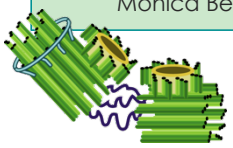
INSTITUTO GULBENKIAN de CIÊNCIA

Perspectives on Cell Cycle; Centrosome & Cilia Biogenesis & Function

Mónica Bettencourt-Dias



1997-2001-PhD- Cell Biology of Heart Regeneration, Prof. J Brockes, University College London, UK

2002-2006-Post-doctoral research- Regulation of Cell Proliferation, Prof. D Glover, University of Cambridge, UK + Diploma Sci. Com. Birkbeck College, UK

Oct-2006-Principal Investigator- Regulation of Cell Proliferation & Centrosome Assembly, Instituto Gulbenkian de Ciência, Portugal

- Perspectives on the Cell Cycle
- Importance of centrioles: their functions
- Centrioles & Cancer
- Centriole Biogenesis & Number Control

- **Perspectives on the Cell Cycle**
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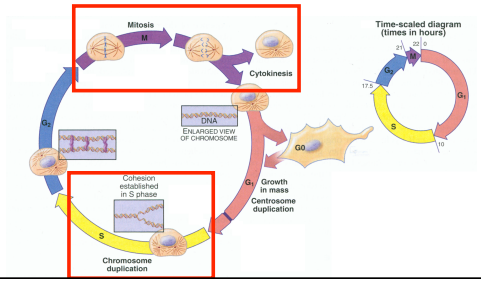
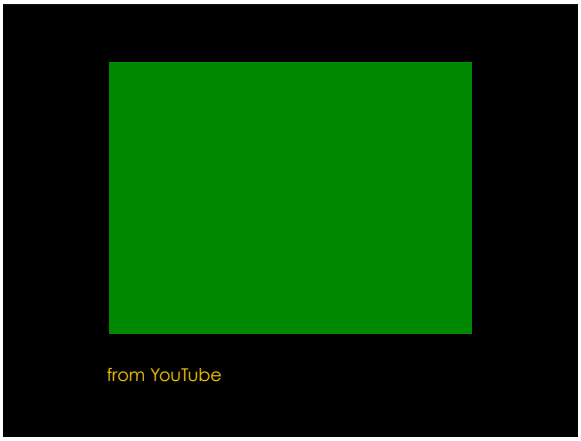
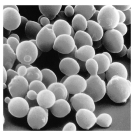
Cell Cycle

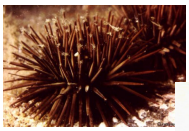
-Cells must replicate their DNA (& organelles)- S phase
-Cells must segregate the DNA equally to the progeny cells in mitosis (& organelles)- Mitosis



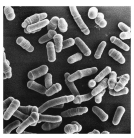


Experimental Systems used in Cell Cycle Research


Saccharomyces cerevisiae

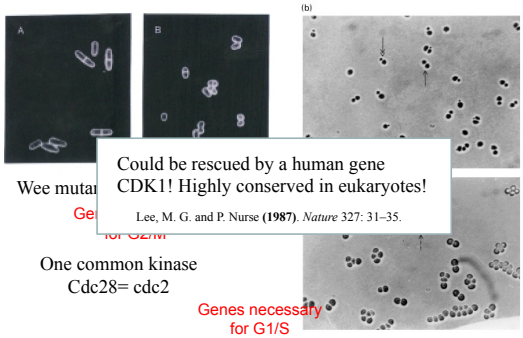

Arbacia punctulata


Drosophila melanogaster


Schizosaccharomyces pombe


Xenopus laevis

Cdc mutants



Wee mutan
Ge
for G2/M

Could be rescued by a human gene CDK1! Highly conserved in eukaryotes!

Lee, M. G. and P. Nurse **(1987)**. *Nature* 327: 31–35.

One common kinase
Cdc28= cdc2

Genes necessary for G1/S

Nurse´s lab in 70´s

Hartwell´s lab in 70´s

Cell, Vol. 33, 389–396, June, 1983, Copyright ©1983 by Cell Press

Cyclin: A Protein Specified by Maternal mRNA in Sea Urchin Eggs That Is Destroyed at Each Cleavage Division

Tom Evans,* Eric T. Rosenthal,† Jim Youngblom,† Dan Distel,† and Tim Hunt‡
Marine Biological Laboratory
Woods Hole, Massachusetts 02543

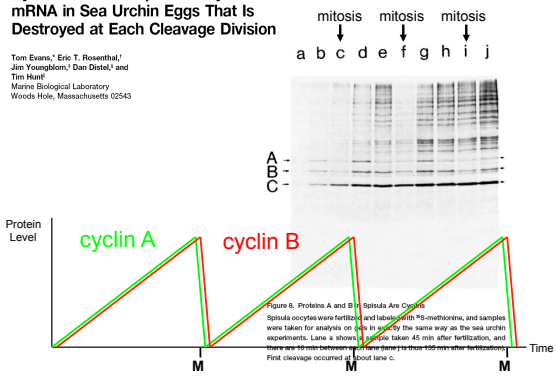


Figure 6. Proteins A and B in Spisula Are Cyclins

Spisula oocytes were fertilized and labeled with ^{35}S-methionine, and samples were taken for analysis on gels in exactly the same way as the sea urchin experiments. Lane a shows oocytes taken 45 min after fertilization, and there are 10 min between each lane (lane j is thus 135 min after fertilization). First cleavage occurred at about lane c.

