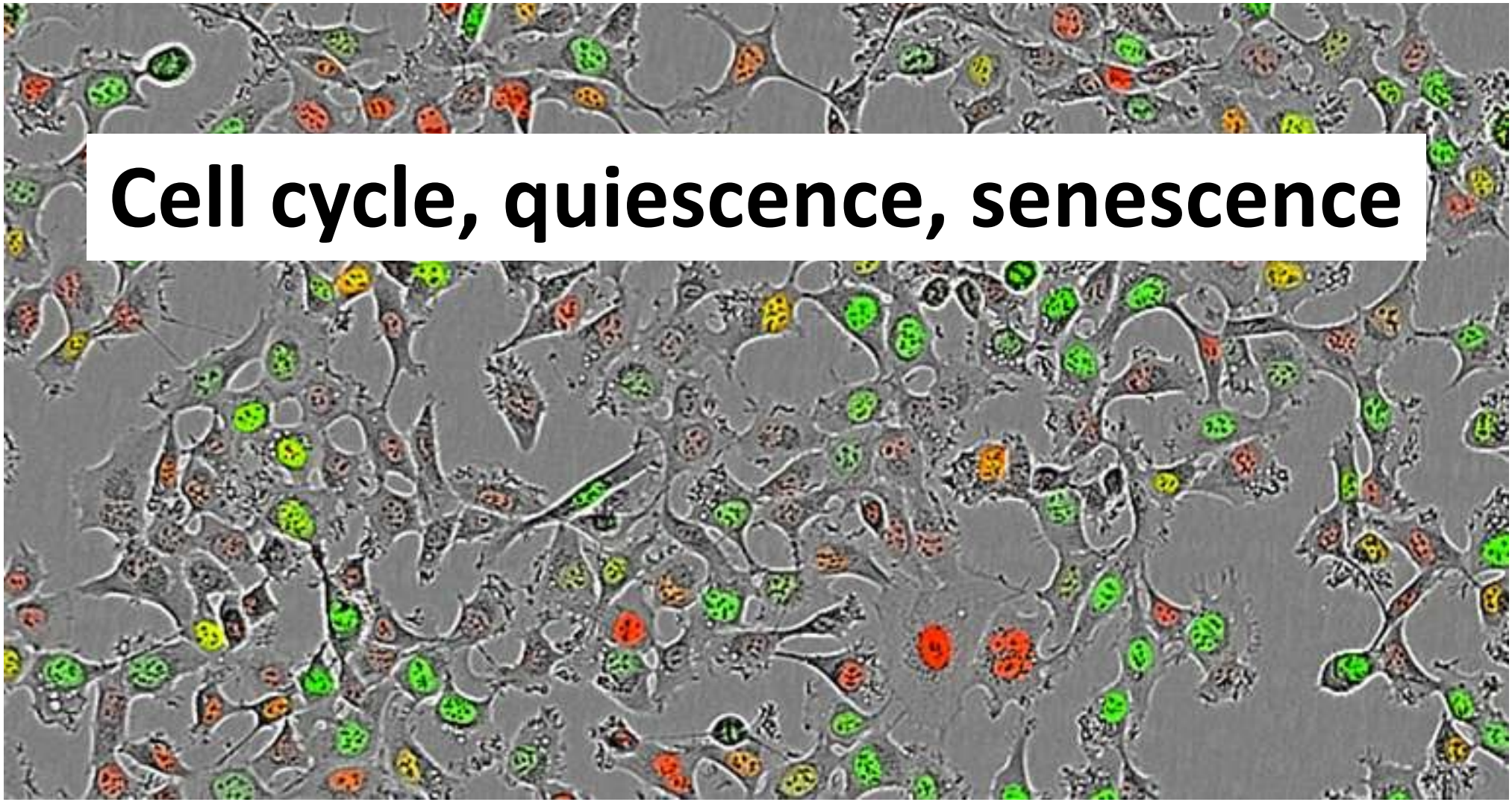
Cell cycle, quiescence, senescence



M1 International, Cancer Cell Biology, TU n°05

université PARIS-SACLAY
GRADUATE SCHOOL
Health and Drug Sciences

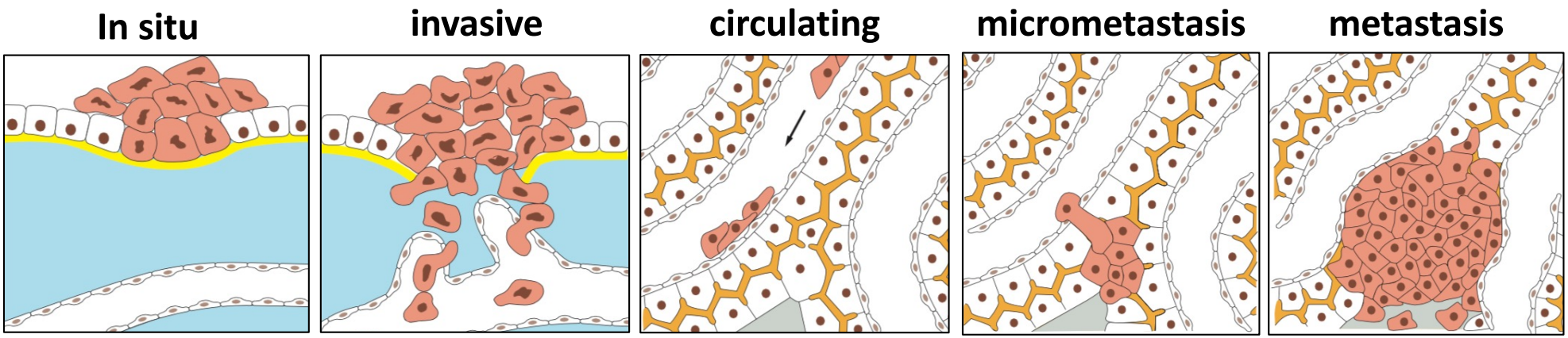
d2lp

université PARIS-SACLAY
FACULTÉ DE PHARMACIE

Definitions

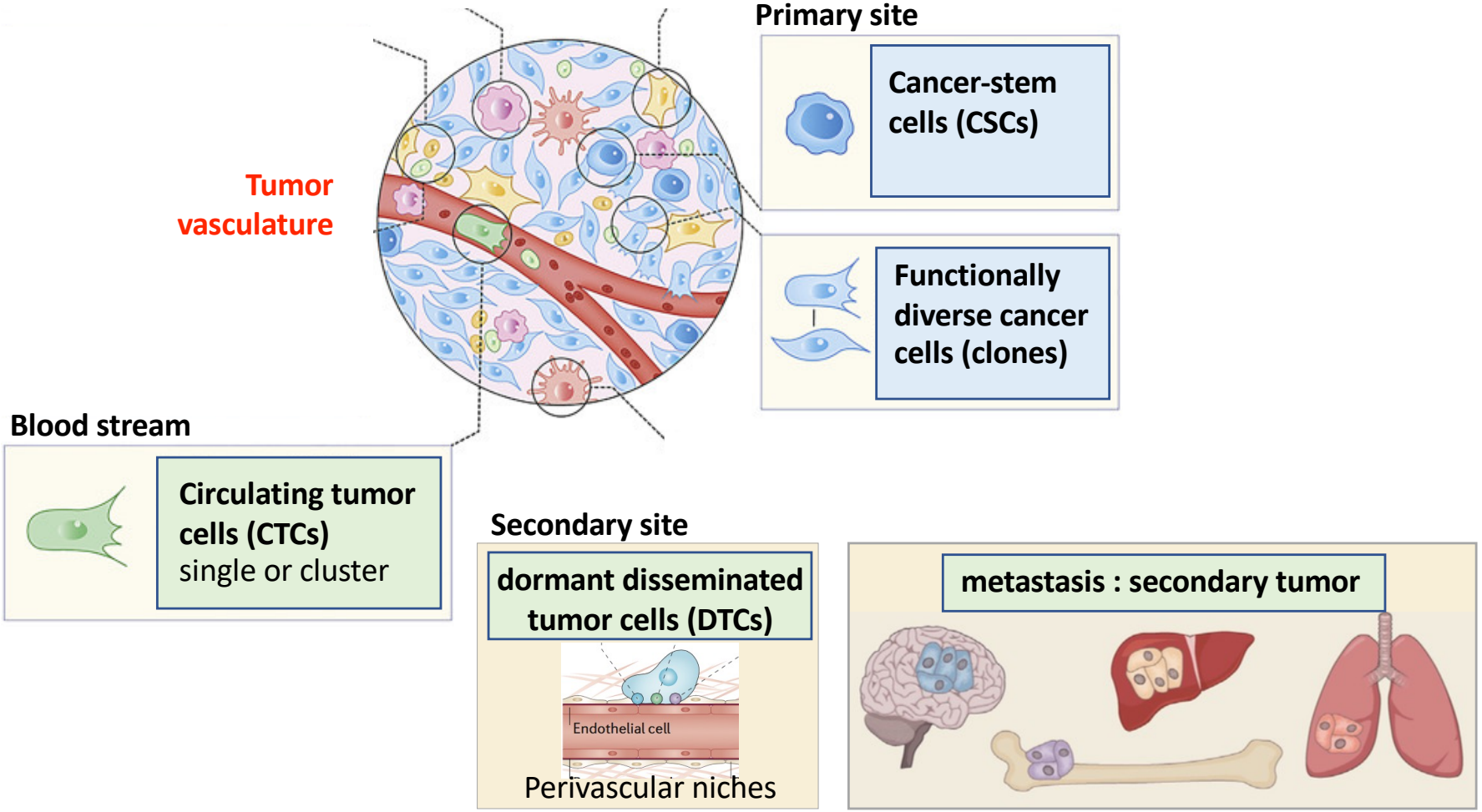
A tumor/neoplasm is a type of abnormal and excessive growth of tissue.
The word tumor comes from the Latin word for swelling.

Tumor/neoplasm can be
- Benign (ex: skin mole)
- In situ (potentially malignant, still in the place where they started)
- Malignant = cancer (focus of oncology)



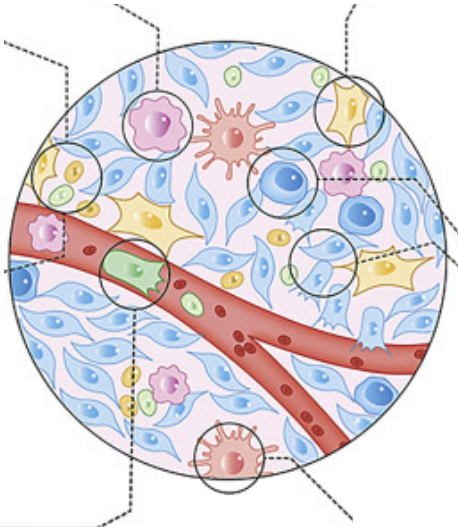
Wikipedia
Adapted from igure 20-16, Molecular Biology of the Cell 6th

Intratumoral heterogeneity



Adapted from Gonzalez-Silva et al, Trends in Cancer, 2020
Ghajar, Nature Cancer Rev., 2015
Lambert et al., Cell, 2016

intratumoral heterogeneity



Tumor vasculature

Primary site

Cancer-stem cells (CSCs)

Cause of recurrence ?

Functionally diverse cancer cells (clones)

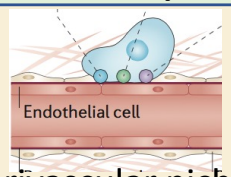
Blood stream

Circulating tumor cells (CTCs)
single or cluster

Cause of recurrence ?

Secondary site

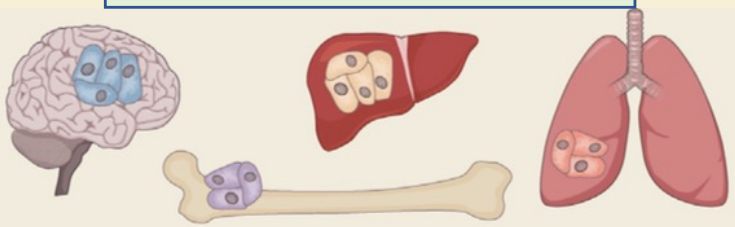
dormant disseminated tumor cells (DTCs)



Perivascular niches

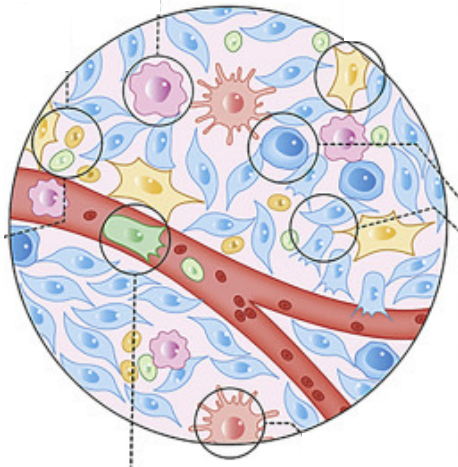
Cause of death

metastasis : secondary tumor



Adapted from Gonzalez-Silva et al, Trends in Cancer, 2020
Ghajar, Nature Cancer Rev., 2015
Lambert et al., Cell, 2016

intratumoral heterogeneity / tumor microenvironment (TME)



Tumor vasculature

Primary site

Cancer-stem cells (CSCs)

Cancer-associated fibroblasts (CAFs)

Functionally diverse cancer cells (clones) some invasive

Tumor-infiltrating immune cells

If immuno-suppressive = protumoral
If anti-cancer immunity = antitumoral

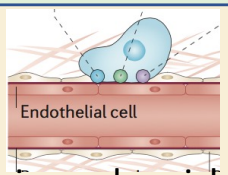
Blood stream

Circulating tumor cells (CTCs) single or cluster

Tumor-educated platelets (TEPs)

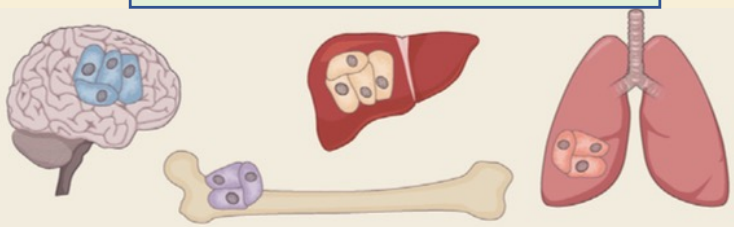
Secondary site

dormant disseminated tumor cells (DTCs)



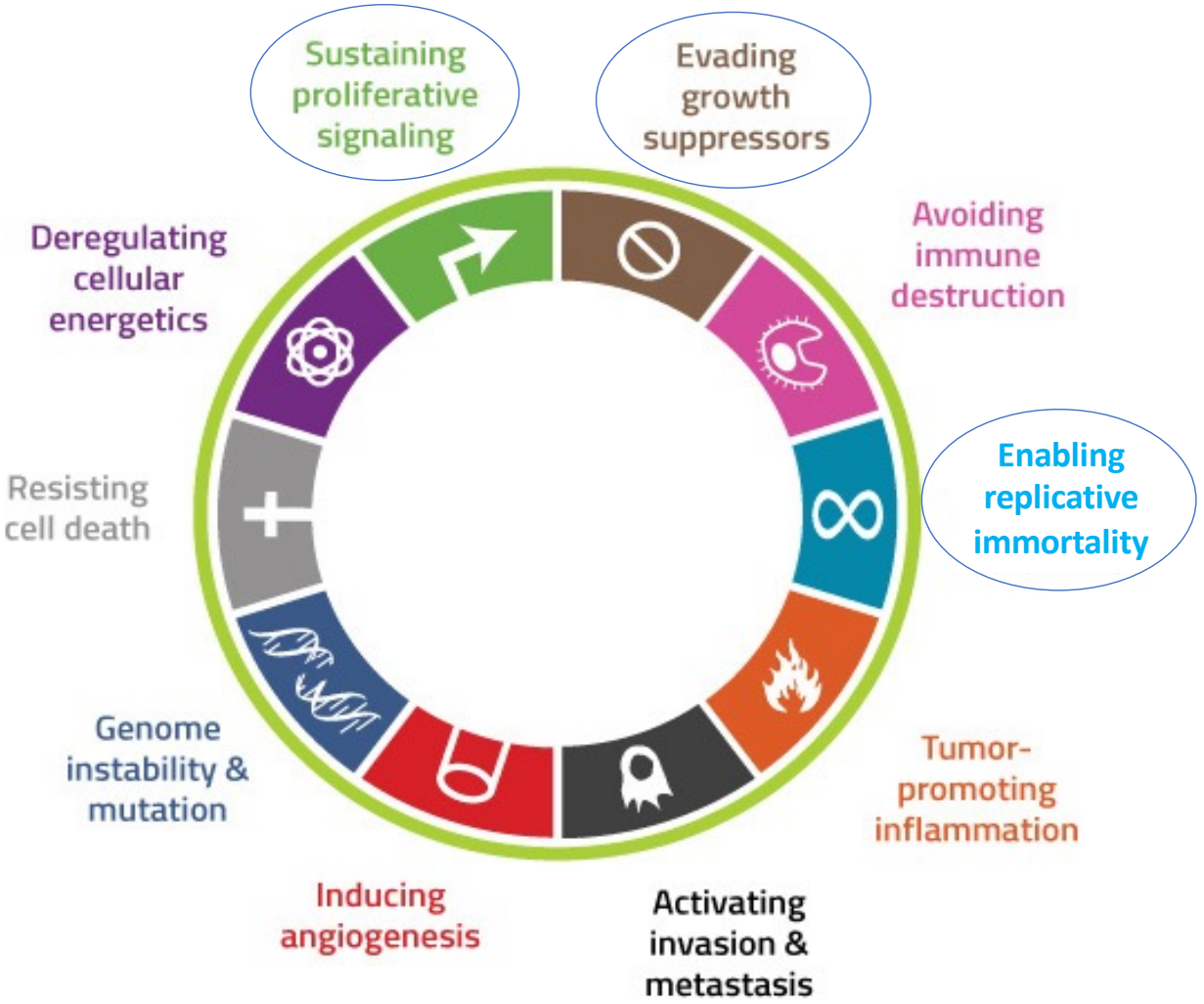
Perivascular niches

metastasis : secondary tumor



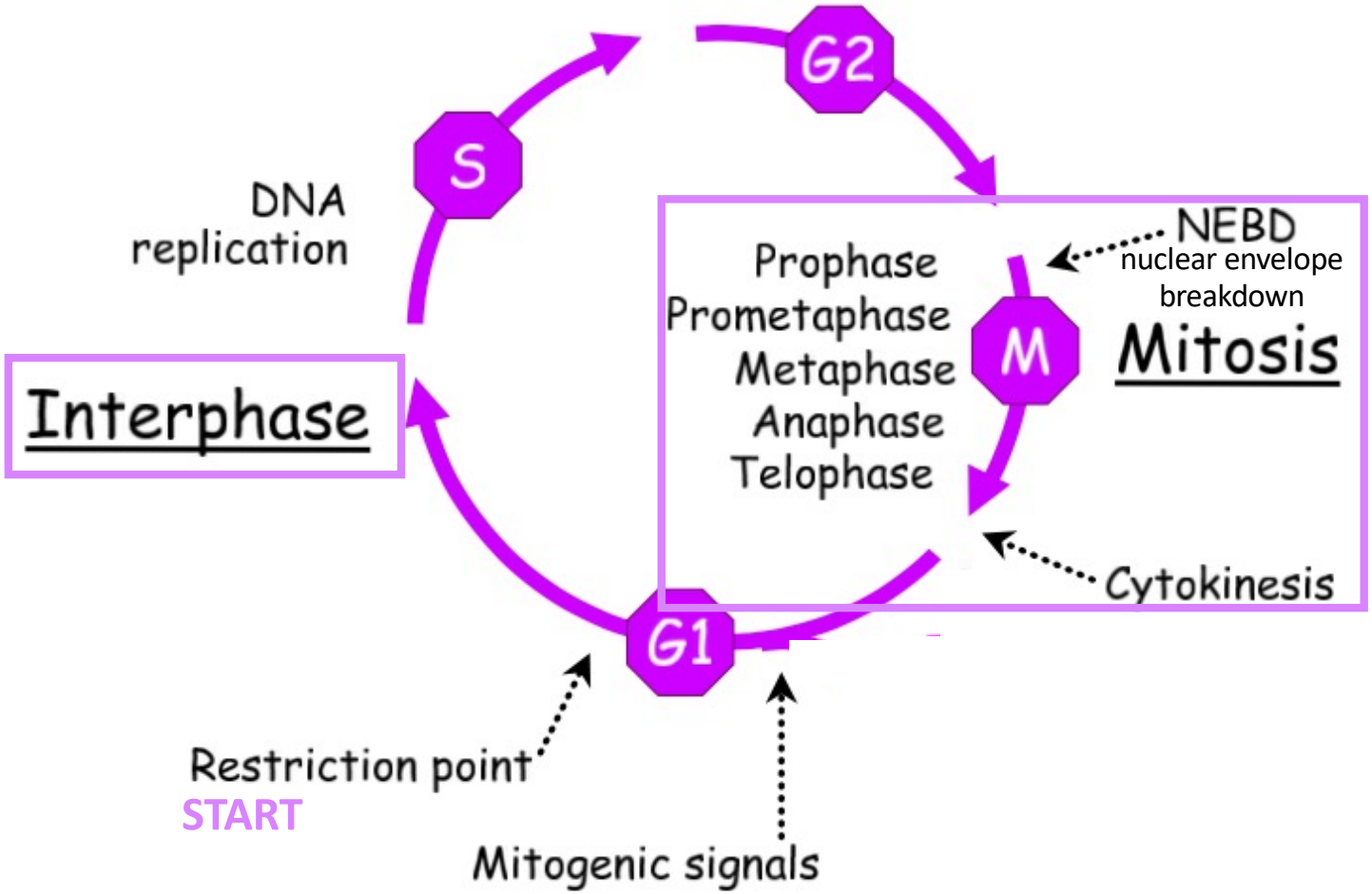
Adapted from Gonzalez-Silva et al, Trends in Cancer, 2020
Ghajar, Nature Cancer Rev., 2015
Lambert et al., Cell, 2016

