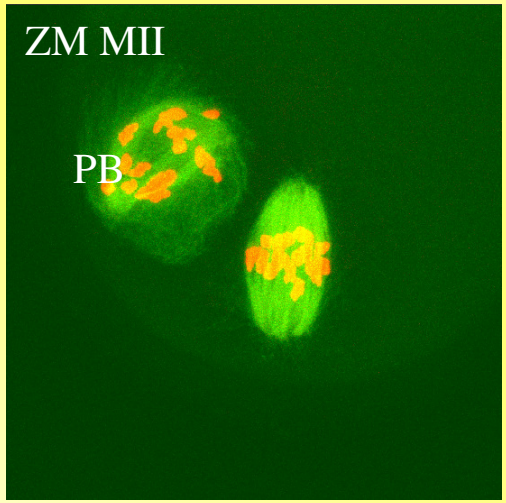
Meiosis I-arrested oocytes frequently possess more than 20 bivalent chromosomes





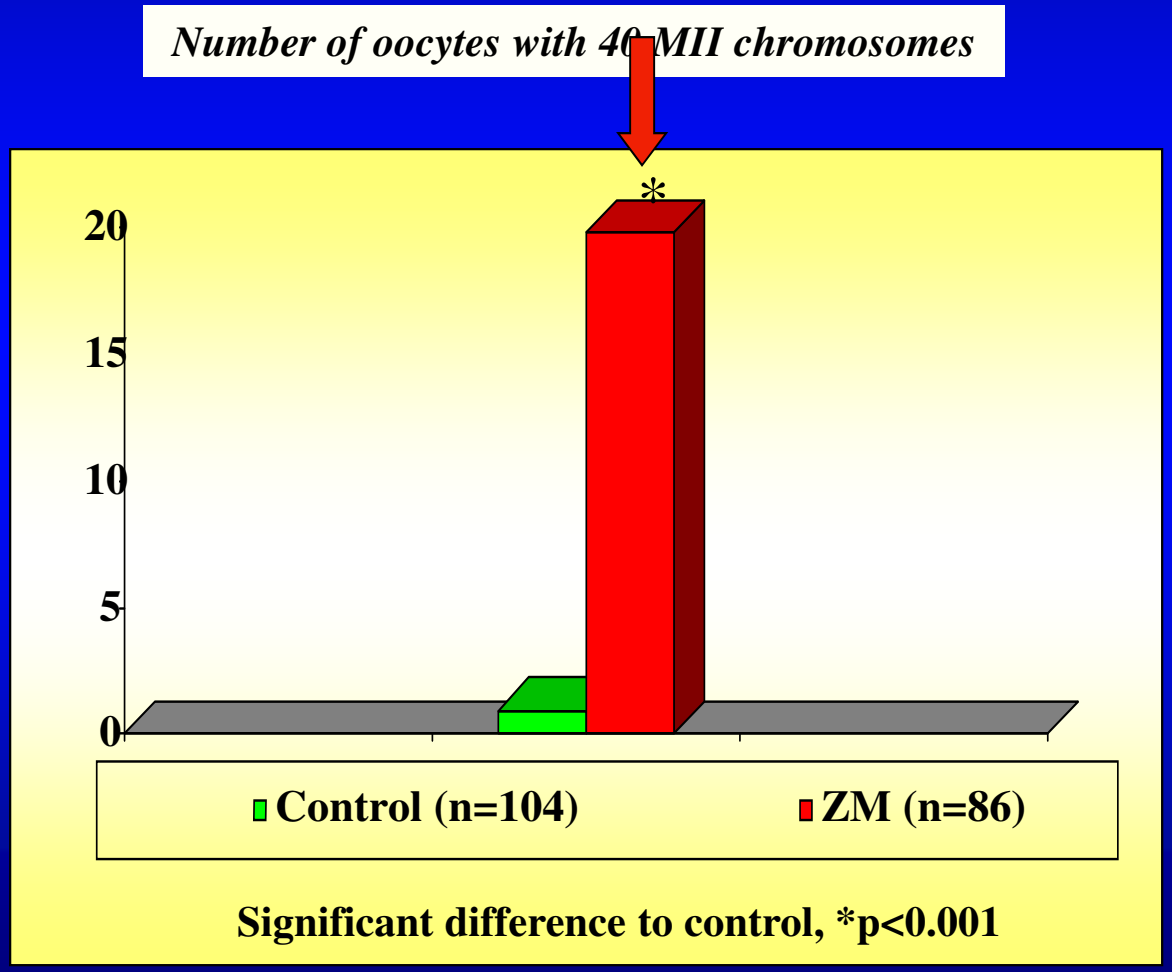


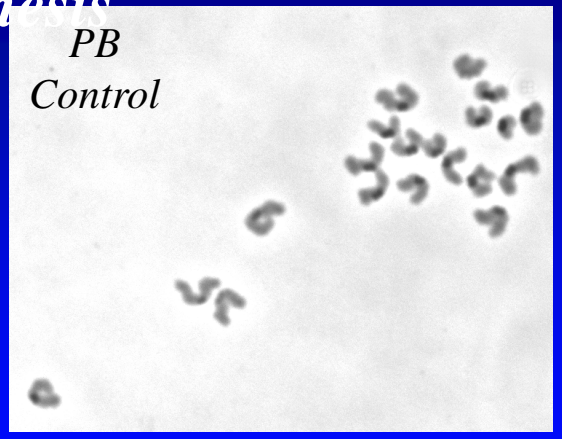


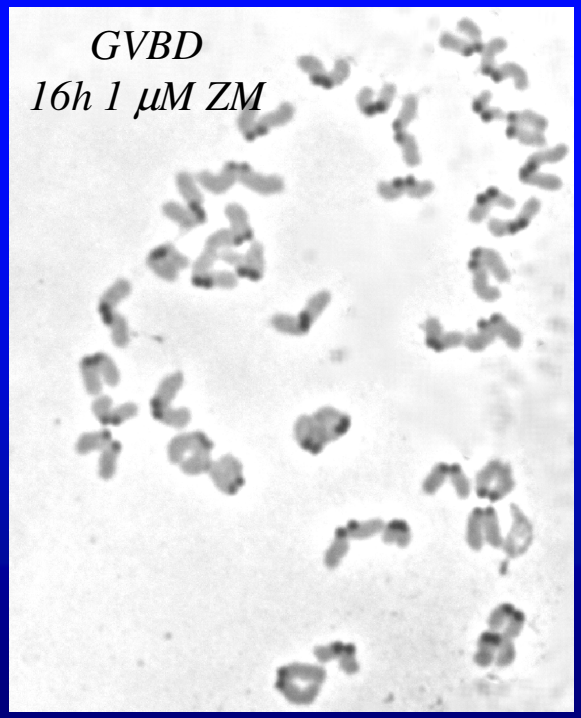


Vogt et al., 2009, RMB Online 19,352-368; 2009

Meiosis I-arrest is leaky and some oocytes separate chromosomes without cytokinesis

