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PLoS one

The N-Terminal Domain of ERK1 Accounts for the Functional Differences with ERK2

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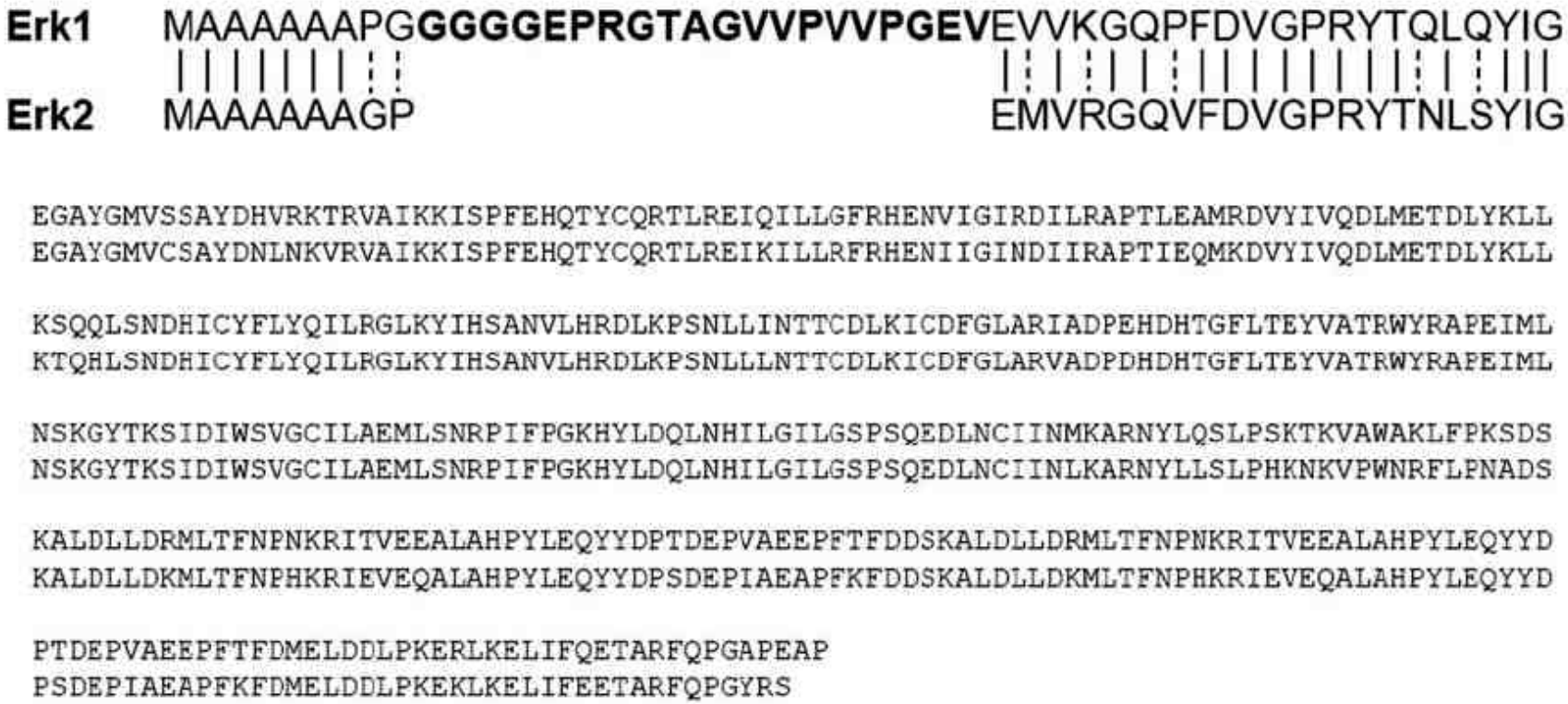
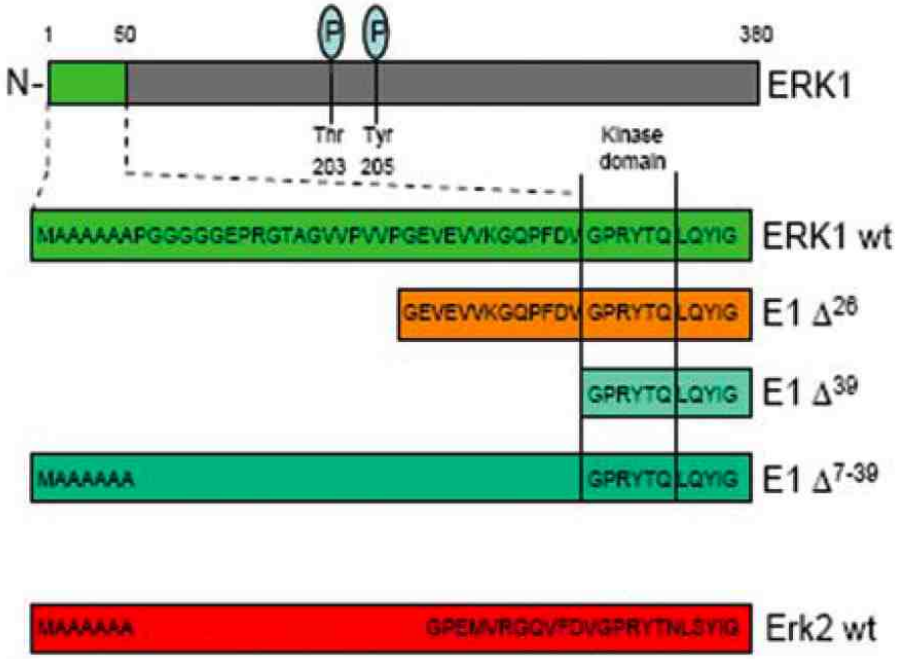
Alignment of the amino acid sequences of rat ERK1 and ERK2.

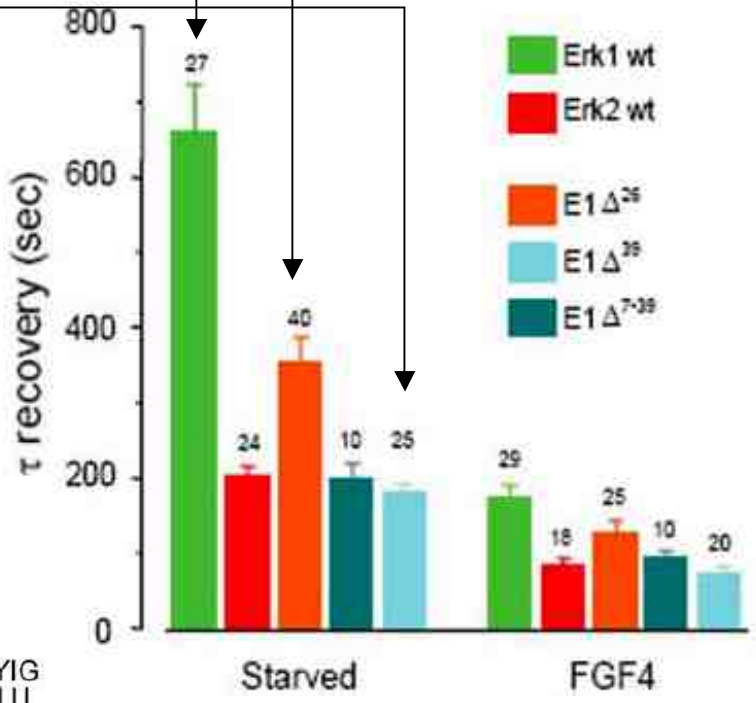
The N-terminus is shown with a larger font. The 20 aa present only in ERK1 are displayed in bold.



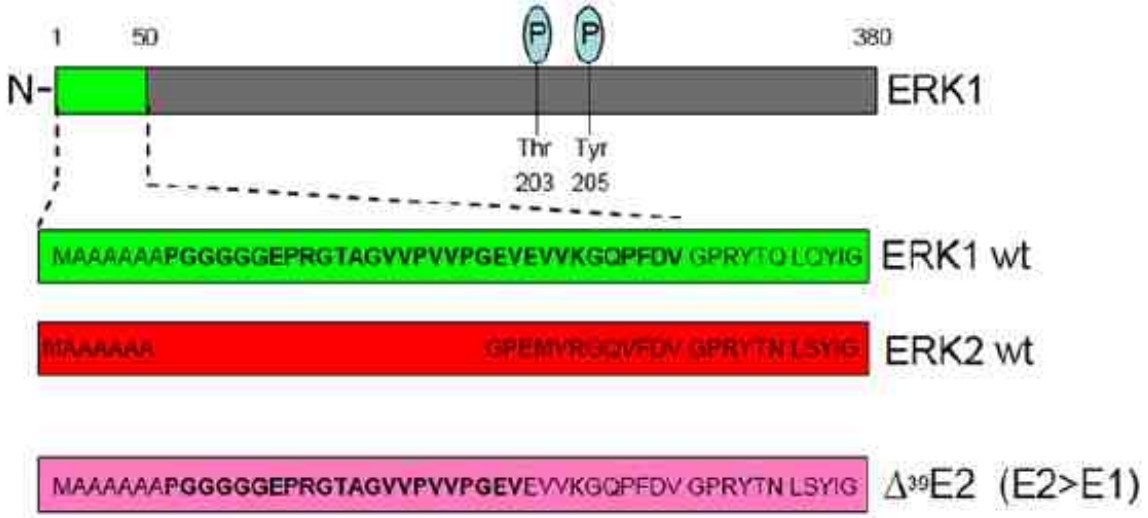
ERK1 deletants:

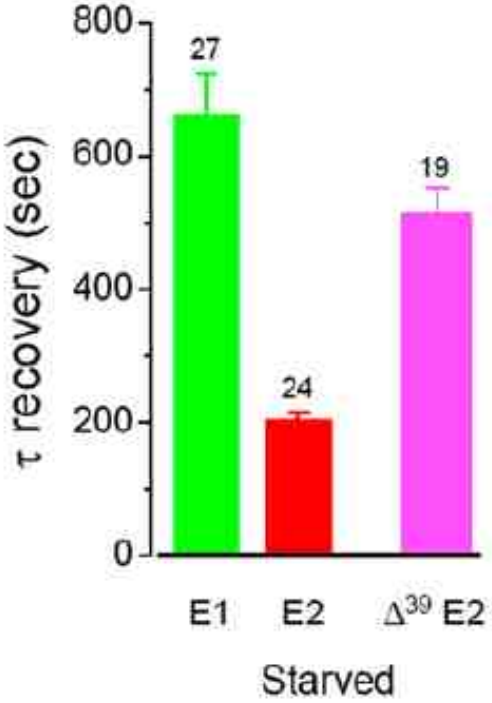
The time constant of the nucleo-cytoplasmic shuttling of ERK1 fusion proteins is strongly affected by the different deletions of the N-terminus

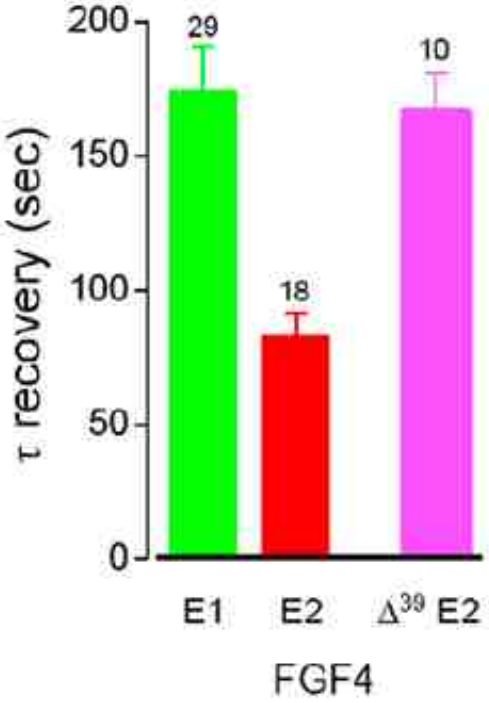




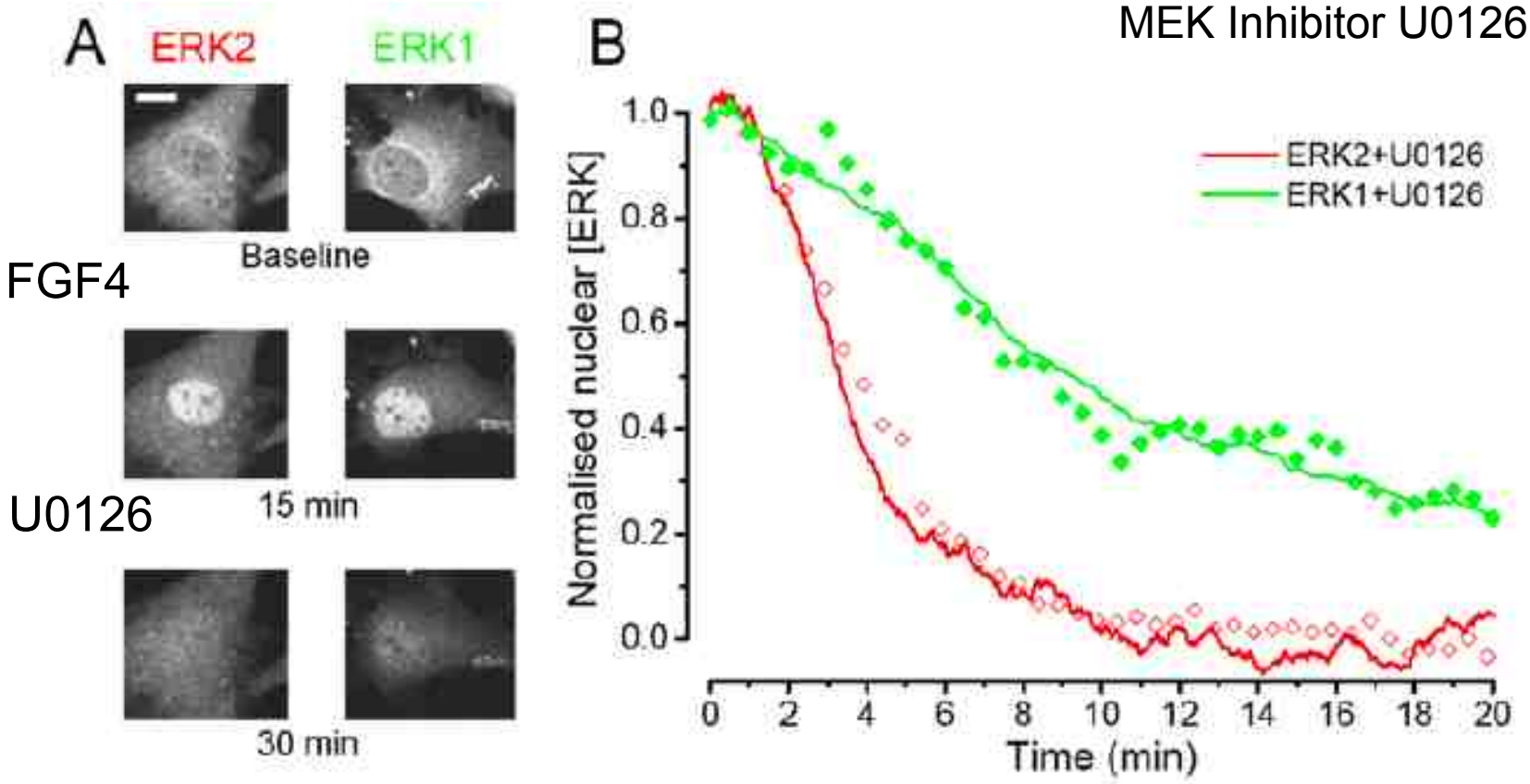
Fusion of ERK2-GFP with the N-terminus of ERK1 (Δ39 E2/E.E1).