Hallmarks of cancer : related to cell cycle



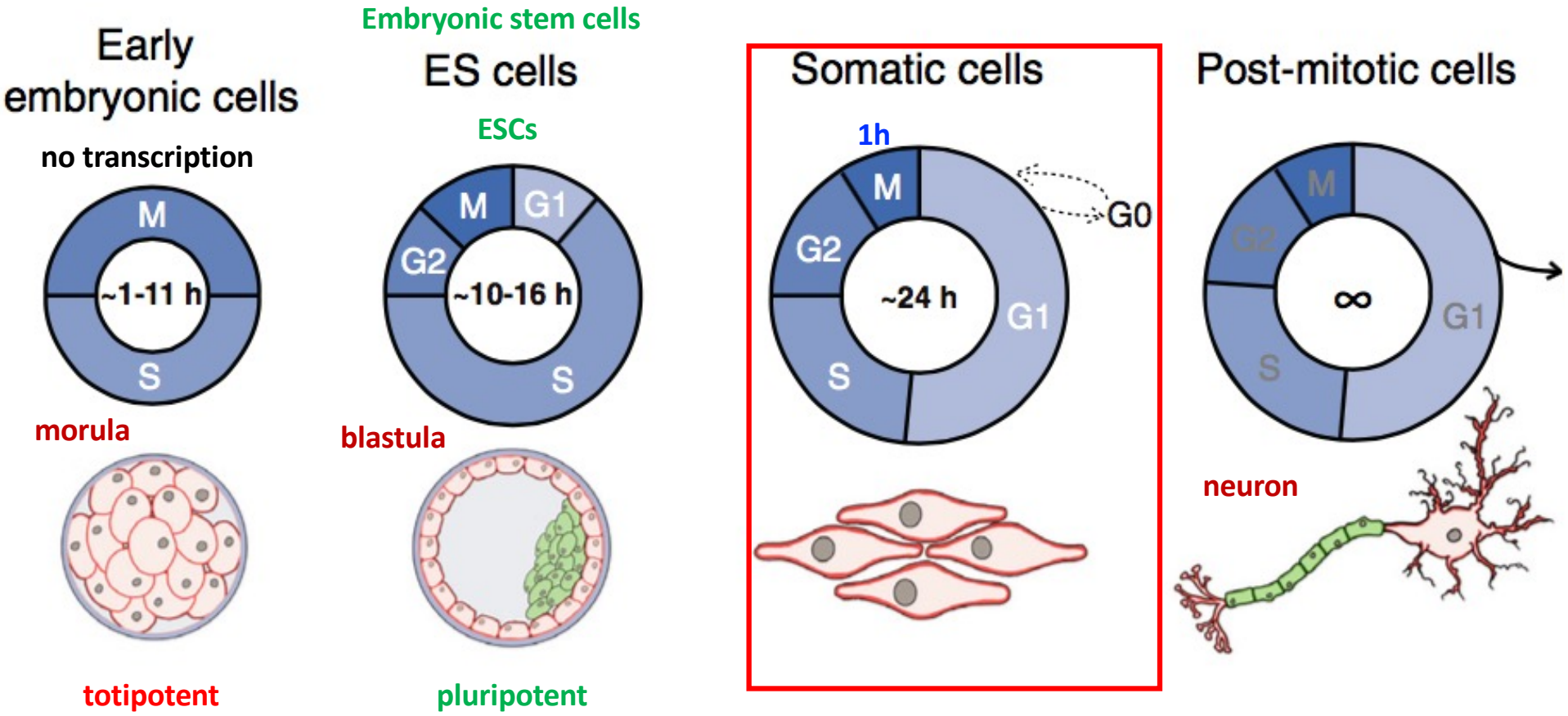
Adapted from Hanahan et Weinberg. Cell 2011

Usually 4 phases in eukaryotic cell cycle



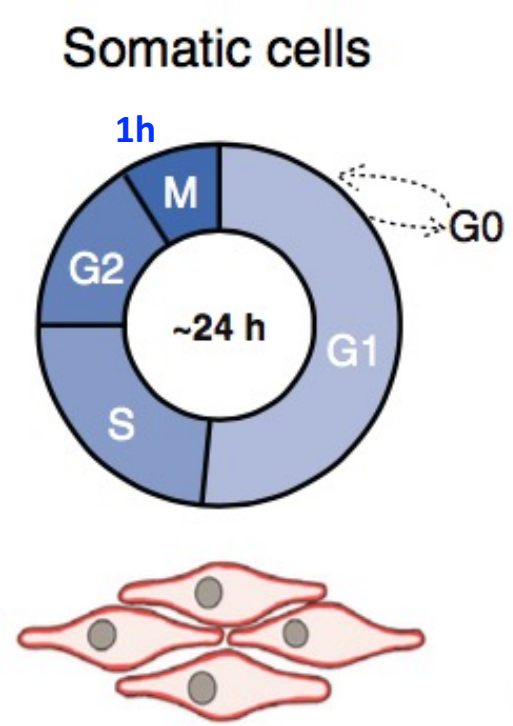
Meijer, Oncology, 2003

Cell cycle variation in different cell types



Padgett & Santos, FEBS letters, 2020

Cell cycle control system in normal cells

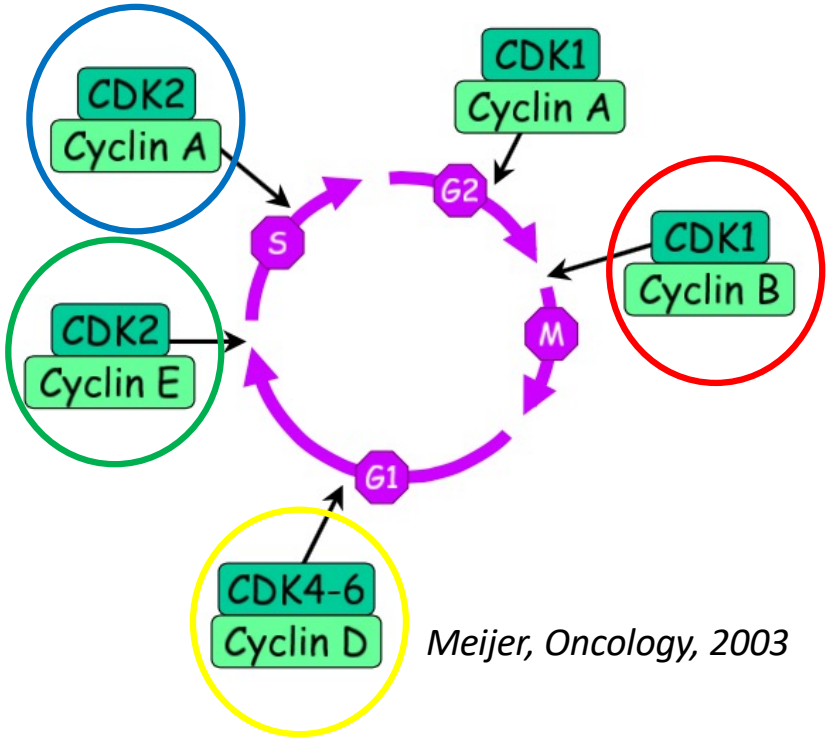


- Orderly sequence of events (4 phases)
- Binary (switches on/off) : complete and irreversible
- Remarkably robust and reliable
- Adaptable

- Reversible exit possible in G0-quiescence
- Permanent exit : terminal differentiation, senescence, death

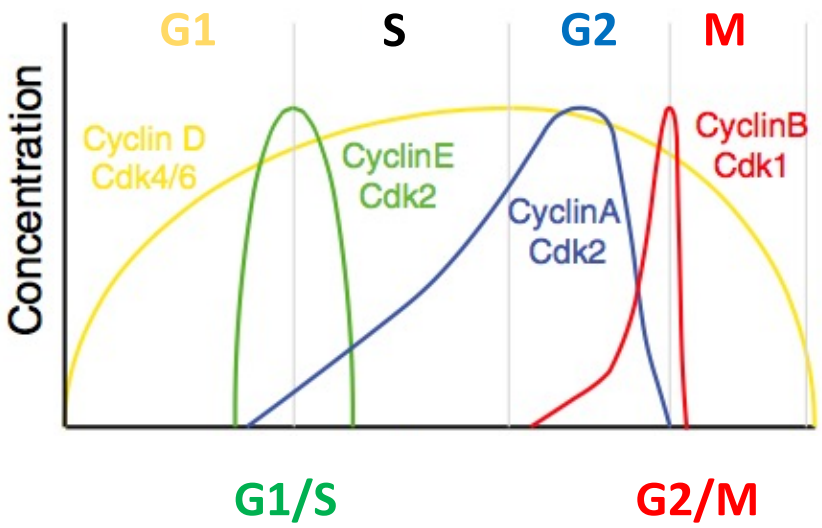
Padgett & Santos, FEBS letters, 2020

Cell cycle regulators : cyclin-dependent kinases



Meijer, Oncology, 2003

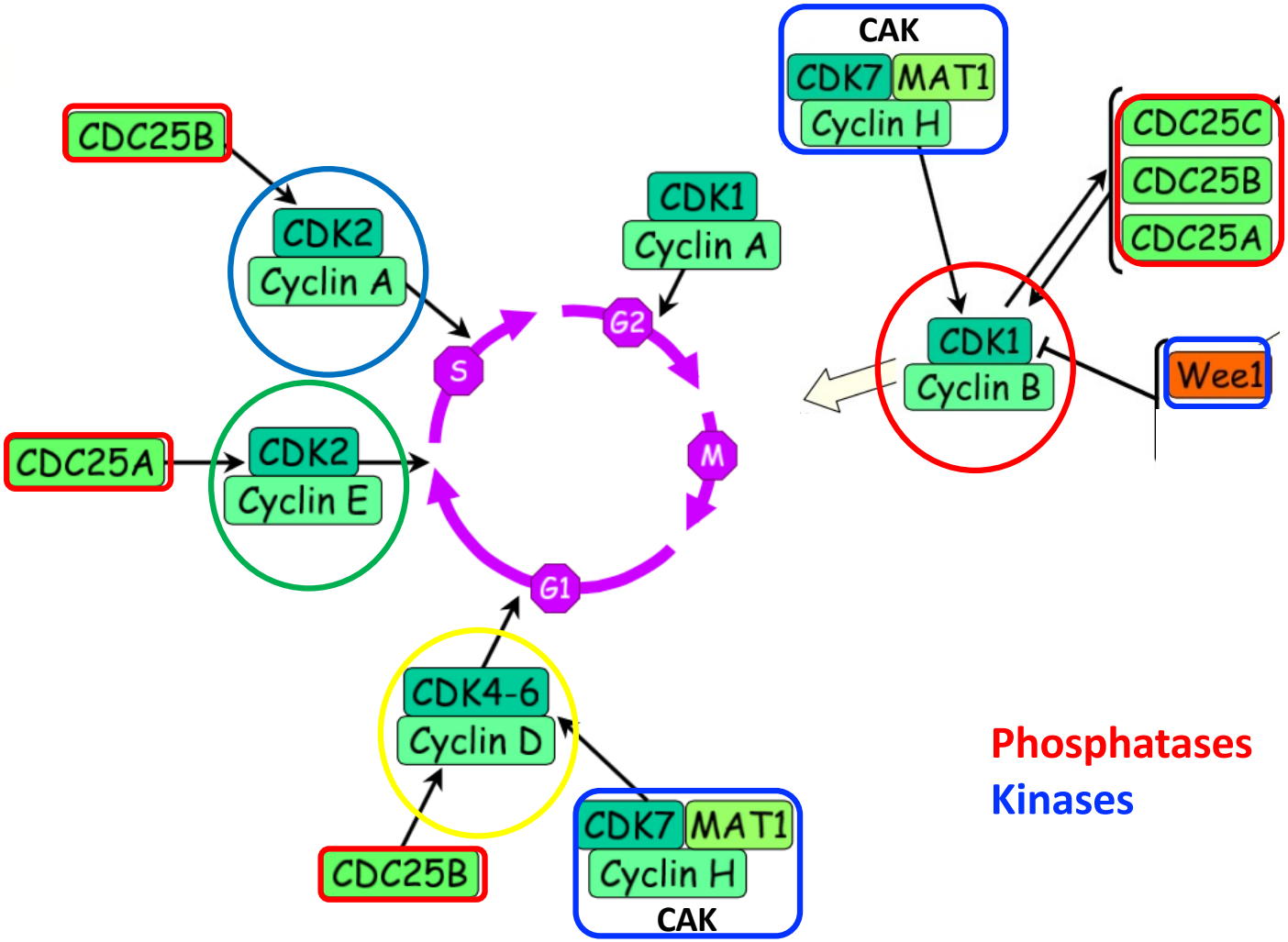
Four classes of cyclin



Padgett & Santos, FEBS letters, 2020

Nobel Prize in Physiology or Medicine 2001, Hartwell, Nurse and Hunt

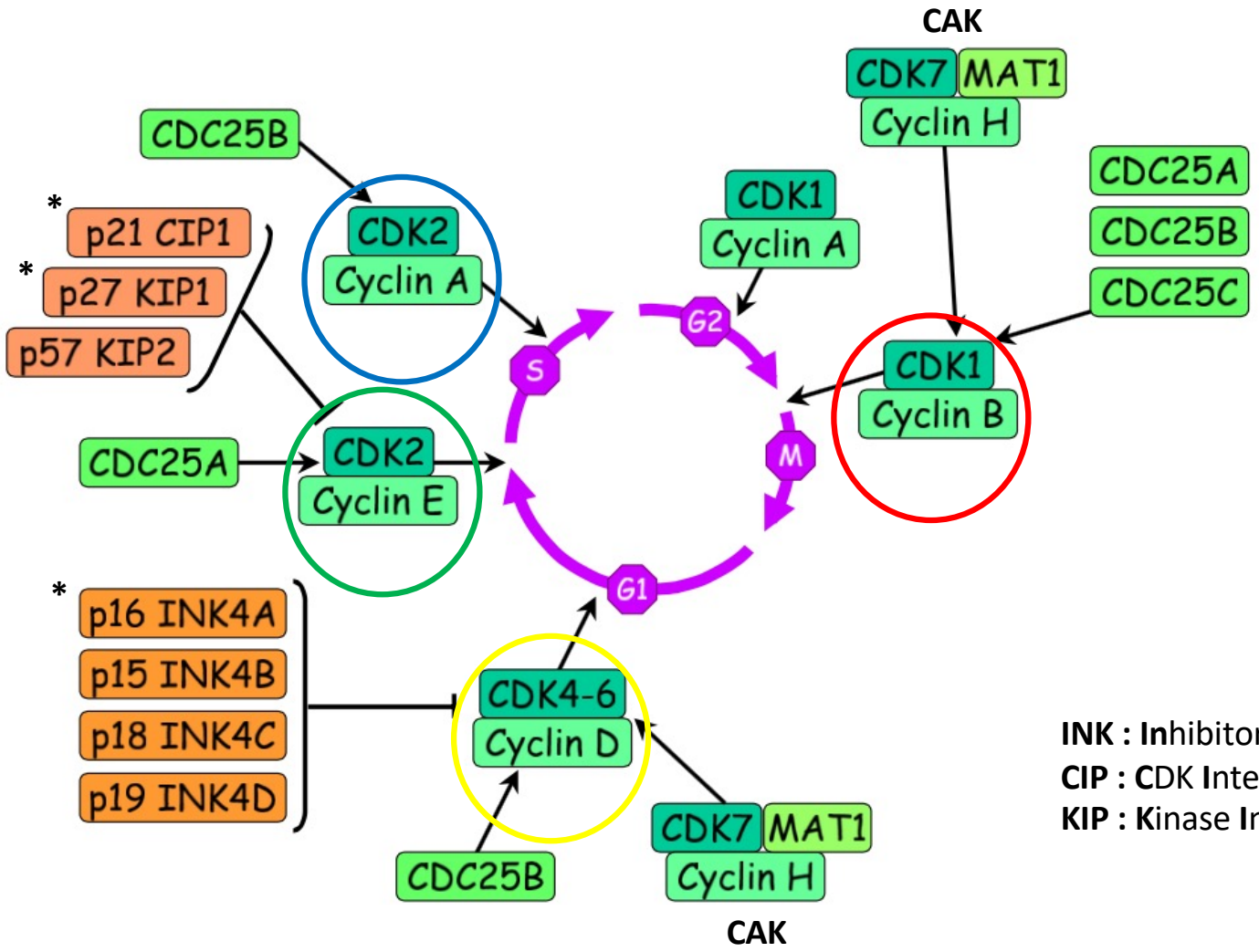
CDKs are regulated by kinases and phosphatases



Adapted from Meijer, Oncology, 2003

CDKs are inhibited by CDKI/CKI/CDIs

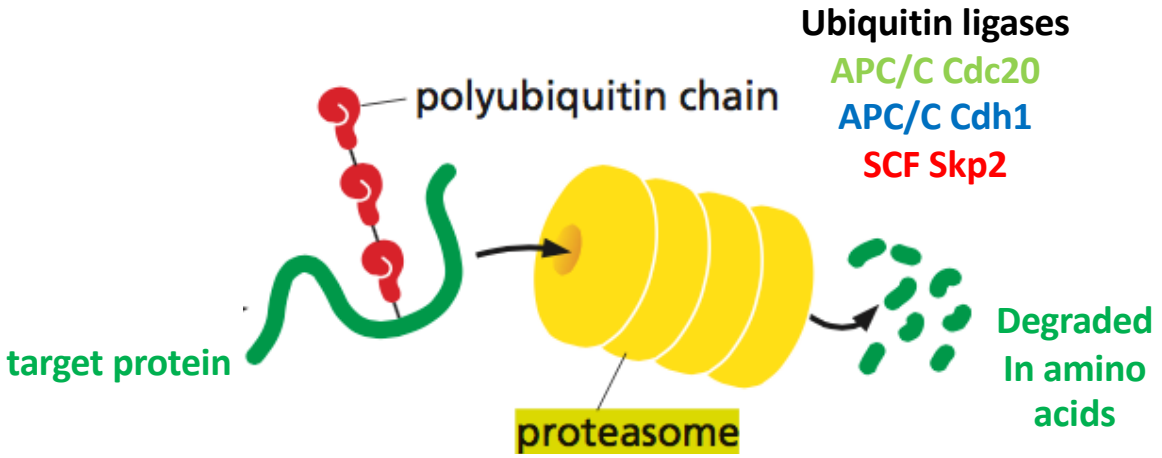
cyclin-dependent kinase inhibitors



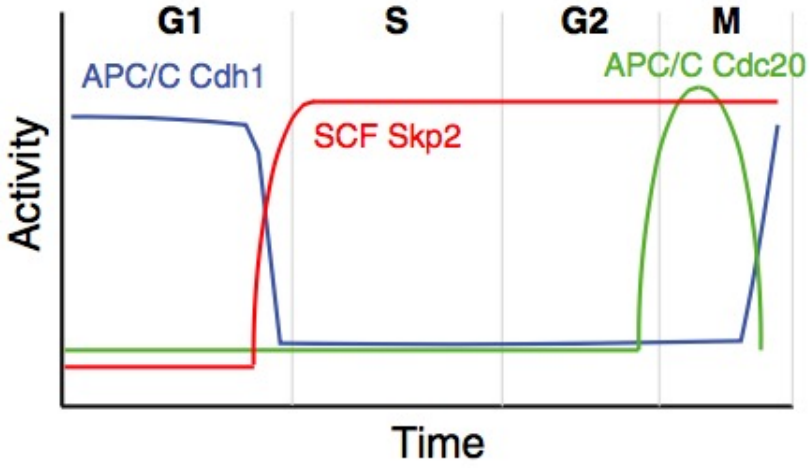
INK : Inhibitor of CD**K**
CIP : CDK **I**nteracting **P**rotein
KIP : Kinase **I**nhibitory **P**rotein

