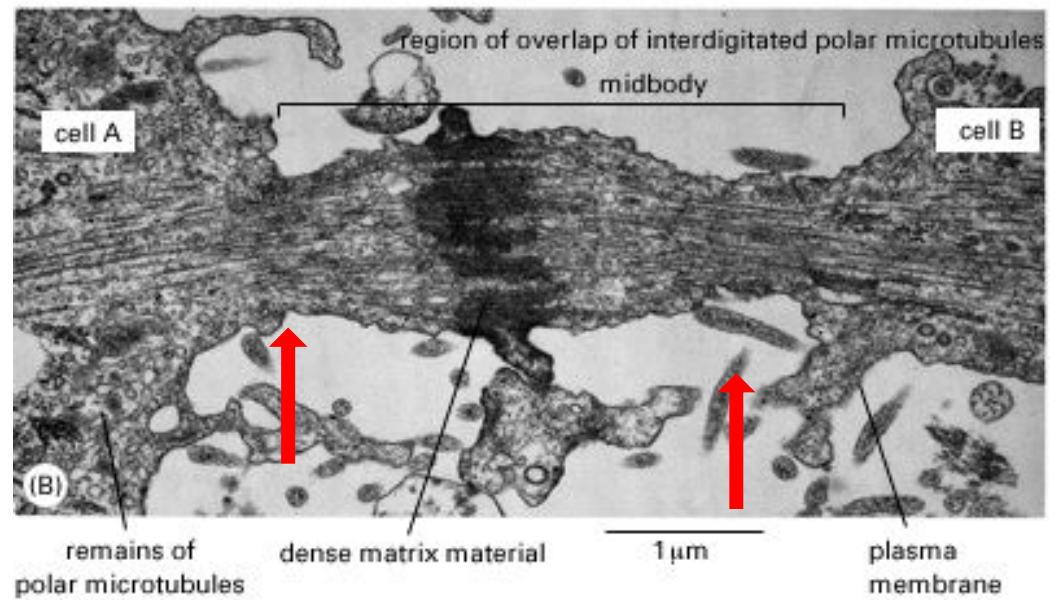
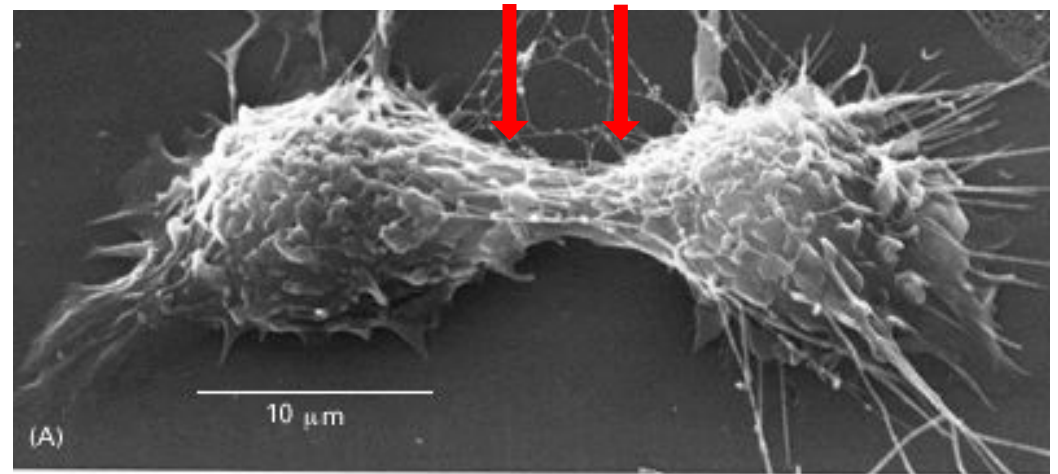


# **Лекция 7**

**Митотические пункты  
контроля, продолжение**

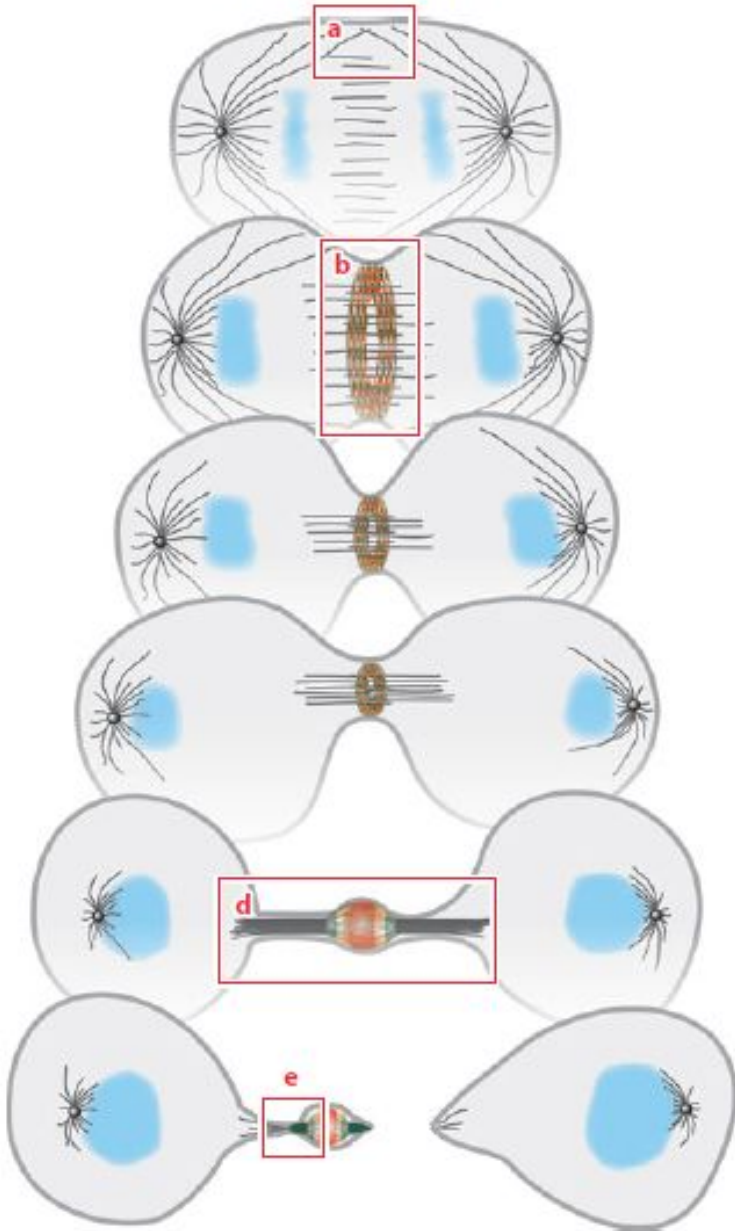
**Старение клеток**

# ЦИТОКИНЕЗ



Тельце Флемминга

Cytokinesis at a glance



# Цитокинез

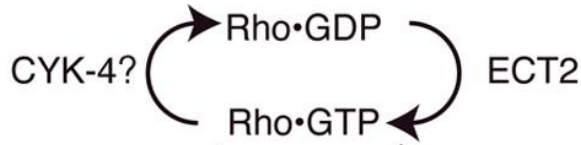
Сжатие мембран происходит с помощью сокращения актомиозинового кольца – его надо сформировать (1) и затем дать сигнал к сокращению (2).

После того, как мембраны сблизятся, должен сработать механизм слияния (3)

# Формирование акто-миозинового кольца

GAP ( GTF-ase activating protein)

GEF (GTF-exchange factor)



ROCK

MYPT

rMLC-PO4

active myosin II

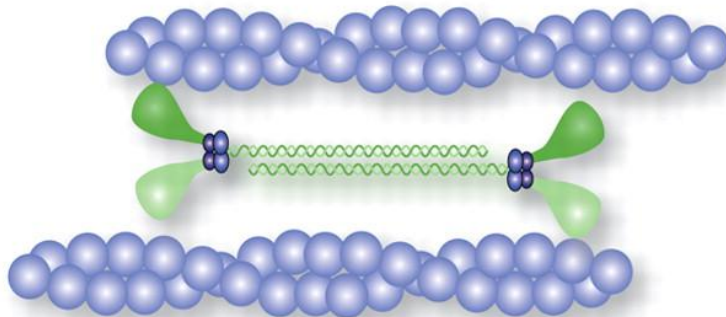
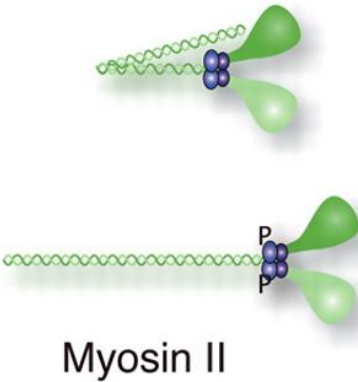
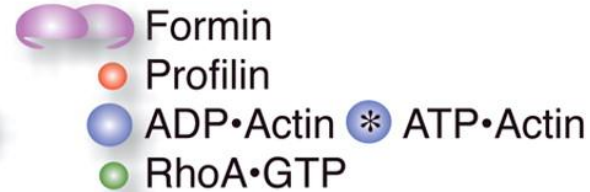
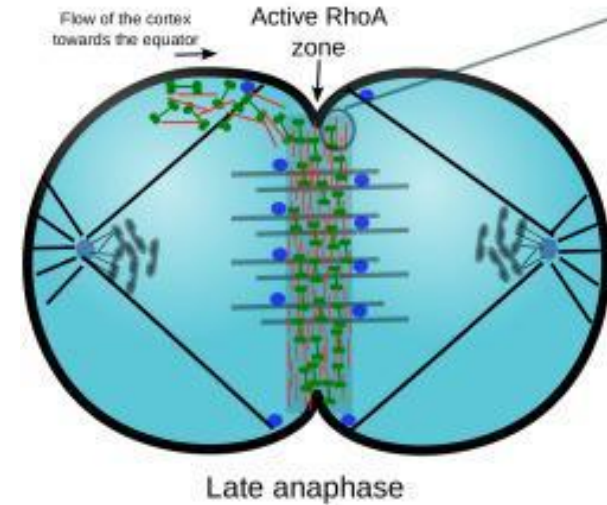
formin-homology proteins

profilin

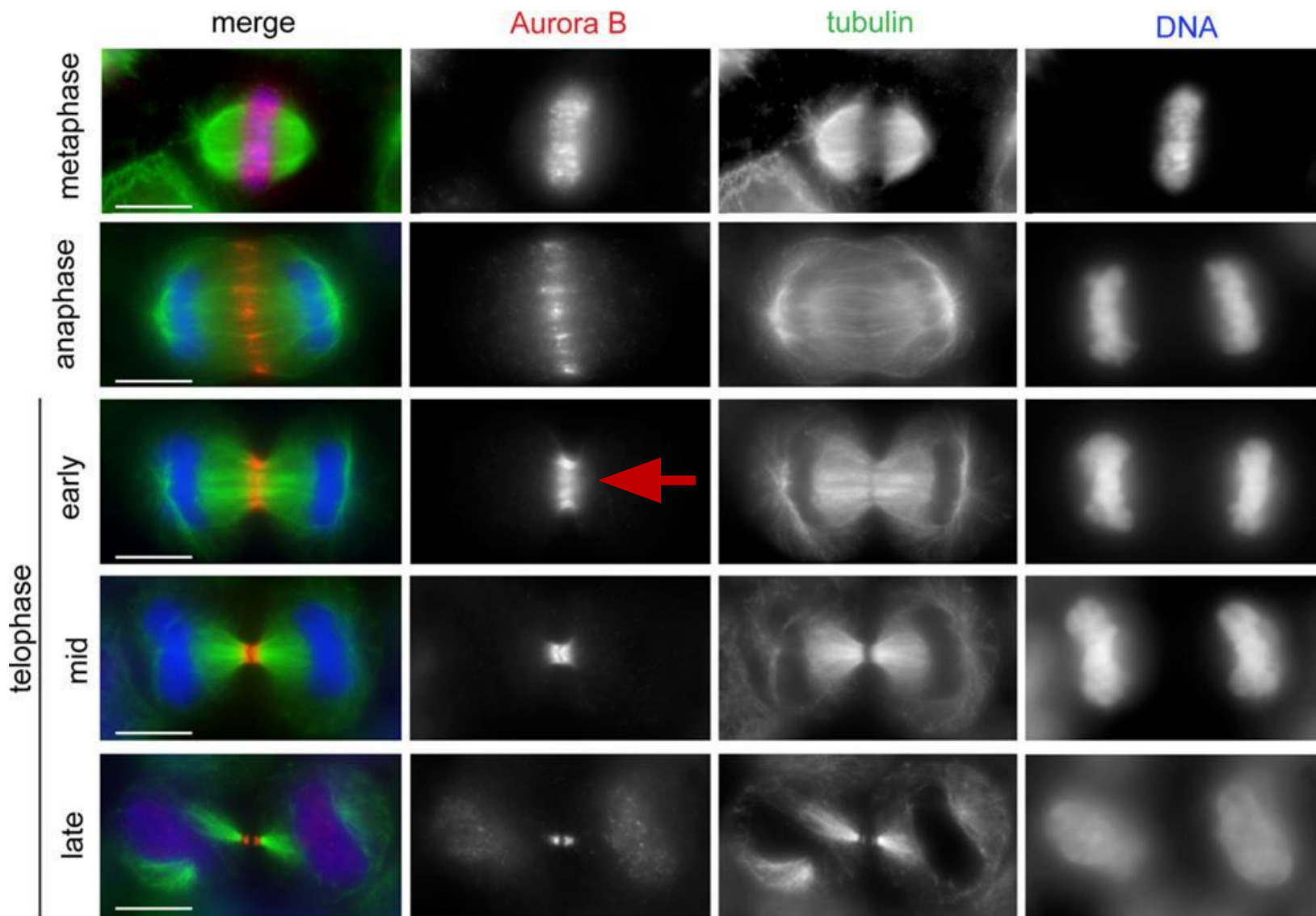
f-actin

contractile ring

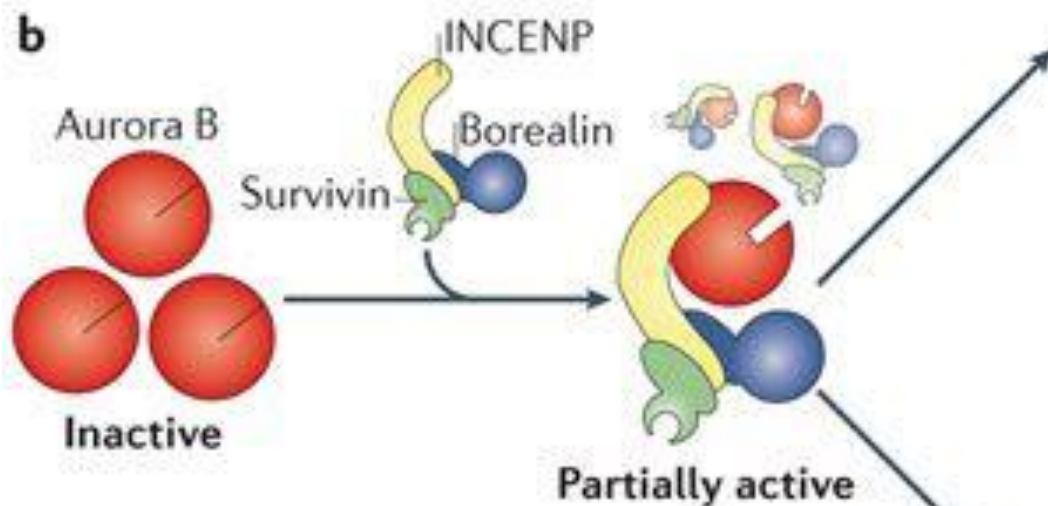
Actin and Myosin II



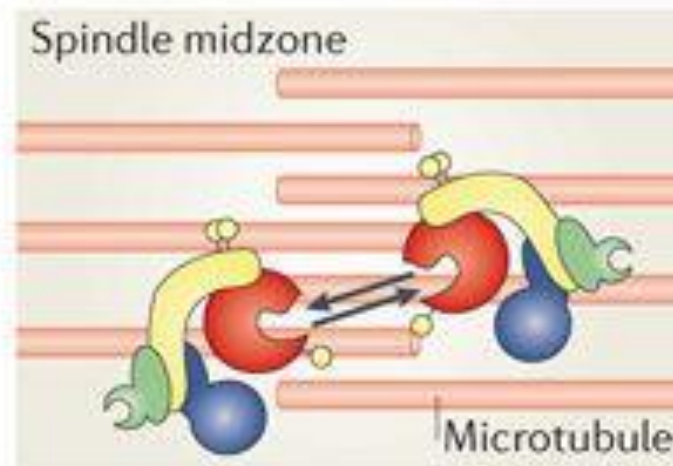
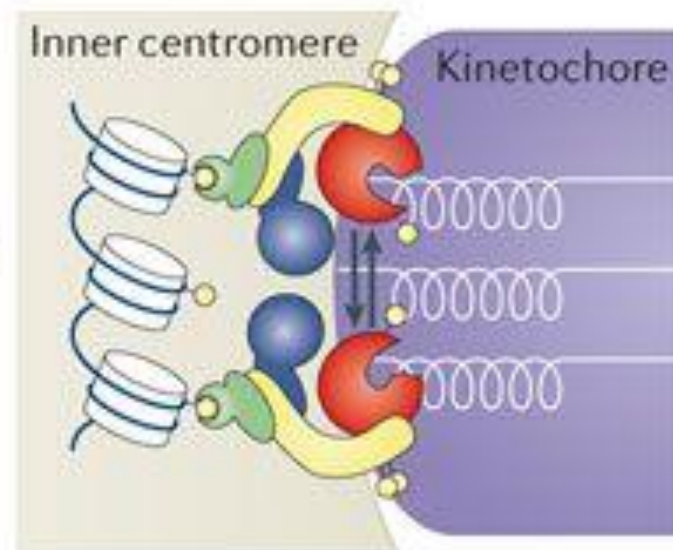
# Локализация Авроры В в митозе



# Перемещение CPC по микротрубочкам



До анафазы

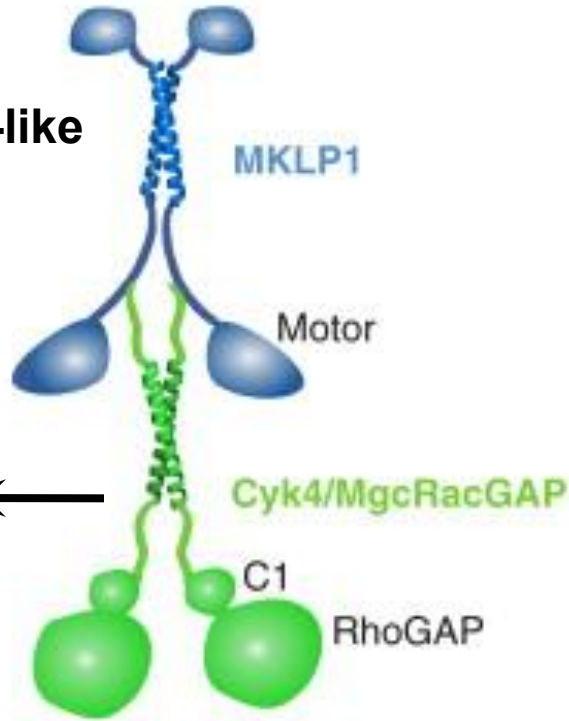


Fully active

**MKLP2** mitotic kinesin-like protein 2 – моторный белок, связывается с INCENP и перемещает весь комплекс CPC по микротрубочкам

