The cell division cycle

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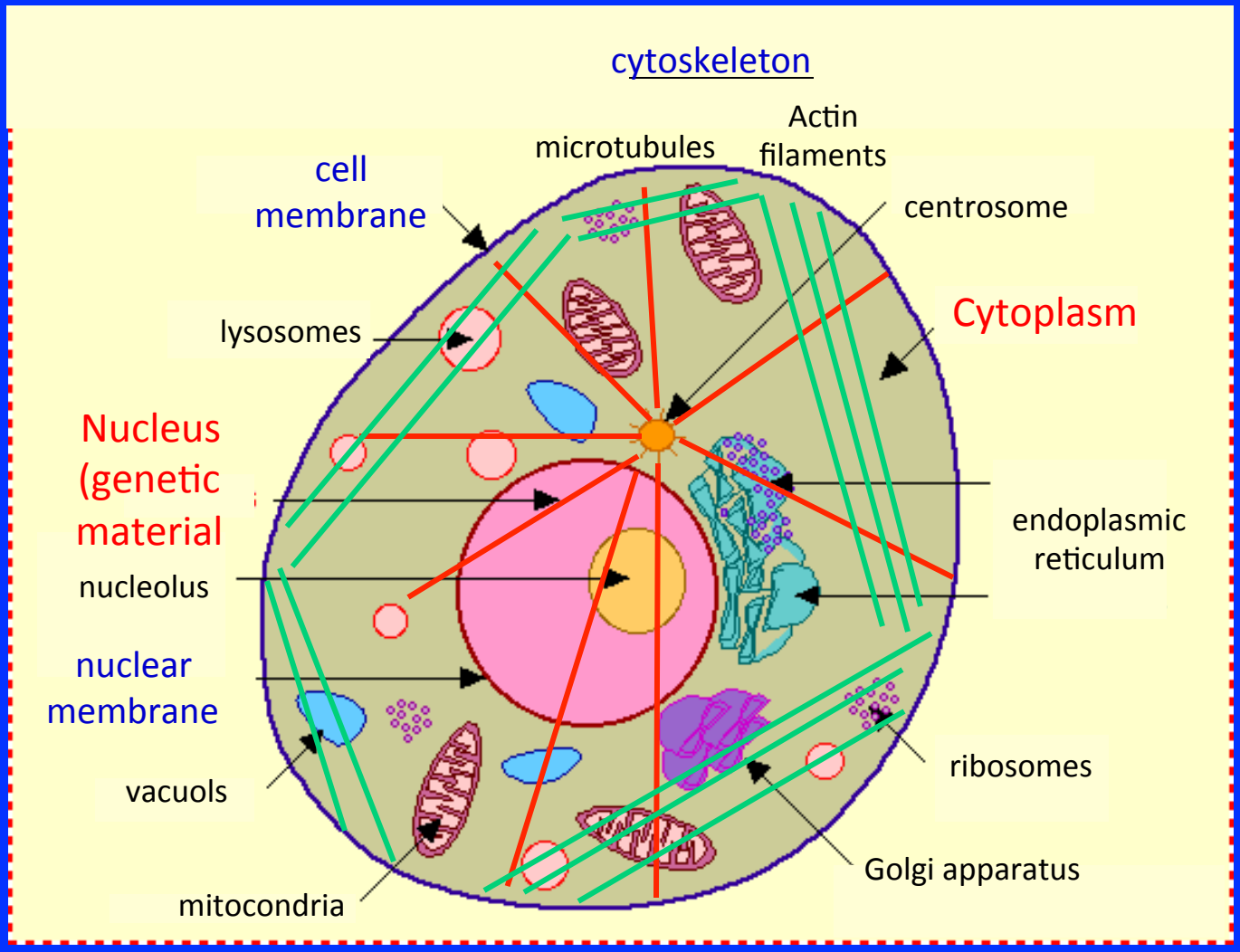
Today's lesson

- The animal cell
- The cell division cycle
- Levels of control of the cell cycle
- Mitosis and its regulatory checkpoints
- Mitosis and cancer

Two methodological approaches for the analysis of mitotic division:
- mathematical models
- fluorescence microscopy

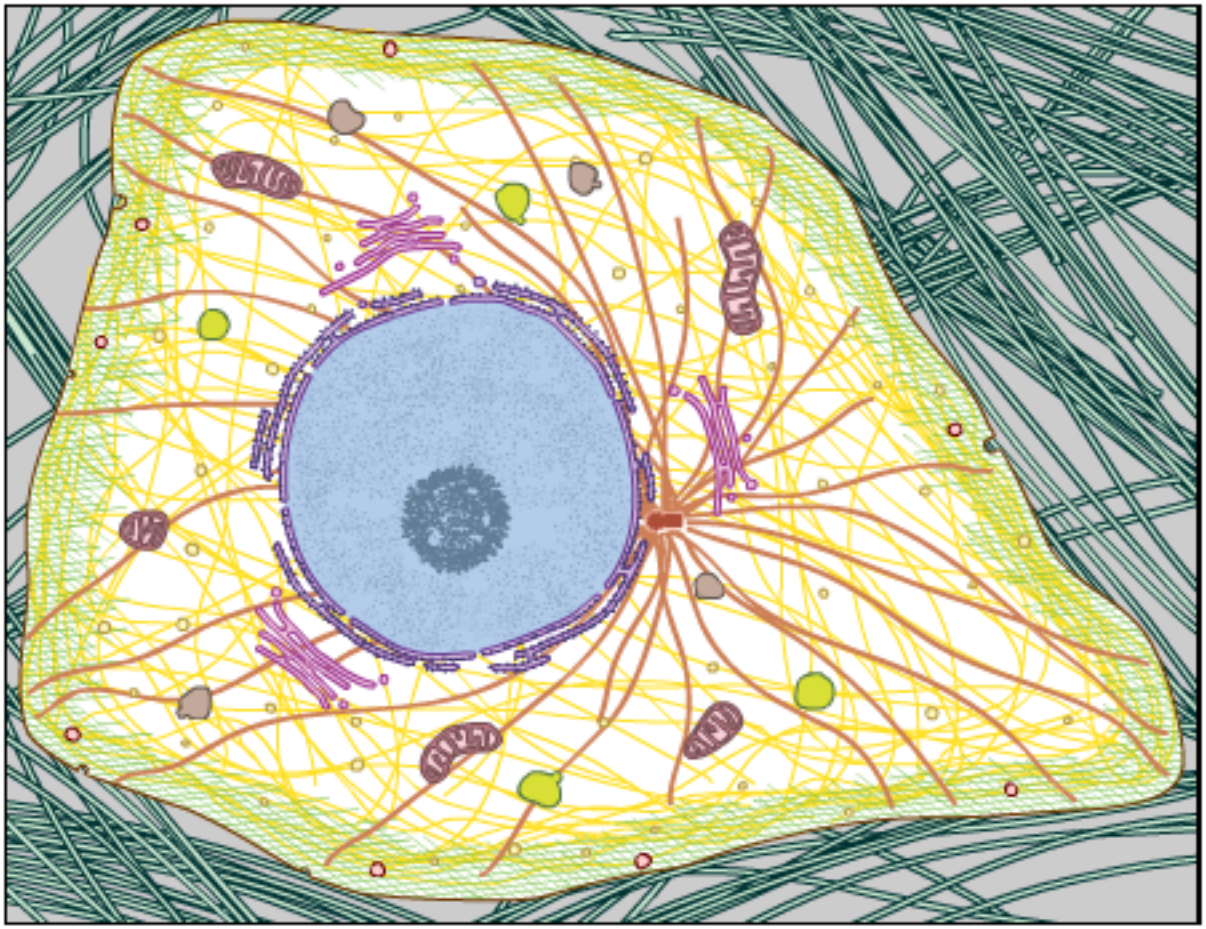
The animal cell



THE CELL™ an image library

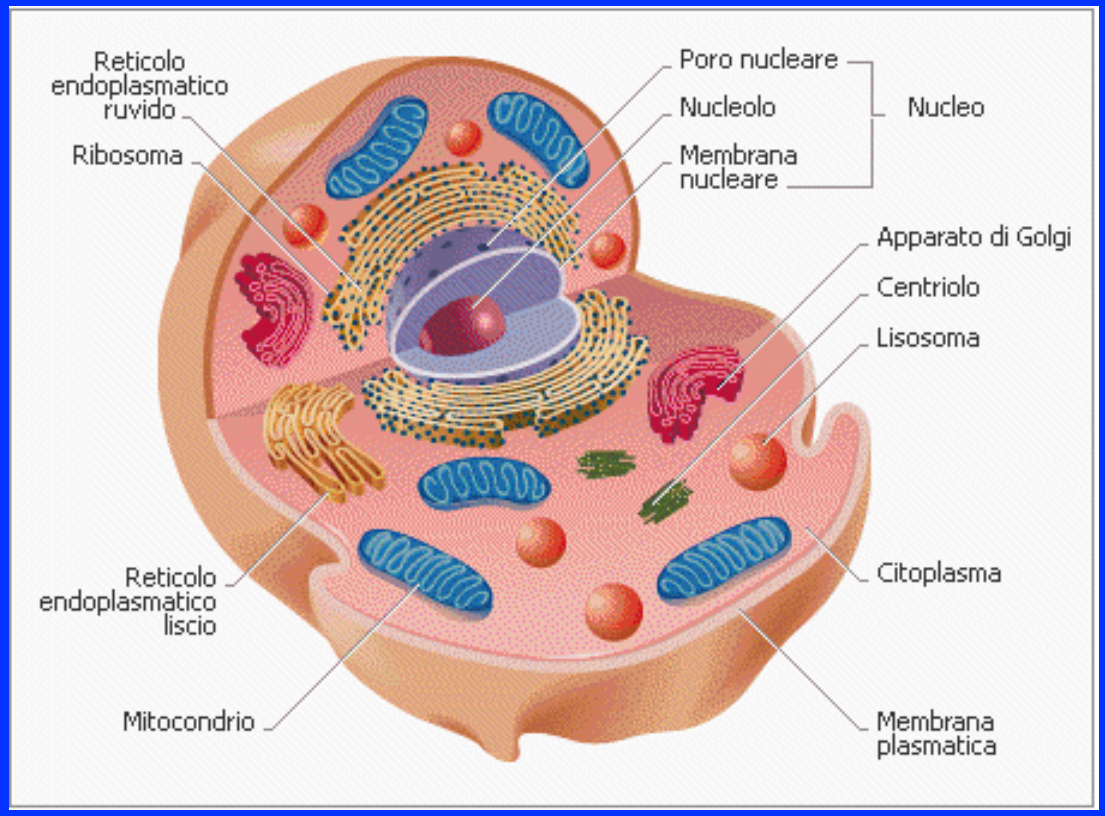
https://www.cellimagelibrary.org/

Explore the Cell

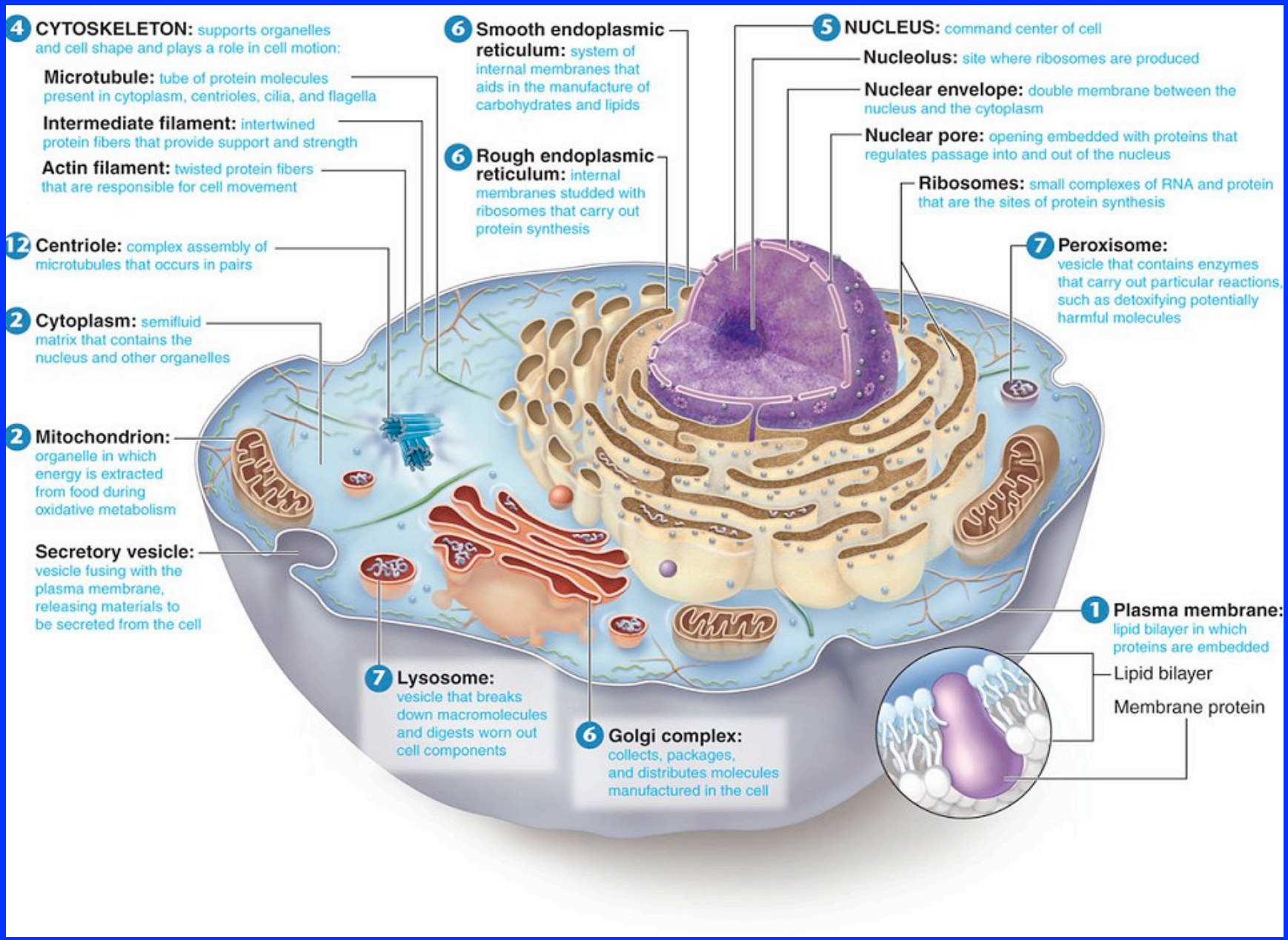


actin cytoskeleton
chromosome
endoplasmic reticulum
endosome
extracellular matrix
Golgi apparatus
intermediate filament cytoskeleton
lysosome
microtubule cytoskeleton
microtubule organizing center
mitochondrion
nuclear envelope
nucleolus
nucleus
plasma membrane
ribosome
vacuole
vesicle

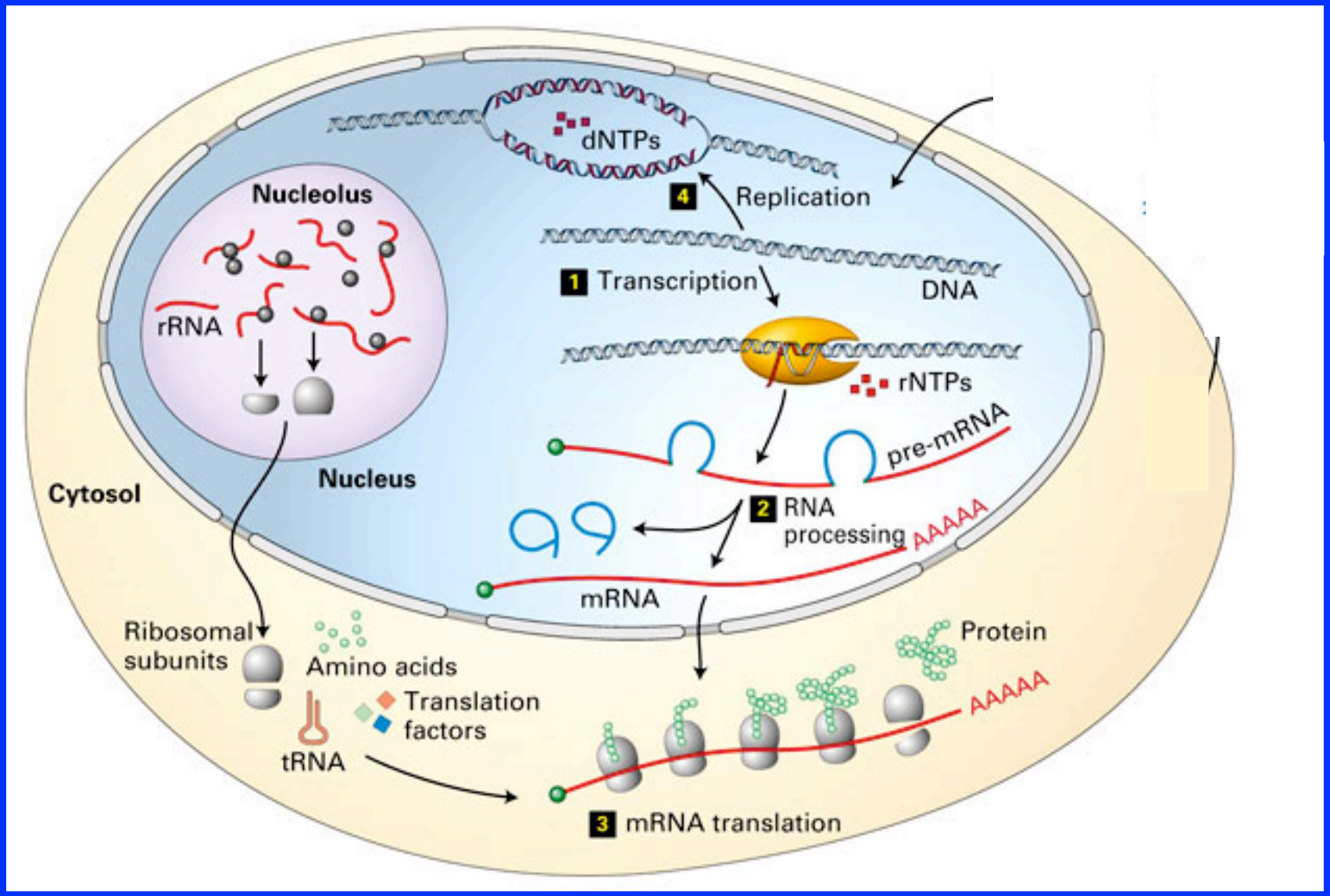
The animal cell (3D)



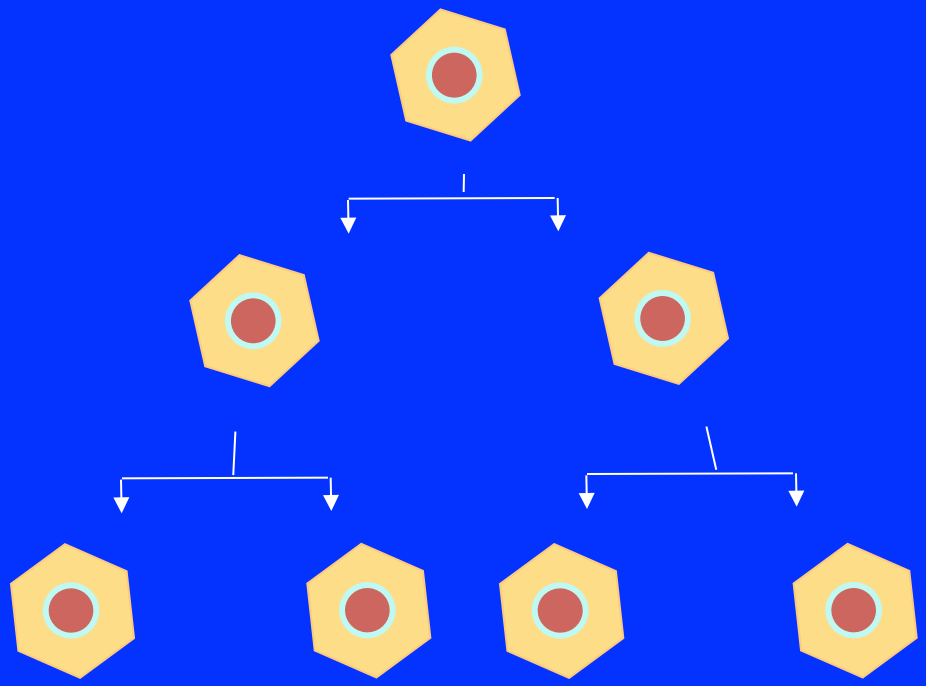
The animal cell (3D): compartmentts and functions



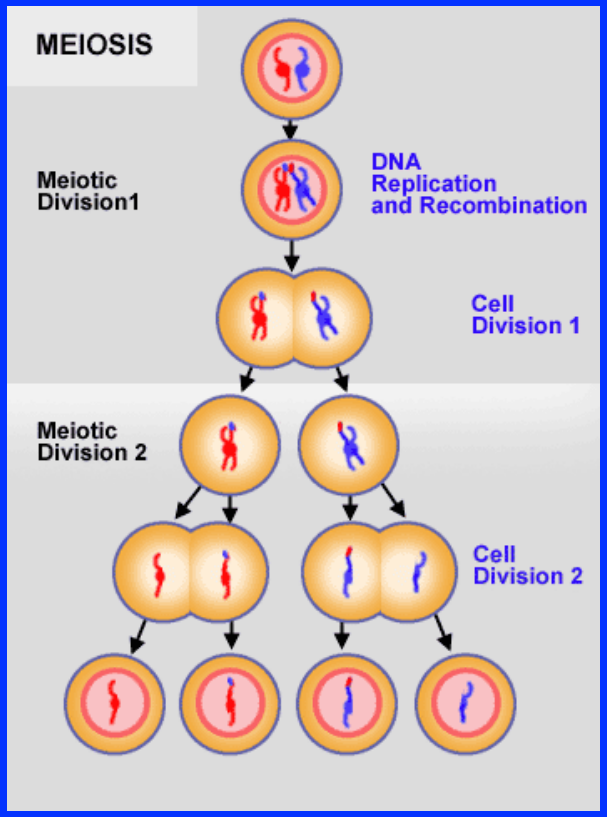
The flux of genetic information: from DNA to proteins



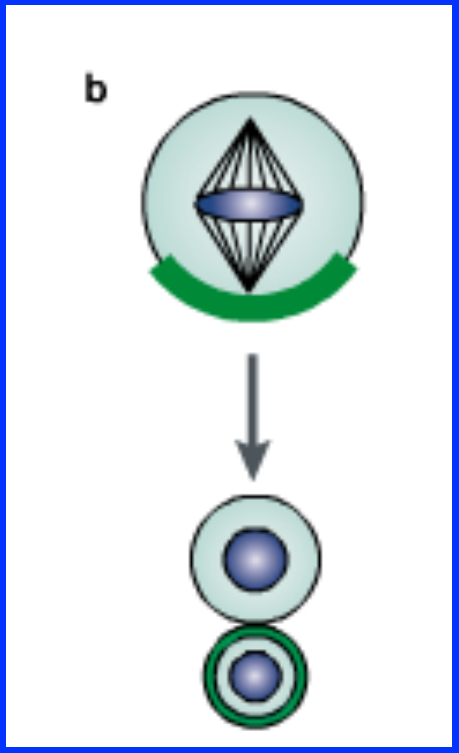
Cell division: generate two daughter cells identical to the mother cell



Two non equivalent divisions



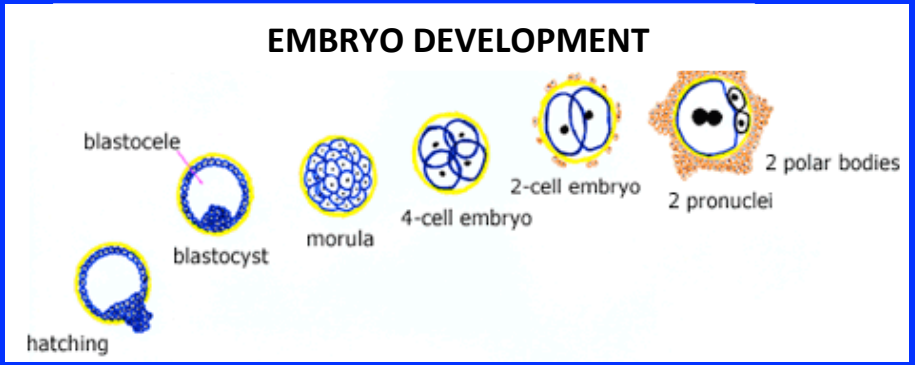
Meiosis II: chromosome segregation without replication



Asymmetric division (stem cells): unequal distribution of cellular components

Cell division in the whole organism: aims

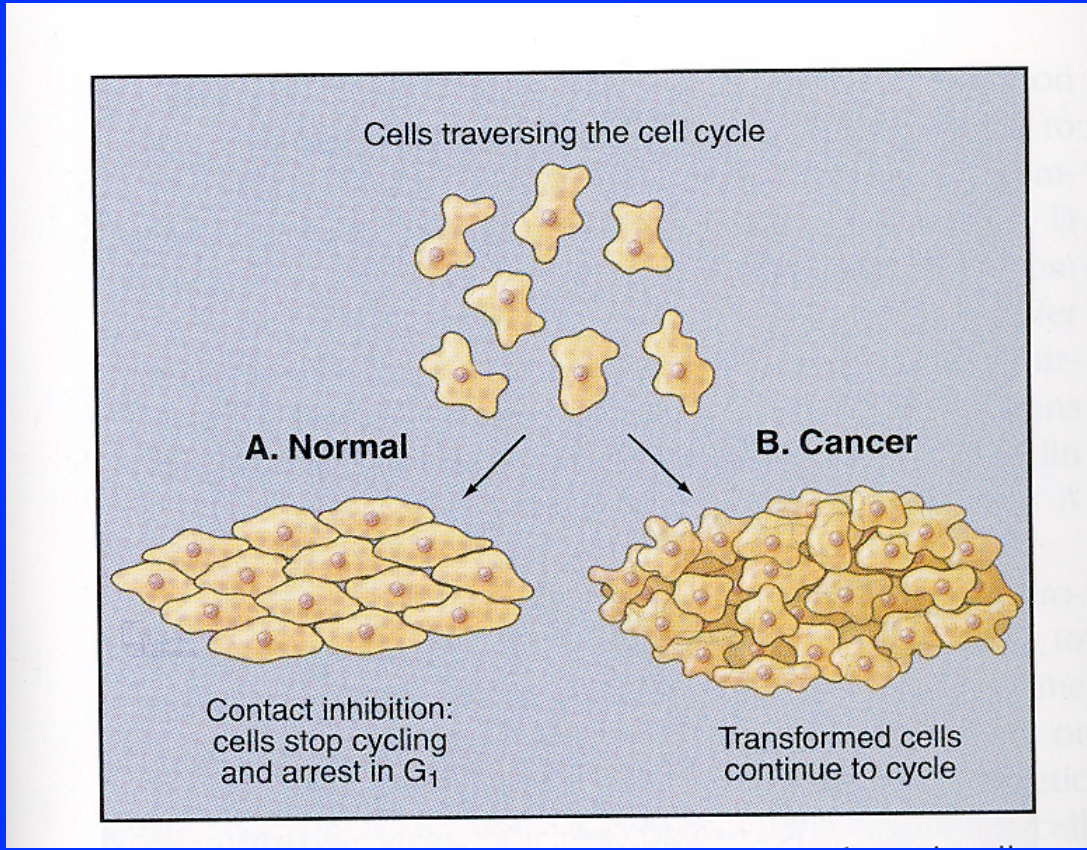
✓ Development and growth: from one single cell (fertilised egg or zygote) to the adult