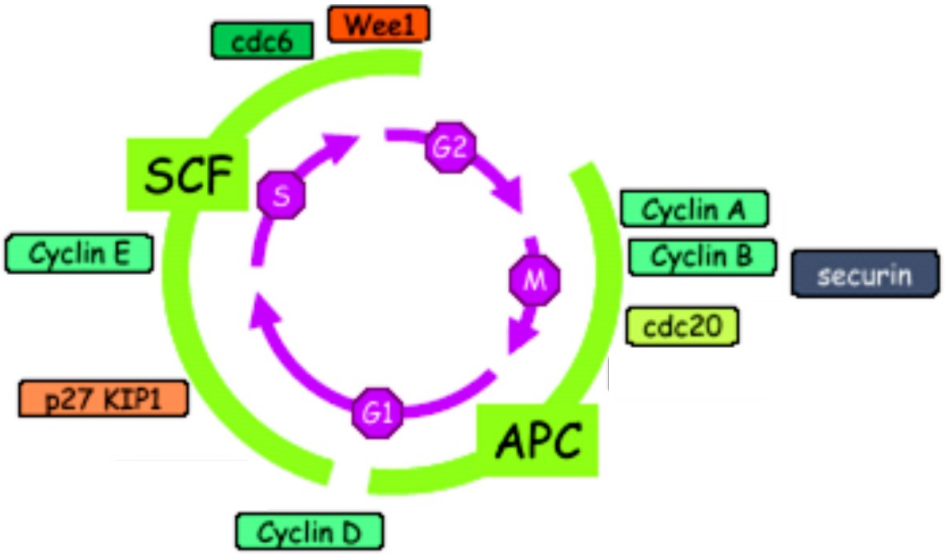
Meijer, Oncology, 2003

The cell cycle is regulated by the proteasome



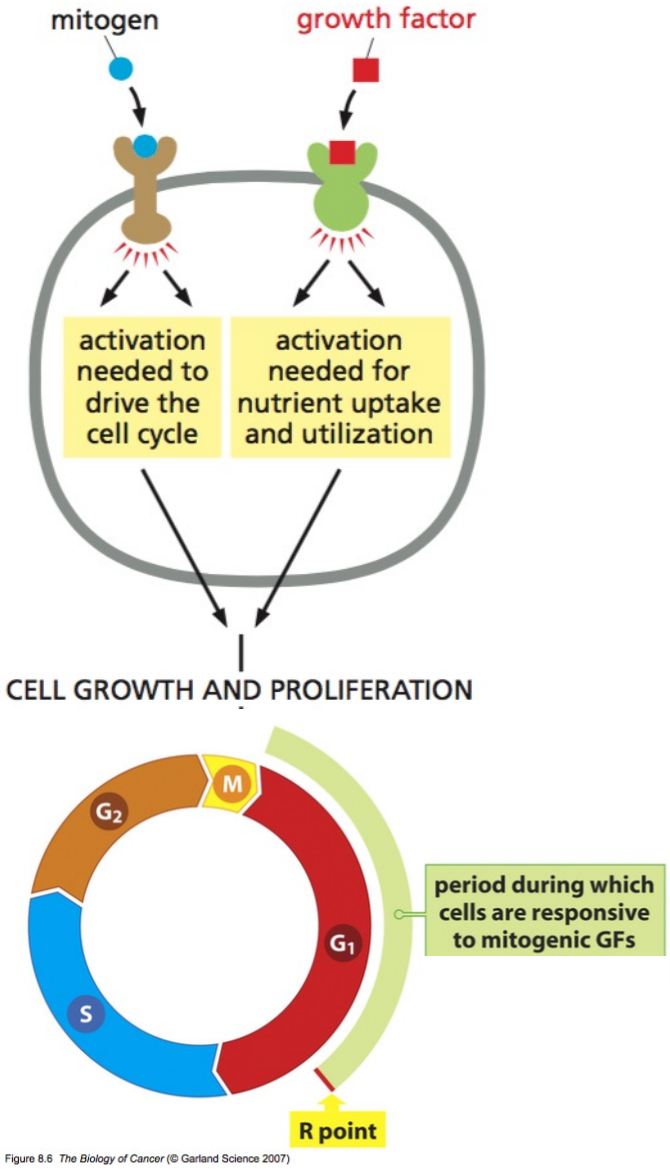
Ubiquitin-mediated degradation
Nobel Prize in Chemistry 2004,
Ciechanover, Hershko & Rose

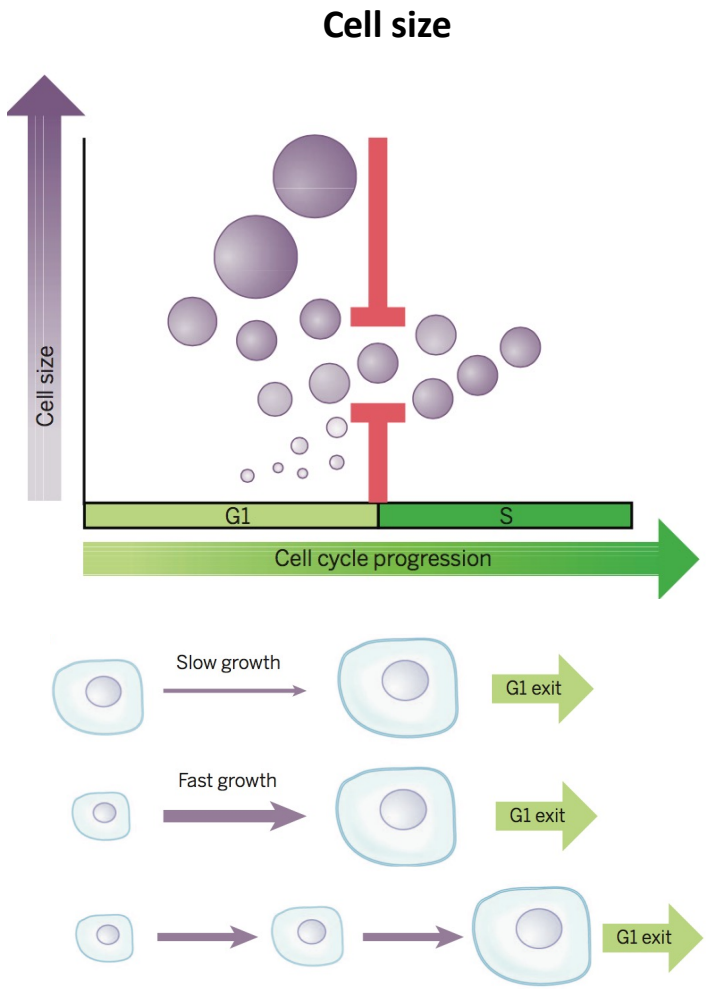




Adpated from figure 12-50, Molecular Biology of the Cell 6th
Padgett & Santos, FEBS letters, 2020 ; Meijer, Oncology, 2003

Restriction point in G1 : commitment to division





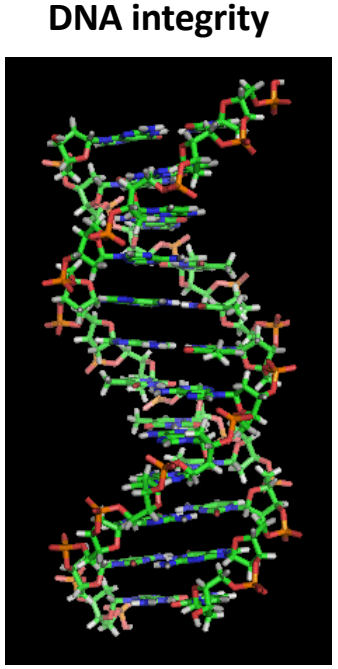
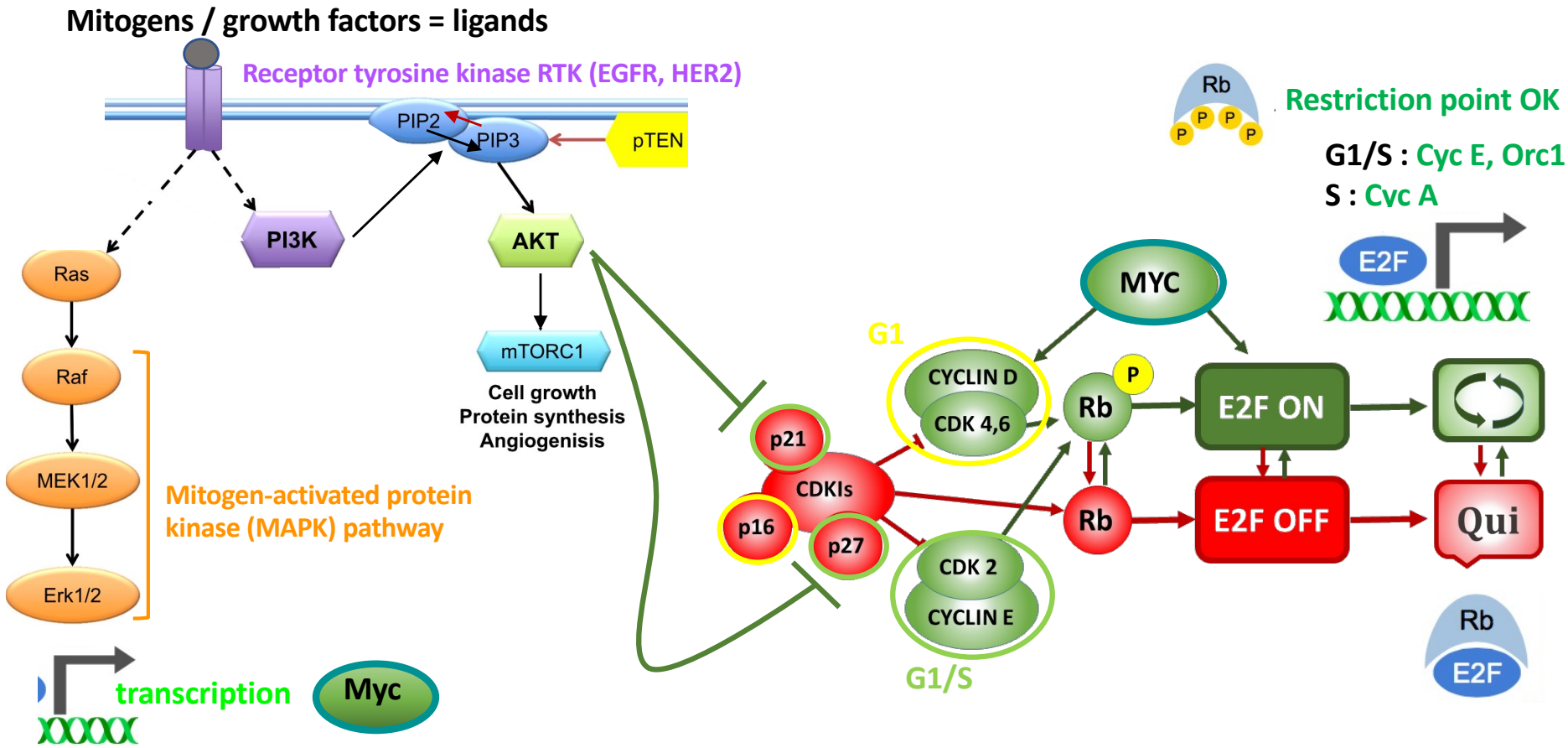


Figure 20-26, Molecular Biology of the Cell 6th
Ginzberg et al., Science, 2015

Progression through the restriction point
- Cell signaling and transcriptional regulation -



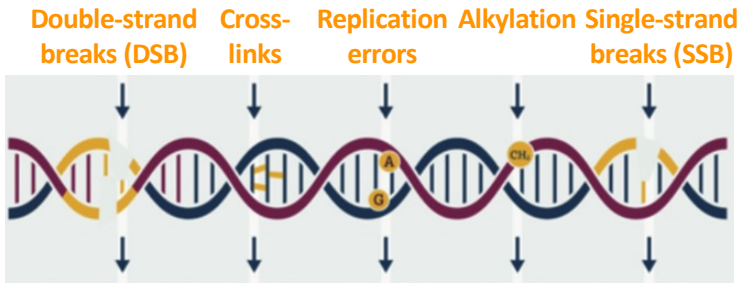
Toss & Cristofanilli, Breast Cancer Res., 2015

GF : Nobel Prize in Physiology or Medicine 1986, Cohen & Levi-Montalcini

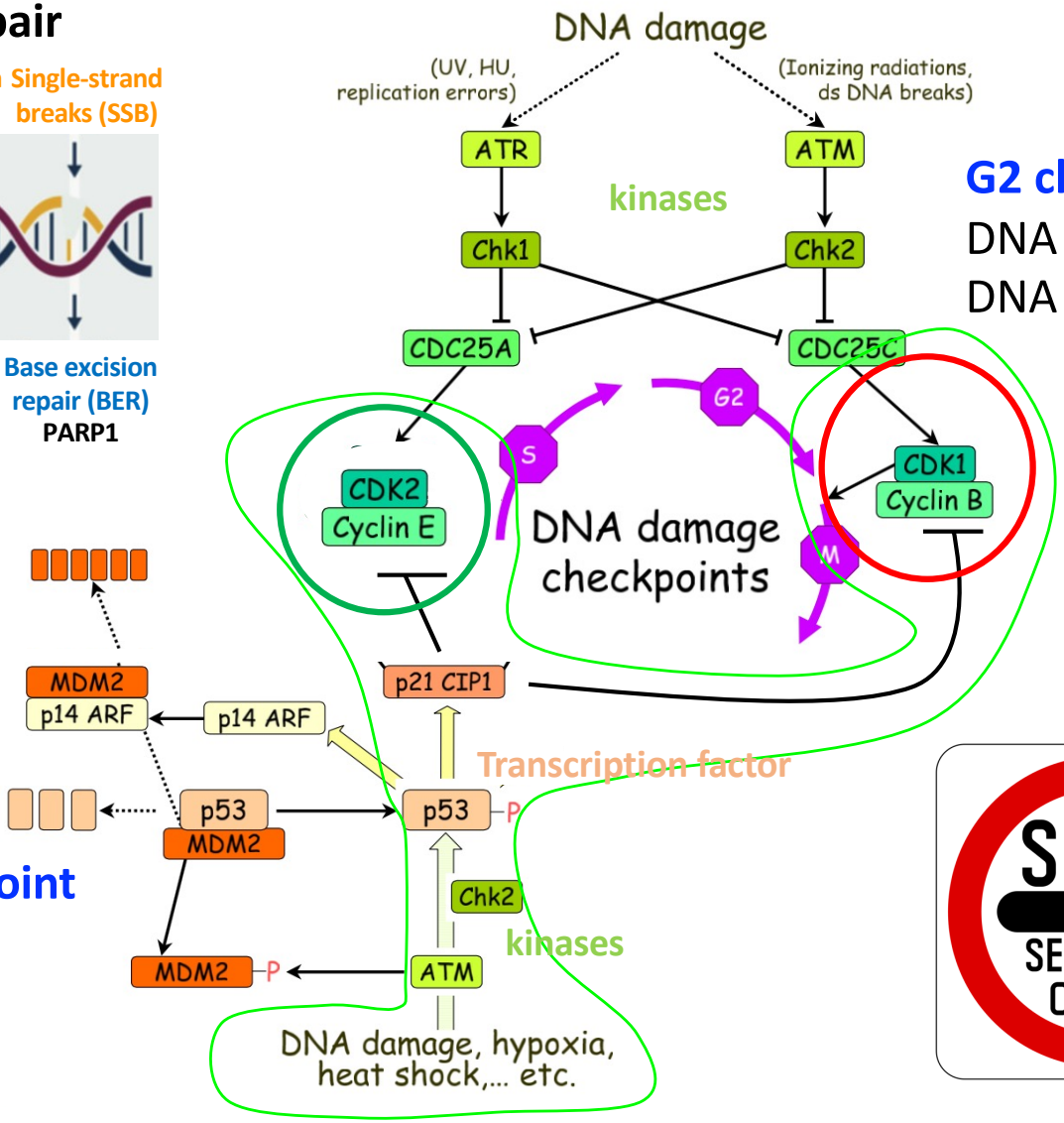
https://www.bethyl.com/content/The-Rb-E2F-Switch-Regulation-of-Cellular-Quiescence ; Lim & Kaldis, Dvpt, 2013

DNA damage prevents cell cycle : role of ATM/ATR, p53

DDR : DNA damage repair



Nobel Prize in Chemistry 2015
T. Lindahl, P. Modrich & A. Sancar

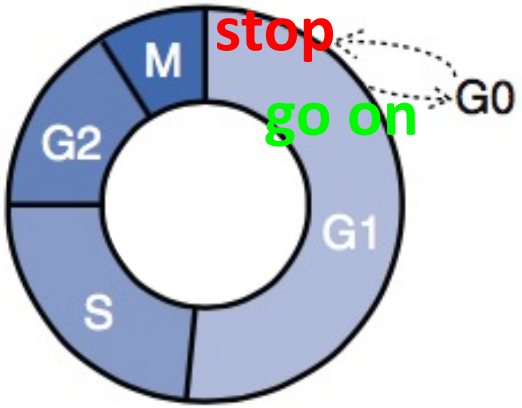


G2 checkpoint
DNA damage
DNA replicated

G1/S checkpoint
DNA damage

Adapted from Meijer, Oncology, 2003

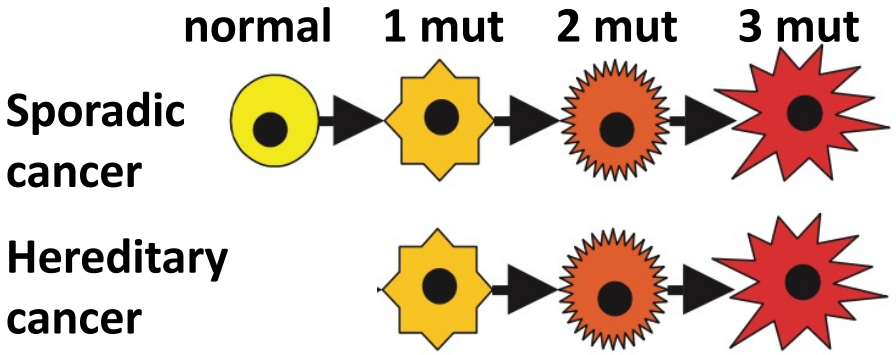
Proto-oncogenes / tumor suppressor genes



	in cancer
Tumor suppressor genes (Rb, p53, p16/INK4, PTEN, BRCA1…)	**lost**
Proto-oncogenes (EGFR, HER2, Ras, Myc, Akt, BRAF, c-Src …)	**activated**
Viral proteins (v-Src, E6, E7 …)	