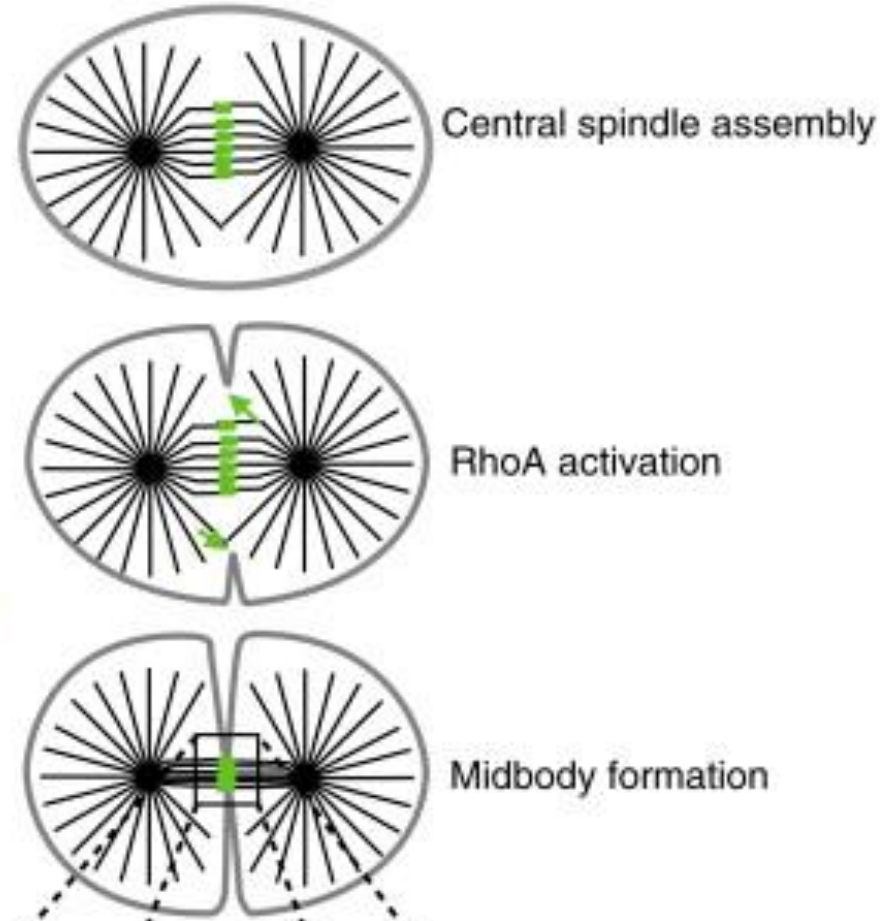
**A** Centralspindlin tetramer

mitotic kinesin-like protein 1

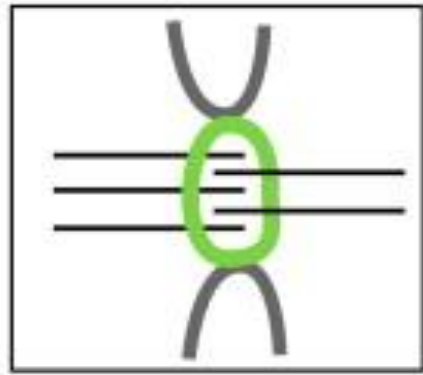


Активация Ect2

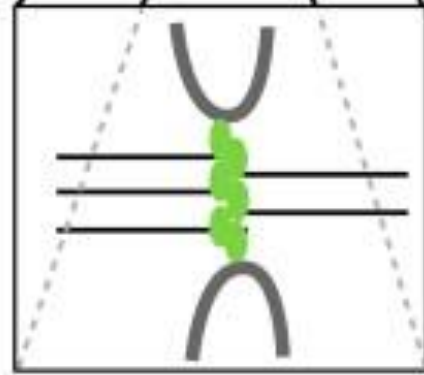
**B**



**C**

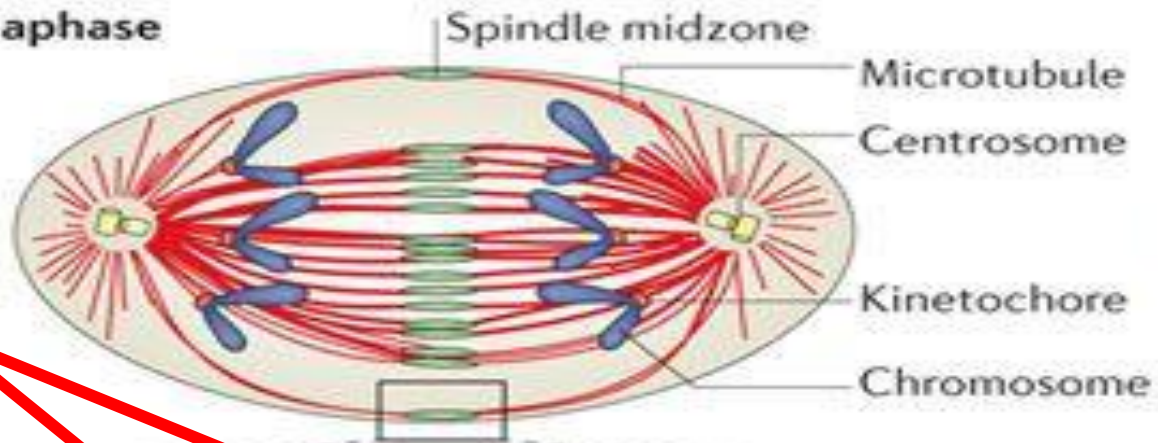


Or

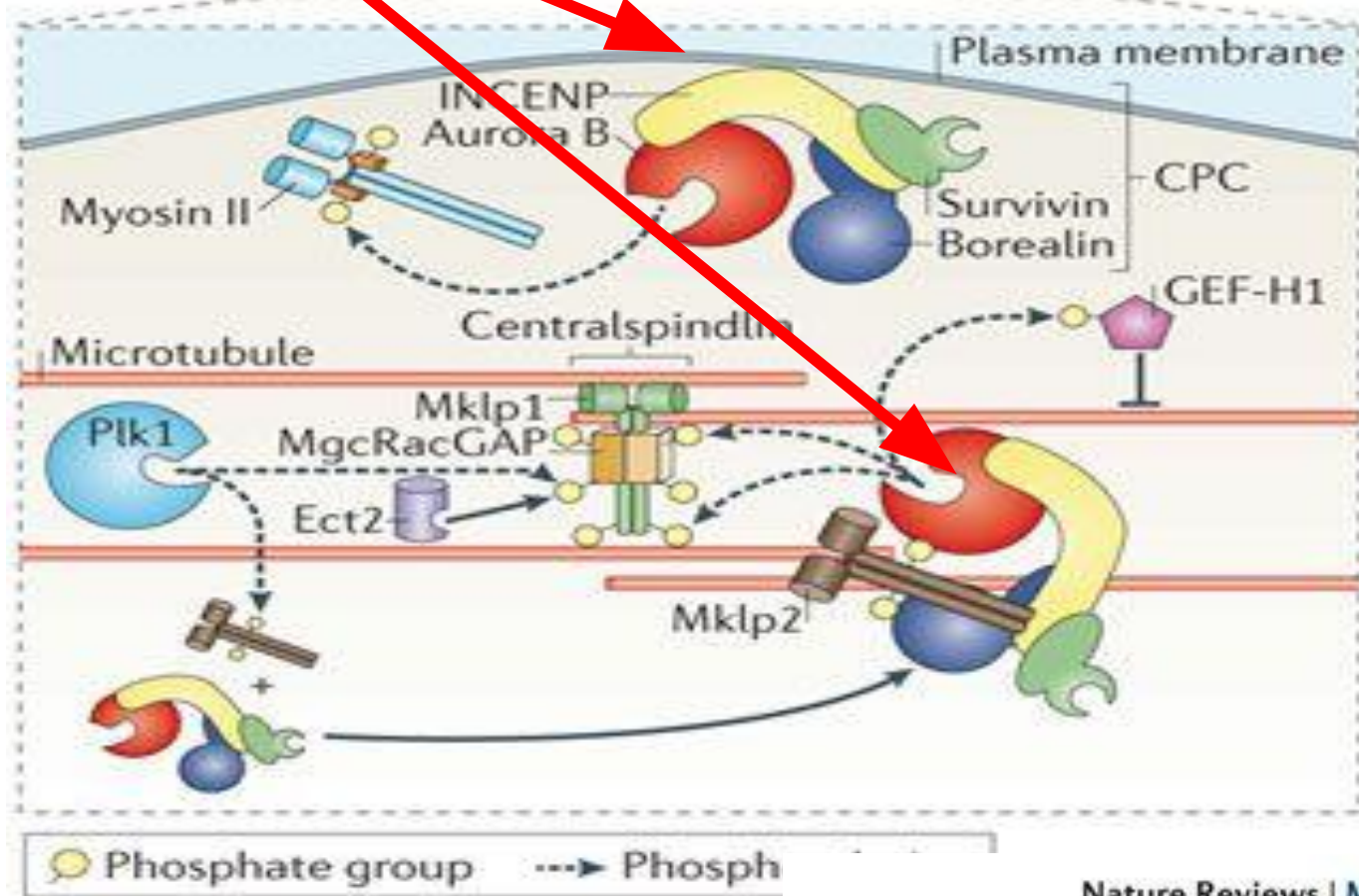


14-3-3

**a Late anaphase**



Два места  
расположения  
CPC в анафазе

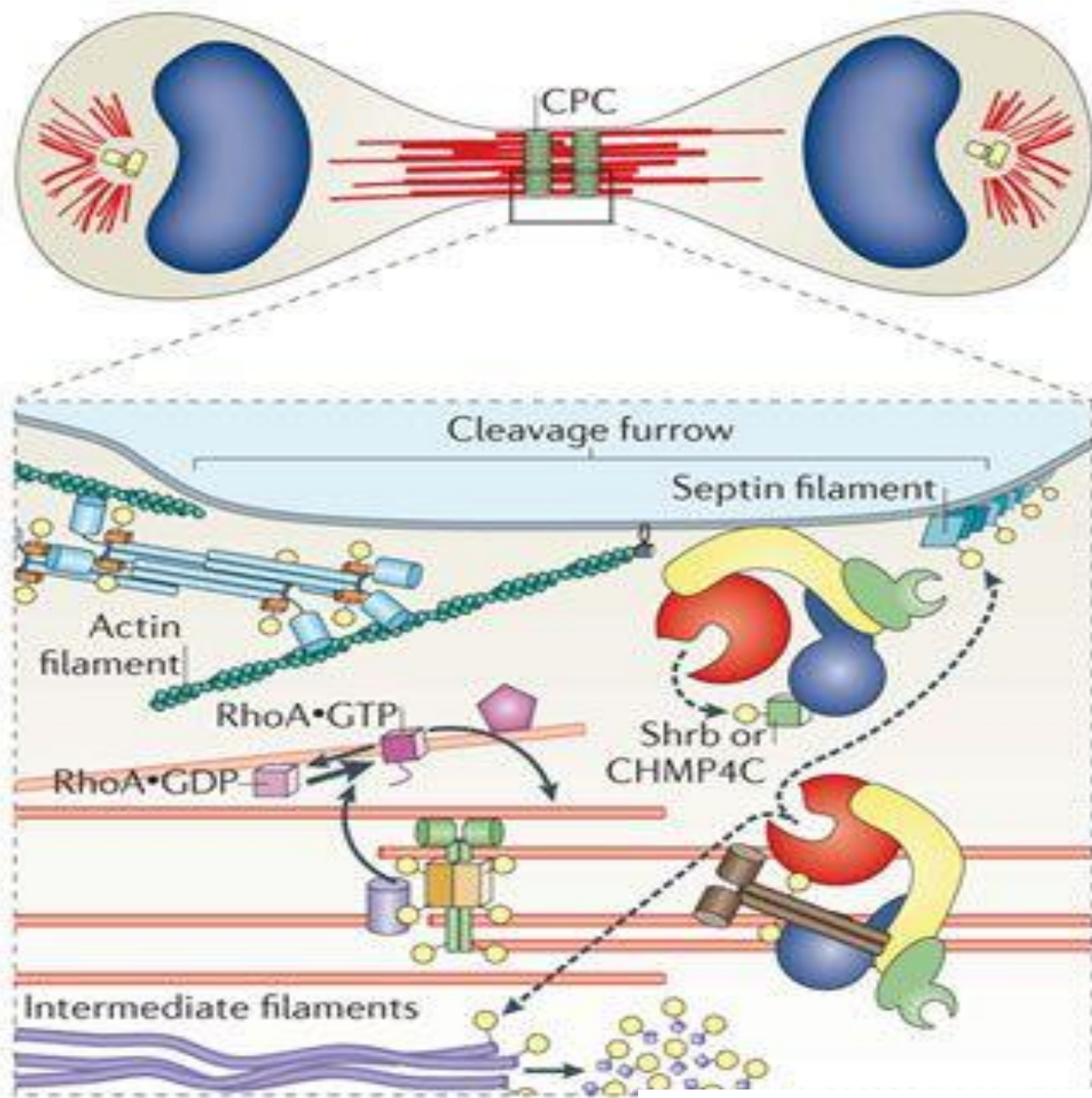


mitotic  
kinesin-like protein  
2

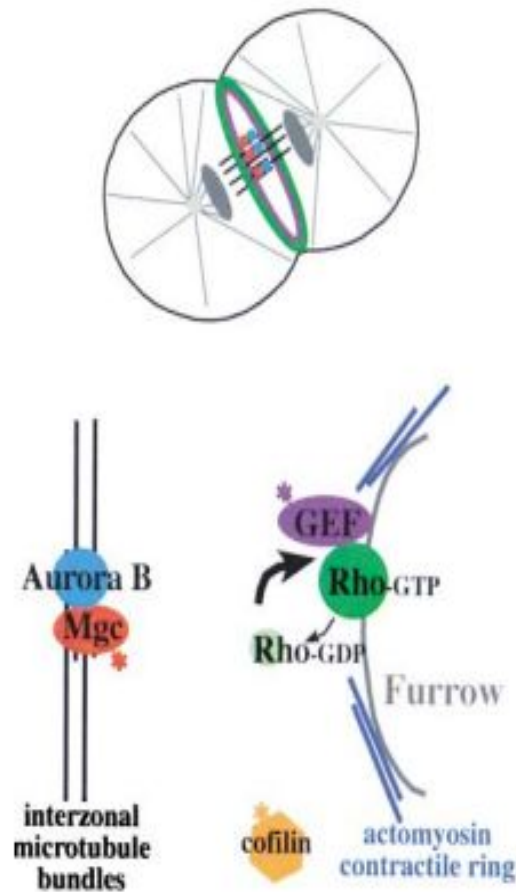
epithelial  
cell-transforming  
2 (Ect2).



## b Telophase and cytokinesis



## A Furrow Ingression



## B Cytokinesis Completion

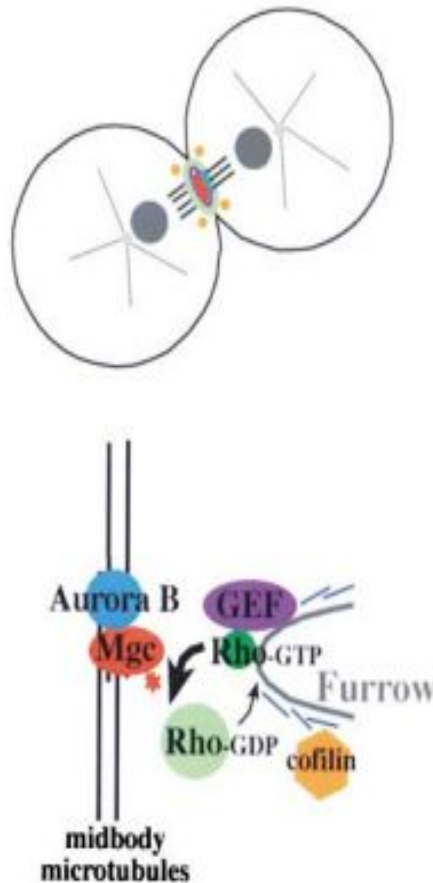


Figure 1. A Model in which Furrow Contraction and Localized RhoA GAP Activity Promote Cleavage Furrow Disassembly during Completion of Cytokinesis

(A) Furrow ingression: Aurora B (blue circle) and MgcRacGAP (in a complex with the MKLP-1 kinesin; red oval) localize to interzonal microtubule bundles that form between the segregating chromosomes during late anaphase. Aurora B phosphorylates Mgc (red asterisk) converting it to a RhoA GAP, but the distance between the microtubule bundles and membrane-bound RhoA and the presence of phosphorylated, active Rho GEF result in the net activation of RhoA (RhoA-GTP; dark green). Active RhoA promotes furrow assembly and contractility and leads to the phosphorylation of cofilin, blocking its actin filament severing activity.

(B) Cytokinesis completion: contraction of the cleavage furrow around the compacted midbody microtubules brings RhoA in close apposition to aurora B-activated MgcRacGAP. This increase in the local concentration of RhoA GAP activity in combination with inactivation of the RhoA GEF by dephosphorylation

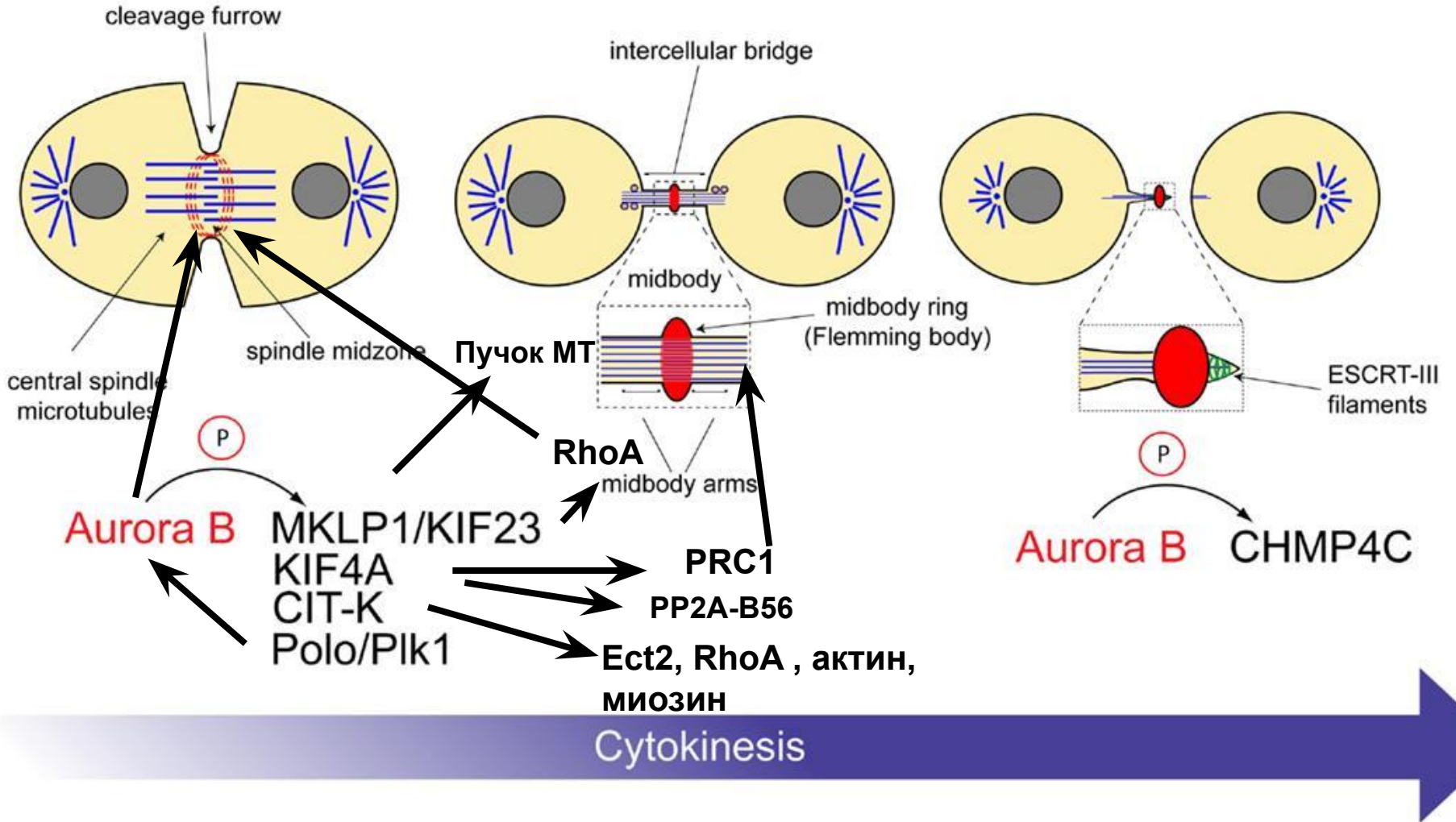
leads to the net inactivation of RhoA (RhoA-GDP; light green). Inactivation of RhoA results in cofilin dephosphorylation and activation. Cofilin-mediated severing of actin filaments contributes to cleavage furrow disassembly.