✓ Tissue renewal, regeneration and repair: to replace cells that die due to aging or to accidental damage (variable frequence in the different cell types)

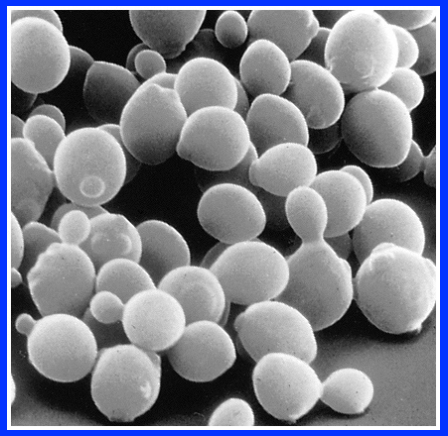
✓ Gametogenesis: meiotic division to form oocytes and spermatozoa

Cell division alterations: pathogenesis

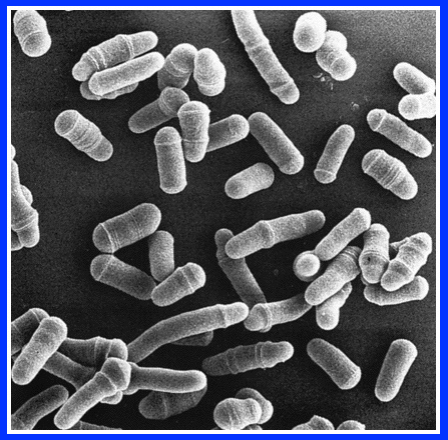


Loss of control of cell division is associated with tumorigenesis

Model systems to study cell division



Saccharomyces cerevisiae



Schizosaccharomyces pombe

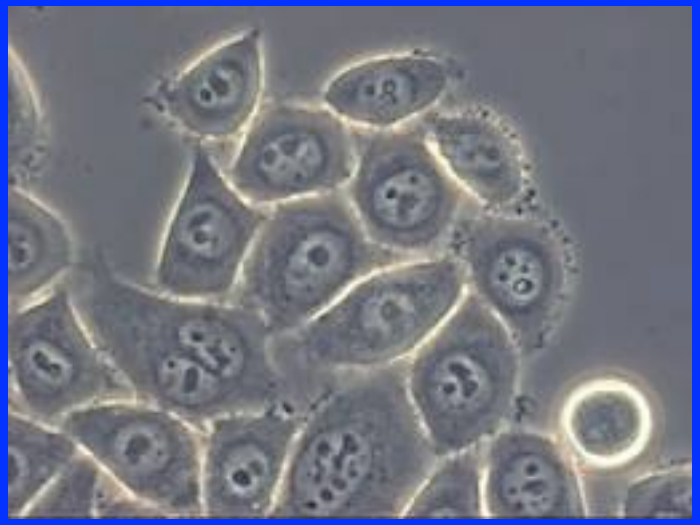
Genetic studies

Arbacia punctulata

Xenopus laevis

Biochemical studies

Importance of cell cultures to study cell division



HeLa cells (from cervical cancer) were the first immortalised human cells (1951): able to divide in 24 hours in culture, to grow indefinitely, resistant to be sent all over the world, they revolutionised research on cell division and on consequences of its alterations.

La vita immortale di Henrietta Lacks (The immortal life of Henrietta Lacks) , Rebecca Skloot, Adelphi

HOW TO FAITHFULLY TRANSMIT THE GENETIC MATERIAL?
Duplicate (without errors!) before dividing

1 cell (mother)

↓ cell division

2 daughter cells genetically identical to the mother

1 set of chromosomes

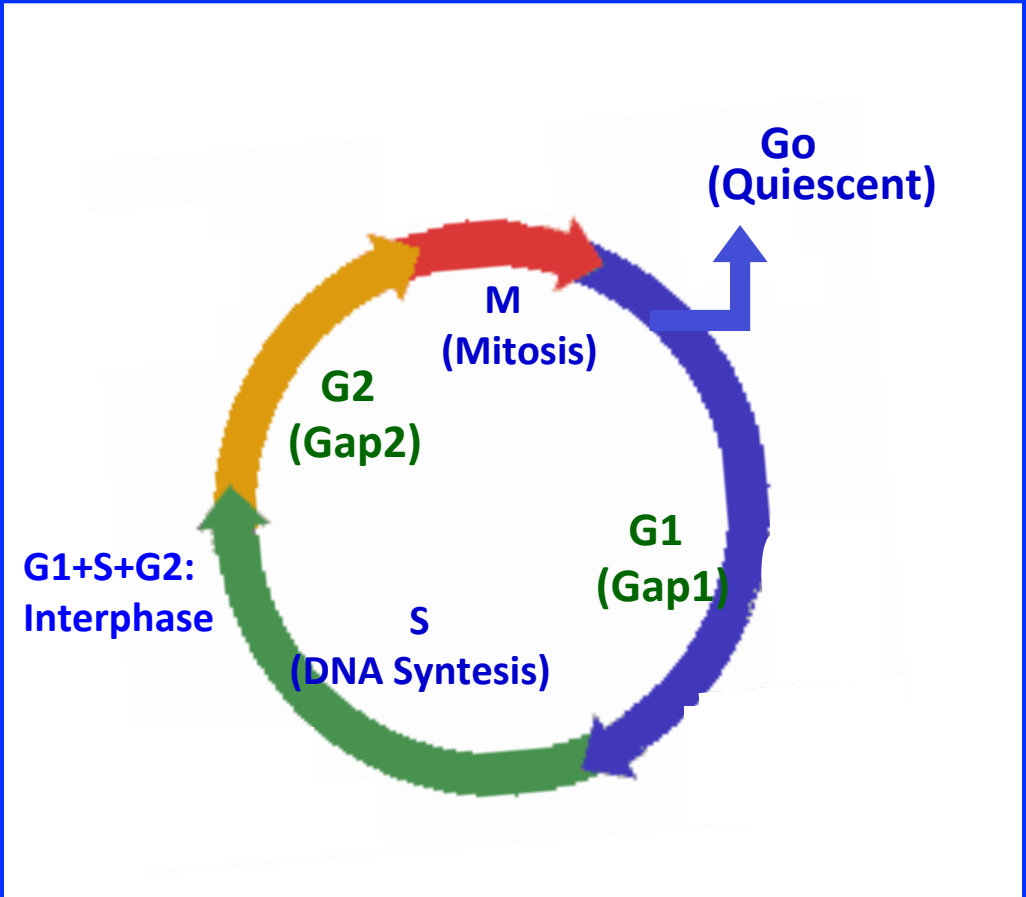
↓ DNA replication

2 identical sets of chromosomes

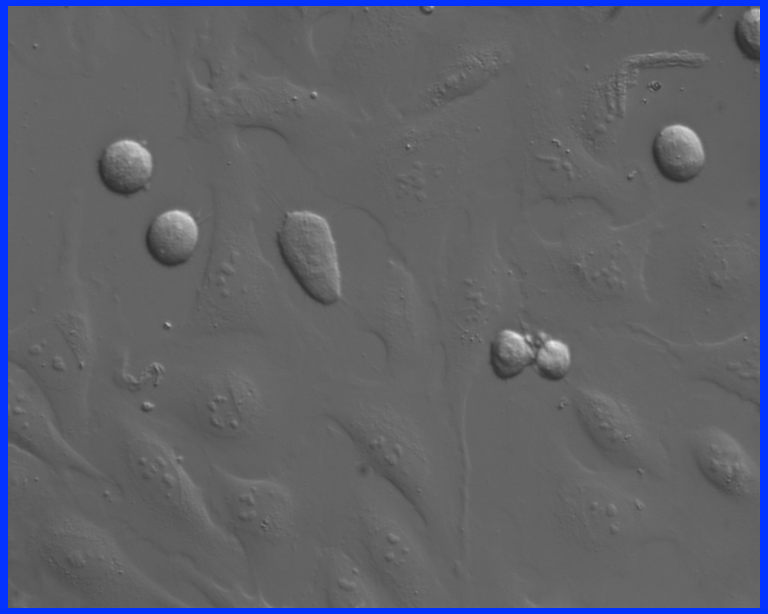
↓ Chromosome segregation (Mitosis)

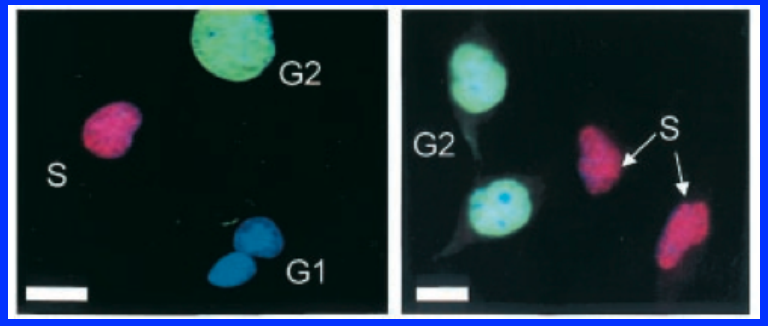
1 complete set of chromosomes in each daughter cell

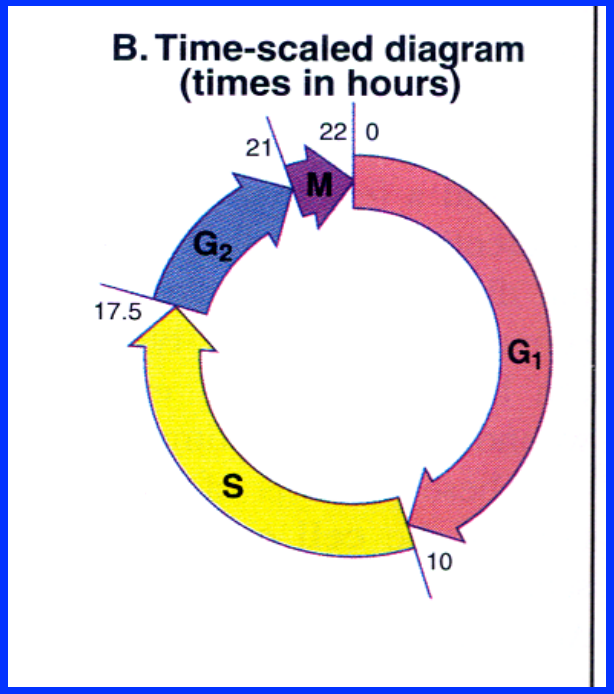
The cell division cycle:
alternation of Synthesis and Mitosis



The cell division cycle: heterogeneity of cell cultures

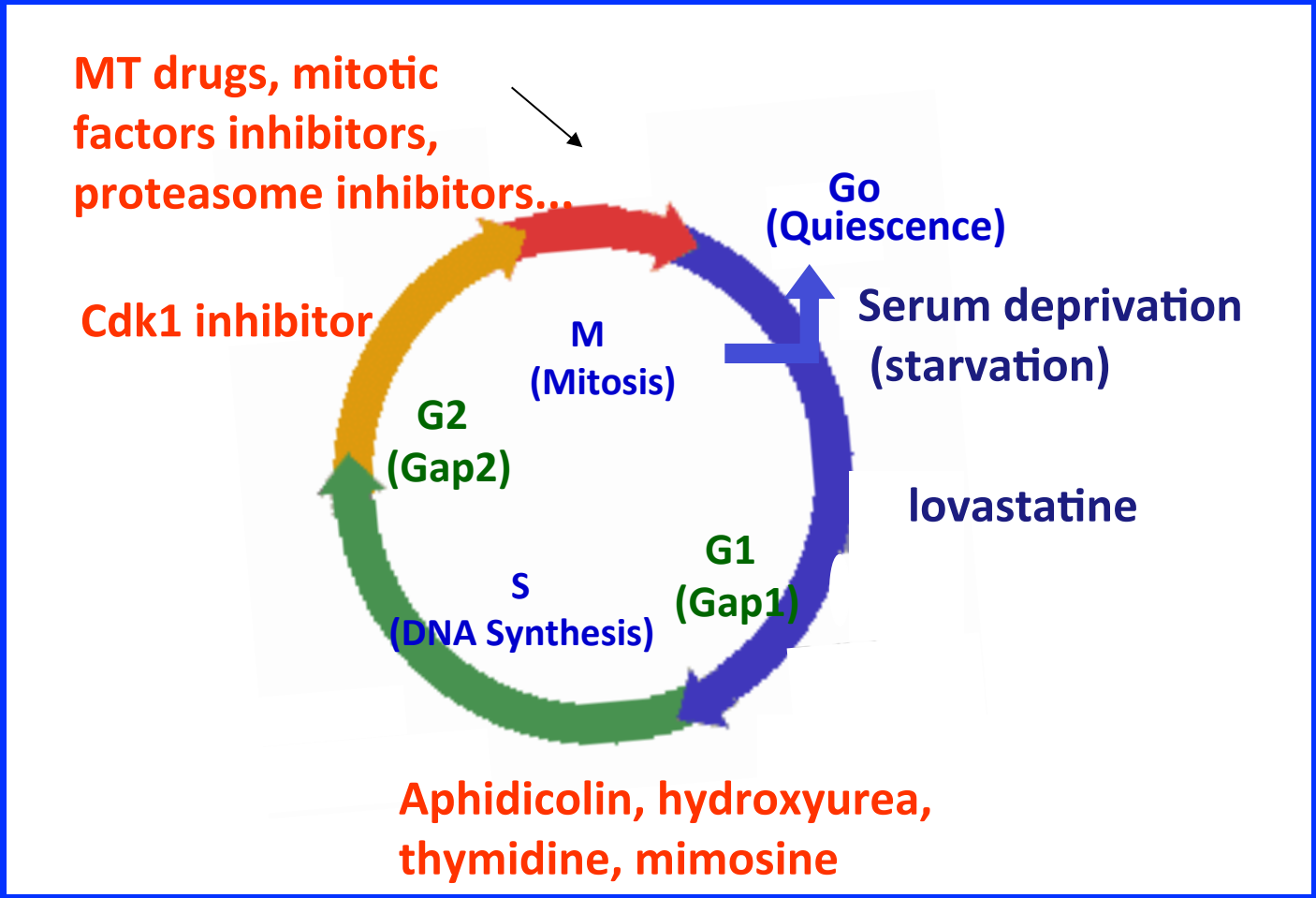






In an asynchronous culture we will find represented all phases of interphase and mitosis, corresponding to their duration

Methods of cell cycle synchronisation



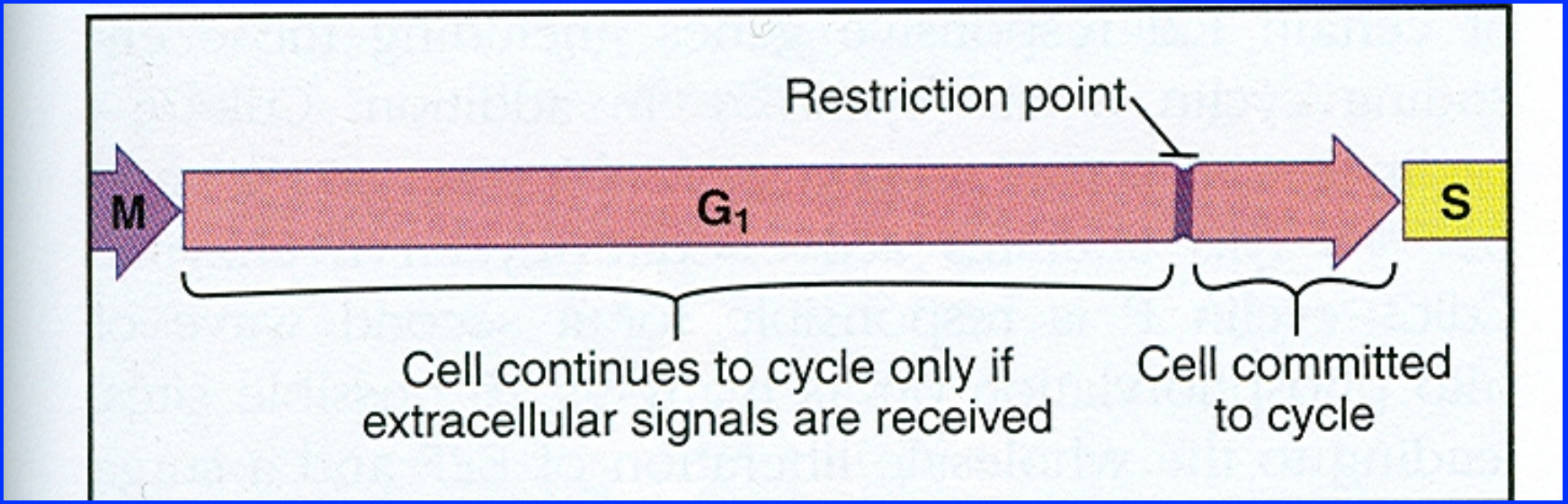
It is possible to arrest and “resume” cell cycle progression at specific points by making use of molecules with reversible action

- **How to decide whether to enter in the division cycle?**

→ Response to external signals

Control of cell cycle entry: the restriction point

At the end of each division, daughter cells "decide" whether to enter a new division cycle or in a quiescent phase.
External signals (growth factors, pro-proliferative stimuli, presence of surrounding cells) are fundamental for the choice of dividing.



The restriction point identifies the moment after which the cell will continue dividing independent of external signals.

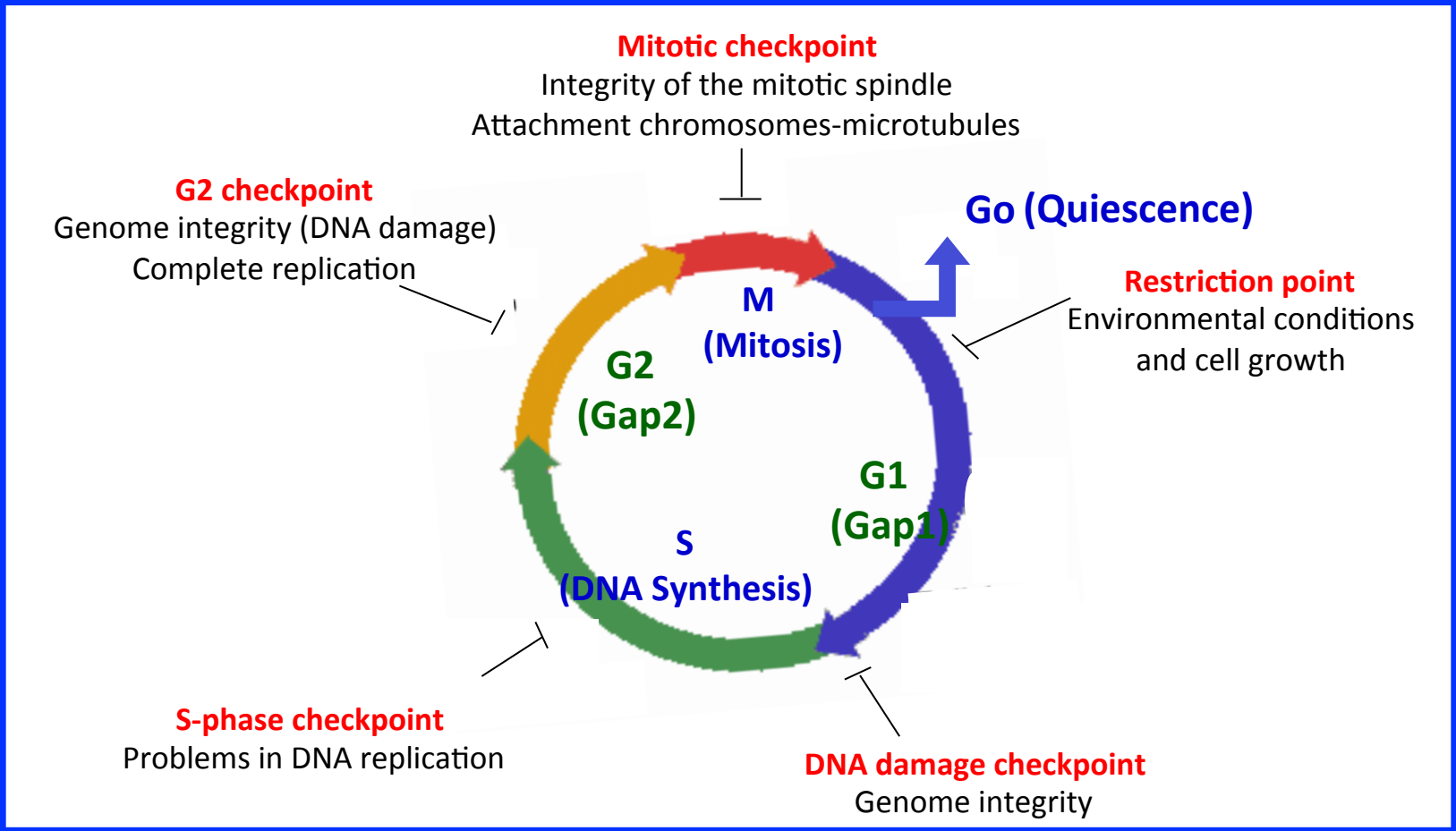
- **How to decide whether to enter in the division cycle?**

→ Response to external signals

- **How to control the temporal unfolding of the division cycle?**

→Checkpoint mechanisms (internal signals)

Cell cycle control: checkpoint mechanisms



Cell cycle checkpoints ensure the correct sequence of events and arrest cell cycle progression in the presence of errors, to enable the cell to complete the process and/or repair the damage. If this is not possible, a programmed cell death process will be activated.

- **How to decide whether to enter in the division cycle?**

→ Response to external signals

- **How to control the temporal sequence of the division cycle?**

→ Checkpoint mechanisms (internal signals)

- **How to ensure the correct progression through the different cell cycle phases?**

→ Regulatory mechanisms (genes, proteins)

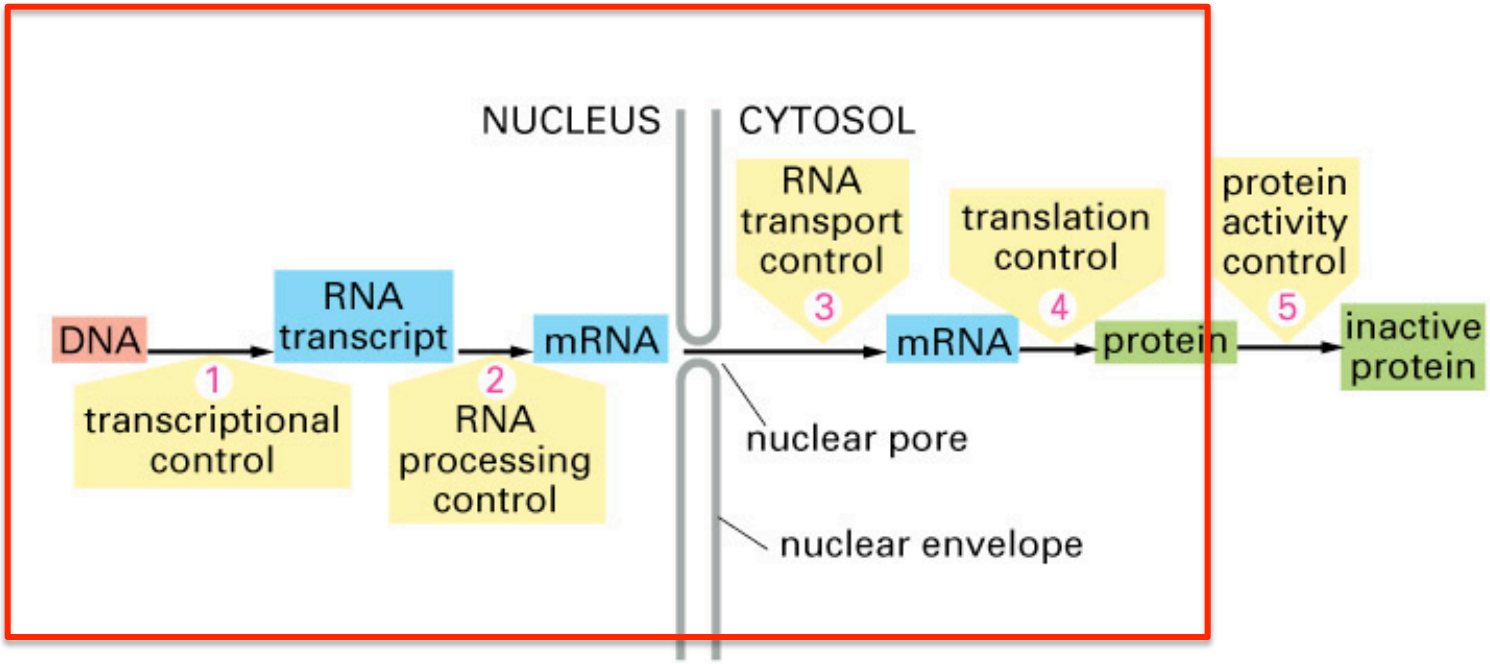
Progression through the cell cycle: regulatory levels

✓ **Gene expression**: activation of specific genes in the moment of the cell cycle when the function of the corresponding protein product is required

✓ **Post-translational modifications** (e.g. **Phosphorylation**): modulation of protein activity in specific cell cycle phases