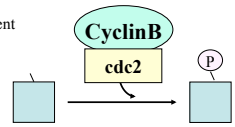
Cyclin + CDK (MPF) - induce entry into meiosis in frog eggs

Maller and Lohka, 1988

Cyclin dependent Kinase

Cyclin: regulatory component

Cdk: catalytic component

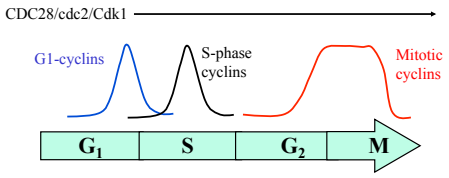


The Nobel Prize in Physiology or Medicine, 2001

"for their discovery of key regulators of the cell cycle"

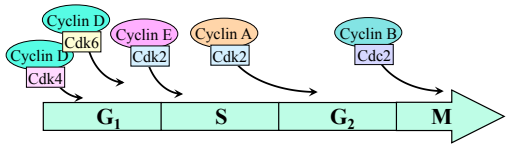
Leland H. Hartwell — R. Timothy (Tim) Hunt — Sir Paul M. Nurse

Cyclin/Cdk complexes drive the cell cycle: different cyclins specify kinase function & different times

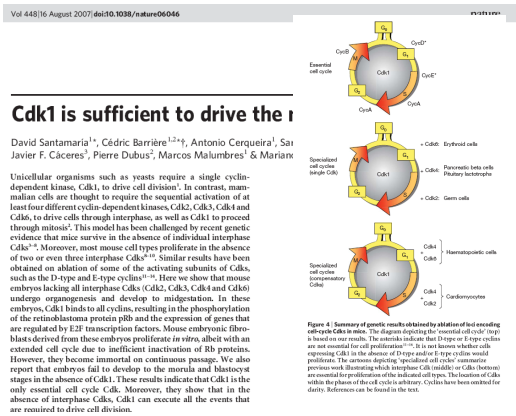


Stage specific control based on:
- Multiple oscillatory cyclins
- Cdk inhibitors
- Additional regulation at the level of Cyclin/Cdk complexes

Multiple Cdks contribute to cell cycle regulation in metazoans



Are they all needed?
What gives direccionality to the cell cycle?



Vol 448|16 August 2007|doi:10.1038/nature06046

Cdk1 is sufficient to drive the

David Santamaría[1]*, Cédric Barrière[1,2]*†, Antonio Cerqueira[1], Sa
Javier F. Cáceres[3], Pierre Dubus[2], Marcos Malumbres[1] & Mariano

Unicellular organisms such as yeasts require a single cyclin-dependent kinase, Cdk1, to drive cell division[1]. In contrast, mammalian cells are thought to require the sequential activation of at least four different cyclin-dependent kinases, Cdk2, Cdk3, Cdk4 and Cdk6, to drive cells through interphase, as well as Cdk1 to proceed through mitosis[2]. This model has been challenged by recent genetic evidence that mice survive in the absence of individual interphase Cdks[3–5]. Moreover, most mouse cell types proliferate in the absence of two or even three interphase Cdks[6–10]. Similar results have been obtained on ablation of some of the activating subunits of Cdks, such as the D-type and E-type cyclins[11–16]. Here we show that mouse embryos lacking all interphase Cdks (Cdk2, Cdk3, Cdk4 and Cdk6) undergo organogenesis and develop to midgestation. In these embryos, Cdk1 binds to all cyclins, resulting in the phosphorylation of the retinoblastoma protein pRb and the expression of genes that are regulated by E2F transcription factors. Mouse embryonic fibroblasts derived from these embryos proliferate *in vitro*, albeit with an extended cell cycle due to inefficient inactivation of Rb proteins. However, they become immortal on continuous passage. We also report that embryos fail to develop to the morula and blastocyst stages in the absence of Cdk1. These results indicate that Cdk1 is the only essential cell cycle Cdk. Moreover, they show that in the absence of interphase Cdks, Cdk1 can execute all the events that are required to drive cell division.

Protein degradation provides directionality

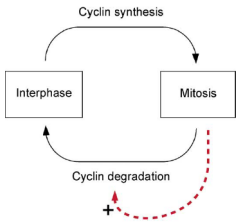


Figure 1. The Original Cyclin-Based Model for the Cell Cycle
The simplest possible model for the cell cycle based on the discovery of cyclin. See text for details.

How do cyclins die?

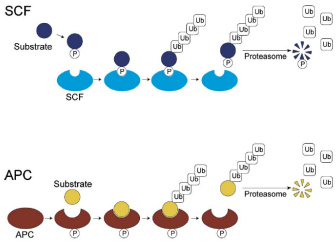
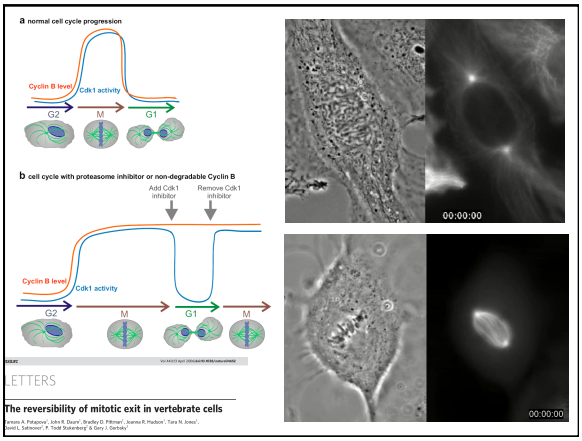
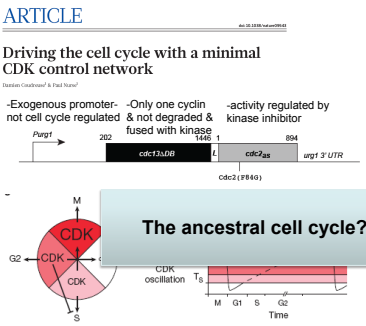