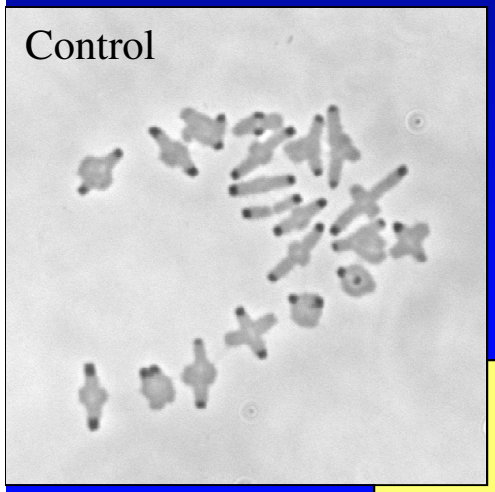


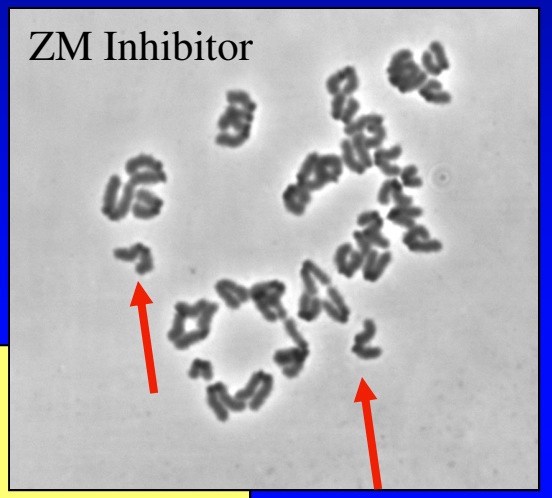


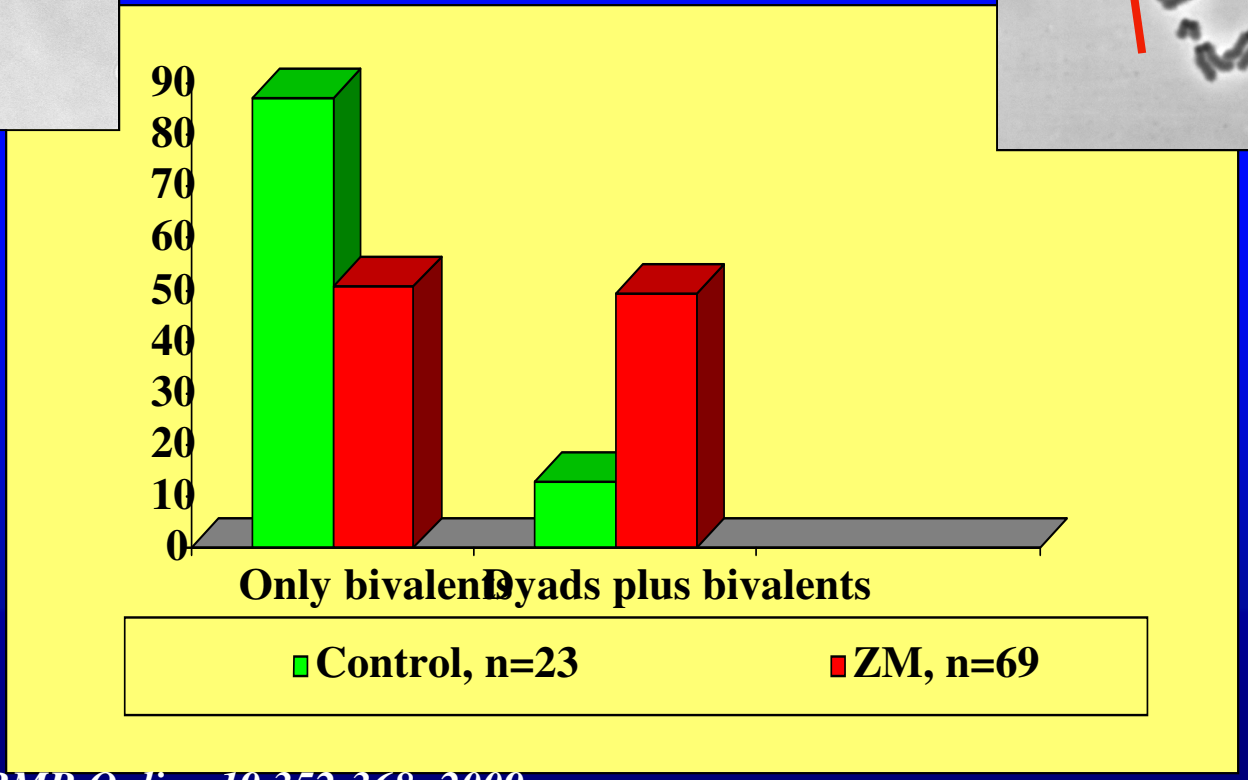


Vogt et al., 2009, RMB Online 19,352-368; 2009

Homologes cannot separate properly and sister chromatid cohesion appears sequentially rather than instantaneously released in oocytes with inactive AURKB







Vogt et al., 2009, RMB Online 19,352-368; 2009

Aurora kinase B is involved in chromosome congression, cytokinesis, chromatin conformation and the regulation of loss of chromatid cohesion and chiasma resolution

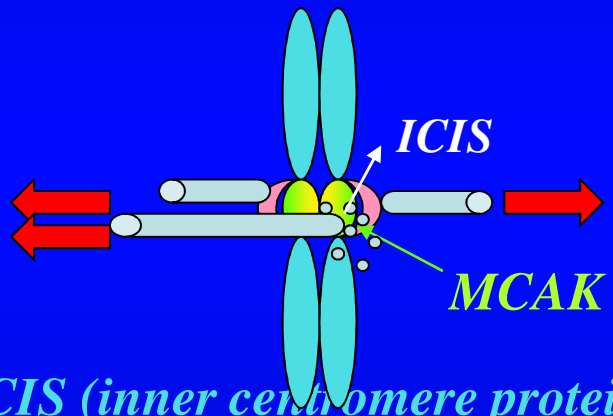
Modelling altered expression in aged oocytes III:

Role of MCAK in oogenesis

MCAK (mitosis centromere associated kinesin) (Kif2C in mouse)
Member of kinesin 13 family with centrally located motor domain

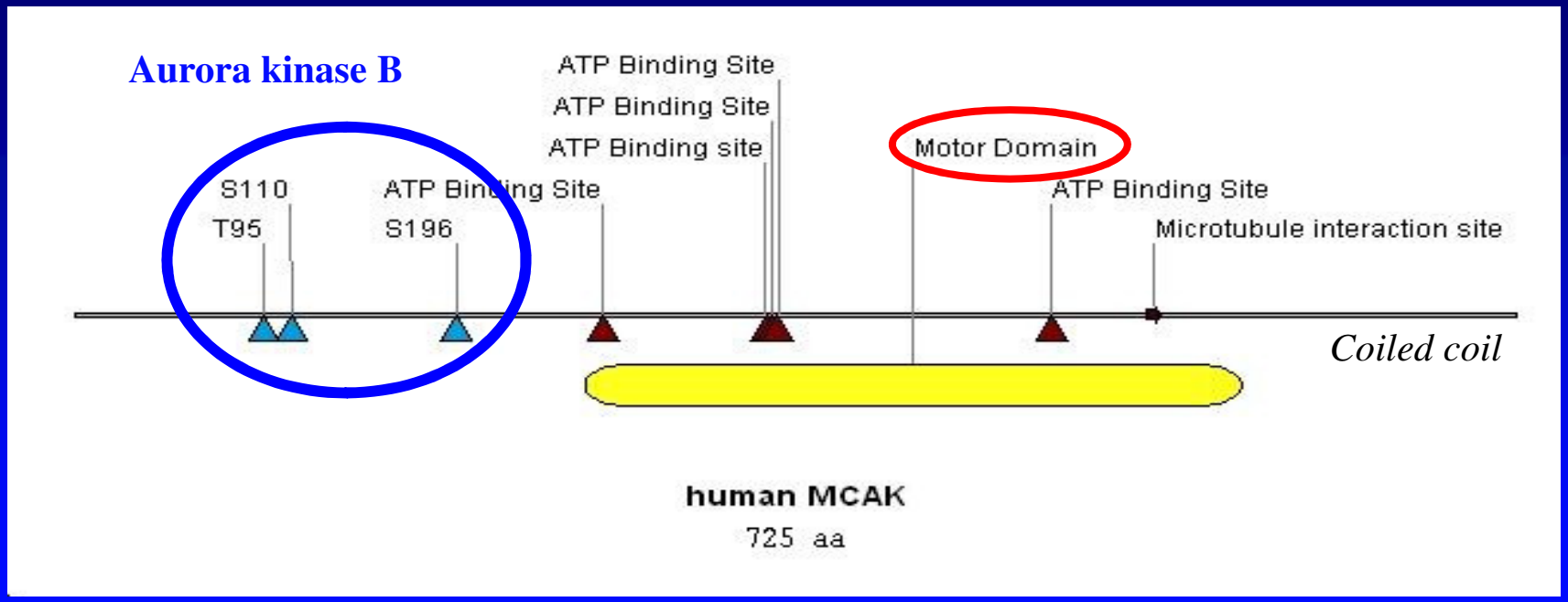
Homodimer of 81.3 kDa (human)