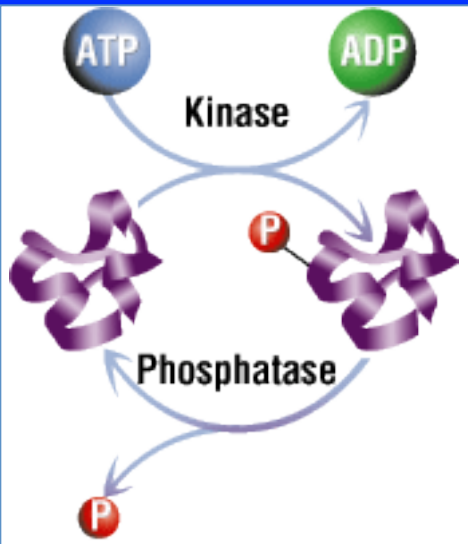
✓ **Protein degradation**: controlled elimination through proteolysis of specific proteins when their function is completed

From genes to proteins: multiple levels of control



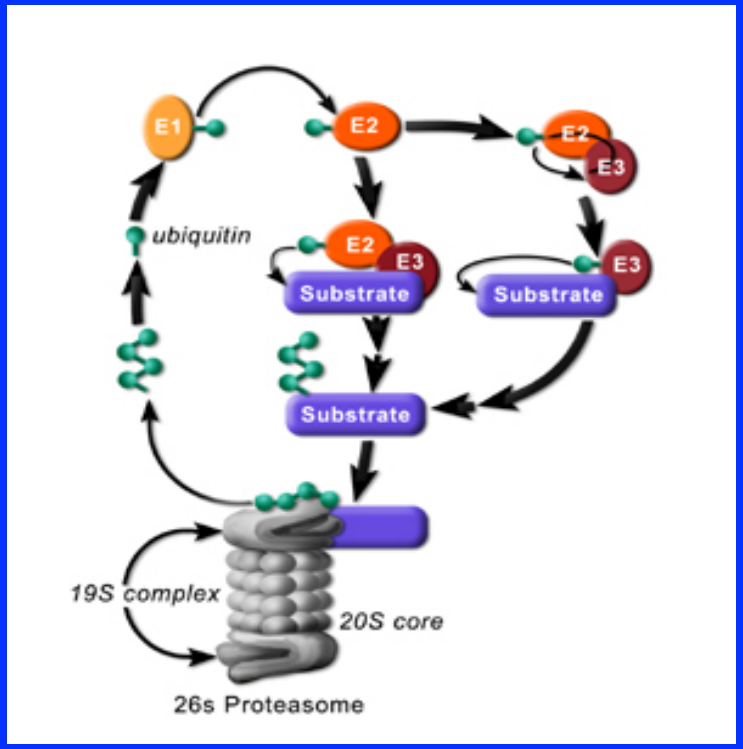
Two important examples of protein activity control

Phosphorylation

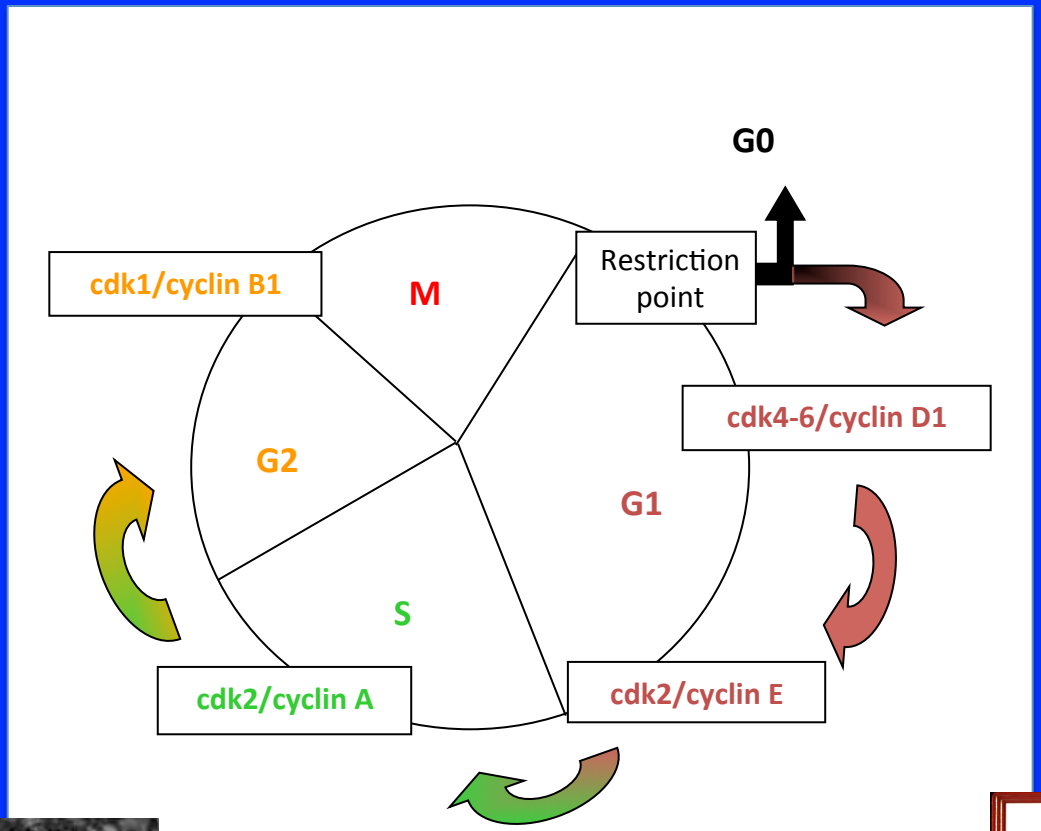


- Conformational changes : activation
- Localization
- Binding to specific partners

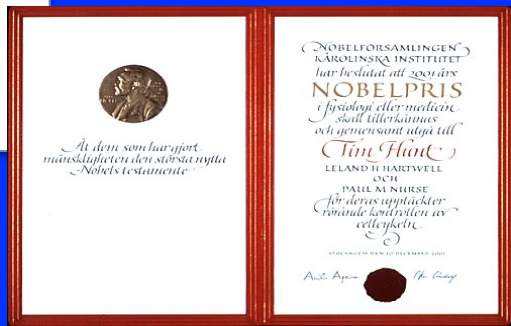
Proteolysis



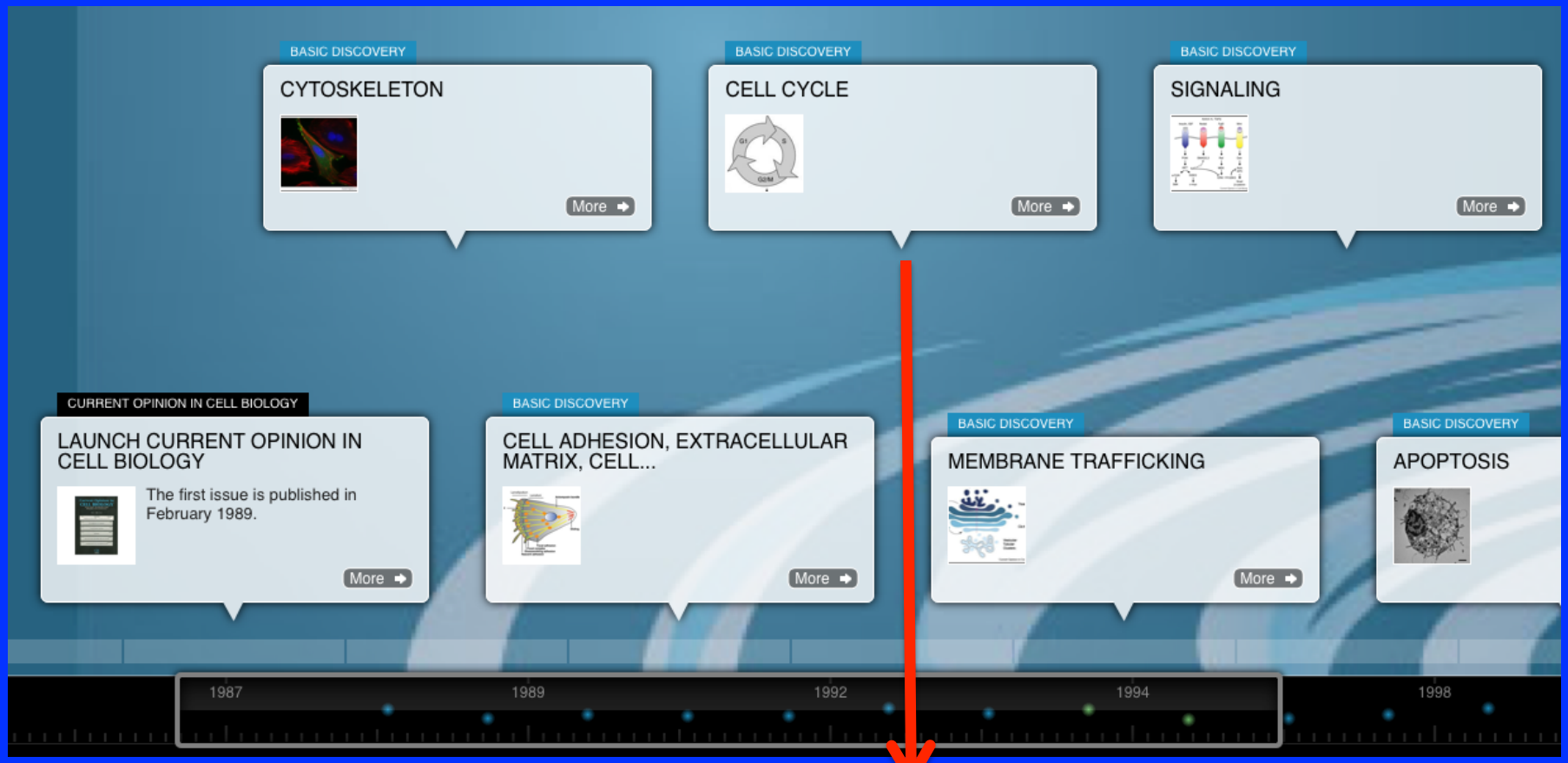
cyclin/cdk complexes are the main "enigines" of the cell cycle



Hunt, Nurse, Hartwell



cyclin/cdk complexes: a milestone in the cell cycle field



Current Opinion in Cell Biology 25 Years

Maturation promoting factor, cyclin and the control of M-phase

T. Hunt

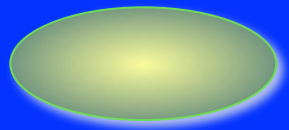
Department of Biochemistry, University of Cambridge, Cambridge, UK

Current Opinion in Cell Biology 1989, 1:268–274

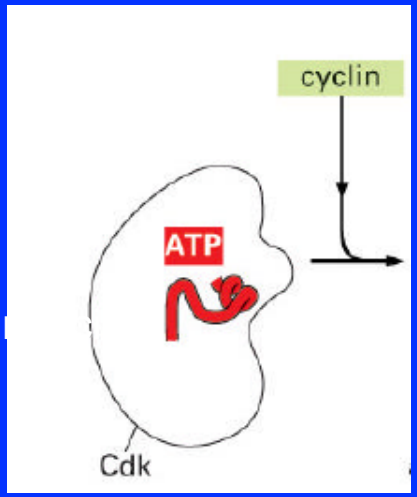
CYCLIN/CDK COMPLEXES: the regulatory mechanism

– regulatory subunit
– periodic expression during the cell cycle

CYCLIN

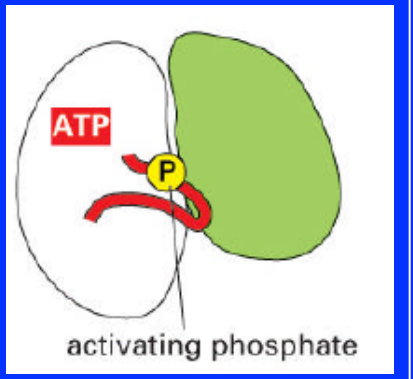


– catalytic subunit
– active only when bound to cyclin
–costitutively expressed during the cell cycle



CYCLIN-DEPENDENT KINASE (CDK)

ACTIVE COMPLEX



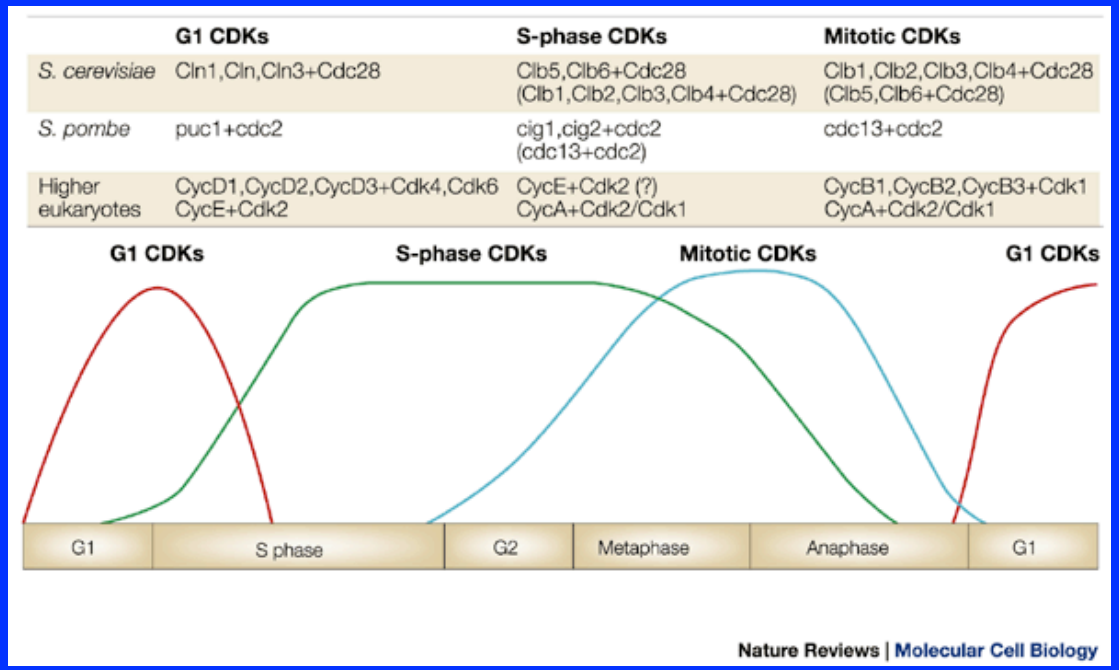
Integration of different levels of control of the cell cycle: the example of cyclin-cdks complexes

✓ cdks are active in complex with their regulatory subunit, cyclin

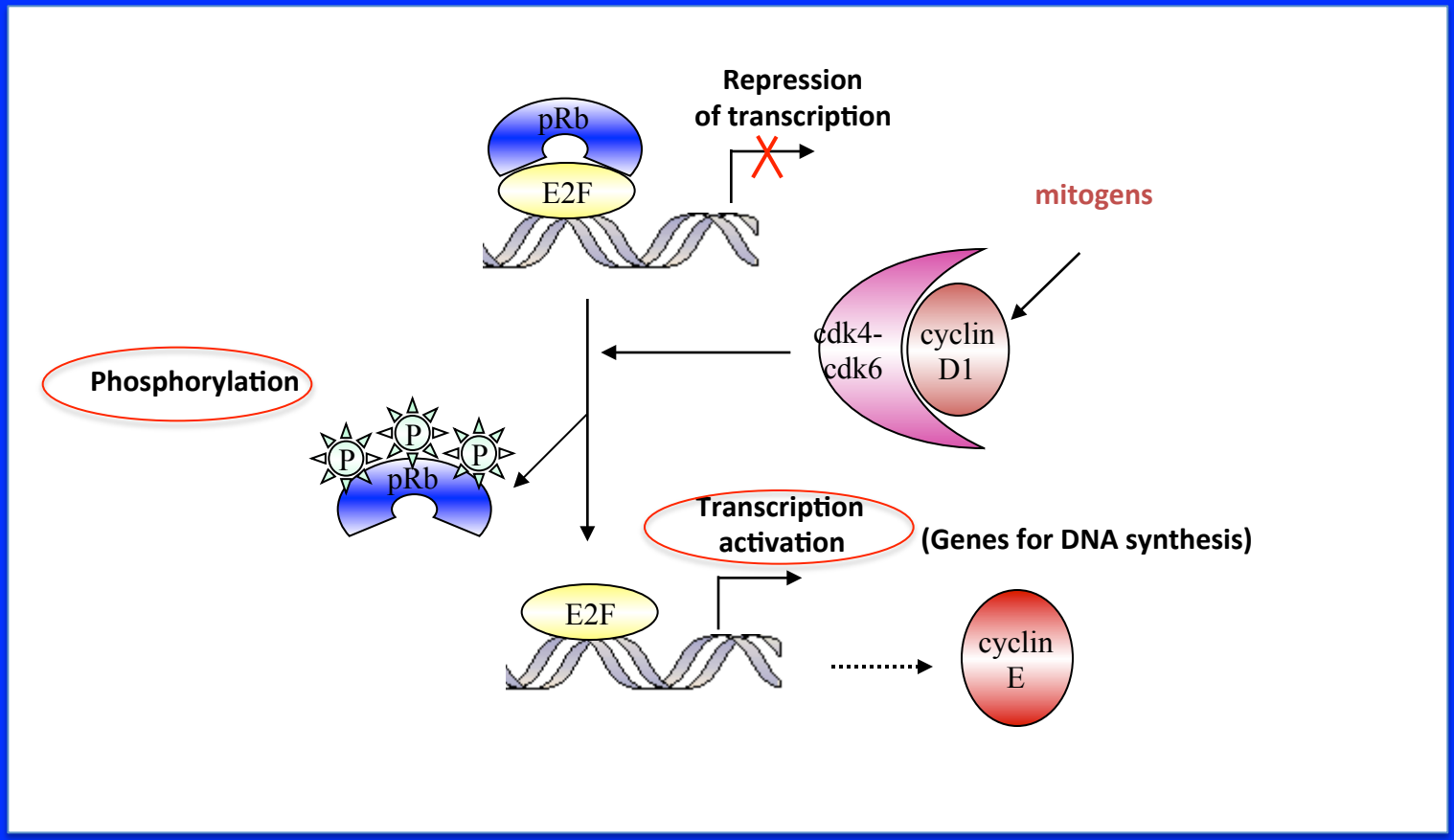
✓ fluctuations of cyclins levels are due to cell cycle-dependent **trascriptional activation** and regulated **degradation**

✓ Specific complexes cyclin/cdk characterize different cell cycle phases and trigger the transition from one phase to the subsequent by **phosphorylating** key downstream substrates

	G1 CDKs	S-phase CDKs	Mitotic CDKs
S. cerevisiae	Cln1,Cln,Cln3+Cdc28	Clb5,Clb6+Cdc28 (Clb1,Clb2,Clb3,Clb4+Cdc28)	Clb1,Clb2,Clb3,Clb4+Cdc28 (Clb5,Clb6+Cdc28)
S. pombe	puc1+cdc2	cig1,cig2+cdc2 (cdc13+cdc2)	cdc13+cdc2
Higher eukaryotes	CycD1,CycD2,CycD3+Cdk4,Cdk6 CycE+Cdk2	CycE+Cdk2 (?) CycA+Cdk2/Cdk1	CycB1,CycB2,CycB3+Cdk1 CycA+Cdk2/Cdk1

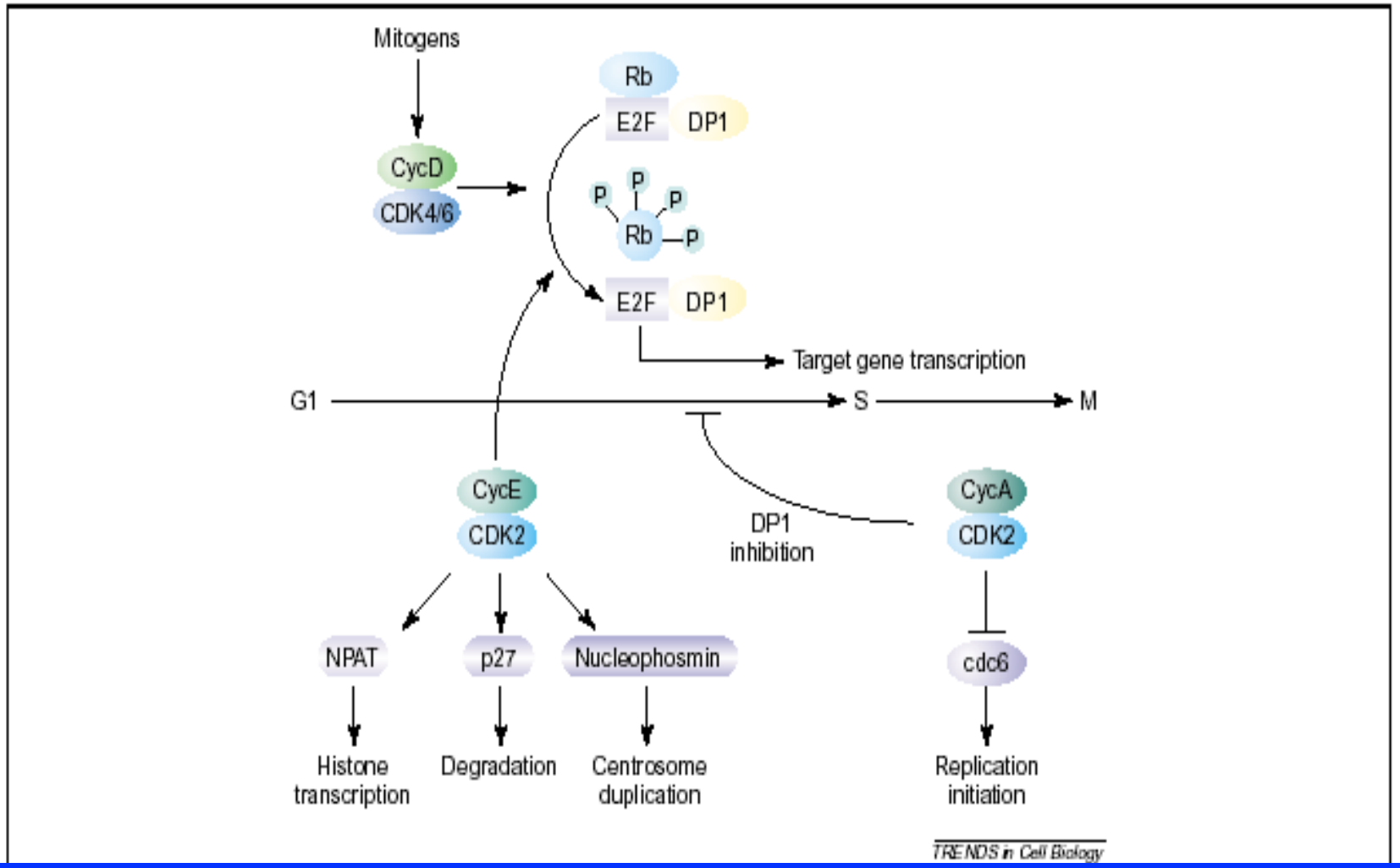


From external signals to transcriptional activation



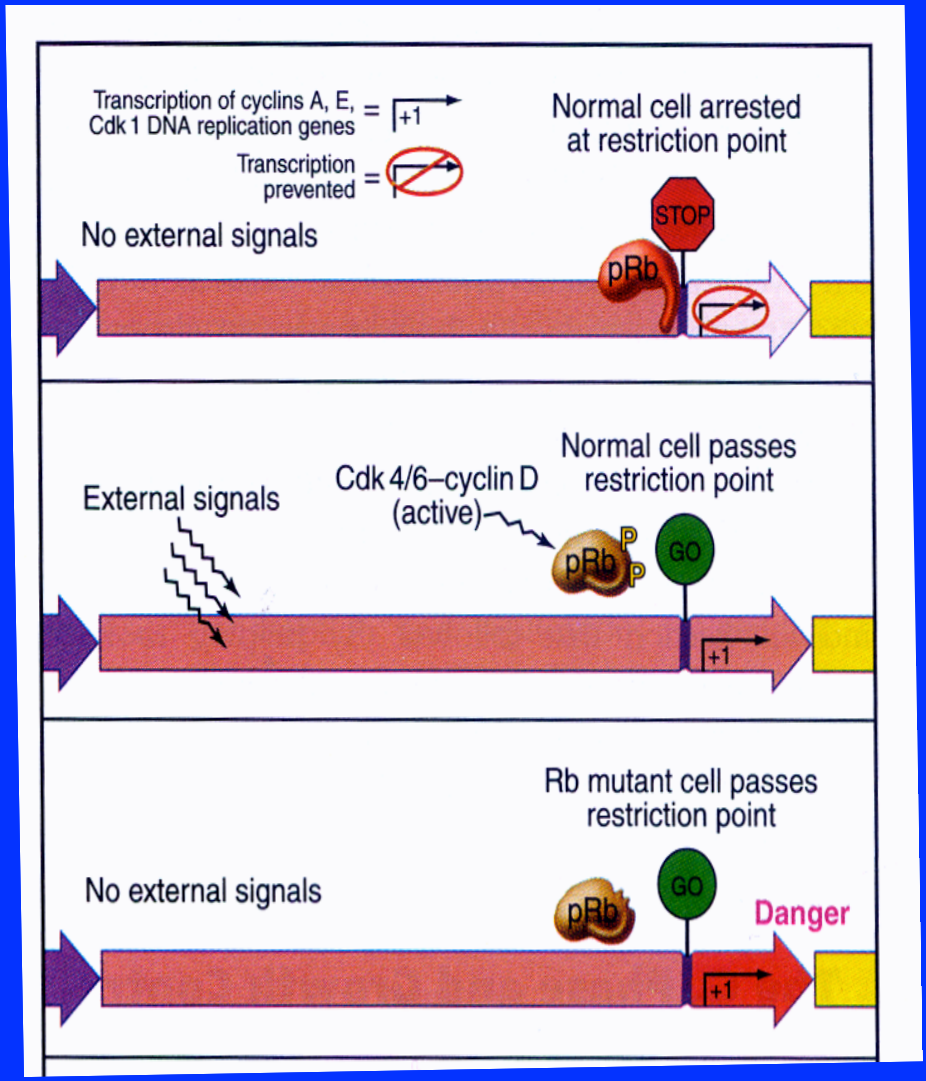
During interphase, downstream of cyclin-cdks, waves of transcriptional activation are observed

Integration of different levels of control of the cell cycle: the transcriptional repressor pRb downstream of cyclin/cdk complexes



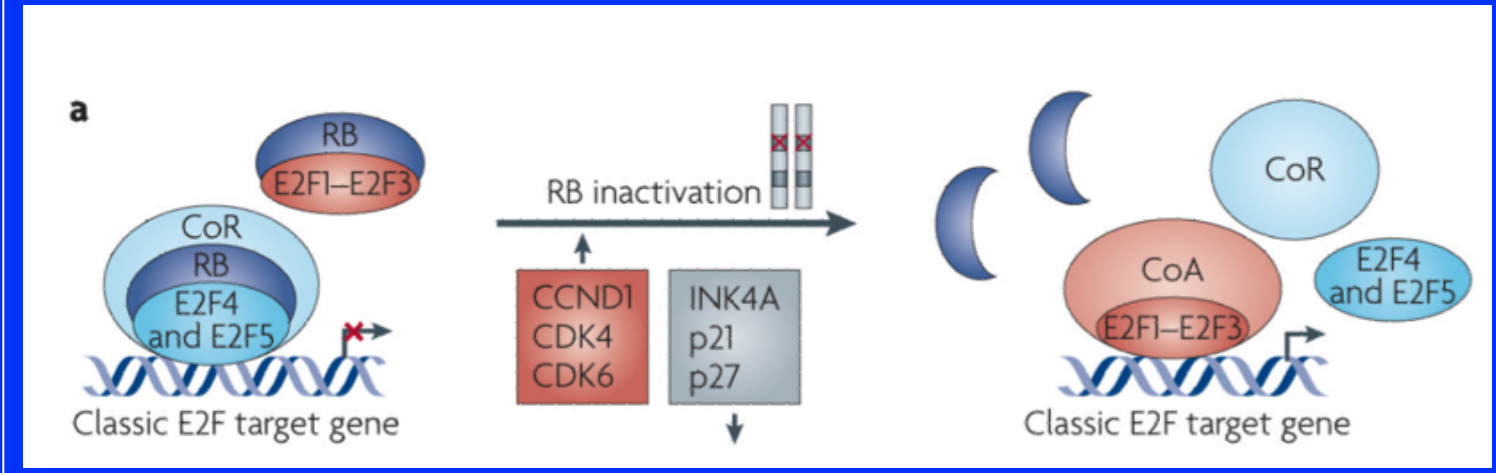
Mutations in the RB-1 gene, restriction point and tumorigenesis

The RB-1 gene, coding for the pRb protein, is mutated in retinoblastoma (child tumor of the retina) and also mutated or functionally inactive in 1/3 of human tumors: tumor suppressor gene

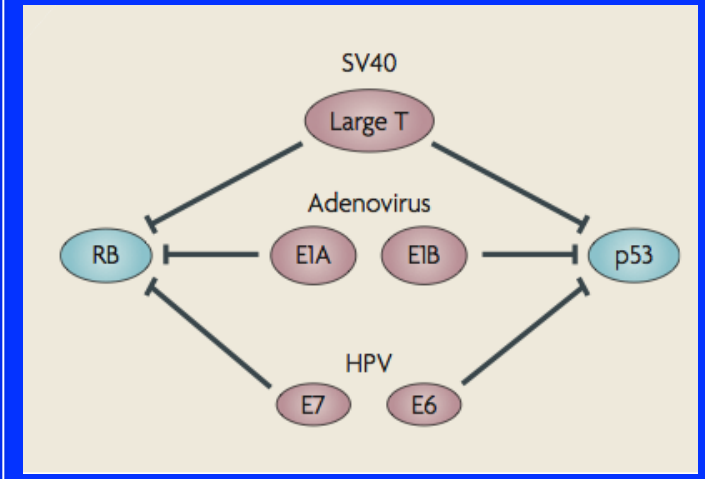


Cells with non functional pRb go through the restriction point even in the absence of the correct external signals.