Figure 5. Regulating Protein Destruction in the Cell Cycle
Cartoons of the two major proteolytic systems that regulate the cell cycle. Both are E3 complexes that facilitate the transfer of ubiquitin from the active site of an E2 enzyme to a substrate that is thus marked for destruction by the proteasome. SCF binds phosphorylated substrates through a variety of accessory proteins, which present them to the complex for ubiquitination. SCF is active throughout the cell cycle, and the stability of individual substrates is regulated by their phosphorylation and the level of the accessory proteins that recognize them. By contrast, the APC appears to recognize substrates directly and its activity is regulated during the cell cycle as a result of phosphorylation by Cdk1 and other kinases.



Is regulating the activity of one CDK enough?



The ancestral cell cycle?

- differential expression, degradation and subcellular localization of CDK subunits, attainment of specific ratios of cyclin to CDK, and cyclin-mediated changes in substrate specificity are not essential for cell cycle progression.
- CDK oscillator provides g and directionality to a le
- it of two activity holds that define independent cell cycle Phases

- Perspectives on the Cell Cycle
- CDKs play an important role- a single one may rule the process, but tissue specificity is ensured by others
- Protein degradation is an important way of ensuring irreversibility
- Is there a minimal cell cycle? Manipulating the activity of a single CDK is able to drive it! Synthetic biology approaches may help us in these questions! Also comparative genomics.

- Perspectives on the Cell Cycle
- **Importance of centrioles: their functions**
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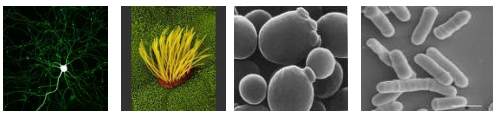
Diversity of cell shapes and functions



The cytoskeleton

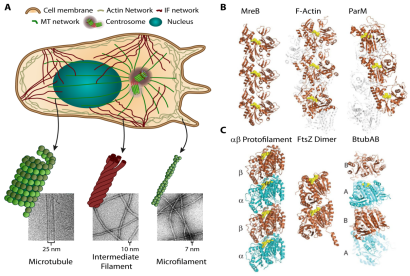
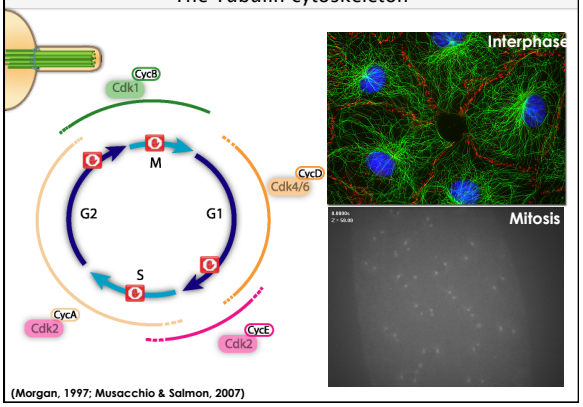


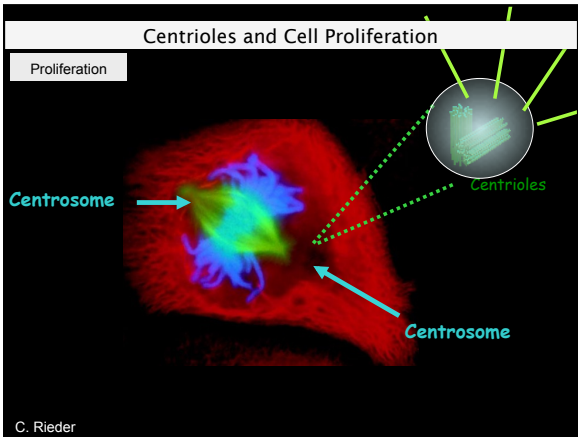
Figure 1.1 - Eukaryotic cytoskeleton and Prokaryotic counterparts. A. The cytoskeleton of a Eukaryotic cell consists of three major cytoskeleton components including microtubules, intermediate filaments and actin filaments. Tubulins assemble into protofilaments that compose the MTs, forming 25 nm tubular structures. It is speculated that intermediate filaments polymerize by lateral association of tetramers that bind end-to-end to generate 10 nm filaments. Actin monomers assemble into helical 7 nm microfilaments (EM micrographs adapted from (Metzler, 2001)). **B.** and **C.** Conserved longitudinal interaction of tubulin-like and actin-like filaments. **B.** MreB forms straight filaments, whereas F-actin is right-handed and double-helical (PDB identifiers 1JCG and 1YAG). ParM forms a left-handed double helix (PDB identifier 1MWM) (adapted from (Lowe and Amos, 2009) **C.** αβ-tubulin is a tight heterodimer with GDP trapped in the middle (PDB identifier, 1JFF). FtsZ assembles from identical monomers (PDB identifier, 1W5A). BtubAB assembles from free A and B subunits, producing an alternating protofilament (PDB identifier, 1BTQ) (adapted from (Lowe and Amos, 2009)).