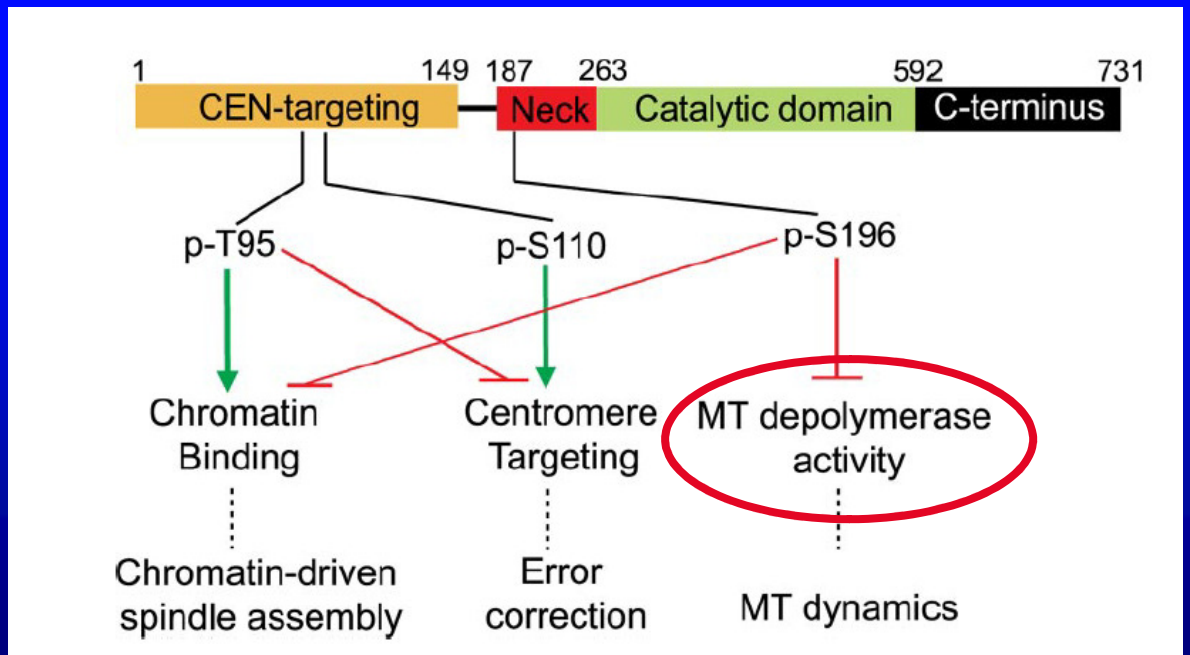
MCAK is implicated in correction of wrong (merotelic) attachment of microtubules to kinetochores (Andrews et al. 2004 Dev Cell)

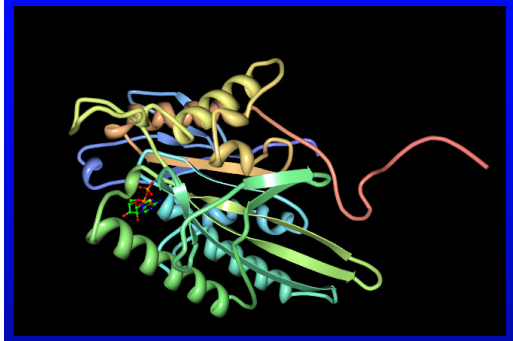


Regulation by ICIS (inner centromere protein,; Ohi et al. 2003)

By phosphorylation by Aurora kinase A (prophase association with centrosomes; Zhang et al., 2008)
By Aurora kinase B (chromatin and centromere targetting and depolymerase activity; Zhang et al., 2007)

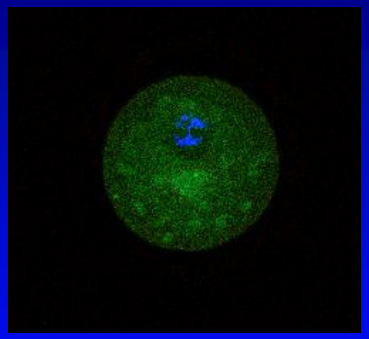




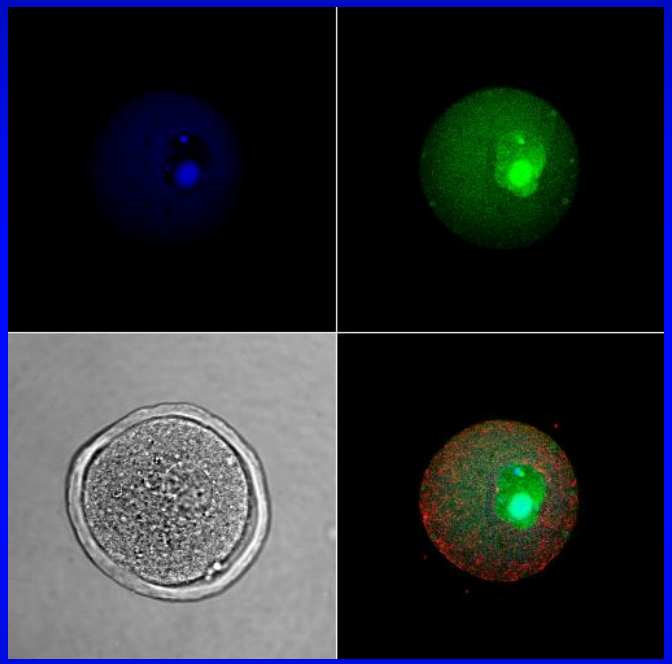


Zhang et al., 2007 Mol Biol Cell 18, 364ff

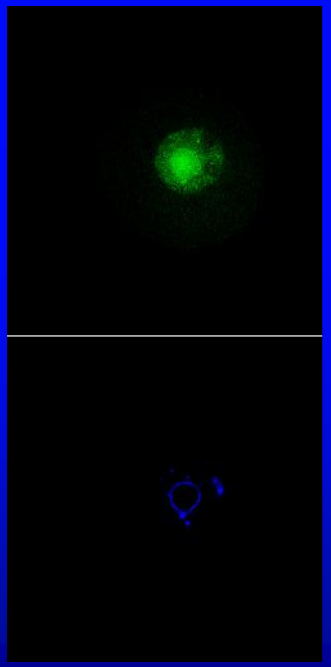
Nuclear MCAK may be marker of oocyte maturity/developmental potential