Altered pRb/E2F pathway and transformation: molecular mechanism



Chen et al., Nat Rev Cancer. 2009



The example of oncoviruses: viral oncoproteins use the pRb/E2F pathway as a target

Polager and Ginsberg, Nat Rev Cancer. 2009

Alterations in E2F genes in tumors

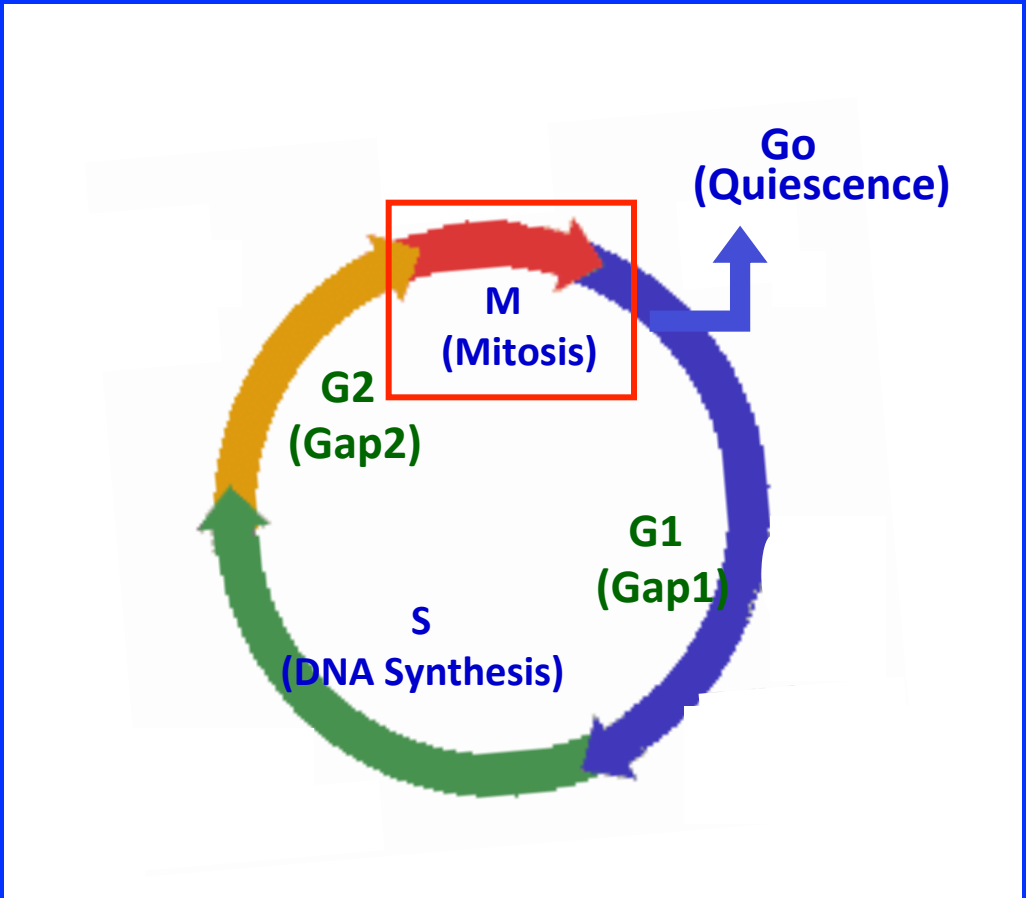
Table 3

Genetic alterations of E2F family members in human cancers

Gene (chromosome)	Genetic alteration	Human cancer	Notes
E2F1 (20q11.2)	Amplification	HCC[96-98], oesophageal SCC[175], NSCLC[176] and cancer[177–179]	None
	Increased expression	NSCLC[111,135,179,180], SCLC[112,181], glioblastoma[109], oesophageal SCC[181,182], HCC[183,184], pancreatic ductal carcinoma[185], and GI stromal[186], breast[187] and ovarian cancer[130,131,188]	None
	Decreased expression	Gastric adenocarcinoma[189], oral SCC[190], and colon[191] and bladder cancer[192]	None
E2F2 (1p36)	Amplification	SCLC[193], alveolar rhabdomyosarcoma[194] and osteosarcoma[195]	Detection of 1p32–1p36 amplicon in SCLC
	Deletion	Neuroblastoma[196], pheochromocytoma[197] and breast cancer[198]	None
	Increased expression	Ovarian cancer[130,131]	None
E2F3 (6p22)	Amplification	Retinoblastoma[105,106,133], uveal melanoma[199], and breast[200,201] and bladder cancer[103,104]	Detection of 6p21.2 amplicon in breast cancer; complete *RB1* inactivation through LOH in retinoblastoma
	Increased expression	NSCLC[111], SCLC[111], and bladder[103,104], breast[118], ovarian[188] and prostate cancer[202]	Decreased *RB1* expression in breast cancer
E2F4 (16q21–q22)	Amplification	Bladder cancer[101]	CGH analysis carried out on 12 transitional cell carcinoma lines
	Deletion	HCC[203] and breast cancer[117,204,205]	None
	Increased expression	Breast[117] and colon cancer[206]	None
	Mutation (AGC repeat)	GI cancer[207–209]	Expanded or reduced polyserine stretch and LOH frequently observed
E2F5 (8q21.2)	Amplification	Osteosarcoma[195], and bladder[102], colon[210] and breast cancer[128,200],	Minimal common region of 8q21.3–8q23 in osteosarcoma; *MOS* and/or *MYC* amplification in breast cancer
	Increased expression	Ovarian[115,188] and breast cancer[200]	None
E2F6 (2p25.1)	Amplification	Neuroblastoma[211] and ganglioneuroblastoma[212]	Detection of 2p25 amplicon in ganglioneuroblastoma; *MYCN* amplification in neuroblastoma
E2F7 (12q21.2)	Increased expression	Cutaneous SCC[132]	None
	Decreased expression	Ovarian cancer[115]	None
E2F8 (11p15.1)	Deletion	Medulloblastoma[213]	LOH of subchromosomal region 11p13–11p15.1
	Increased expression	Ovarian cancer[114]	None

Chen et al., Nat Rev Cancer. 2009

The cell division cycle:
alternation of di Synthesis e Mitosis



- **How to decide whether to enter in the division cycle?**

→ Response to external signals

- **How to control the temporal sequence of the division cycle?**

→ Checkpoint mechanisms (internal signals)

- **How to ensure the correct progression through the different cell cycle phases**

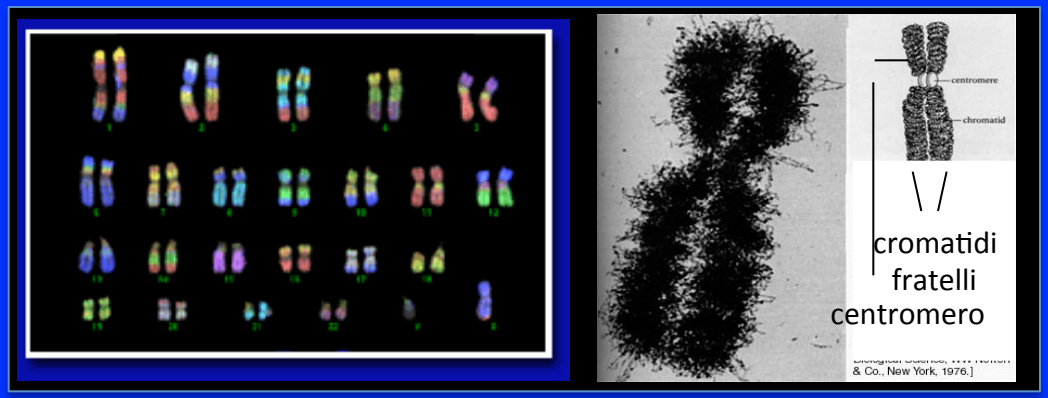
→ Regulatory mechanisms (genes, proteins)

- **How to distribute duplicated genetic material?**

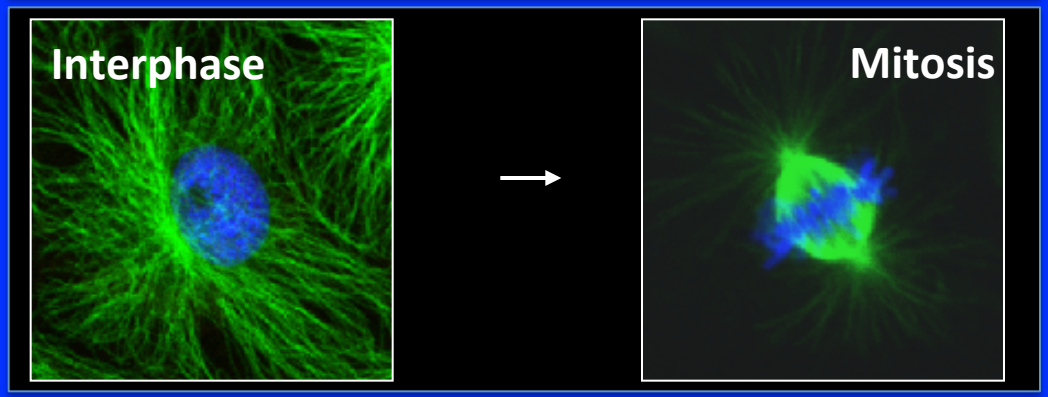
→ Mitosis

Mitotic entry: cellular changes

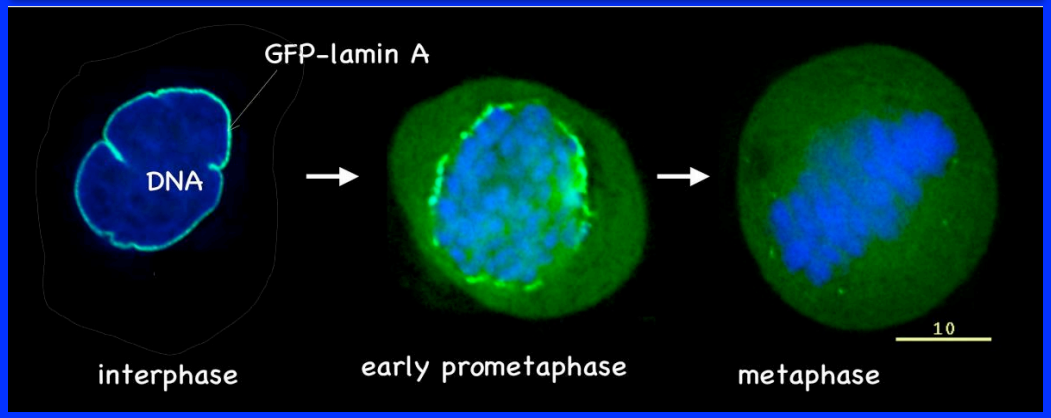
cdk1/cyclin B1 MPF



Chromosome condensation



Cytoskeletal changes

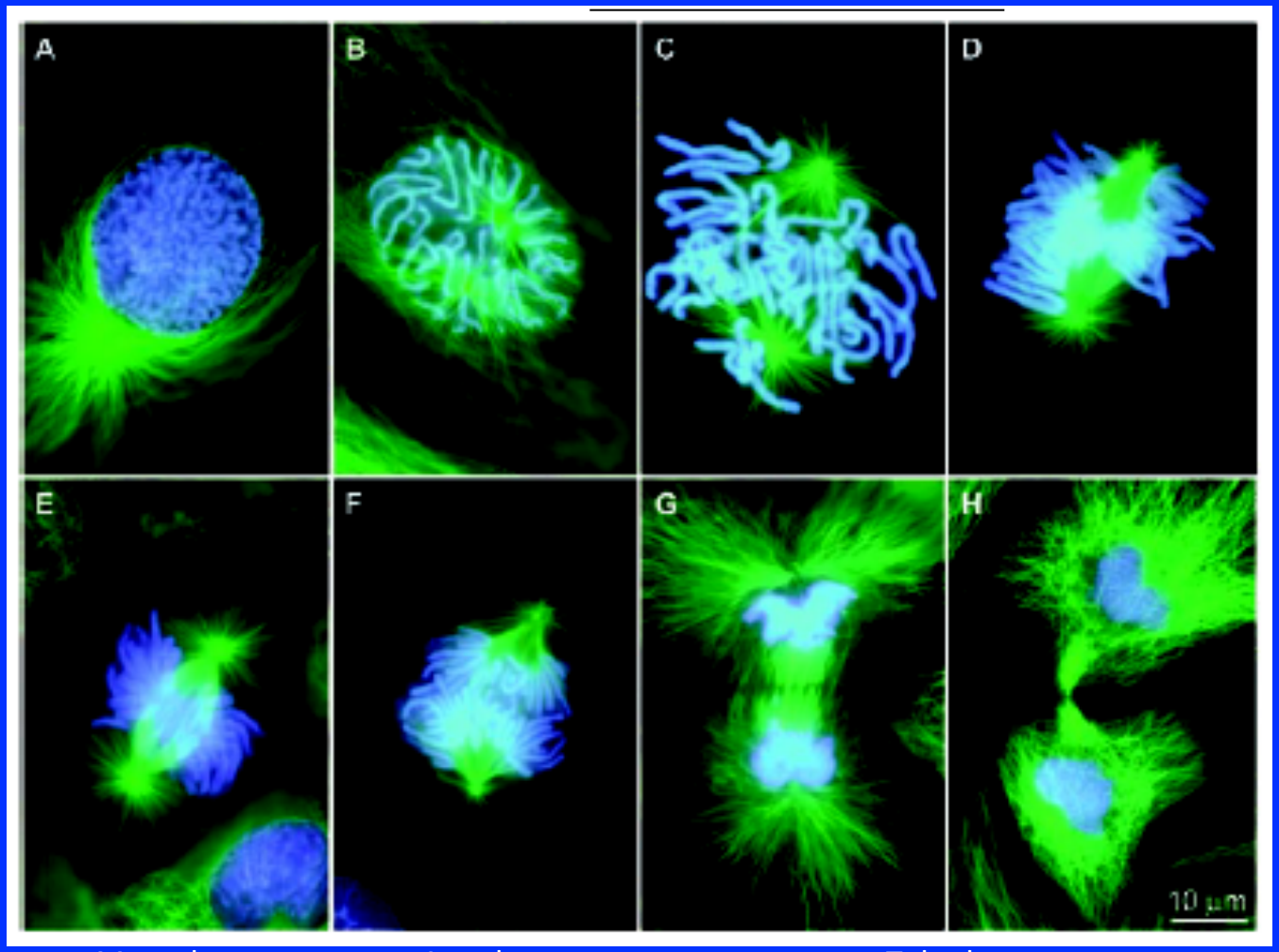


Nuclear envelope breakdown

Mitotic division

Prophase

Prometaphase



Metaphase

Anaphase

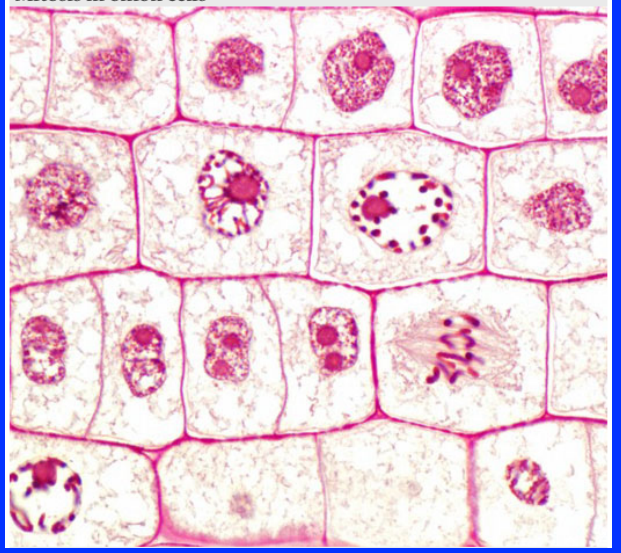
Telophase

microtubules
DNA

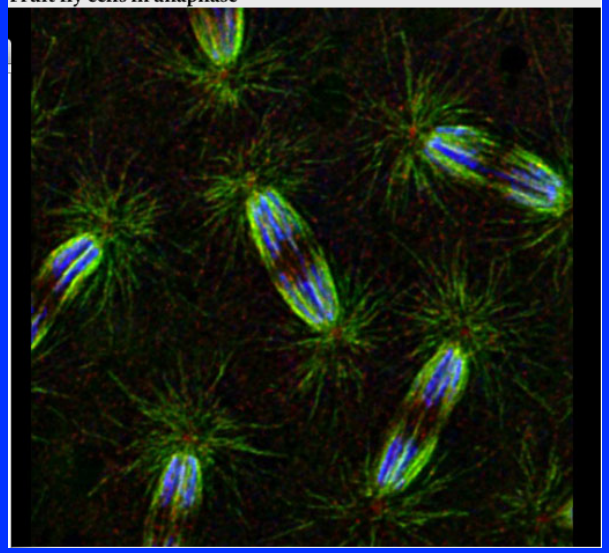
Rieder and Khodjakov, 2003 Science **300:** 91-96

"Seing" division

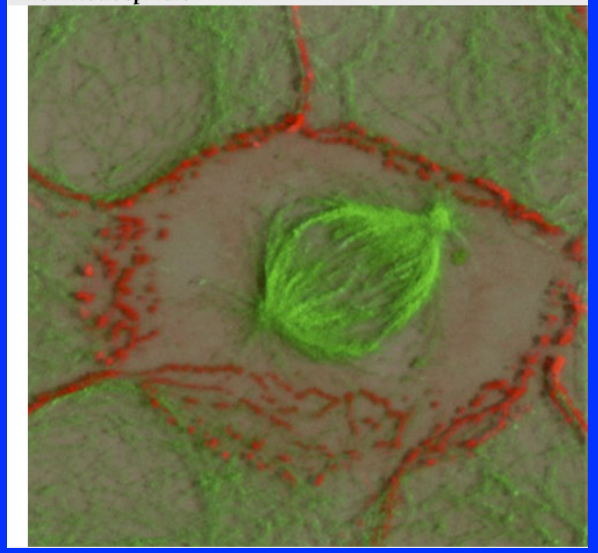
Mitosis in onion cells



Fruit fly cells in anaphase

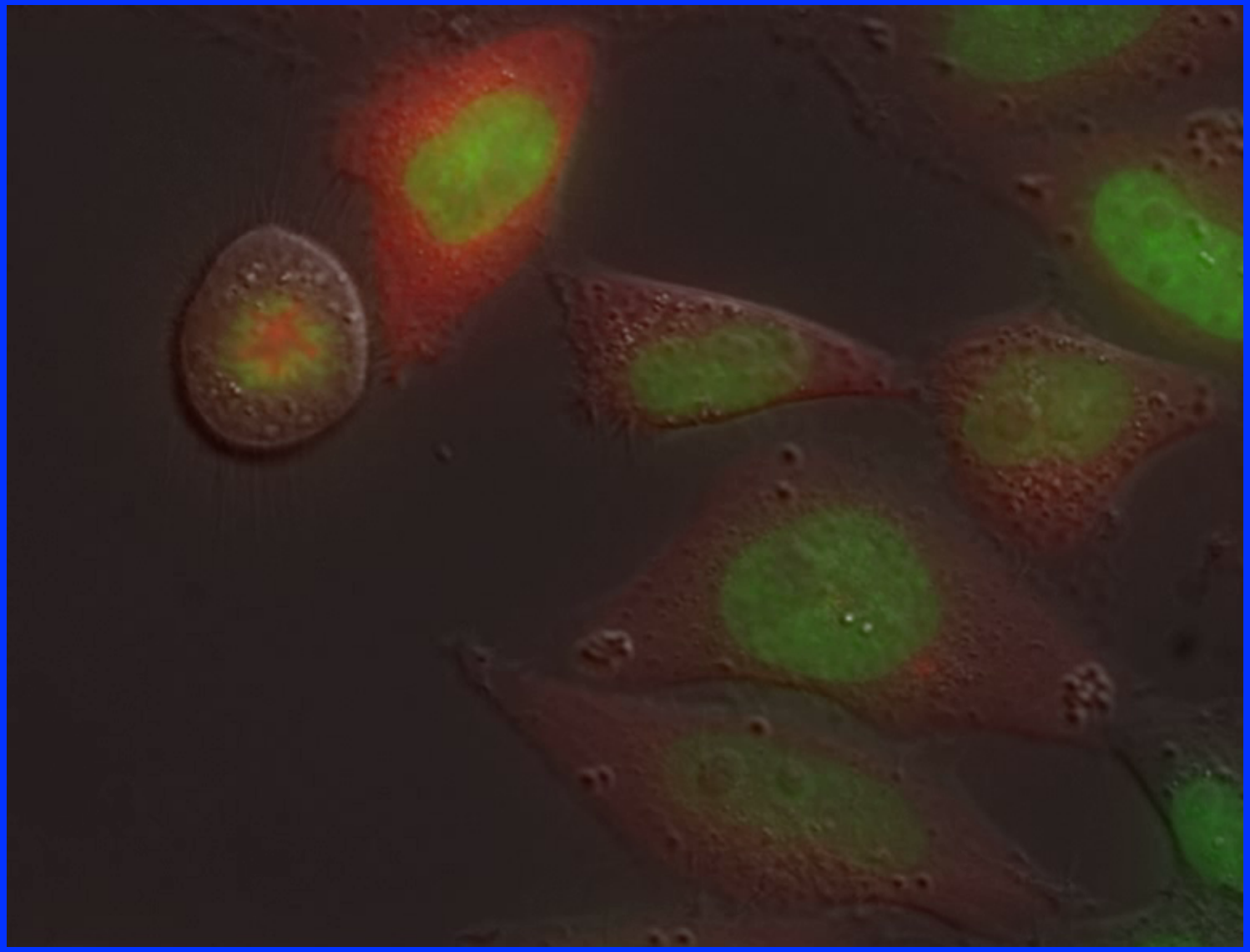


The mitotic spindle

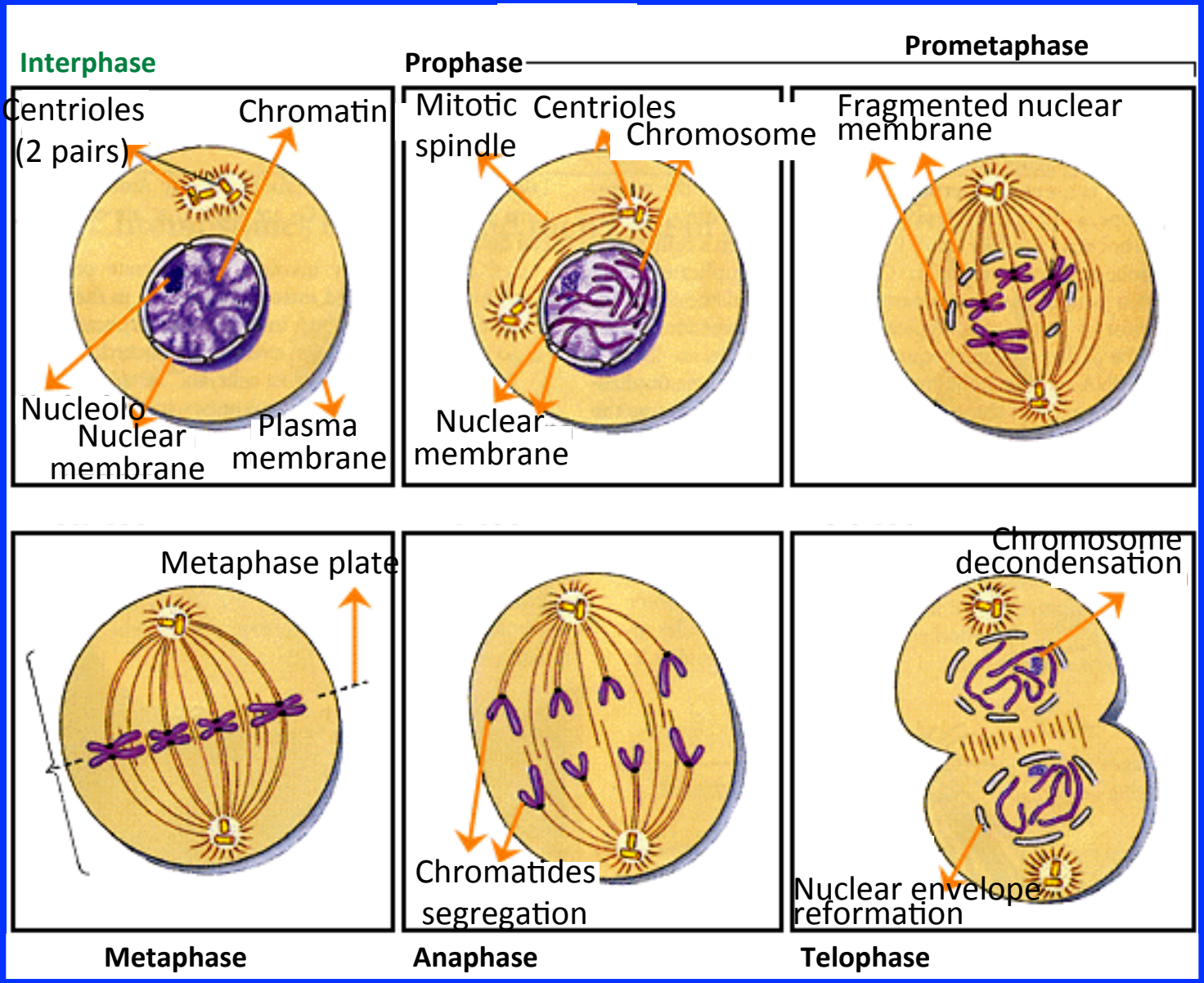


Wellcome Trust Image Gallery "Cell division: mitosis and cytokinesis"
http://bigpictureeducation.com/cell-division-images