c-Src : Nobel Prize in Physiology or Medicine 1989, J. Bishop & H. Varmus

Carcinogenesis
Multi-step mutations acquisition

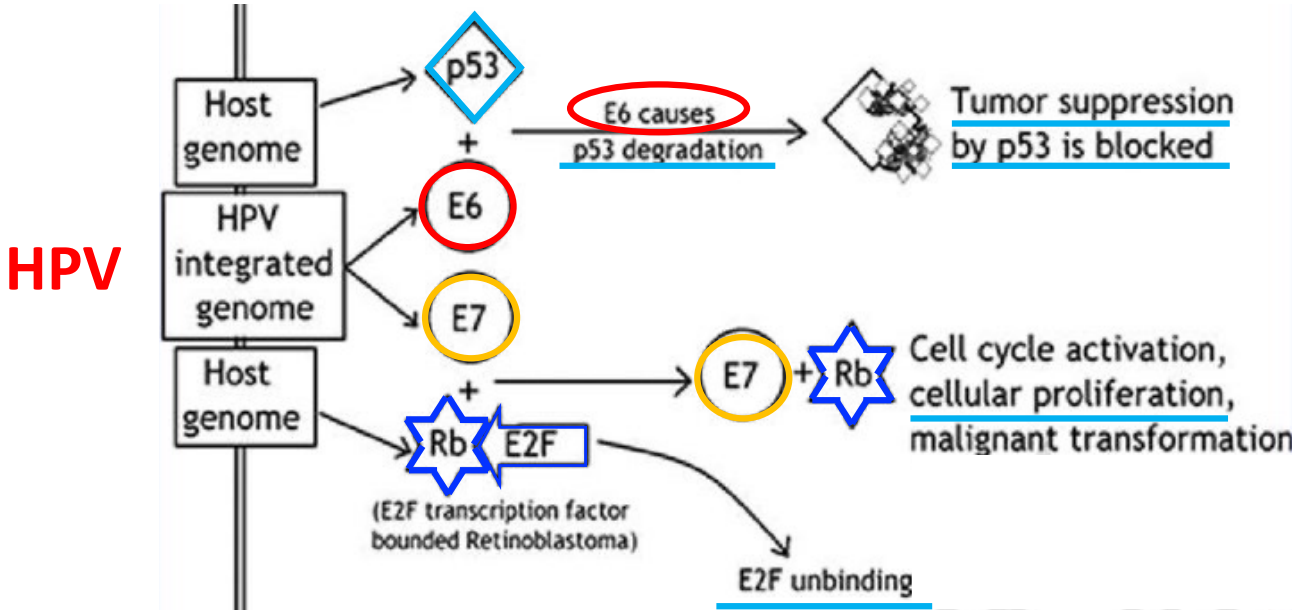


Tumor mutational burden = TMB

Adapted from Ashkenazi et al., Neoplosia, 2008

Oncoviruses

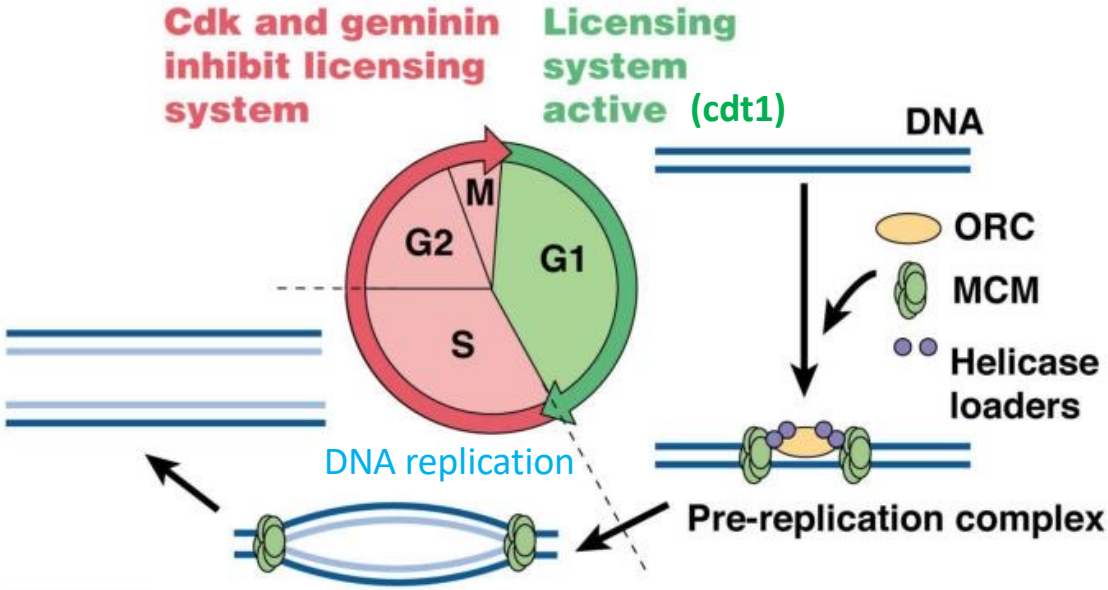
• Papillomavirus (HPV)	cervix cancer	(DNA virus)
• Hepatitis B (HBV)	liver cancer	(DNA virus)
• Hepatitis C (HCV)	liver cancer	(RNA virus)
• Epstein-Barr (EBV)	lymphoma	(DNA virus)
• HIV	kaposi sarcoma	(RNA virus)
• HTLV	leukemia/lymphoma	(RNA virus)



HPV / cancer : Nobel Prize in Physiology or Medicine 2008, Harald zur Hausen

Akram et al., Viral Immuno., 2016

One genome replication per cell cycle



2012 Pearson Education, Inc

ORC : origin recognition complex
MCM : helicase

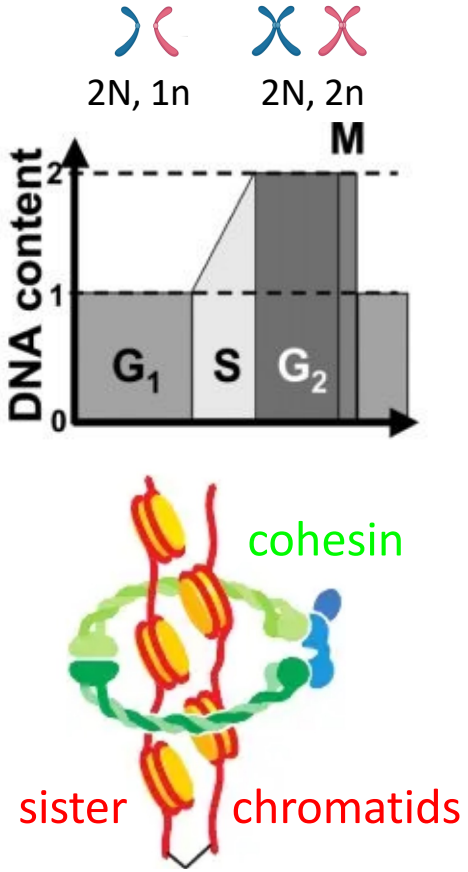
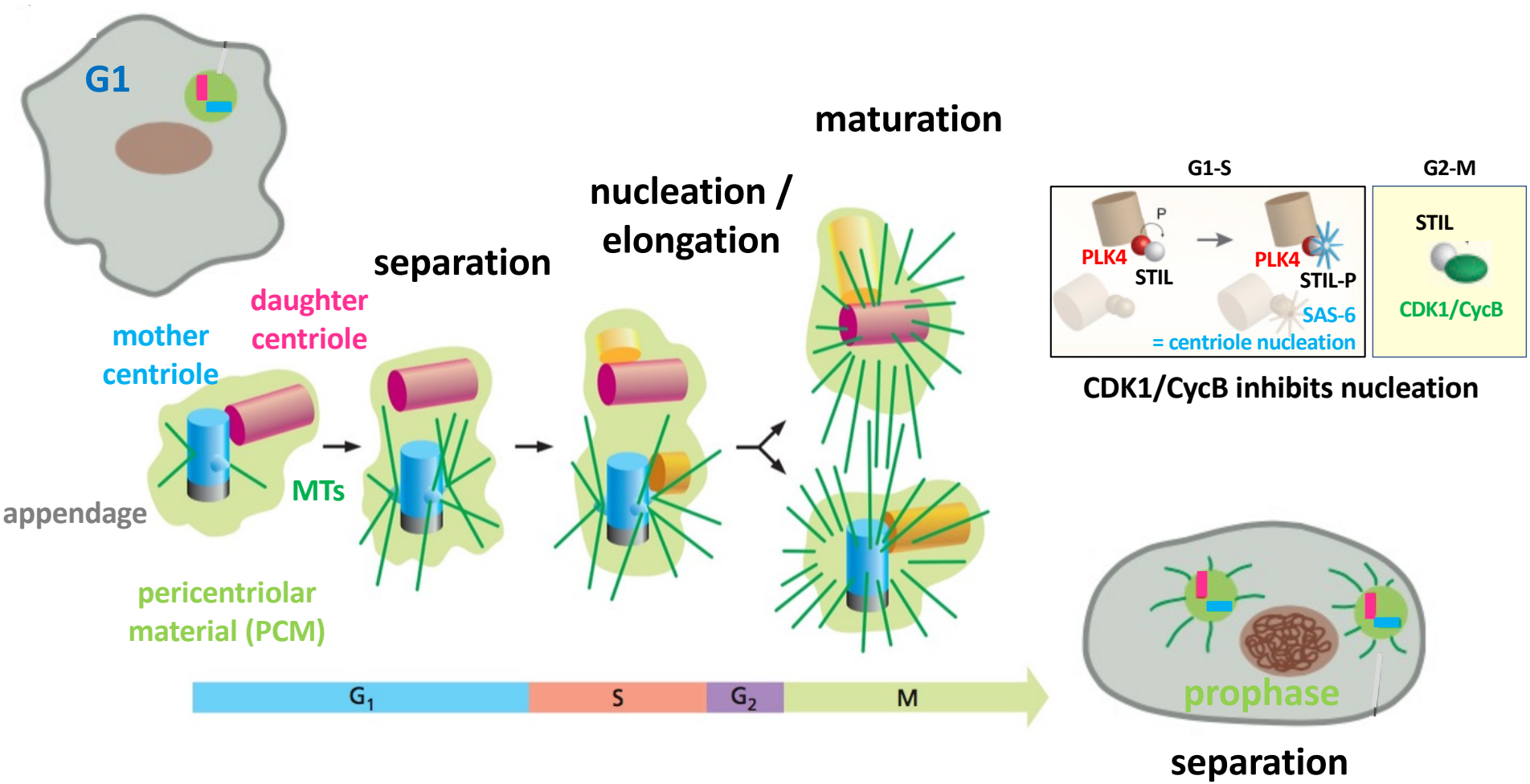


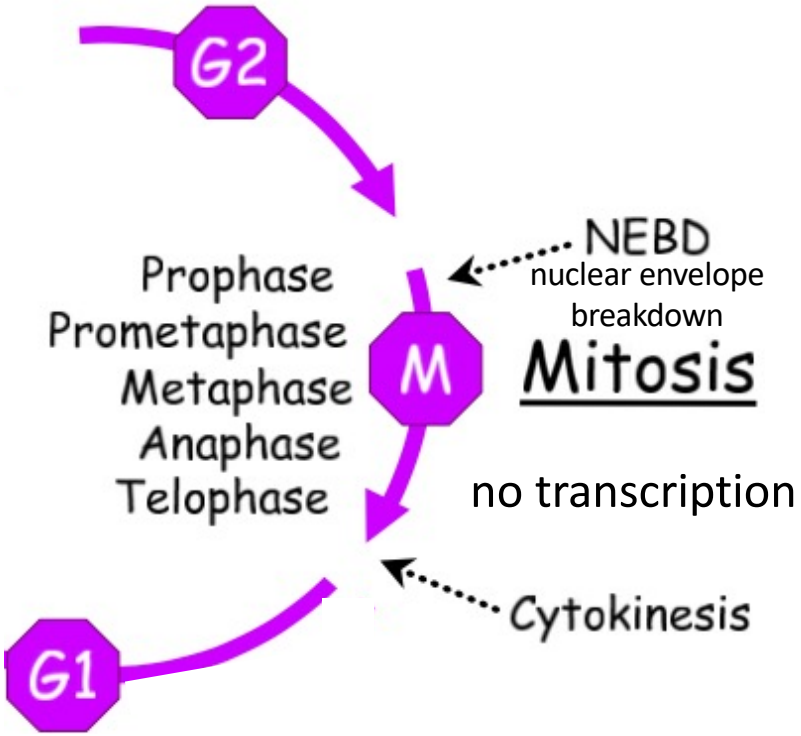
Figure 17-19, Molecular Biology of the Cell 6th

One centrosome duplication per cell cycle

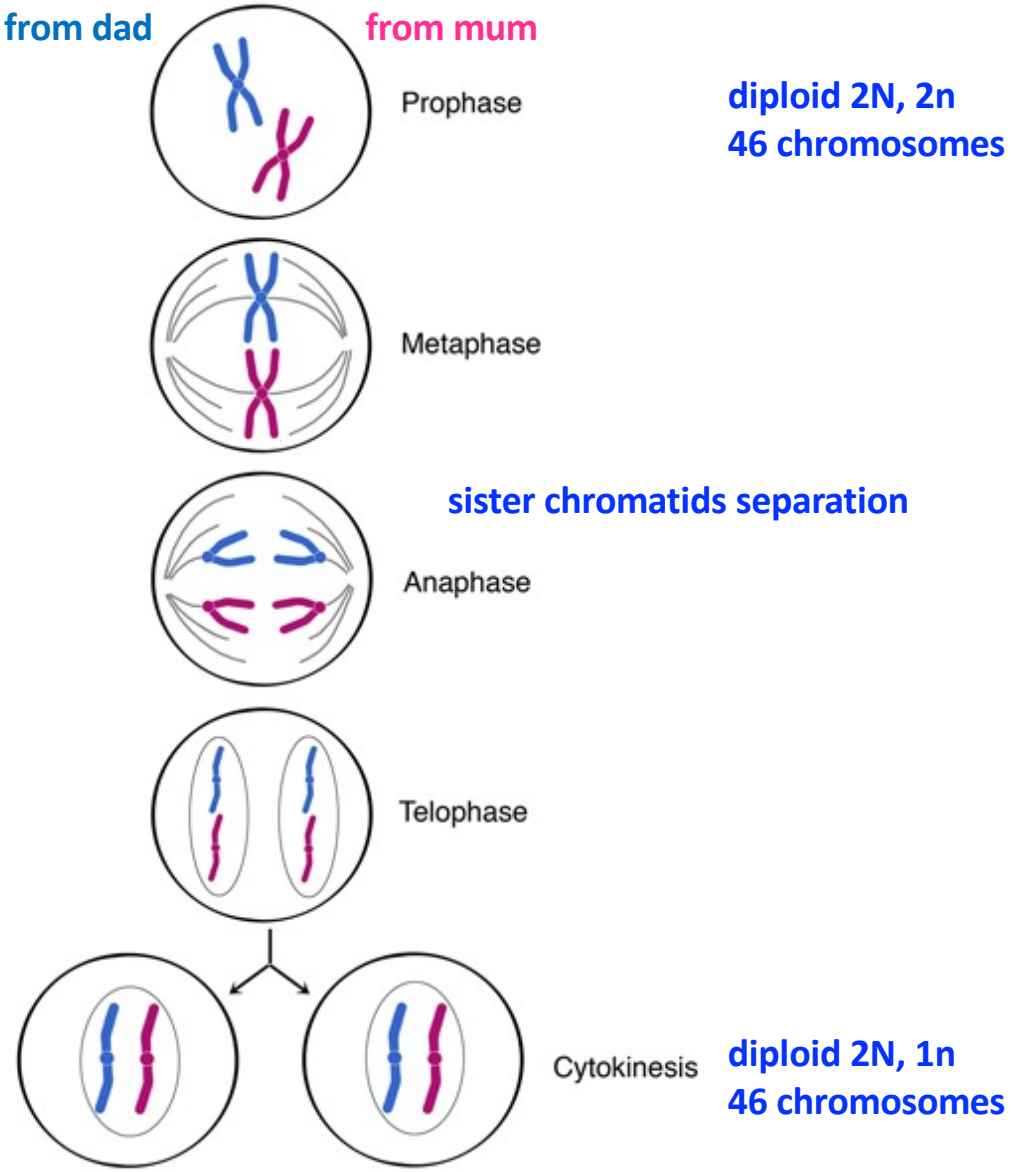


Adapted from Figure 17-26 & 16-66, Molecular Biology of the Cell 6th
Zitpuni et al, Current Biol., 2016

One mitosis per cell cycle

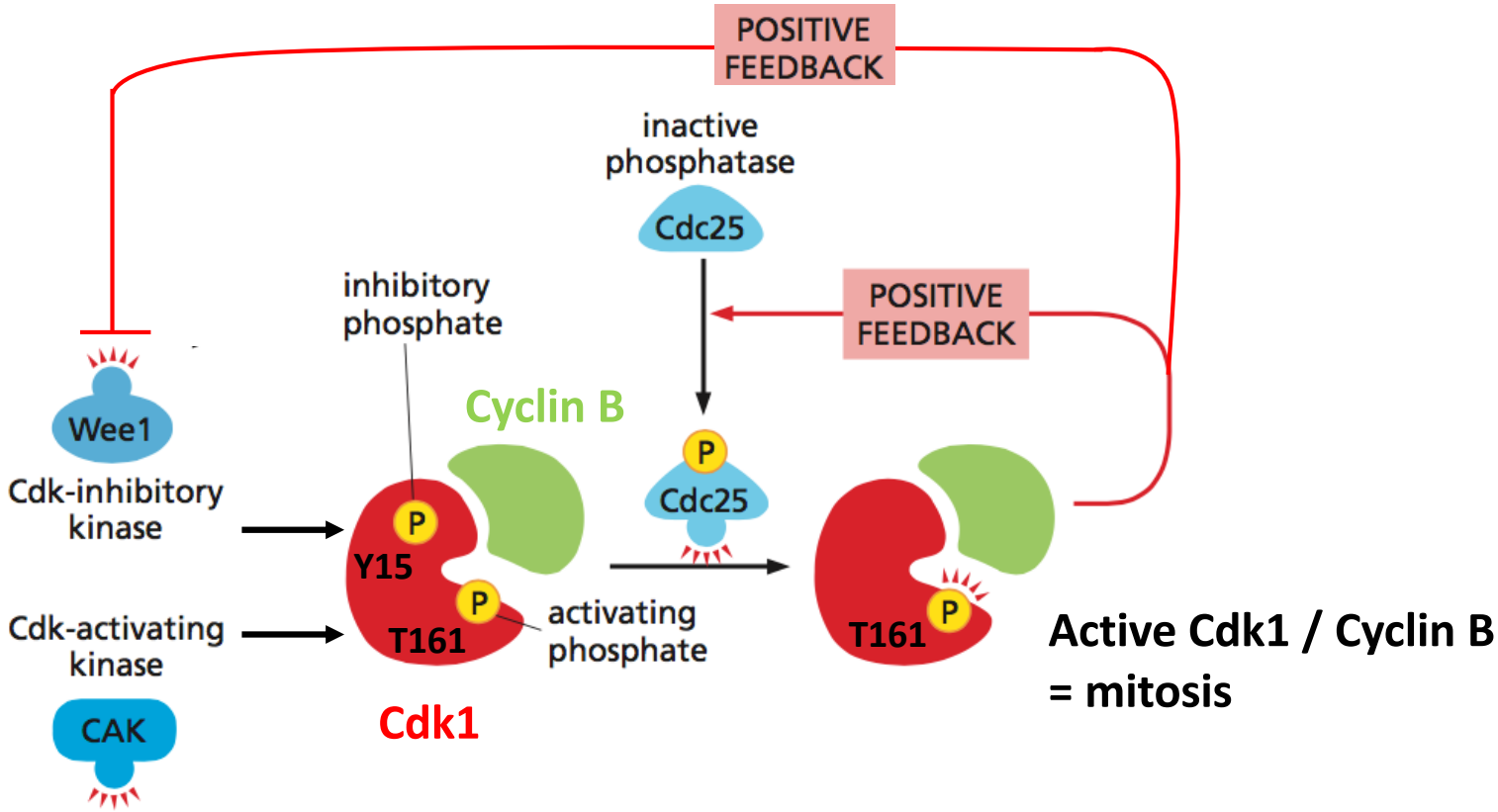


Meijer, Oncology, 2003



Adapted from http://cyberbridge.mcb.harvard.edu/

Cdk1-cyclin B activation at G2/M : kinase / phosphatase

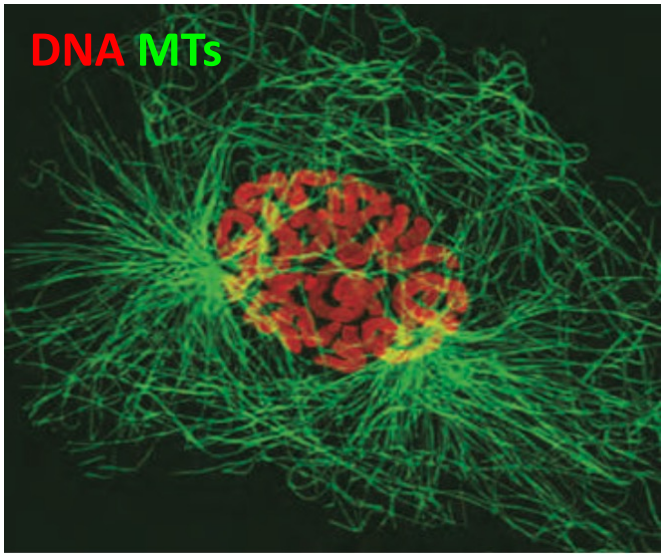


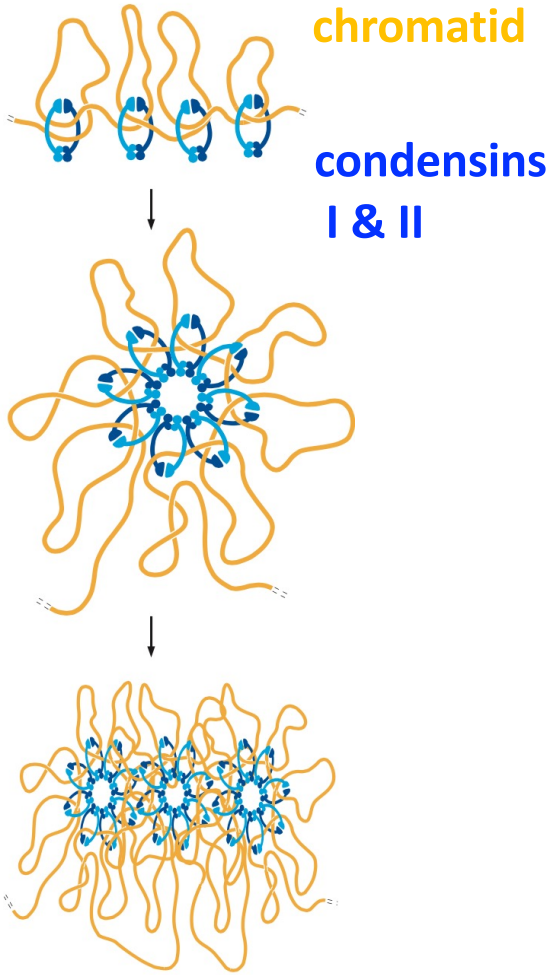
Binary (switches on/off) : complete and irreversible