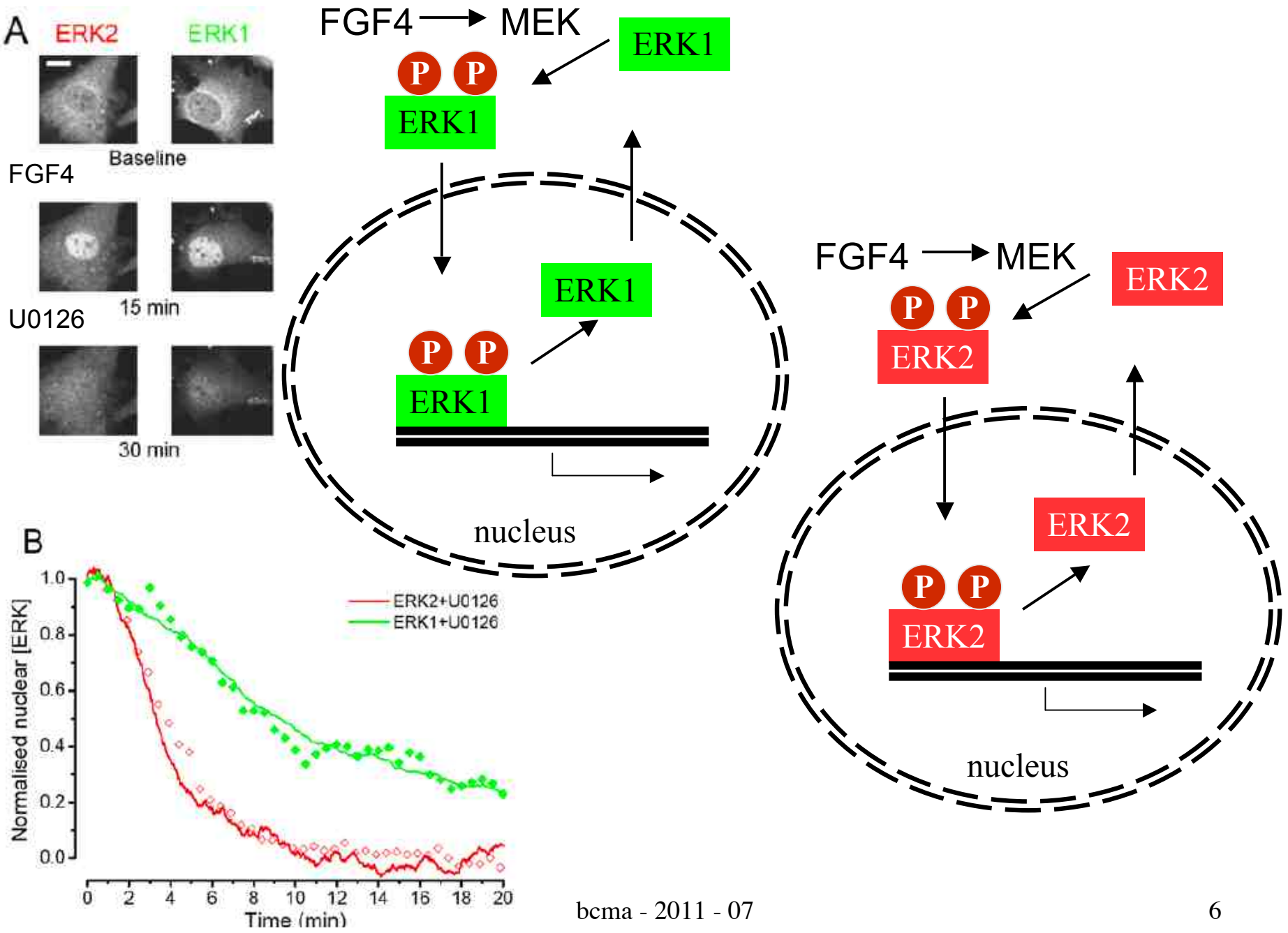


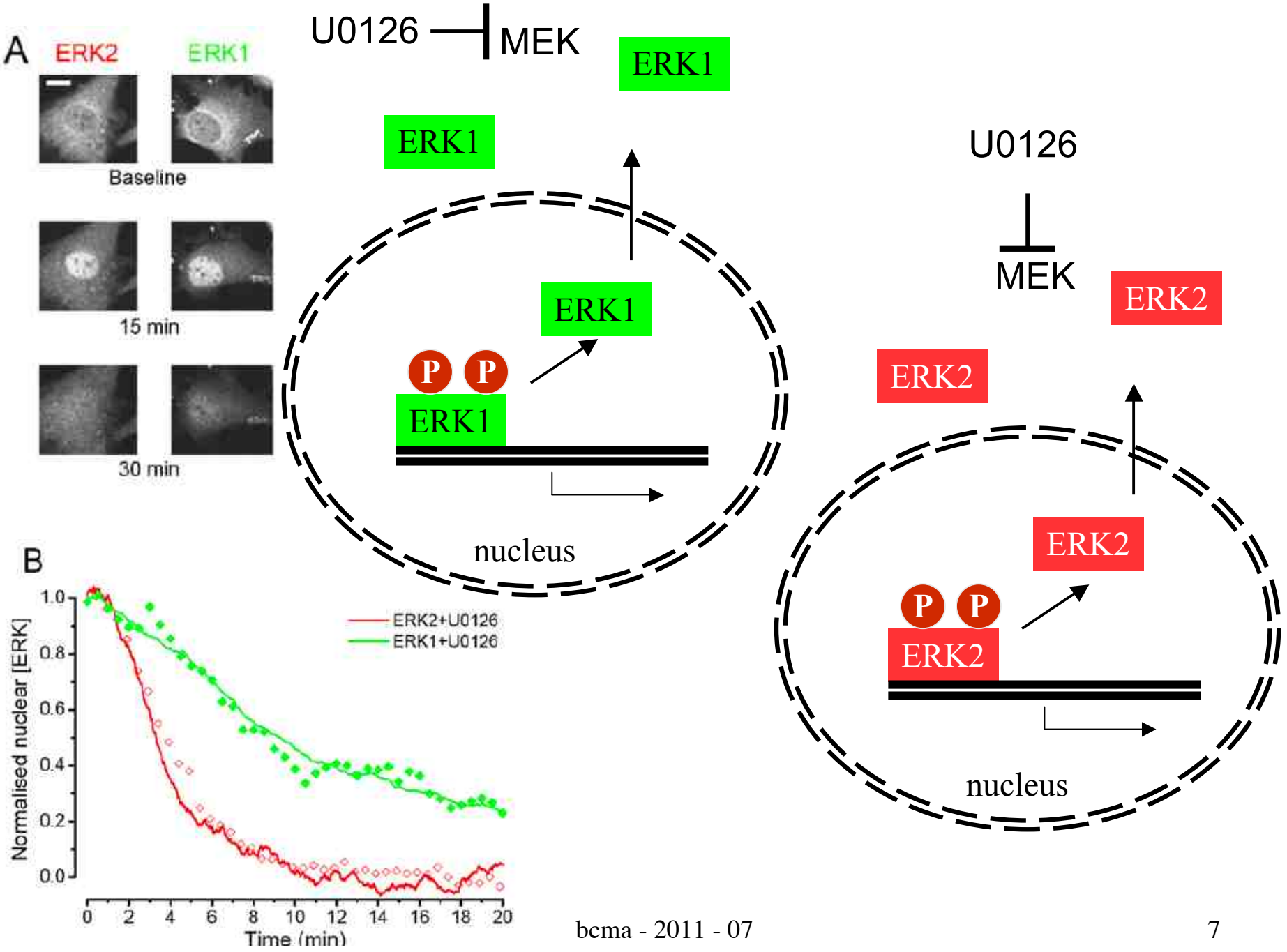


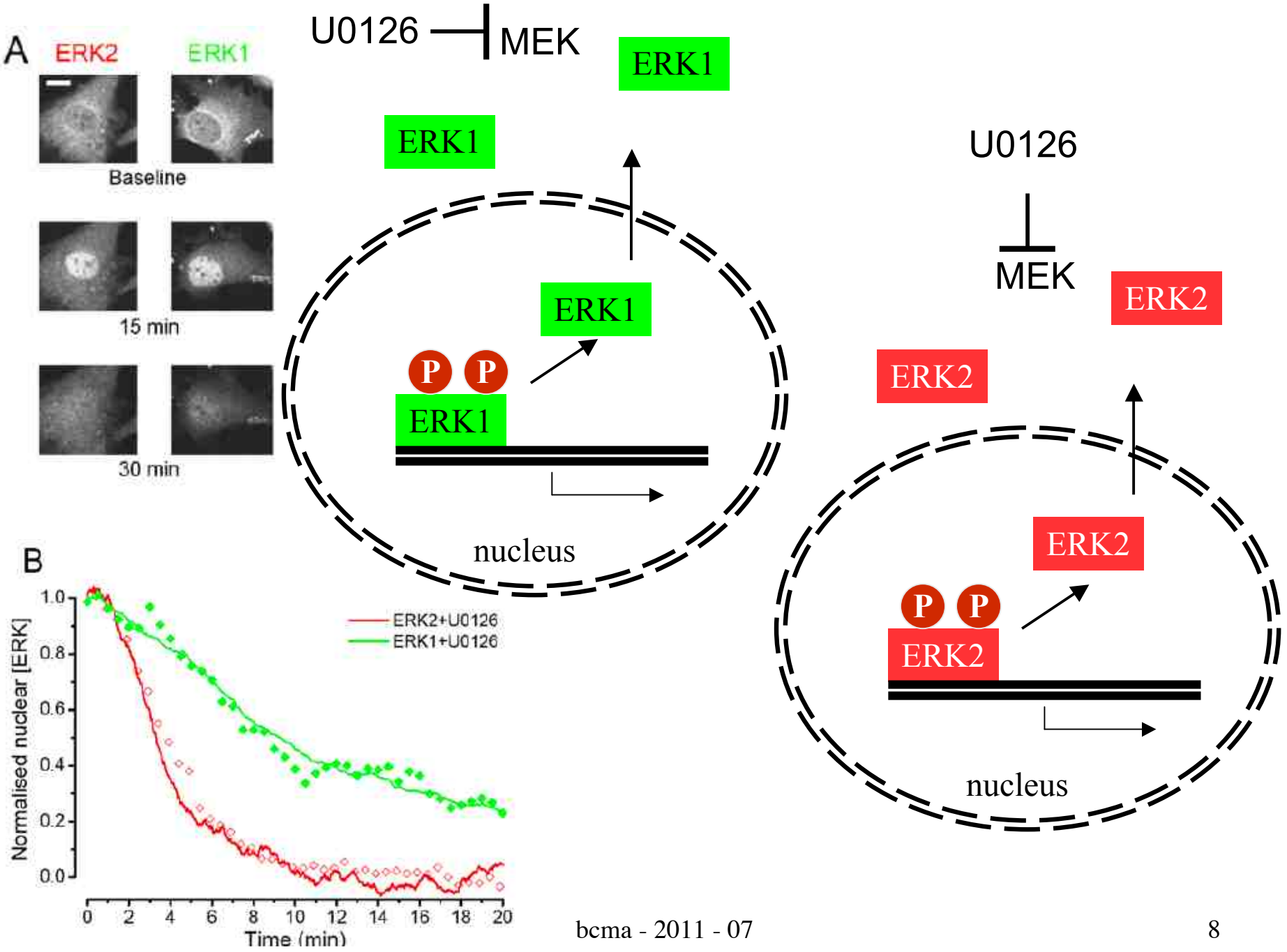


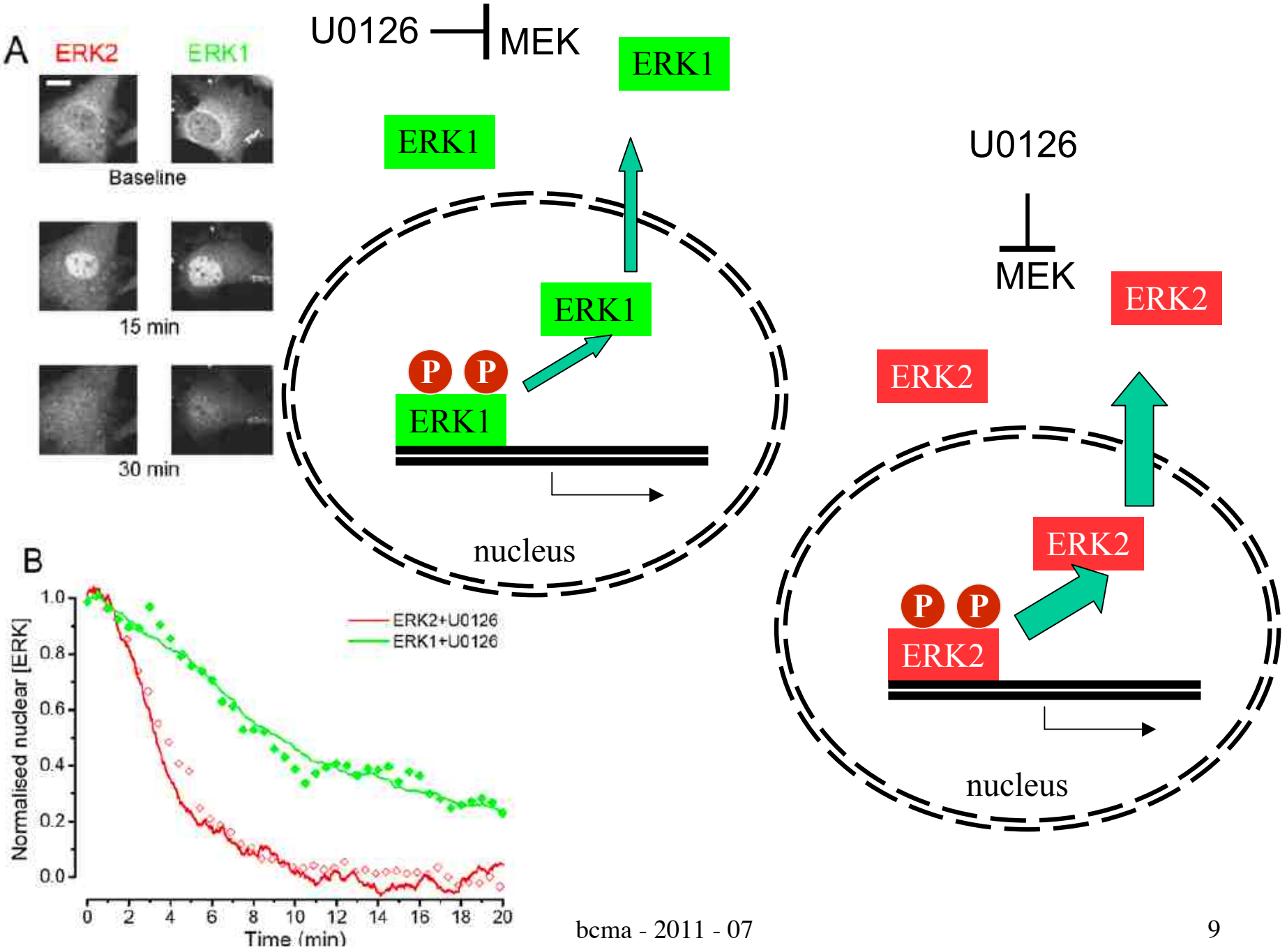
Cells transfected with ERK1-GFP and ERK2-GFP were treated with FGF4 for 15 min to allow complete nuclear translocation and then with the ERK blocker U0126. The inactivation of the ERK pathway caused the immediate loss of nuclear accumulation of ERK-GFP unmasking the action of nuclear dephosphorylation.