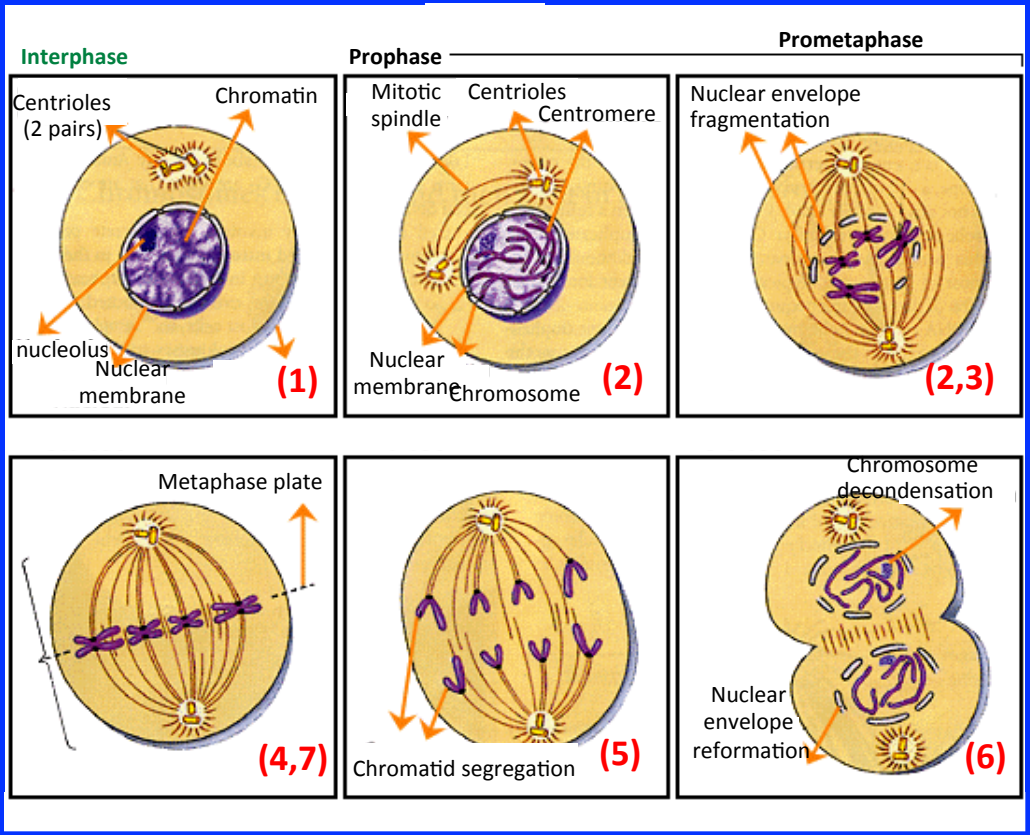
Mitotic division: a dynamic view



Mitotic division: a schematization



Control of mitosis



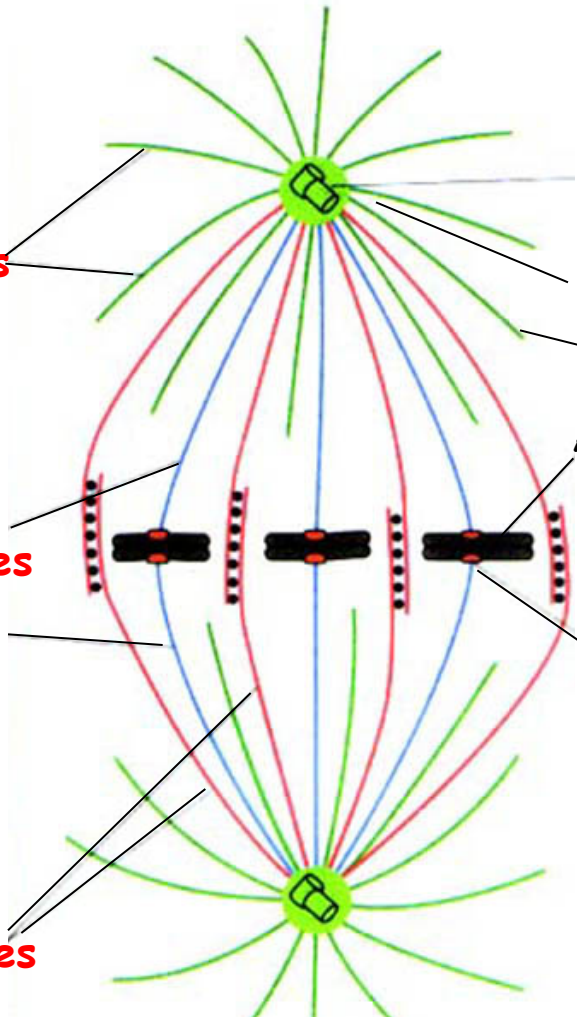
Regulation in space:

- chromosome condensation (1)
- mitotic spindle organization (2)
- contact microtubules /chromosomes (3)
- chromosome alignment (4)
- separation of sister chromatids (5)
- cytoplam division in the central region (6)
- mitotic spindle orientation (7)

Regulation in time:

This series of events must occur in a Coordinated manner and in a precise temporal sequence

The mitotic spindle

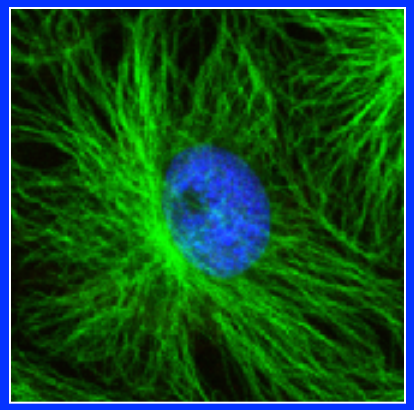


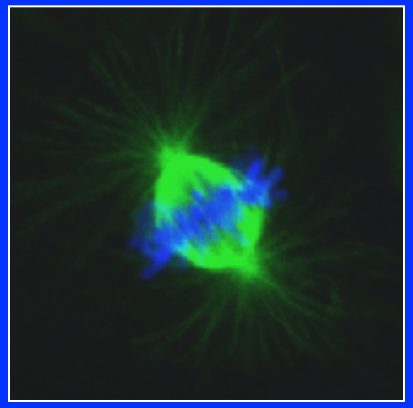
astral microtubules
Division plane

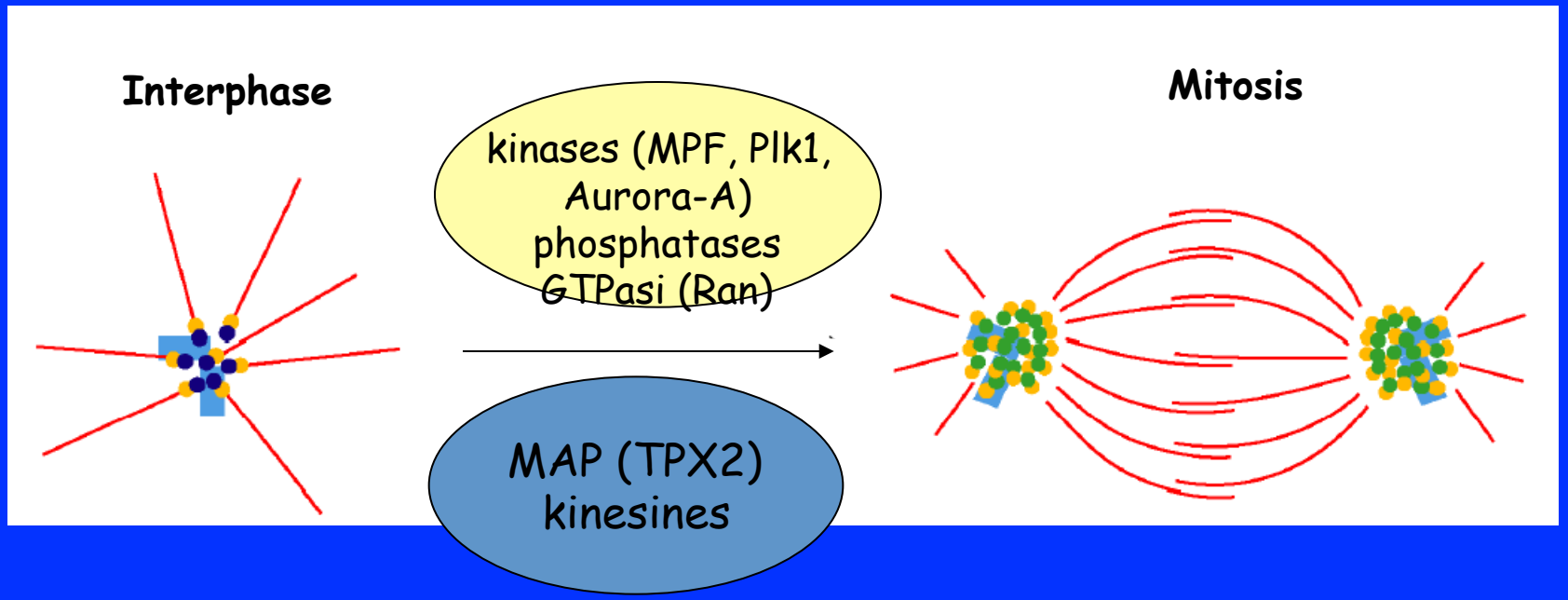
Kinetochore microtubules
chromosome alignment
in a metaphase plate

interpolar microtubules
Spindle bipolarity

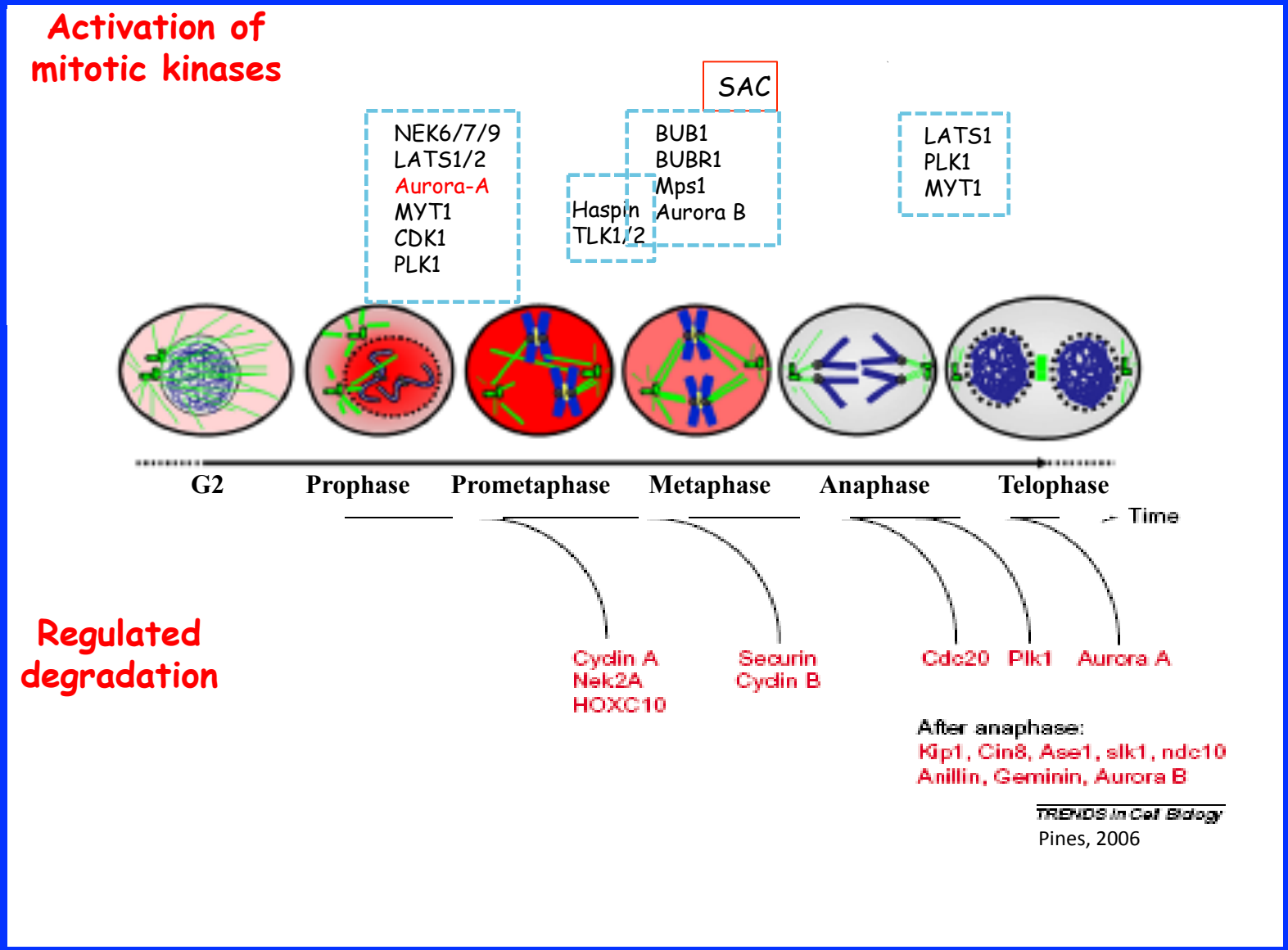
How to move from an interphase cytoskeleton to the mitotic spindle? The regulators







Phosphorylation and proteolysis control mitotic progression

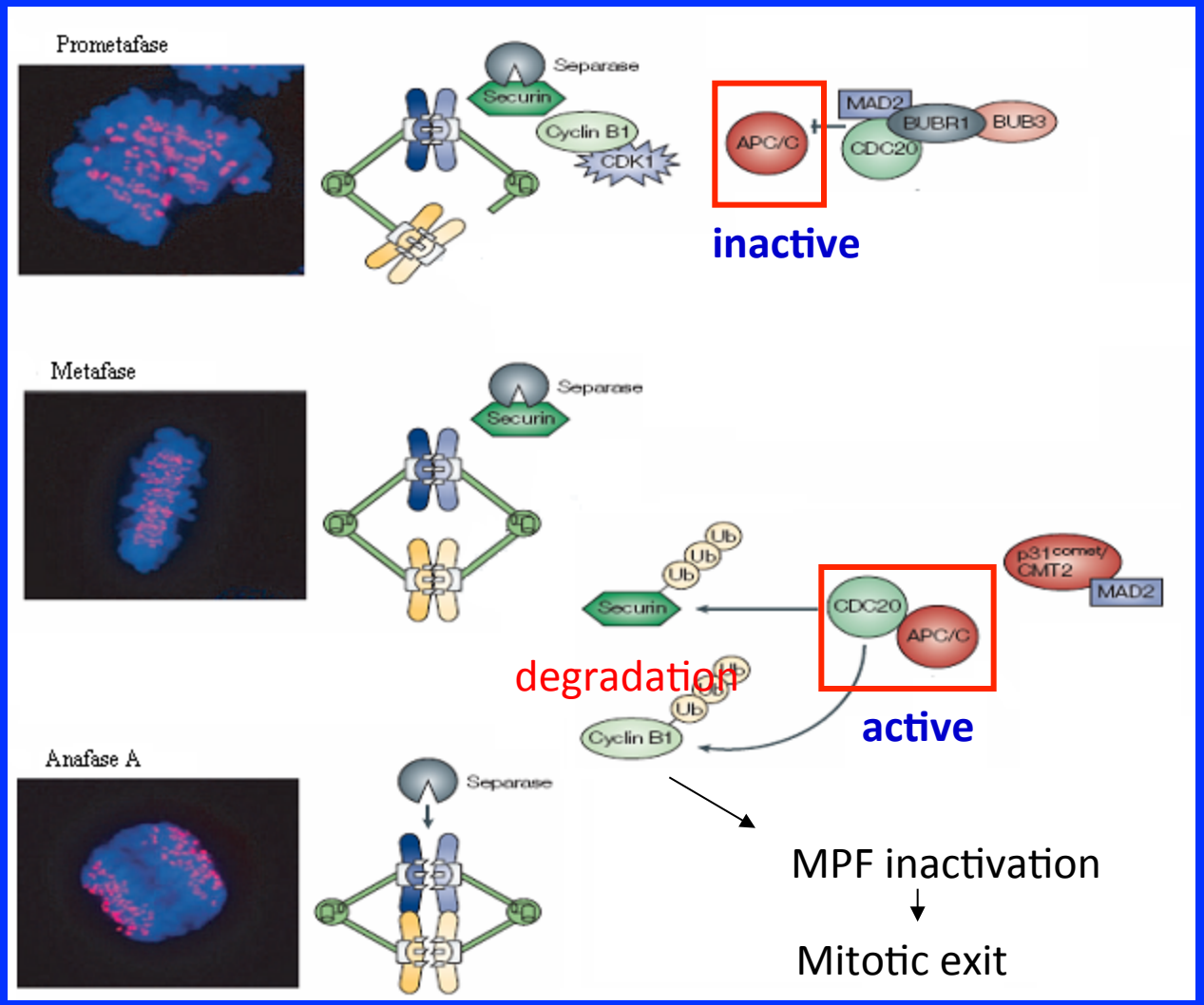


The mitotic checkpoint : chromatids separation only when all chromosomes are aligned!

presence of unattached chromosomes

all chromosomes attached to the spindle

Chromosome segregation

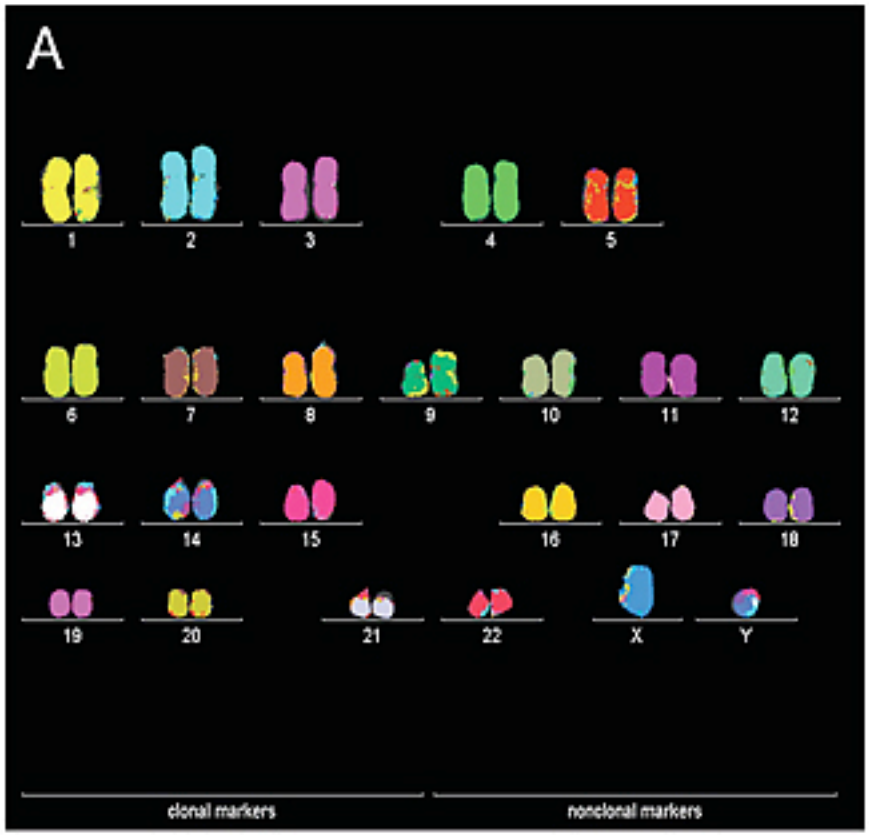


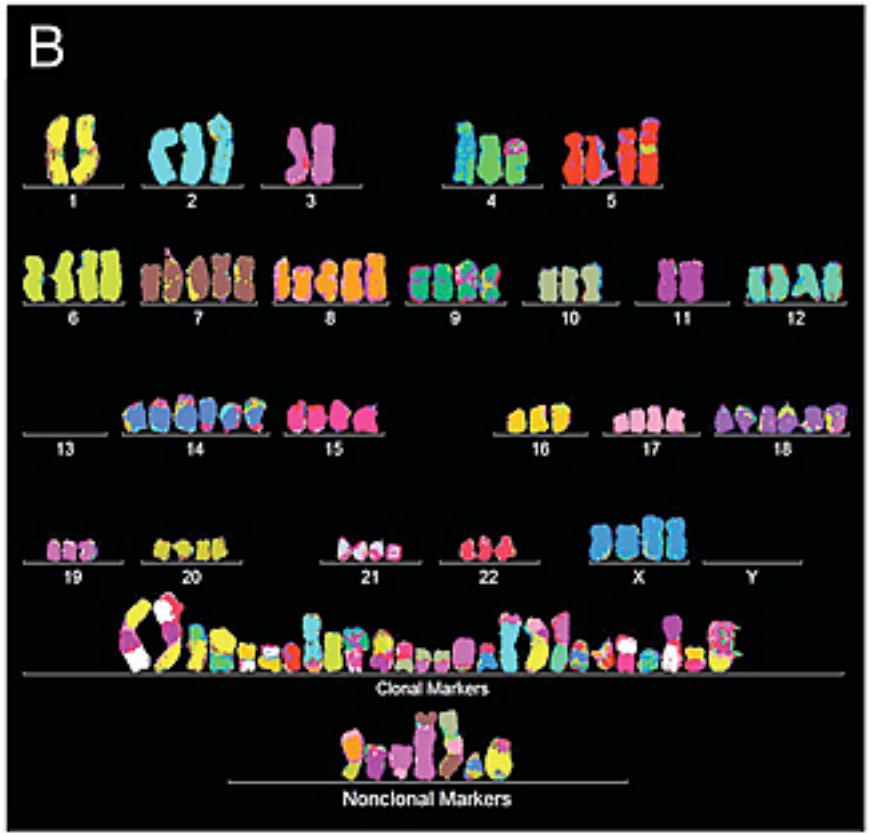
Kops et al., 2005

Loss of control of mitosis: neoplastic transformation

- cancer is caused by alterations in cell division
- the mitotic spindle is the apparatus that ensures balanced chromosome segregation
- centrosomes are the major organizers of the mitotic spindle in somatic cells

Tumor cells are strongly aneuploid (loss or gain of chromosomes)



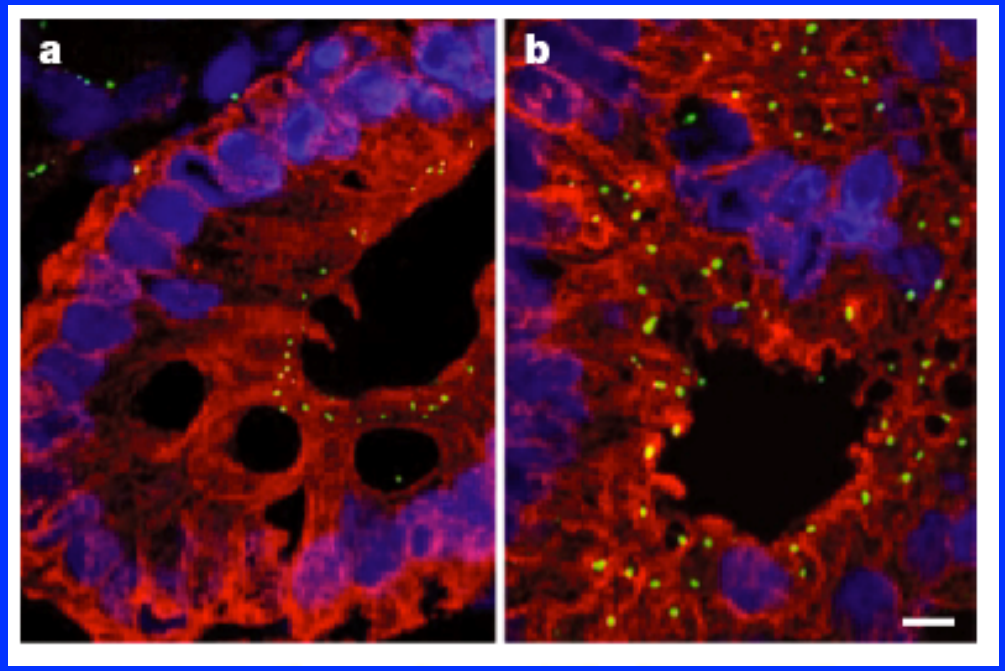


Normal cell

Tumor cell

Centrosome abnormalities and tumorigenesis (I): tumor tissues display abnormal centrosome number and activity

colon epithelium



Nigg, 2002

Normal tissue

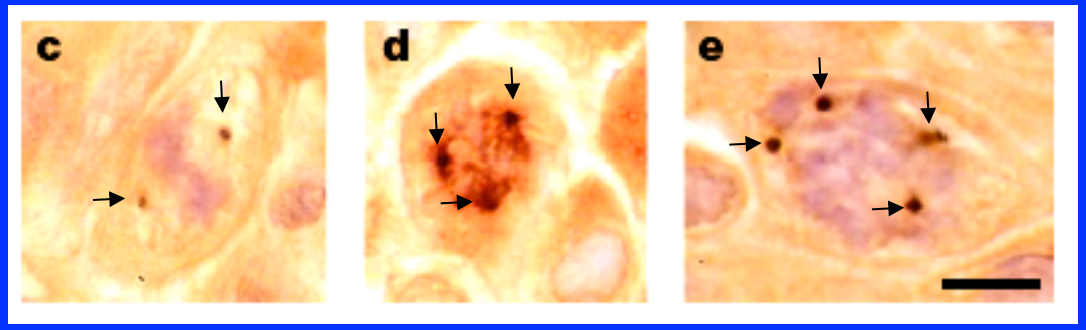
Tumor tissue

Centrosome abnormalities and tumorigenesis (II)

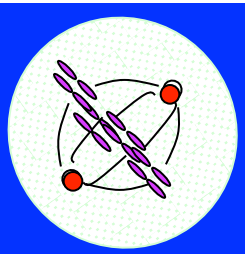
-Centrosomal defects can yield abnormal mitoses and chromosome mis-segregation...