NSN

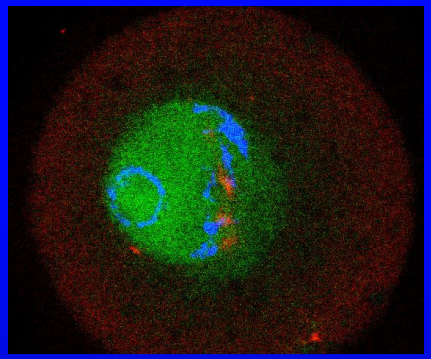


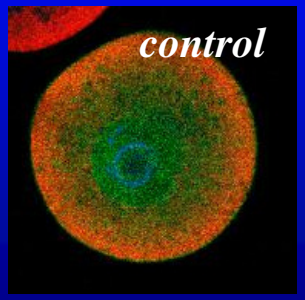
Transition to SN



SN

SN





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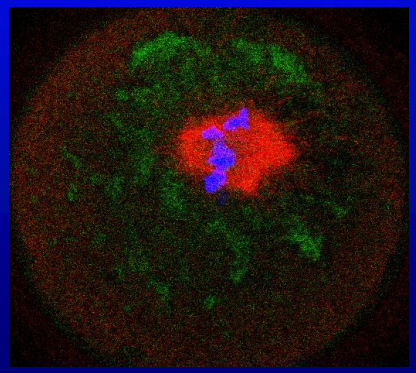
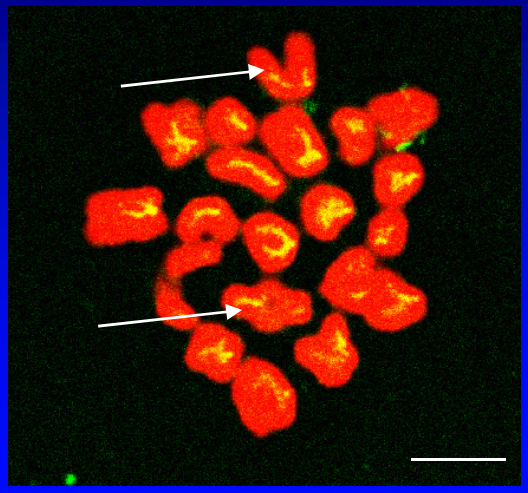
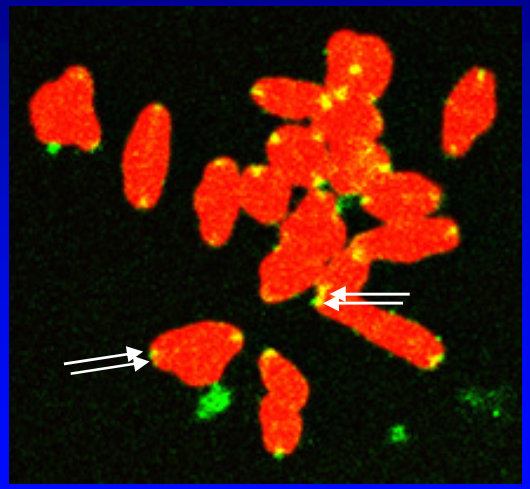


GVBD

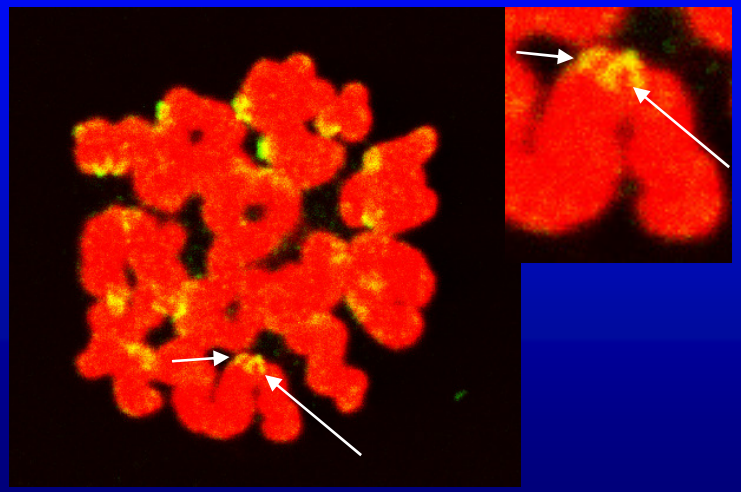
MCAK is recruited from chromatid arms to centromeres during prometaphase I at meiotic maturation: role in chromosome cohesion?