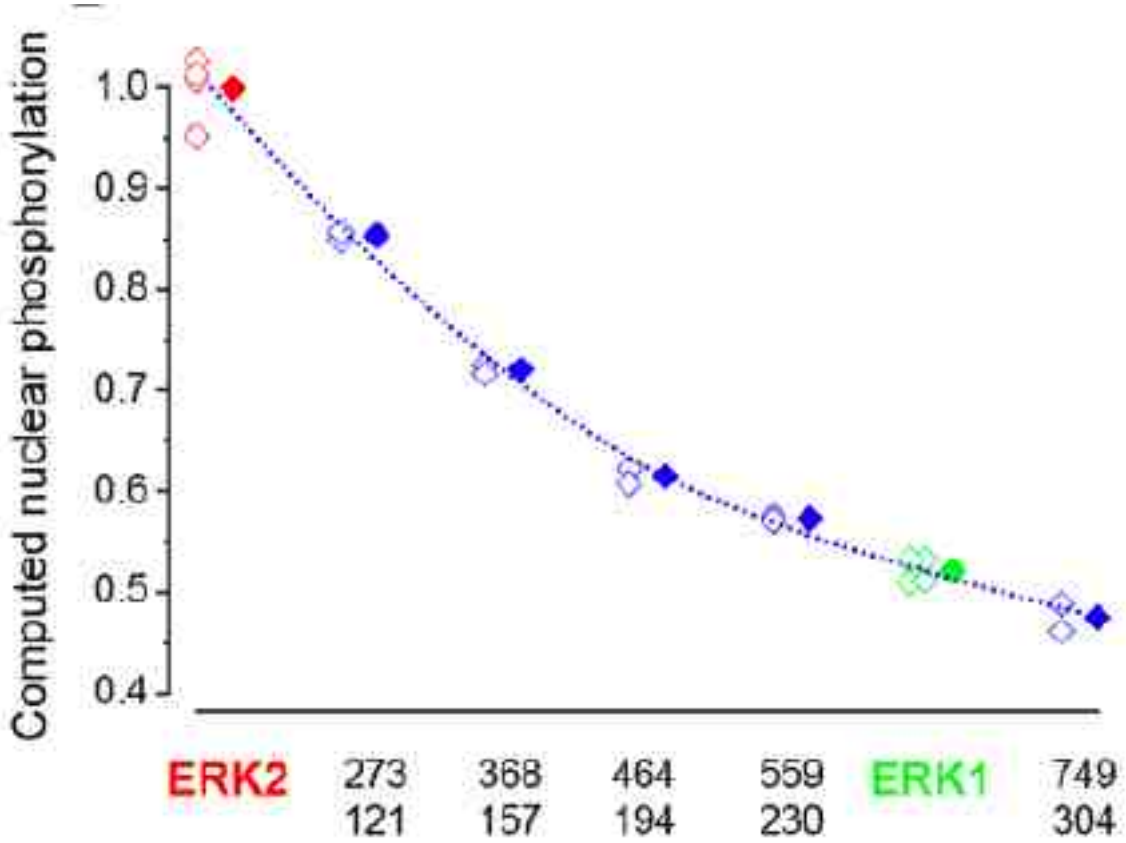

A
ERK2
ERK1
Baseline
FGF4
15 min
U0126
30 min
FGF4 → MEK
ERK1
P P
ERK1
ERK1
P P
ERK1
nucleus
FGF4 → MEK
ERK2
P P
ERK2
ERK2
P P
ERK2
nucleus
B
ERK2+U0126
ERK1+U0126
Normalised nuclear [ERK]
1.0
0.8
0.6
0.4
0.2
0.0
0 2 4 6 8 10 12 14 16 18 20
Time (min)


A
ERK2
ERK1
Baseline
15 min
30 min
U0126
MEK
ERK1
ERK1
ERK1
P
P
ERK1
nucleus
U0126
MEK
ERK2
ERK2
ERK2
P
P
ERK2
nucleus
B
Normalised nuclear [ERK]
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
16
18
20
Time (min)
ERK2+U0126
ERK1+U0126

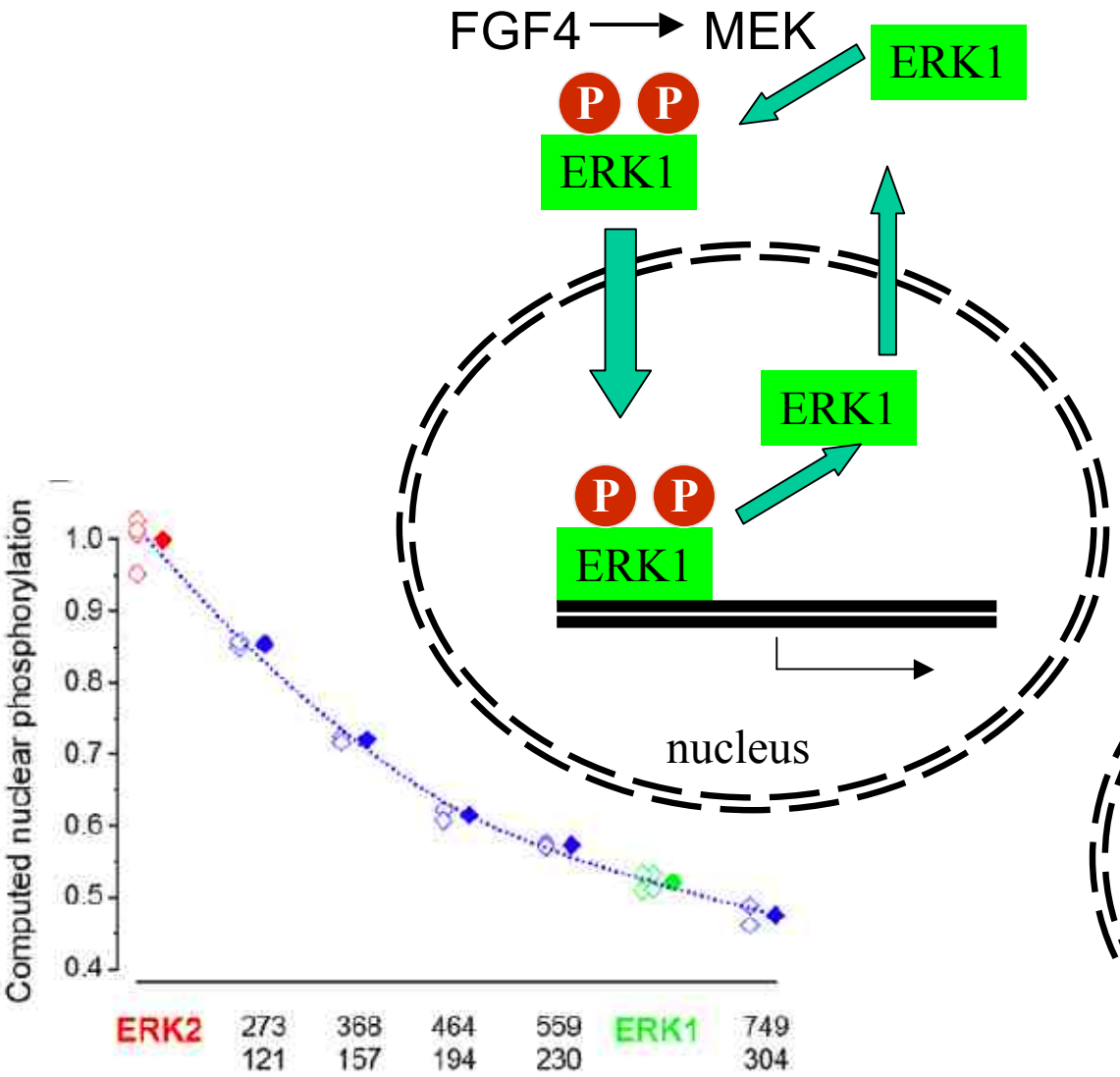

A
ERK2
ERK1
Baseline
15 min
30 min
U0126
MEK
ERK1
ERK1
ERK1
P
P
ERK1
nucleus
U0126
MEK
ERK2
ERK2
ERK2
P
P
ERK2
nucleus
B
Normalised nuclear [ERK]
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
16
18
20
Time (min)
ERK2+U0126
ERK1+U0126

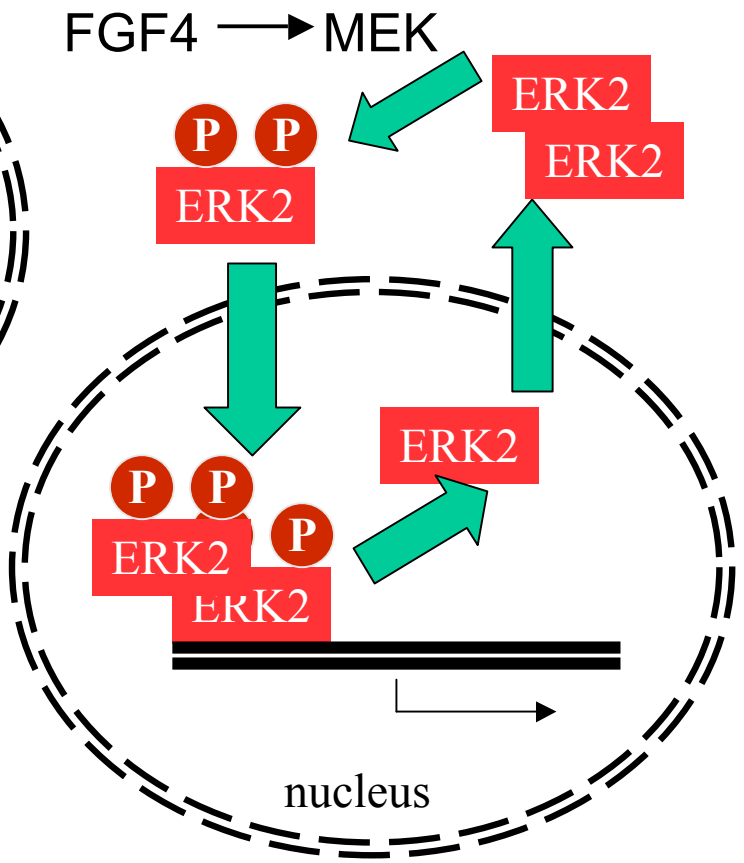

A
ERK2
ERK1
Baseline
15 min
30 min
U0126
MEK
ERK1
ERK1
ERK1
P
P
ERK1
nucleus
U0126
MEK
ERK2
ERK2
ERK2
P
P
ERK2
nucleus
B
Normalised nuclear [ERK]
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
16
18
20
Time (min)
ERK2+U0126
ERK1+U0126

Phosphorylation in the nucleus as a function of the shuttling speed

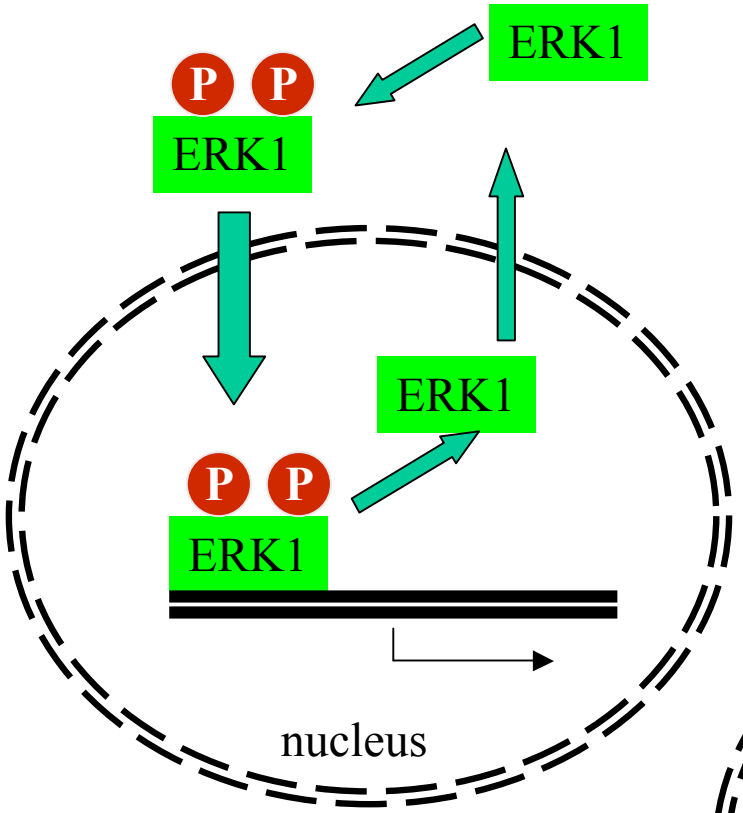


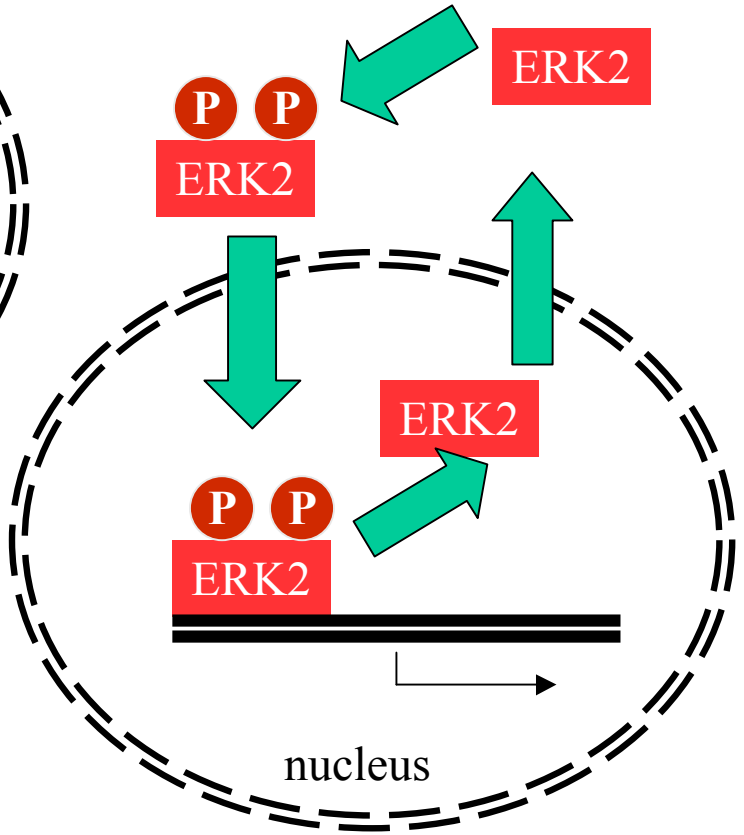
The total level of phosphorylation of ERK1 is only about half of ERK2