# Telophase

## Early

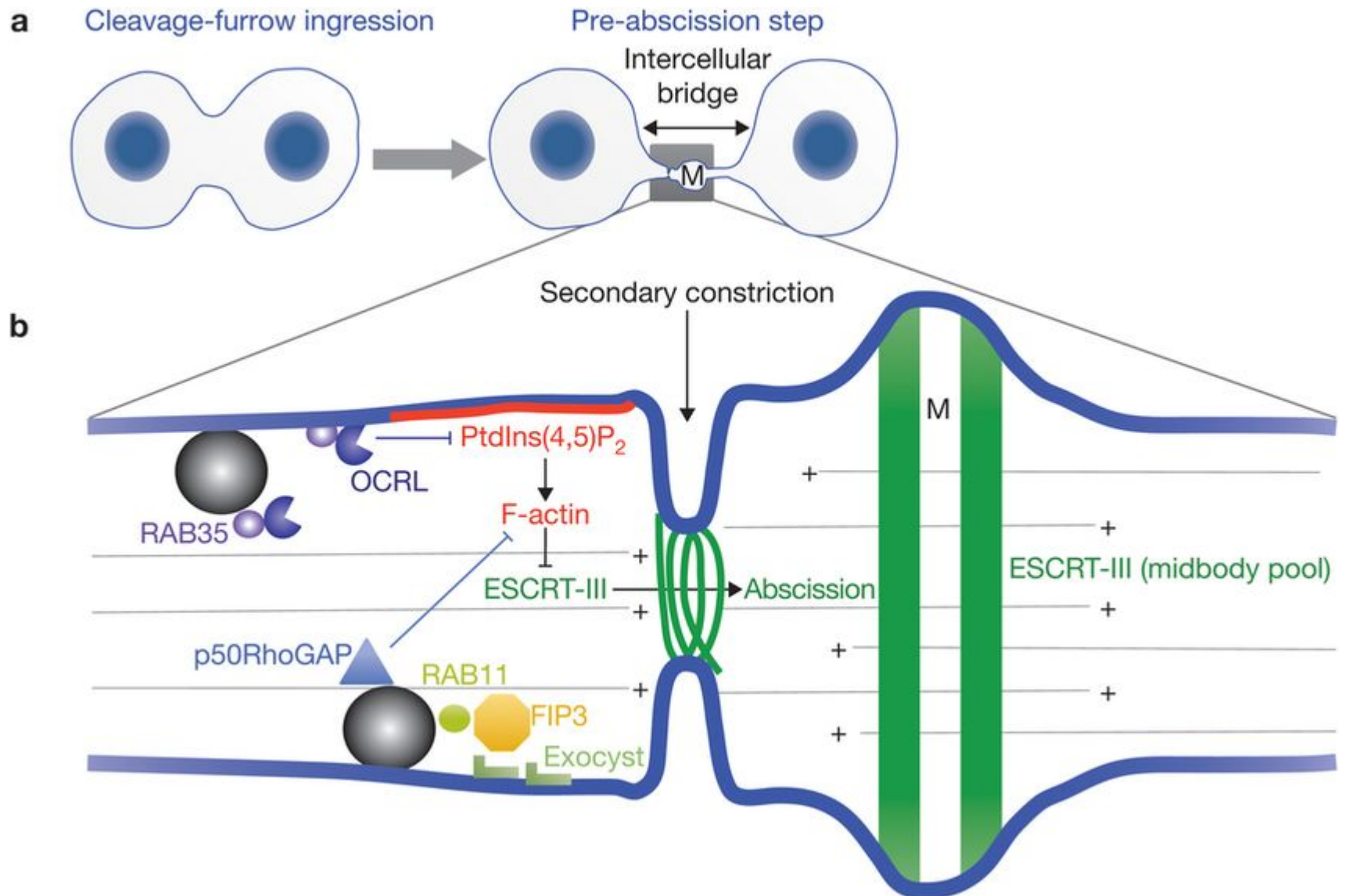
## Late

## Abscission

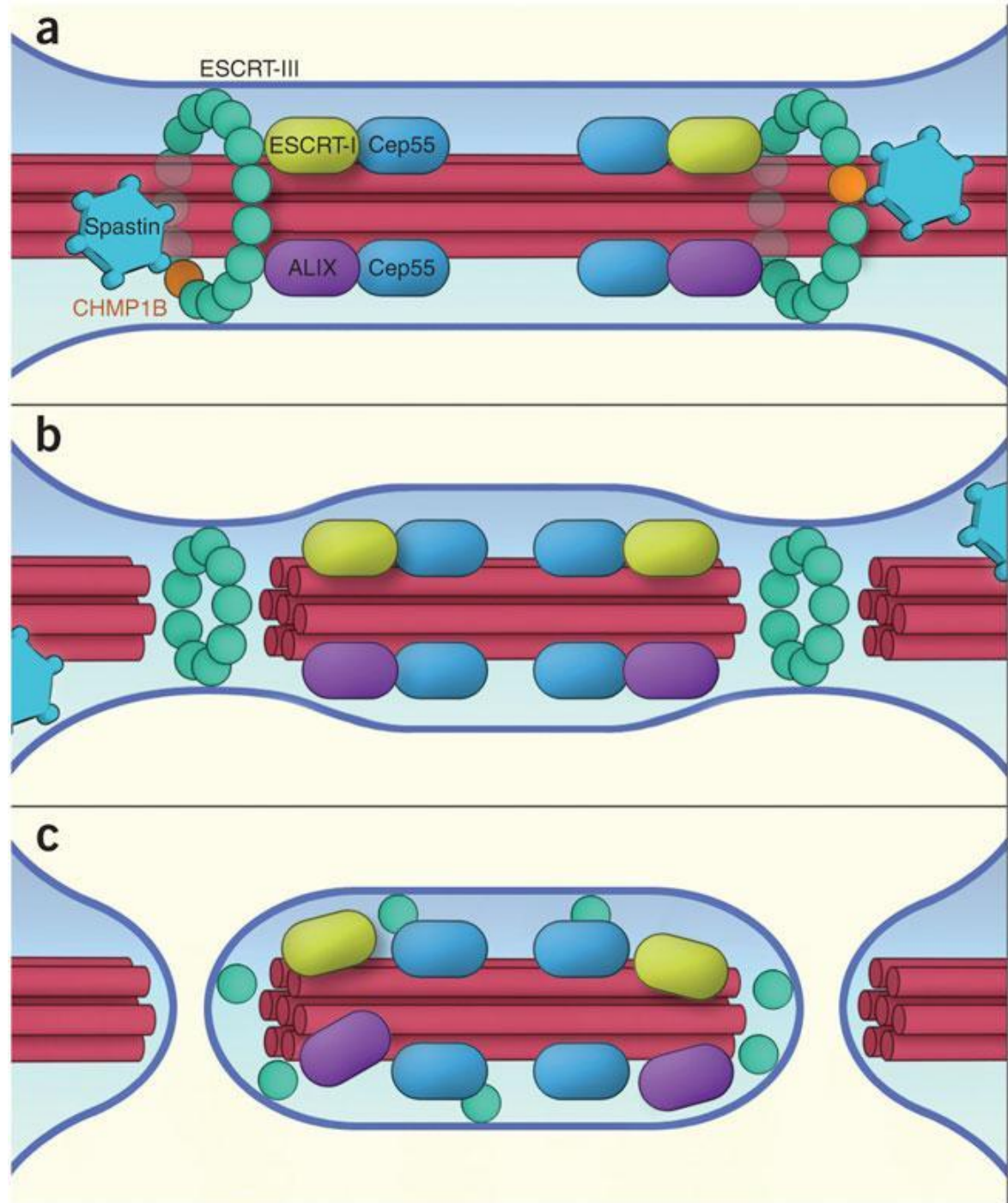


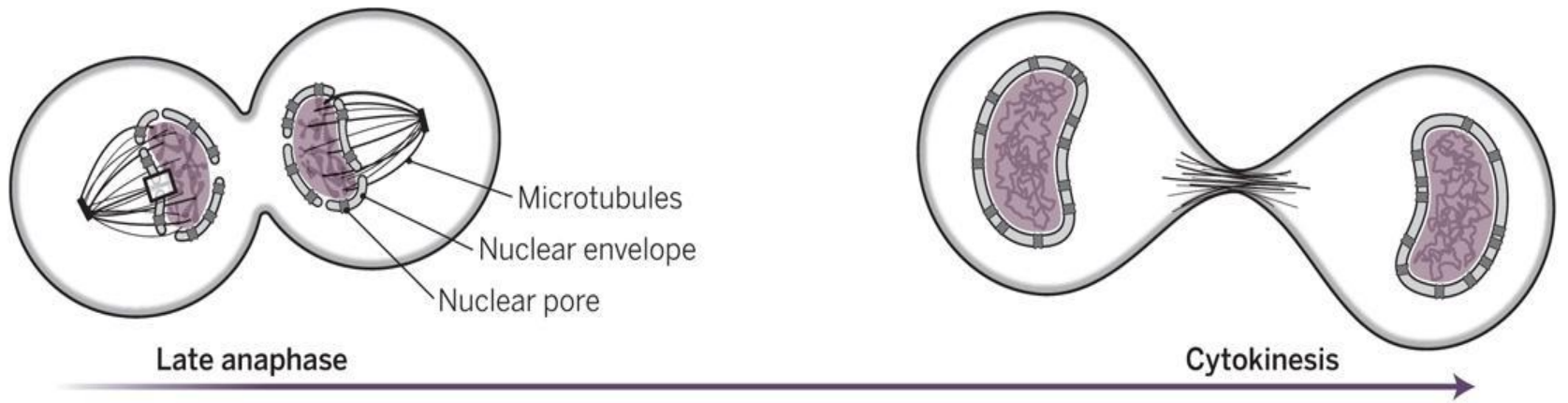
Цитронкиназа CIT-K

# Цитокинез

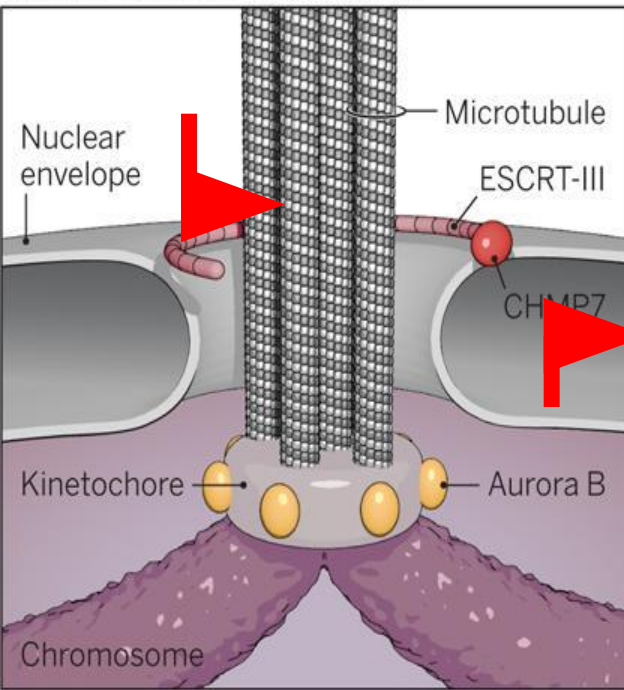


# Цитокинез

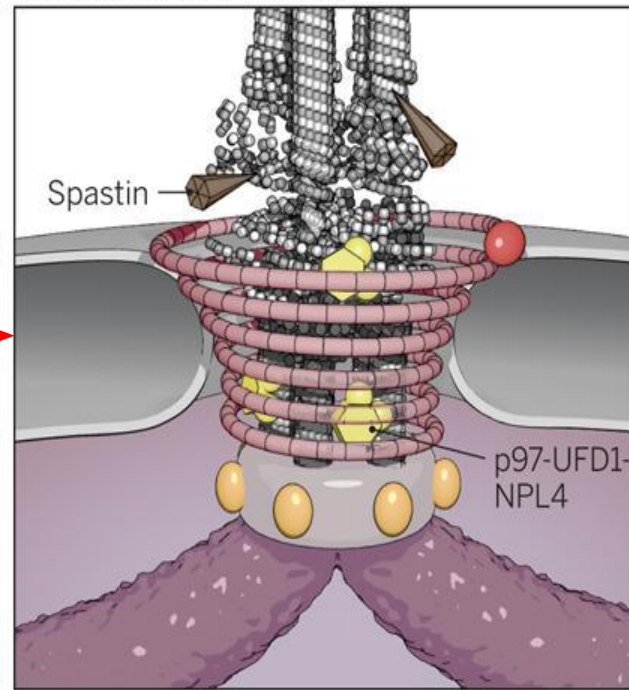




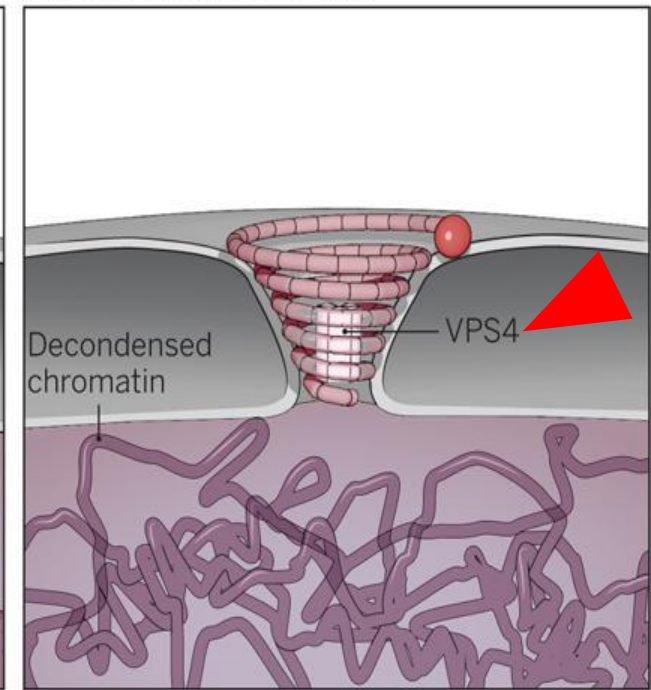
### Nuclear envelope fenestration

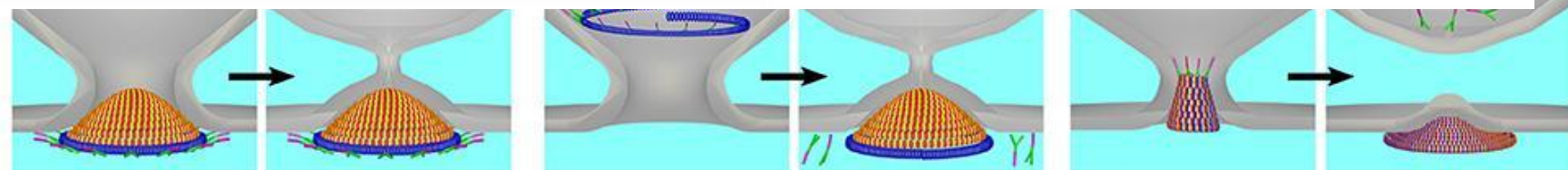
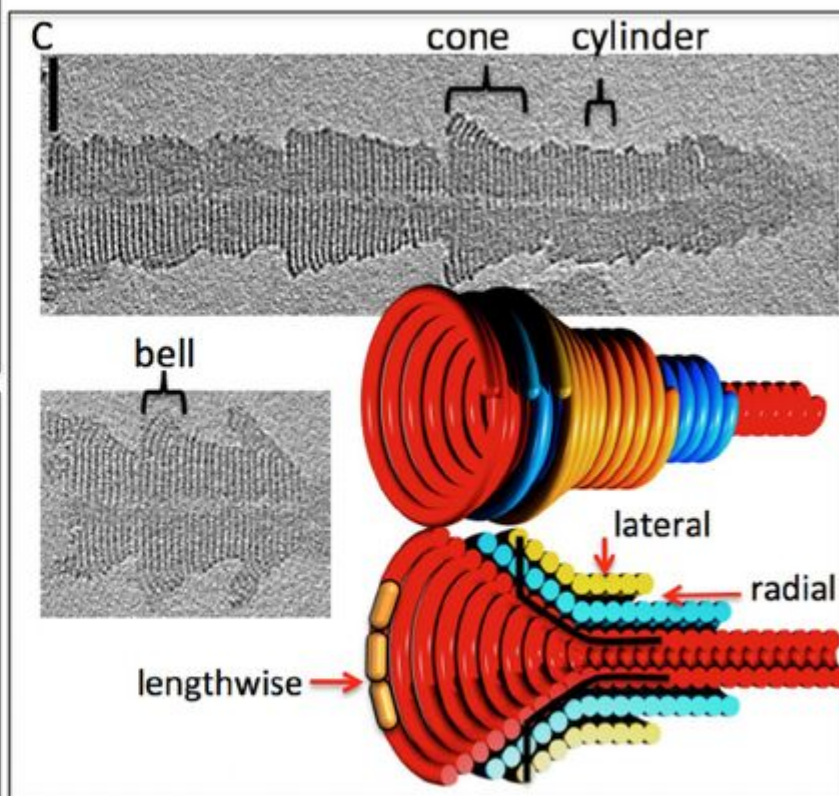
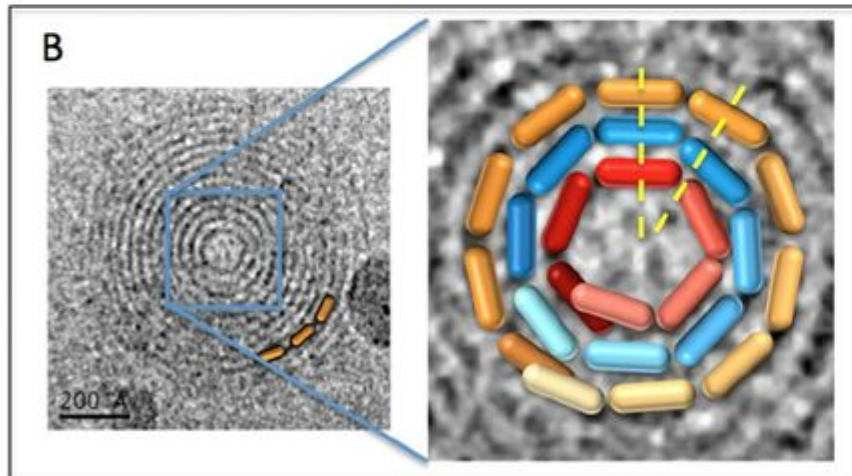
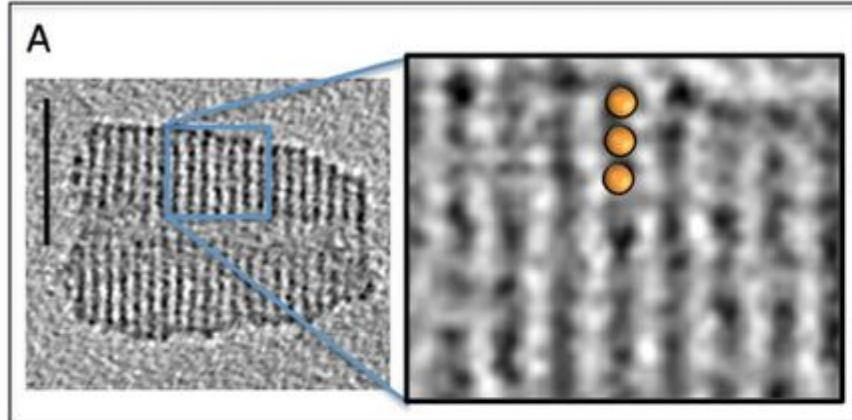


### Microtubule remodeling

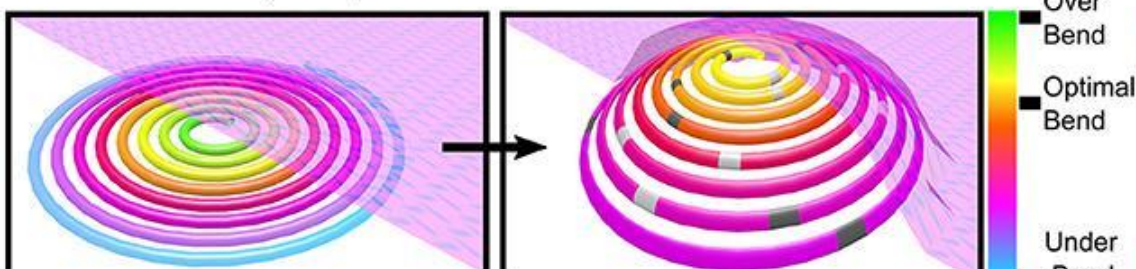


### Nuclear envelope closure

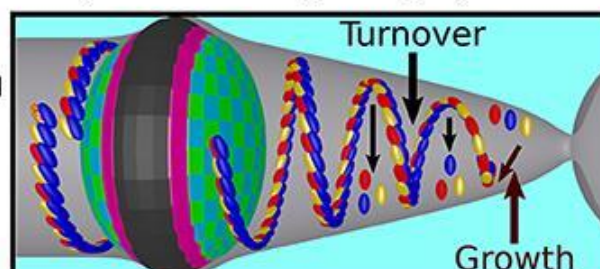




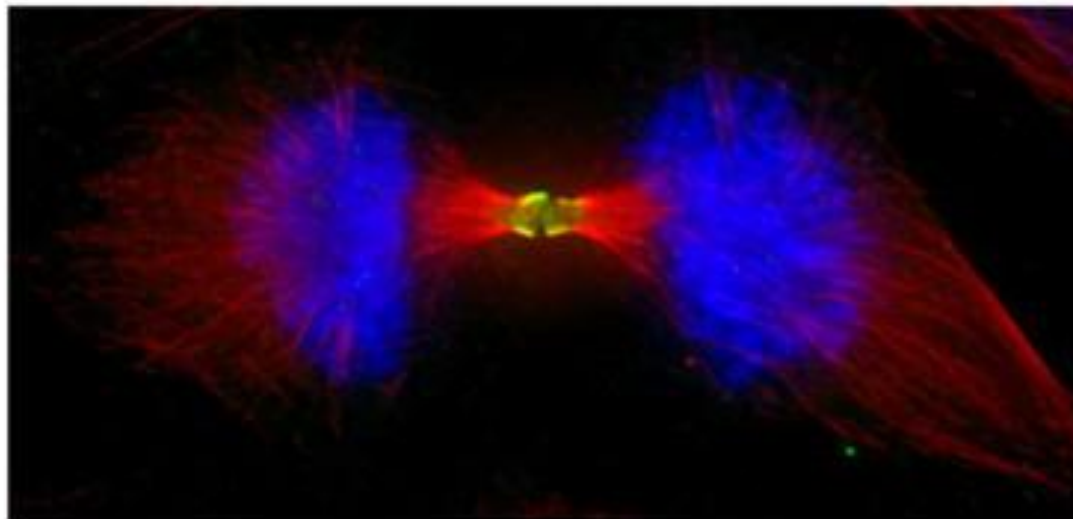
**D** Vps2/Vps24 Induced Deformation



**E** Dynamical Sliding during Cytokinesis



(a)



APC/Cdh1

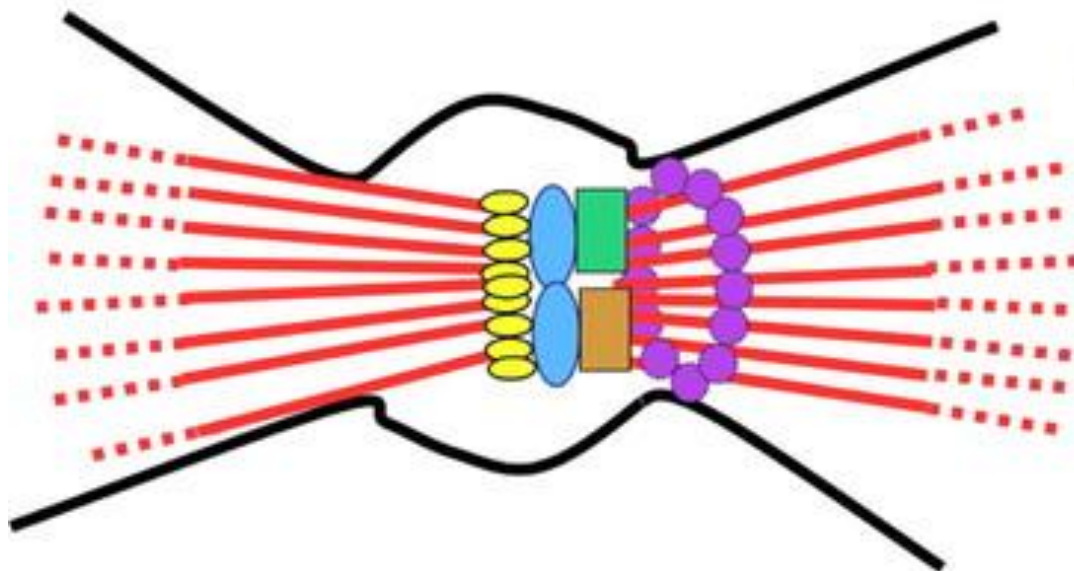


Plk1

AuroraB



(b)