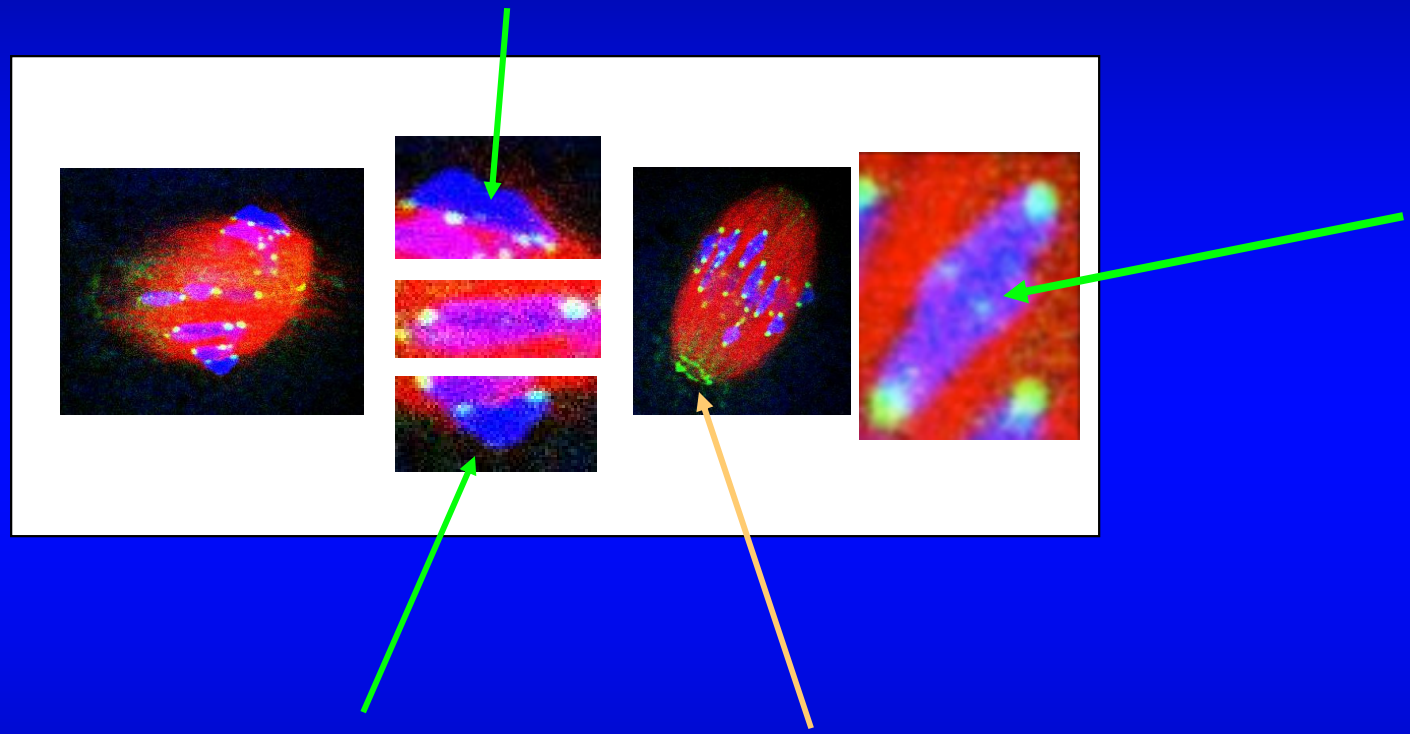
MCAK at chromatid arms after GVBD



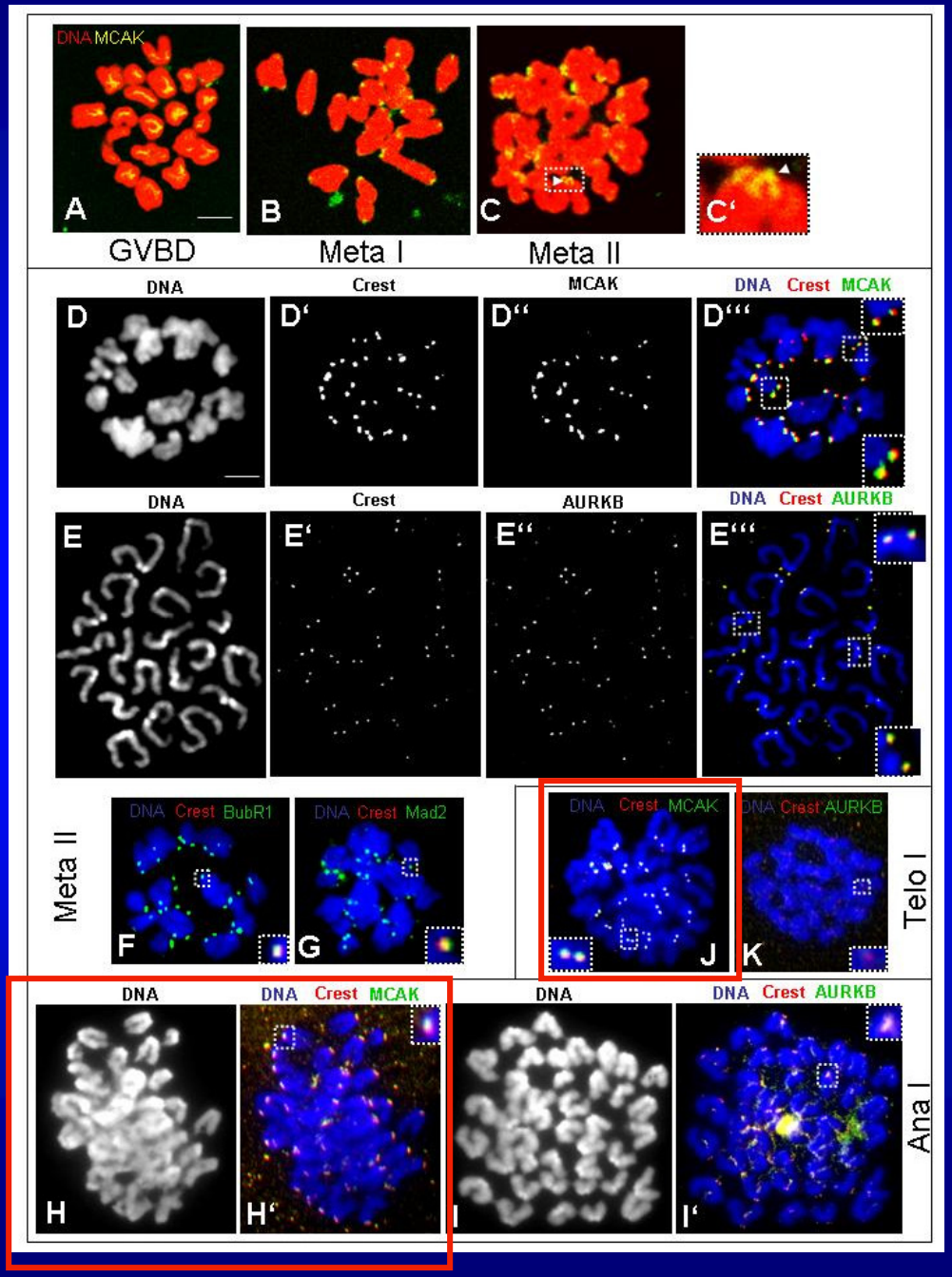
MCAK at centromeres of both sister chromatids at metaphase I



MCAK is present at spindle poles/centrosomes (focusing?) and at sites of exchanges/chiasmata on bivalents at prometaphase I (role in chiasma resolution?)