The Tubulin cytoskeleton

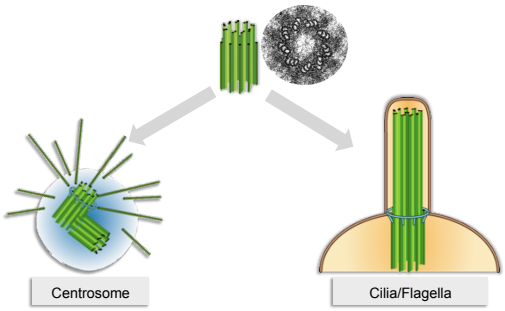


(Morgan, 1997; Musacchio & Salmon, 2007)

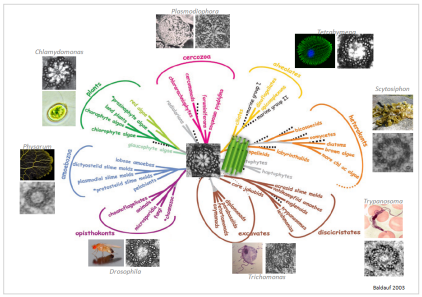
Centrioles and Cell Proliferation



The centriole has a variety of different functions

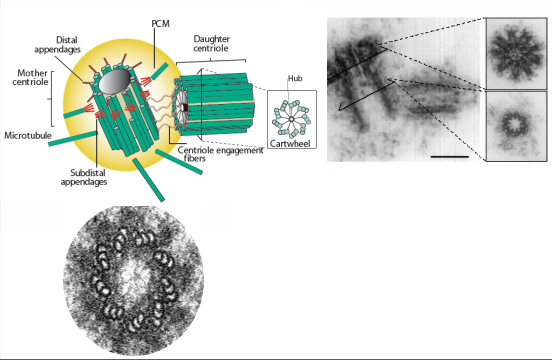


The centriole is an ancient eukaryotic feature



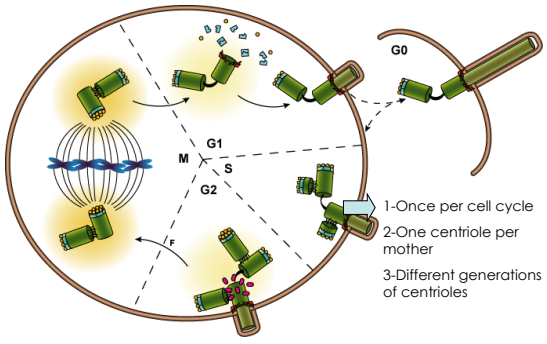
Carvalho-Santos et al. JCS 2010

Centrosome & Centrioles

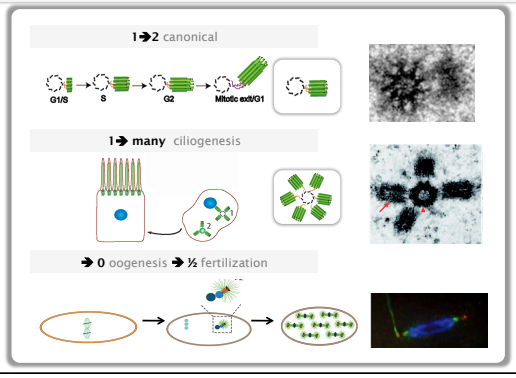


Bettencourt-Dias & Glover, 2007, NRMCB

Control of centriole fate and number



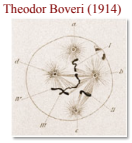
Centriole Number



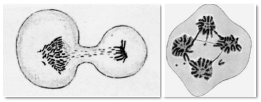
(Riparbelli and Callaini, 2010)
Anderson & Brenner, JCB, 1971

Concerning the origin of malignant tumors

Theodor Boveri (1914)



Galeotti, 1893; Hansemann, 1890



Aneuploidy induced by multipolar mitosis could have a causative role in tumorigenesis

Centrosome Changes in Human Tumors

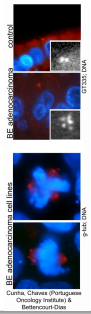
Zyss & Gergely, 2009

Table 1. Centrosome abnormalities in human cancers[a]

Cancer type	Centrosome analysis		Centrosome abnormalities	Early lesions	Correlation		Prognostic	Refs
	Marker	Method			p53	CIN		
Bladder cancer	γ-tubulin Aurora-A	IHC	N			✓		[129]
Breast (invasive)	Centrin γ-tubulin	IHC, EM	N, S		✗			[18]
Breast (DCIS)	Centrin	IHC	N	✓	✗	✓		[12]
Breast (DCIS, invasive)	γ-tubulin	IHC		✓		✓	✓	[130]
Hepatocellular carcinoma	Pericentrin	IHC	N, S	✓	✓	✓		[131]
Non-small cell lung cancer	Pericentrin γ-tubulin	IHC	N, S		✗		✗	[132]
Pancreatic DC	Pericentrin γ-tubulin	IHC	N, S	✓				[133]
Prostate cancer	Pericentrin	IHC	N, S			✓		[134]
Breast, prostate, lung, colon	Pericentrin	IHC	N, S		✗	✓		[135]
Breast, cervix, prostate (pre-invasive)	Pericentrin	IHC	N, S	✓				[19]
Myeloma	Centrin γ-tubulin Pericentrin	ICC	N	✓		✓	✓	[25]
B-CLL	γ-tubulin	ICC				✗	✗	[21,136]
CML	Pericentrin	ICC	N, S	✓		✓		[137]
AML	Pericentrin	ICC	S			✓		[22]
Non-Hodgkin's lymphoma	γ-tubulin Pericentrin	ICC	S			✓		[23]
Burkitt's lymphoma	γ-tubulin	ICC	N, S			✗	✗	[138]

