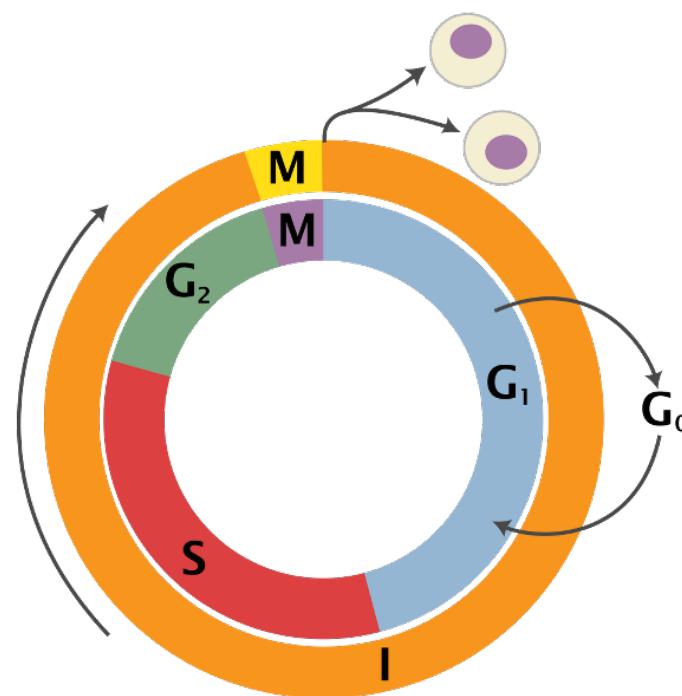


Introduction to Molecular and Cellular Biology

LECTURES 23-24:

Cell cycle II



LECTURES 23-24: CELL CYCLE II

➤ Ubiquitylation and proteosome degradation

➤ APC/C in the cell cycle:

- molecular mechanisms

- structure-function relationship

➤ Cancer:

- microevolutionary process

- causes of cancer

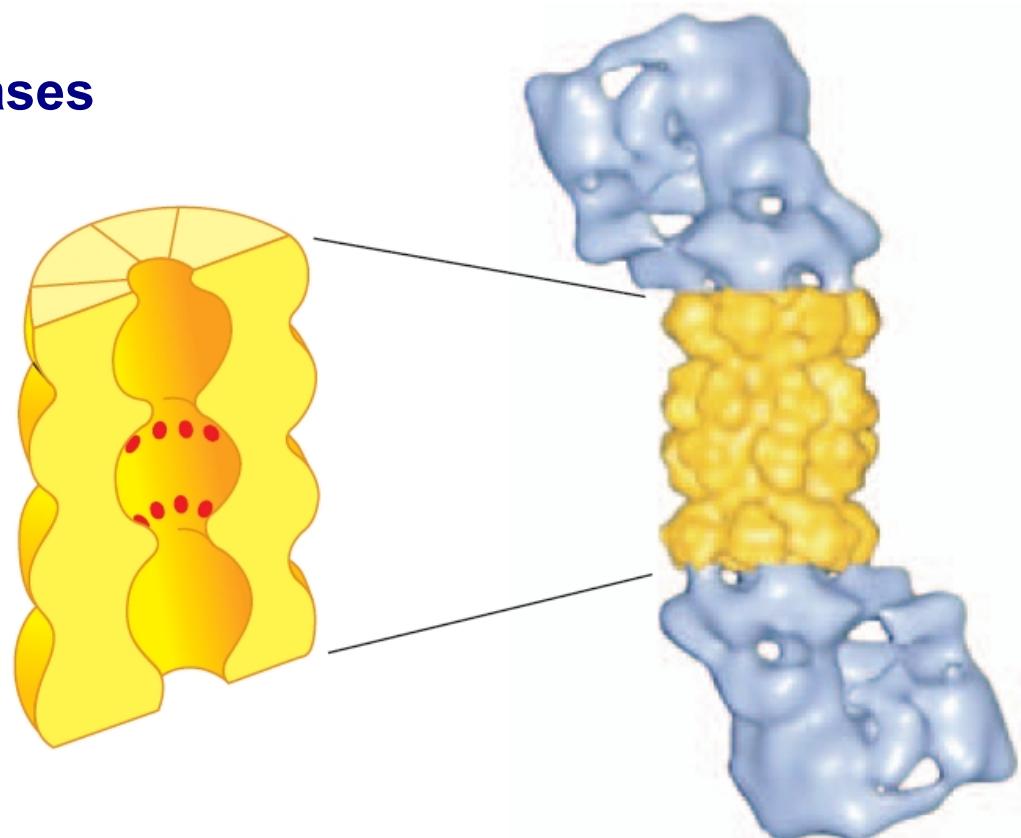
- oncogens and molecular basis

- treatment

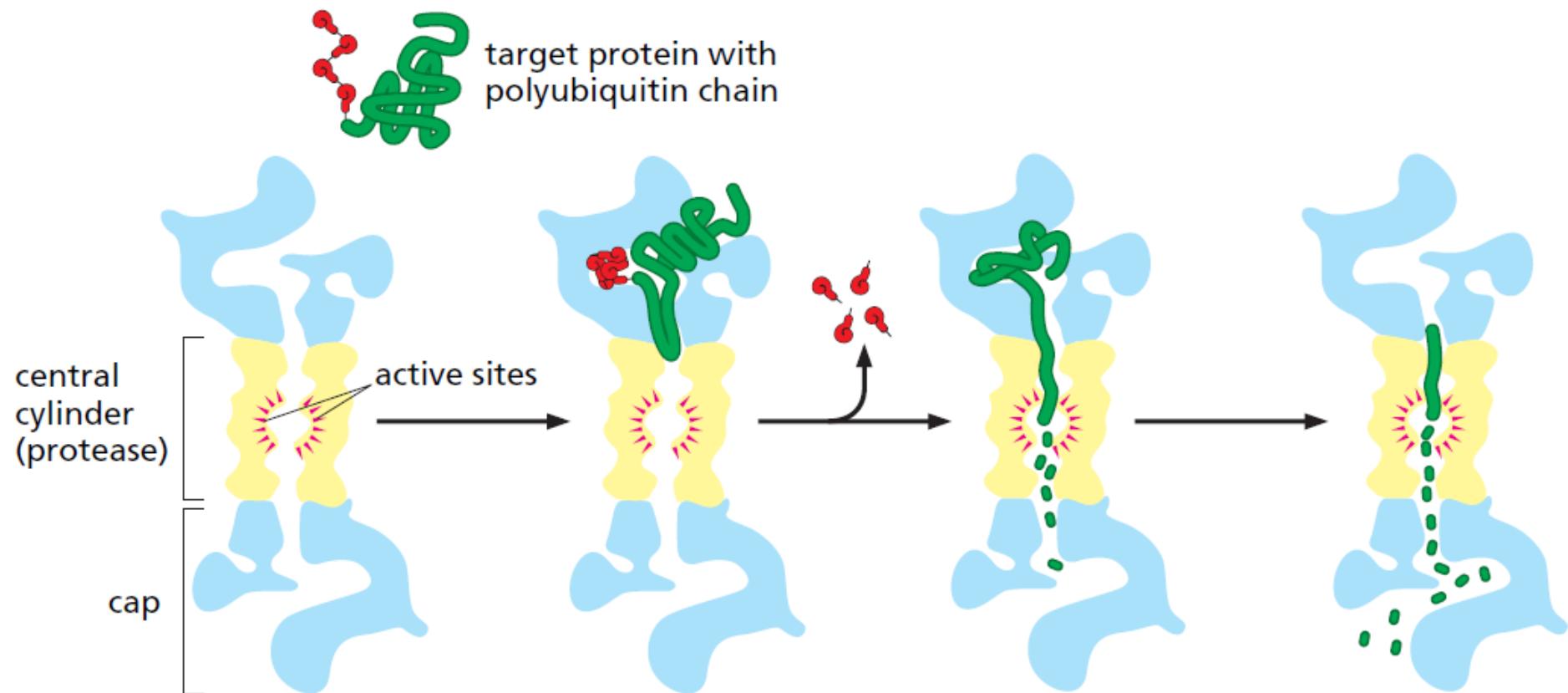


PROTEOSOME

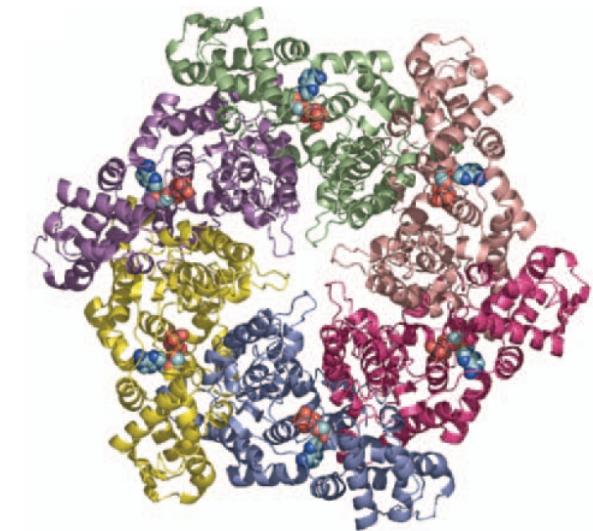
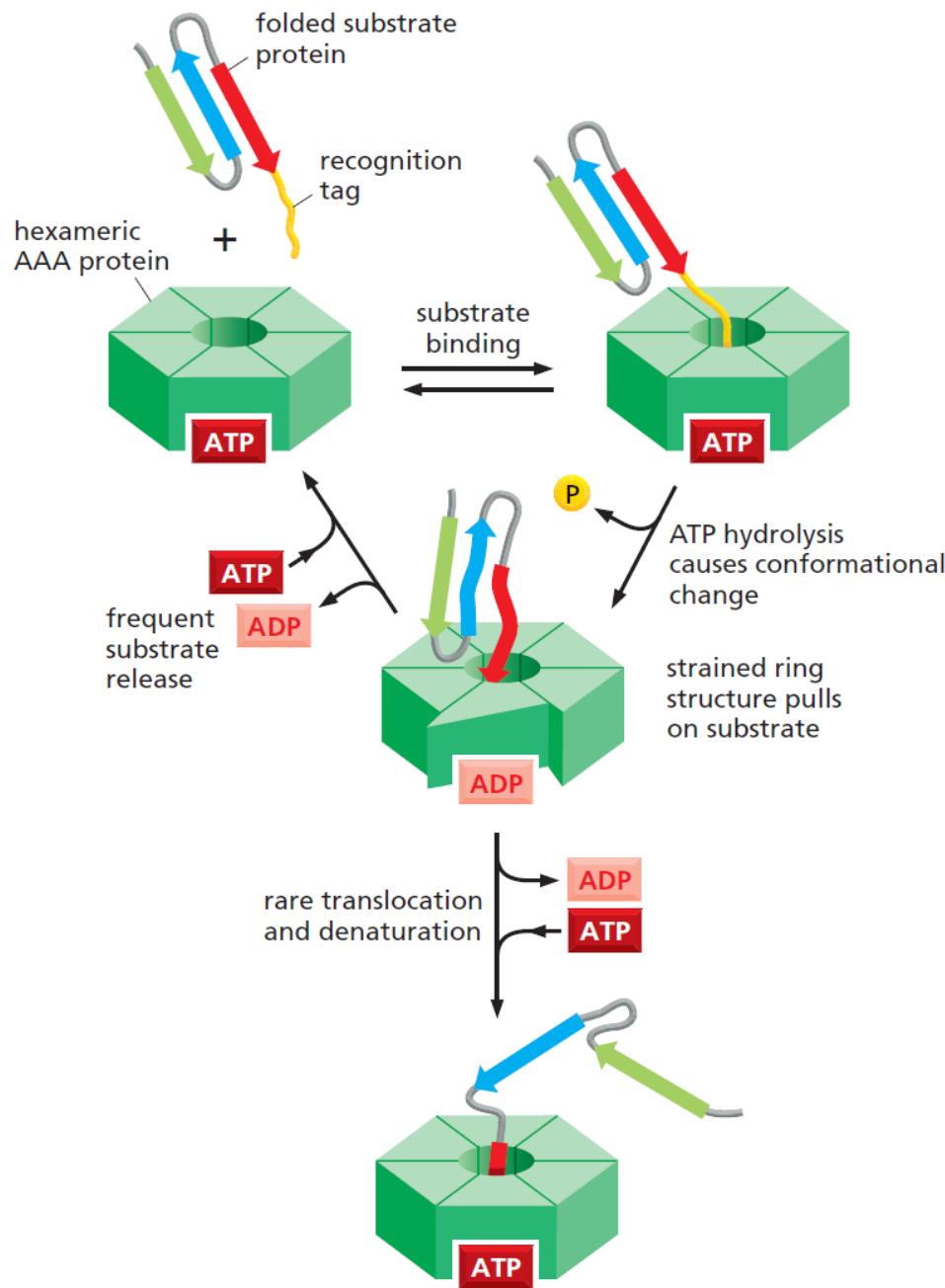
- Big protein complex with ATP-dependent proteases that degrade proteins:
 - unfolded
 - unneeded for the cell
- 1% of the whole cell
- Subunits:
 - central 20S hollow cylinder: proteases
 - 19S cap
- Mechanism steps:
 - unfolding (AAA-family)
 - proteolytic degradation



PROTEOSOME: MECHANISM

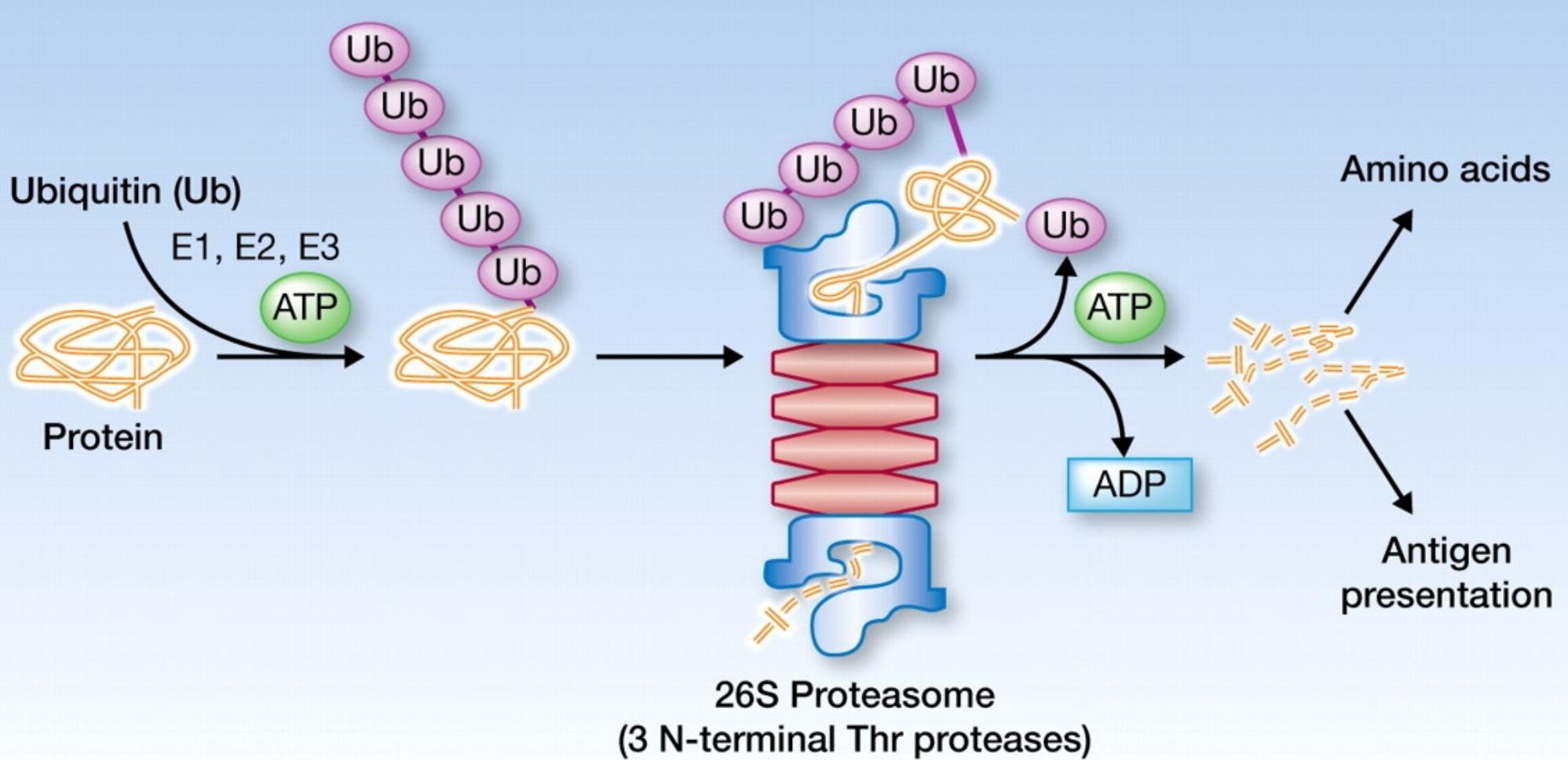


PROTEOSOME: MECHANISM



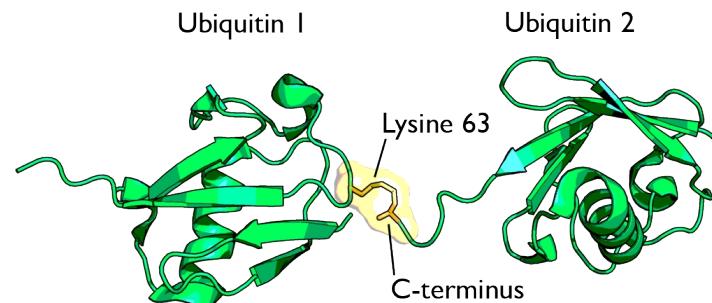
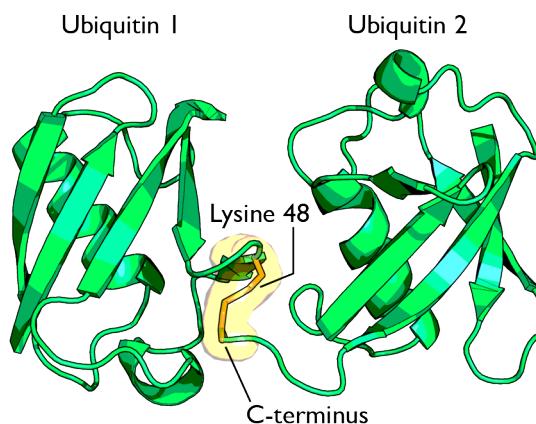
AAA hexamer