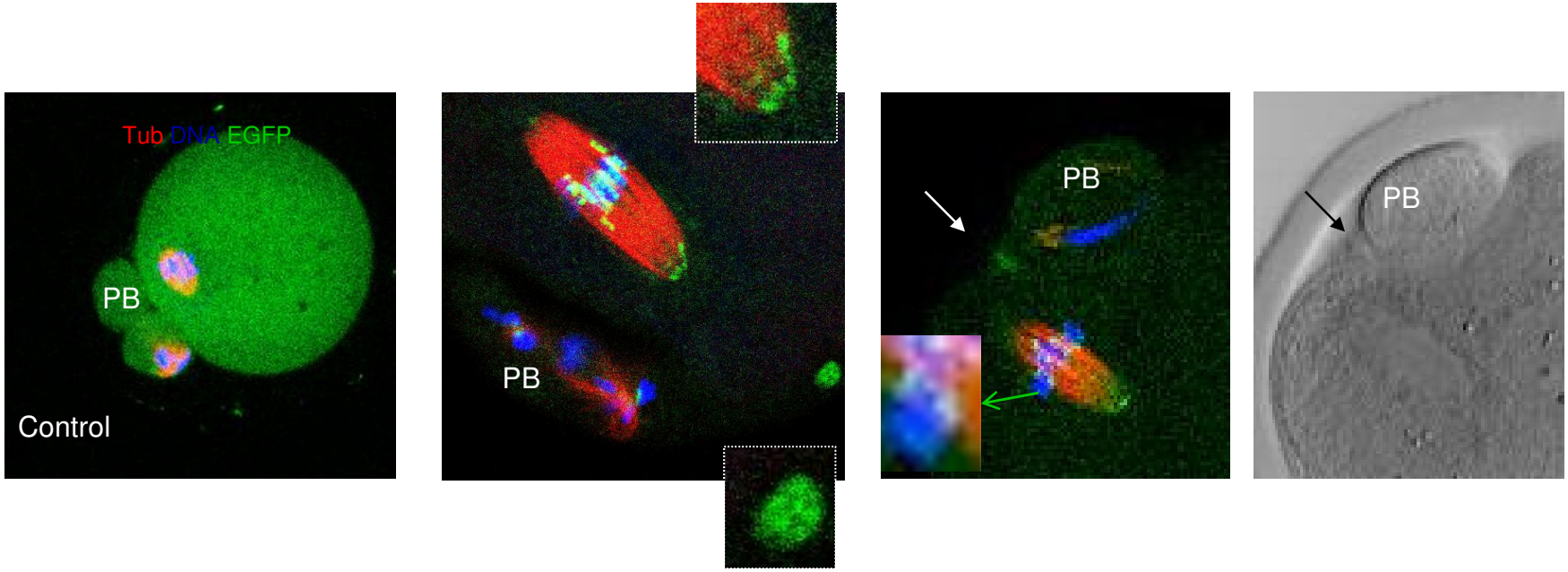


MCAK is present at centromeres up to telophase I unlike in spermatocytes

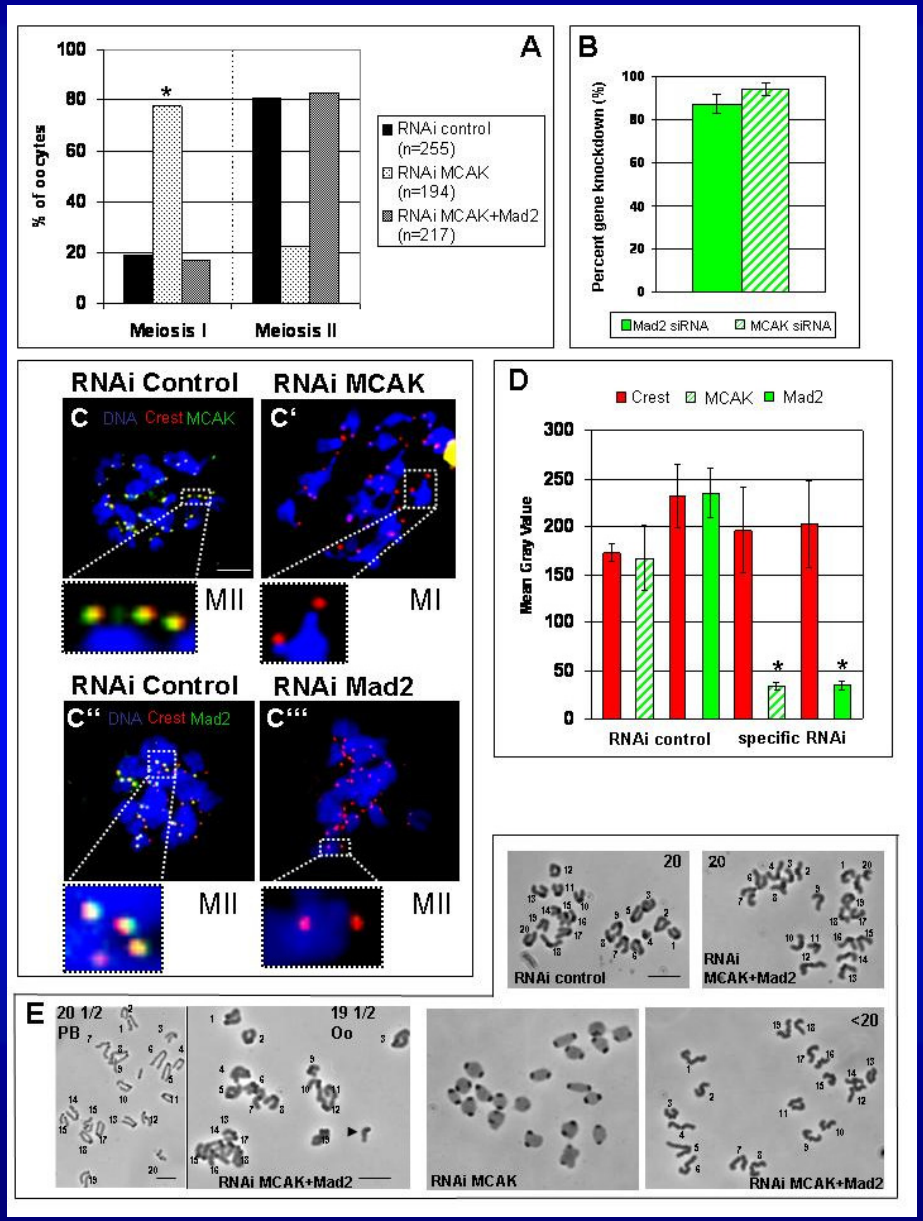


MCAK is associated with centromeres of metaphase II chromosomes but is also present at the cytokinesis furrow, in contrast to cytoplasmic EGFP protein in the control:

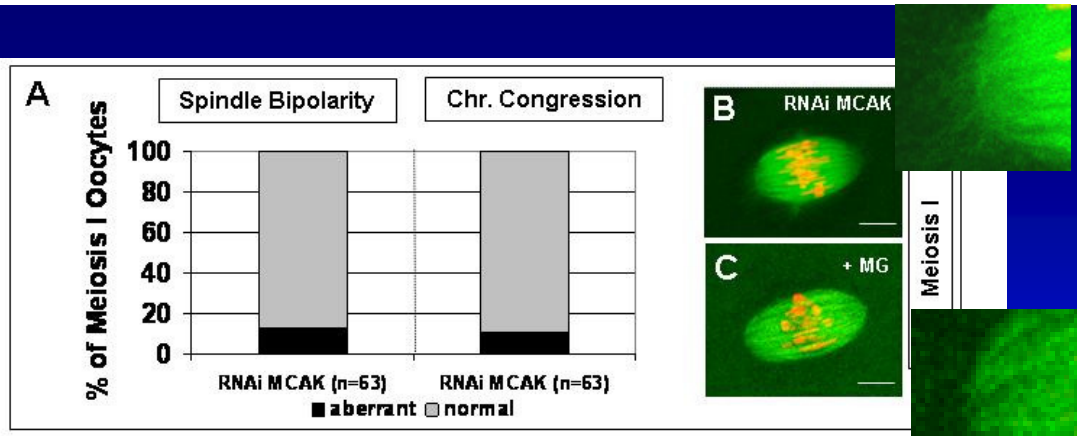
Role in depolymerisation of microtubules of the midbody or preventing lagging?