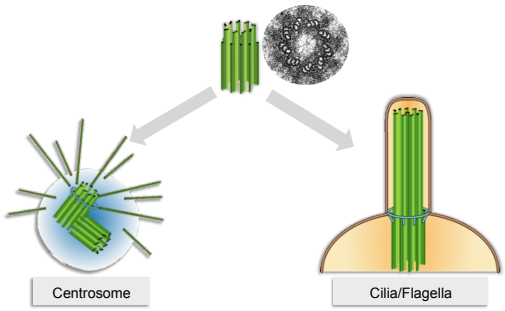
[a]Abbreviations: AML, acute myeloid leukaemia; B-CLL, B cell chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; DC, ductal carcinoma; DCIS, ductal carcinoma in situ; EM, electron microscopy; IHC, immunohistochemistry; ICC, immunocytochemistry; N, numerical; S, structural; ✗, investigated and shown to be false; ✓, investigated and confirmed.

Multiple centrioles in cancer



Cunha, Chaves (Portuguese Oncology Institute) & Bettencourt-Dias

The centriole has a variety of different functions

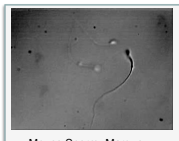


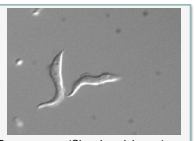
Centrioles and Cell Motility

Proliferation

Motility

CELL MOTILITY:
PROPULSION


Mouse Sperm- Marquez

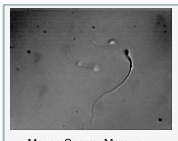

Trypanosome (Sleeping sickness)

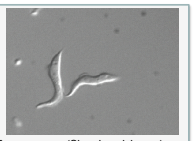
Centrioles and Cell Motility

Proliferation

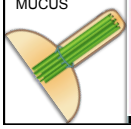
Motility

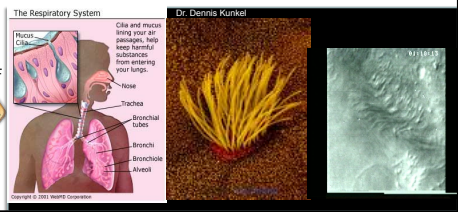
CELL MOTILITY:
PROPULSION


Mouse Sperm- Marquez


Trypanosome (Sleeping sickness)

FIXED CELL
MOTILITY:
MOVEMENT OF
MUCUS



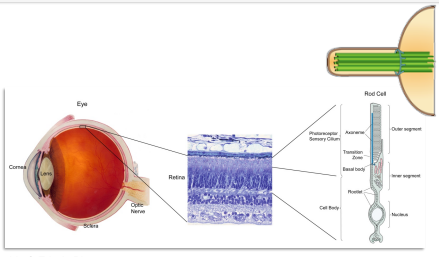


Centrioles & Cell Sensing

Proliferation

Motility

Sensing



Qin Liu & Eric A. Pierce


Reiter

Most of Our Cells

-Sense Fluid Flow
-Sense whether we have eaten enough
-Determine what is Left/Right
.....

Cilia/Flagella formation

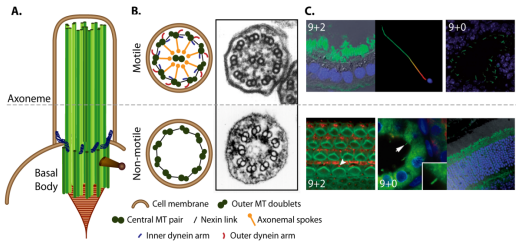


Figure 2.8 – Structure of Cilia and Flagella. A. A schematic representation of the cilia/flagella structure. The basal body docks at the cell membrane through transitional fibres and grow the axoneme of cilia and flagella, surrounded by the ciliary membrane. **B.** Schematic representation of cross section through the axoneme of motile and non-motile cilia. EM micrograph of tracheal motile cilia is represented on top while renal non-motile primary cilia at the bottom (adapted from (Sate and Christensen, 2007) and http://www.kidneyresearchcenter.org/). Note that differences between these two types of cilia include the lack of central pair and dynein arms in primary cilia. **C.** Examples of mammalian ciliated cells and their axonemal configuration. Top panels represent cells bearing motile cilia, including ependymal cells, sperm and the node of mouse embryo. Bottom panels include cells that bear non-motile cilia such as cells in the inner ear, renal cells and eye photoreceptor cells (adapted from (Fliegauf et al., 2007; Verghese et al., 2009)).