PROTEOSOME: MECHANISM



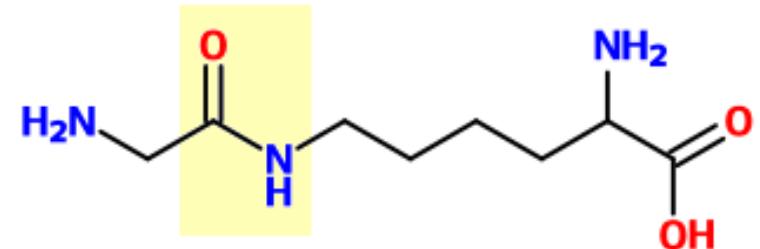
UBIQUITIN

- 76 residues
- In all tissues => *ubiquitously*
- Very conservative (human vs. yeast: 95%)
- 4 genes in mammals
- 7 Lys at N-terminus => sites for covalent binding:
 - Lys29,48 => proteolysis
 - Lys63 unknown



UBIQUITYLATION

- Monoubiquitylation
- Multiubiquitylation
- Polyubiquitylation



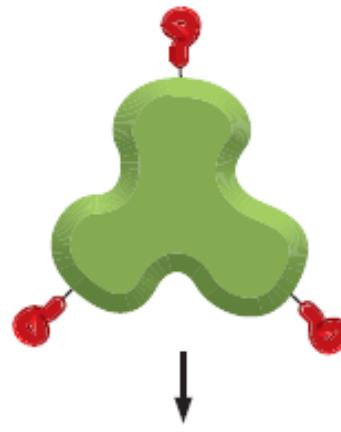
Gly_{ubiquitin}-Lys_{target} side-chain covalent bond

MONOUBIQUITYLATION



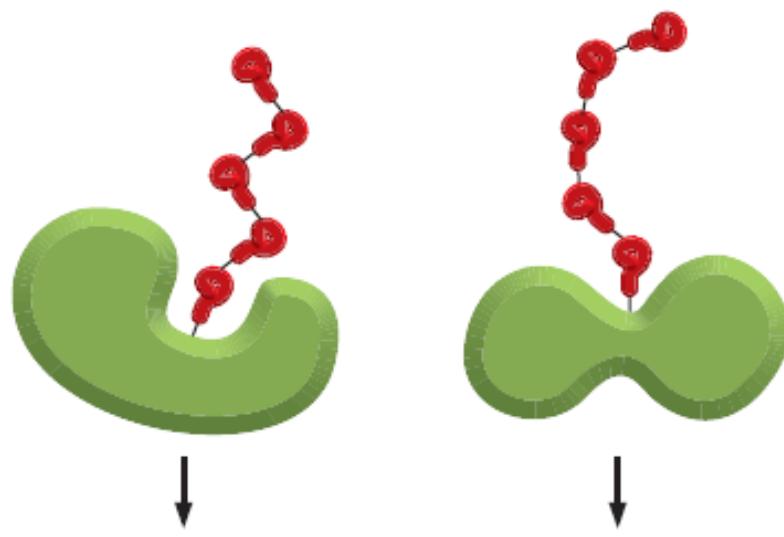
histone regulation

MULTIUBIQUITYLATION



endocytosis

POLYUBIQUITYLATION

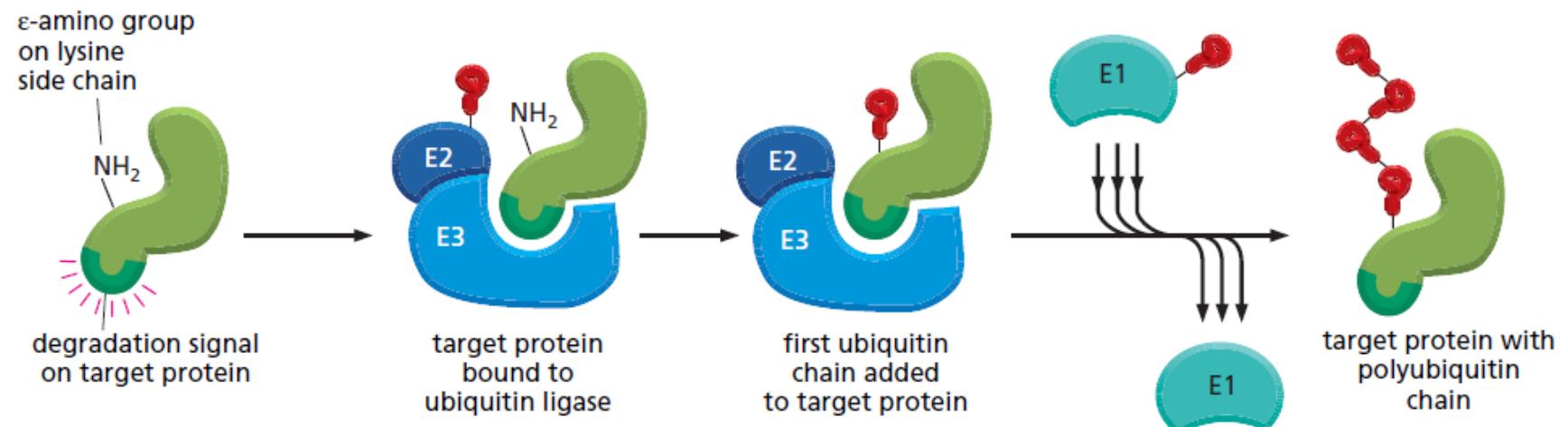
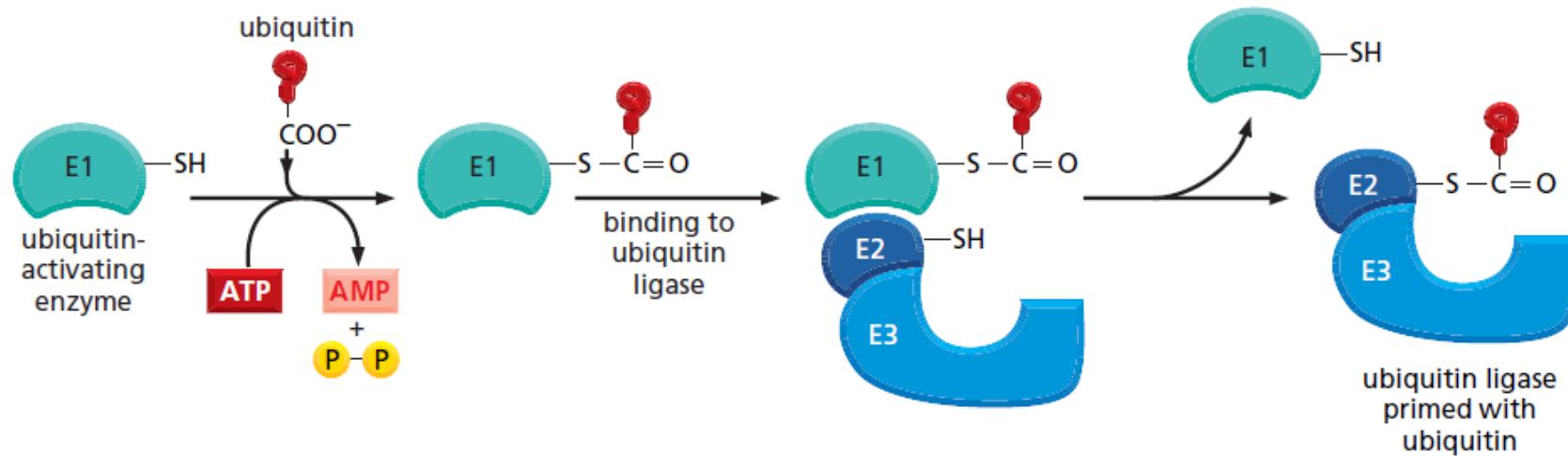


proteasomal degradation
DNA repair

UBIQUITYLATION

➤ Ubiquitin-conjugating system:

- E1 ubiquitin activating enzyme
- E2 ubiquitin conjugating enzyme, ~30 different
- E3 ubiquitin ligase (binding to signal: degron), ~100 different



REGULATED PROTEIN DESTRUCTION

➤ Proteins destructed by proteosomes through ubiquitin pathway:

- abnormally folded

- conditionally short-lived

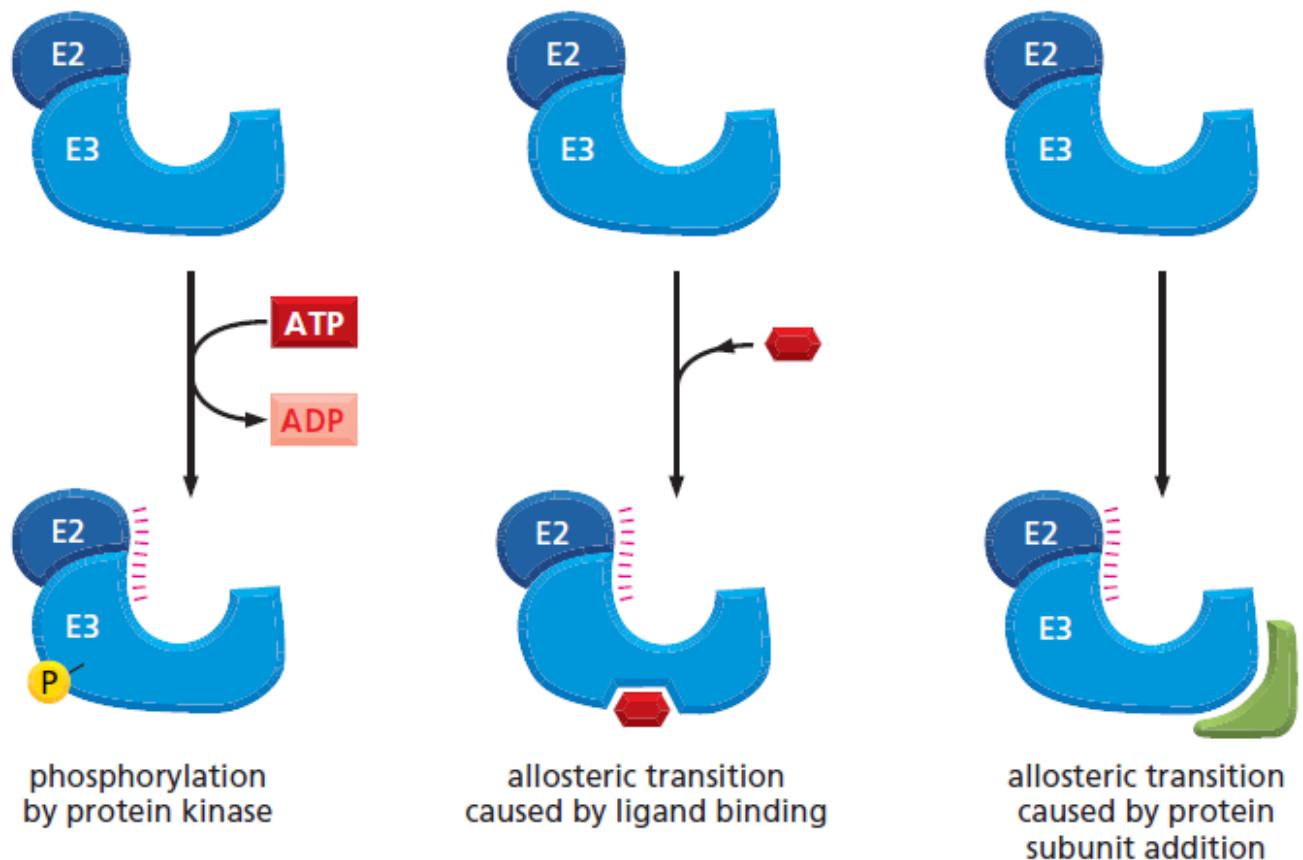
➤ E3 regulation:

- phosphorylation

- allosteric regulation

- addition of a subunit

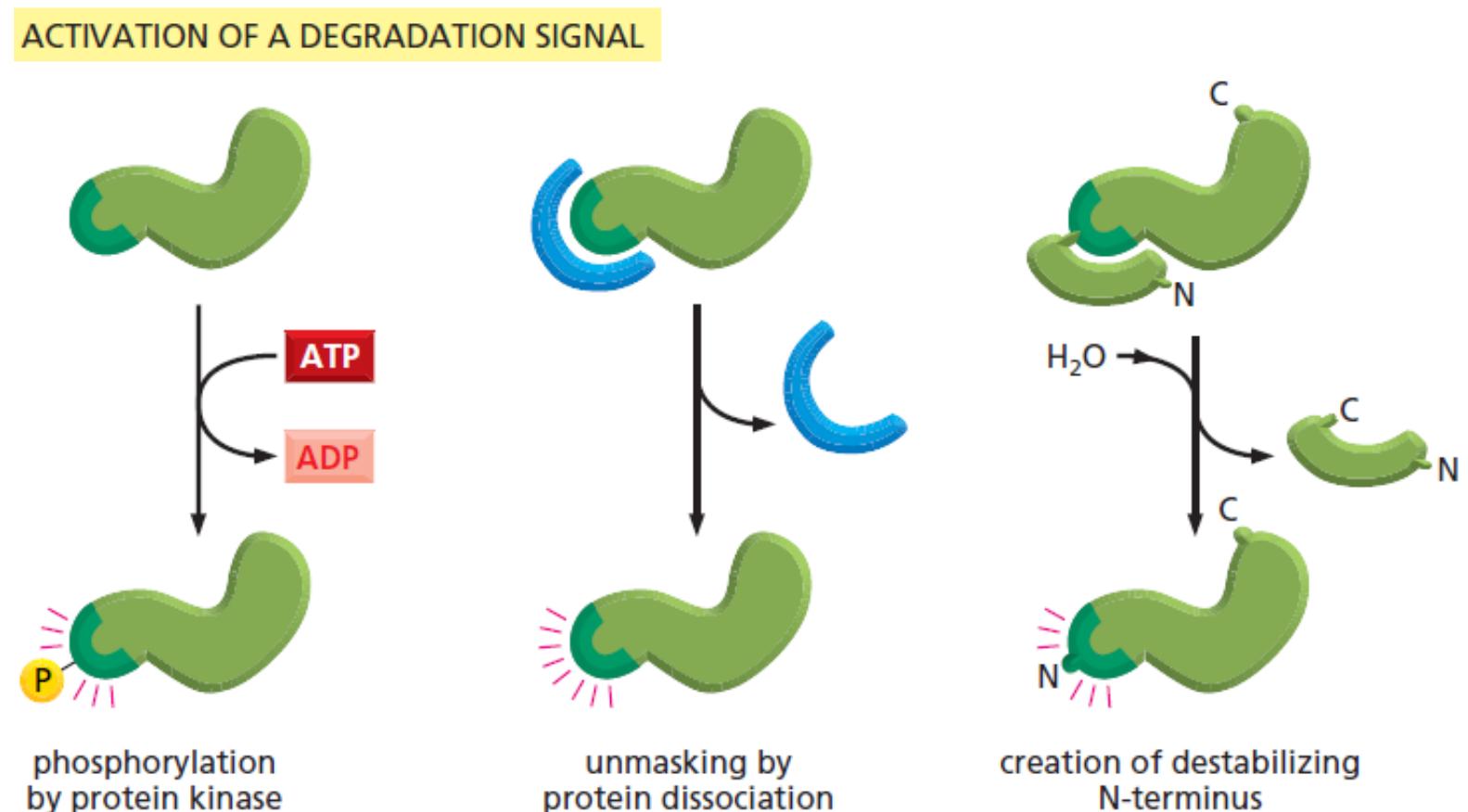
ACTIVATION OF A UBIQUITIN LIGASE



REGULATED PROTEIN DESTRUCTION

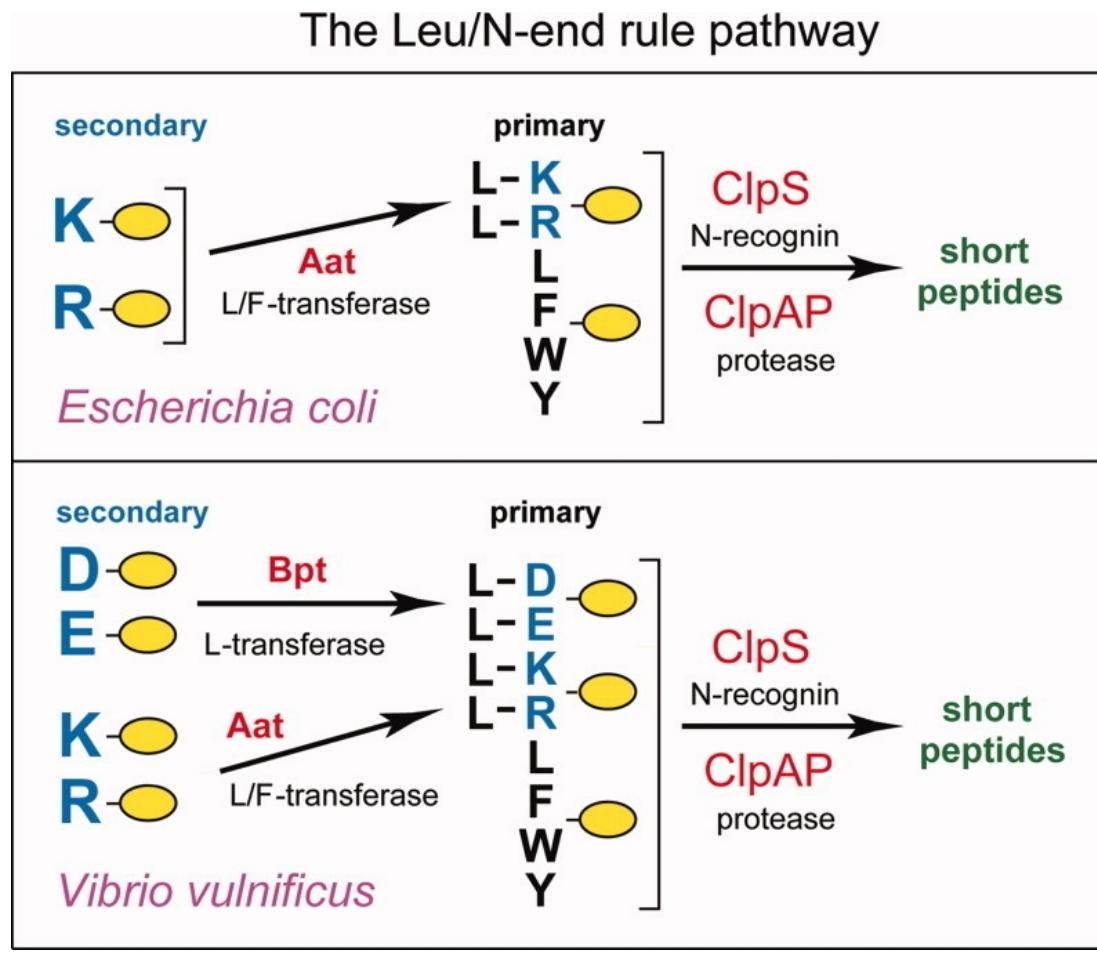
➤ Signal:

- phosphorylation
- unfolded state => unmasking
- cleavage (destabilizing N-terminal residue, “N-end rule”)



N-END RULE

- Arg, Lys, His, Phe, Leu, Tyr, Trp, Ile, Asp, Glu, Asn, Gln => destabilization (yeast)
- Methionine aminopeptidases
- Several N-rule pathways: Arg, Asn, Leu
- Example: cohesin

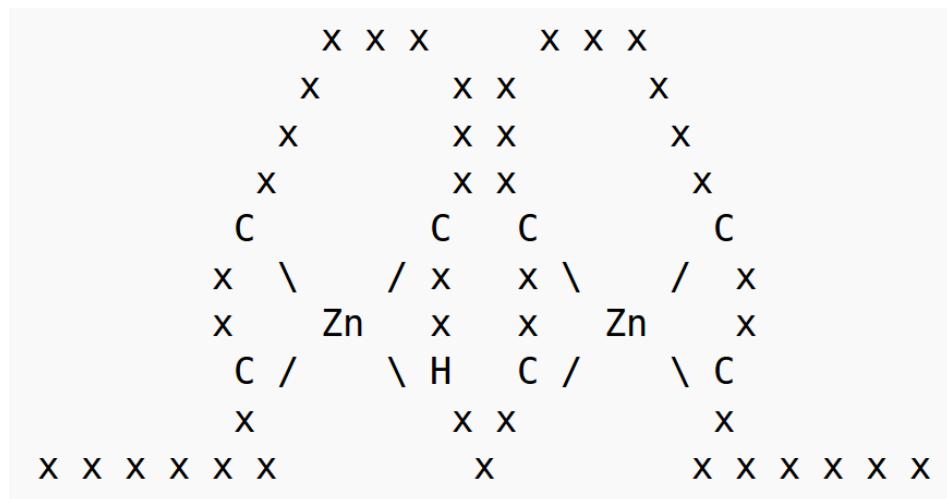
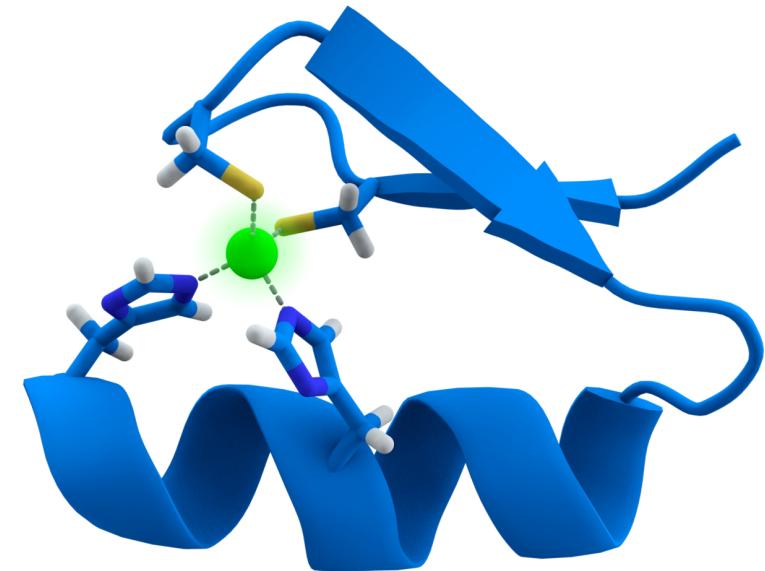


- Val -> 100h
- Met, Gly -> 30h
- Pro -> 20h
- Ile -> 20h
- Thr -> 7.2h
- Leu -> 5.5h
- Ala -> 4.4h
- His -> 3.5h
- Trp -> 2.8h
- Tyr -> 2.8h
- Ser -> 1.9h
- Asn -> 1.4h
- Lys -> 1.3h
- Cys -> 1.2h
- Asp -> 1.1h
- Phe -> 1.1h
- Glu -> 1.0h
- Arg -> 1.0h
- Gln -> 0.8h

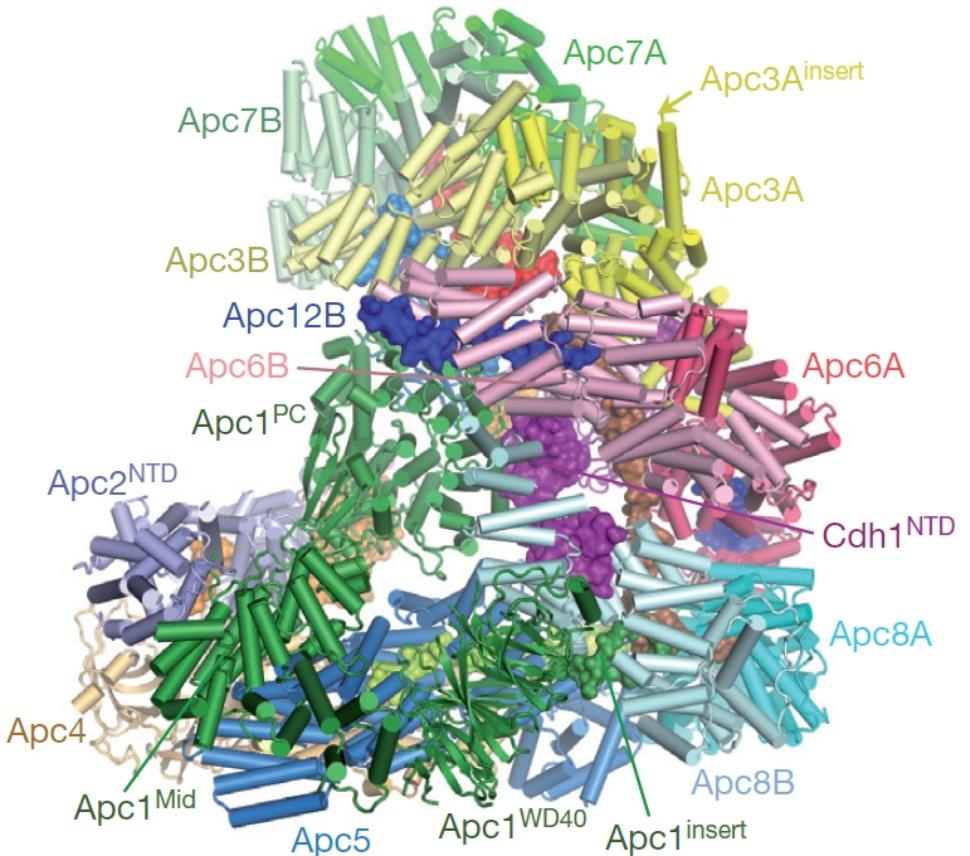
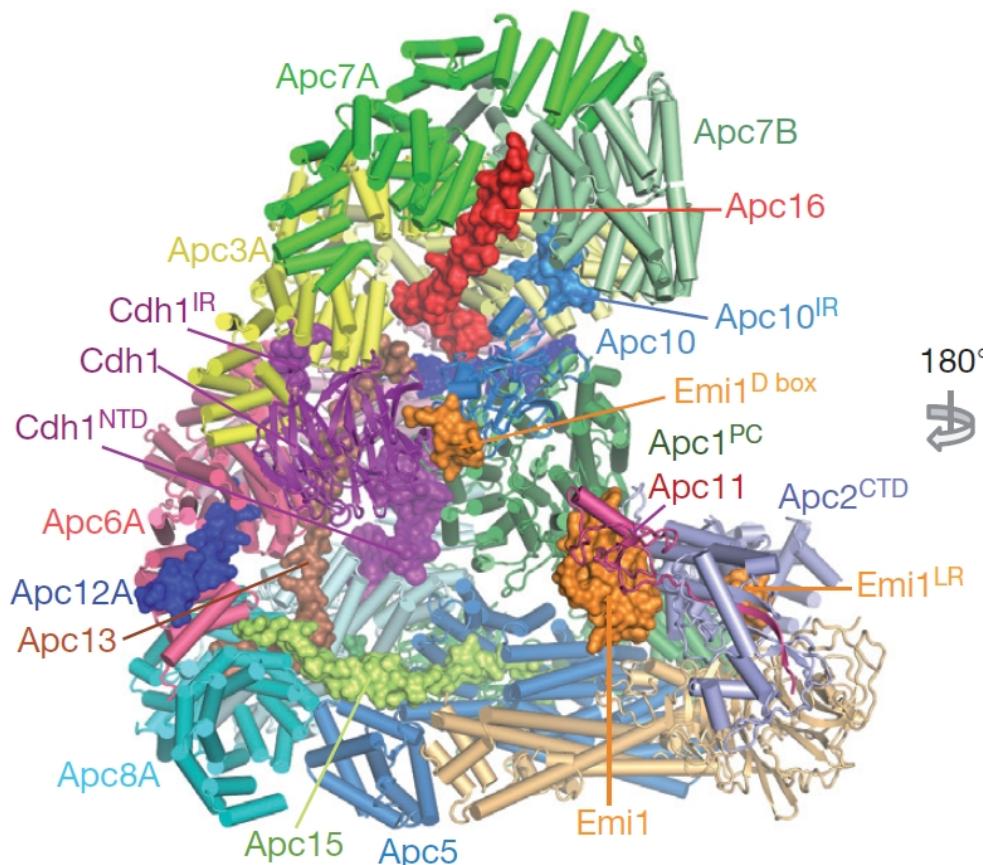
$t_{1/2}$ mammals