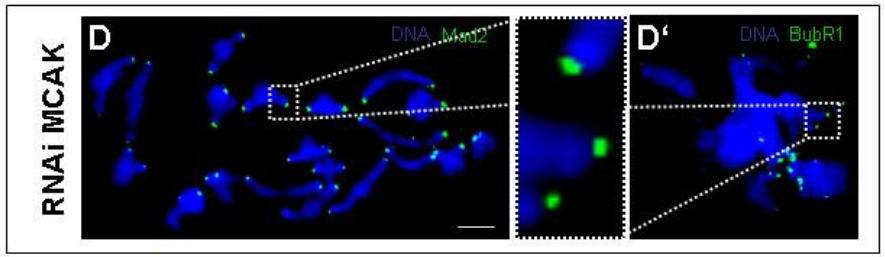


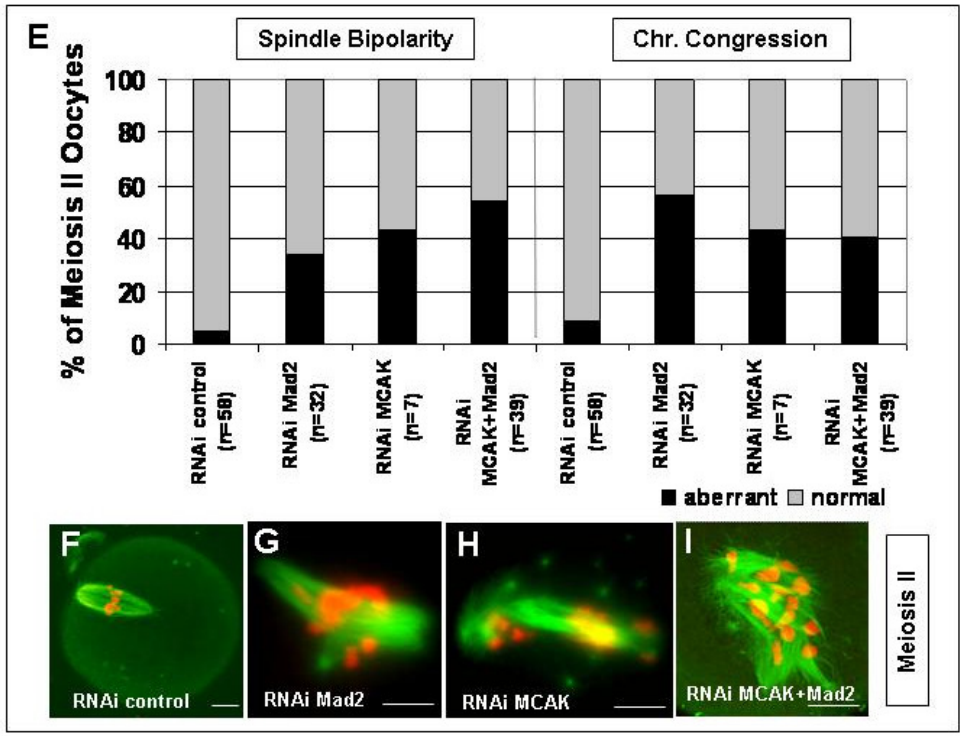
Knockdown of MCAK induces meiotic arrest that can be overcome by knockdown of Mad2 checkpoint expression: necessary to silence the SAC



MCAK mRNA and protein is significantly reduced by injection of specific RNAi



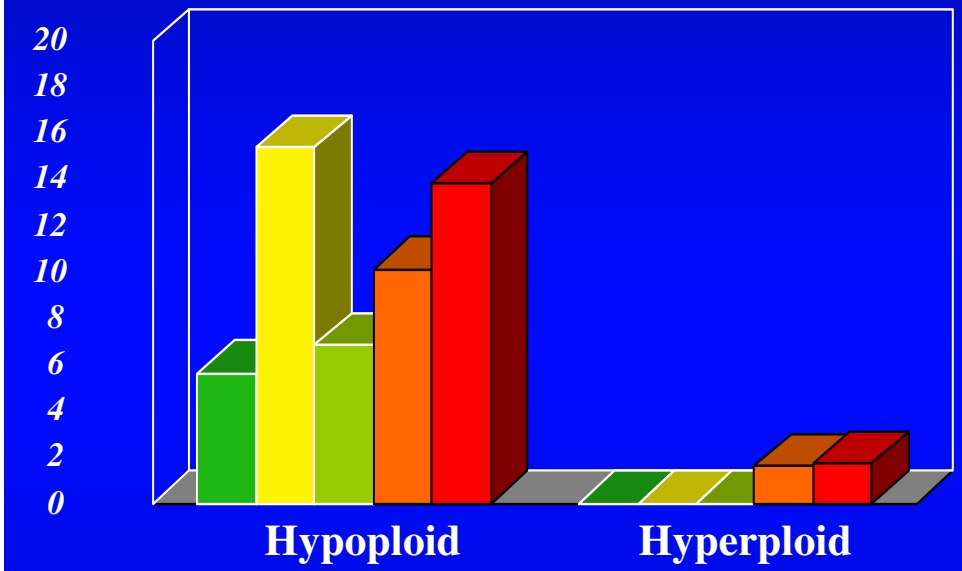




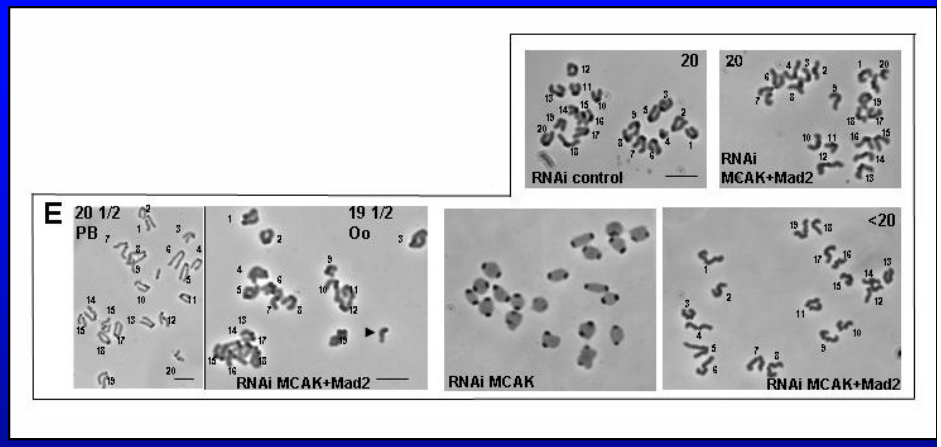
Prolonged culture (16h) results in failures of pole focussing while chromosomes become aligned.

Double knockdown of MCAK and Mad2 results in severe spindle aberrations whereas chromosome congression failure at MII is not increased over MAD2 RNAi

Knockdown of MCAK and Mad2 induces an increase in hypoploidy but not in hyperploidy of MII oocytes



- Control, n = 126
- 1.5uM ZM from 7h, n = 104
- Control, unspec RNAi, n = 59
- RNAi Mad2, n = 129
- RNAi MCAK & Mad2, n = 58



Role of MCAK in resolution of merotelic attachments of lagging chromosomes at anaphase/telophase I of oogenesis?

In aged oocytes multiple factors are deregulated: Expression AURKB⇑, MCAK ⇓and mad2/bubR1⇓(checkpoint components)

Multiple changes may synergistically increase risk for predisposition to first and second meiotic errors and loss of cell cycle control!

Genetics and Molecular Biology:

What is the molecular basis of activities of AURKB and MCAK?

What can be seen at the protein level?

Embryologist and Clinican:

What is at the basis of the changes in expression?

Can we supplement aged oocytes to have normal maturation and gene expression?


Edgar Vogt


Alexandra Kipp


Ilse Betzendahl


Mouhrad Sanhaji


Wolgang Klein

Gene Technology/Microbiology
University of Bielefeld
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Linda Wordemann
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University of Washington School of Medicine
Seattle

Stephen Taylor
School of Biological Sciences
University of Manchester

MTG, Altendorf: Octax Eyewear™ MX

MCAK is present in unique pericentromeric ring on meiotic chromosomes in spermatocytes

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A Perikinetochoric Ring Defined by MCAK and Aurora-B as a Novel Centromere Domain