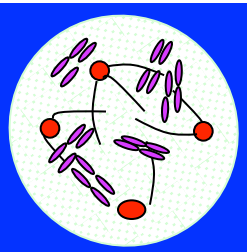
normal cell (prostate)

transformed cell (prostate)



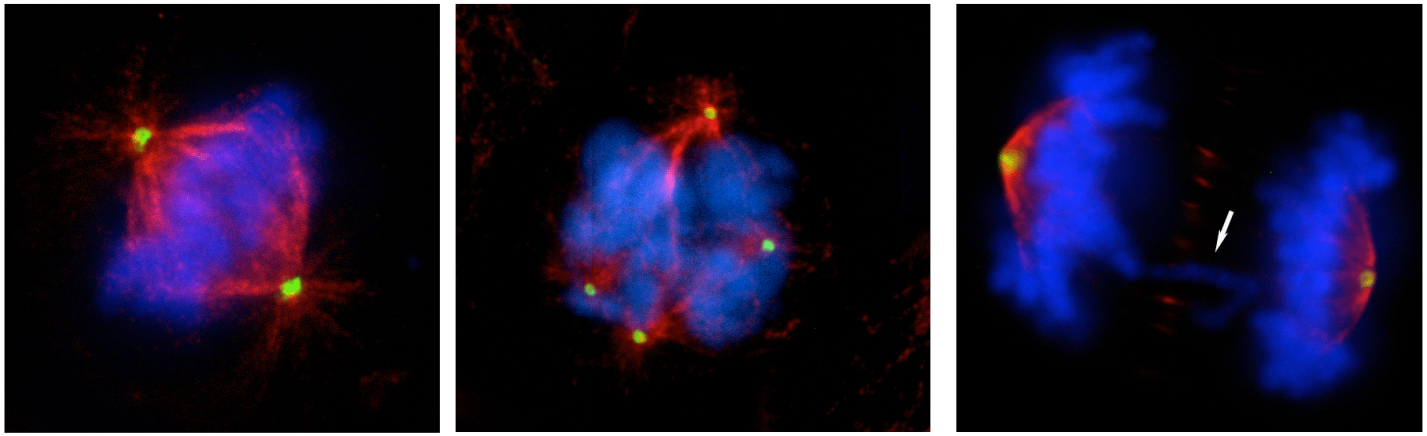
Nigg, 2002





... and thus contribute to generation of aneuploid cells

Mitotic spindle defects and mis-segregation



normal spindle | multipolar spindle | anaphase with lagging chromosome

(Lia Asteriti)

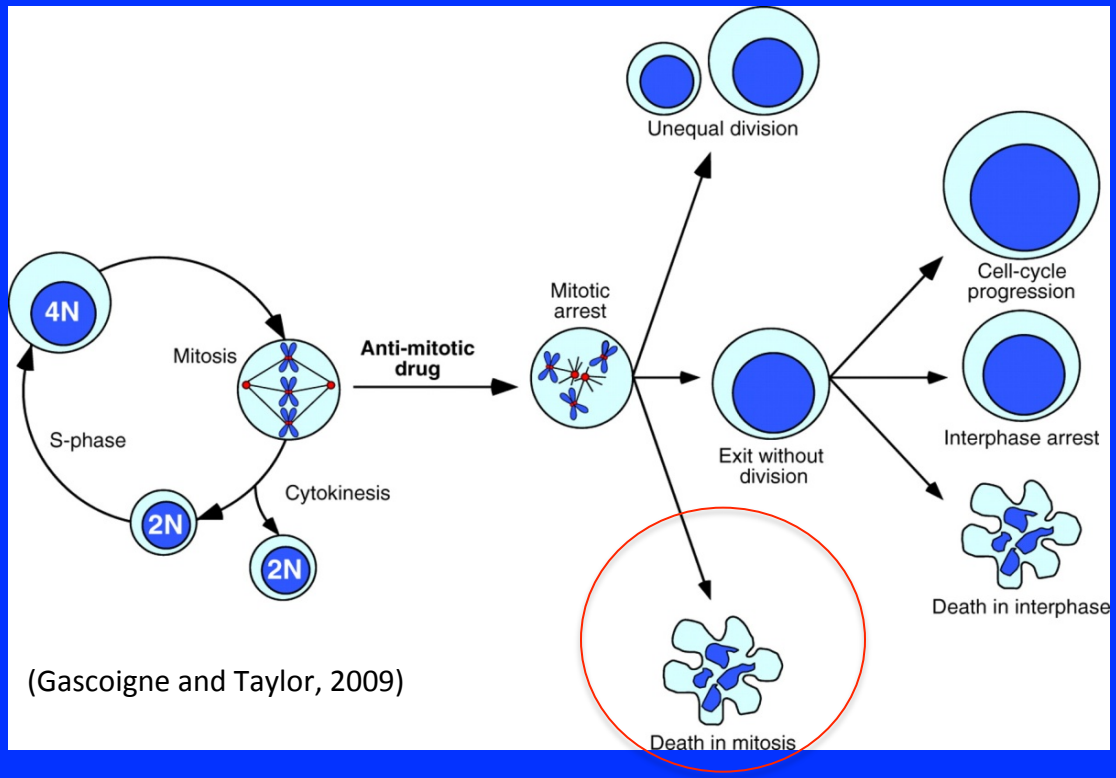
An abnormal number of spindle poles is not sensed by the mitotic checkpoint and can induce defective chromosome segregation

Mitosis as a therapeutic target

✓ mitosis is a selective target since tumor cells are dividing cells

✓ anti-microtubule compounds are used in chemotherapies

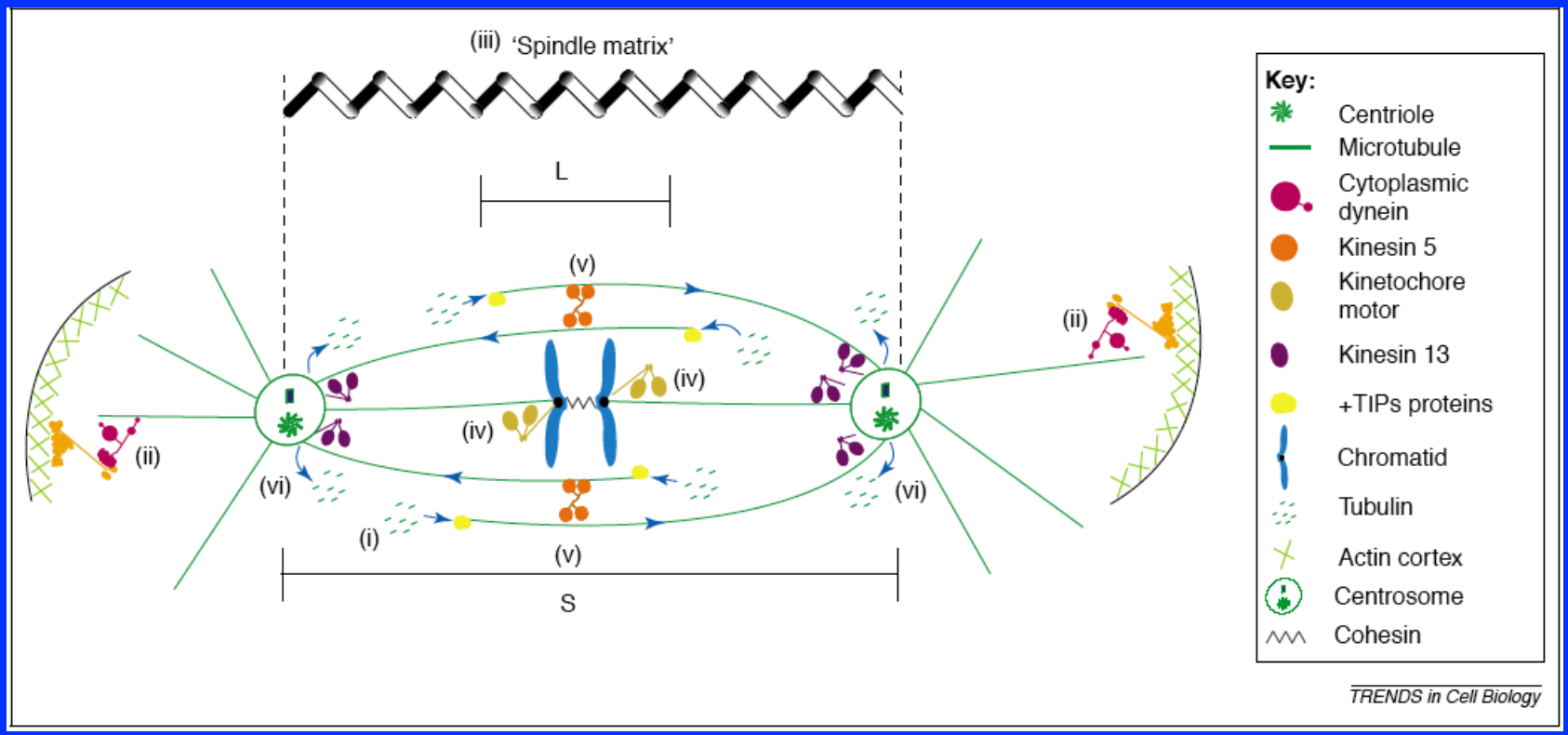
✓ mitotic regulators are expressed only in dividing cells and are often deregulated in cancer: development of specific inhibitors



(Gascoigne and Taylor, 2009)

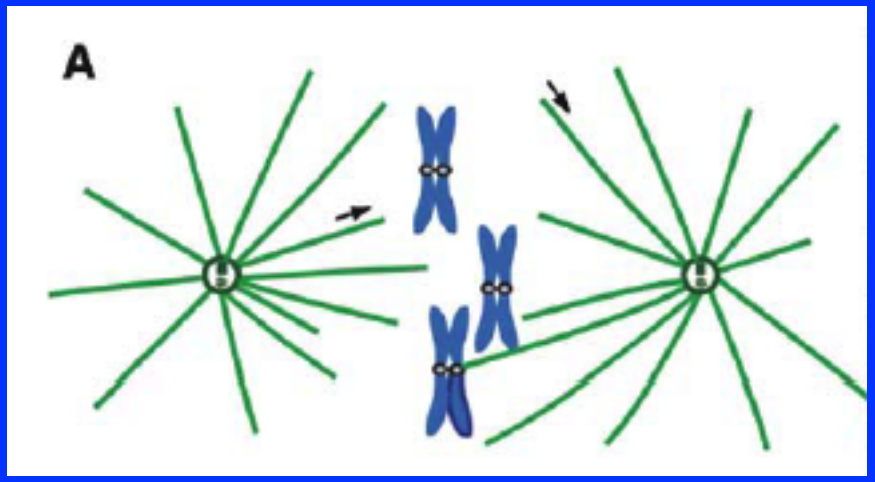
Matematical models of mitotic processes

(Modeling mitosis, Mogilner et al., 2006)



- Microtubule dynamics
- Mitotic spindle elongation
- Metaphase spindle length
- Spindle positioning
- Interaction microtubules/kinetochores
- Mitotic spindle checkpoint

Interaction chromosomes/microtubules: matematical model of search and capture

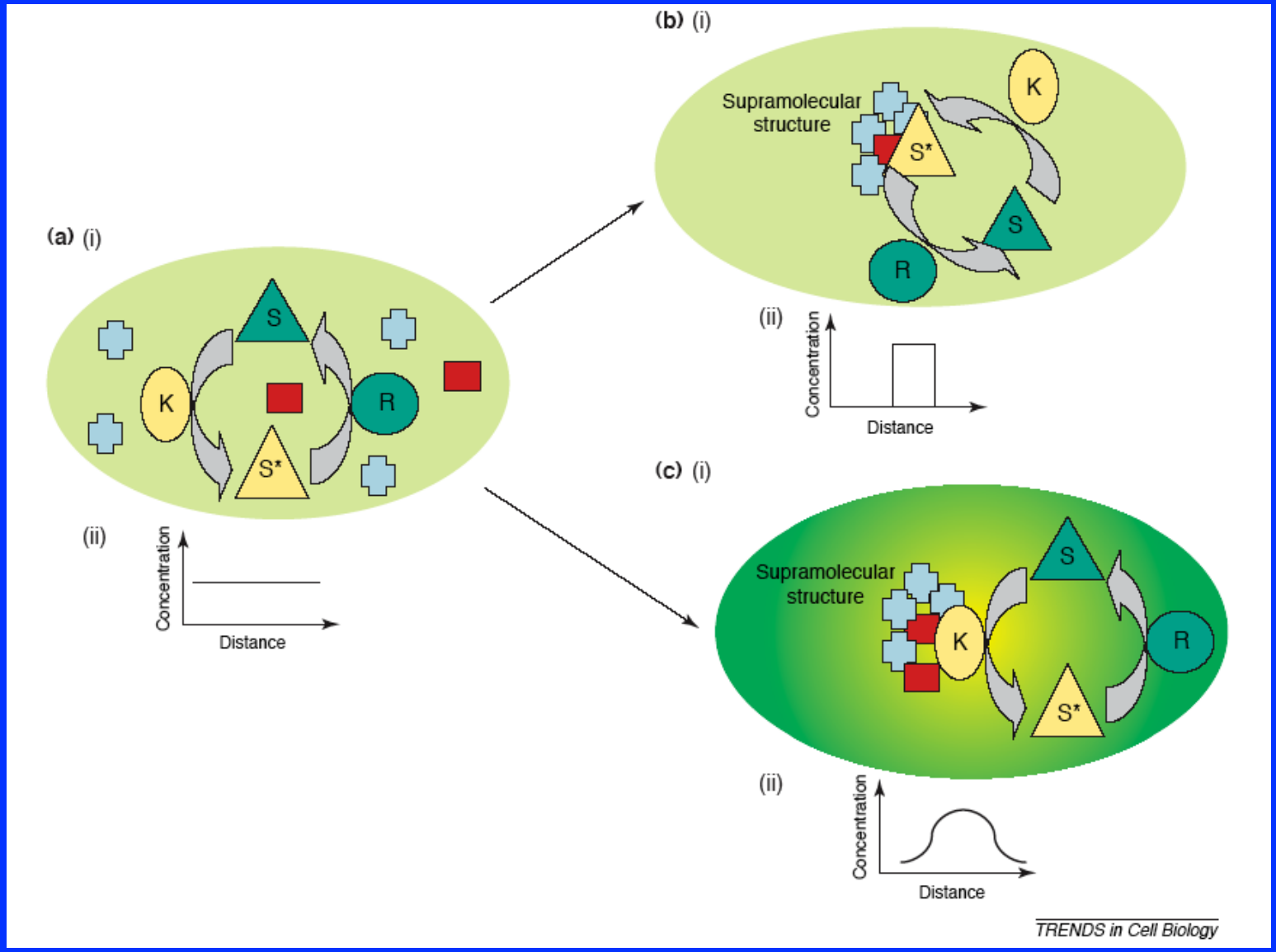


Modeling: if the process is random times are far too long repsect to those observed in living cells (20-30 minutes)

Hypothesis: a “bias” near chromosomes facilitates the process. Simulation under these conditions is consistent with times observed in cells.

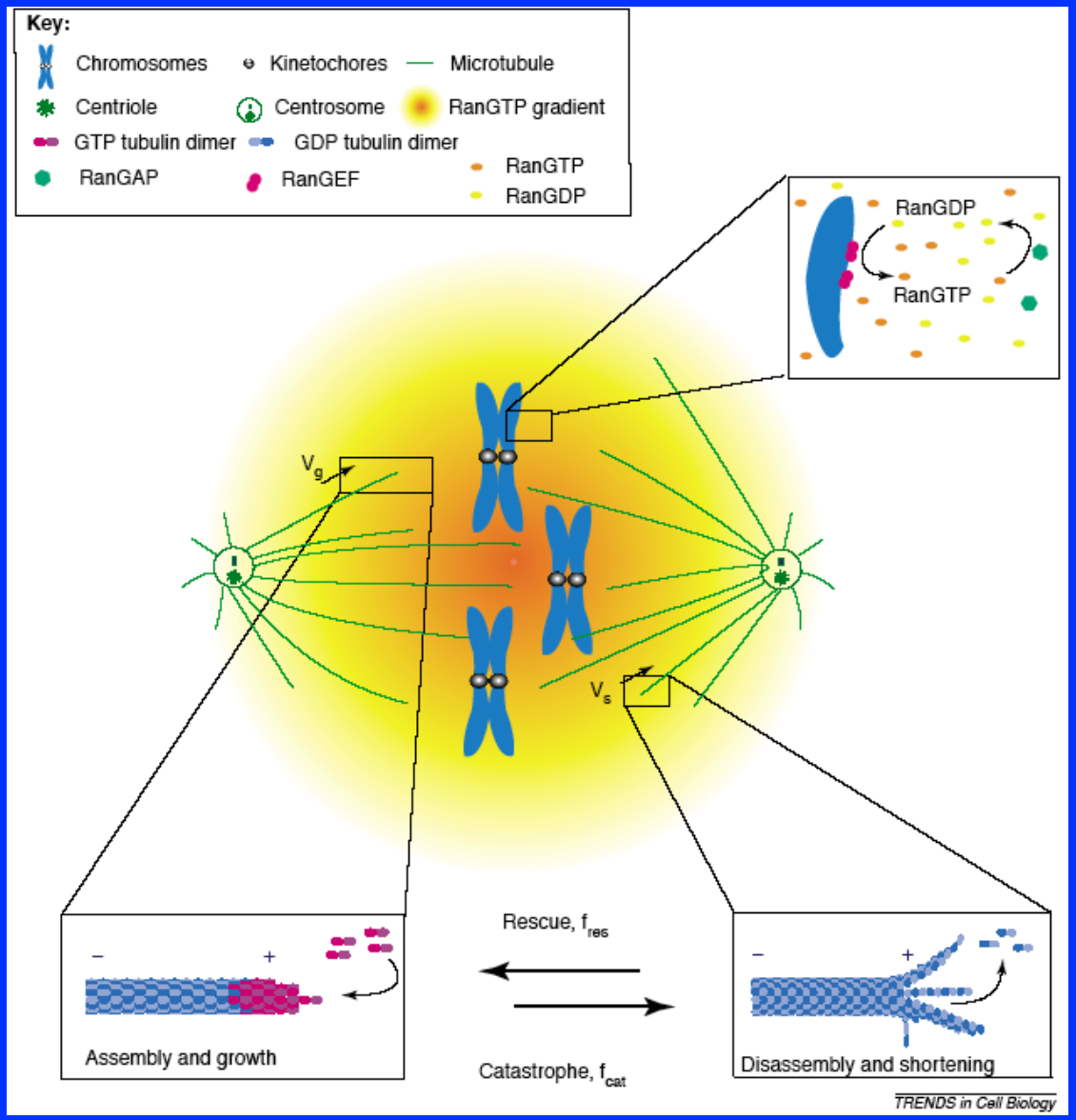
Wollman et al., 2005

Introduction of the gradient concept in spatial organization of the mitotic spindle

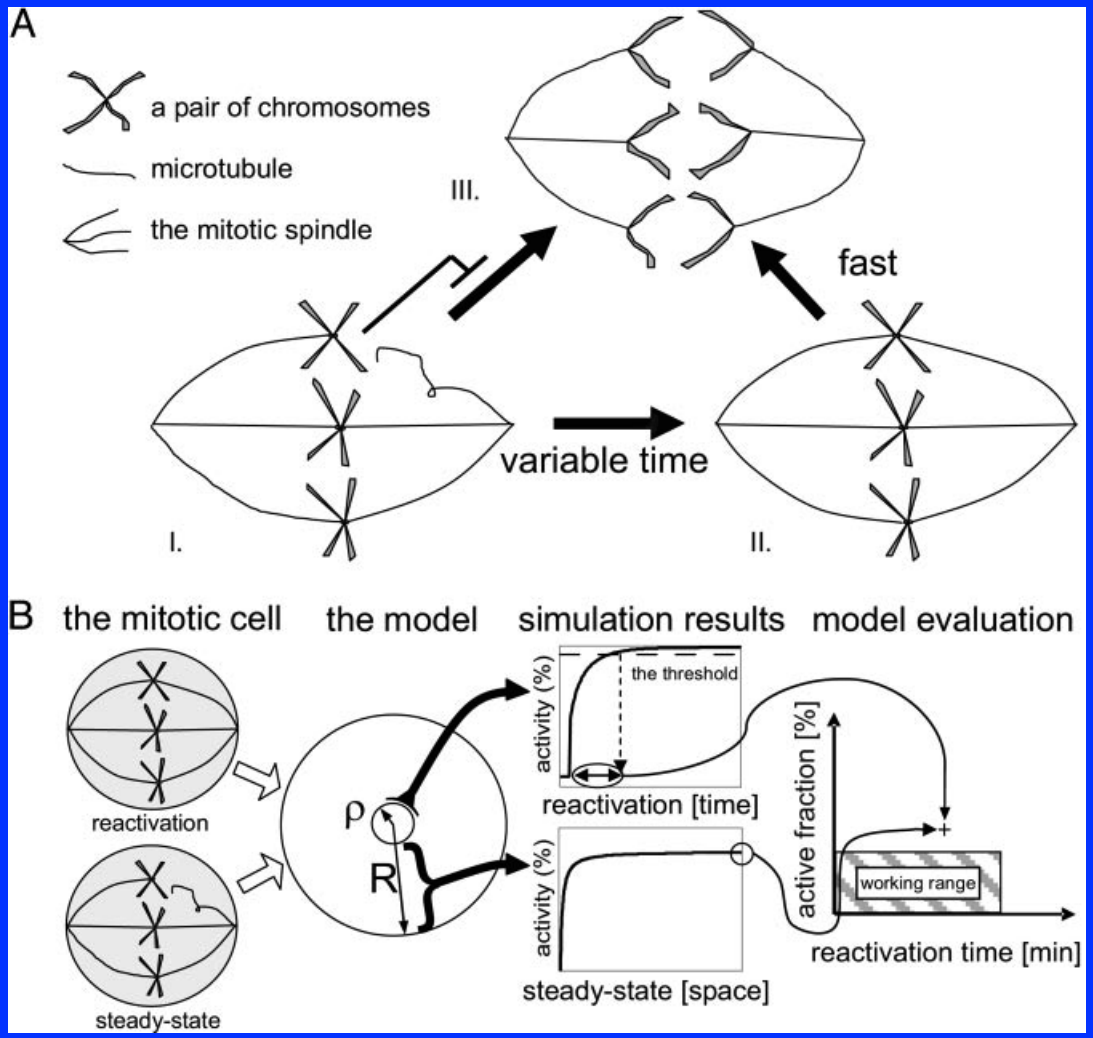


Bastiaens et al., 2006 - Gradients in the self-organization of the mitotic spindle

The "bias" in the search-and-capture model: the RanGTP gradient



Matematical model of the mitotic checkpoint



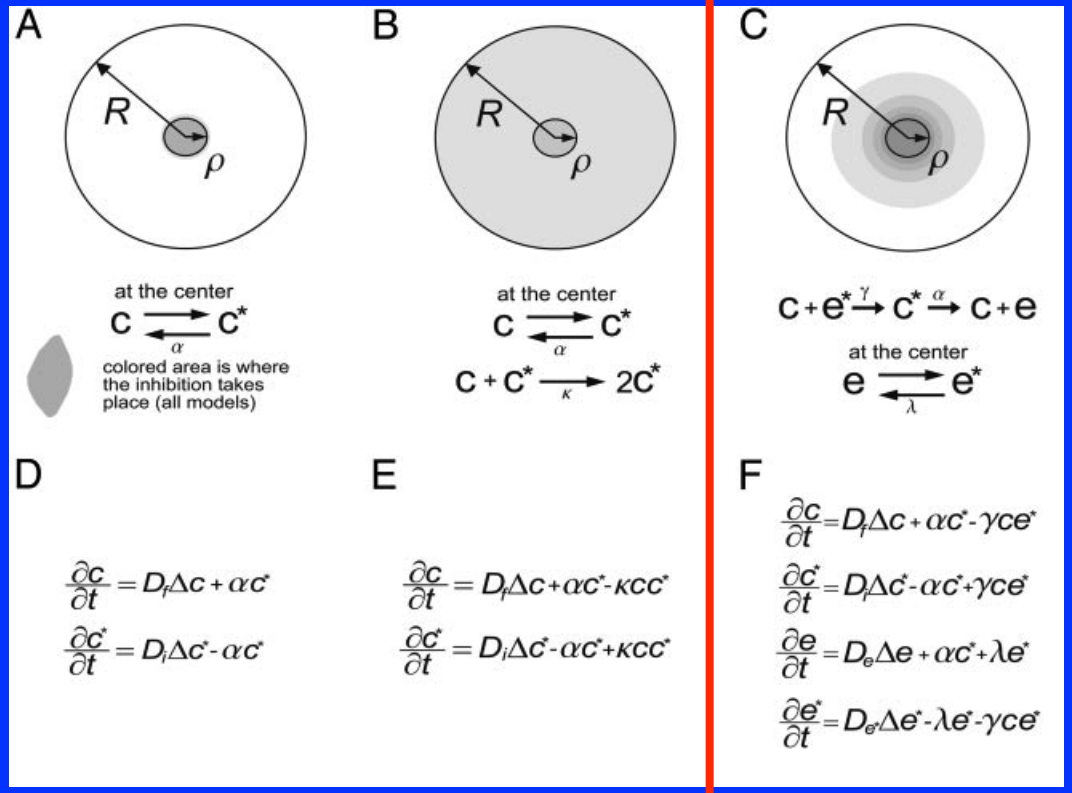
Physical constants (S. cerevisiae)