APC/C: ANAPHASE PROMOTING COMPLEX

- Multimeric E3-RING ligase
- RING:
 - 40-60 aa
 - C-X₂-C-X_[9-39]-C-X_[1-3]-H-X_[2-3]-C-X₂-C-X_[4-48]-C-X₂-C
- High conservation
- 13 subunits
 - catalytic core (Apc2, Apc11)
 - scaffold proteins
 - many repeats within the subunits



APC/C STRUCTURE

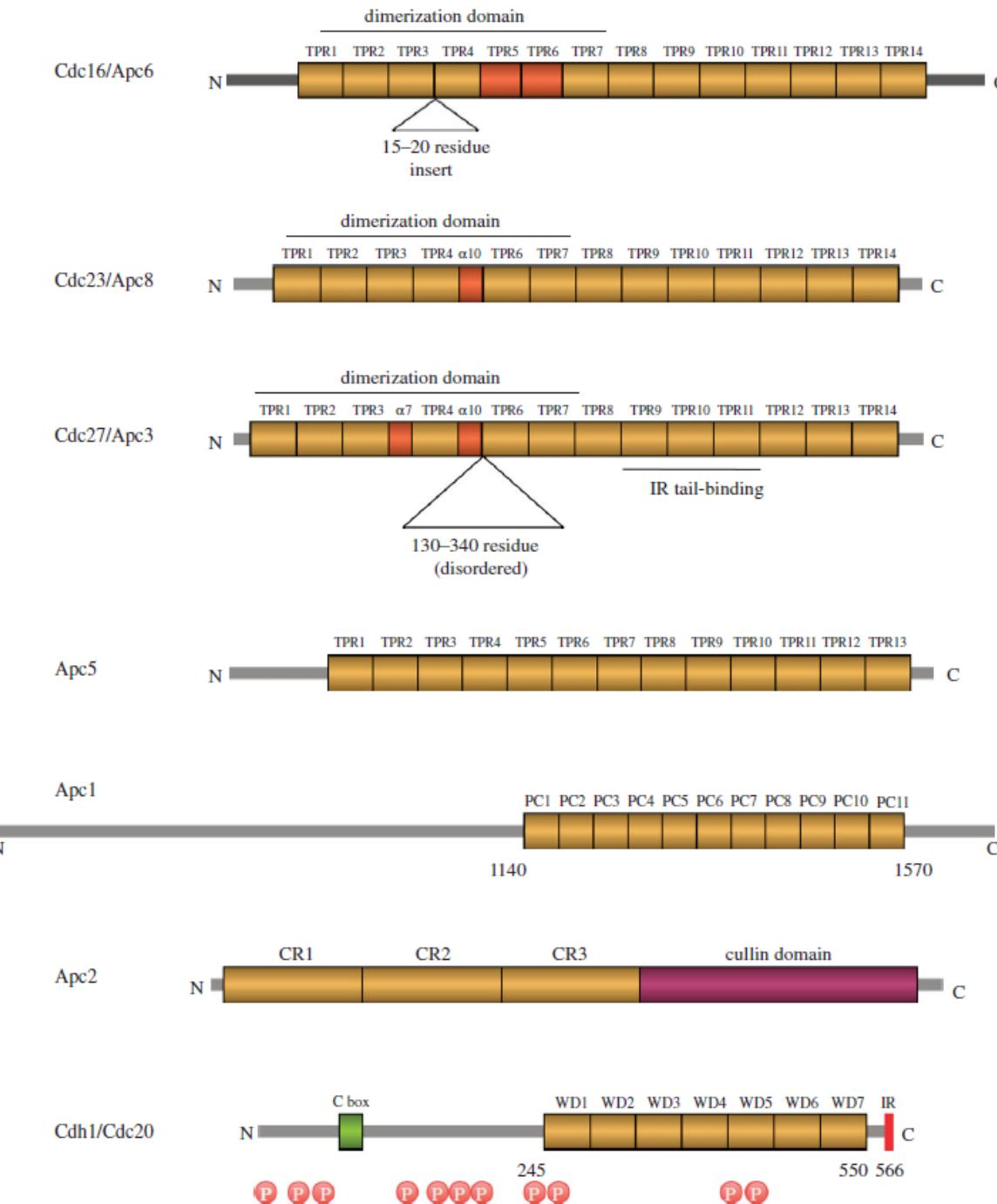


APC/C in complex with Emi1

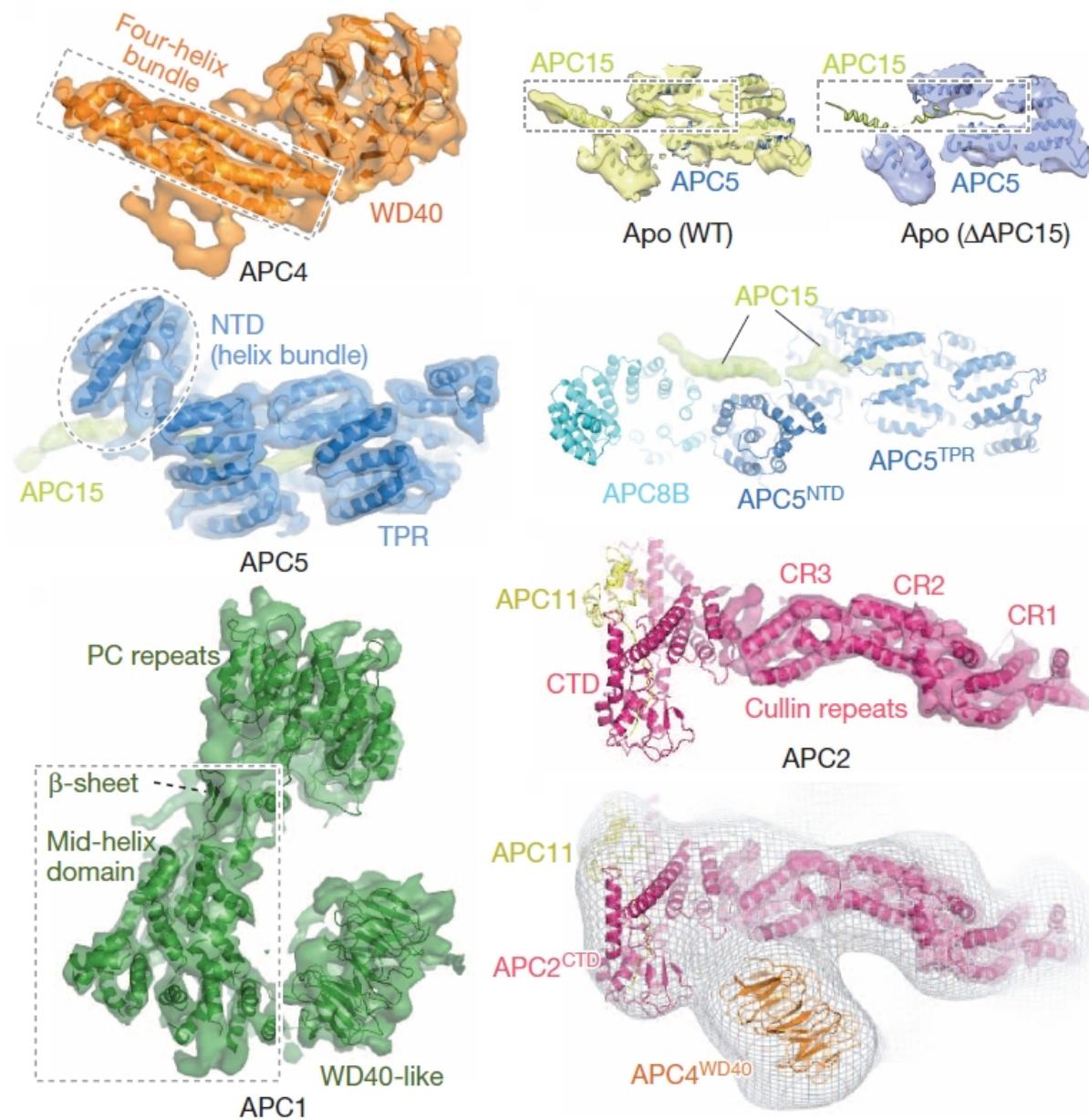
APC/C SUBUNITS

<i>S. cerevisiae</i>	mol. mass (kDa)	stoichiometry	<i>H. sapiens</i>	<i>S. pombe</i>	structural motif	function
core subunits						
Apc1	196.1	1	Apc1	Cut4	PC repeats	scaffolding subunit
Apc2	100.0	1	Apc2	Apc2	cullin homology	catalytic centre
Cdc16	95.0	2	Apc6	Cut9	TPR	scaffolding subunit
Cdc27	85.4	2	Apc3	Nuc2	TPR	Cdh1/Doc1 binding
—			Apc7	—	TPR	Cdh1/Doc1 binding
Apc4	75.3	1	Apc4	Lid1	unknown	scaffolding subunit
Apc5	79.3	1	Apc5	Apc5	extended TPR	scaffolding subunit
Cdc23	73.1	2	Apc8	Cut23	TPR	Scaffolding subunit
Mnd2	42.8	1	—	—	unknown	Ama1 inhibitor
Apc9	30.9	1 or 2	—	—	unstructured	Cdc27 stabilizing