MKLP1

Cep-55

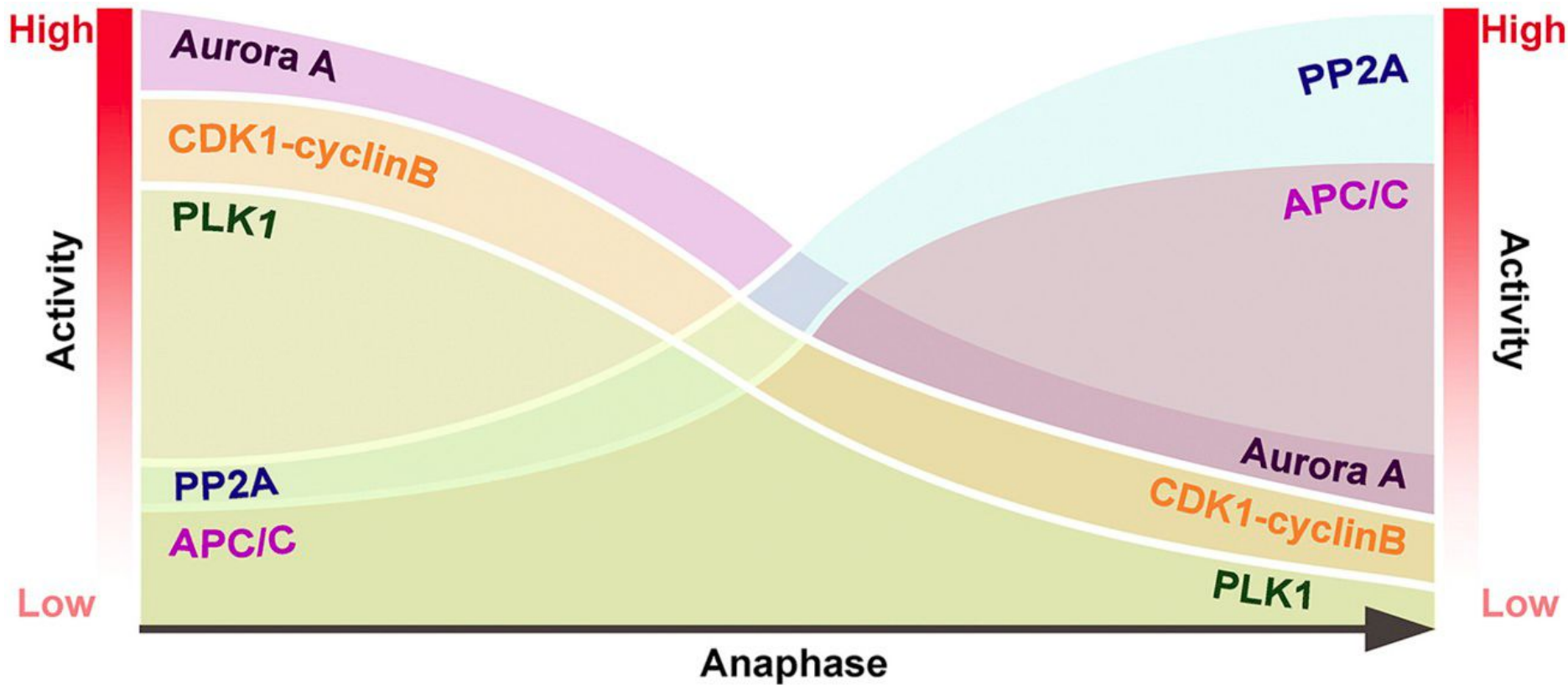
Tsg101

ALIX

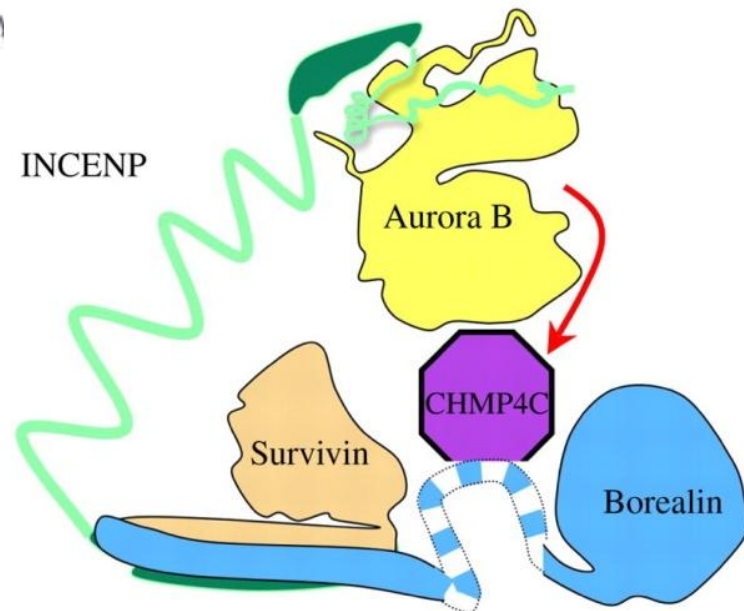
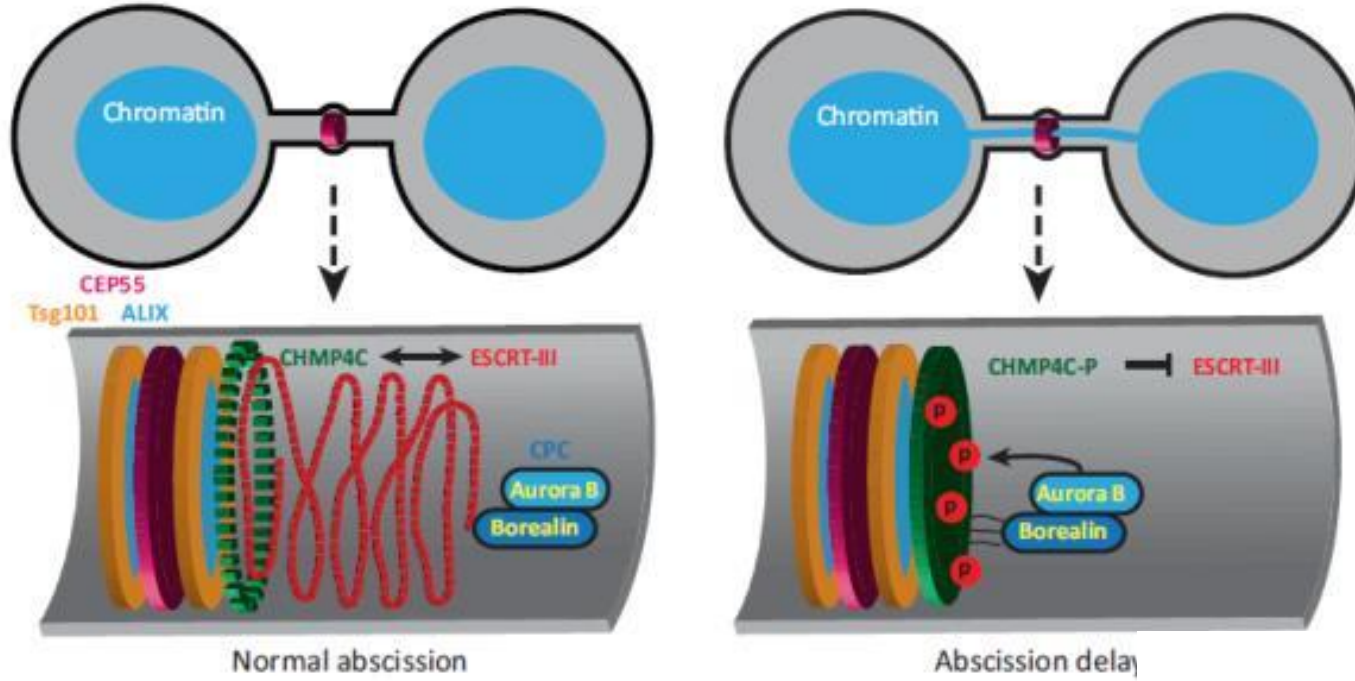
ESCRT-III



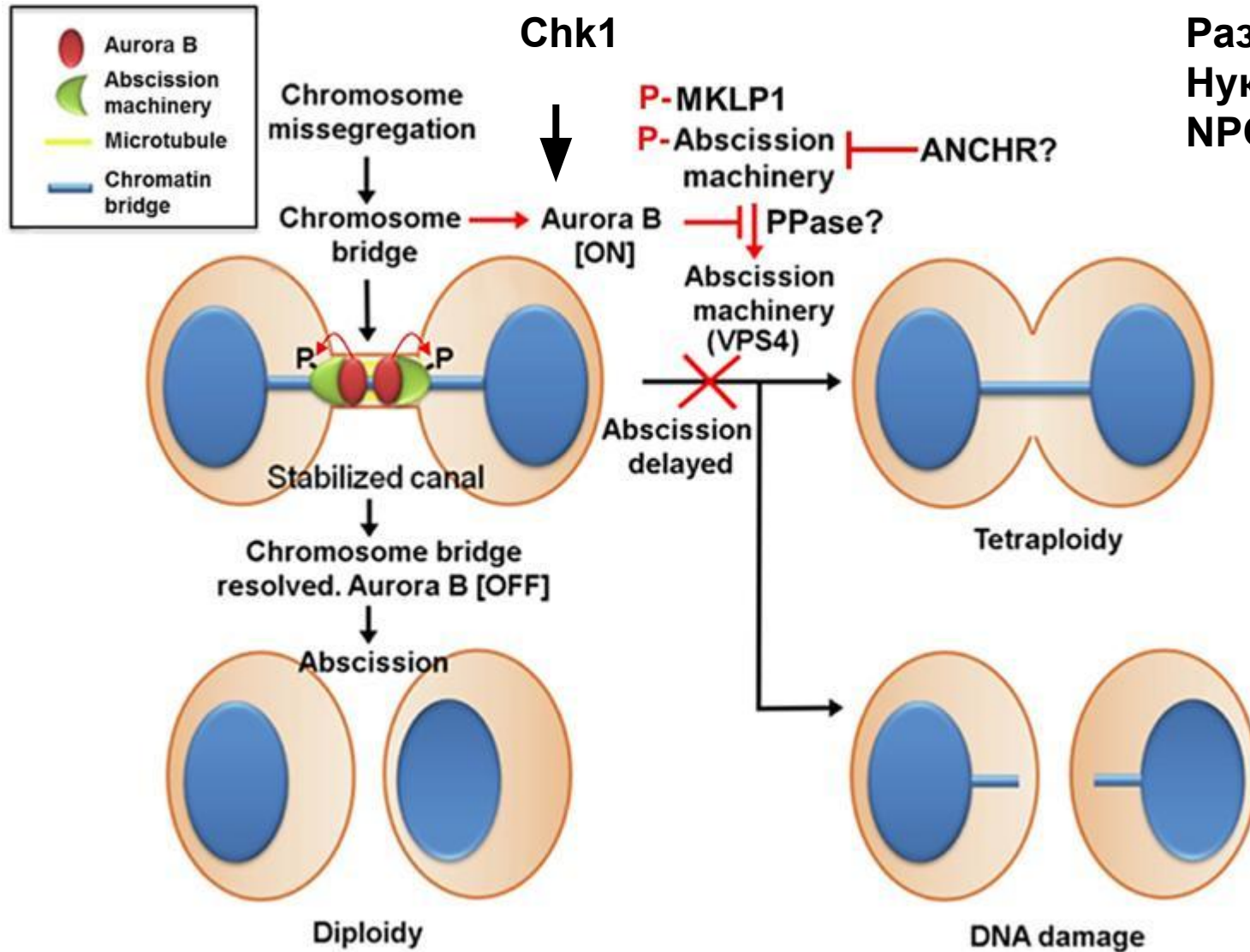
# Изменение активности киназ и фосфатаз в анафазе



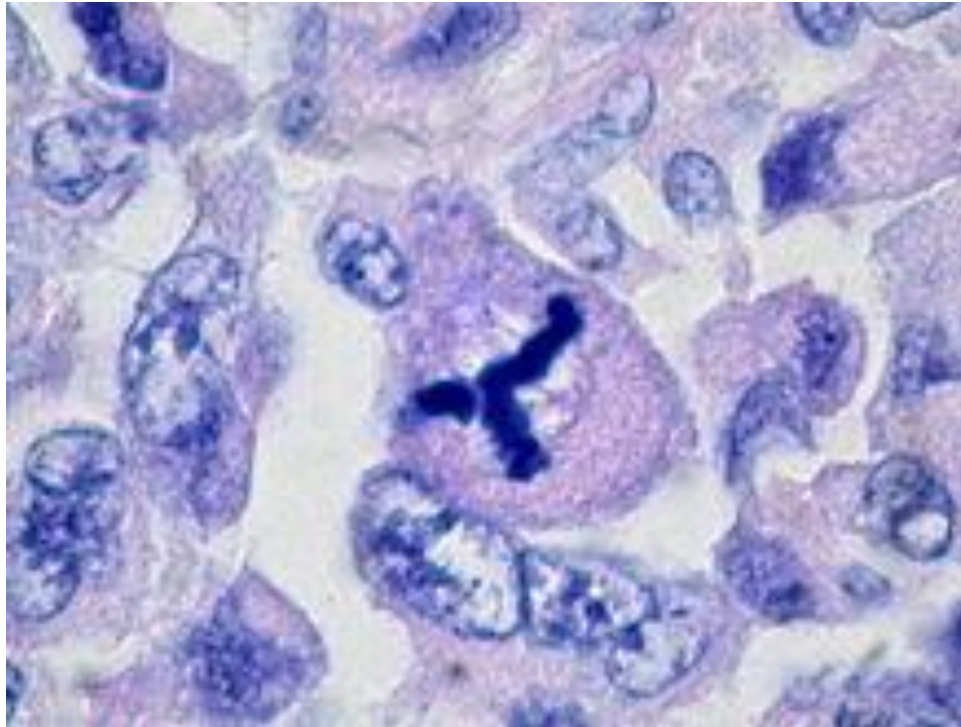
# The abscission checkpoint



# При наличии хромосомного моста включается чек-поинт The abscission checkpoint



# Патологические митозы



Повреждение ДНК

Нарушение числа полюсов

Нарушение прикрепления МТ к кинетохору

Нарушение формирования веретена

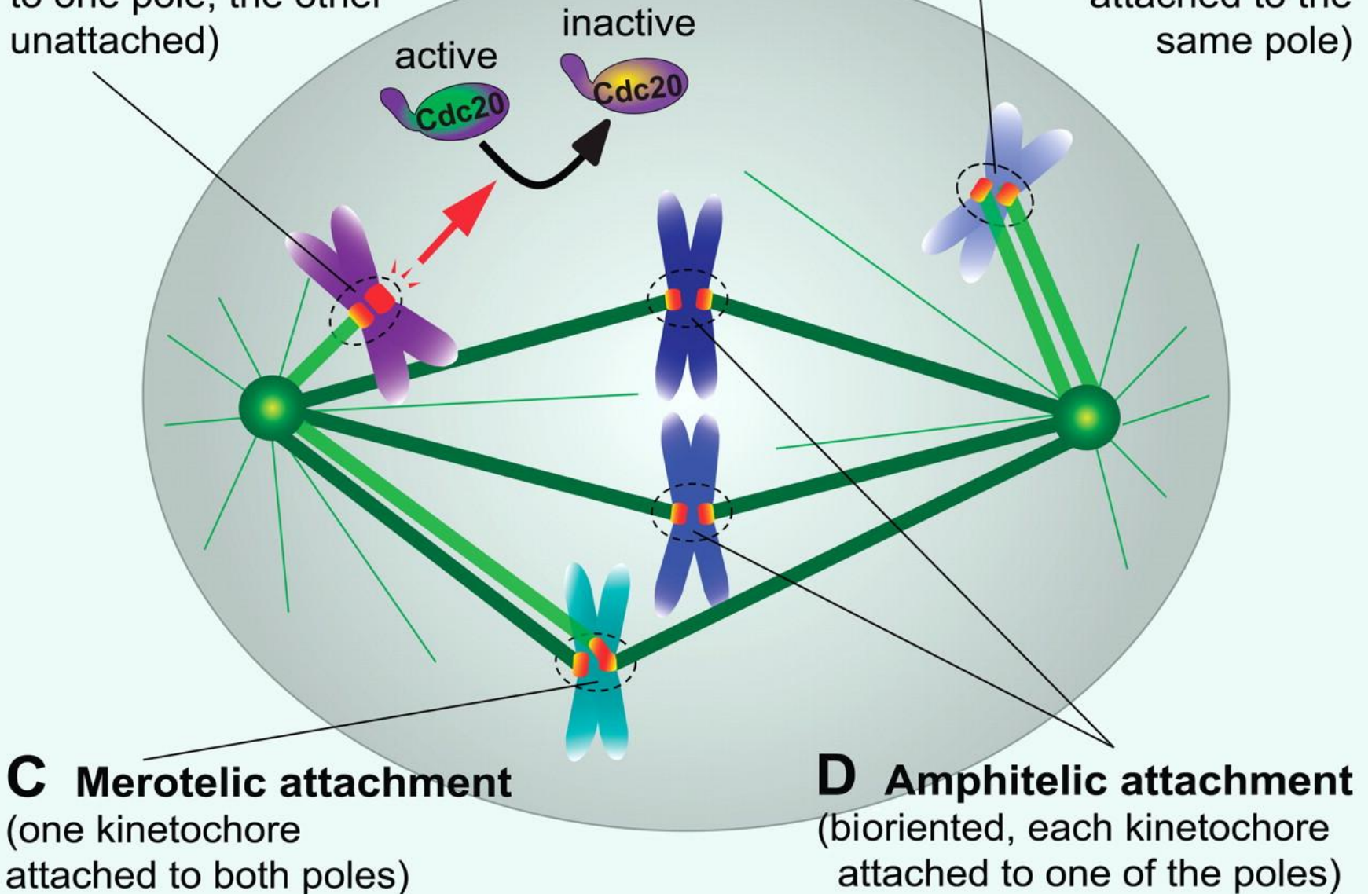
Нарушение цитотомии

## A Monotelic attachment

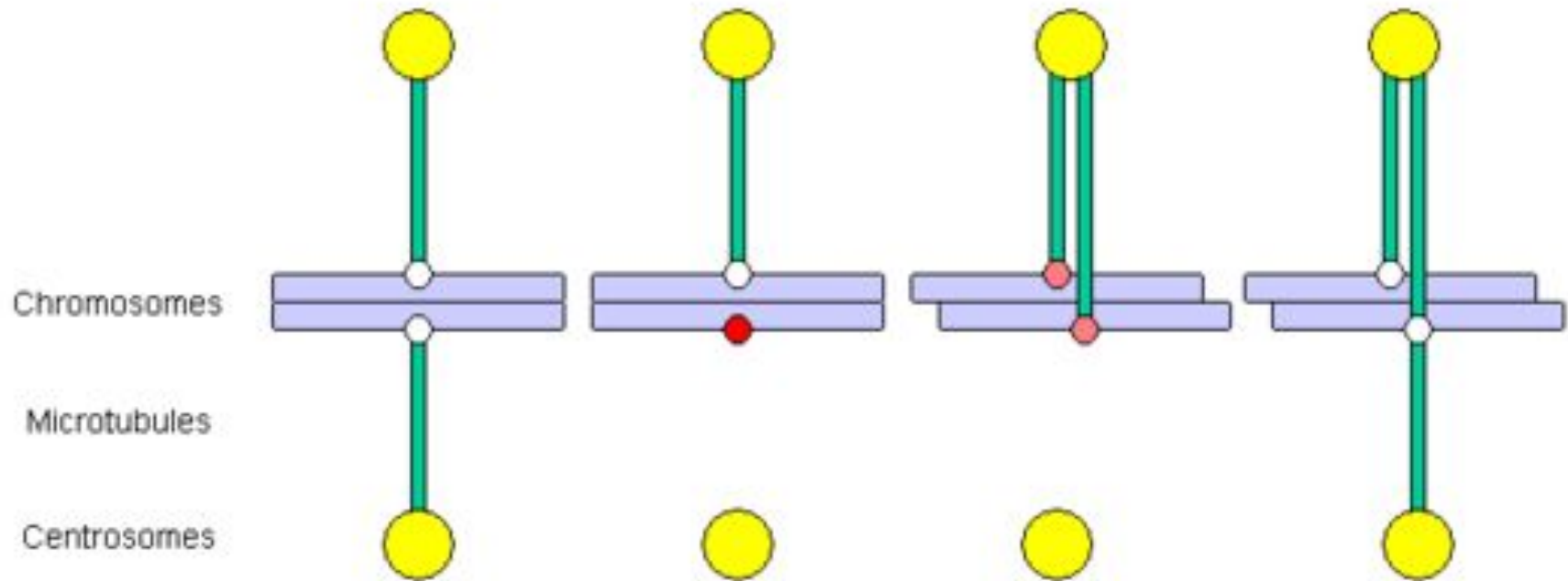
(one kinetochore attached to one pole, the other unattached)

## B Syntelic attachment

(both kinetochores attached to the same pole)

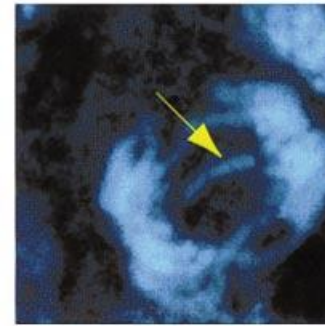
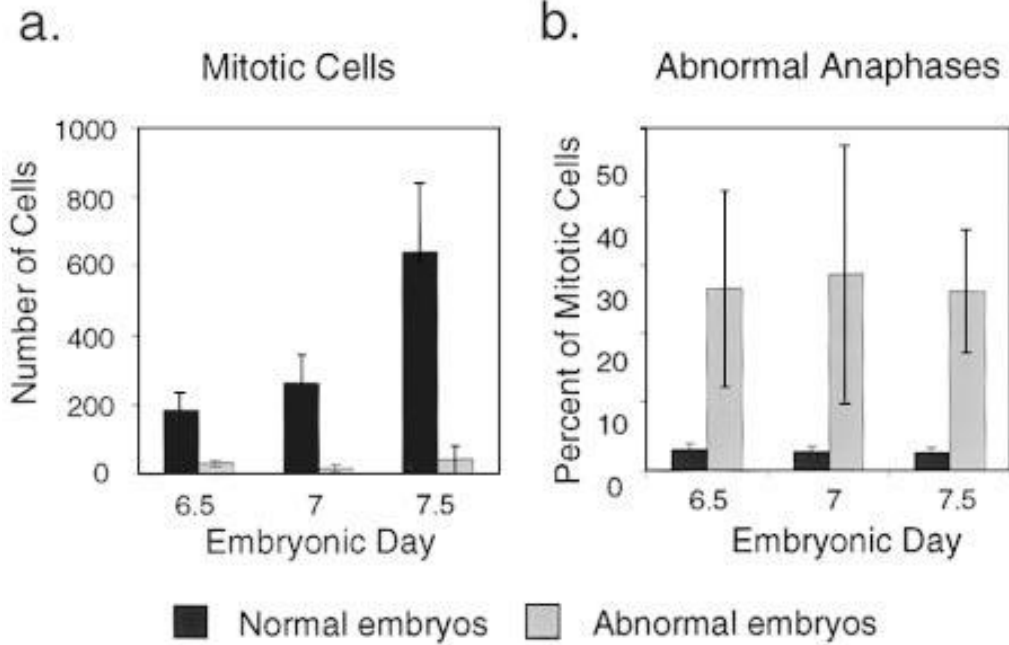


# Варианты нарушений взаимодействия кинетохоров и микротрубочек

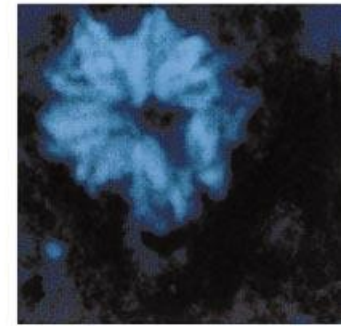


Configuration	Amphitelic	Monotelic	Syntelic	Merotelic
Centromeric tension	Yes	No	No	Yes
Spindle checkpoint	Inactive	Active	Active	Inactive

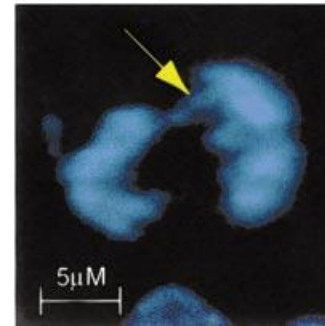
# Analysis of Chromosome Missegregation in *Mad22/2* Cells



d.



f.

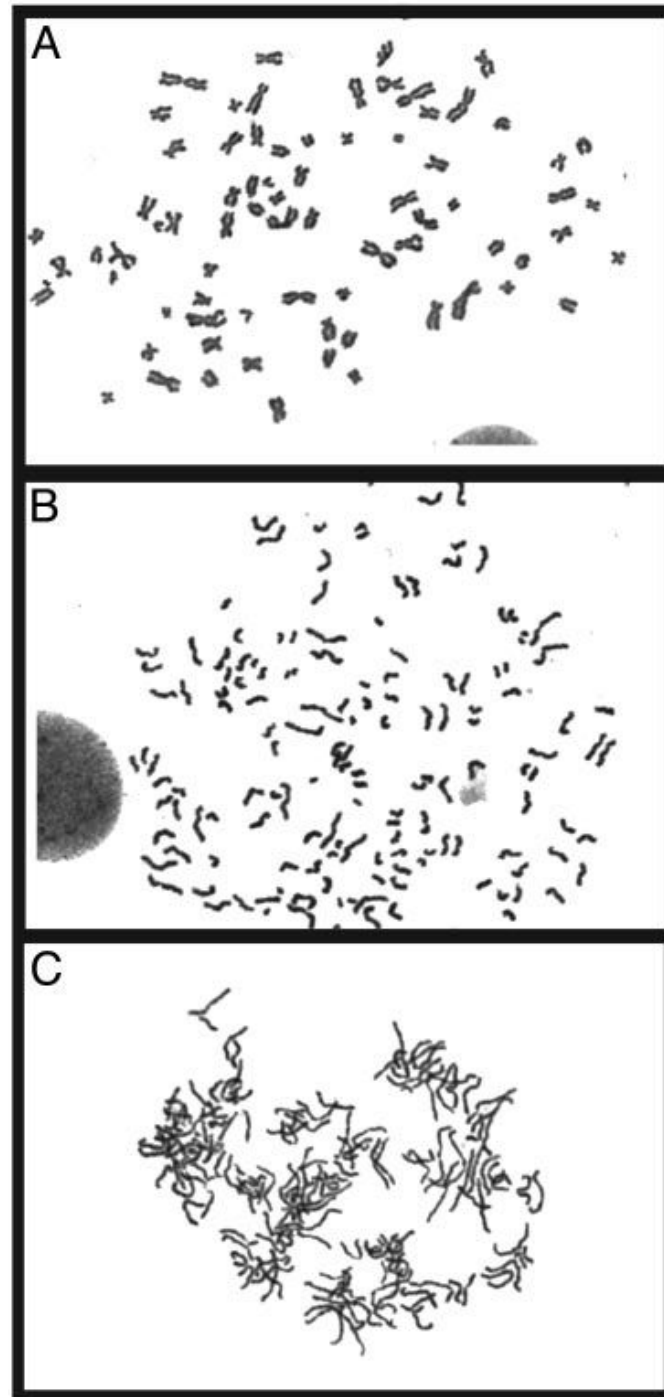


**Altered chromosome morphology from severe MAD2 depletion.**

Shown are normal metaphase spread from a lamin-transfected cell (A) .