Adapted from Figure 17-20, Molecular Biology of the Cell 6th

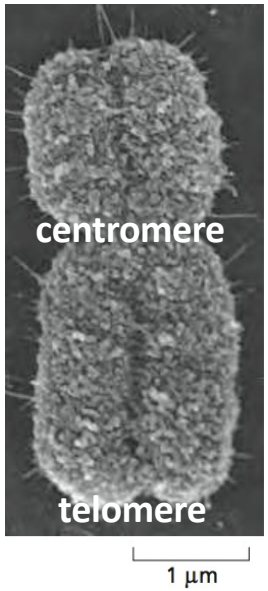
Prophase : chromosomes condensation

Immunofluorescence





scanning electron microscopy of a condensed chromosome

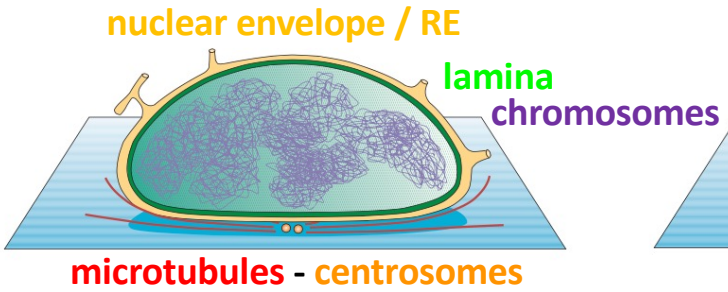


2 sister chromatids

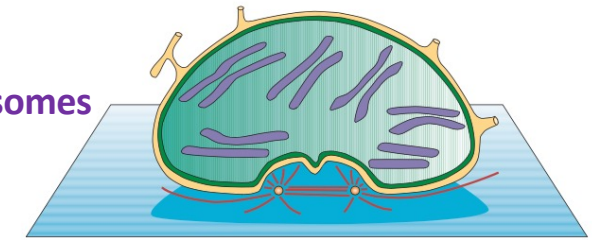
Panel 17-1 & figure 17-21, 22, Molecular Biology of the Cell 6th

Prometaphase : nuclear envelope breakdown (NEBD)

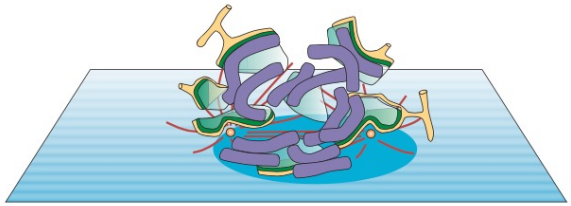
Interphase



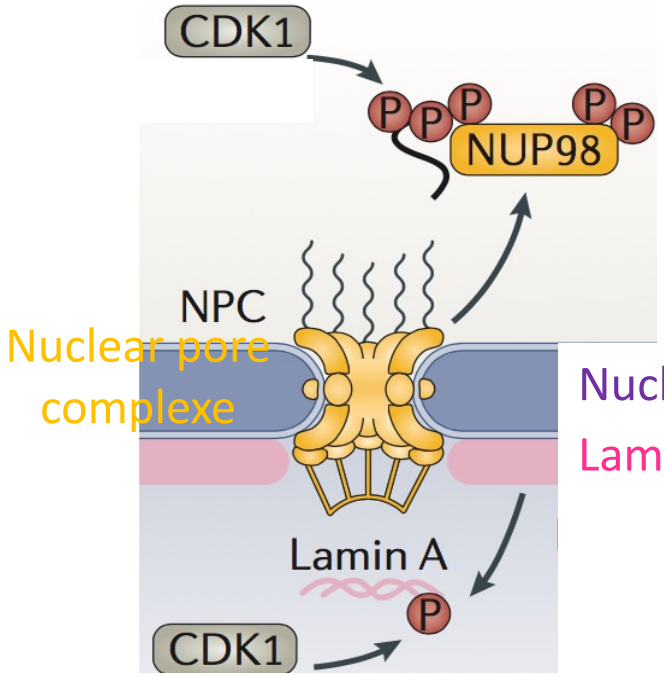
Prophase



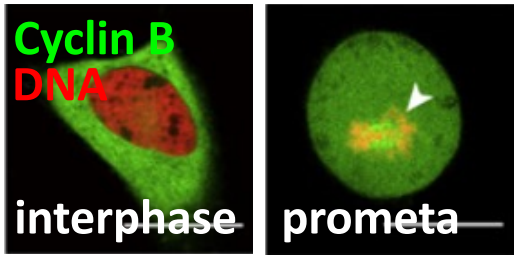
Prometaphase



Burke & Ellenberg, Mol Cell Biol, 2002



Immuno-fluorescence



Adapted from Ungricht & Kutay, Mol Cell Biol, 2017
Santos et al., Cell, 2012

Metaphase : mitotic spindle

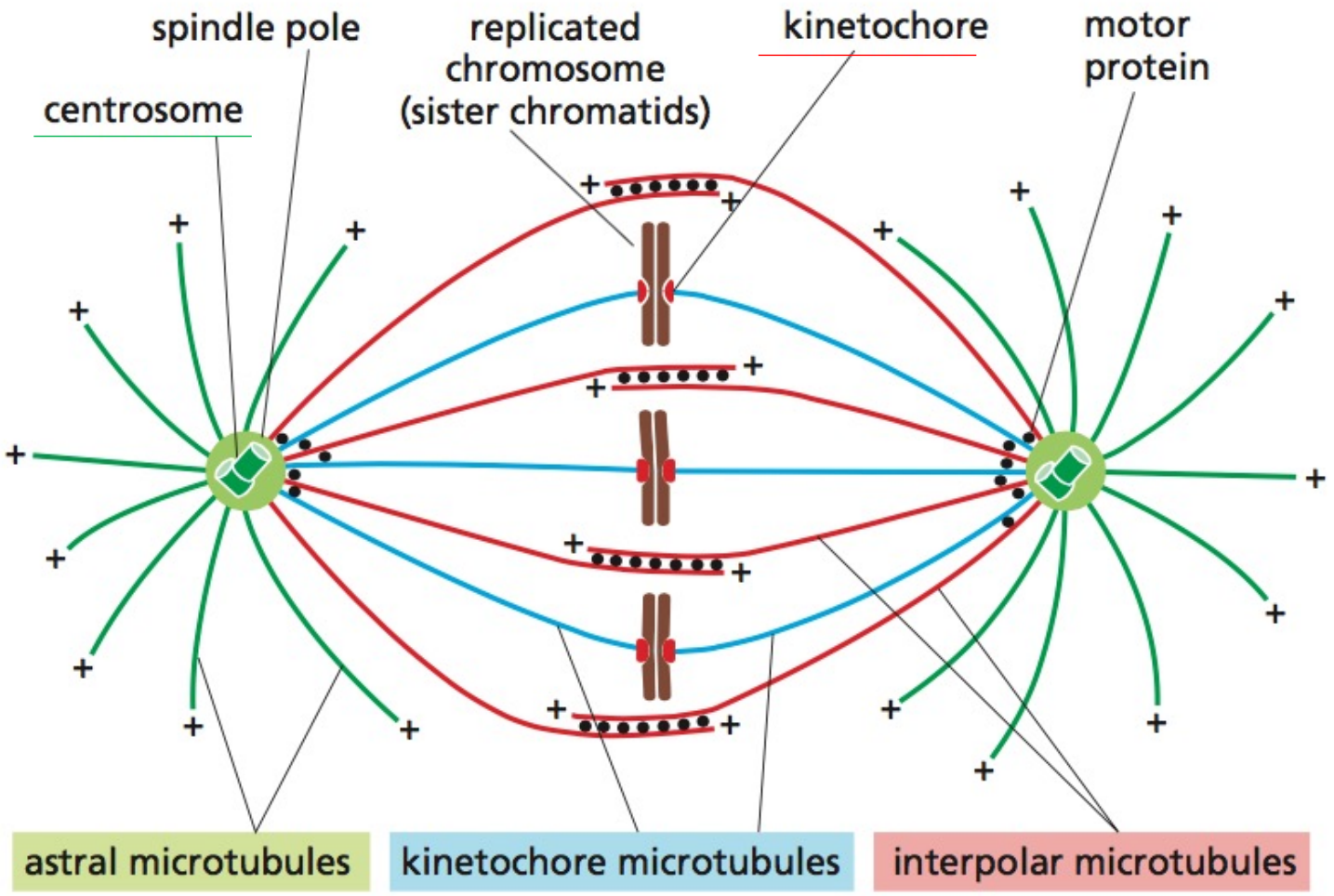
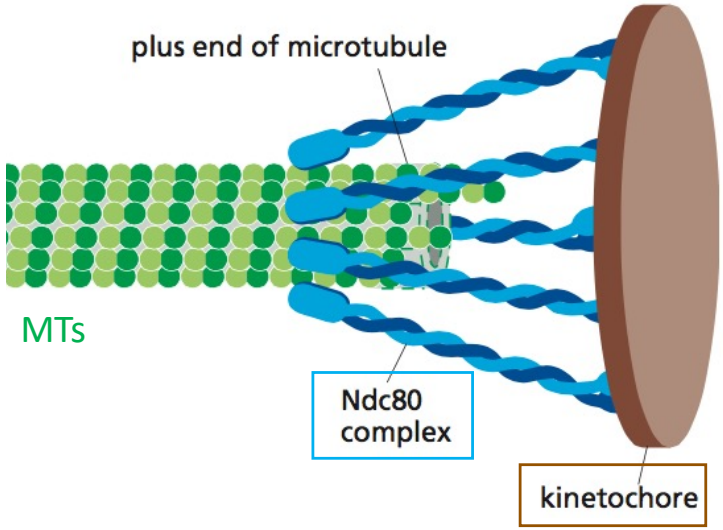


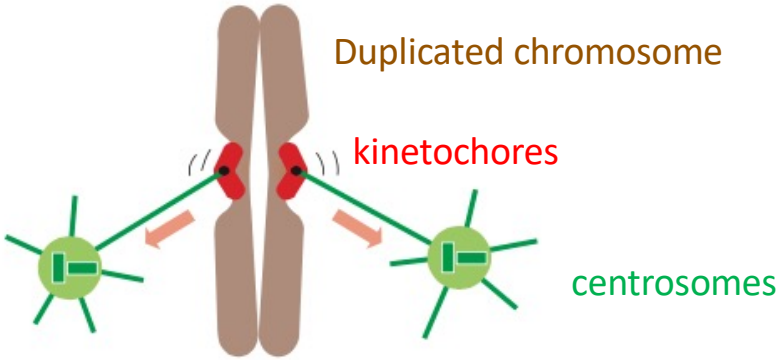
Figure 17-23, Molecular Biology of the Cell 6th

Kinetochores attach sister chromatids to the spindle

end-on attachment

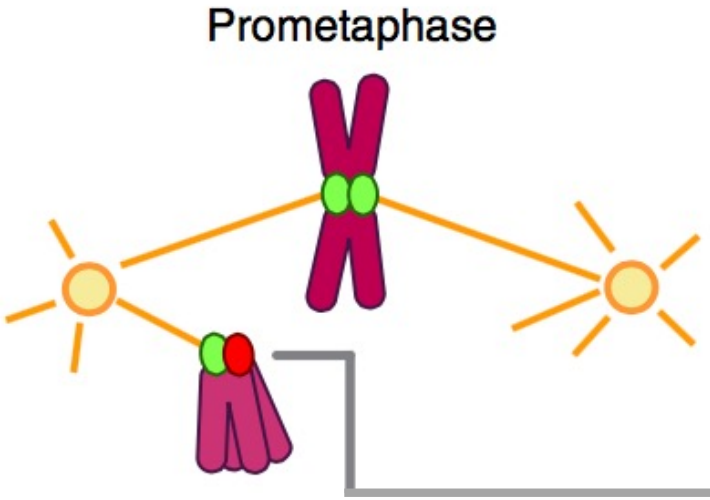


hight tension = STABLE attachment

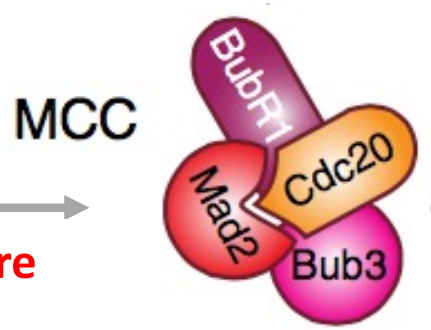


Adapted from figure 17-31, 33, 34, Molecular Biology of the Cell 6th

Spindle assembly checkpoint (SAC)
Chromosome / spindle attachment : a big deal



Mitotic checkpoint complex (MCC):
Inhibits Cdc20 (APC/C ubiquitin ligase)
(APC/C Anaphase Promoting Complex/Cyclosome)

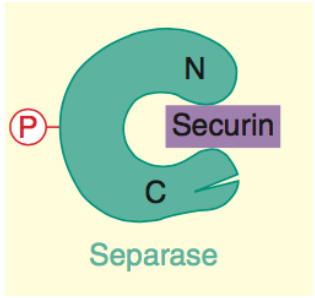


Unattached kinetochore

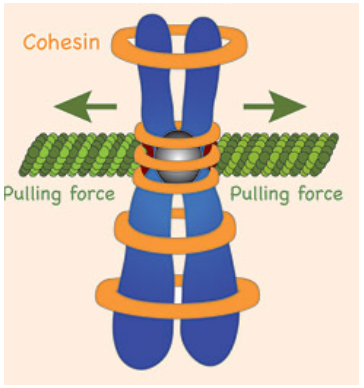
no anaphase until all kinetochores are attached

Adapted from Lara-Gonzalez et al., Current Biol., 2012

Anaphase : pull the chromatids to opposite ends



Uhlmann, Curr. Biol., 2003



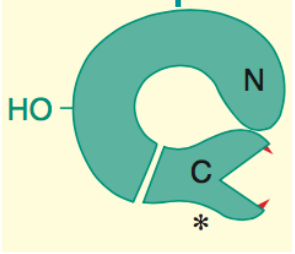
Visintin

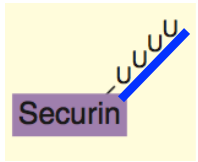
APC/C-Cdc20

Cyclin B-uuuu / cdk1

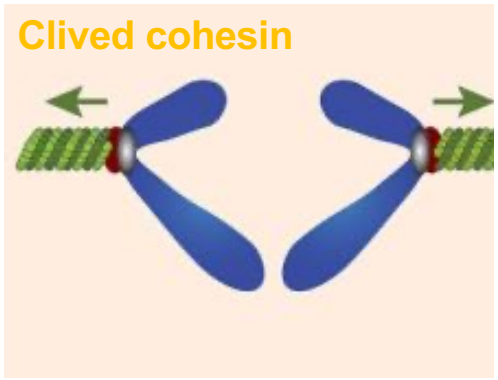
degradation by proteasome

Active separase





Clived cohesin



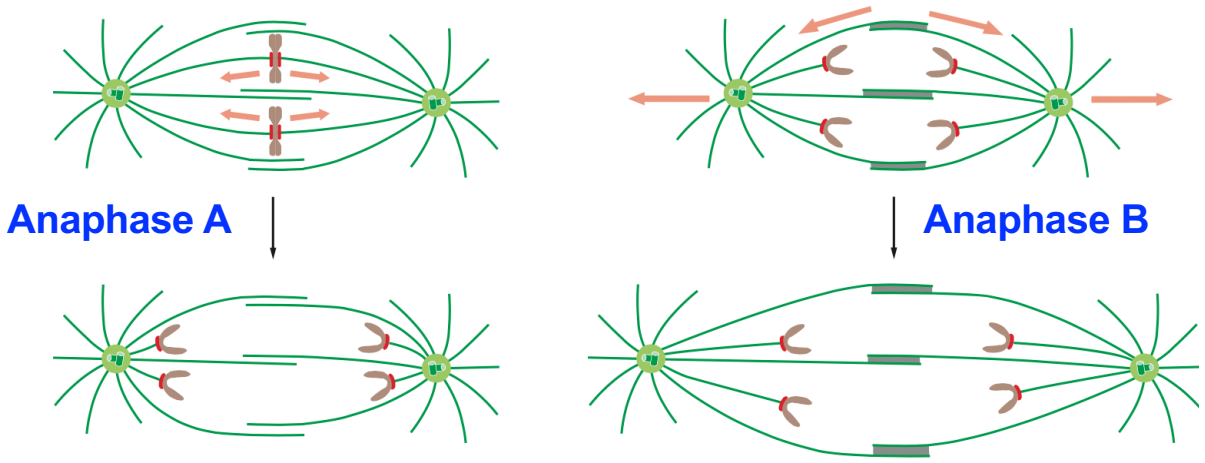
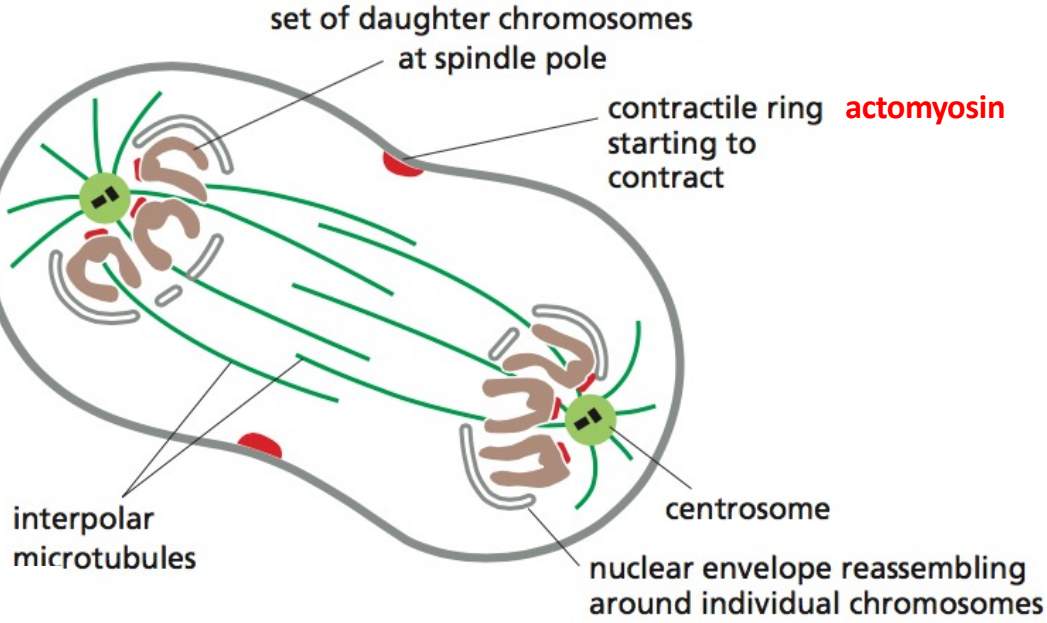
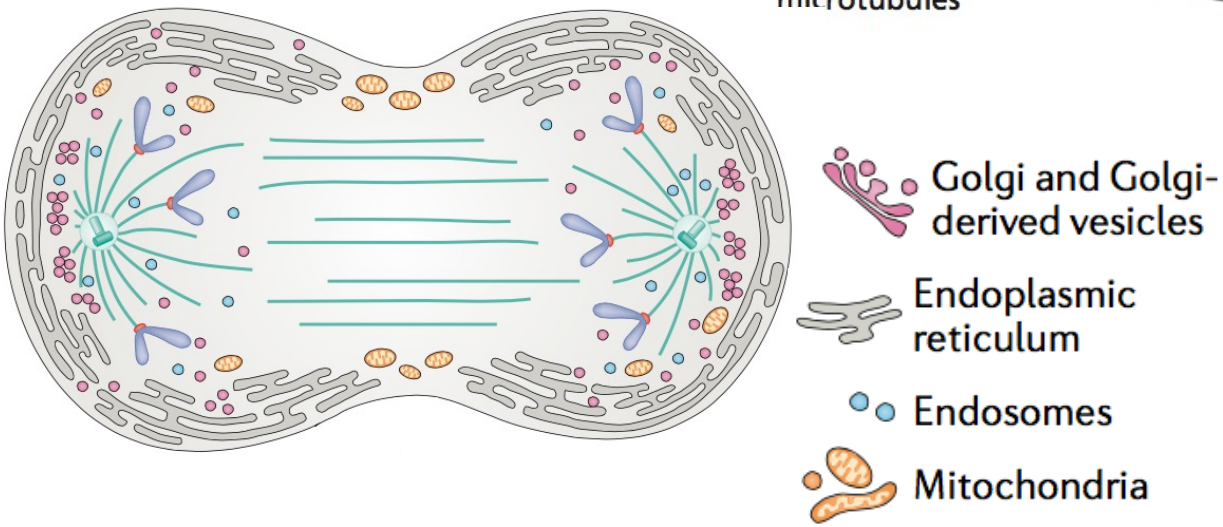