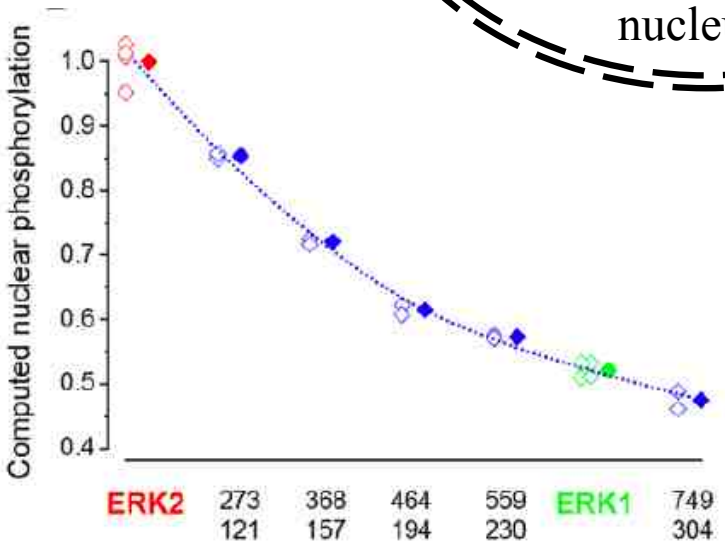


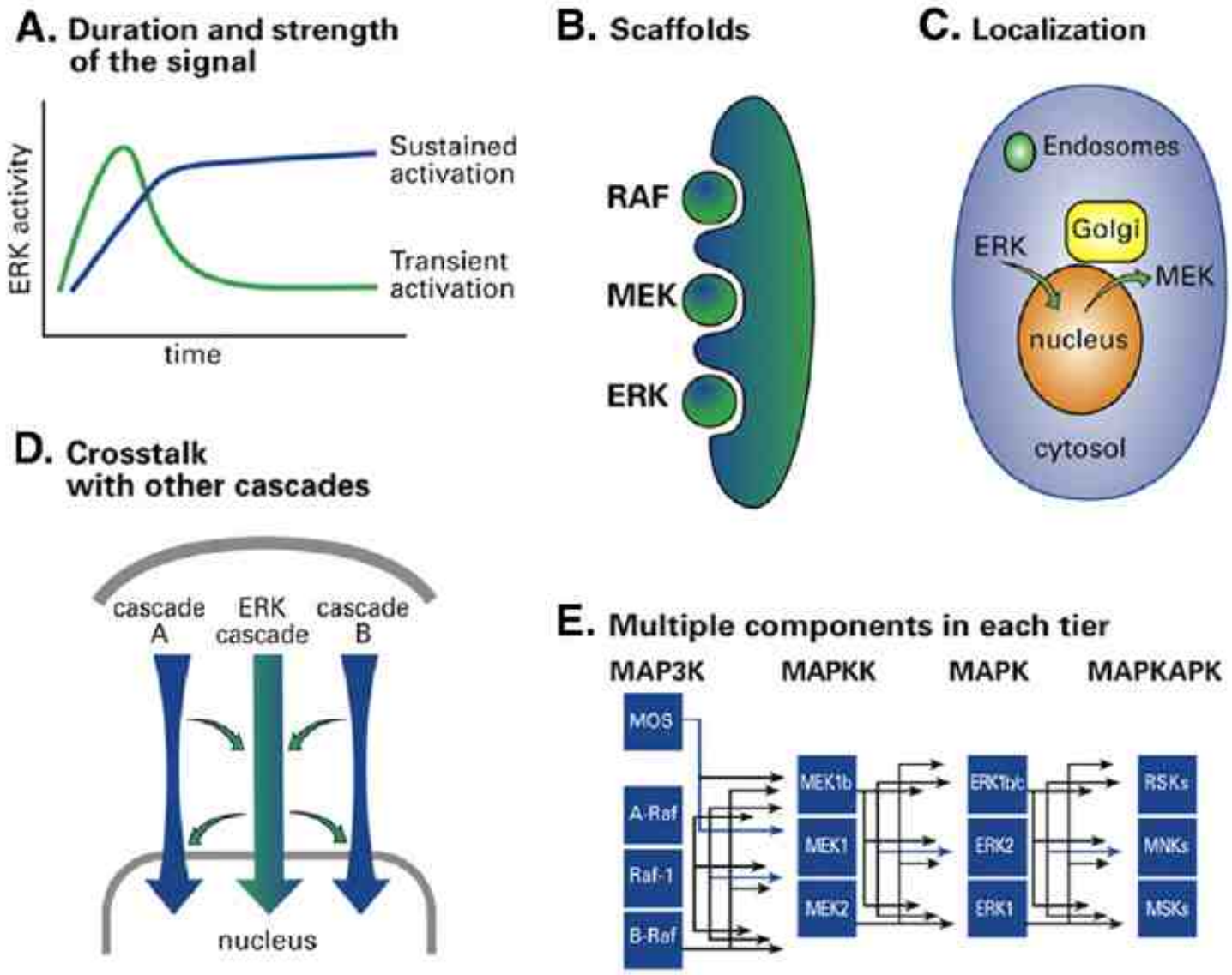


The total level of nuclear phosphorylation of ERK1 is only about half of ERK2


ERK1
P
P
ERK1
ERK1
P
P
ERK1
nucleus


ERK2
P
P
ERK2
ERK2
P
P
ERK2
nucleus

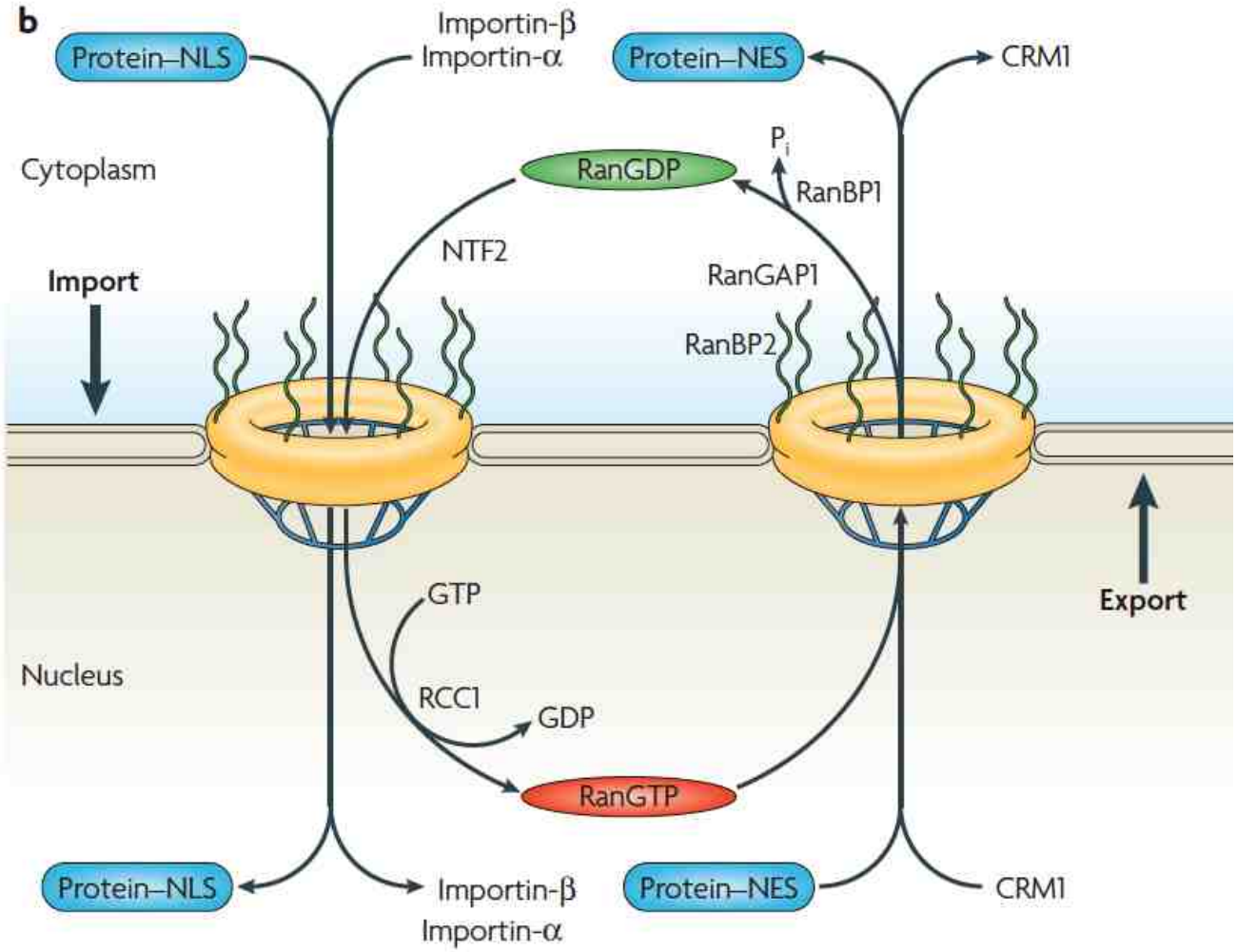

Computed nuclear phosphorylation
1.0
0.9
0.8
0.7
0.6
0.5
0.4
ERK2
273
121
368
157
464
194
559
230
ERK1
749
304



Biochimica et Biophysica Acta 1773 (2007) 1213–1226

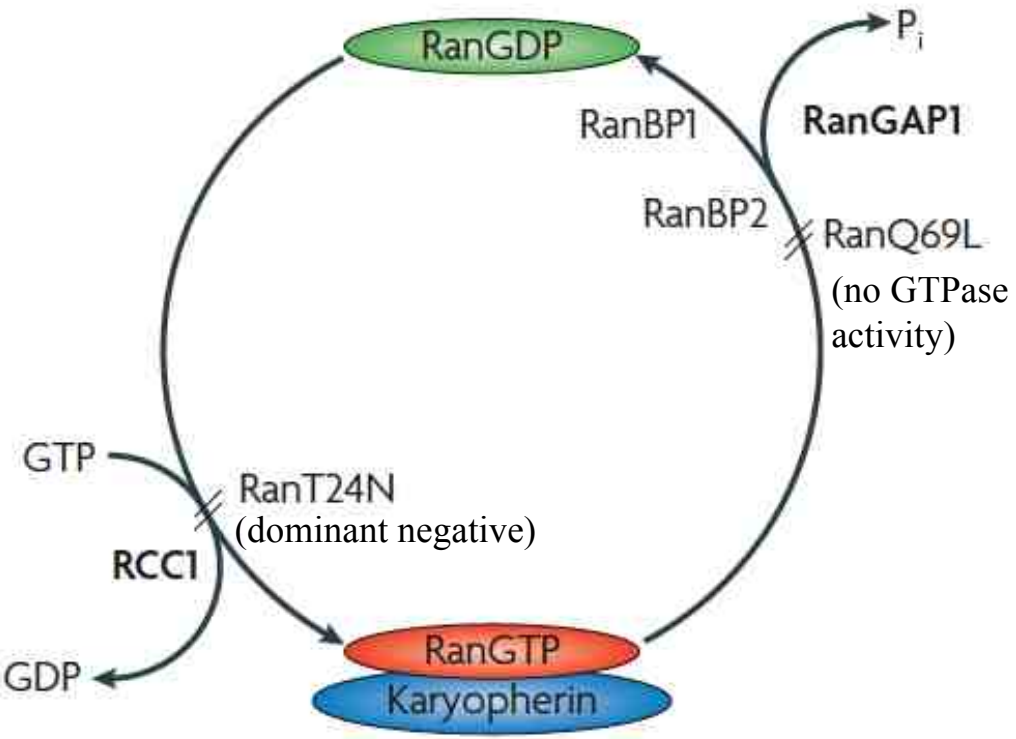
Ran function in nucleocytoplasmic transport, spindle assembly and DNA replication.

Ran directs nucleocytoplasmic transport



The GTP–GDP cycle of Ran.

Ran is loaded with GTP by the guanine nucleotide-exchange factor RCC1. RanGTP adopts a distinct conformation that allows it to interact with a transport factor from the importin-β superfamily, also known as the karyopherins. Hydrolysis of GTP to GDP by Ran requires the interaction of a Ran GTPase-activating protein, RanGAP1, and is stimulated by Ran-binding protein-1 (RanBP1) or RanBP2. RanGDP has a different conformation that does not interact strongly with karyopherin and can be considered inactive. Mutants of Ran block the GTP–GDP cycle: RanT24N has a reduced affinity for nucleotides and forms a stable complex with RCC1, thereby blocking RanGTP formation, whereas RanQ69L cannot hydrolyse GTP and is locked in the GTP-bound conformation.



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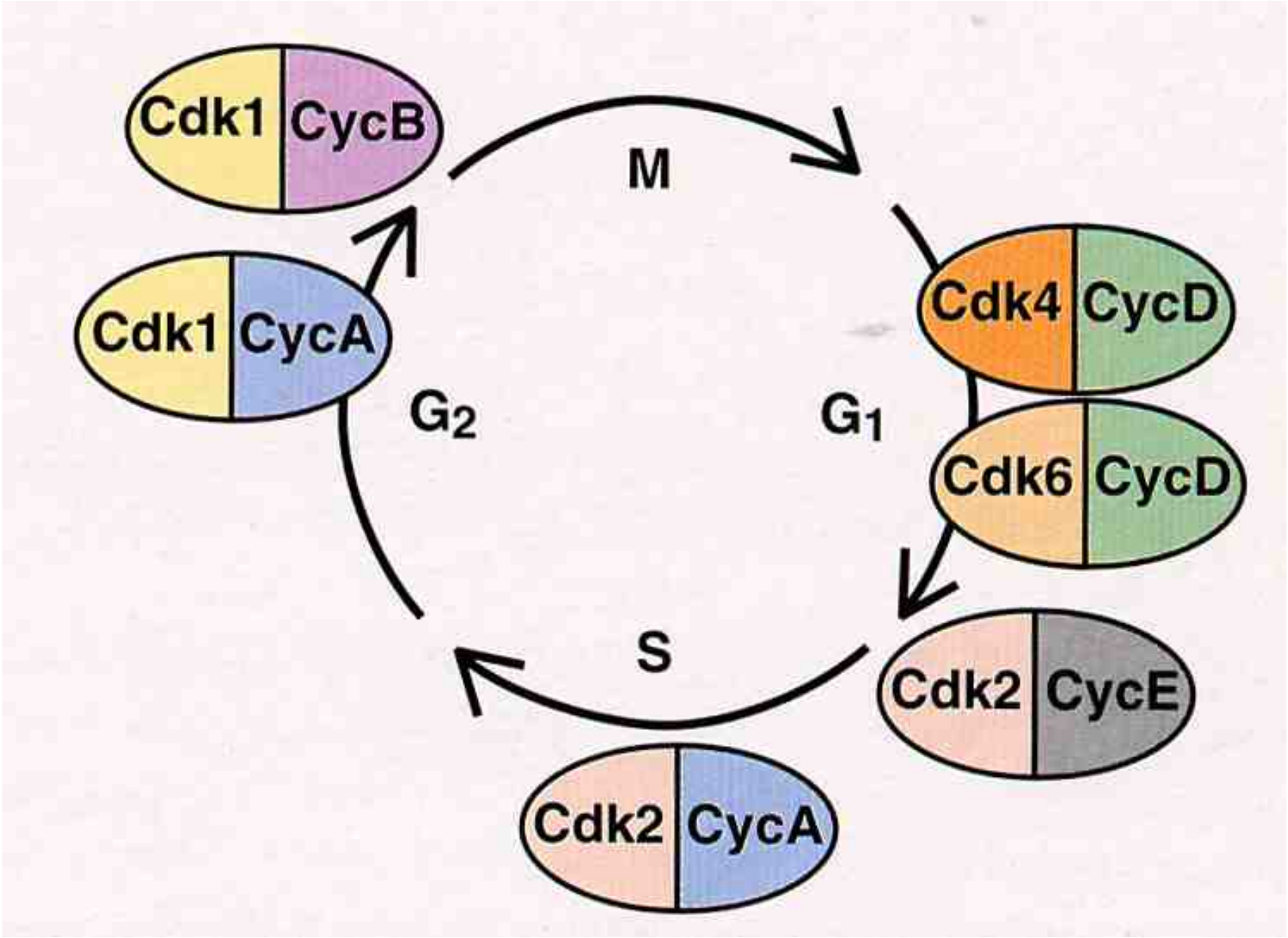
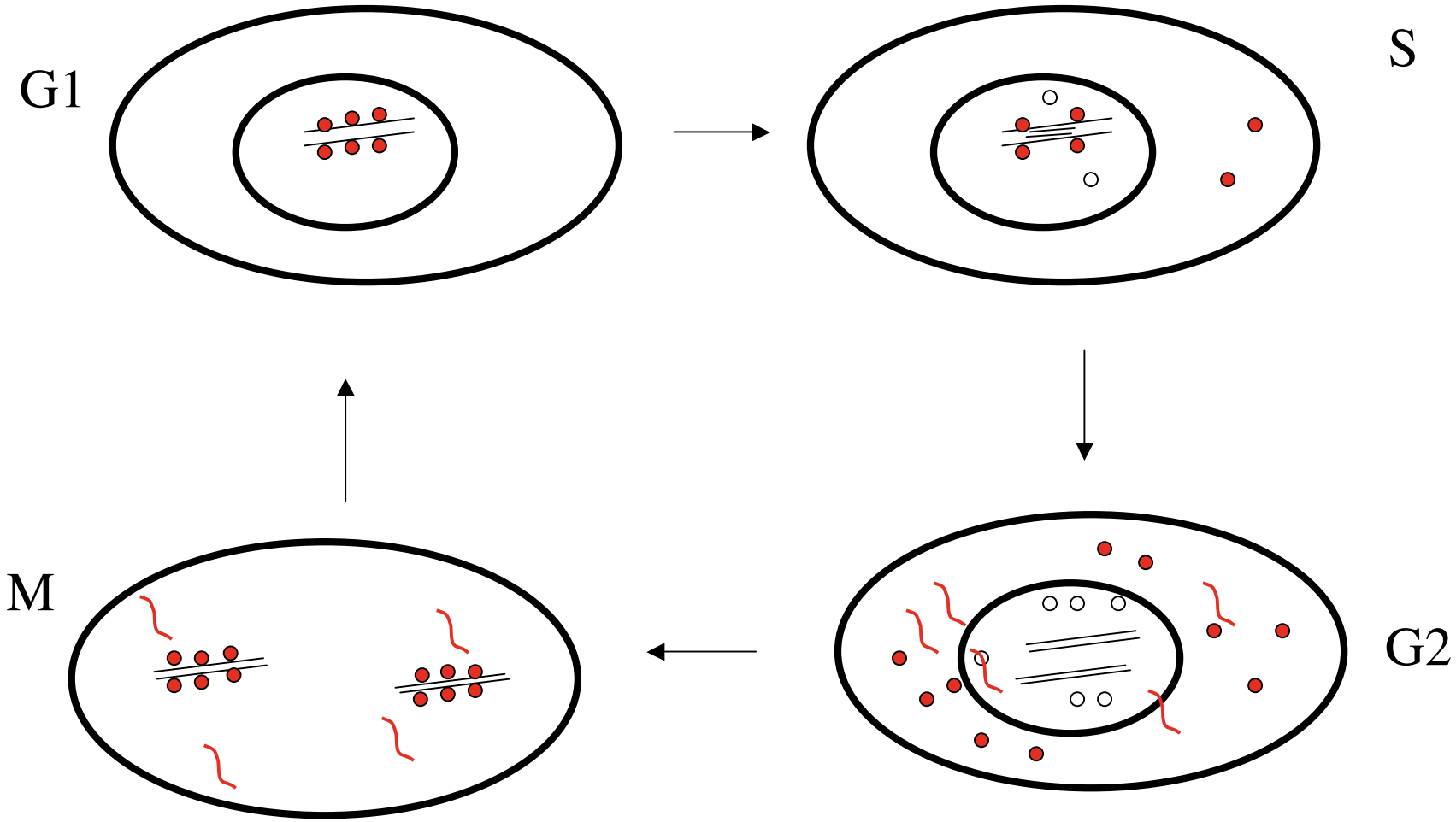
In addition to its characterized roles in nucleocytoplasmic transport Ran, a small GTPase of the Ras superfamily, has functions in:

-DNA reduplication inhibition
-centrosome duplication and microtubule dynamics
-kinetochore attachment of microtubules and chromosome alignment
-nuclear-envelope dynamics and NPC formation

DNA reduplication inhibition

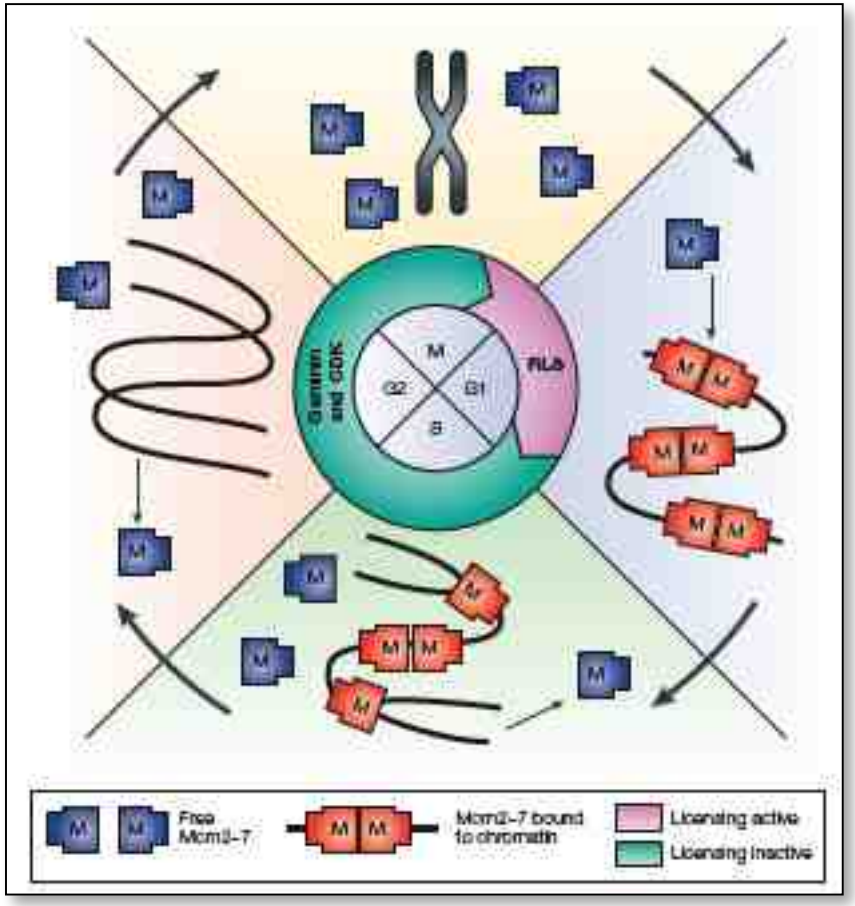

Cdk1
CycB
M
Cdk4
CycD
Cdk1
CycA
G2
G1
Cdk6
CycD
S
Cdk2
CycE
Cdk2
CycA



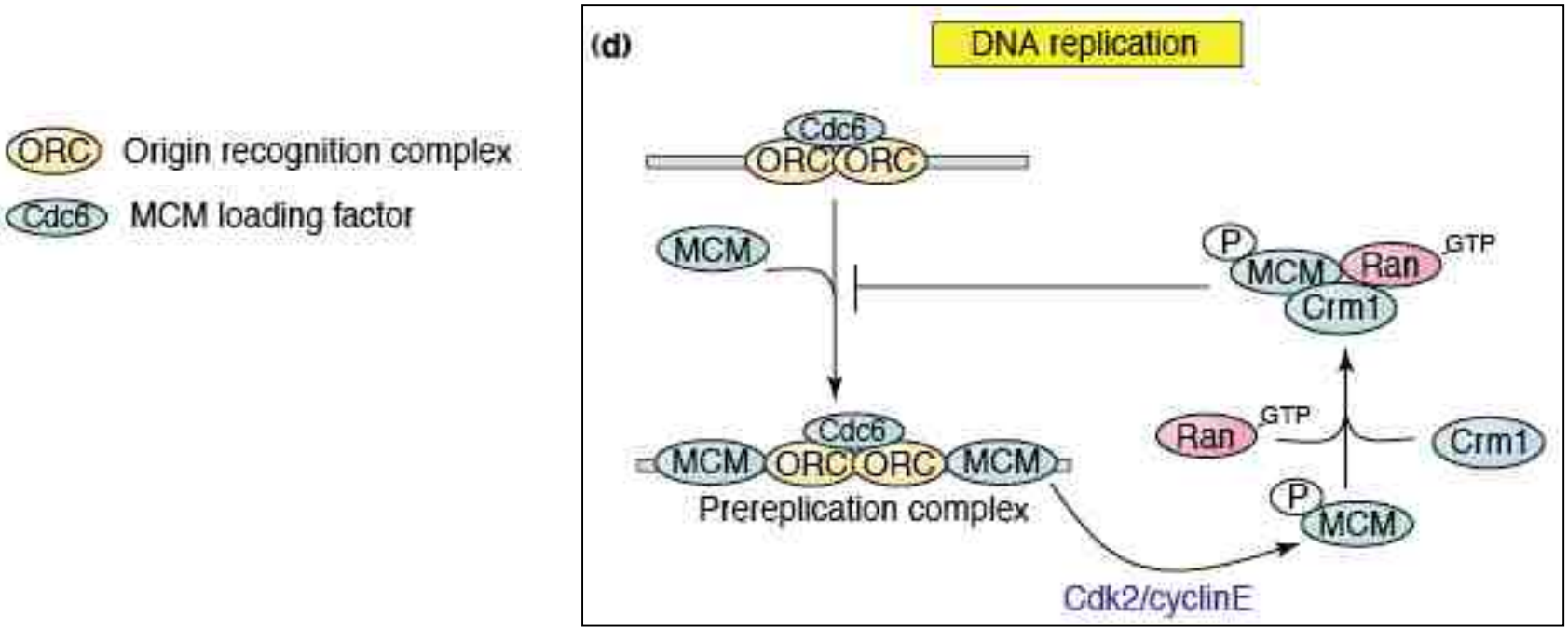
● *Licensing factors* ○ *Licensing factors* degradati

mRNA che codifica per licensing factor

Figure 1 | Regulated loading and unloading of Mcm2–7 during the cell cycle. A small segment of chromosomal DNA that encompasses three replication origins is shown. At the end of mitosis (M), the replication licensing system (RLS) is activated, which causes minichromosome maintenance (Mcm) 2–7 proteins (blue shapes marked 'M') to be loaded onto potential replication origins (origin licensing). The licensing system is turned off at the end of G1 by inhibition by cyclin-dependent kinases (CDKs) and/or geminin. During S phase, the Mcm2–7 complexes are displaced from replicated DNA by moving ahead of the replication fork, and are removed from DNA at fork termination. In this way, replicated DNA cannot undergo further initiation events until passage through mitosis.



(d) **RanGTP inhibits DNA reduplication in the same cell cycle**. During S phase, high cyclin-dependent kinase (Cdk) activity phosphorylates MCM helicase. This promotes the interaction of MCM with both RanGTP and Crm1 in the nucleus, thereby preventing MCM from participating in DNA reduplication.



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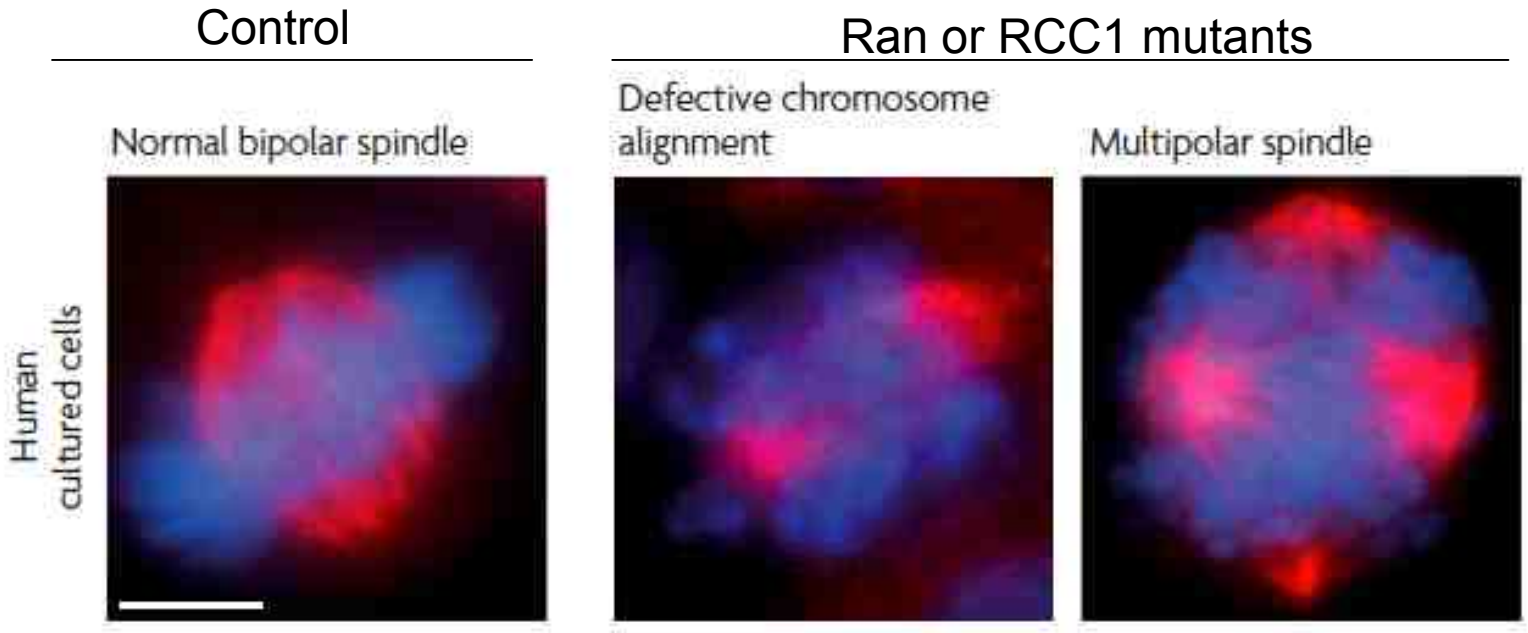
RAN in mitosis

→ centrosome duplication and microtubule dynamics

kinetochore attachment of microtubules and chromosome alignment

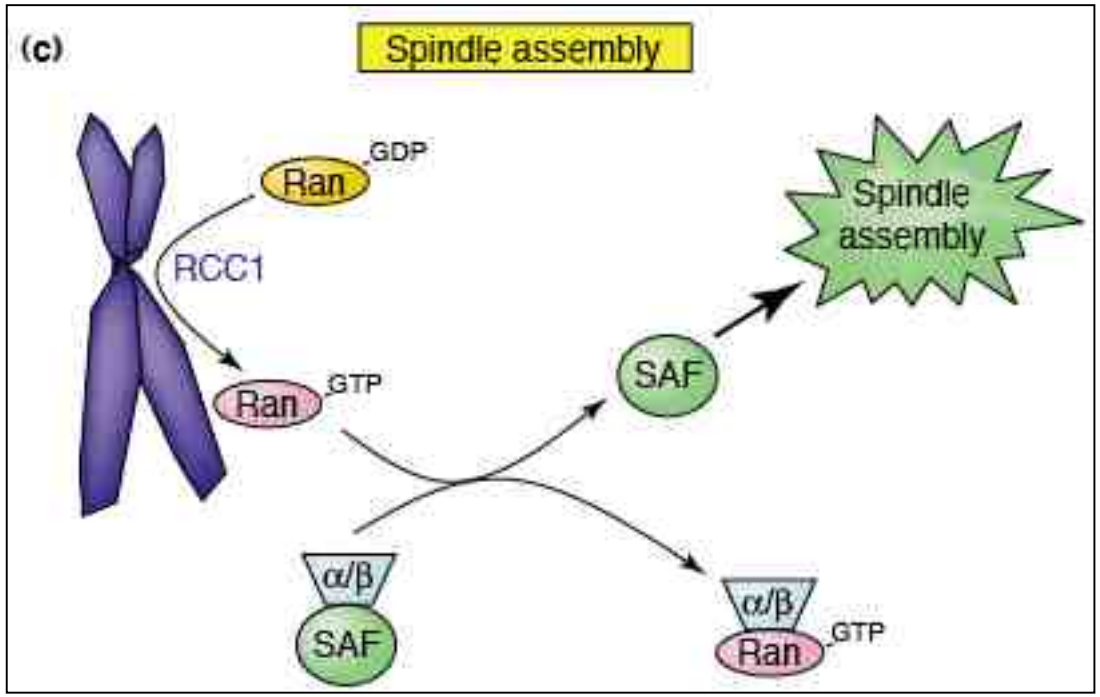
nuclear-envelope dynamics and NPC formation

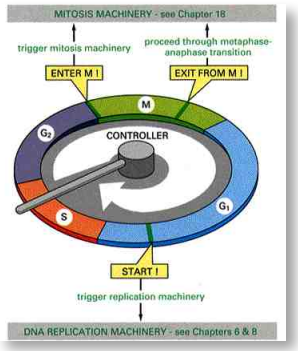
Disruption of the GTP–GDP cycle of Ran causes aberrant mitotic spindle assembly.



Expression of Ran mutants or mislocalization of the guanine nucleotide exchange factor RCC1 from the chromosomes produces defects in chromosome alignment at metaphase or multipolar spindles. Scale bars, 10 μm.

(c) **RanGTP stimulates spindle assembly during mitosis**. A high RanGTP concentration is generated on mitotic chromosomes by chromosome-bound RCC1. This RanGTP locally stimulates the release of spindle assembly factors (SAF) from the inhibitory binding of importin a/b to promote spindle assembly towards chromosomes.