**A MAD2knockdown HaCaT cell showing premature sister chromatid separation with normal condensation (B).**

**(C) Metaphase spread from a MAD2 knockdown HaCaT cell displaying both premature sister chromatid separation and incompletely condensed chromosomes**





# ПАТОЛОГИЯ МИТОЗА

"отставшие хромосомы"

МНОГОПОЛЮСНОСТЬ

неравномерное расхождение хромосом  
-анеуплоидия

ПОЛИПЛОИДИЯ

нарушение расхождения  
хромосом

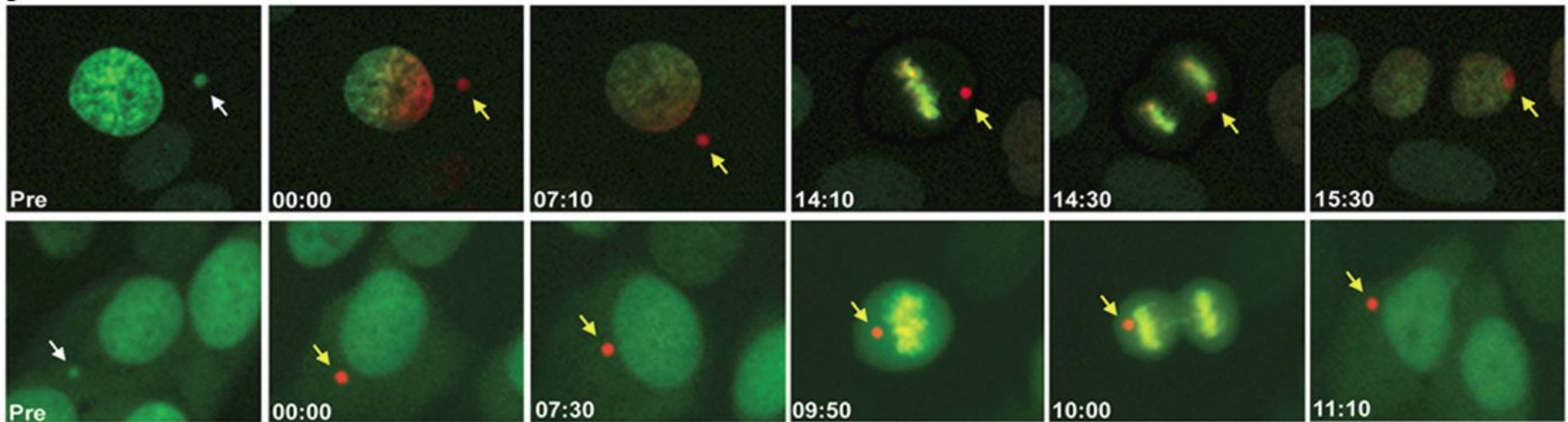
нарушение цитокинеза



# Судьба микроядер.

Микроядро вошло в состав основного ядра

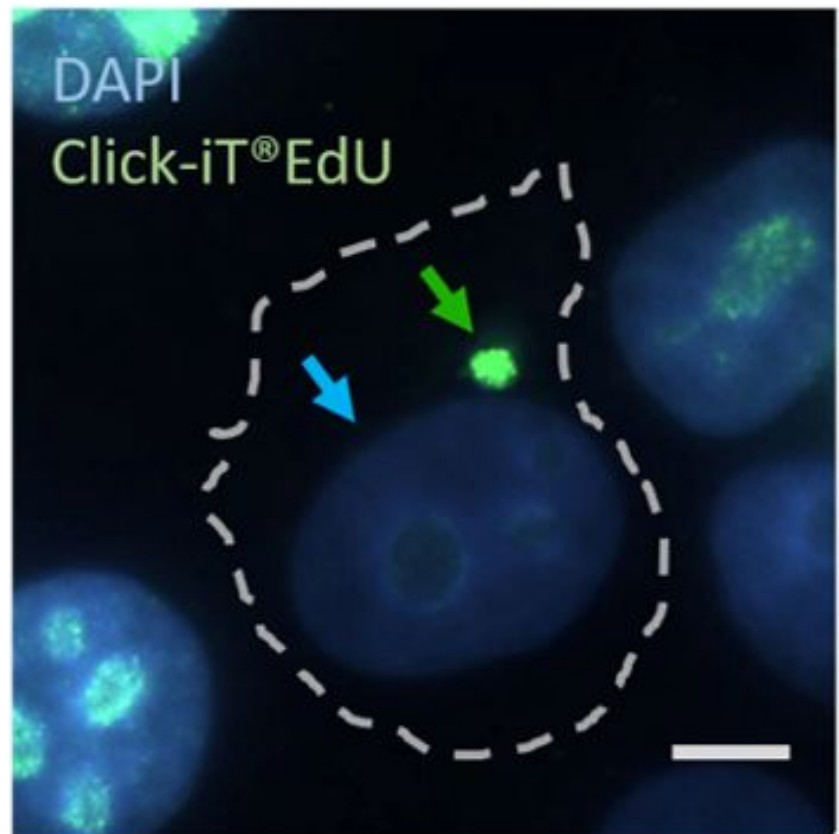
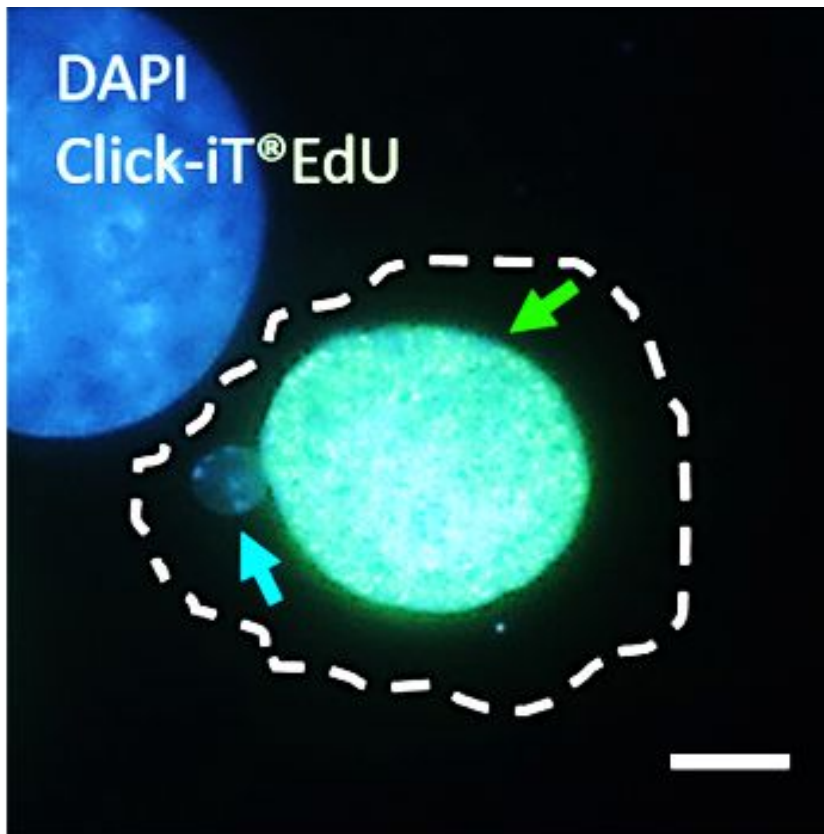
j



Микроядро осталось в цитоплазме

# Асинхронность процессов репликации в основном ядре и микроядре

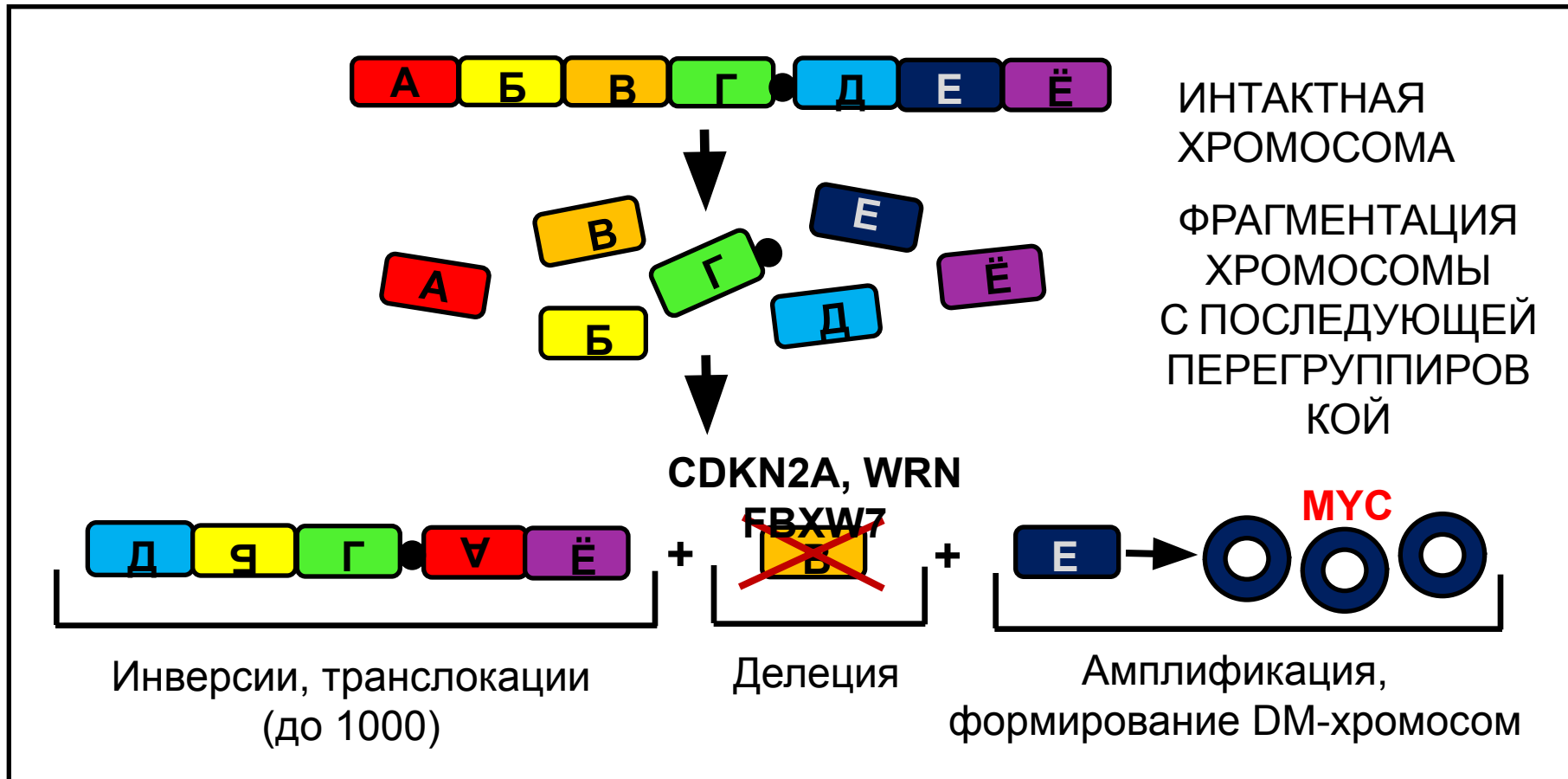
Асинхронное протекание процессов репликации:  
 $87,8 \pm 0,9\%$  (A431) –  $91,2 \pm 0,7\%$  (MCF-7)

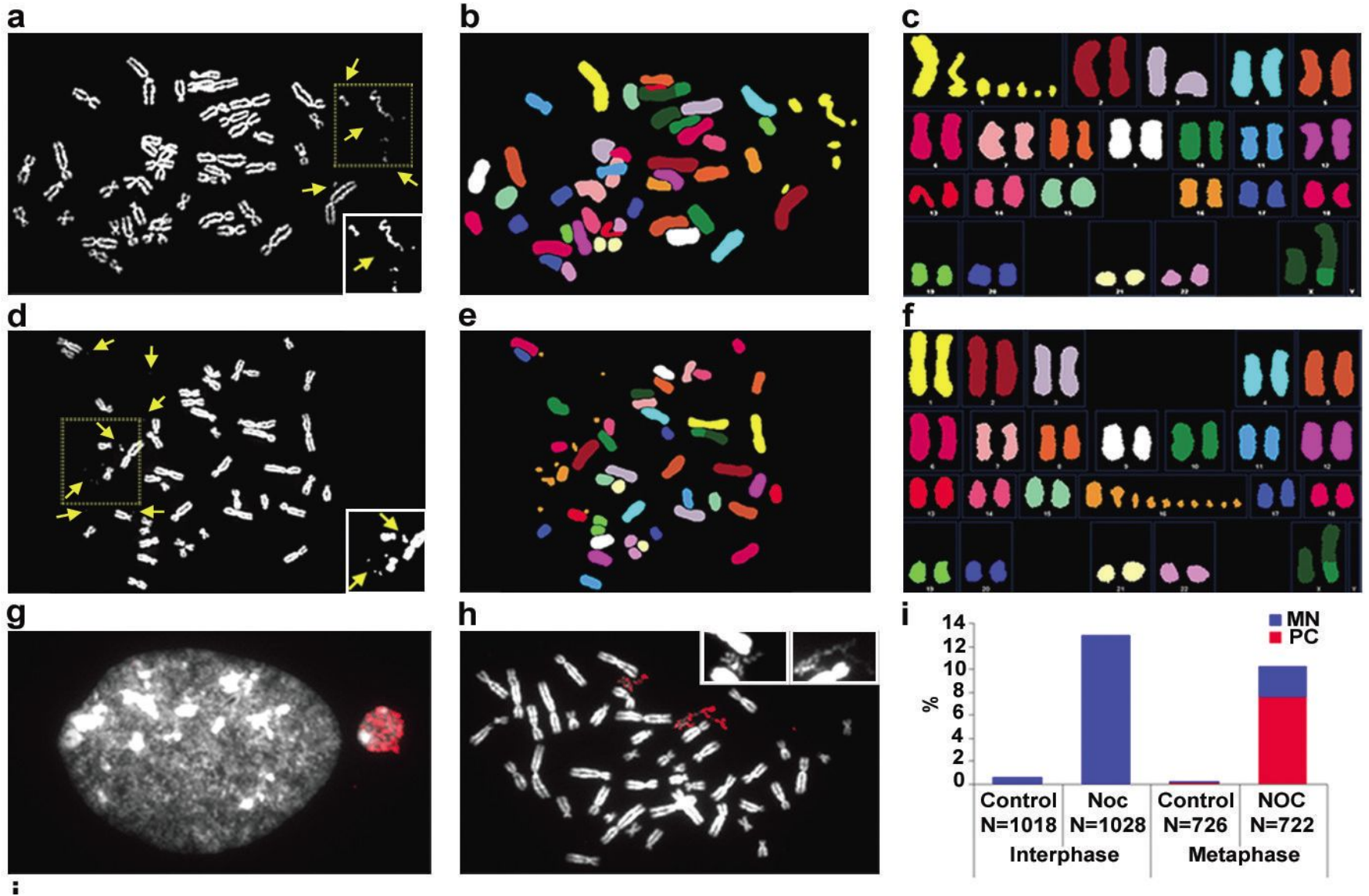


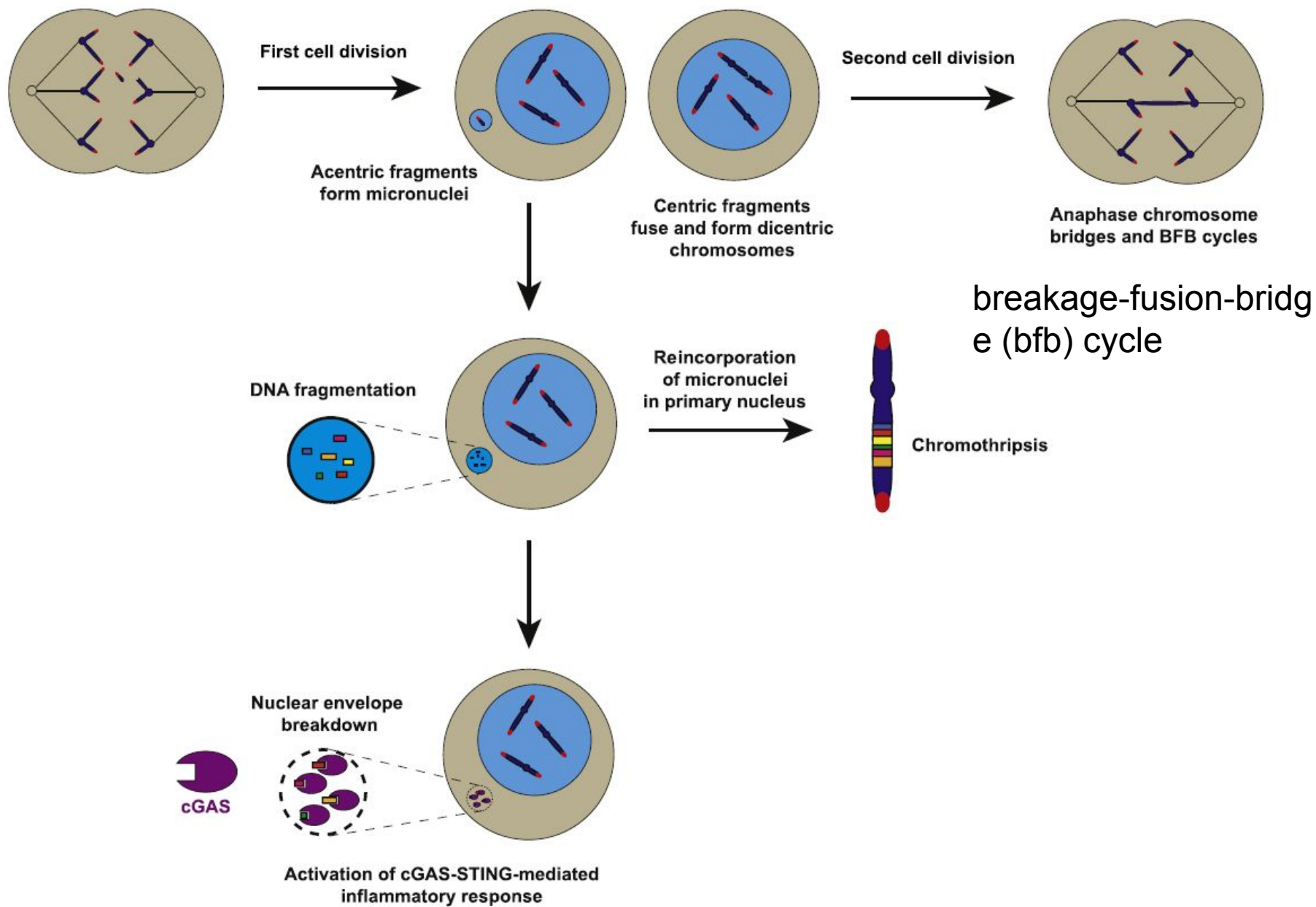
*MCF-7, масштабный отрезок – 10 мкм*

# Процесс хромотрипсиса и его вклад в канцерогенез

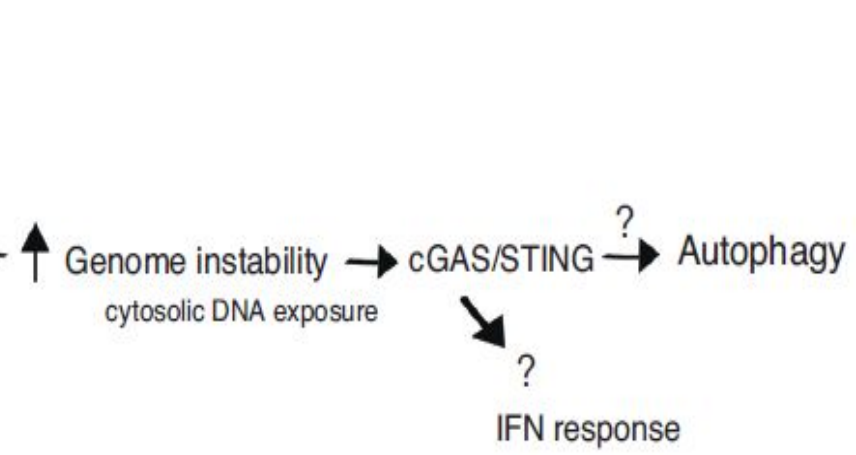
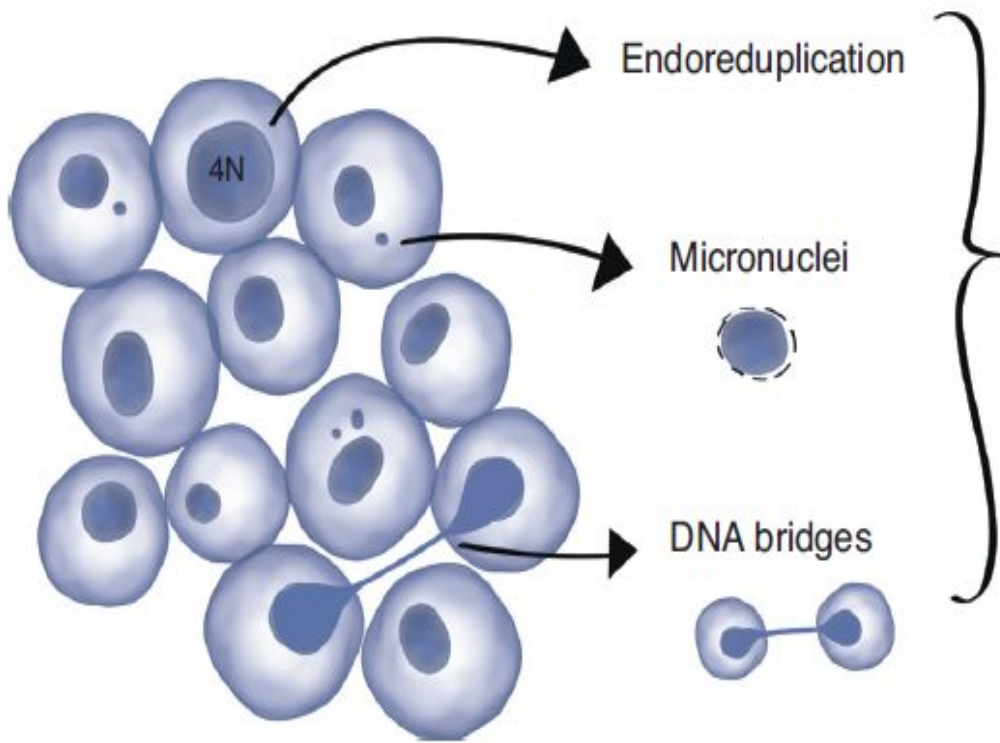
Все зло от хромотрипсиса, а хромотрипсис случается в микроядрах!