Figure 17-40, Molecular Biology of the Cell 6th; Uhlmann, Curr. Biol., 2003

Telophase : returning to an interphase state

- spindle disassembly
- formation of nuclear envelope
- import of nuclear proteins
- chromosomes decondensation
- transcription



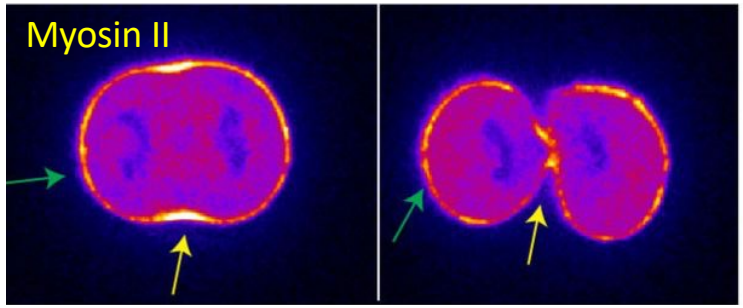


Organelles symmetrically inherited

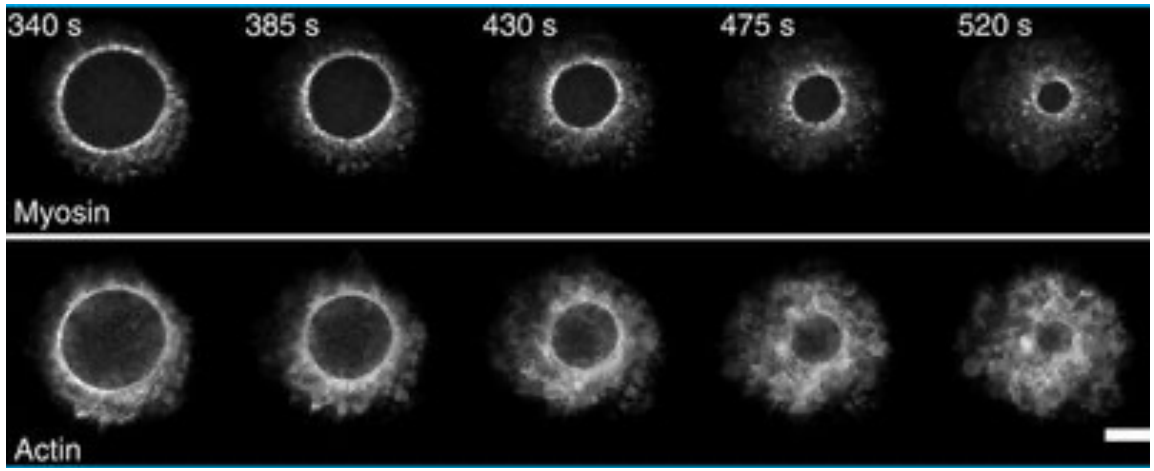
Carlton et al., Mol Cell Biol, 2020
Panel 17-1, Molecular Biology of the Cell 6th

Cytokinesis : splitting cytoplasm into 2 cells

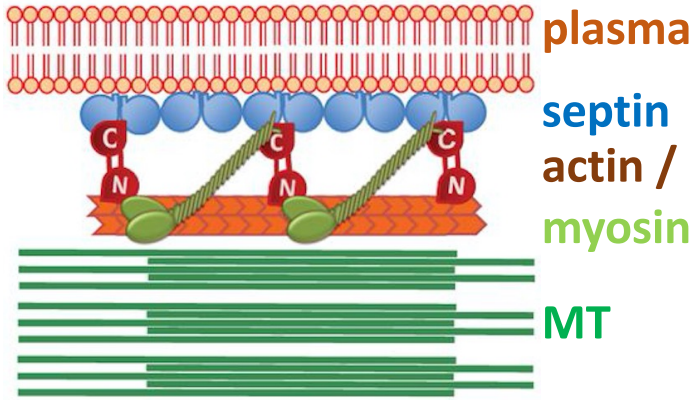
Immuno-fluorescence



Taneja et al., BioRxiv., 2019



Wollrab et al., Nat. Comm., 2015



Menon & Gaestel, J Cell Sci., 2015

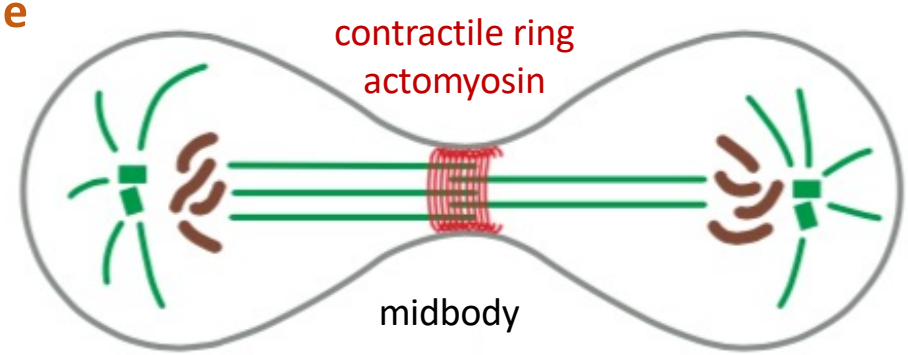
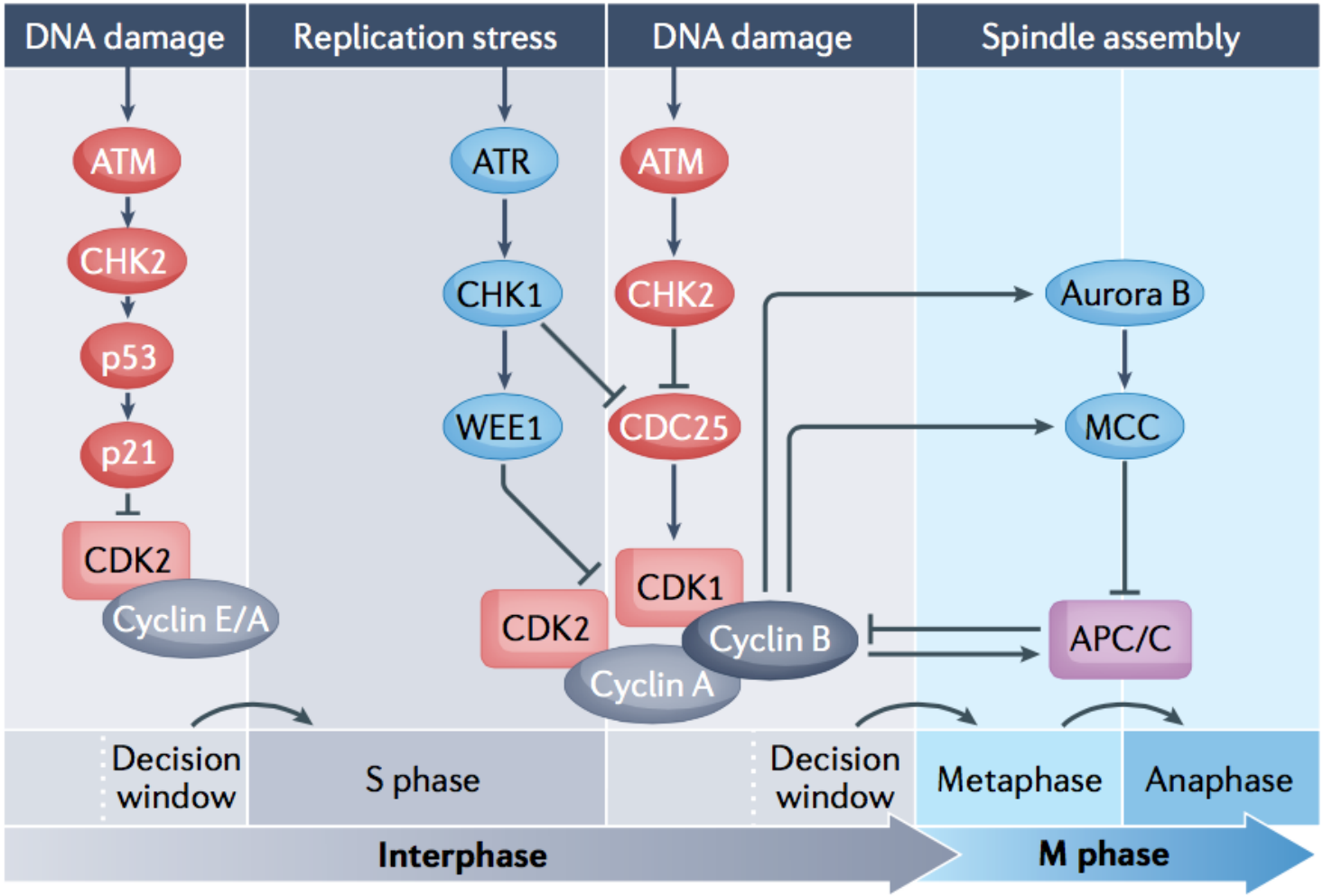


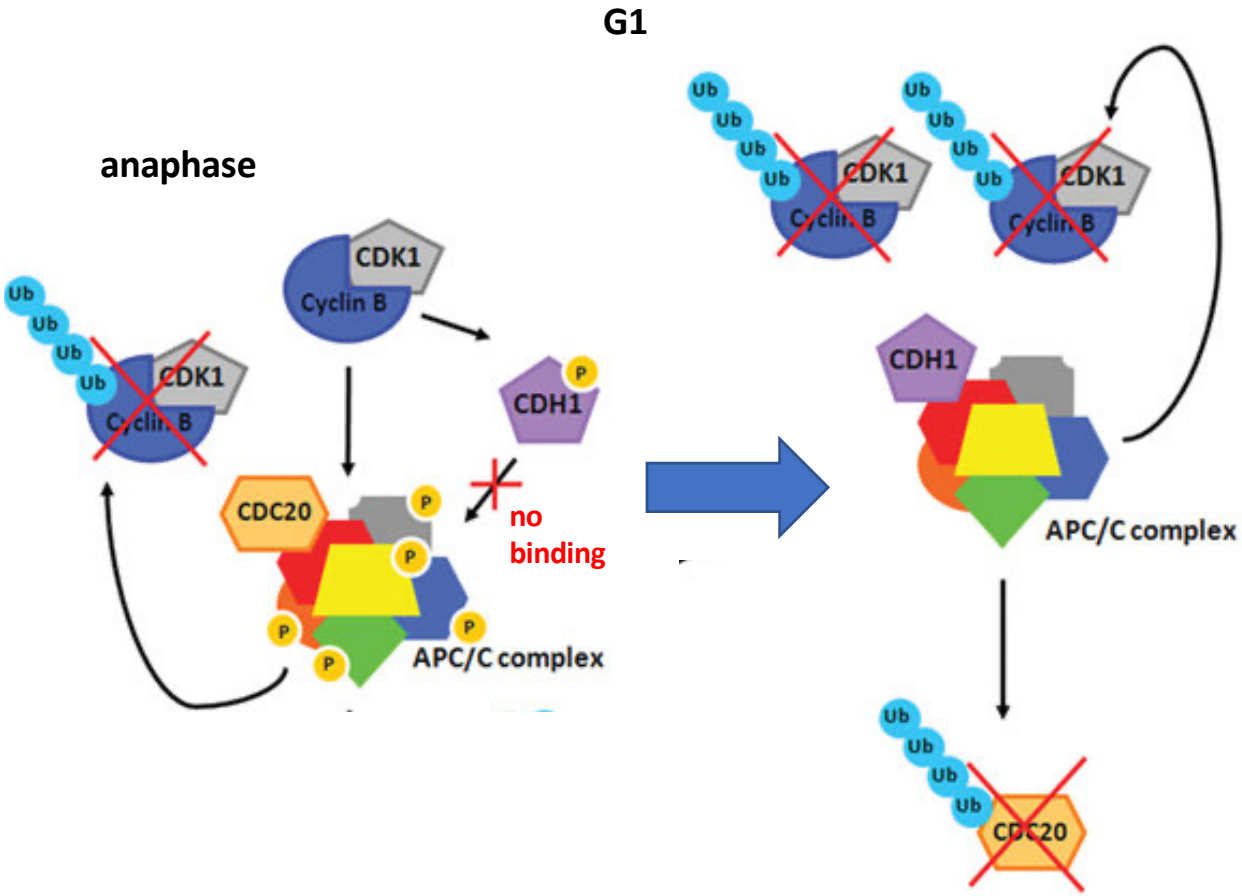
Figure 16-2, Molecular Biology of the Cell 6th

The cell cycle checkpoints : prevent genetic errors

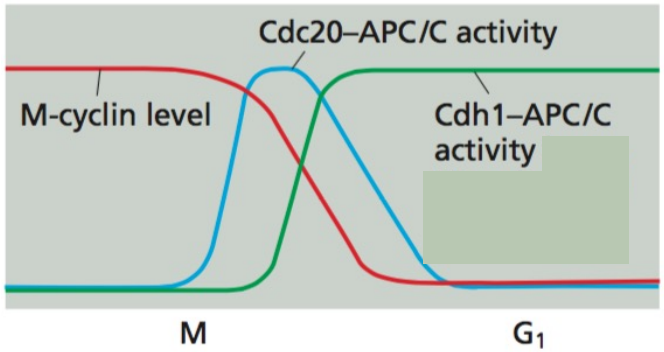


Matthews et al., Nature Reviews, Mol Cell Biol, 2022

Early G1 : preventing precocious re-entry into mitosis

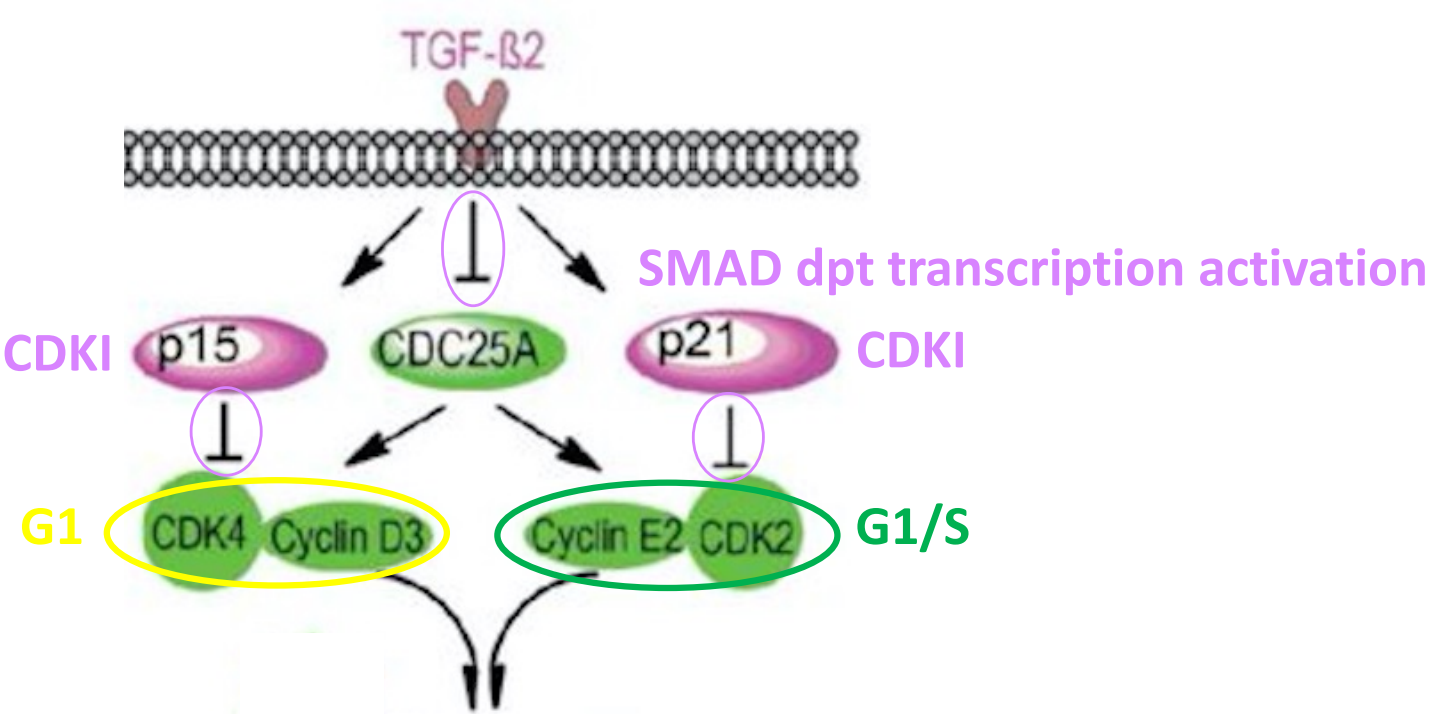


Switch from
Cdc20-APC/C to Cdh1–APC/C
ubiquitin ligase activity
= total Cyclin B degradation



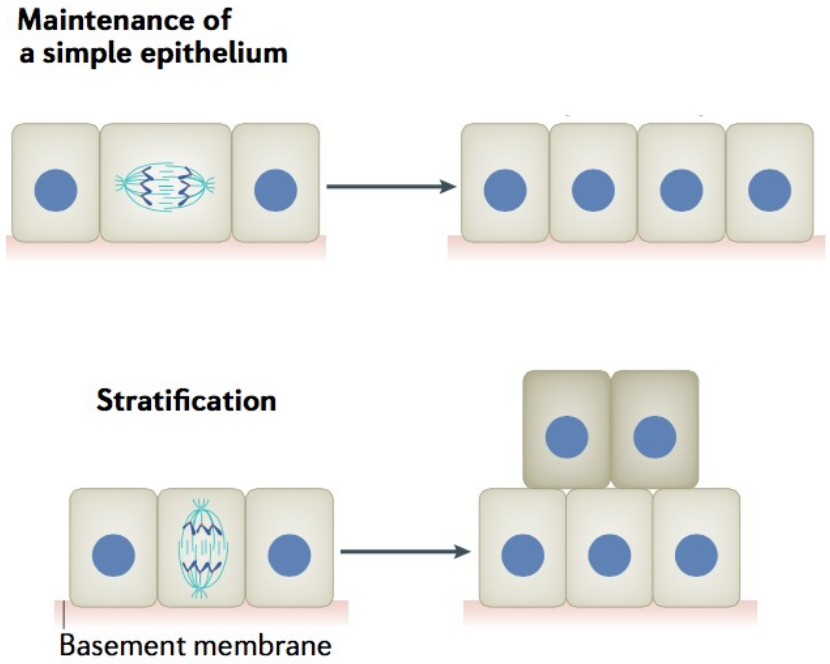
Suryadinata et al., Biosci. Rep., 2010
Figure 17-52, Molecular Biology of the Cell 6th

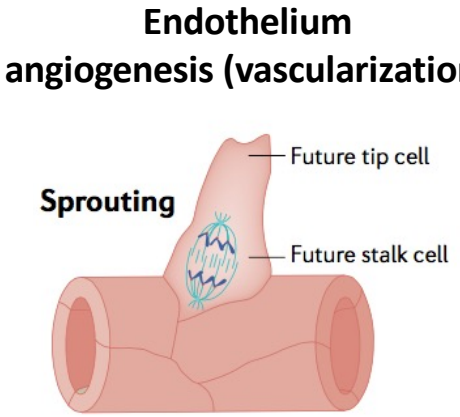
TGF-β2 in G1 : preventing a new precocious cycle