Size of the nuclear radius	**R**	1μm
Size of the kinetochore radius	**ρ**	0.01μm
Intracellular diffusion	**D**	1μm2 s-1
Time between final attachment and beginning of the anaphase	**Tb**	3 min

da Doncic et al., 2005

Modeling mitosis

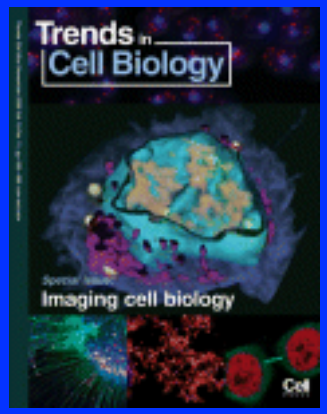
	Direct inactivation	Auto-propagating inactivation	Emitted inactivation
% inhibited molecules	low	OK	OK
Recovery time	OK	slow	OK



da Doncic et al., 2005

"Microscopic marvels"

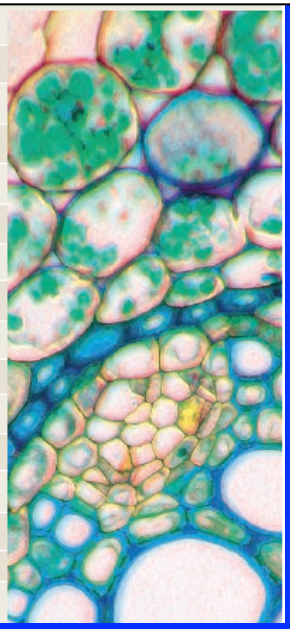
June, 2009



November, 2009

Milestones timeline

1595	invention of the microscope
1858	first histological stain
1871	synthesis of fluorescein
1873	diffraction limit theory
1911	first fluorescent microscopy
1929	first epifluorescent microscope
1935	phase contrast microscopy
1939	polarisation microscopy
1942	immunofluorescence
1955	differential interference contrast
1961	concept of confocal microscopy
1967	the dichroic mirror
1972	fluorescence correlation spettroscopy
1976	FRET FRAP
1980	calcium probes

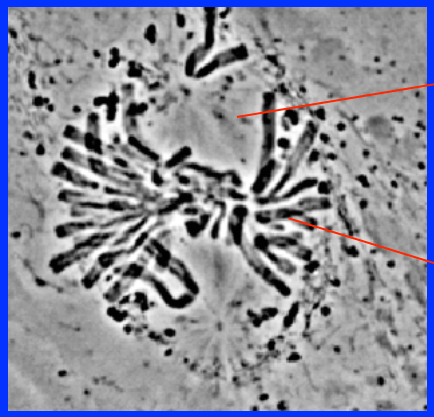


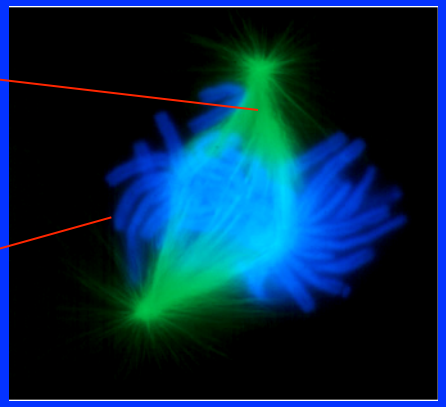
1981	Video-enhancement diff. interf. contrast
	TIRF microscopy
1983	deconvolution microscopy
1987	Realization of confocal microscopy
1990	Two-photon microscopy
1993	Light-sheet microscopy
	Single molecule microscopy
1994	GFP
1997	Fluorescent protein-based biosensors
1999	Red fluorescent proteins
2000	Breaking the diffraction limit: STED
2002	Photoactivatable fluorescent proteins
2006	Breaking the diffraction limit: PALM/STORM

Microscopy methods for studying mitosis

	Phase contrast	Fluorescence
Light source	visible	particularly rich in UV e blue (mercury lamp)
Sample	transparent	Labelled with fluorochromes
Image	Dense and contrasted regions appear dark	High resolution signal of labelled structure

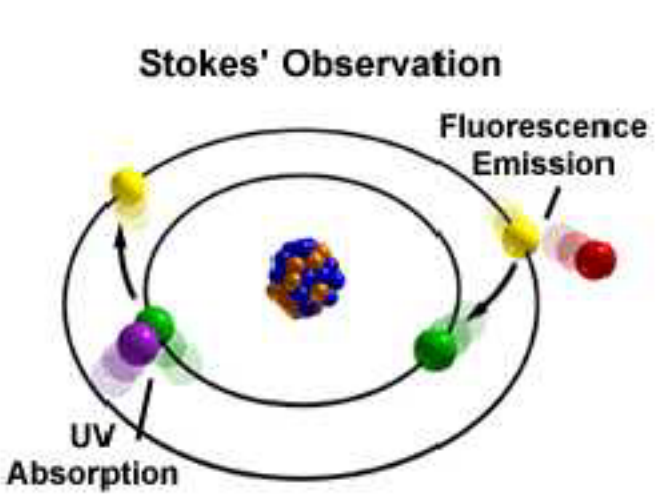
Exemple: metaphase in amphibian cells

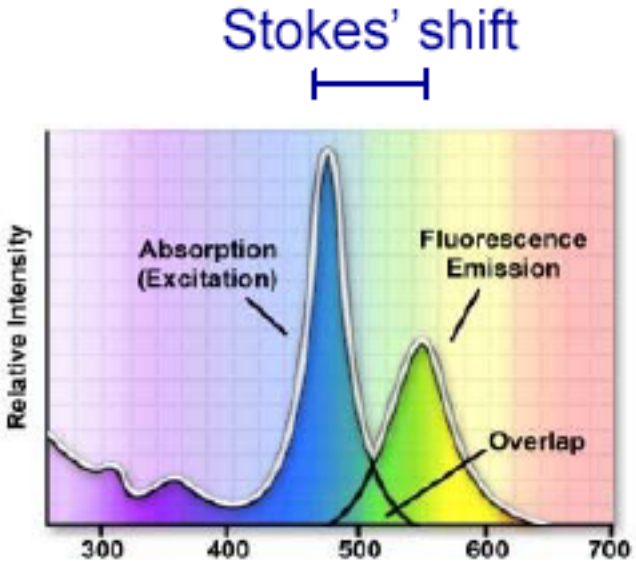




Fluorescence microscopy

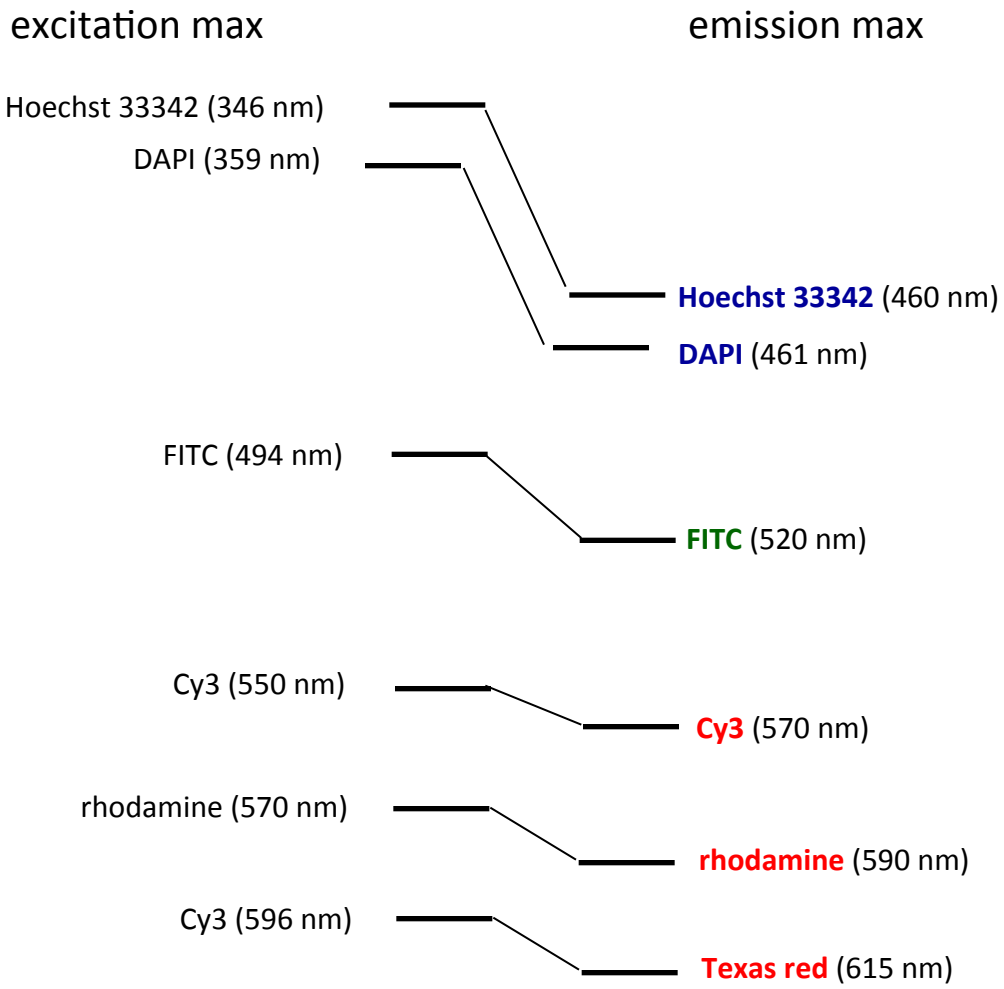
Fluorophores absorb light of a specific wavelength and emit light at higher wavelengths



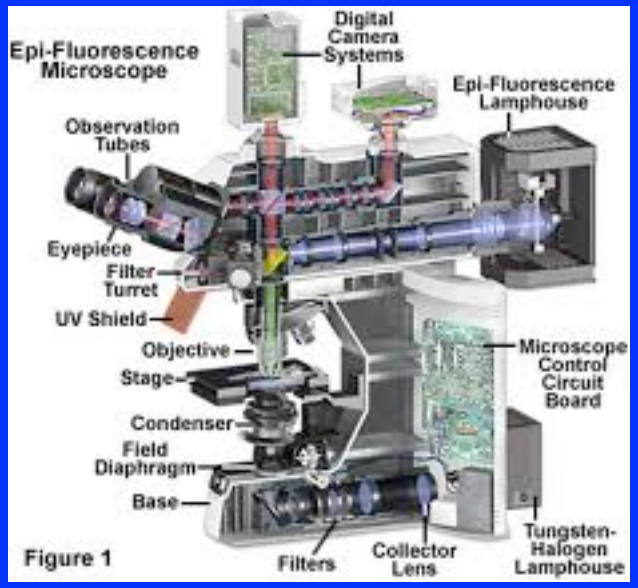


Absorbed light	Emitted light
Invisible (UV)	BLUE
BLUE	GREEN
GREEN	RED

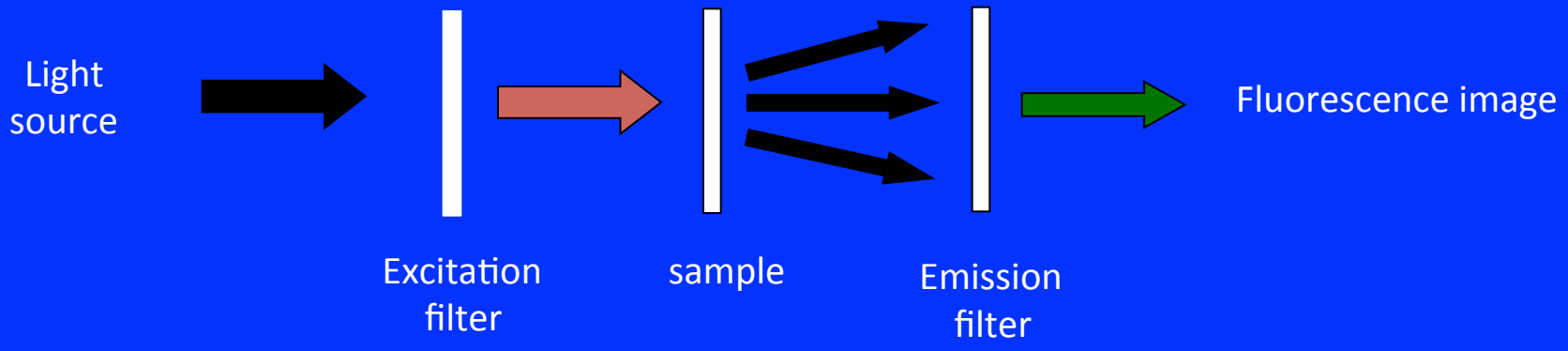
COMMONLY USED FLUOROPHORES



Microscopy

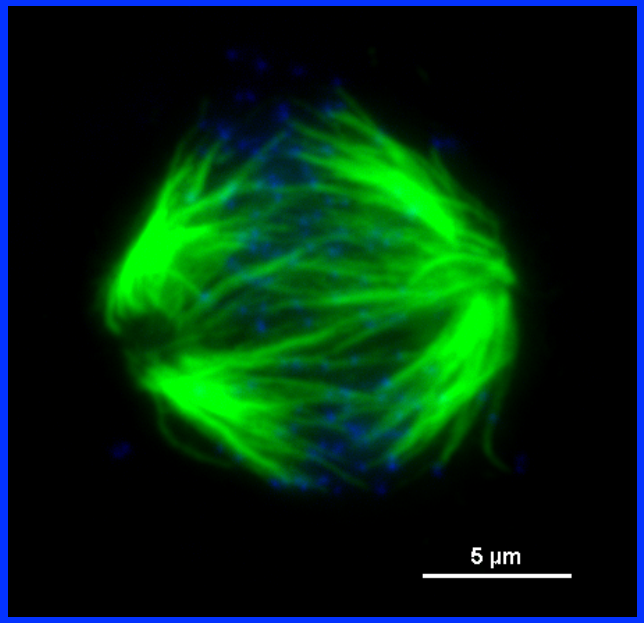


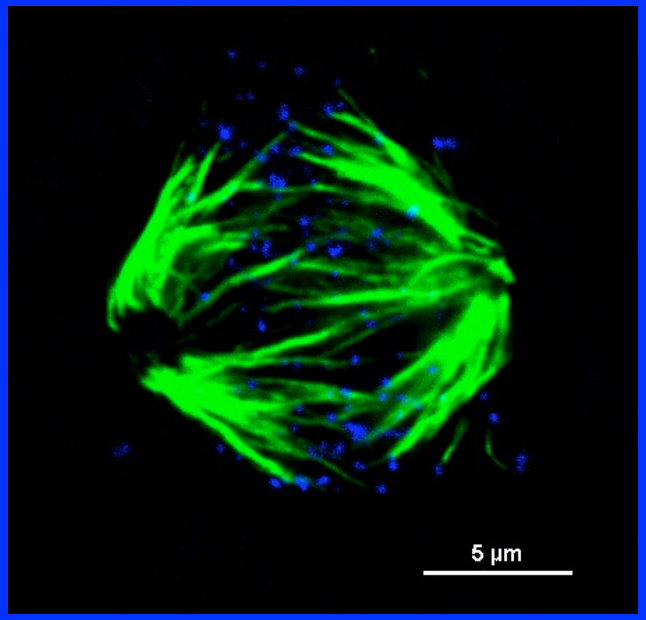
Fluorescence microscopy



Filters = barriers: only specific wavelengths pass through

Visualisation of the mitotic spindle and associated structures in a highly defined manner



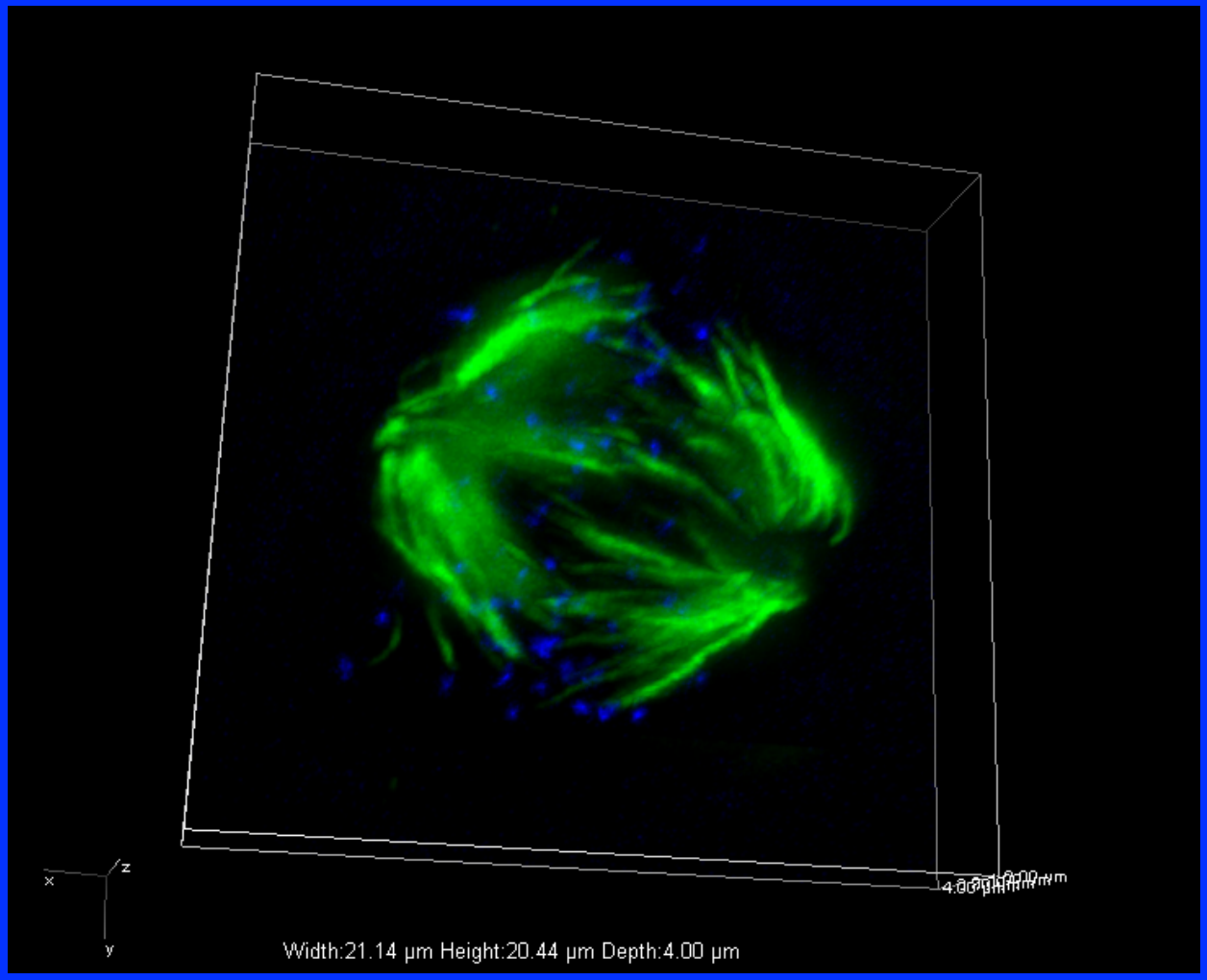


microtubules
kinetochores

Images elaborated with different mathematicial algorithms to obtain the best definition

(Valeria de Turris)

We can collect information along the z-axis and reconstruct a 3D image



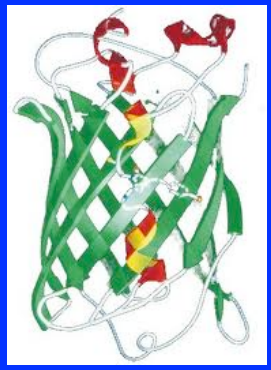
(Valeria de Turris)

√ methodological developments have transformed microscopy from a descriptive approach to a **quantitative, high-content** experimental tool

√ This is accompanied by the increase in computer power and the development of sophisticated imaging softwares that have introduced the **high-throughput level** in microscopy.

√ The discovery of fluorescent proteins, first of all the green fluorescent protein (GFP) which was recognised with the Nobel Prize in 2008, has boosted important developments for **live imaging**

Green Fluorescent Protein
GFP (Nobel Prize for Chemistry in 2008)



√ Produced by the Aequorea jellyfish; fluoresces in green

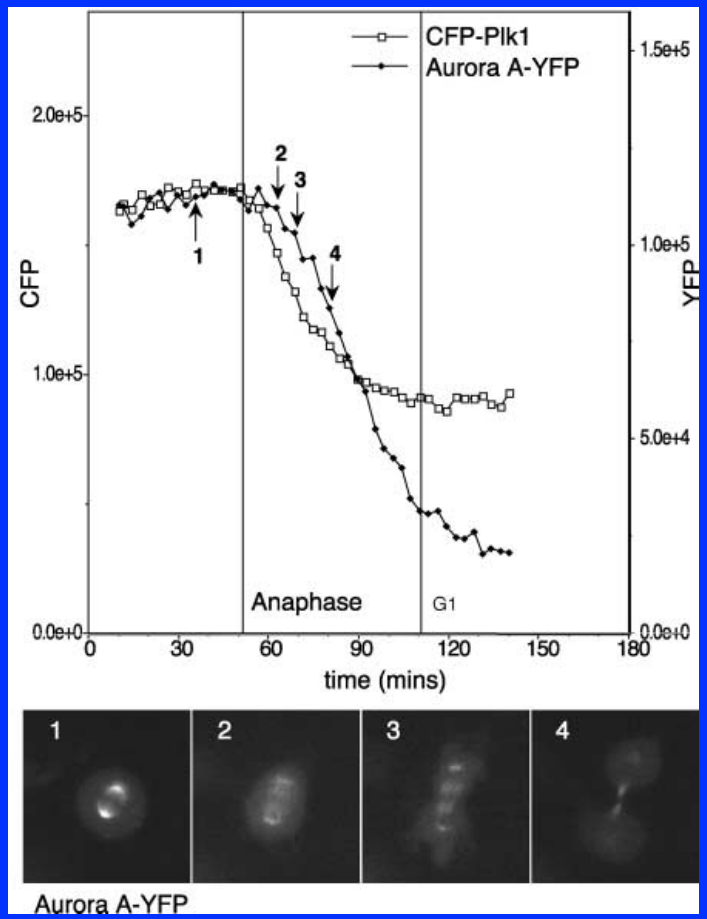
√ The gene has been cloned and is used to produce fluorescent versions of proteins of interest (fusion proteins)

√ Different GFP variants have been produced, with emission at distinct wavelenghts, for multicolor imaging: BFP (blu), CFP (ciano), YFP (giallo); new fluorescent proteins have also been isolated (e.g. Red Fluorescent protein RFP)

Live cell imaging now allows to combine the **molecular level** (by looking at single proteins, structures or interactions) with a **temporal and spatial resolution** that cannot be obtained with other approaches.

We con observe processes in live cells! Dynamic parameters.

Live cell imaging is performed at the single cell-level

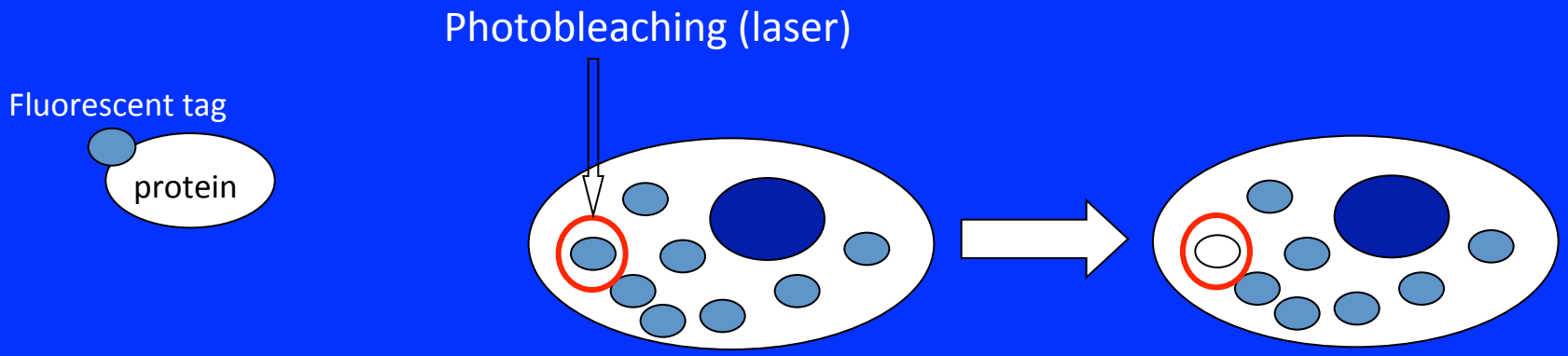


(Lindon and Pines, 2004)

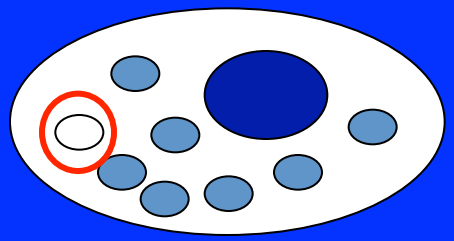
This makes it possible to attribute specific molecular events to the cell under investigation and hence to directly correlate molecular events with changes occurring at the cellular level.