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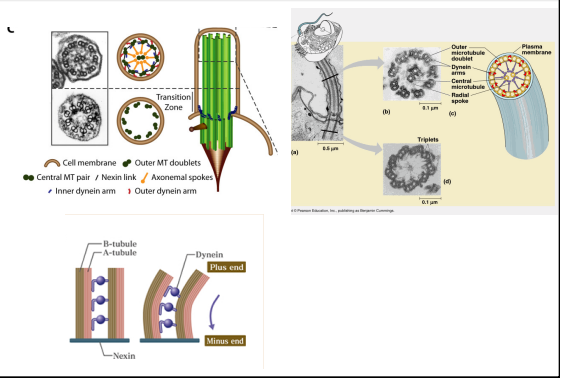
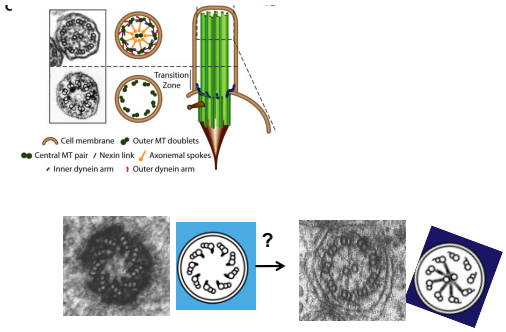
Motile Cilia/Flagella formation



Cilia/Flagella formation



Cilia/Flagella formation: many links with other cellular processes

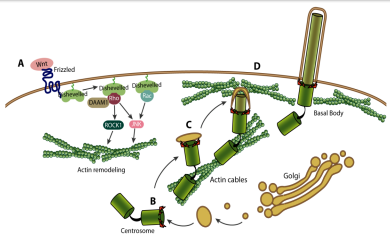


Figure 2.9 – The Different Steps Comprising Ciliogenesis. A. Planar cell polarity cues, regulated by Frizzled and Dishevelled, are important in both anchoring and orientation of the basal bodies at the cell membrane. Some core polarity cues are probably set before basal bodies start migrating. **B.** Vesicles originated from the Golgi seem to anchor at the basal body distal region while it is still in the cytoplasm. This vesicle will form the ciliary sheath and will fuse with the plasma membrane once the basal body anchors (Dirksen, 1971; Sorokin, 1962; Sorokin, 1968). **C.** Basal bodies migrate towards the cell membrane in a process which is apparently dependent on actin cables (Boisvieux-Ulrich et al., 1990; Tamm and Tamm, 1988). **D.** The step of ciliogenesis involves anchoring of the basal body at the cell membrane, in a process which also seems to involve the actin cytoskeleton and further elongation of the axoneme in an IFT dependent manner

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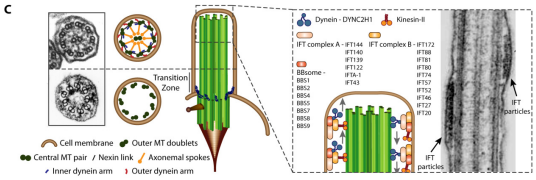
Cilia/Flagella formation



-Diffusion Barriers?
-Pores at tranzition area?

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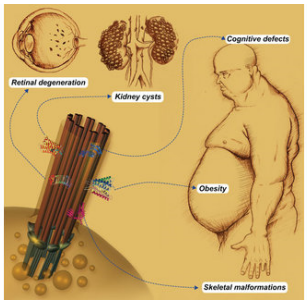
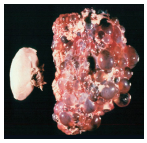
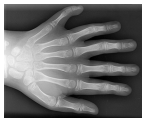
Cilia Number & Structure & Human Disease







Max Nachury

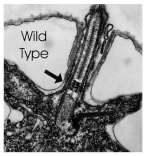
Bardet-Biedl Syndrome

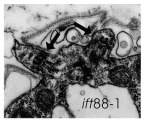
Polycystic Kidney disease and cilia

Chlamydomonas IFT88 and Its Mouse Homologue, Polycystic Kidney Disease Gene _Tg737_, Are Required for Assembly of Cilia and Flagella

Gregory J. Pazour,* Bethany L. Dickert,* Yvonne Vucica,‡ E. Scott Seeley,‡ Joel L. Rosenbaum,‡ George B. Witman,* and Douglas G. Cole§

*Department of Cell Biology, University of Massachusetts Medical School, Worcester, Massachusetts 01655; ‡Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06520; and §Department of Microbiology, Molecular Biology, and Biochemistry, University of Idaho, Moscow, Idaho 83844





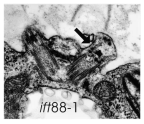


Figure 3. Ultrastructure of the *ift*88-1 flagella. The flagella on *ift*88-1 mutant cells are very short and the microtubules do not extend beyond the transition zone (arrows). The microtubules in wild-type cells start at the basal body, extend through the transition zone, and continue on to the flagellar tip. The wild-type flagellum shown here leaves the plane of section shortly after passing through the cell wall.

Pazour et al, 2000, JCB

Link between polycystic disease & Cilia

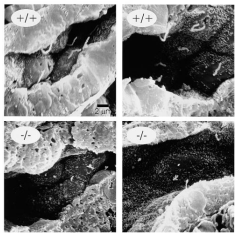
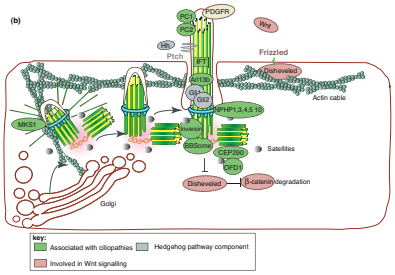


Figure 6. Primary cilia in the kidney of Tg737 mutant mice are shorter than normal. (a)