# Telomere Crisis



# СТАРЕНИЕ КЛЕТОК И Клеточный цикл





# Cell senescence and aging



<https://www.jci.org/articles/view/95148>

<https://nrccrm.ru/patient-guide/live-healthy/age-keys/>

<https://biomolecula.ru/articles/zachem-kletki-stareiut>

**Существует несколько механизмов  
старения клеток**

## Replicative senescence

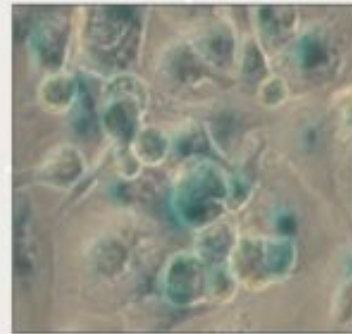


Telomere shortening (ageing)

## Physiological senescence



- Embryonic development
- Wound healing
- Tissue remodelling
- Tumour protection
- Brain homeostasis



## Stress-induced premature senescence



- Activation of oncogenes
- Inhibition of tumour suppressor genes
- DNA damage
- ROS
- Metabolic stress
- Epigenetic stress
- Spindle stress
- Nucleolar stress
- Brain disorders, liver, muscle and cardiovascular diseases

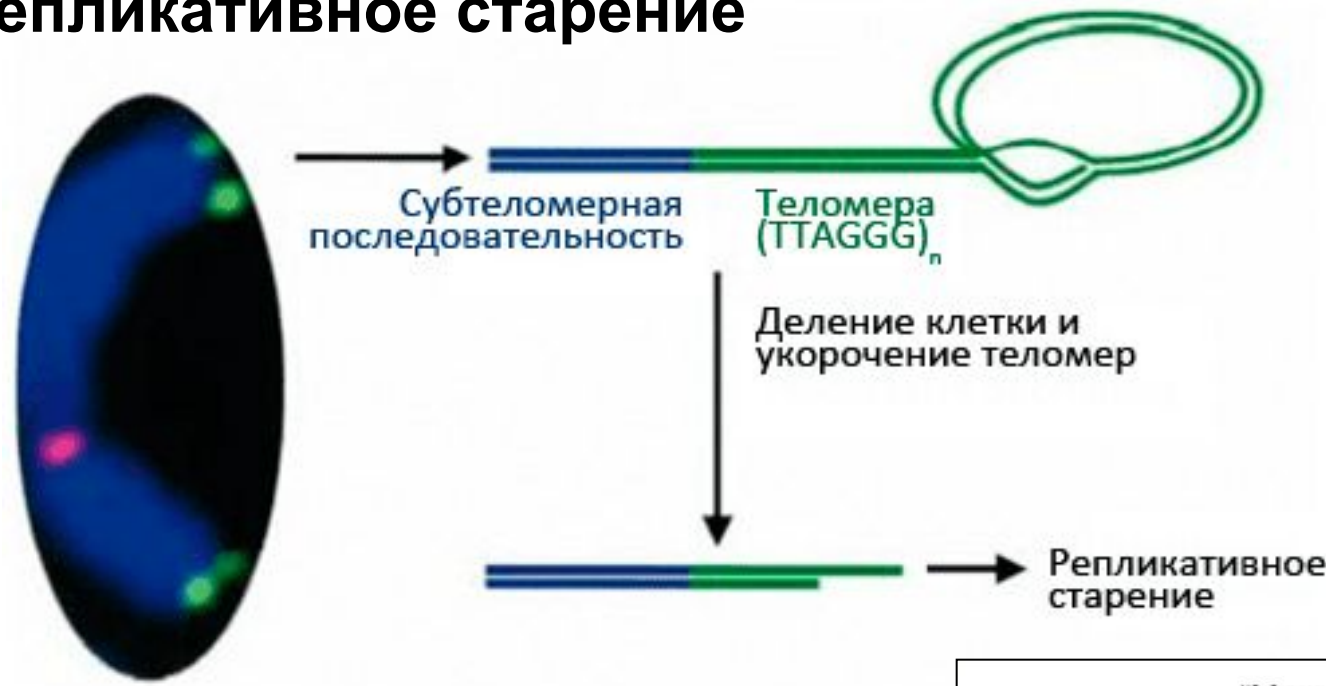
## Drug-induced senescence (chemical-induced SIPS)



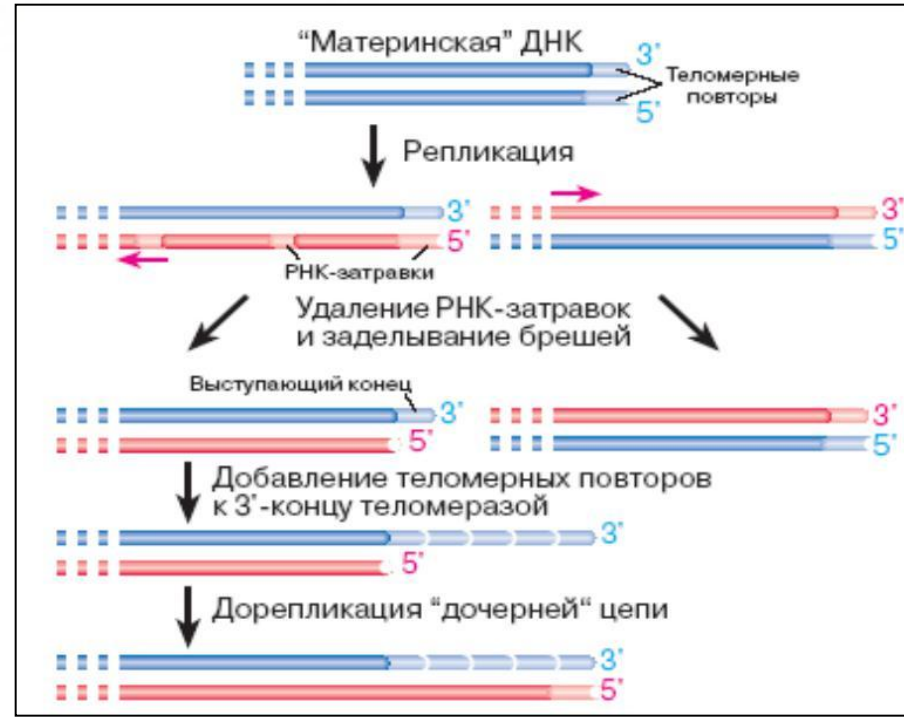
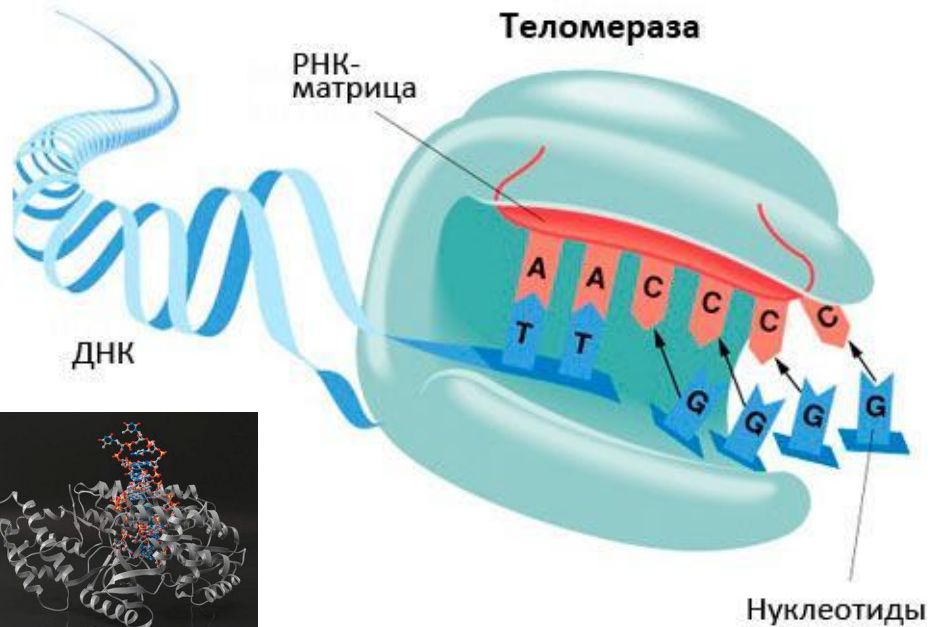
- CDK inhibitors
- HAT/HDAC inhibitors
- PKC activators
- Genotoxic drugs



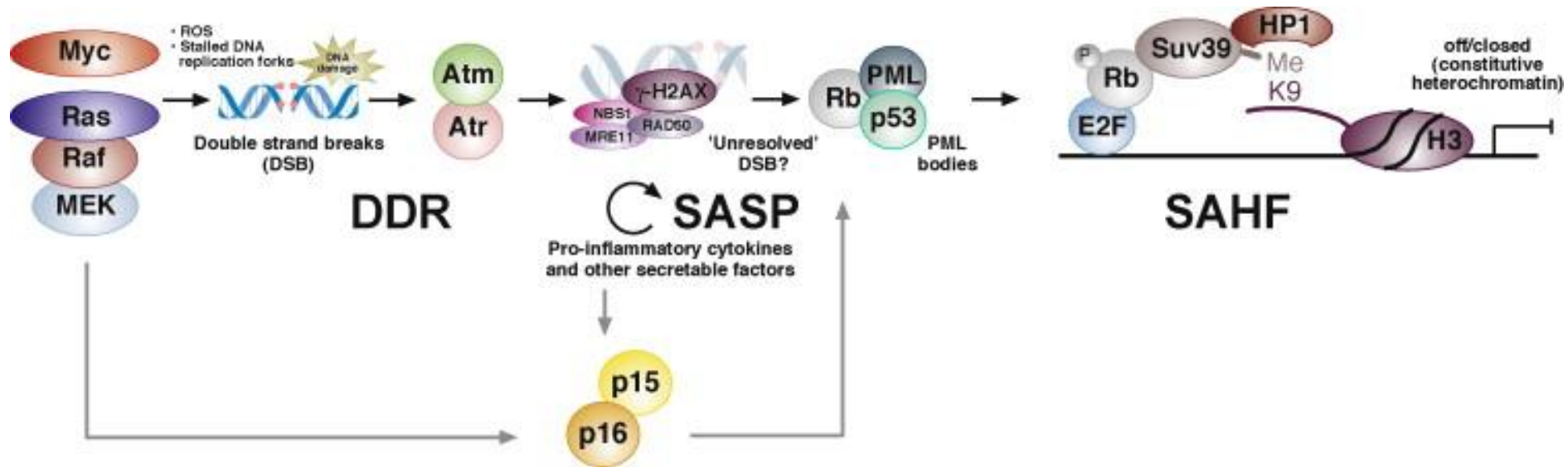
# Репликативное старение



Научно-популярно для интересующихся на биомолекуле:  
Старение — плата за подавление раковых опухолей?  
<https://biomolecula.ru/articles/starenie-plata-za-podavlenie-rakovykh-opukholei>

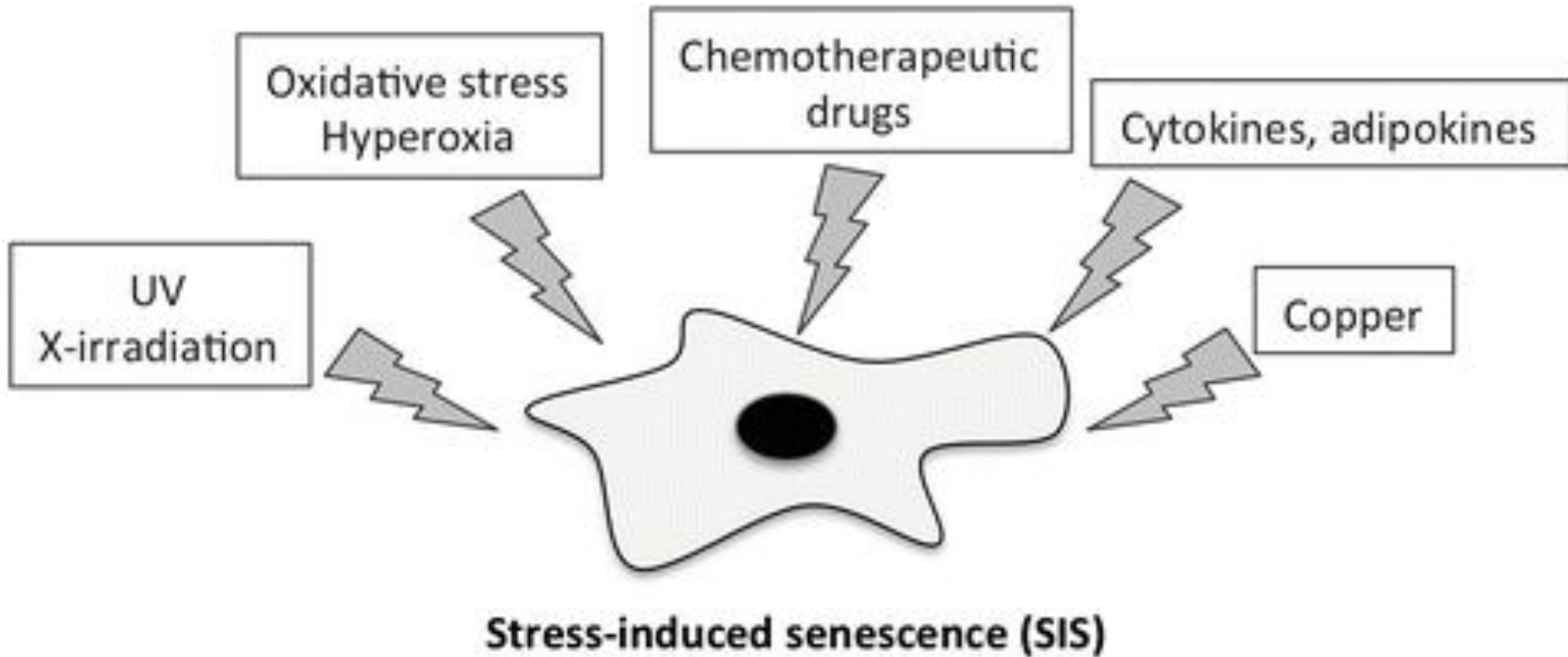


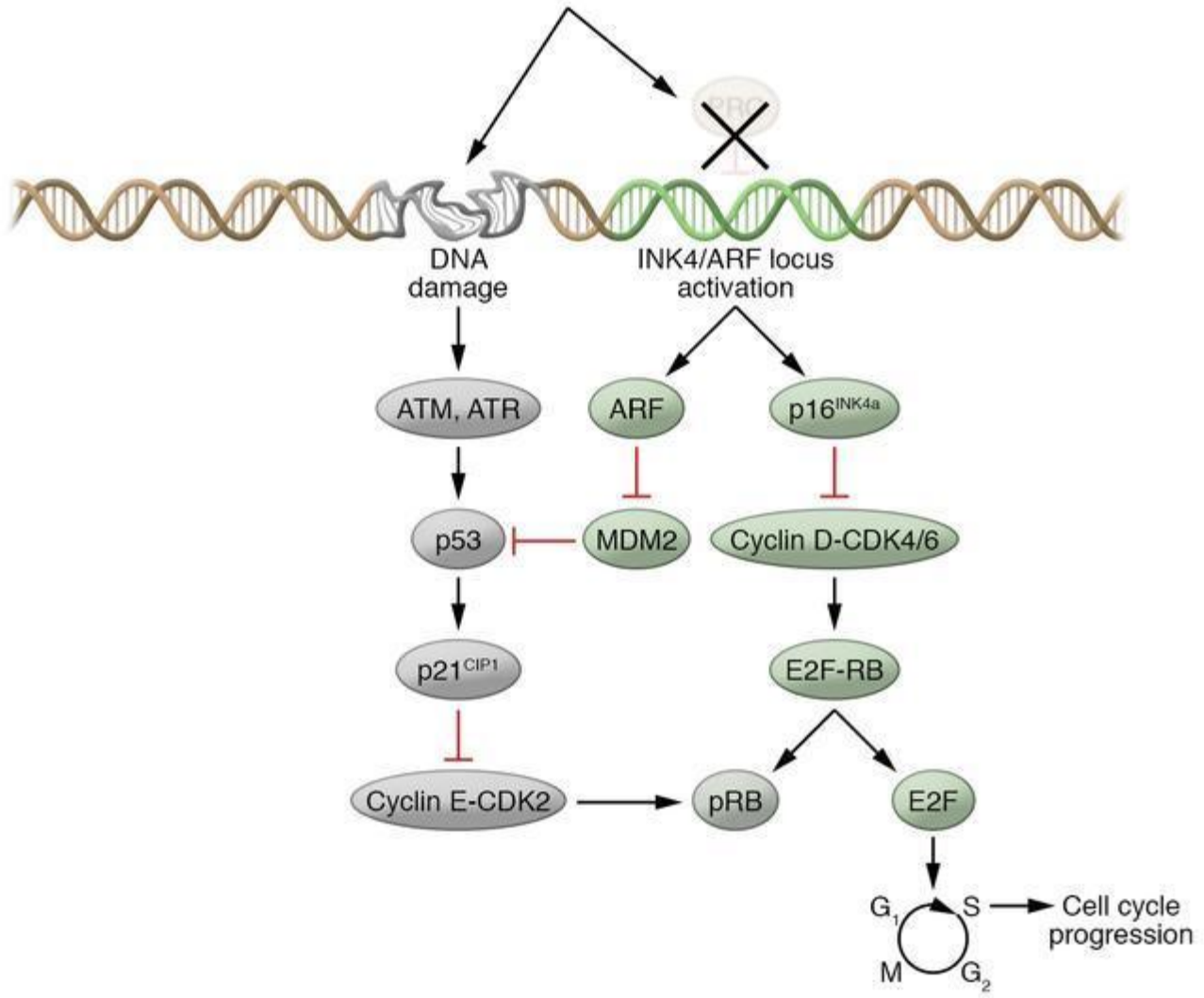
# Онкоген-индуцированным клеточным старением (oncogene-induced senescence,



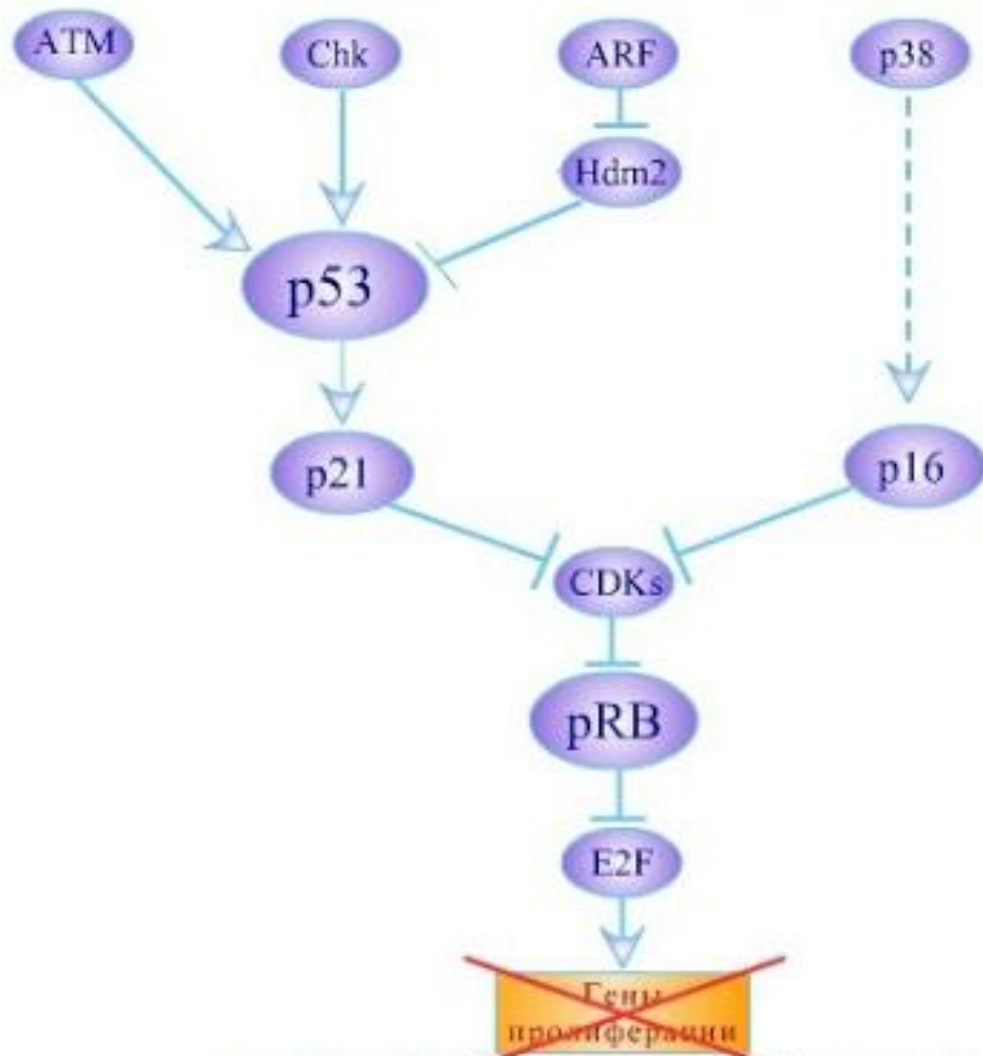
Prototypic oncogene-induced senescence (OIS) by Ras/Raf/Mek. Activated Ras/Raf/Mek oncogenes damage DNA, thereby triggering cellular DNA damage response (DDR) signaling involving ATM/ATR kinases and various components of the double strand break (DSB) repair machinery. Signals from unresolved DSB are relayed to the tumor suppressor p53, PML and pRB, which eventually promote a dynamic process of local, senescence-associated heterochromatin foci (SAHF) formation with the help of histone methyltransferases (such as Suv39h1) in the vicinity of E2F-responsive target genes, thereby transcriptionally silencing E2F-dependent S-phase genes. Moreover, persistent DSB may also trigger a second senescence-associated response, the massive production of largely pro-inflammatory cytokines and other secretable factors (termed "senescence-associated secretory phenotype [SASP]"), considered to reinforce the senescent arrest. Notably, the Myc oncogene is also known to evoke reactive oxygen species (ROS) and DNA replication stress like Ras/Raf-type oncogenes, and, a small fraction of Myc-activated cells directly enter senescence in a cell-autonomous fashion.