α/β Importin α/β

SAF Spindle assembly factors

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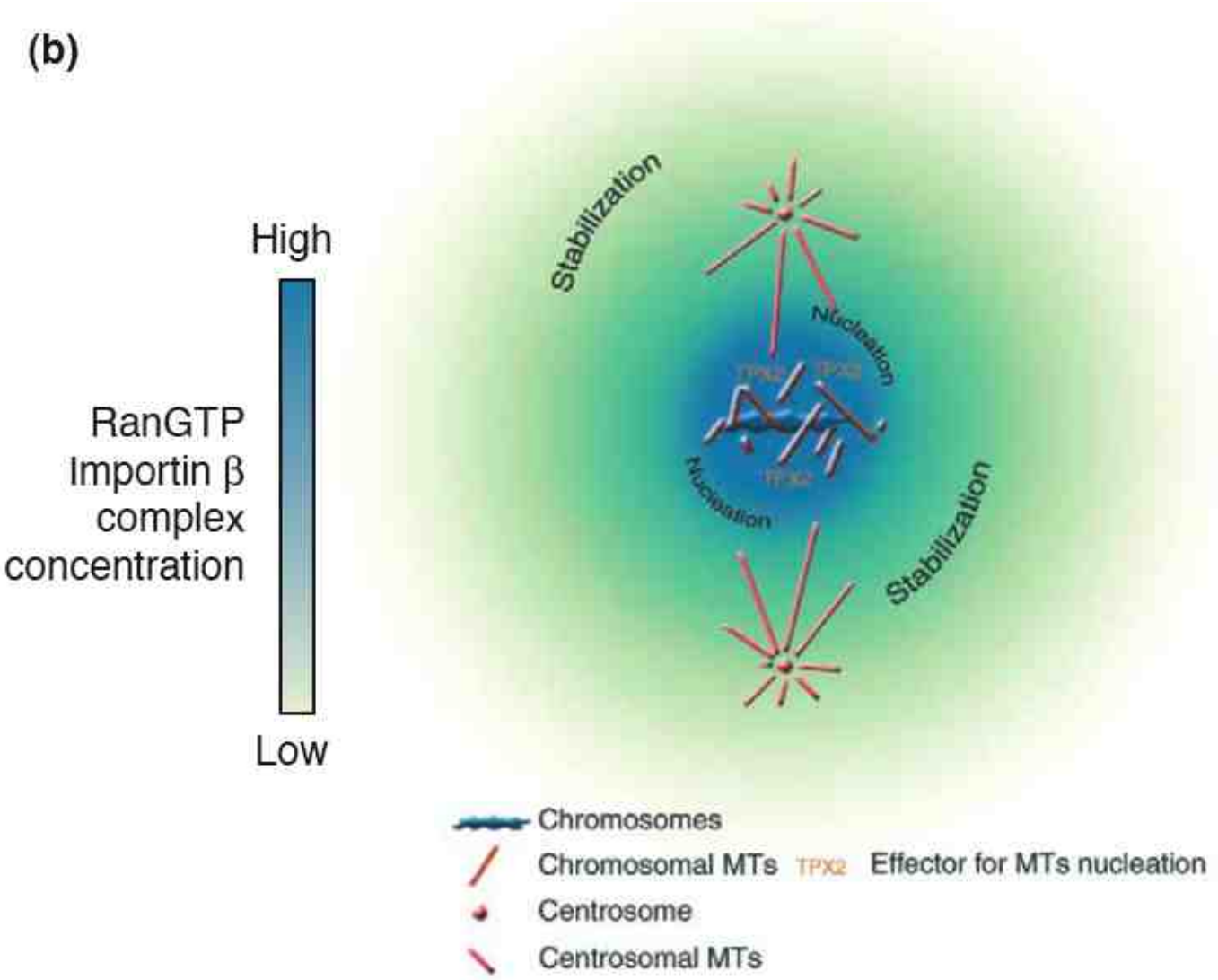
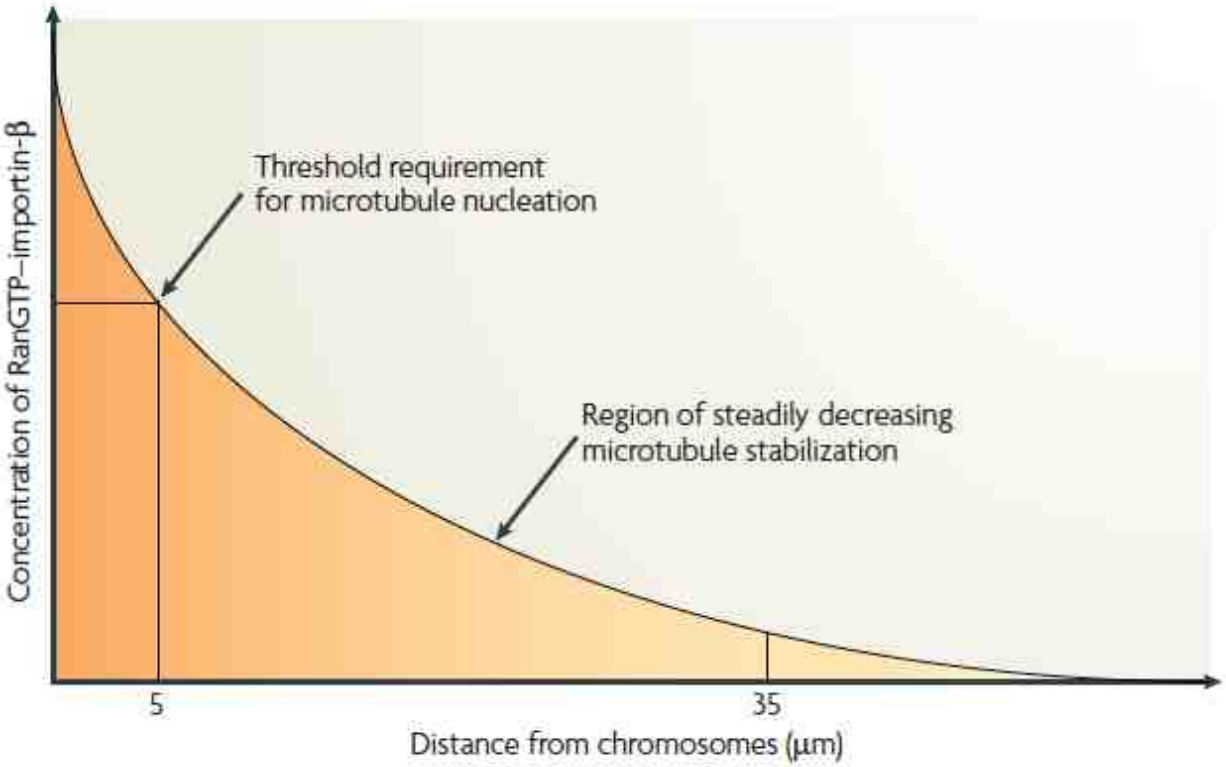
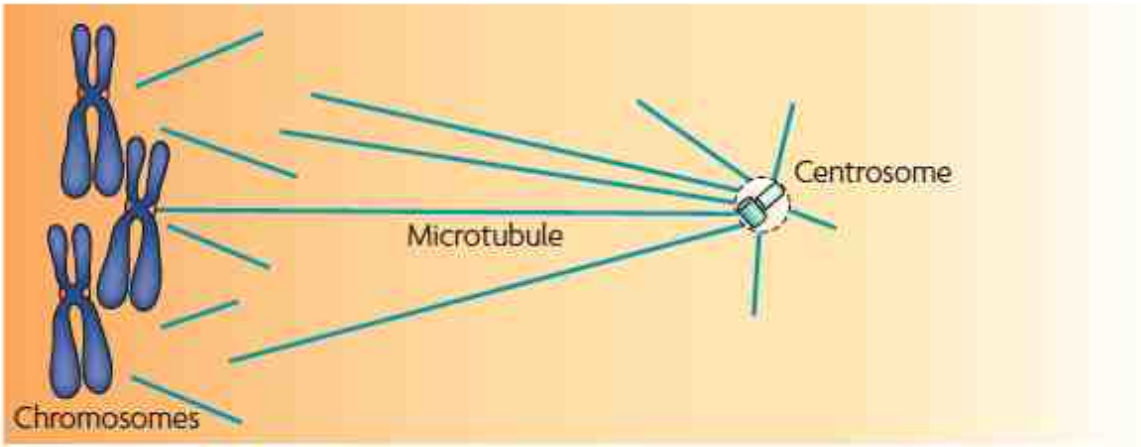
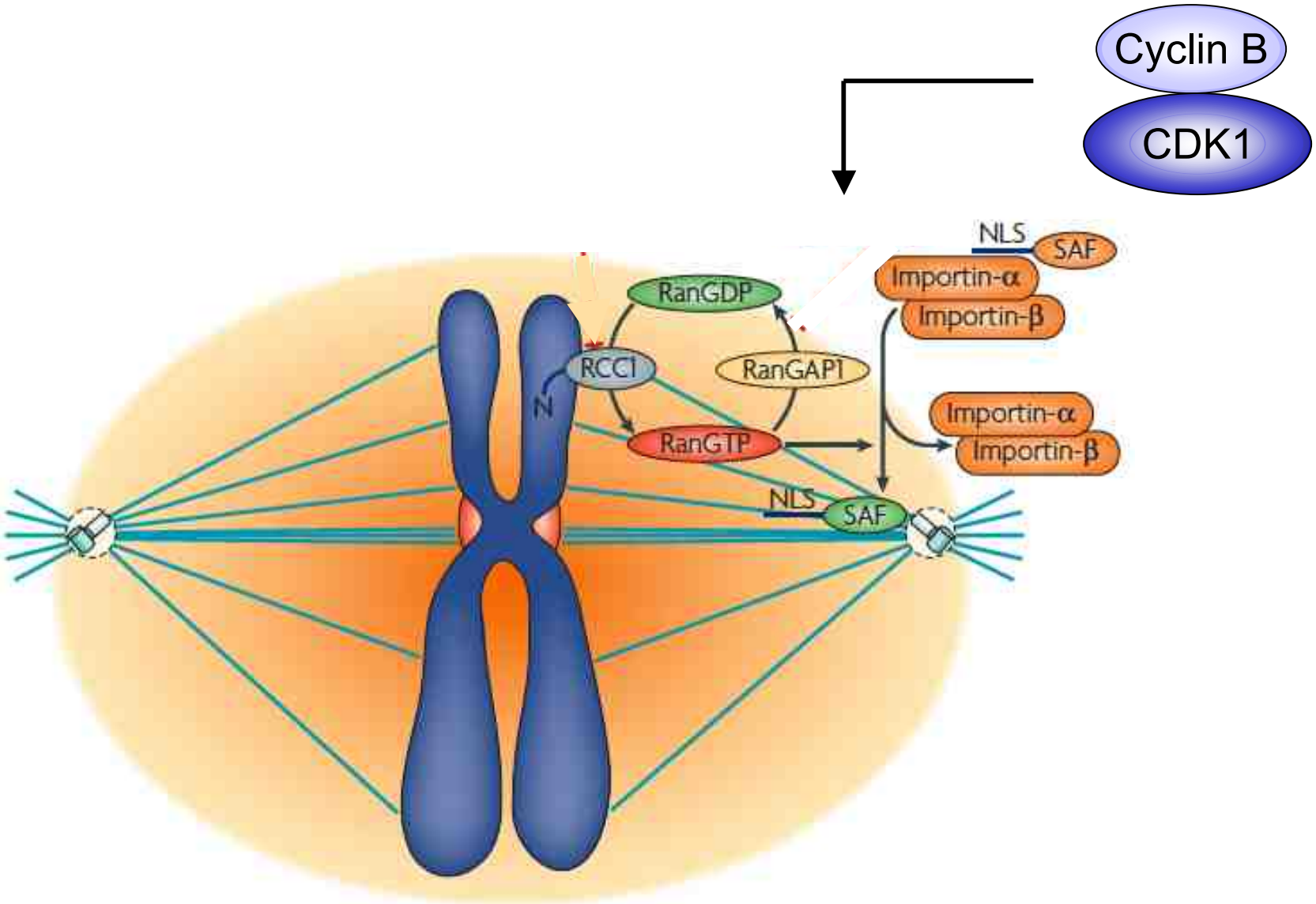
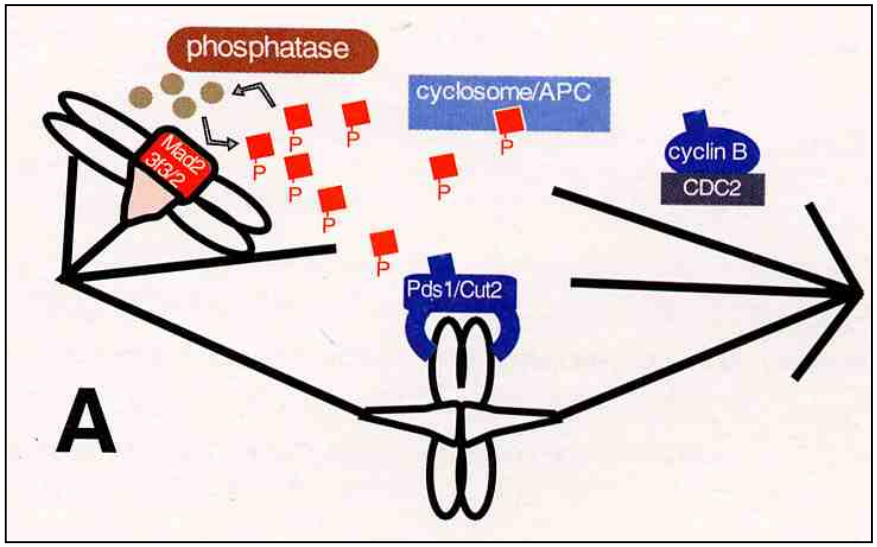

(b)
High
RanGTP
Importin β
complex
concentration
Low
Stabilization
Nucleation
Nucleation
Stabilization
Chromosomes
Chromosomal MTs
TPX2
Effector for MTs nucleation
Centrosome
Centrosomal MTs

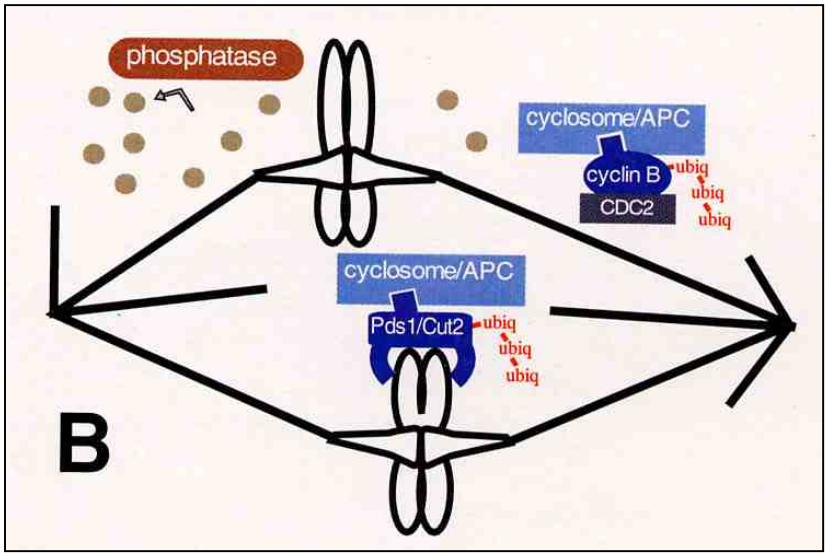

Concentration of RanGTP–importin-β
Threshold requirement
for microtubule nucleation
Region of steadily decreasing
microtubule stabilization
5
35
Distance from chromosomes (μm)

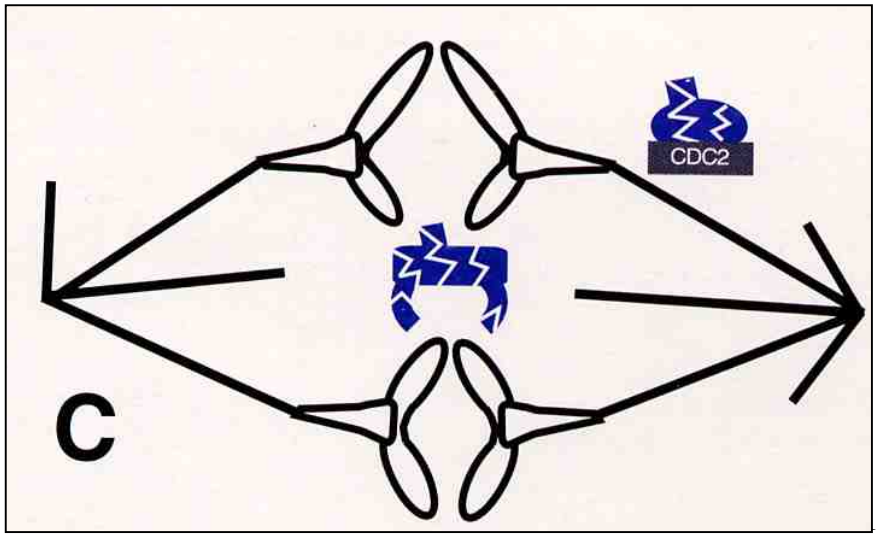

Centrosome
Microtubule
Chromosomes


Cyclin B
CDK1
NLS
SAF
Importin-α
Importin-β
RanGDP
RCC1
RanGAP1
N
RanGTP
Importin-α
Importin-β
NLS
SAF

Metaphase:Inhibition of Anaphase Promoting Complex (APC) by kinase activity of non-aligned chromosomes.



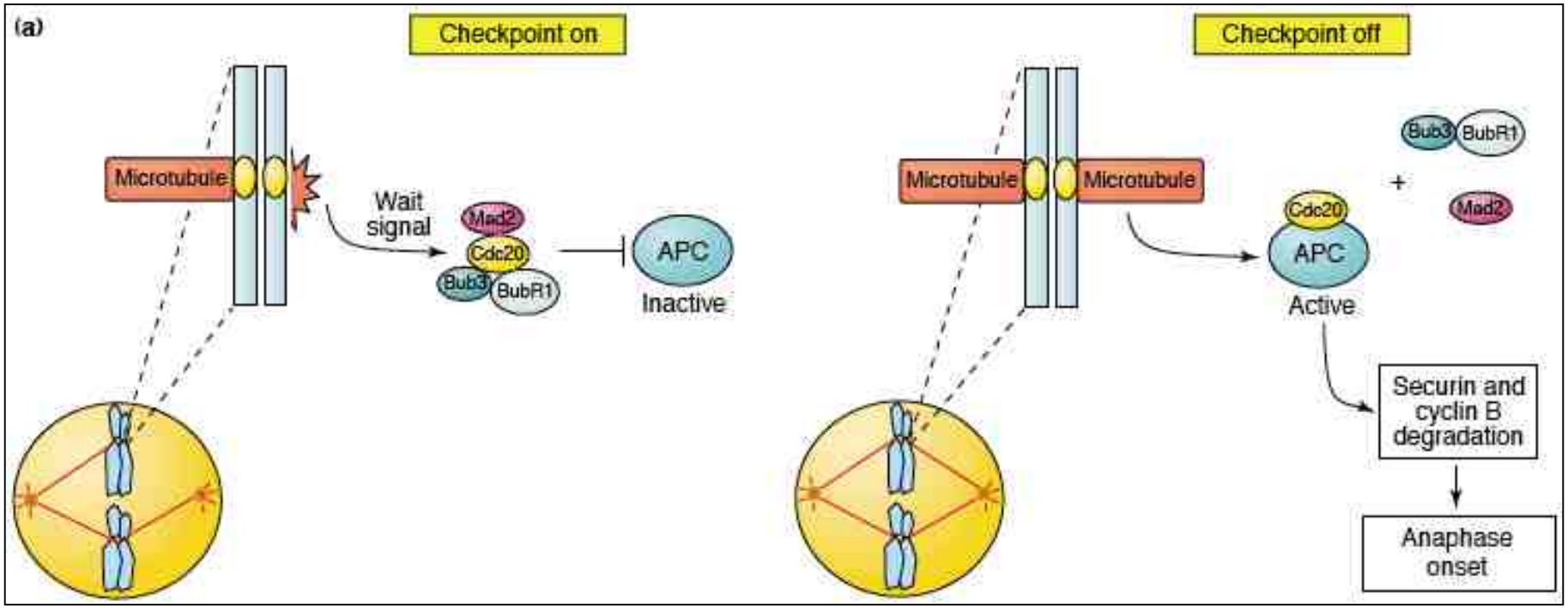




Tension: inhibition of the kinase activity responsible for APC inhibition

Anaphase: synchronization of chromatid separation

Activation and silencing of spindle checkpoint during mitosis.