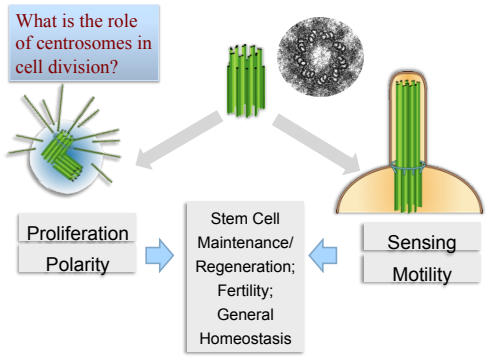
Pazour et al, 2000, JCB

Cilia Number & Structure & Human Disease

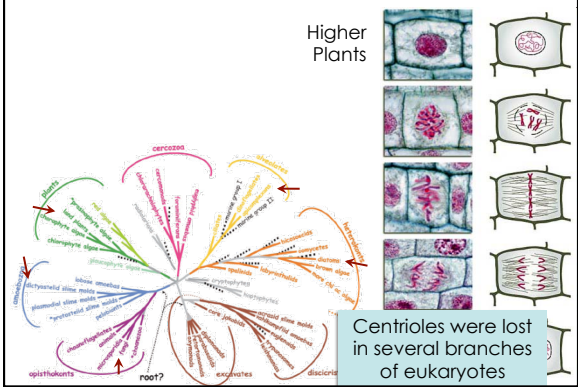


Bettencourt-Dias et al, TIGS, 2011

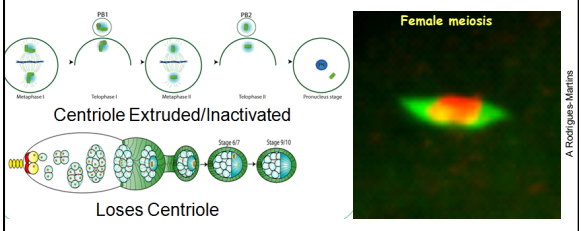
Centrioles have a diversity of cellular functions



Are centrioles needed for cell division?



Are centrioles needed for cell division?



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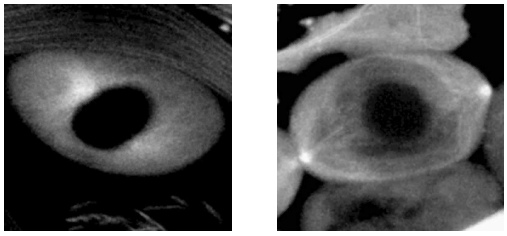
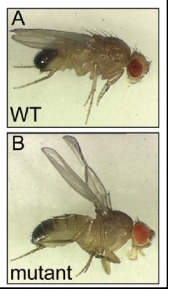


Rebollo et al, 2004

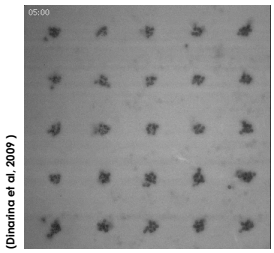
Are centrioles needed for cell division?

Flies without Centrioles

Renata Basto,[1] Joyce Lau,[1] Tatiana Vinogradova,[2,3] Alejandra Gardiol,[1] C. Geoffrey Woods,[4] Alexey Khodjakov,[2,3] and Jordan W. Raff[1,*]
[1] The Gurdon Institute, Tennis Court Road, Cambridge CB2 1QN, UK
[2] Wadsworth Center, New York State Department of Health, Albany, NY 12201, USA
[3] Department of Biomedical Sciences, State University of New York, Albany, NY 12222, USA
[4] Department of Medical Genetics, Cambridge Institute of Medical Research, Cambridge CB2 2XY, UK
*Contact: j.raff@gurdon.cam.ac.uk
DOI 10.1016/j.cell.2006.05.025



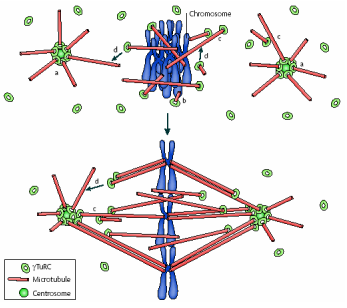
Other ways of nucleating microtubules



(Dinarina et al. 2009)

Chromatin beads and xenopus extracts

Other ways of nucleating microtubules



Stearns 2007, NRMCB

Golgi, etc

What are centrioles needed for?

Flies without Centrioles

Renata Basto,[1] Joyce Lau,[1] Tatiana Vinogradova,[2,3] Alejandra Gardiol,[1] C. Geoffrey Woods,[4] Alexey Khodjakov,[2,3] and Jordan W. Raff[1,*]
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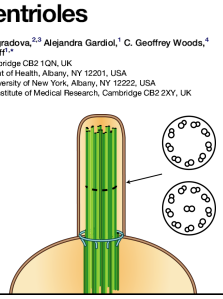
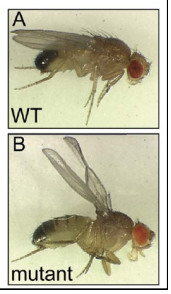
DOI 10.1016/j.cell.2006.05.025

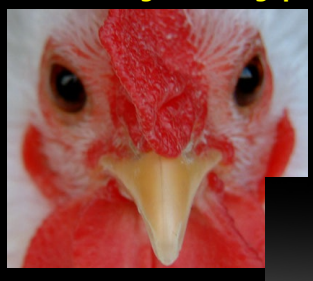




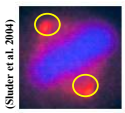
- **Importance of centrioles: their functions-** *Perhaps most important function is to make flagella/cilia- more to discuss in conference talk*

- Perspectives on the Cell Cycle
- Importance of centrioles: their functions
- **Centrioles & Cancer**
- Centriole Biogenesis & Number Control

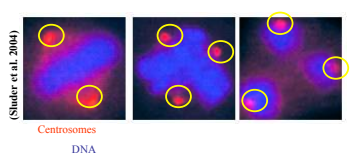
Leading to the big question…..