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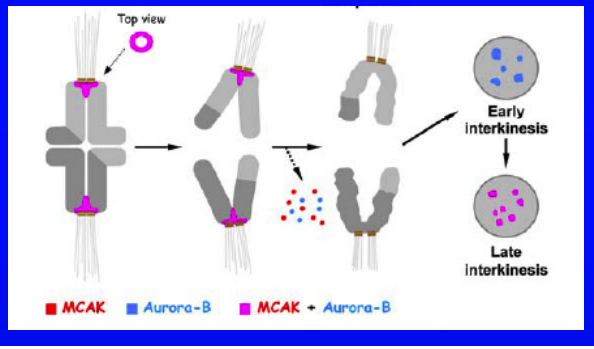
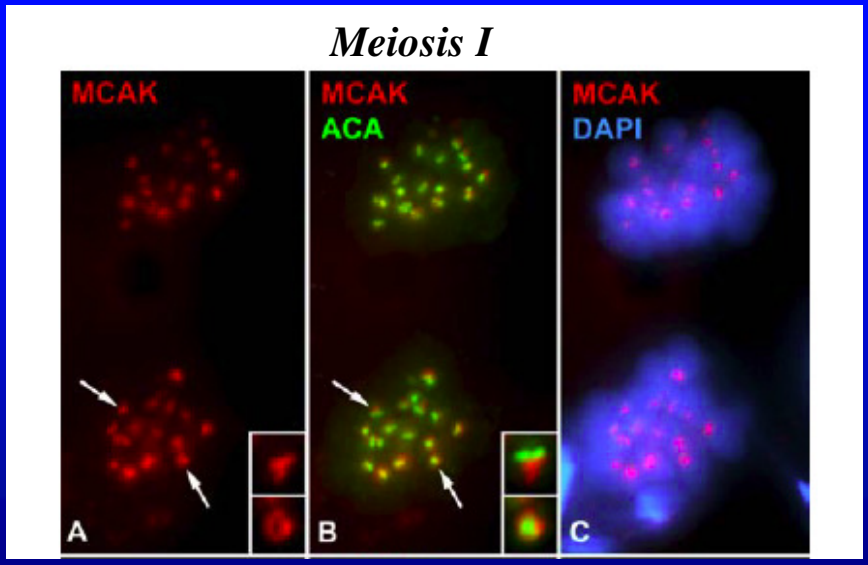
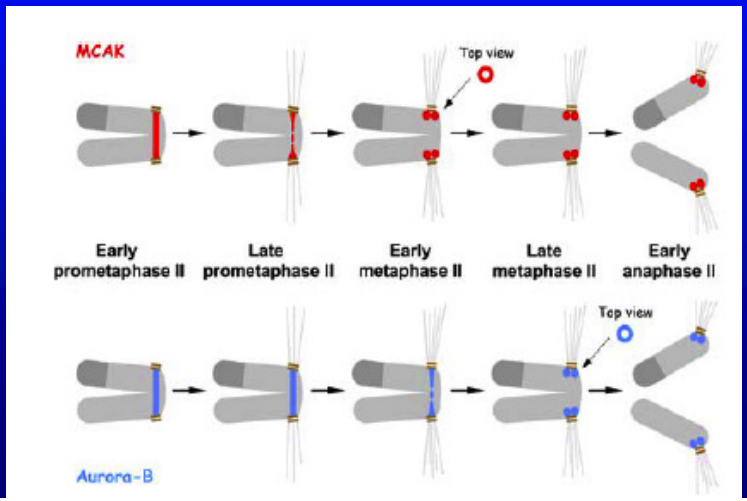
1 Departamento de Biología, Universidad Autónoma de Madrid, Madrid, Spain 2 Department of Physiology and Biophysics, School of Medicine, University of Washington, Seattle, Washington, United States of America



Meiosis I

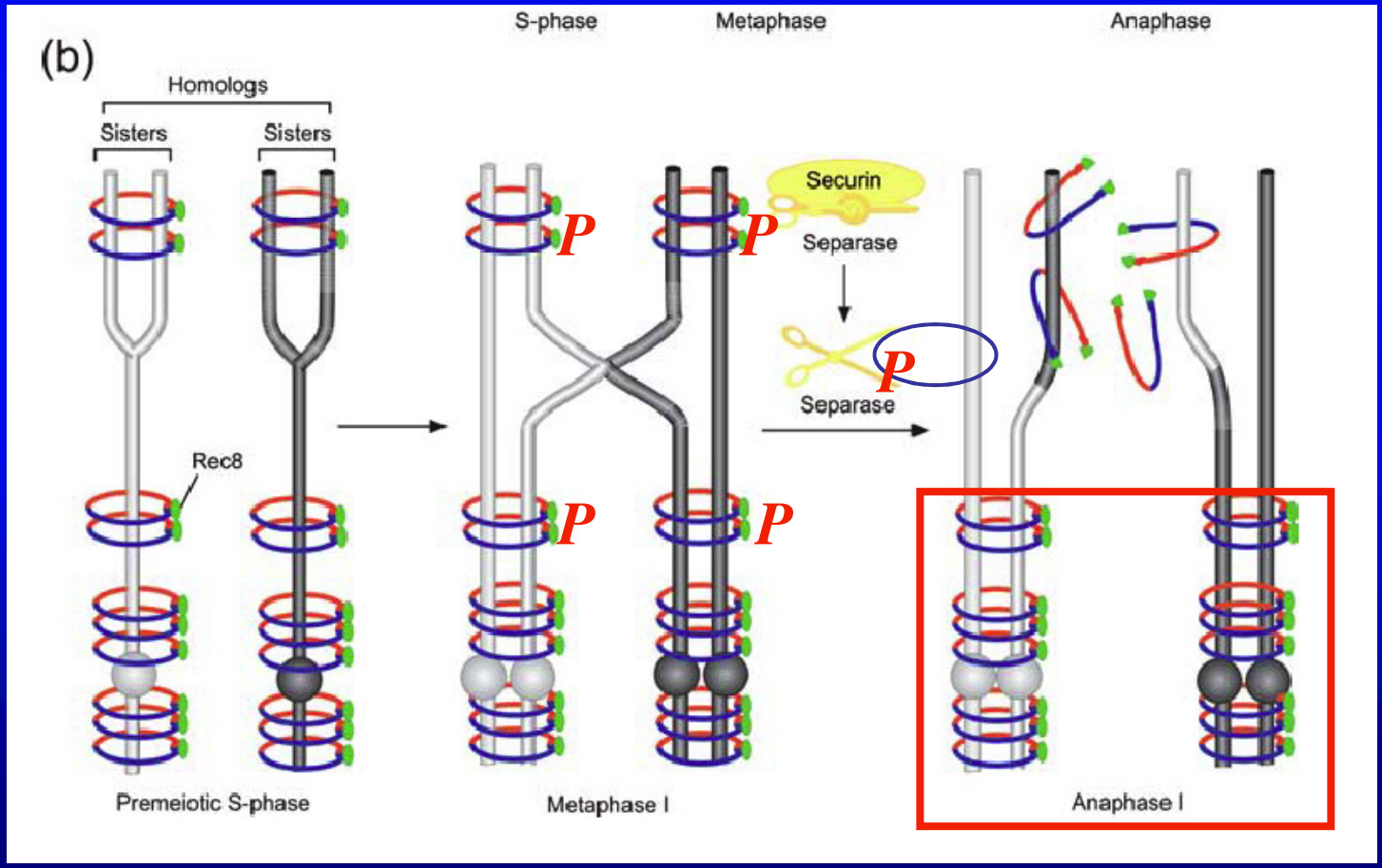




Para et al., PLOS Genet 2006 and 2009

Current working hypothesis:

Aurora kinase B influences phosphorylation of Rec8 cohesin protein at chromosome arms targetting the cohesin for proteolysis by separase (similar to C. elegans AIR-2;Ksitna et al., 2002; Rogers et al., 2002)



Nasmyth & Haering, Ann.Rev.Biochem. 75,595-648 (2005)