# Стресс-индуцированное преждевременное клеточное старение (stress-induced premature senescence, SIPS).



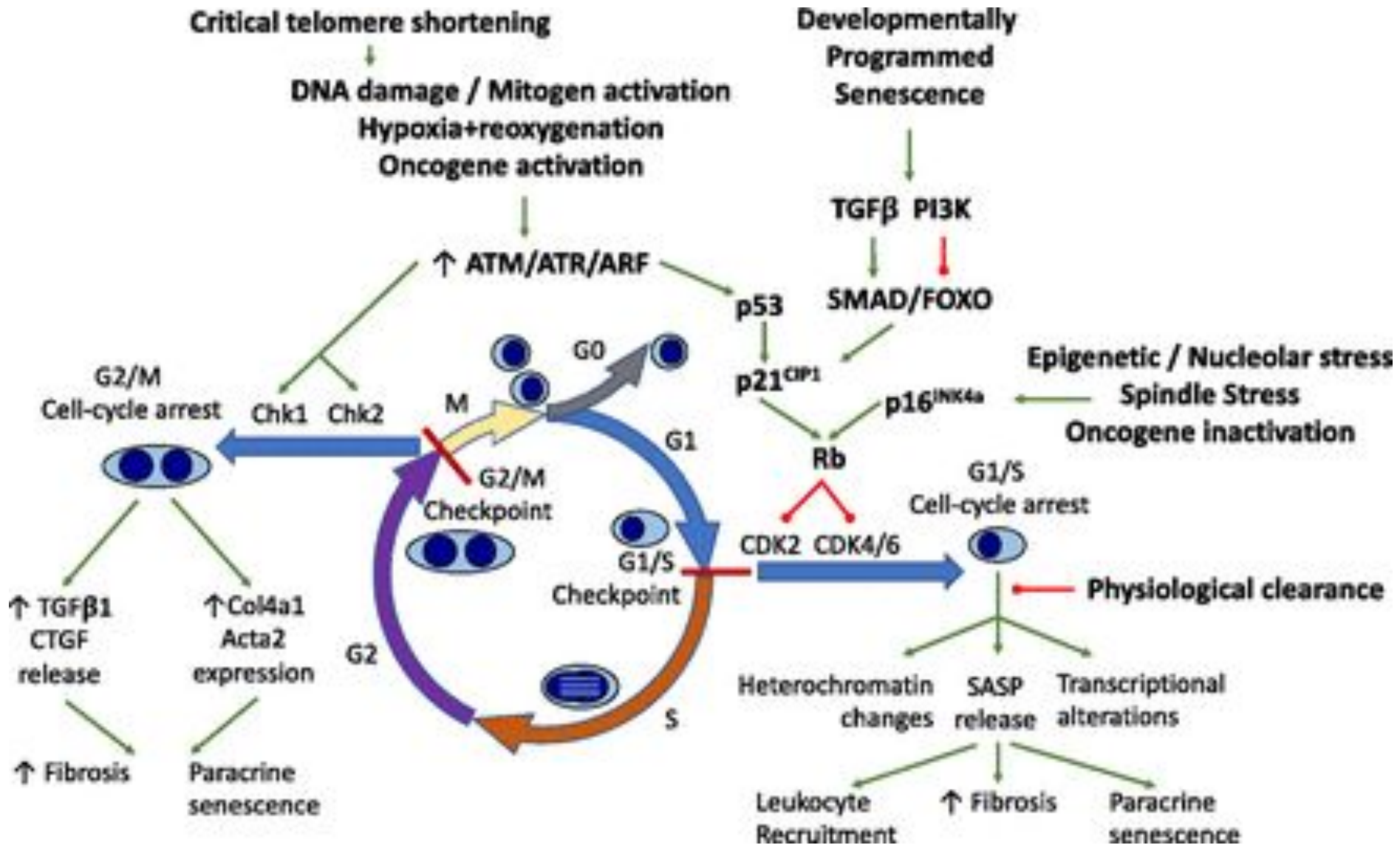






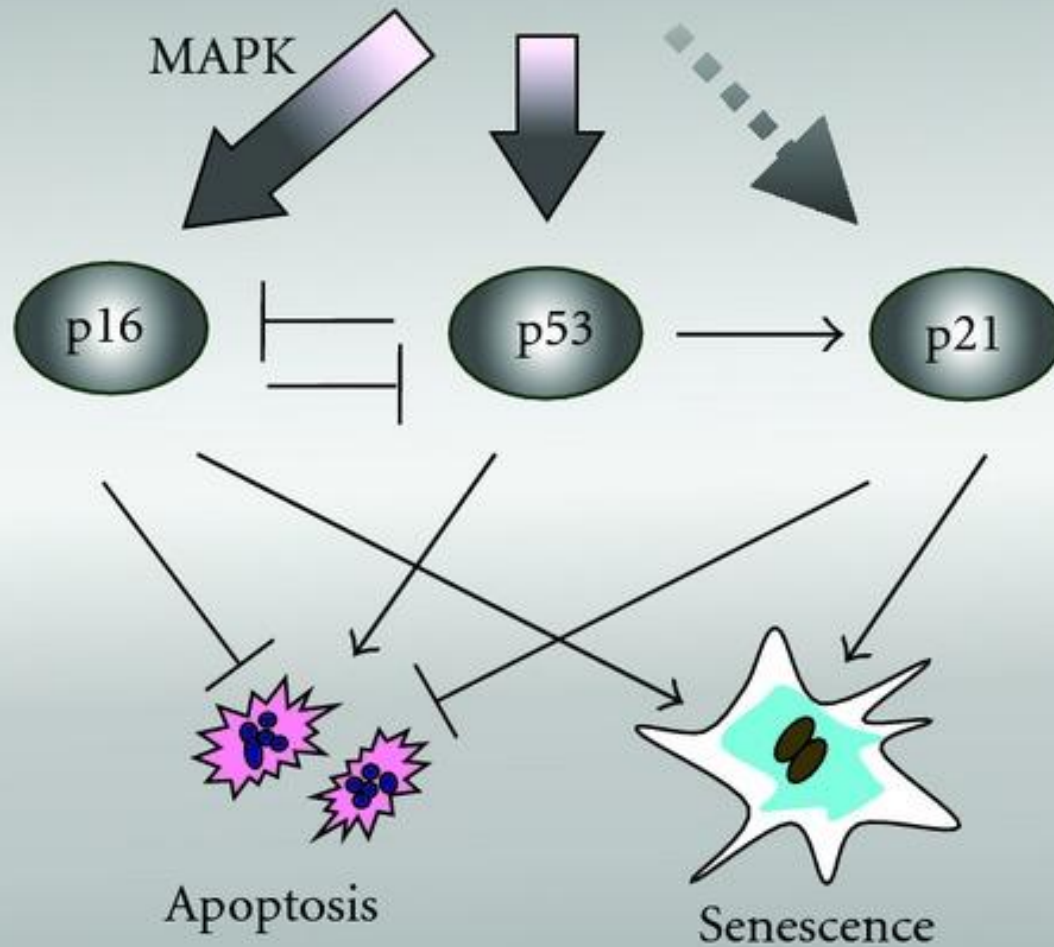
**РЕПЛИКАТИВНОЕ СТАРЕНИЕ**

# В почках- остановка G1 или G2

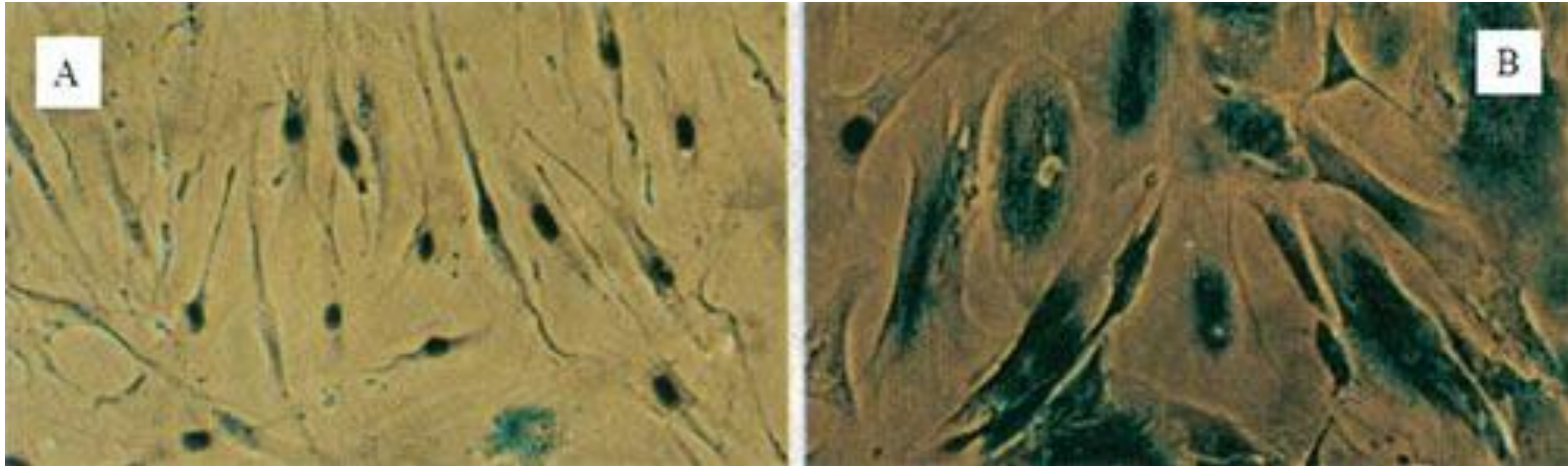


Stress

(Culture age; ionizing radiation)



# Senescent cells



Фенотип стареющих клеток:

Увеличение размеров

Увеличение размера  
ядра/многоядерность

Увеличение размера аппарата

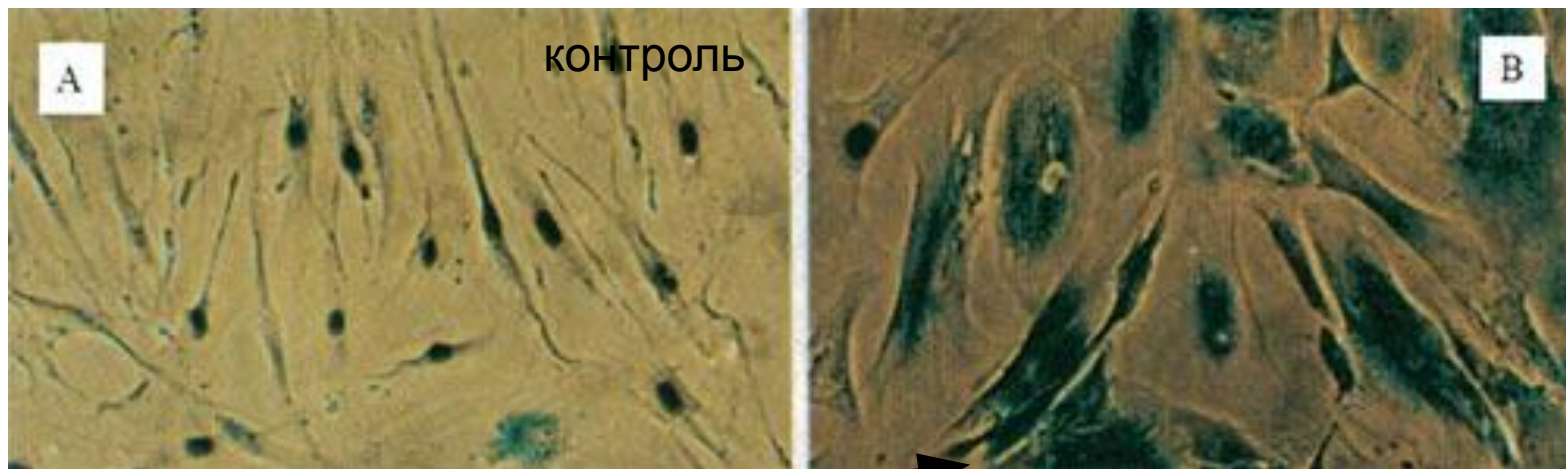
Гольджи

Вакуолизация цитоплазмы (стресс  
ЭПР)

*Биомаркеры клеточного старения.*

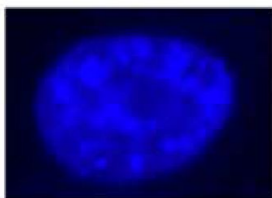
1. Ассоциированная с клеточным старением  $\beta$ -галактозидаза (senescence-associated beta-galactosidase, SA- $\beta$ -Gal).
2. Белок HMGB1 (high-mobility group protein B1).
3. Фосфорилированный гистон  $\gamma$ H2AX.
4. Ассоциированные с клеточным старением гетерохроматиновые фокусы (SAHF).
5. Белок промиелоцитарного лейкоза (PML).

# Маркеры стареющих клеток

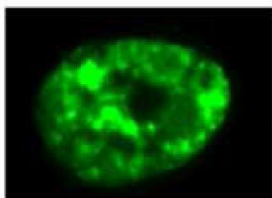


SA-β-галактозидаза

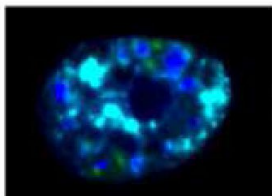
стареющие



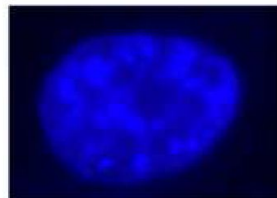
SAHF  
DAPI



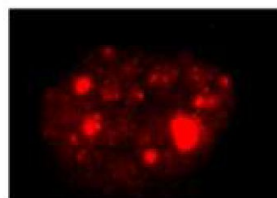
H3K9meth



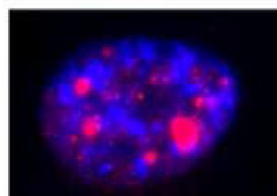
merged



DAPI

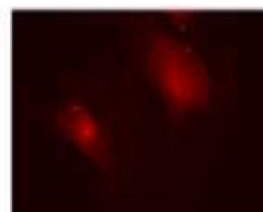


γH2AX



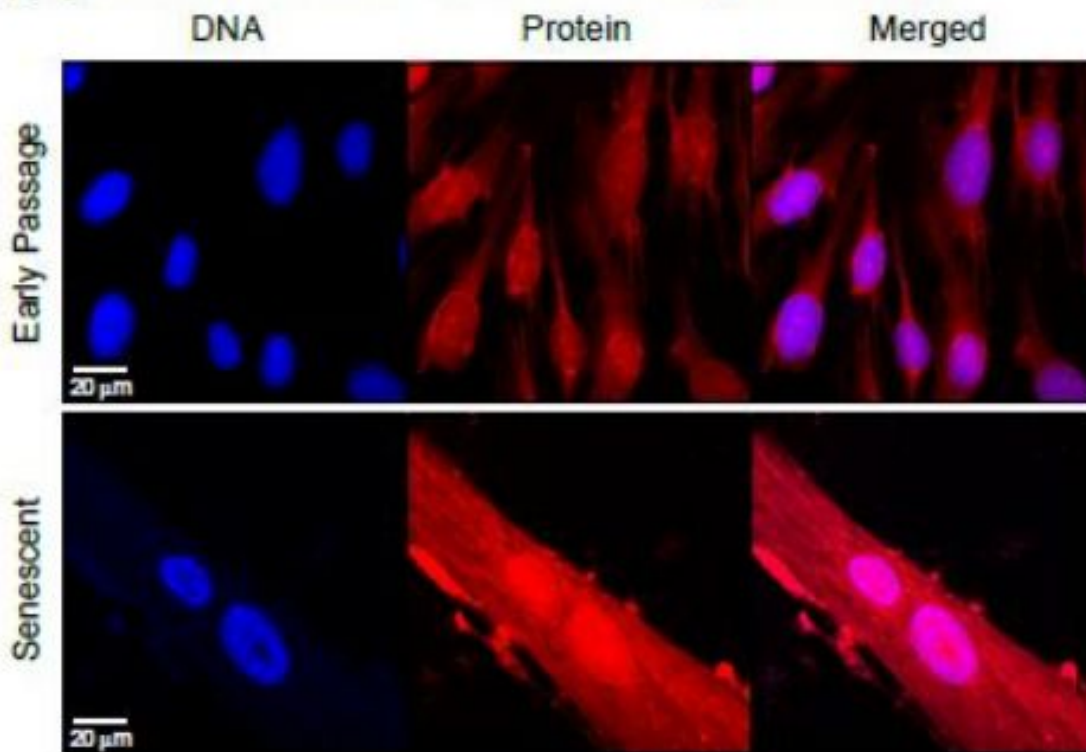
merged

стареющие    контроль

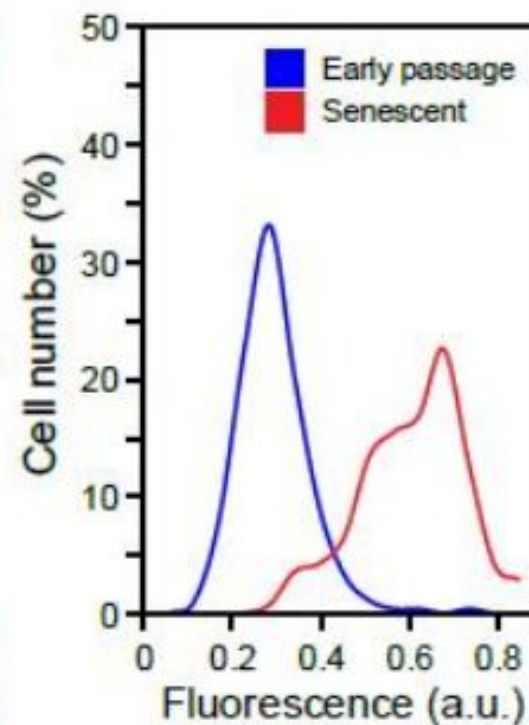


p16<sup>INK4a</sup>

### (A) Human fibroblasts, NanoOrange staining



### (B) Histograms, NO

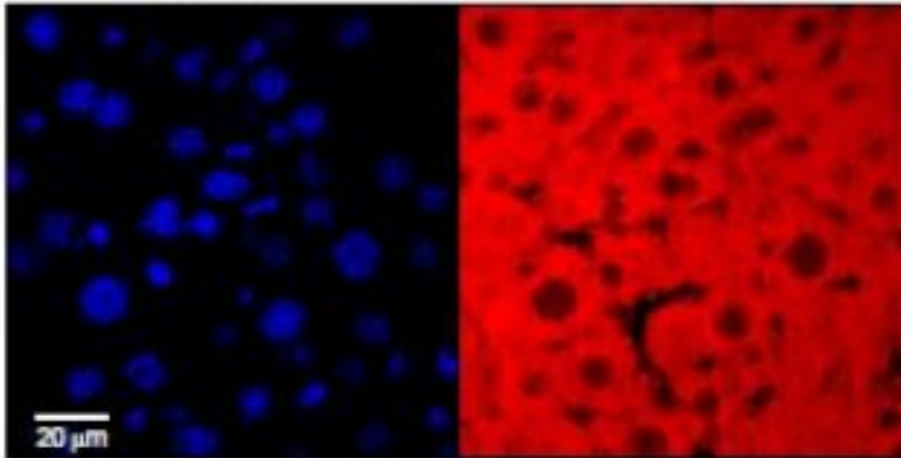


Еще один маркер – увеличение содержание тотального белка в клетках  
Это мышинные фибробласты на разных пассажах.