Apc10/Doc1	28.8	1	Apc10	Apc10	Doc homology/IR motif	substrate recognition
Apc11	18.9	1	Apc11	Apc11	RING H2	catalytic centre
Cdc26	14.1	2	Cdc26	Hcn1	unstructured	Cdc16 stabilizing
Apc13/Swm1	19.4	2	Apc13	Apc13	unstructured	Cdc23 stabilizing
—	—		—	Apc14	unknown	unknown
—	—		—	Apc15	unknown	unknown
—	—		Apc16	—	unknown	unknown
	1127–1158	17–18				
co-activators						
Cdc20	67.4	1	Cdc20		WD40/IR motif	substrate recognition
Cdh1	62.8	1	Cdh1		WD40/IR motif	substrate recognition
Ama1	67.0	1	—		WD40/IR motif	substrate recognition
inhibitors						
MCC			MCC	MCC		APC/C inhibitor
Mad2			Mad2	Mad2	Mad2 fold	APC/C inhibitor
Mad3/BubR1			Mad3/ BubR1	Mad3/ BubR1	TPR/pseudo- substrate	APC/C inhibitor
Bub3			Bub3	Bub3	WD40	APC/C inhibitor
—			Emi1/Emi2	—	pseudo-substrate	APC/C inhibitor
Acml			—	—	pseudo-substrate	APC/C inhibitor

APC/C SUBUNITS ARCHITECTURE

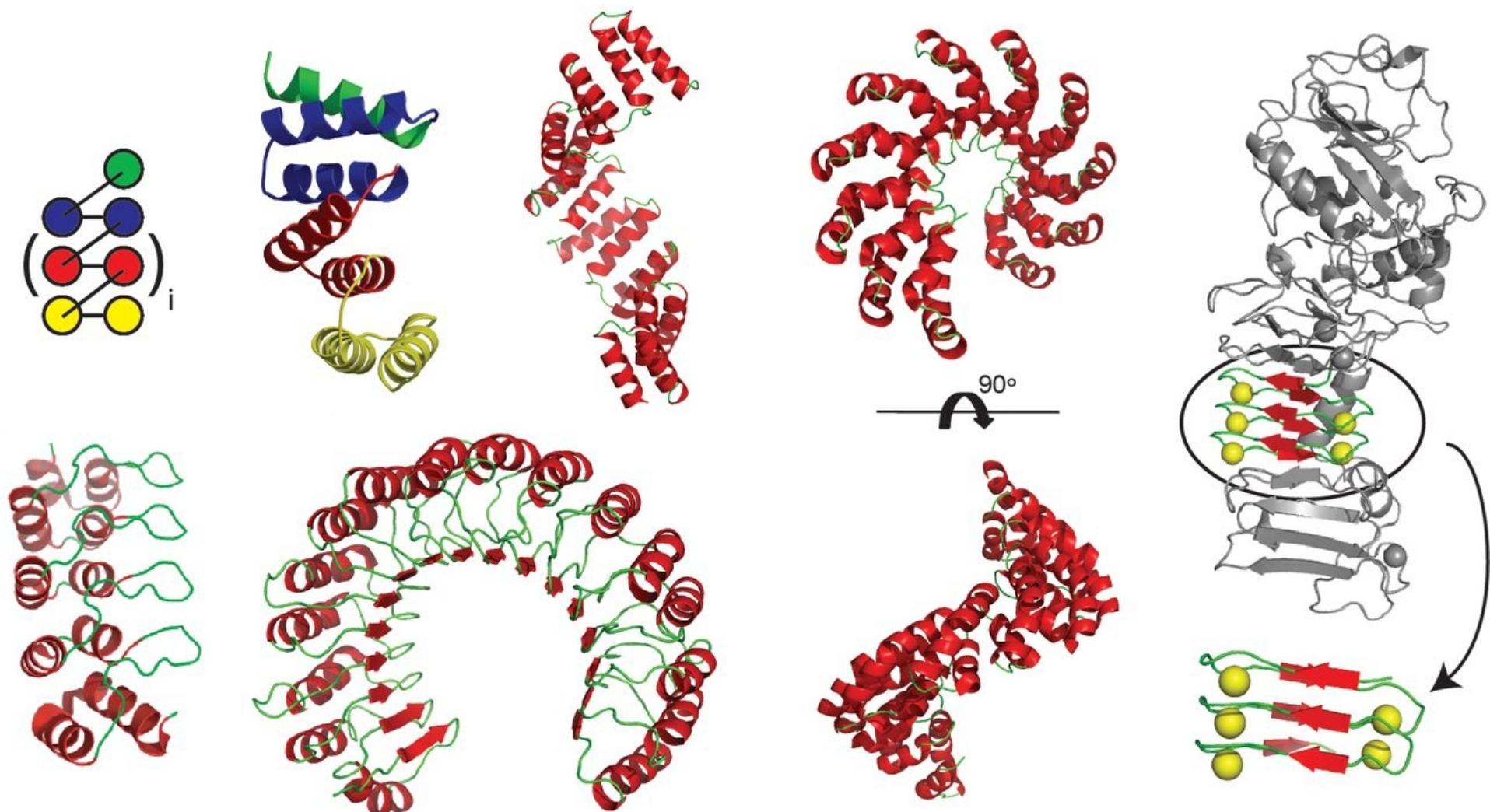


APC/C SUBUNITS ARCHITECTURE



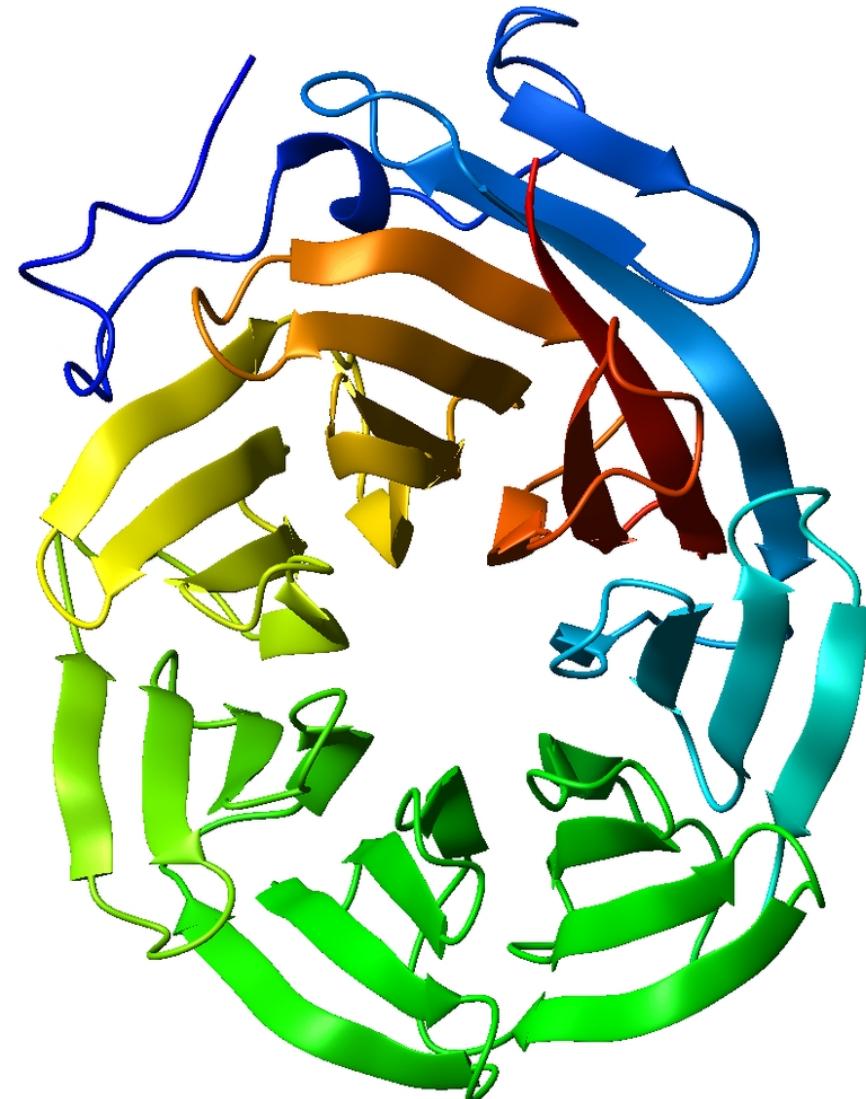
SCAFFOLD: TPR (TETRATRICOPEPTIDE)