(a) An unattached kinetochore sends out a 'wait' signal to halt cell-cycle progression at the metaphase–anaphase transition. This wait signal is generated by dynamic association of the spindle-checkpoint components with the unattached kinetochore, which leads to the inhibition of Cdc20/FZY by the checkpoint components. As a result, anaphase-promoting complex (APC) is inactivated and the cells are arrested at metaphase. Upon proper attachment of microtubules to all kinetochores, checkpoint components dissociate from Cdc20/FZY, allowing the activation of APC by Cdc20/FZY. Ubiquitination of securin and cyclin B by APC leads to their destruction, thereby triggering chromosome segregation and anaphase onset.

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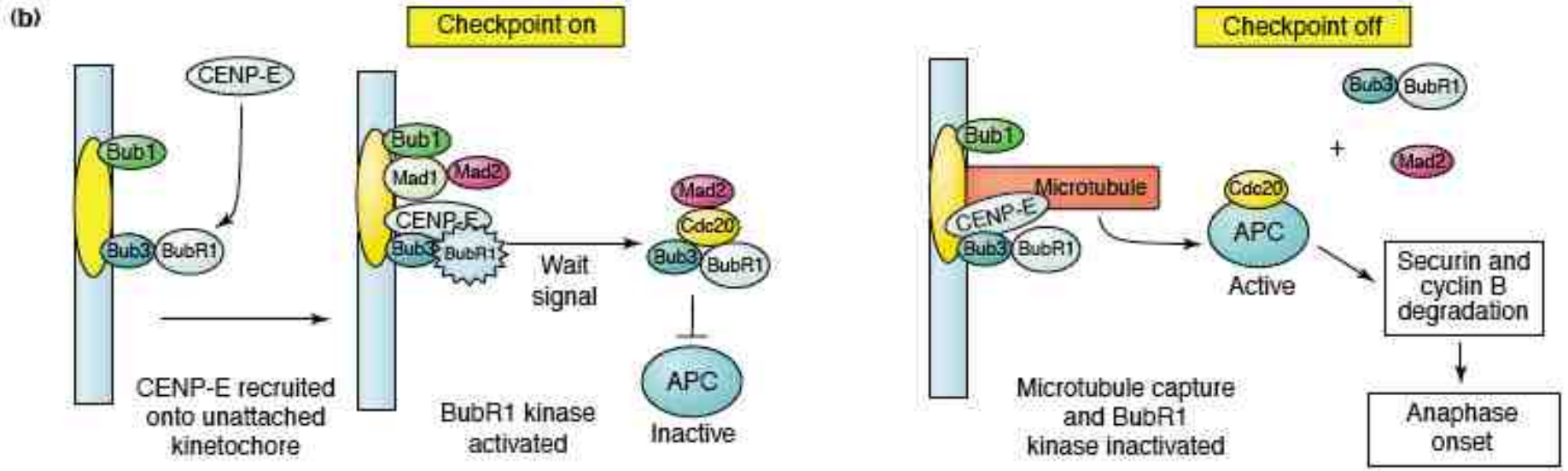

(b). CENP-E (kinetochore-associated kinesin)-dependent BubR1 kinase is required for turning on and off of the spindle-checkpoint signal. BubR1 is required for recruiting CENP-E to the unattached kinetochore. Direct association of CENP-E and BubR1 activates BubR1 kinase activity, which is essential for mitotic checkpoint signaling. The spindle checkpoint is silenced by CENP-E dependent capturing of microtubule, which alters or disrupts the interaction of CENP-E with BubR1 and inactivates BubR1 kinase.

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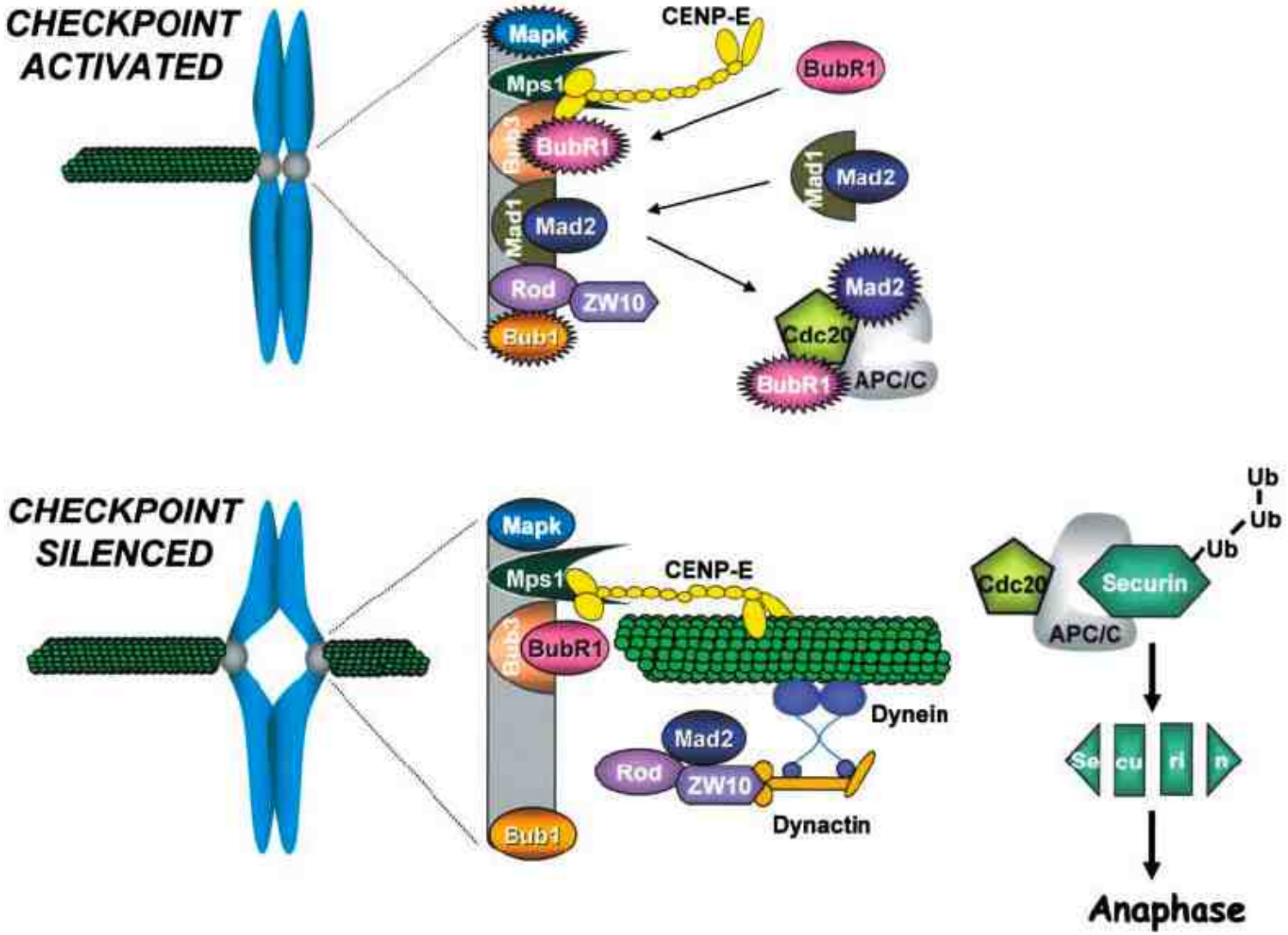
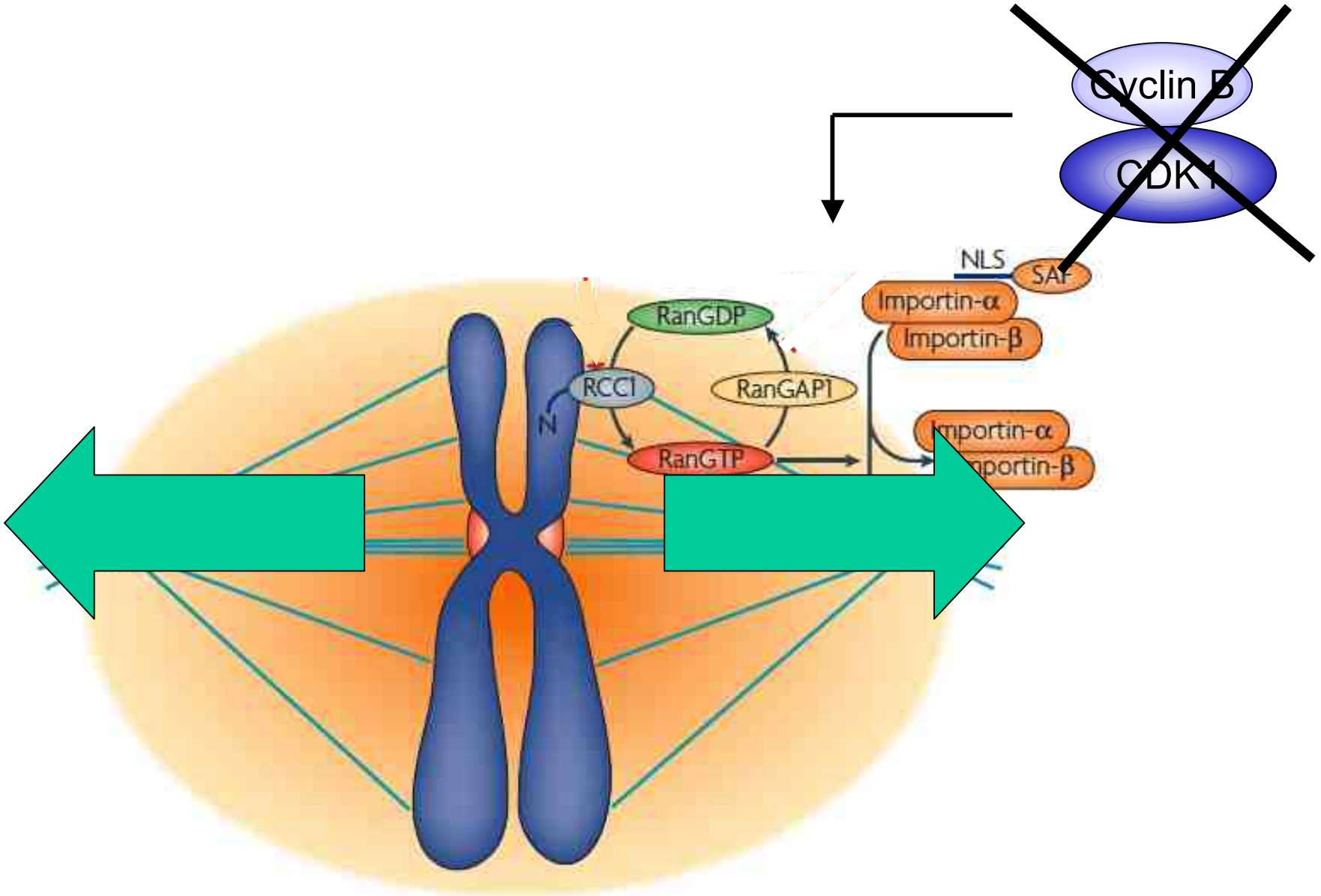

CHECKPOINT ACTIVATED
Mapk
Mps1
Bub3
BubR1
Mad1
Mad2
Rod
ZW10
Bub1
CENP-E
BubR1
Mad1
Mad2
Mad2
Cdc20
BubR1
APC/C
CHECKPOINT SILENCED
Mapk
Mps1
Bub3
BubR1
CENP-E
Dynein
Mad2
Rod
ZW10
Dynactin
Bub1
Ub
Ub
Ub
Cdc20
Securin
APC/C
Anaphase


Cyclin B
CDK1
NLS
SAF
Importin-α
Importin-β
RanGDP
RCC1
RanGAP1
RanGTP
N
Importin-α
Importin-β

RAN in mitosis

centrosome duplication and microtubule dynamics

kinetochore attachment of microtubules and chromosome alignment

→ nuclear-envelope dynamics and NPC formation

nuclear-envelope dynamics and NPC formation

NPC life cycle Assembly

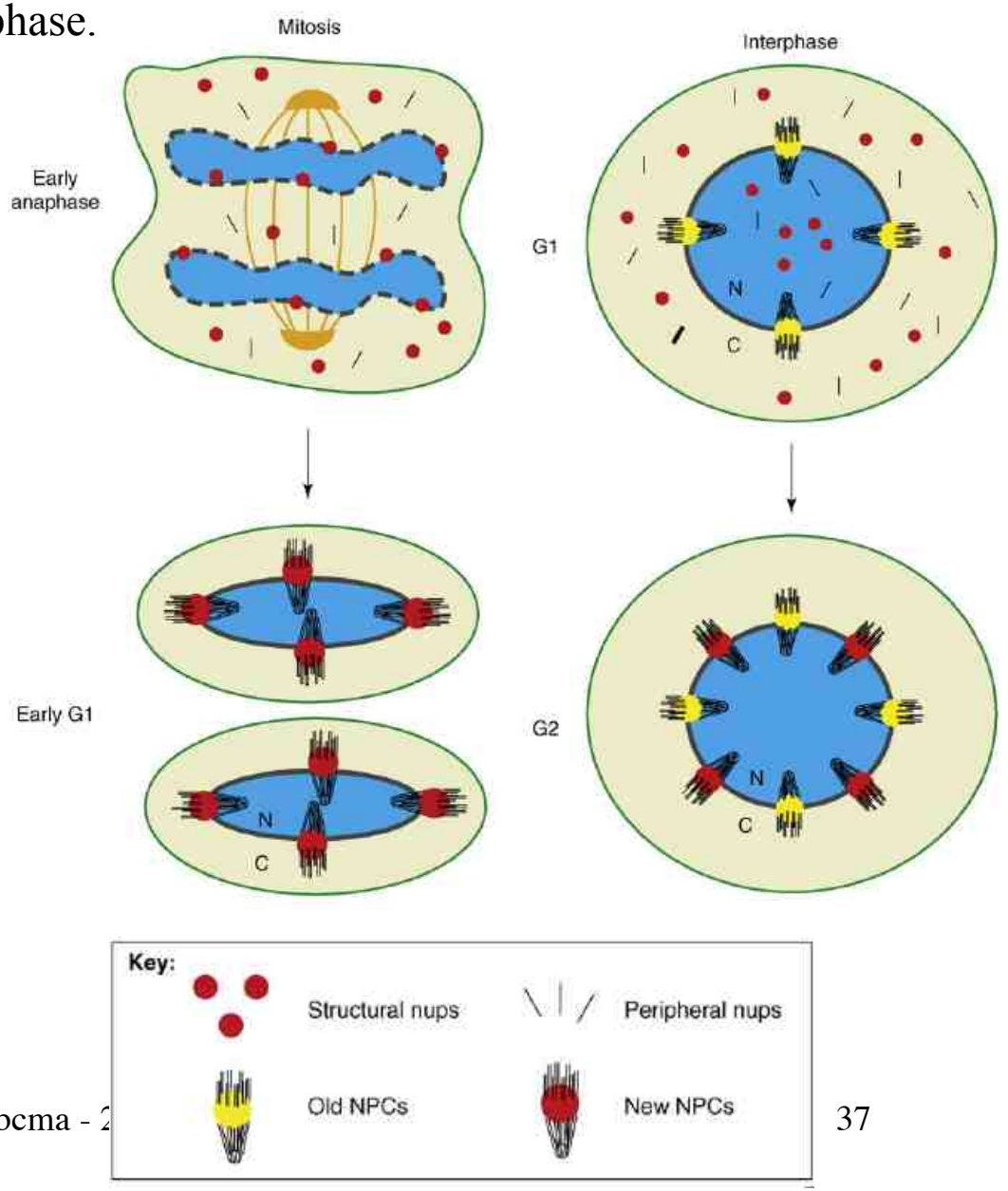
The biogenesis of nuclear pores is essential for cell survival and proliferation. There are two phases during the metazoan cell cycle in which NPCs need to be assembled: first, at the end of mitosis, when theNEreforms around the segregated chromosomes and second, during interphase in which the cells double their number of pores in preparation for the next round of division.