**(A) Mouse liver, young**

DNA

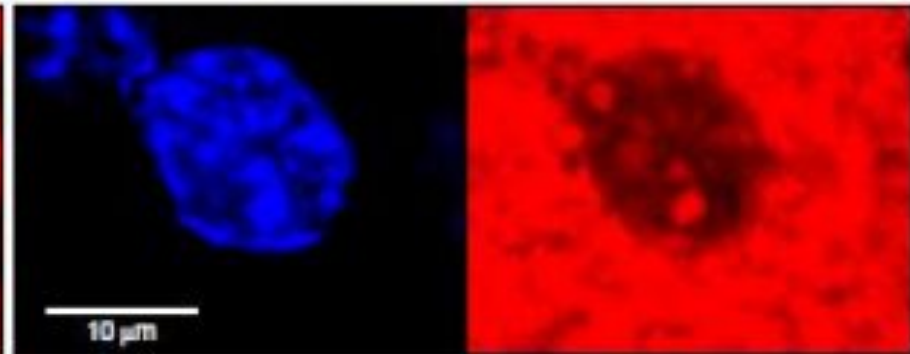
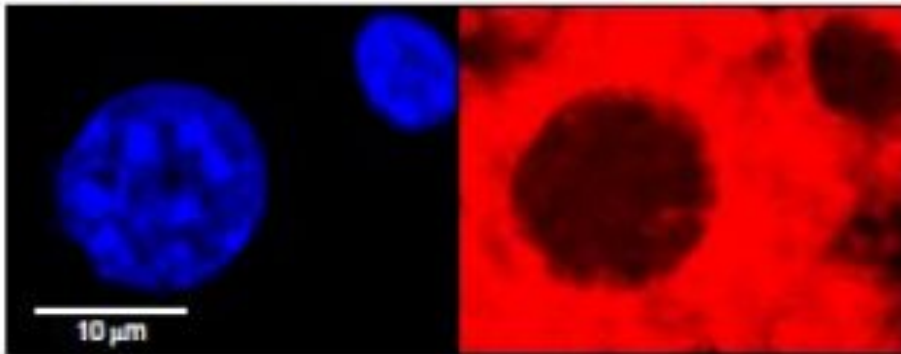
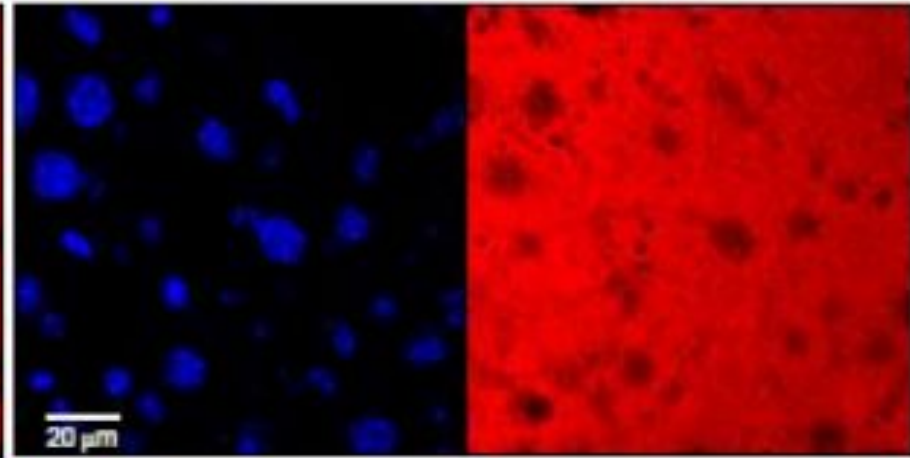
Protein



**(B) Mouse liver, old**

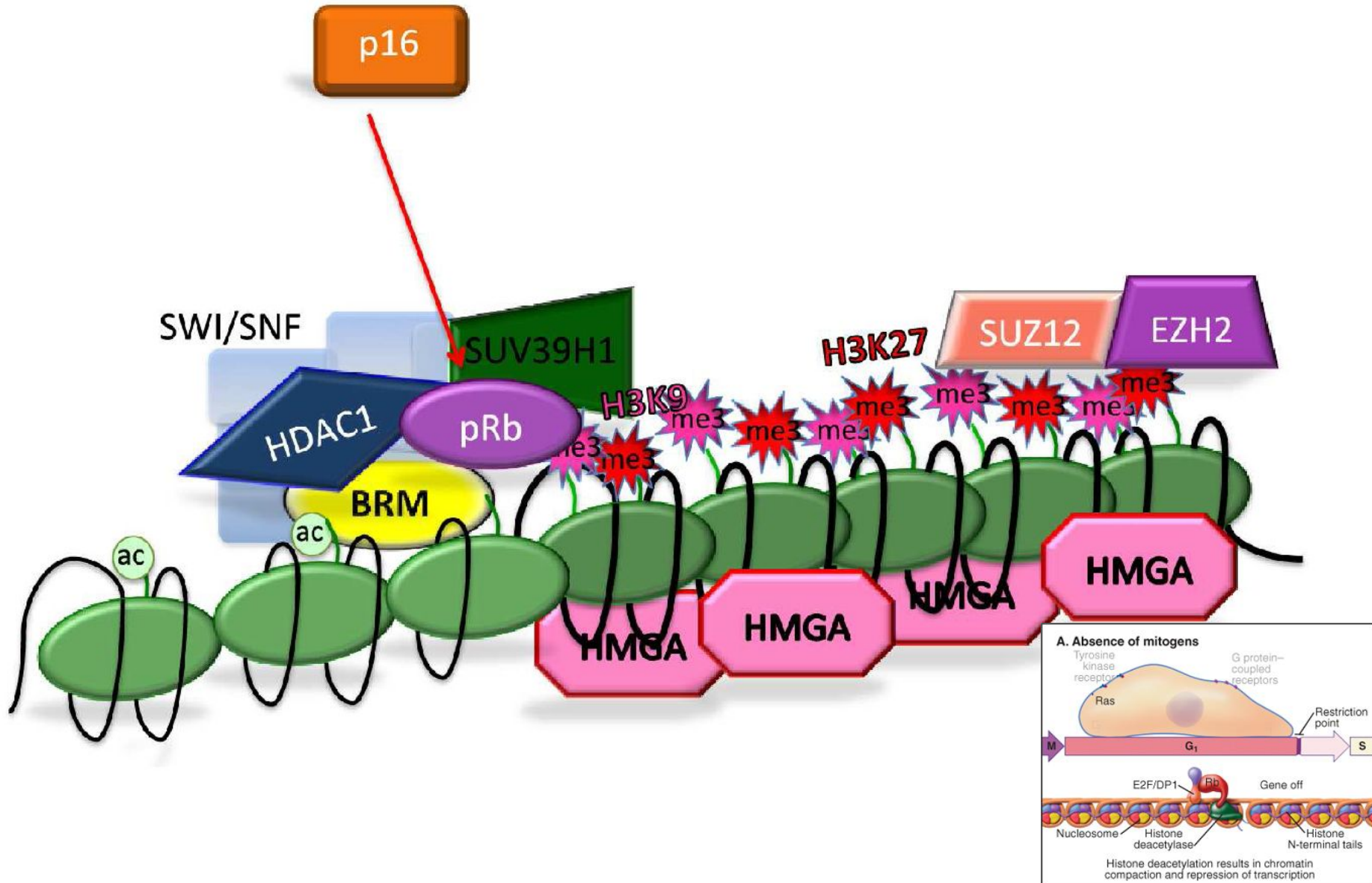
DNA

Protein



А это гепатоциты молодых и старых мышек. Увеличение содержания белка не так выражено. Однако видно накопление белка в ядре гепатоцита.

# Ассоциированные с клеточным старением гетерохроматиновые фокусы (SAHF).



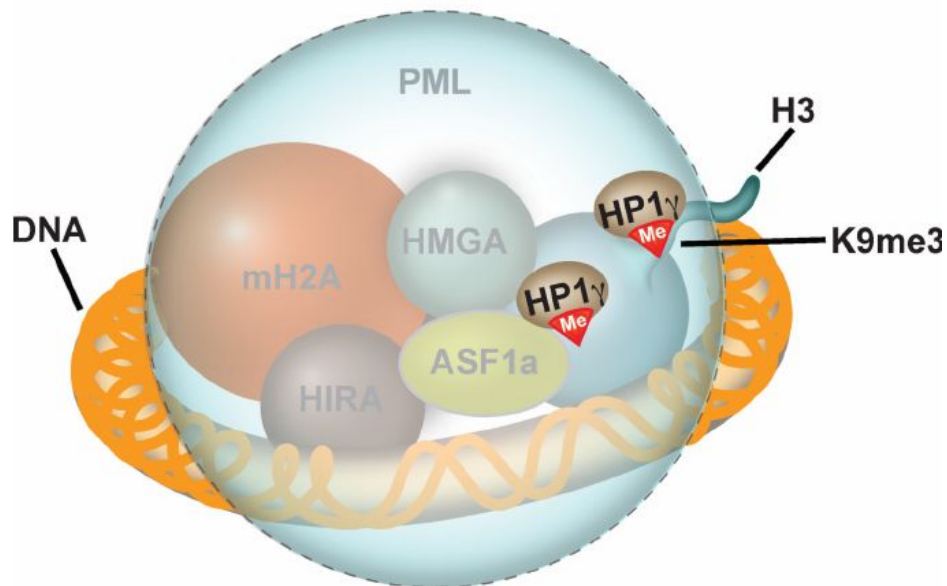


# PML

Oncogenic Expression  
(H-RAS V12, N-RAS, BRAF)



hyper-replication/ATR checkpoint  
p16<sup>INK4A</sup>/p53 upregulation



DNA Damage  
Tumourigenesis

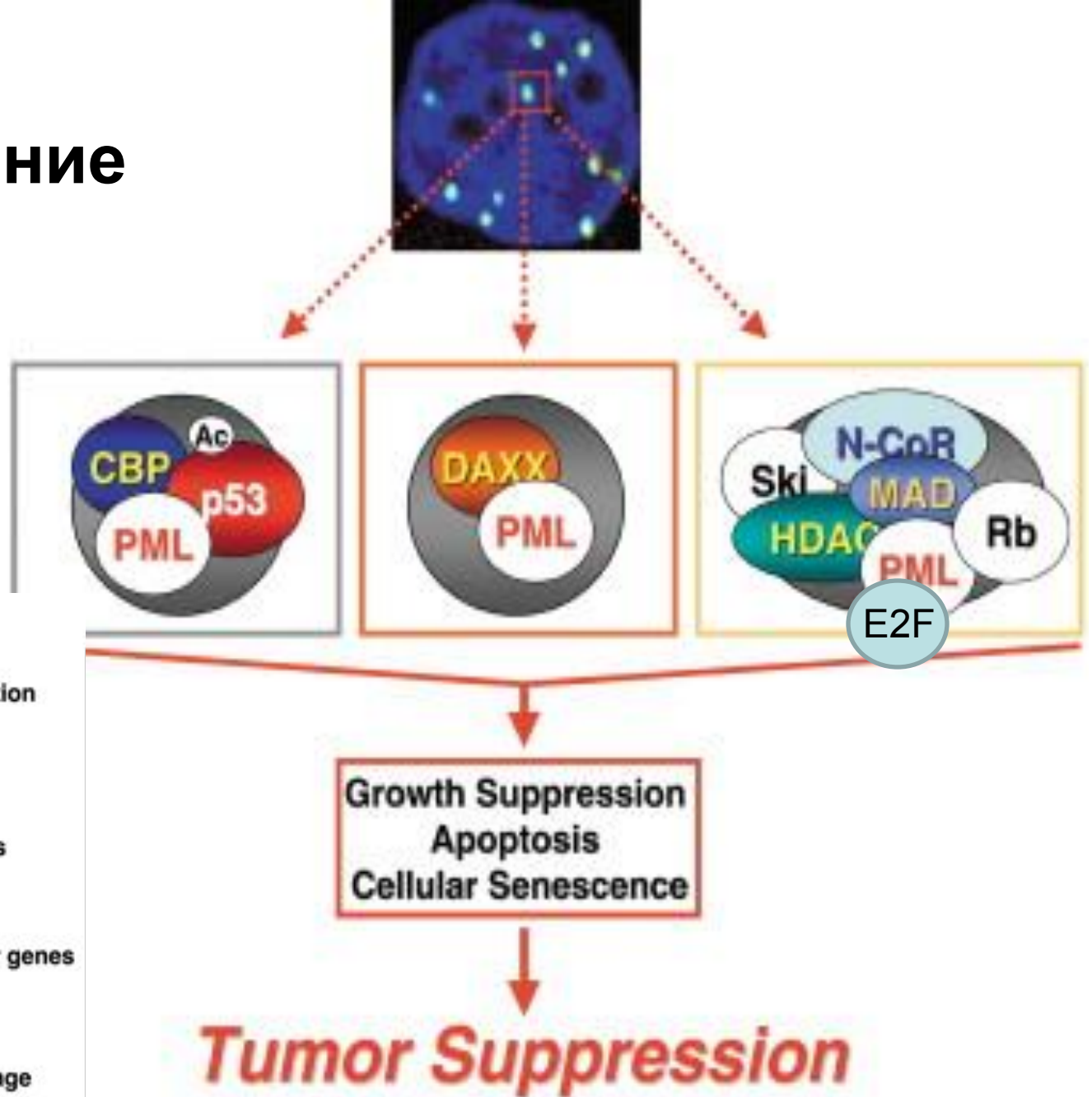
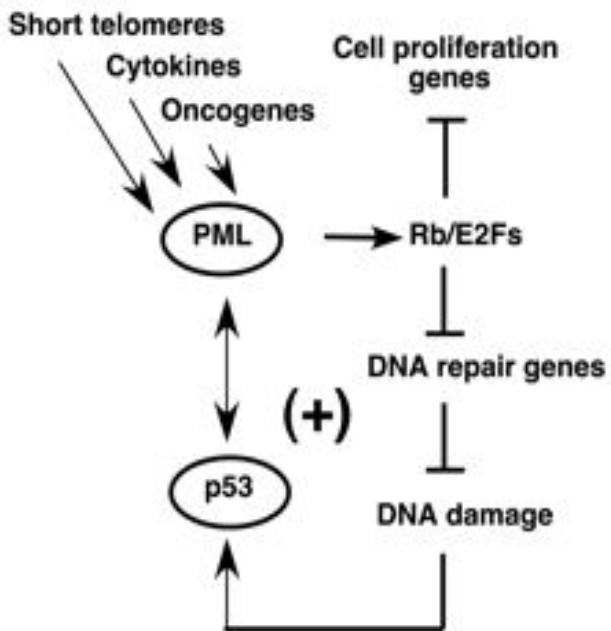
[https://doi.org/10.1016/S0092-8674\(02\)00626-8](https://doi.org/10.1016/S0092-8674(02)00626-8)

<http://genesdev.cshlp.org/content/25/1/41.full>

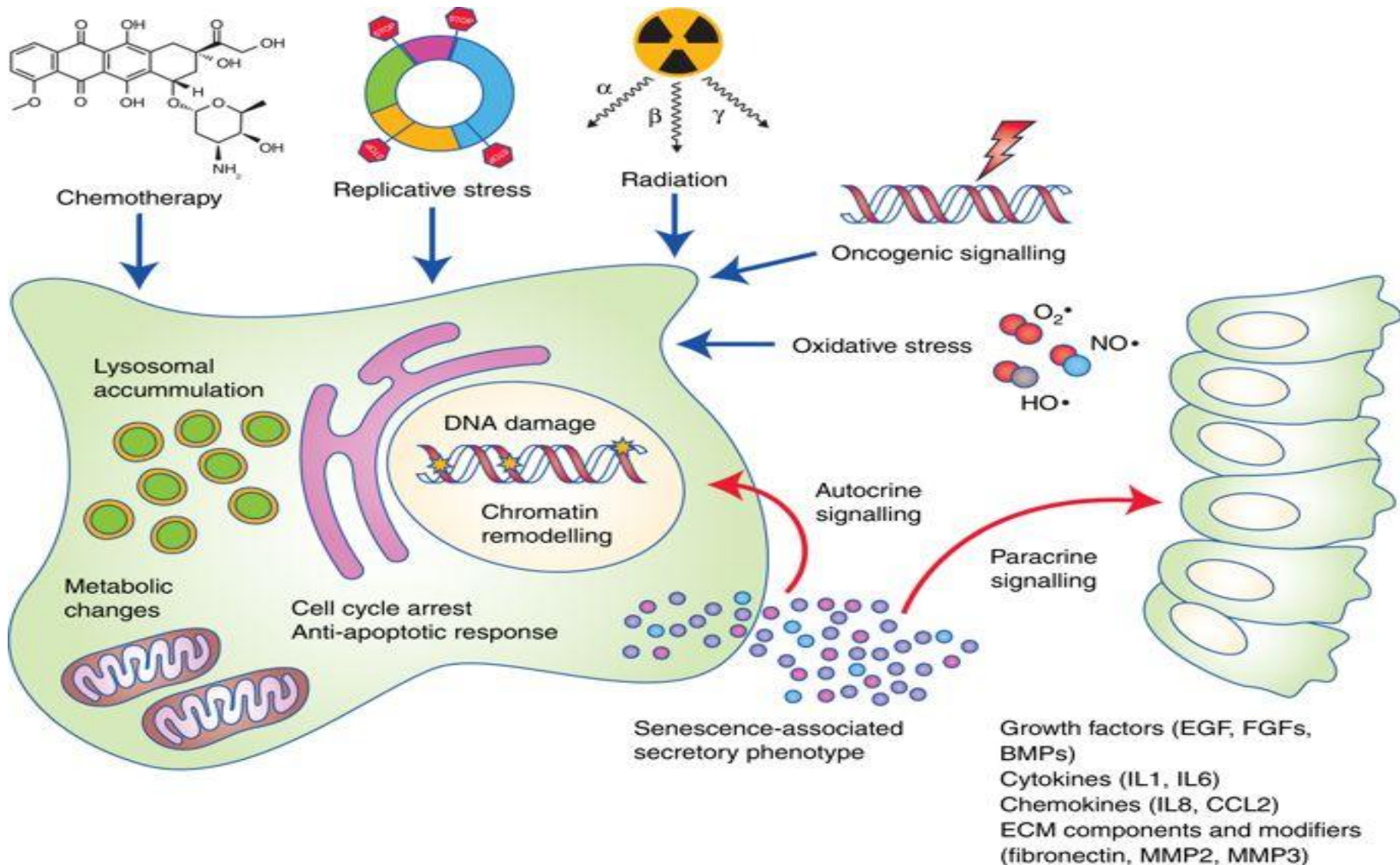
Белок промиелоцитарного лейкоза  
(PML)

# PML- ингибирование генов

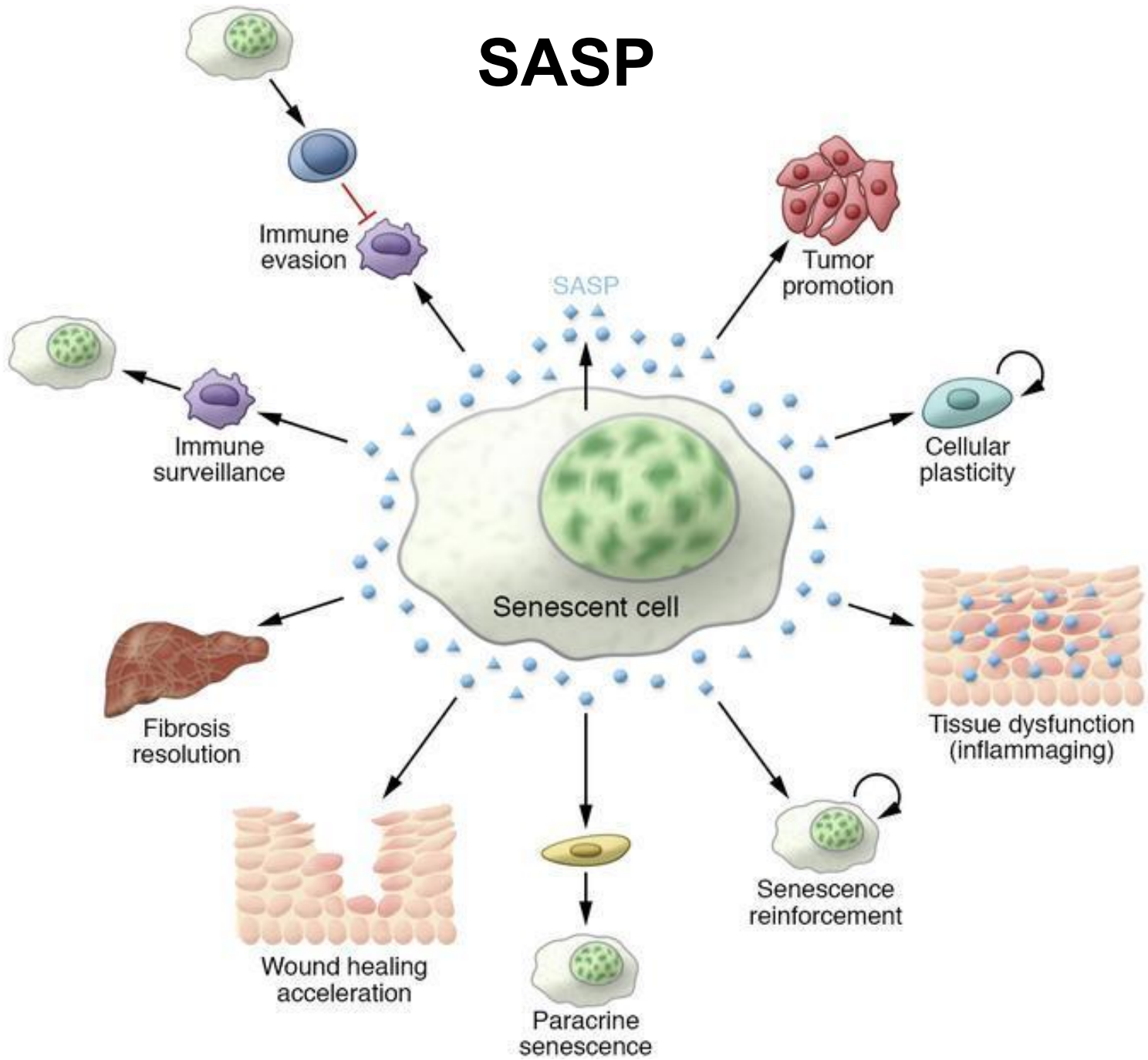
A



# Ассоциированный с клеточным старением секреторный фенотип (senescence associated secretory phenotype, SASP)



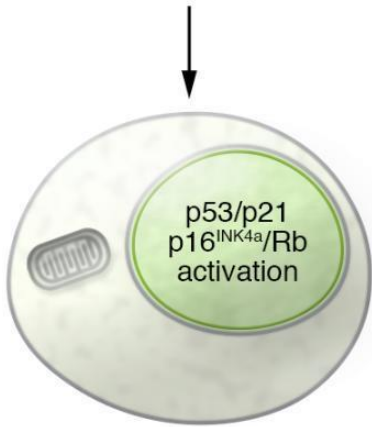
# SASP



# Старение - это динамичный многоступенчатый процесс

## Senescence initiation

Senescence-inducing signals, e.g., oncogene activation, DNA damage

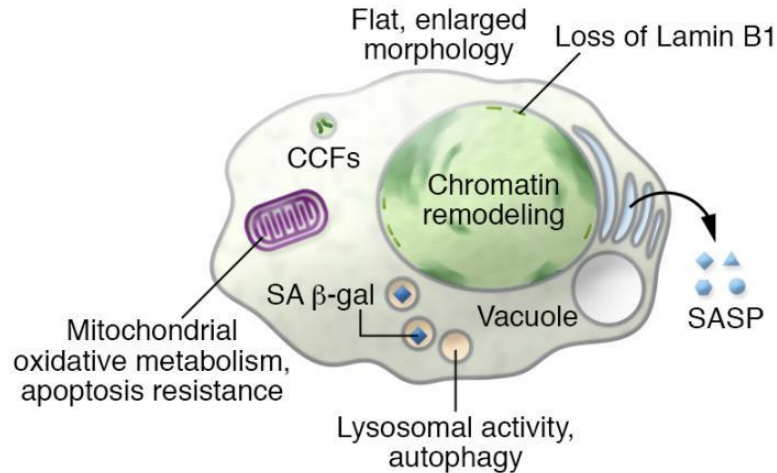


Cell cycle exit

**Быстрое старение**

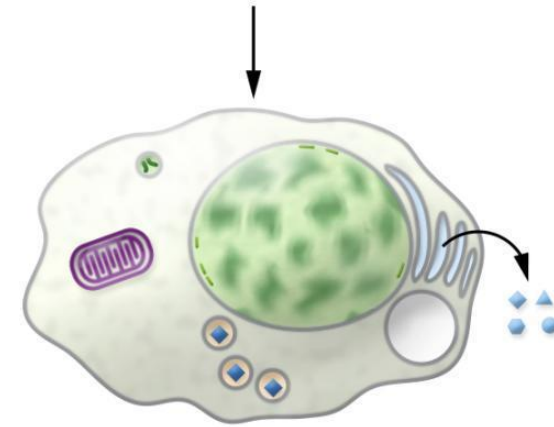
## Early senescence

Progressive chromatin remodeling, implementation of senescence program



## Late senescence

Triggered by aging or long-term, unscheduled damage



Chronic inflammation ("inflammaging"), diversification of senescent phenotype

**Хроническое старение**

**А теперь задание!**

**Я старалась, но может где и ошиблась 😊 ))**

Можно изобразить на листочке,  
сфотографировать и отправить



# Цитокинез

a

**Midbody core**  
KIF4, PRC1 (centralspindlin,  
Cep55, ALIX?)

Secondary  
ingression  
zone

~0.2  $\mu\text{m}$

~1-2  $\mu\text{m}$

**Midbody flank**  
MKLP2, Aurora B,  
CENP-E

Midbody  
MTs

b

**Midbody ring**  
Ect2, anillin, RhoA, ARF6,  
centralspindlin, Cep55, ALIX

ESCRT-I

ESCRT-III

**Abscission site**  
Spastin, ESCRT-III,  
VPS4, RhoA, anillin