Wu et al., Cell Cycle, 2016

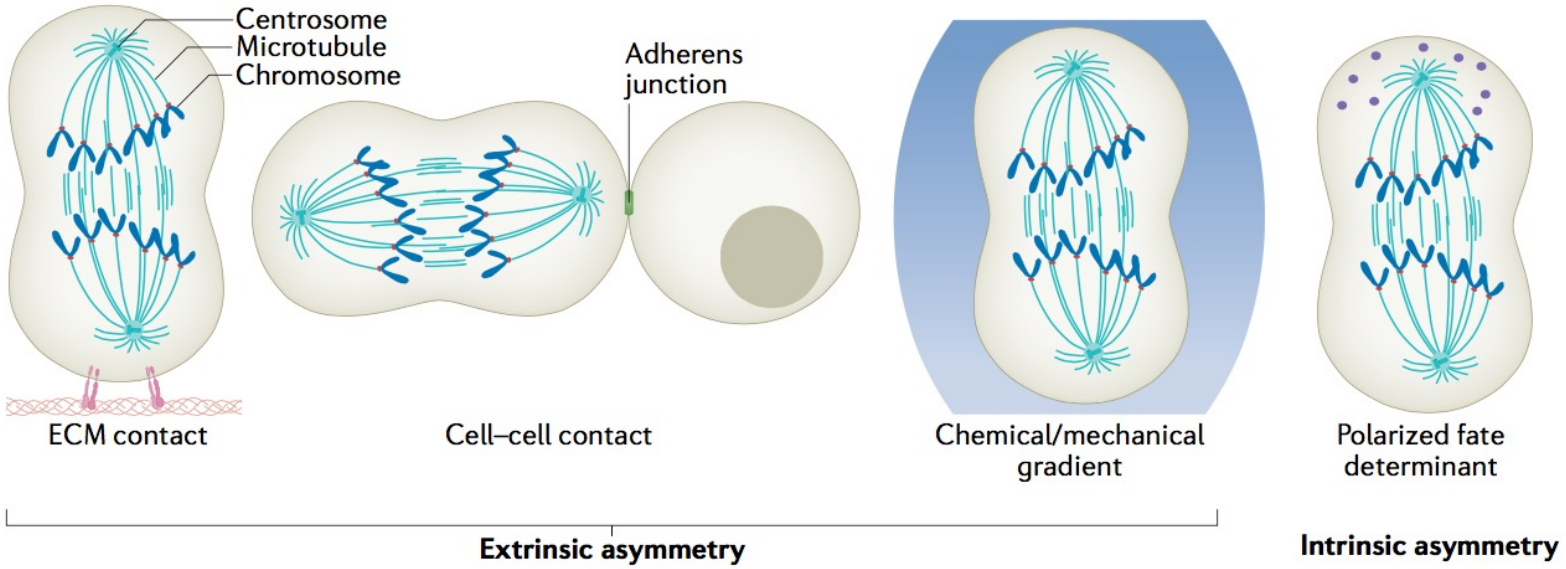
Spindle orientation and tissue organization

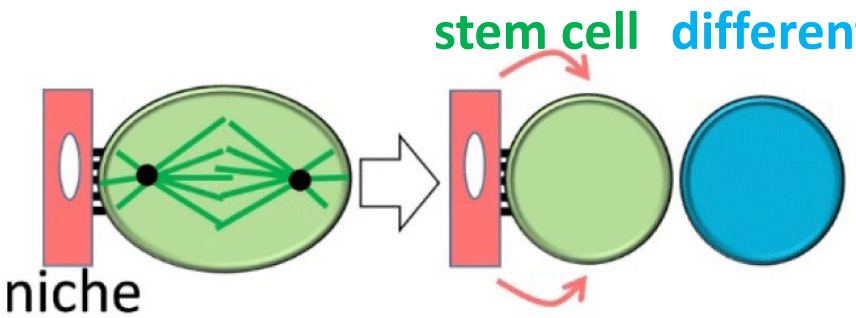


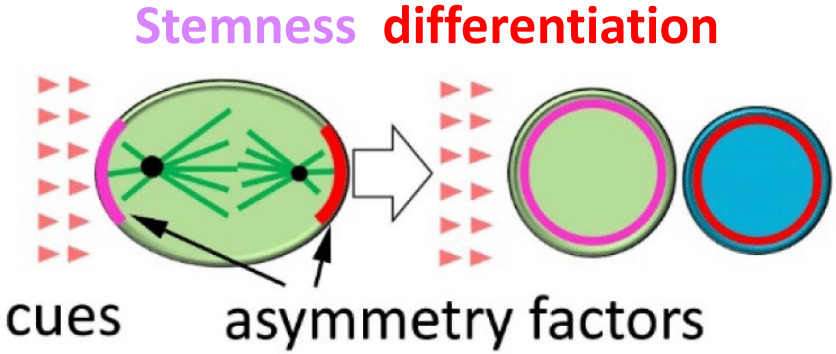


Lechler & Marina Mapelli, Mol. Cell Biol., 2021

Asymmetric division : two different daughter cells
Example of stem cells

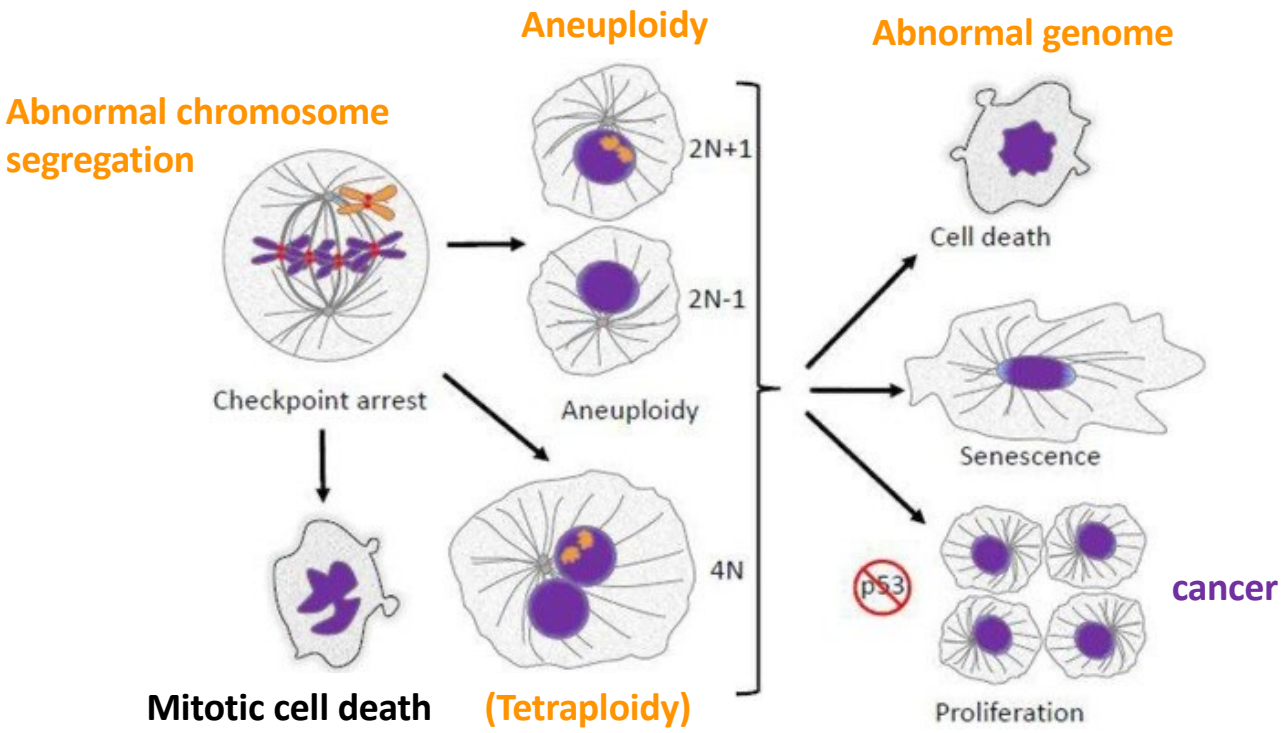




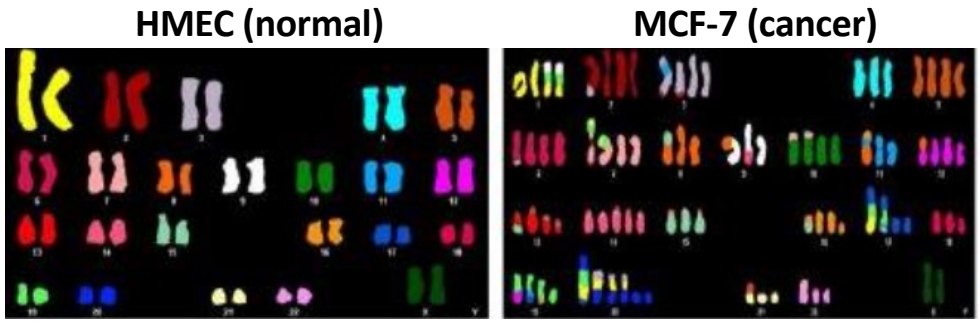


Lechler & Marina Mapelli, Mol. Cell Biol., 2021
Majumdar & Liu, AIMS Mol. Sci., 2020

Mitotic defects : aneuploidy

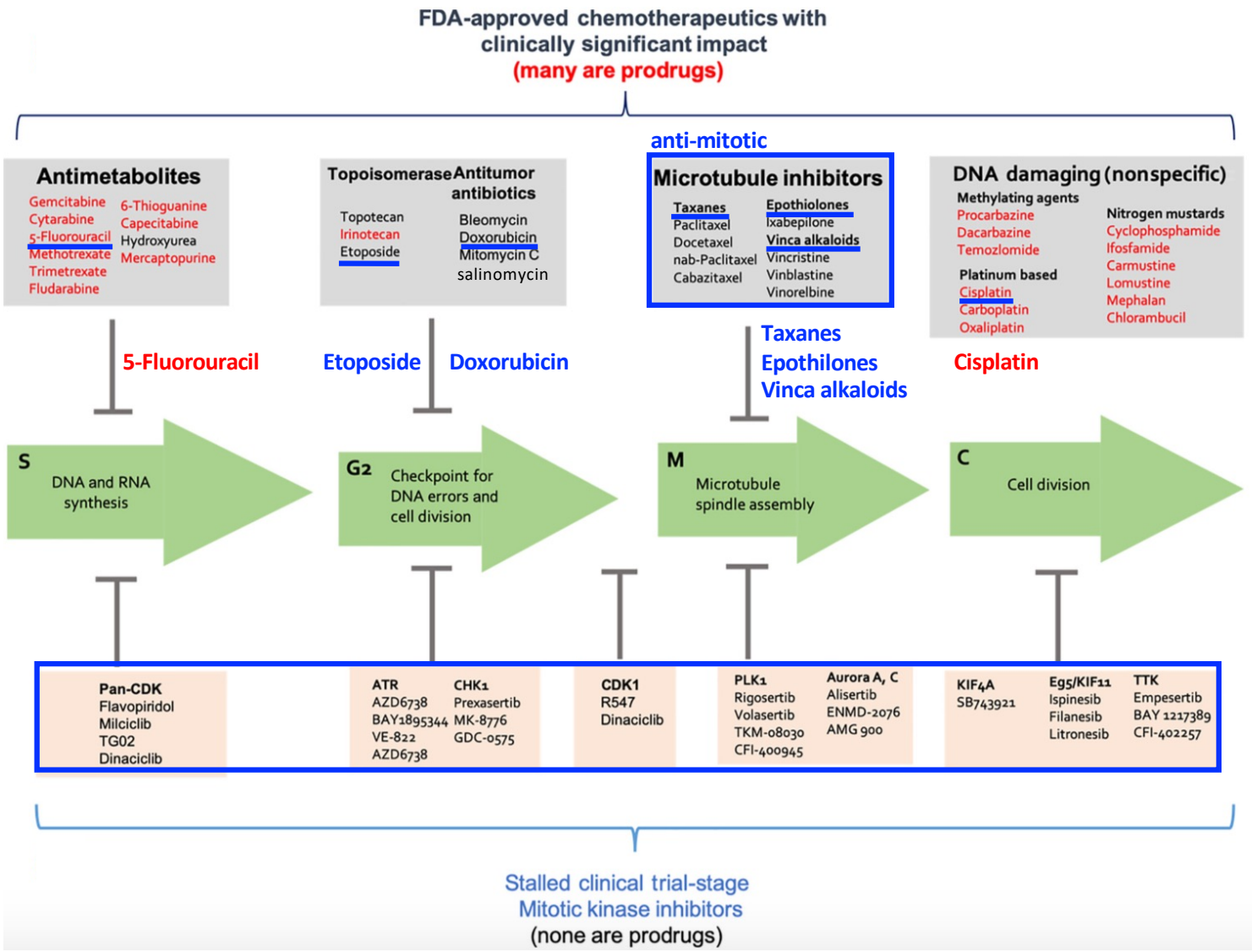


Karyotype of human breast cell lines



Potapova & Gorbsky, Biology, 2017

Cancer drugs and cell cycle : failure in recent clinical trials



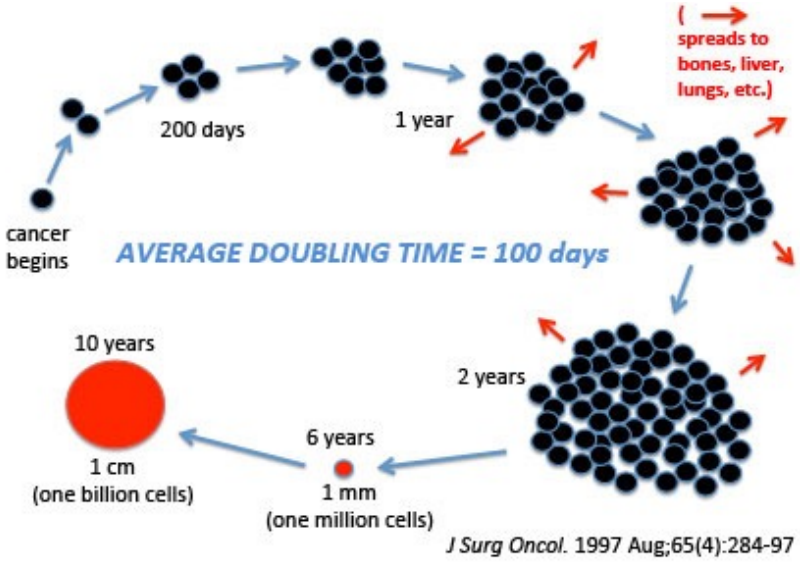
Working but …
- Side effects
- Recurrence

Not working

Adapted from Yan et al., Trends in Cancer, 2020

Proliferation of cancer cells can be lower than normal tissues

Cell division in cancer



cell division in tissues

From days to lifetime depending on tissue

Small intestine	2-3 days
Lung	8 days
Platelets	10 days
Epidermis	10-30 days
Hepatocyte	1/2 year
Fat cells	8 years
Neuron	lifetime

Side effects on fast dividing tissues and low efficiency in oncotherapy

G0 / quiescence : reversible arrest of cell cycle

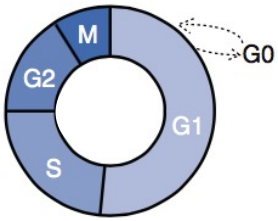
Quiescence/G0 :

- State of reversible cell cycle arrest, with active mechanisms to protect cells from damage.
- Adult stem cells (hematopoietic, muscle, neural, hair) and differentiated cells (fibrocyte, lymphocyte, hepatocyte)
- Reenter the cell cycle when confronted with the appropriate stimulus (tissue repair, wound healing, immunity).

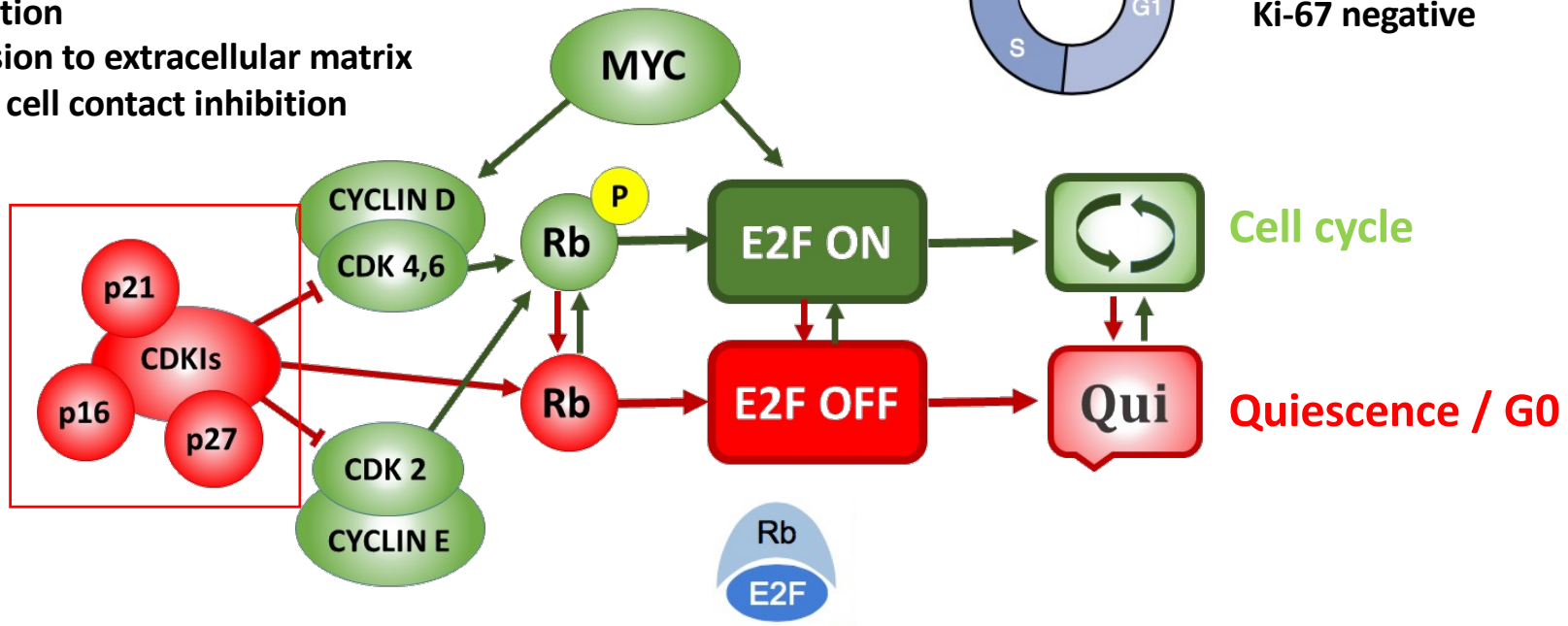
Long term tissue maintenance and regeneration

***In vitro* induction of quiescence from G1**

- Serum starvation
- Loss of adhesion to extracellular matrix
- Confluence = cell contact inhibition



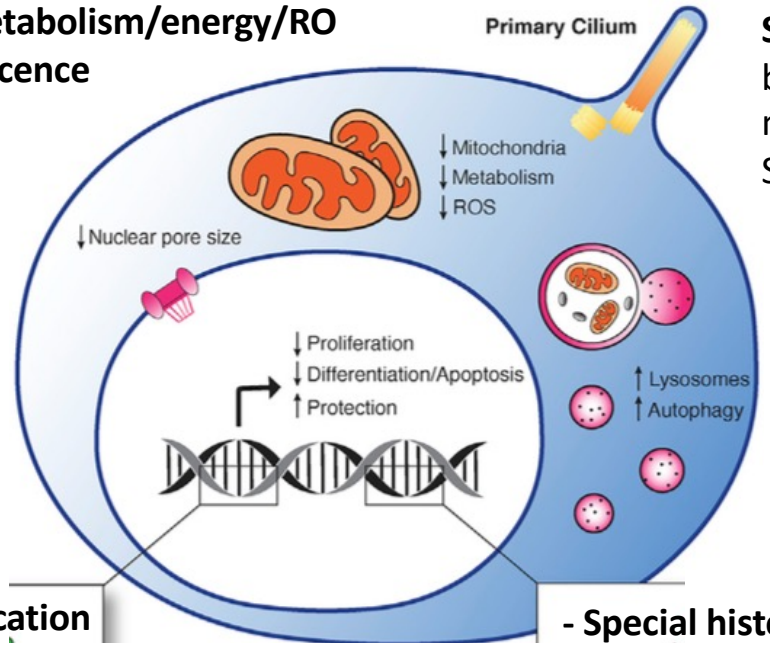
G0 quiecsence
Ki-67 negative



https://www.bethyl.com/content/The-Rb-E2F-Switch-Regulation-of-Cellular-Quiescence

Properties of G0 quiescent cells

- **Low transcription/translation/metabolism/energy/RO**
- **No proliferation/apoptosis/senescence**



Sensory structure
basal body + axoneme (MTs)
mechano / chemo sensor
Signaling hub

- **High lysosomes**
- **High autophagy**

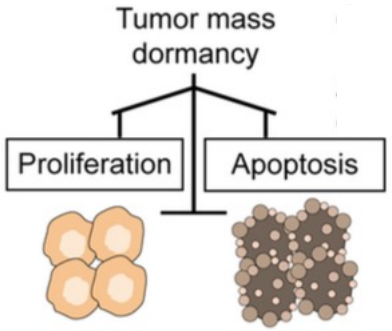
- **No DNA replication**
- **DNA methylation**

- **Special histone (centromere integrity)**
- **Limit DNA damage**

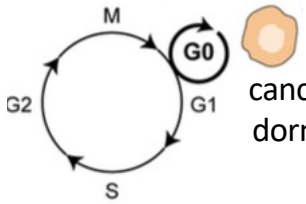
Ready to re-enter cell cycle when needed !