Even though both processes lead to the same final structure, they occur under very different conditions. Mitotic-NPC assembly takes place concomitantly with reformation of the nuclear membrane around segregated chromosomes. At this time, NPCs are rebuilt from disassembled subcomplexes that were dispersed into the cytoplasm during breakdown of the NE. By contrast, NPC assembly during interphase occurs in an intactNEusing newly synthesized nups and in a cellular environment in which the nucleus and the cytoplasm are physically separated.

Interphase assembly is the only existing mechanism in organisms, such as yeasts, that undergo closed mitosis (i.e. their NE does not break down during cell division). Although accumulating evidence indicates that both types of pore assembly might occur through similar processes, owing to their very different environments it is likely that differences will be uncovered.

NPC assembly during mitosis and interphase.

Mitotic-NPC assembly occurs concomitantly with the formation of new nuclear envelopes (NEs) around chromatin. During this time, NPCs assemble by recycling subcomplexes that were dispersed into the cytoplasm during NPC and NE breakdown. Note that, during mitosis, the cytoplasmic and nuclear contents are mixed together. Mitotic assembly is a step-wise process that begins with the recruitment of structural nups to chromatin during early anaphase. By contrast, during interphase, NPCs assemble into an intact NE when the nucleus and cytoplasm are physically separated. During this process, NPCs use newly synthesized nucleoporins present on both sides of the nuclear envelope.



Concentration of Ran on chromatin induces decondensation, nuclear envelope formation and nuclear pore complex assembly

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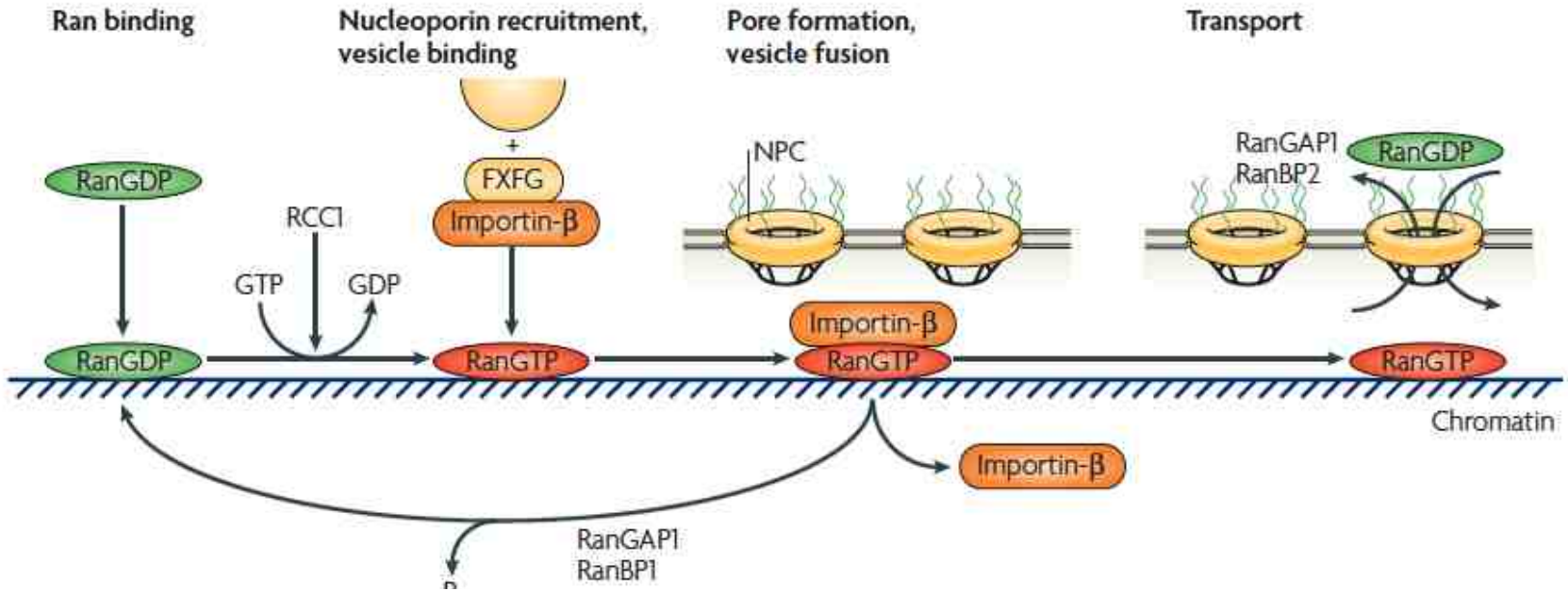
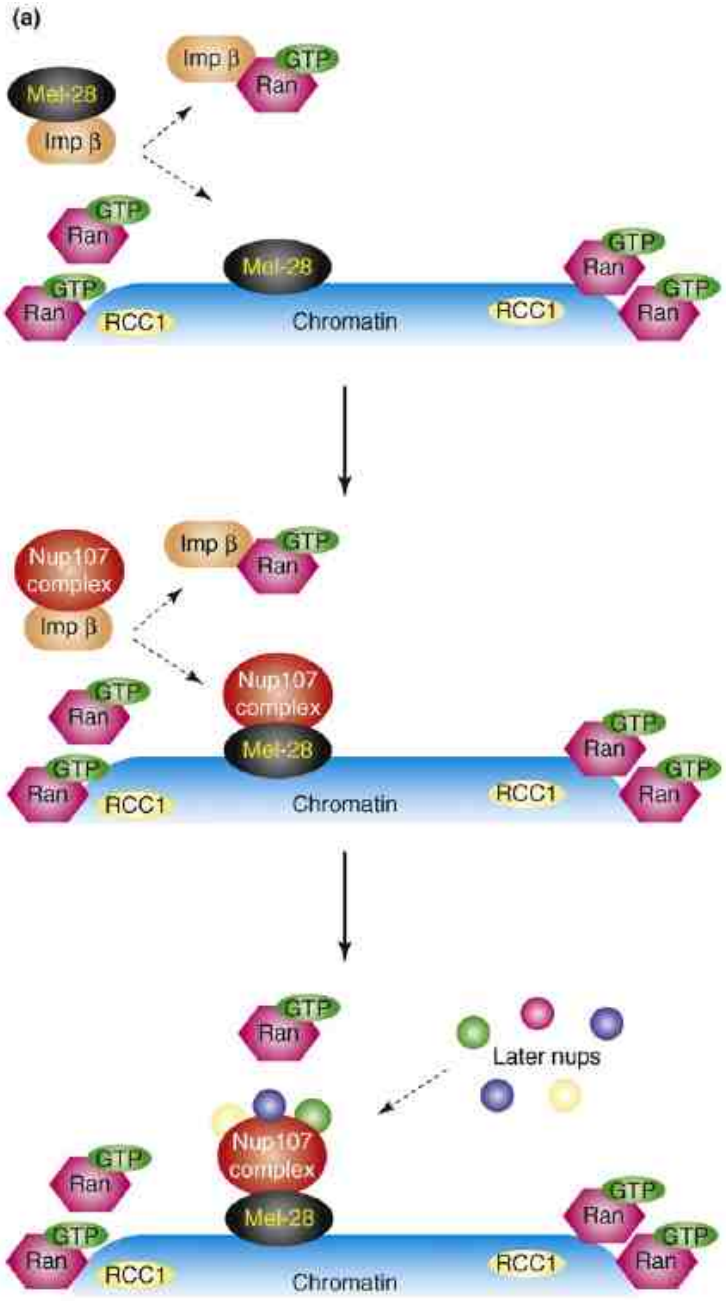


A simple model for the role of Ran in nuclear-envelope assembly. In telophase, Ran (probably in its GDP bound form) becomes recruited and concentrated on the surface of chromatin. Localized generation of RanGTP by the guanine nucleotide-exchange factor RCC1 recruits nucleoporins and vesicles through importin-β, which acts as a localization factor. The interaction of RanGTP with importin-β complexes causes their local release and assembly into nuclear pore complexes (NPCs), the fusion of vesicles to form a double membrane and the restarting of nuclear transport. Importin-β is released from Ran by the action of the Ran GTPase-activating proteins RanGAP1 and RanBP1 (Ran binding protein-1) or RanBP2, which stimulate GTP hydrolysis by Ran. FXFG represents a Phe-Gly (FG) repeat sequence.

Importin b and RanGTPase regulation of NPC assembly.

During mitosis, importin b binds and sequesters the Elys/Mel-28 nucleoporin (Mel-28), preventing its interaction with chromatin. When the importin-b–Mel-28 complexes are in the proximity of DNA, where there is a high concentration of RanGTP owing to the chromatin association of the Ran GDP–GTP exchange factor [regulator of chromosome condensation (RCC1)], RanGTP binds to the transport receptor, thereby releasing Mel-28 and enabling it to bind to chromatin. Following the same mechanism, the importin- b-bound Nup107–160 complex is released by RanGTP in the proximity of DNA and recruited to chromatin through Mel-28. The chromatin-bound Nup107–160 complex can then recruit other nucleoporins in a step-wise manner.



Nuclear Pores Form de Novo from Both Sides of the Nuclear Envelope

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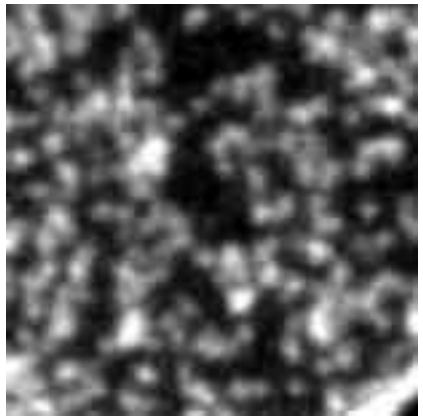
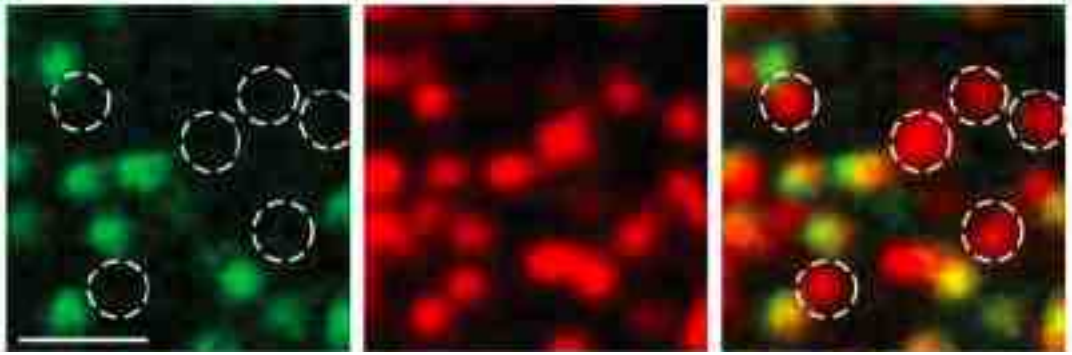
Nuclear pore complexes are multiprotein channels that span the double lipid bilayer of the nuclear envelope. How new pores are inserted into the intact nuclear envelope of proliferating and differentiating eukaryotic cells is unknown. We found that the Nup107-160 complex was incorporated into assembly sites in the nuclear envelope from both the nucleoplasmic and the cytoplasmic sides. Nuclear pore insertion required the generation of Ran guanosine triphosphate in the nuclear and cytoplasmic compartments. Newly formed nuclear pore complexes did not contain structural components of preexisting pores, suggesting that they can form de novo.





Experiments using in vitro-assembled nuclei have indicated that NPCs can form de novo, without using subunits from pre-existing pores

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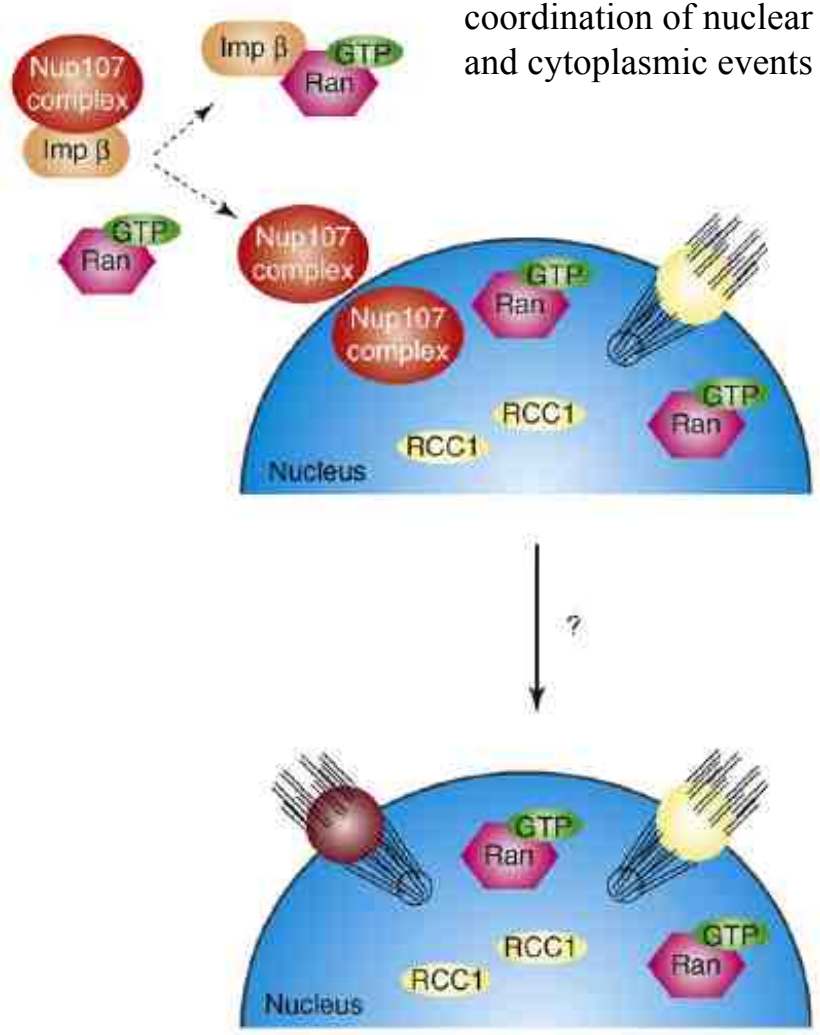
Importin b and RanGTPase regulation of NPC assembly.

Interphase assembly: During interphase, the number of pores doubles to prepare the cells for re-entering mitosis.
Previously, it was believed that NPC doubling was restricted to S-phase; however, recent studies have indicated that NPCs are assembled continuously from G1 to G2 phases.
NPC assembly during interphase requires the RanGTP-dependent release of the Nup107–160 complex from importin b on the cytoplasmic and nuclear side of the NE. How the released complexes coordinate the formation of a functional NPC from both sides of the nuclear envelope is still unclear.



interphase NPC assembly requires the coordination of nuclear and cytoplasmic events

Interphase NPC assembly form independently of pre-existing pores and from both sides of the nuclear envelope, following a mechanism that is at least partially conserved with mitotic-NPC assembly: This raises the question of whether the chromatin-bound intermediates observed during mitosis indeed represent that part of the pore that is inserted into the NE from the nucleoplasmic side during interphase.
If so, the mitotic- and interphase-assembly mechanisms would involve a coordinated interaction between chromatin- bound subcomplexes, cytoplasmic subcomplexes and transmembrane NPC components to assemble the multiprotein NPC into the double membrane of the NE.

Disassembly

NPC disassembly has only been described during mitosis and there is no evidence of pores being dismantled during interphase.

Similar to the assembly process, NPC disassembly takes place through an ordered process.