Measuring the mobility of a specific molecule within the cell

Tecnique: FRAP (Fluorescence Recovery After Photobleaching)

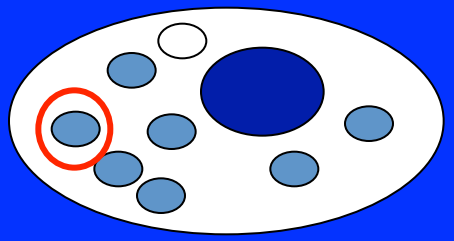


Non- mobile molecules



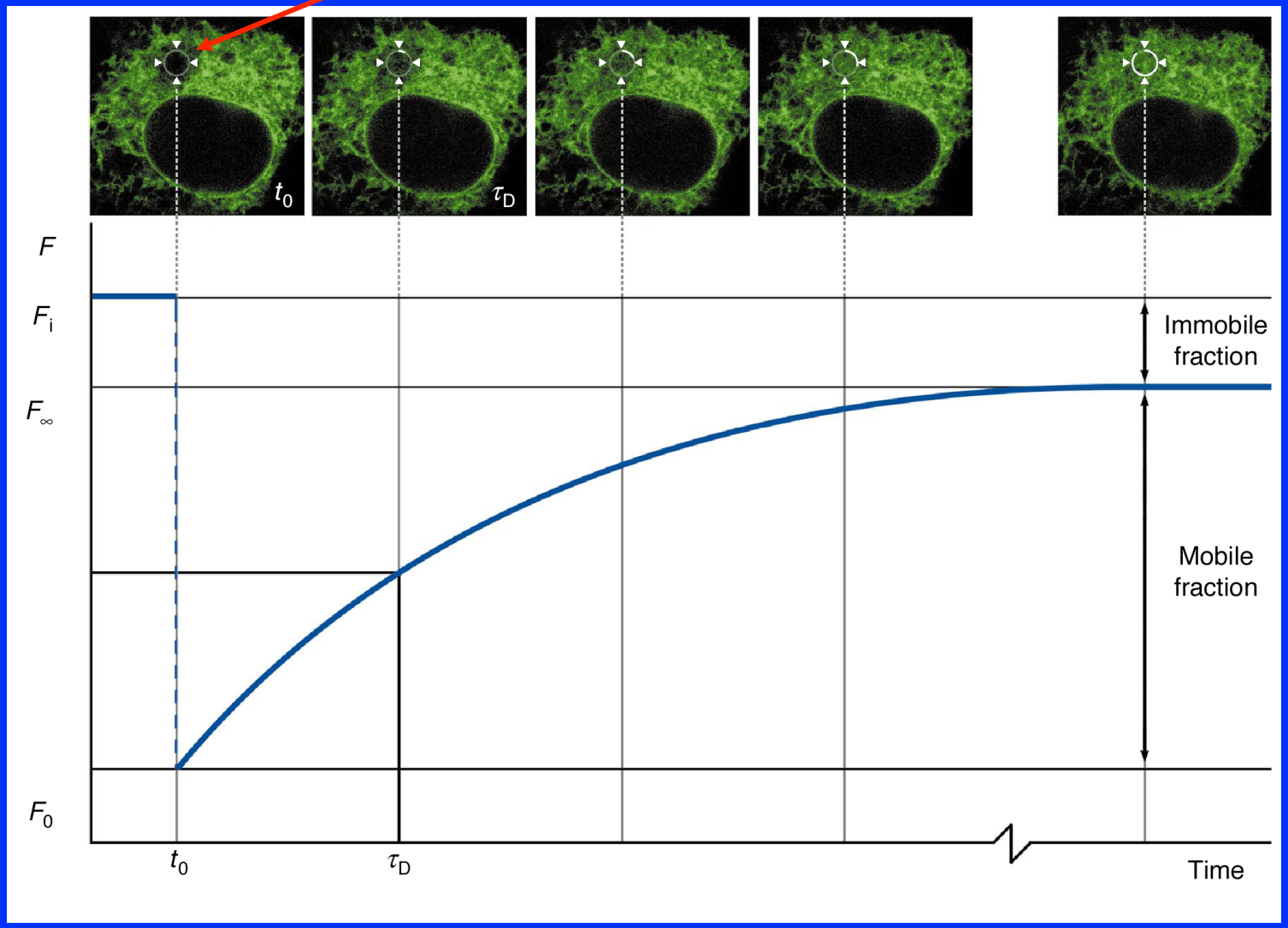
No fluorescence recovery

Mobile molecules

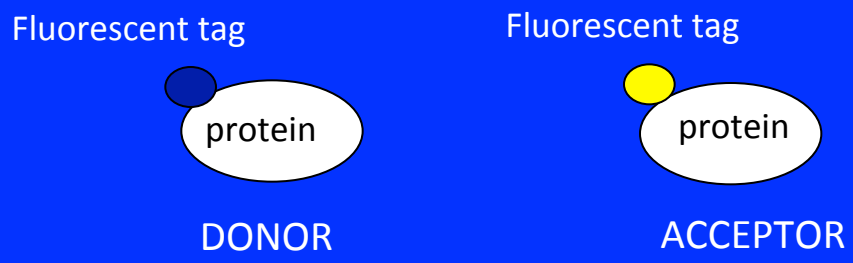


Fluorescence recovery

FRAP: an example



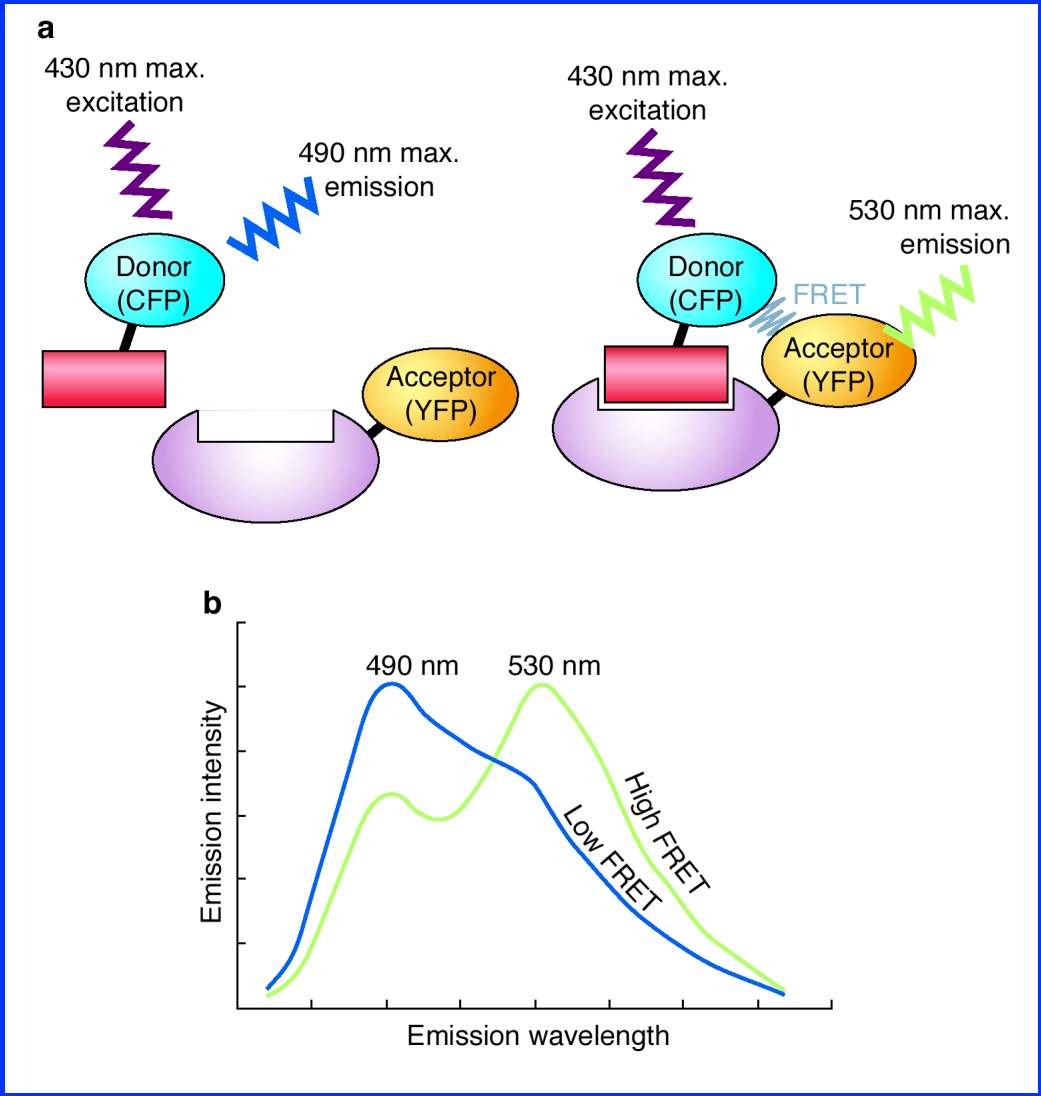
Technique: **FRET (Fluorescence Resonance Energy Transfer)**



Energy transfer between two fluorescent molecules with different exitation spectra. The emission spectrum of the donor must overlap with the exitation one of the acceptor.

Fluorescence microscopy

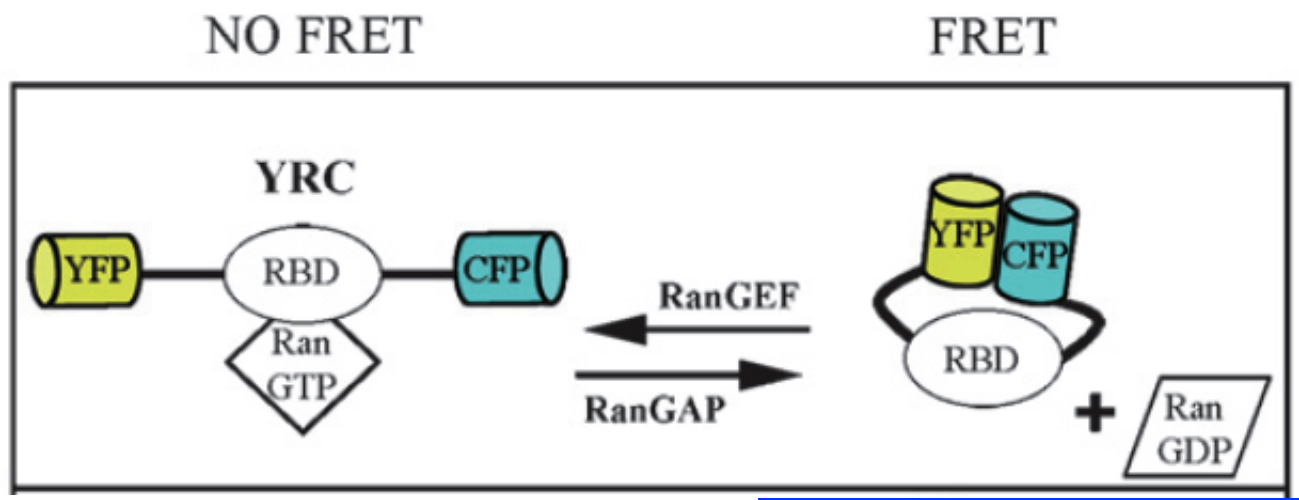
Dynamic information

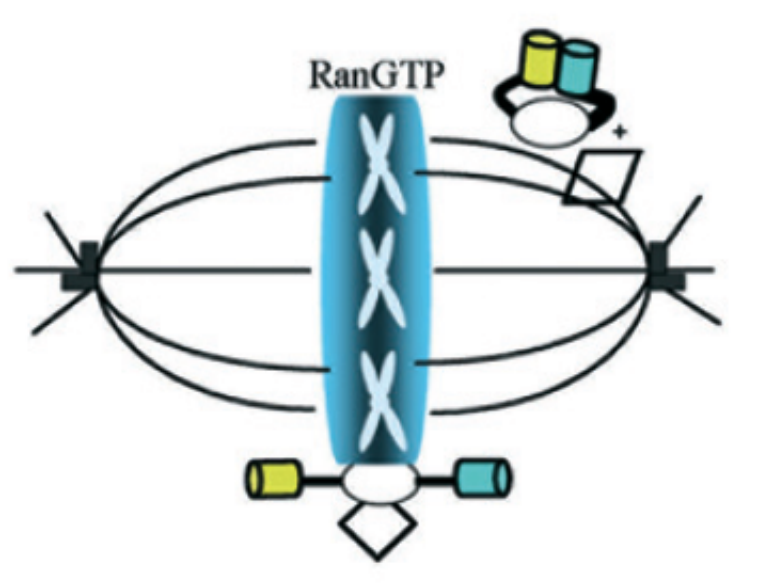


da van Roessel and Brand 2001

FRET occurs only when the 2 molecules are at 60 A or less, indicative of a direct or indirect interaction

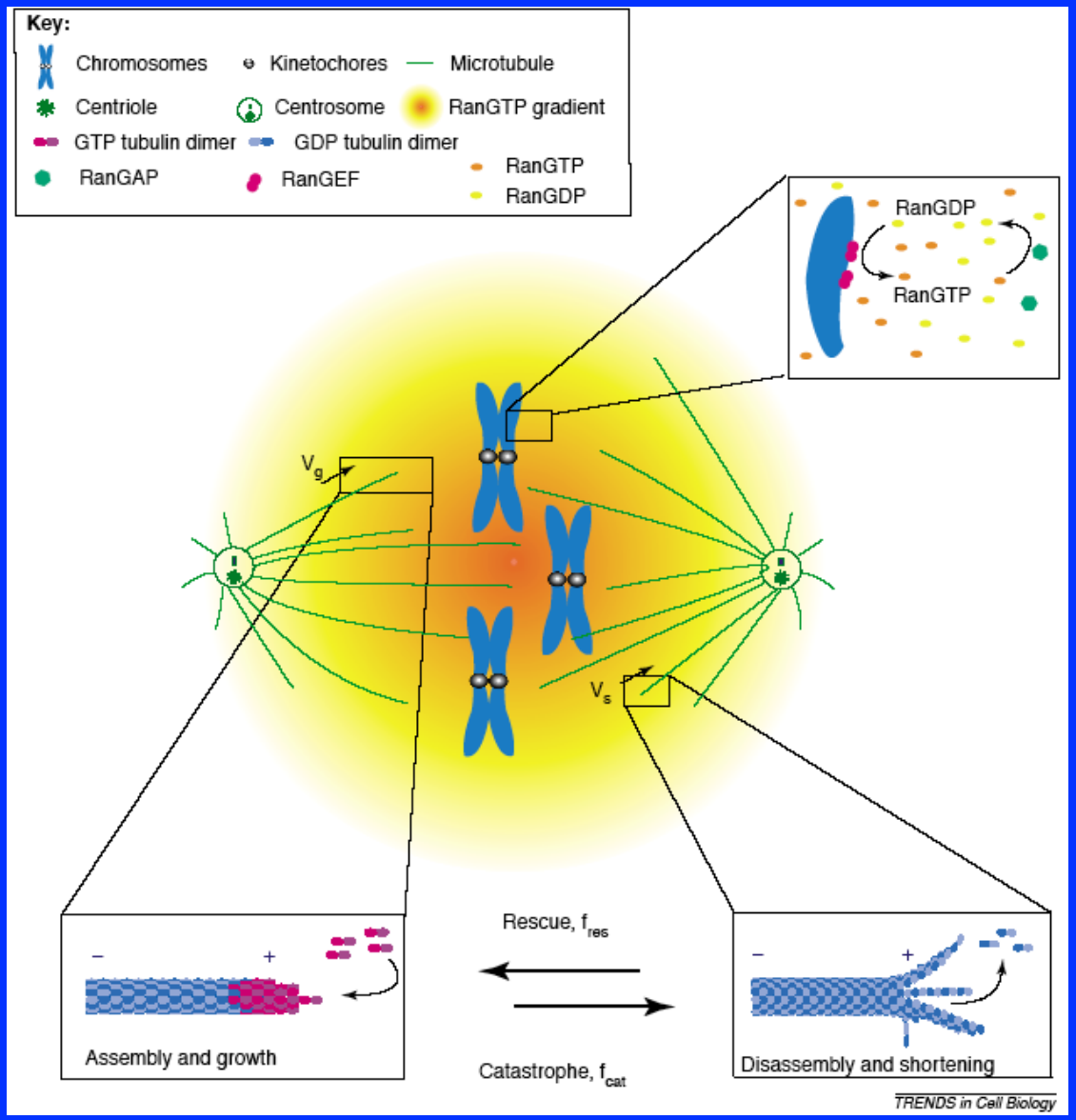
FRET to demonstrate high local concentration of RanGTP within the cell





Ciciarello et al., 2007 - Cell Mol Life Science
Spatial control of mitosis by the GTPase Ran

The "bias" in the search-and-capture model: the RanGTP gradient



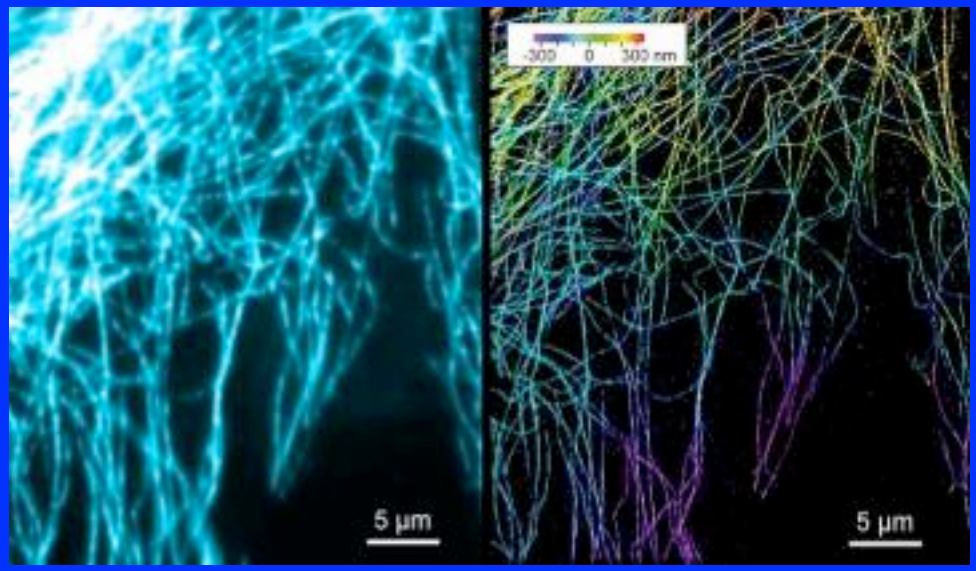
Mogilner et al., 2006 - Modeling mitosis

Towards future application…..

A recent example of sinergy between biology and phisics: “from micro to nano”

News Feature - Nature Methods 6, 15 - 18 (2009)
Super-resolution microscopy: breaking the limits

Imaging techniques to overcome the limit of optical resolution!
Observation of single molecules.



Super-resolution microscopy: overcoming the physical diffraction limit

Abbe Resolution x,y = λ/2NA Abbe Resolution z = 2λ/NA2

Es. Green light : 550 nm. 100 X Obj. NA: 1,4

Resolution limit xy= 550/2x1,4= 200 nm

Resolution limit z= 1100/1,96= 550 nm

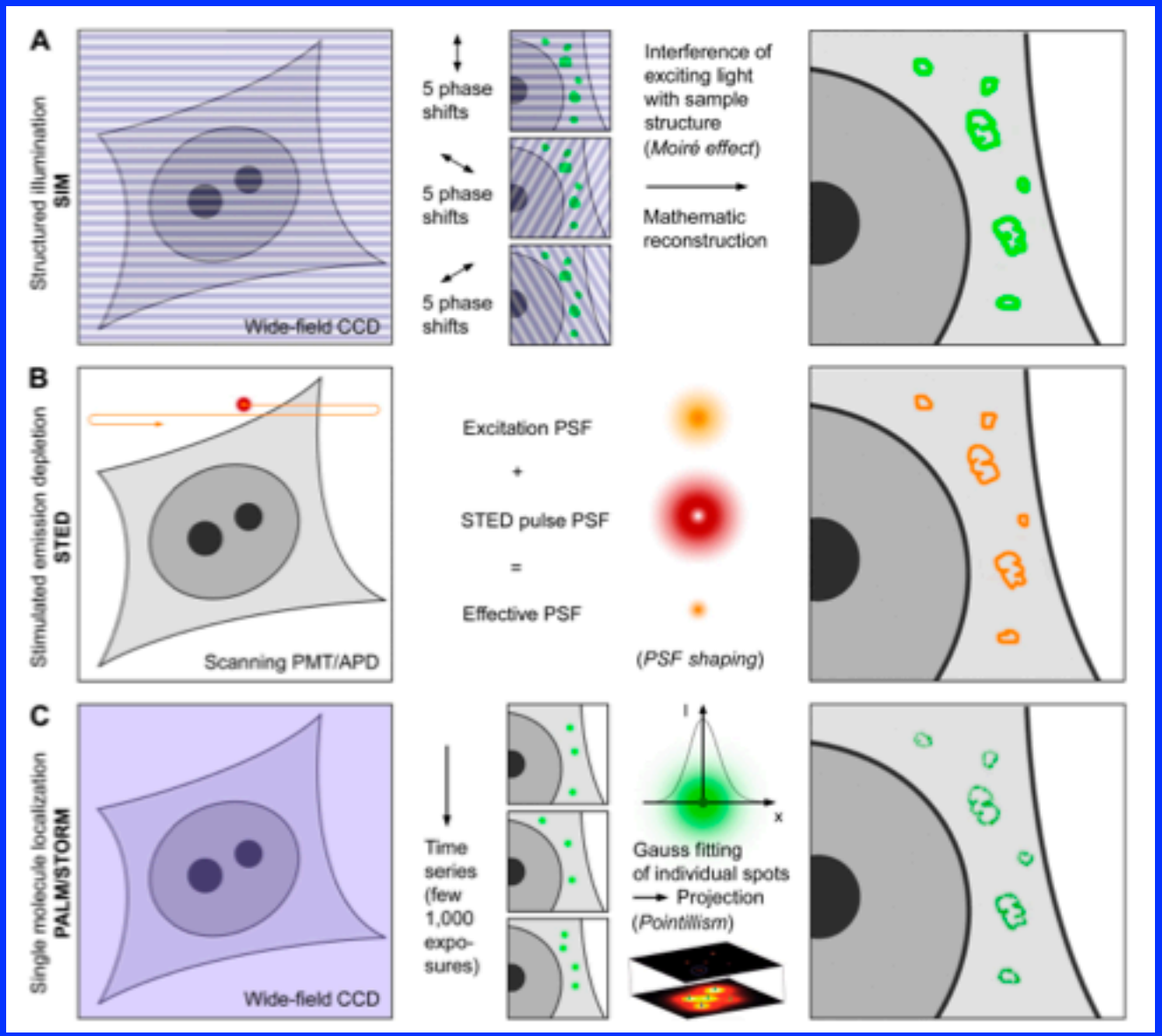
2 objects closer than 200/250 nm cannot be separated

Table I. **Super-resolution light microscopy methods**

	Near-field		Far-field					
Principle	Small aperture scanning (no lens)	Evanescent wave illumination	Wide-field + deconvolution	Confocal laser scanning	Moiré effect with structured illumination		PSF shaping with saturated emission depletion	Photoswitching and localization of single molecules (pointillism)
Acronym	**SNOM/NSOM**	**TIRFM**		**CLSM**	**SIM** (HELM, PEM) **3D-SIM**	**SSIM** (SPEM)	**STED/CW-STED**	**PALM/FPALM/STORM/ dSTORM/PALMIRA**
Illumination-emission dependence	Linear	Linear	Linear	Linear	Linear	Non-linear	Non-linear	Linear
Detector	Scanning PMT/APD	Wide-field CCD/ CMOS	Wide-field CCD/ CMOS	Scanning PMT/ APD	Wide-field CCD/ CMOS	Wide-field CCD/ CMOS	Scanning PMT/APD	Wide-field CCD/CMOS
XY-resolution	20–120 nm	200–300 nm	180–250 nm	180–250 nm	100–130 nm	50 nm	20–100 nm	20–50 nm
Z-resolution	10 nm (near-field range)	100 nm (near-field range)	500–700 nm	500–700 nm	250–350 nm	N.D.	560 nm (CW-STED) to 700 nm (100 nm with z-phase mask)	100 nm (TIRF) 20–30 nm (3D-STORM, TIRF) 75 nm (BP-FPALM, in plane)

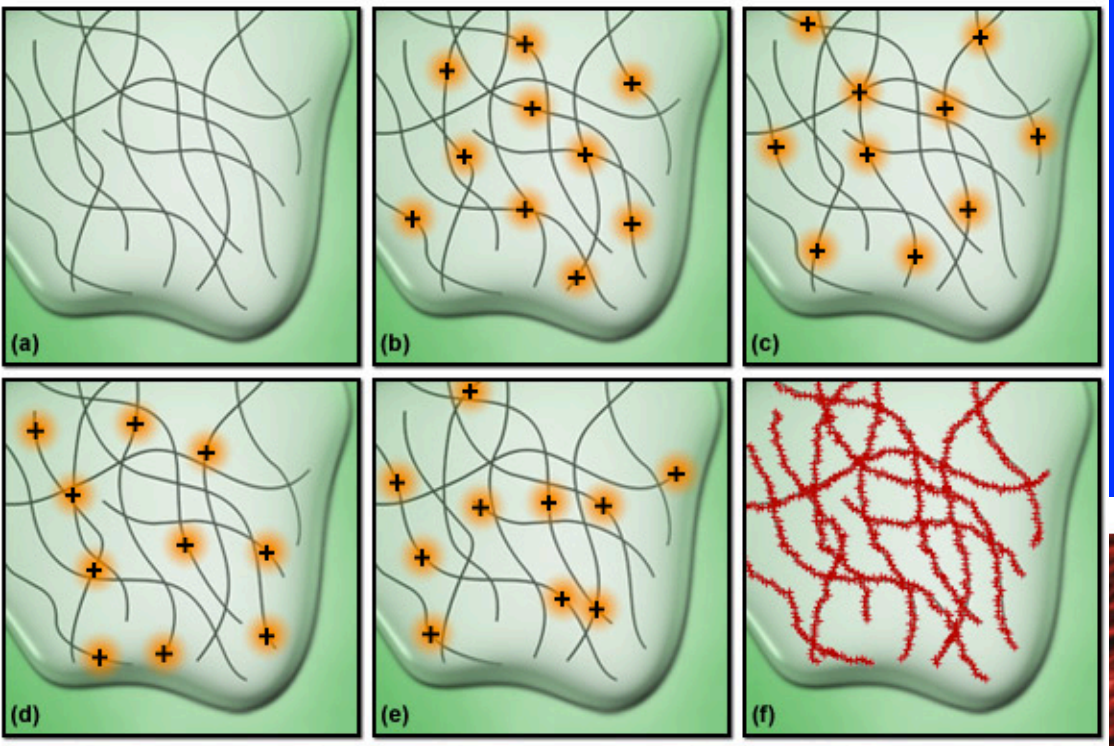
(A guide to super-resolution fluorescent microscopy. Schermelleh et al. JCB, 2010
Localisation microscopy coming of age: from concepts to biological impact. Sauer, JCS, 2013)

Super-resolution microscopy: overcoming the physical diffraction limit



Photoswitchable fluorescent proteins

Basic Principle of STORM Superresolution Imaging



Superresolution Imaging of Microtubules with STORM