Adapted from Marescal & Cheeseman, Dvpt Cell, 2020

Cancer dormancy and recurrence



cancer stem cells (CSC)
dormant disseminated tumor cell (DTC)
Quiescent / slow cycling

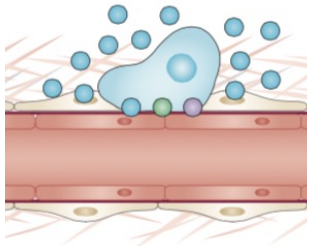


cancer cell dormancy

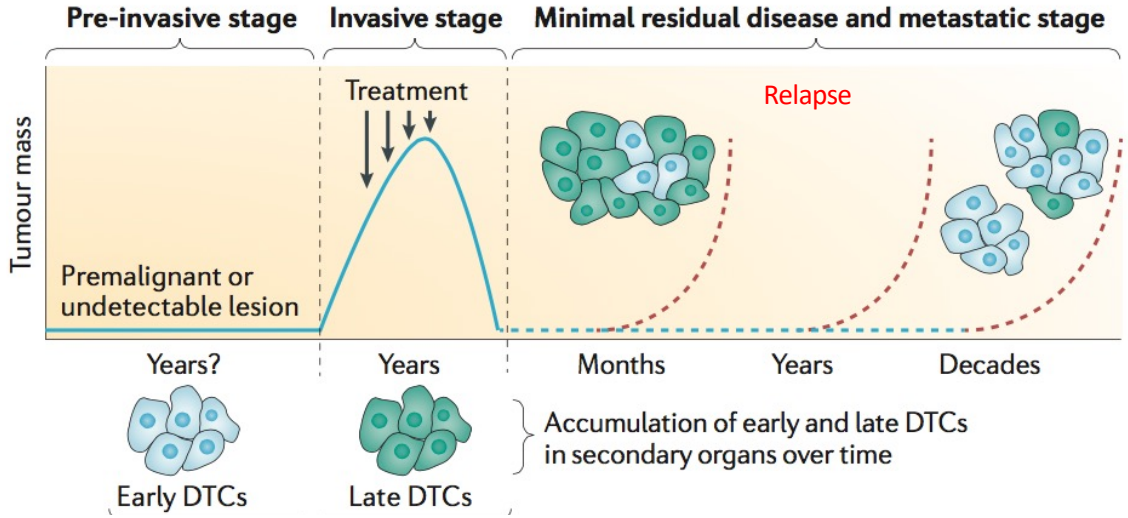
Entry of cancer cells into G0 for decades
- **Surviving harsh environment, therapy**
- **Immune evasion**

If cell-cycle re-entry
- **Seed new tumor formation**

Perivascular niches
Bone marrow, lungs, brain, liver



Keep quiescence of stem cells & dormancy of DTC

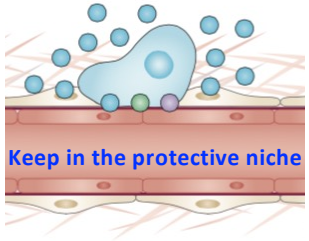


Sistigu et al., Front. In Immuno, 2020
Ghajar, Nature Cancer Rev., 2015
Sosa et al., Nature Review Cancer, 2014

Possible therapeutic strategies related to DTC

Perivascular niches
Bone marrow, lung, brain, liver
DTC : disseminated tumor cell

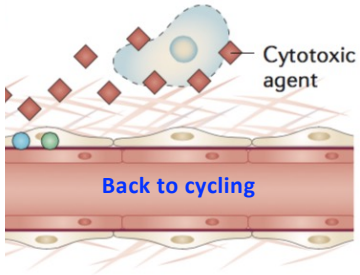
Lock-in strategy = keep sleepy DTC
Long term toxicity ?
Ex : tamoxifen breast cancer



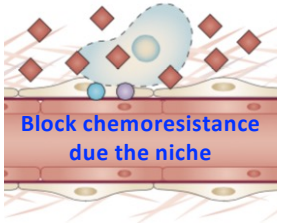
lifetime treatment

healthy lifestyle

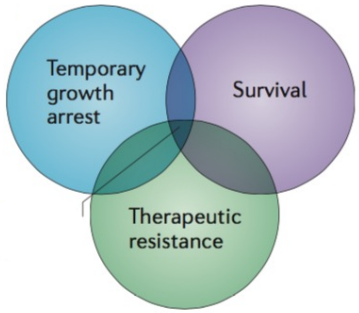
Lock-out strategy = awakening DTC
to be killed by conventional therapy



Eliminating dormant DTC
to be killed by conventional therapy



with (neo)adjuvent therapy



Ghajar, Nature Cancer Rev., 2015

Senescence : a permanent cell cycle arrest

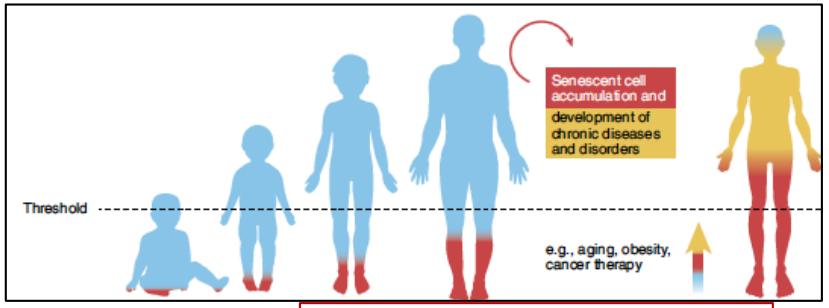
Senescence :

- State of irreversible cell cycle arrest

- Role in embryonic development, wound healing, host immunity, tumor suppression

- Immune clearance of senescent cells is possible

- Hallmarks of aging (age related diseases)



Triggers :

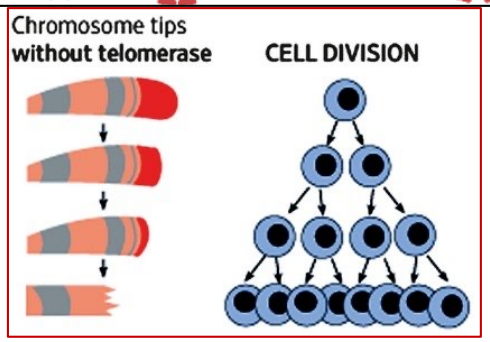
- Telomere shortening (mitotic timer / aging) = replicative senescence

Telomere : Nobel Prize in Physiology or Medicine 2009, Blackburn, Greider & Szostak

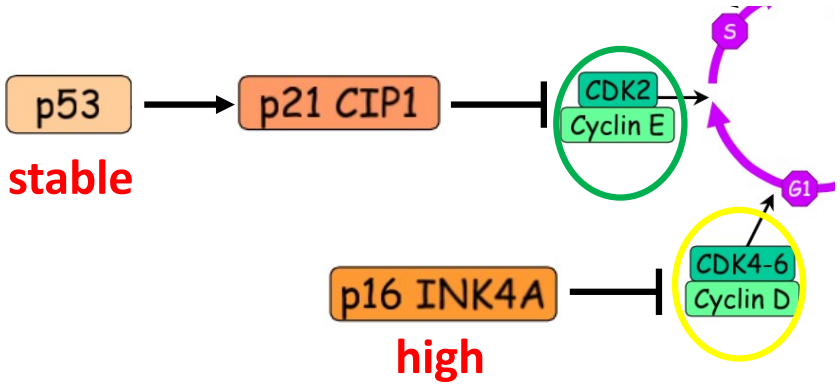
- Damages = premature senescence

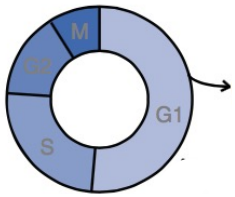
DNA damage, mitochondrial dysfunction, inflammation, ROS, epigenetic alteration….

In cancer : oncogene or therapy induced senescence (OIS or TIS)



Annika Röhl

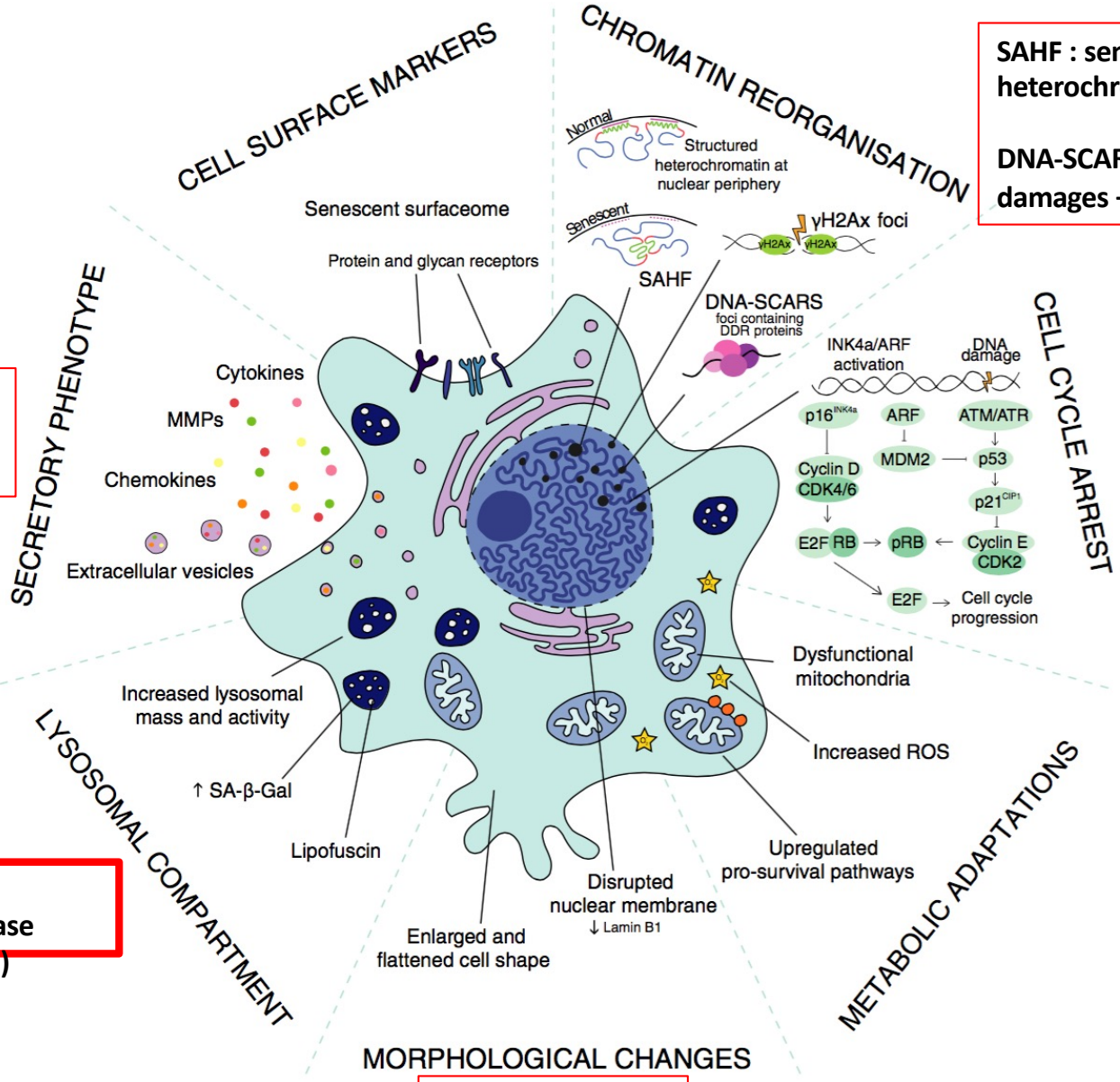




Exit = senescence
Ki-67 negative

Chaib et al, Nature Med., 2022

Hallmarks of senescent cells



SAHF : senescent-associated heterochromatin foci

DNA-SCARS: persistent DNA damages + sensors

SASP : senescent-associated secretory phenotype

Paracrine senescence

. high p16
. Ki-67 negative

SA-β-galactosidase (lysosomal)

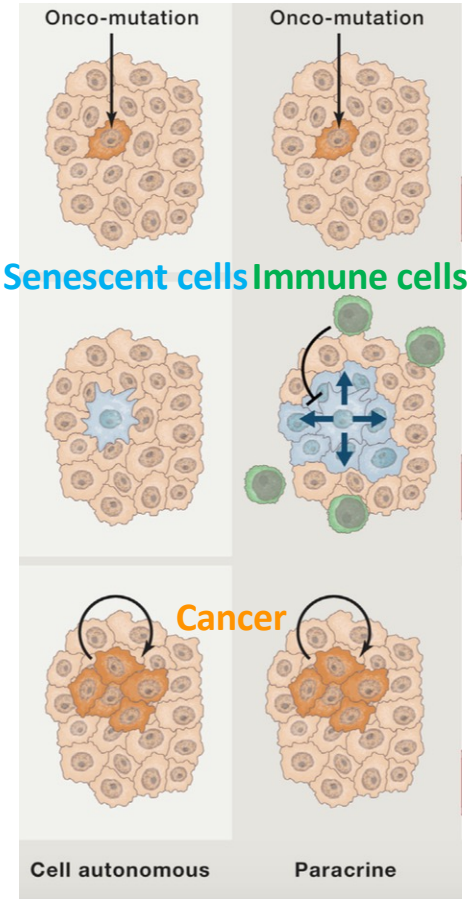
. Giant cell
. Disrupted lamina

Gonzalez-Gualda et al., FEBS J, 2020

Senescence and cancer

Senescence prevents cancer

loss of p16/INK4a and/or p53 function = most common genetic event in human cancers

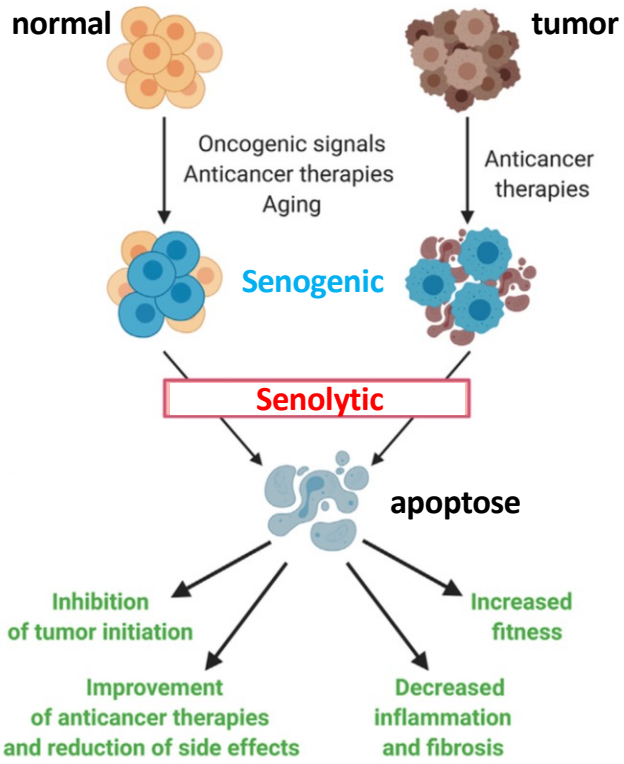


Senescent cell clearance prevents cancer

loss of senescent cell clearance promotes cancer

He & Sharpless., Cell, 2017

Possible therapeutic strategy



Example of senolytic agents
Cardiac glycosides : inhibitors of Na/K ATPase pump (treatment of heart failure, cardiac arrhythmia)

Martin et al., Trends Mol Med, 2020

P53 the guardian of the genome

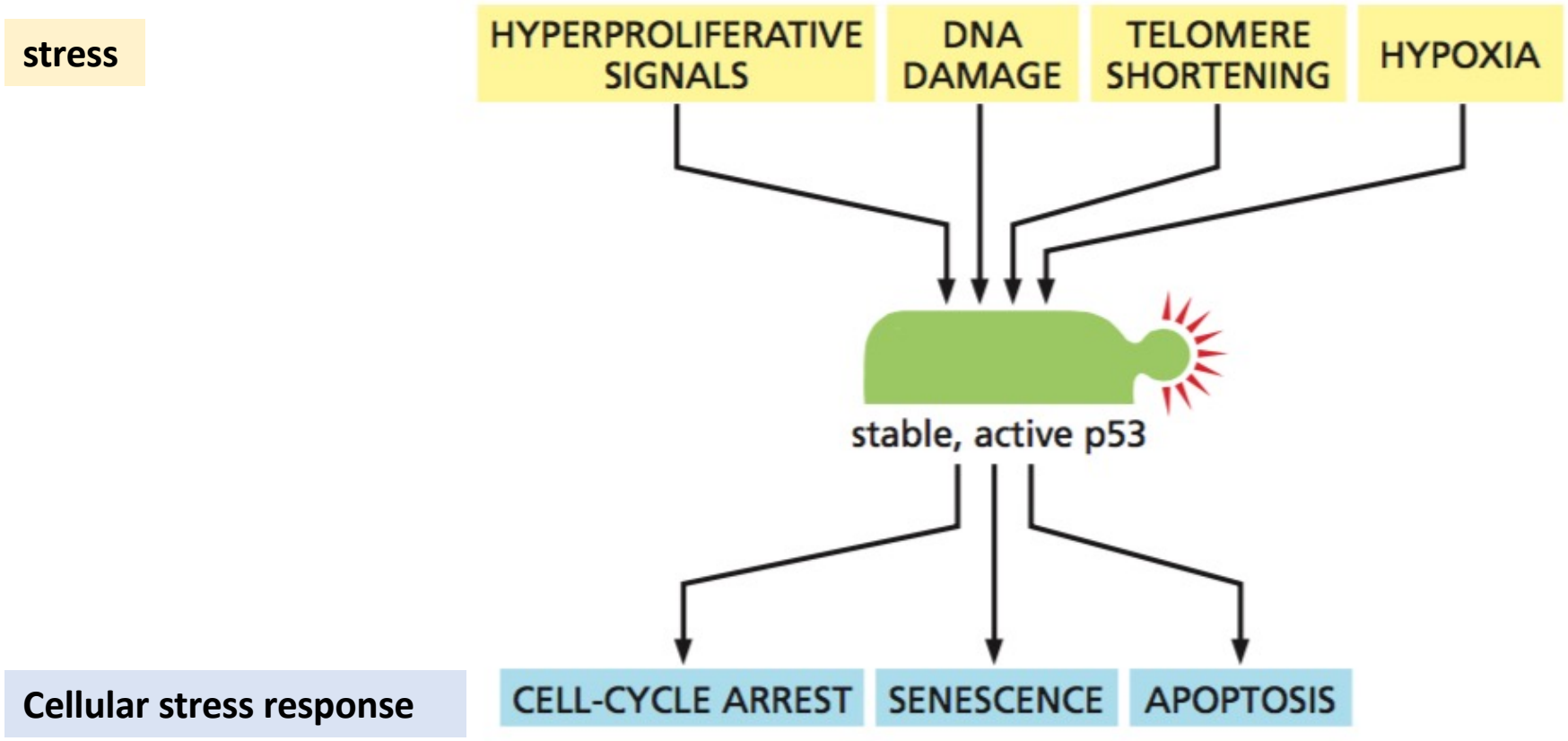


Figure 20-27, Molecular Biology of the Cell 6th