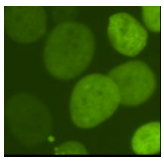


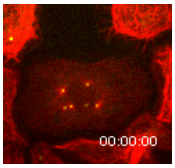
Centrosomes are dominant when present



Centrosomes are dominant when present

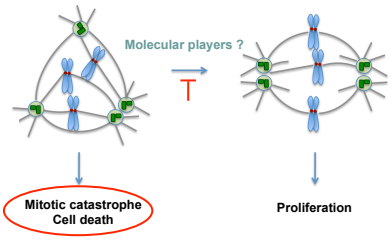






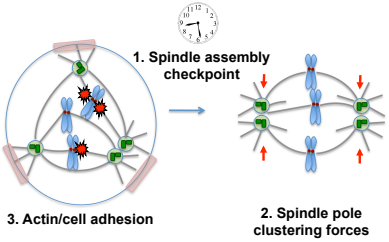
Kwon, Godinho et al, 2008

Centrosome Clustering



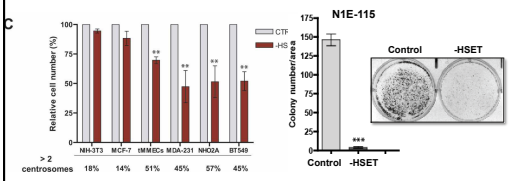
Ring et al., 1982;
Lingle and Salisbury, 1999;
Duensing, 2005; Brinkley, 2001;
Nigg, 2002
Quintyne et al., Science, 2005

Mechanisms of Centrosome Clustering



Kwon*,Godinho* et al., Gen & Dev, 2008
Reviewed in Godinho et al., Cancer&Metastasis Rev., 2009

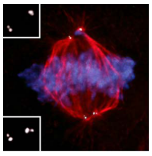
Centrosome De-Clustering can target cancer cells



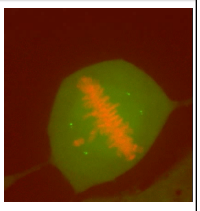
- HSET is not essential in normal cells
- Depletion of HSET leads to cell death proportionally to the percentage of cells with extra centrosomes
-And high levels of HSET associated with bad prognosis in breast cancer (Jose Pereira-Leal´s group)

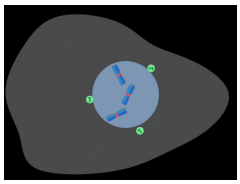
Kwon*,Godinho* et al., Gen & Dev, 2008

How could extra centrosomes lead to aneuploidy?



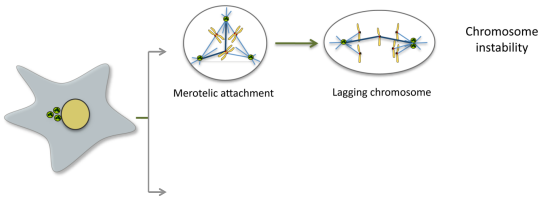
Colon cancer cell line
Godinho et al, 2008





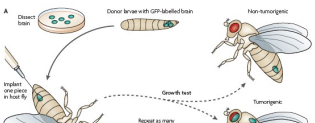
Ganem, Godinho et al, 2009

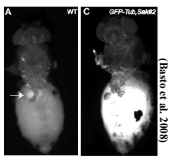
How could extra centrosomes lead to cancer?



(Bettencourt-Dias et al. 2011)

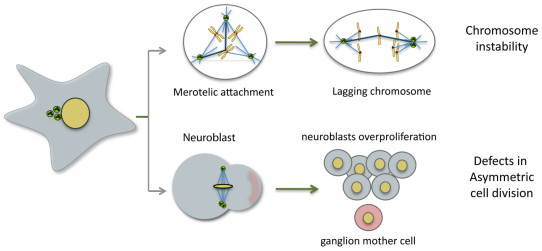
Extra Centrosomes Can Generate Tumors in Flies





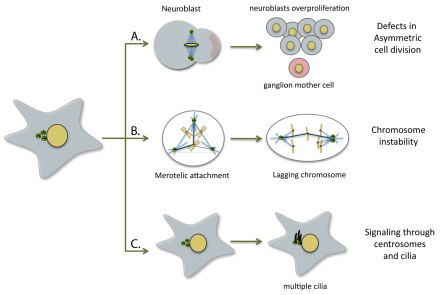
(Basto et al. 2008)

How could extra centrosomes lead to cancer?



(Bettencourt-Dias et al. 2011)

Centrioles & cancer hypotheses



- Centrioles & Cancer: *Jury is still out there but various possibilities – as centrosome and cilia!*

Summary

- Perspectives on the Cell Cycle and *the ancestral machinery*
- Importance of centrioles: their functions- *ancestral function is likely to be flagella but more to discuss during conference*
- Centrioles & Cancer: *they may have several roles… but we know little at this point*
- Centriole Biogenesis & Number Control: *how PLK4 plays an important role in triggering and regulating centriole number*