- 34 aa
- 8 very conserved residues



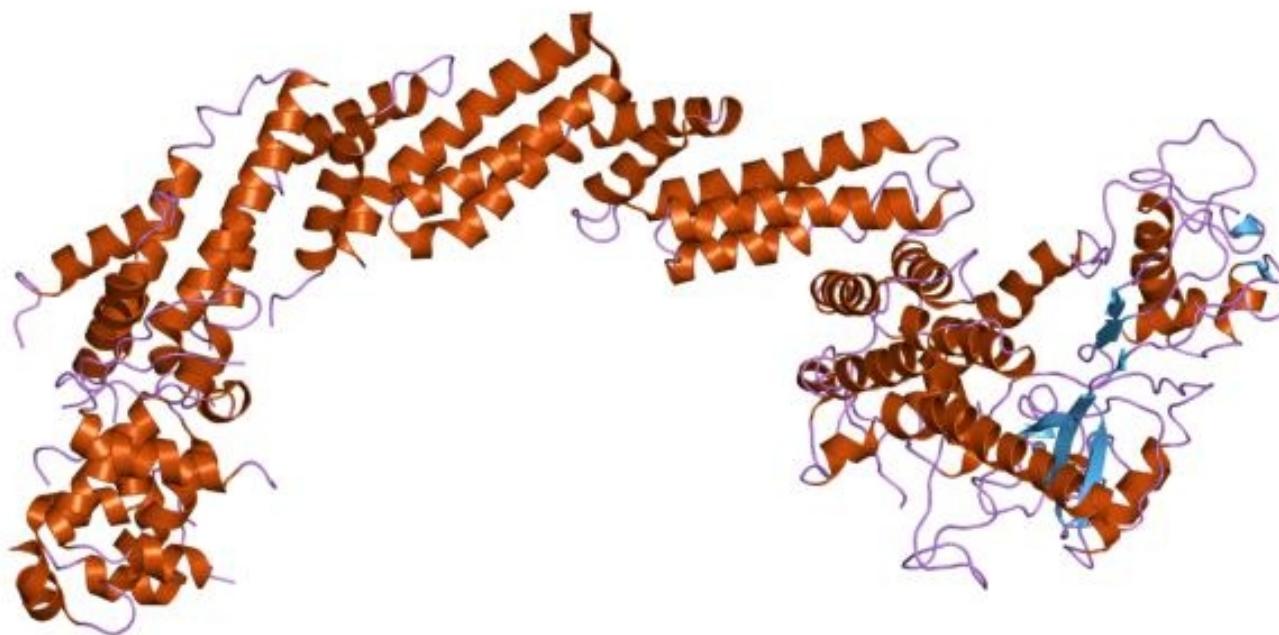
SCAFFOLD: WD40

- 40 aa
- Beta-propeller structure



SCAFFOLD: CULLIN

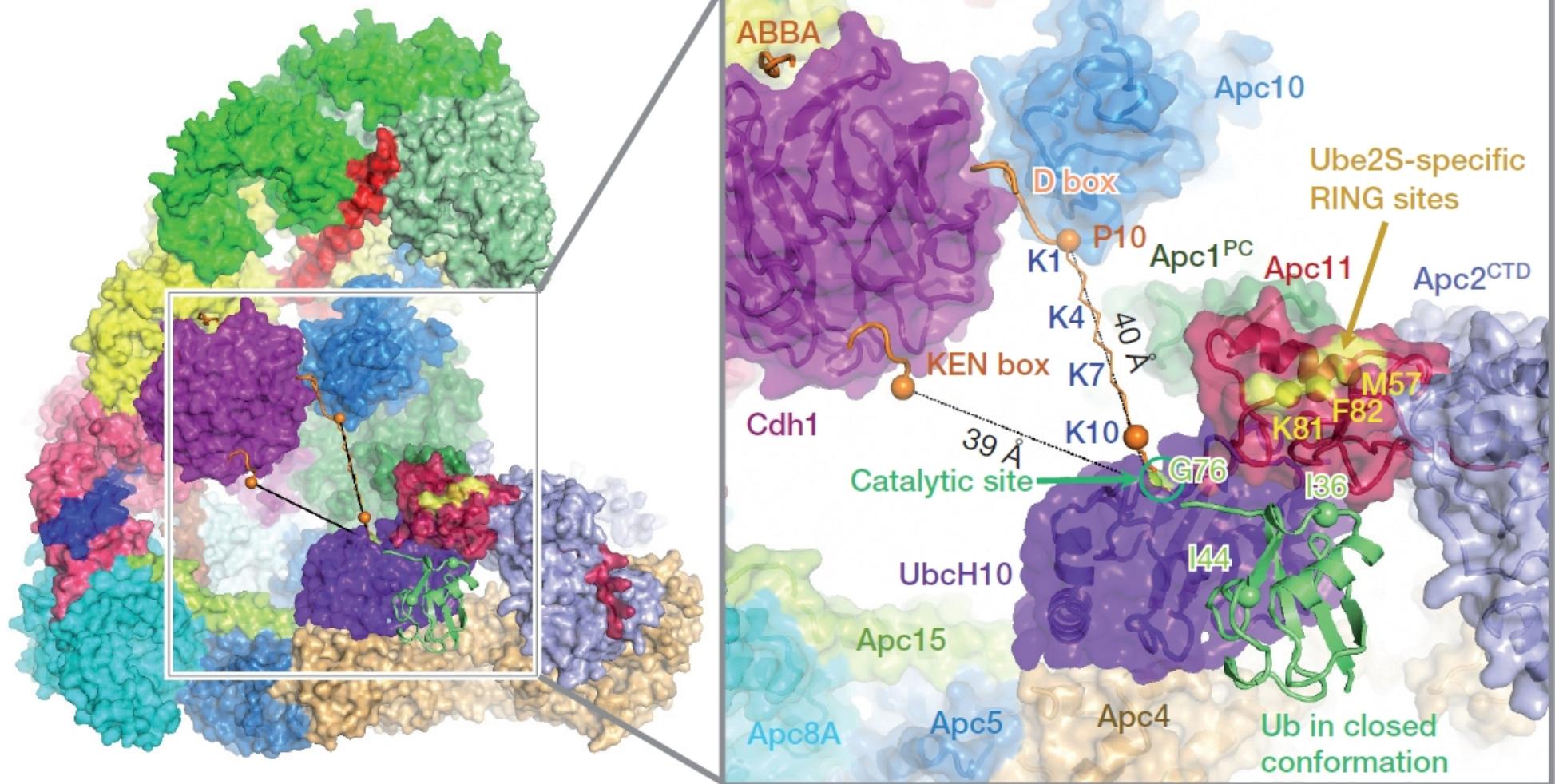
- ~ 750 aa
- Very hydrophobic



APC/C SUBSTRATE RECOGNITION

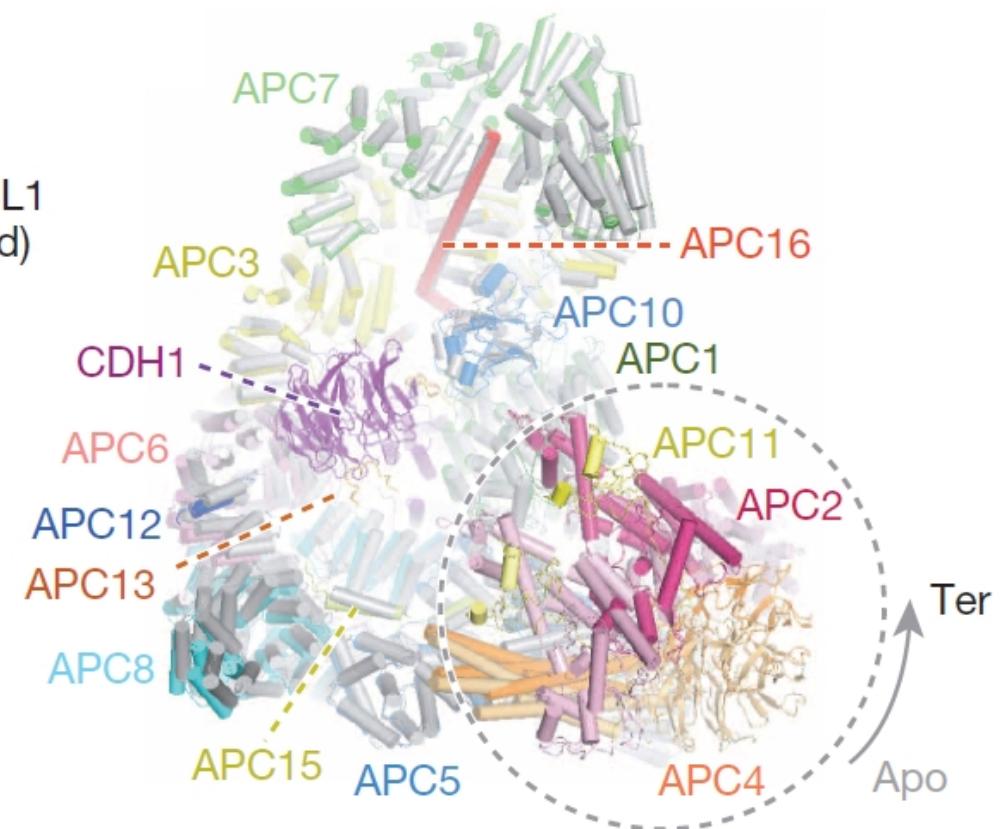
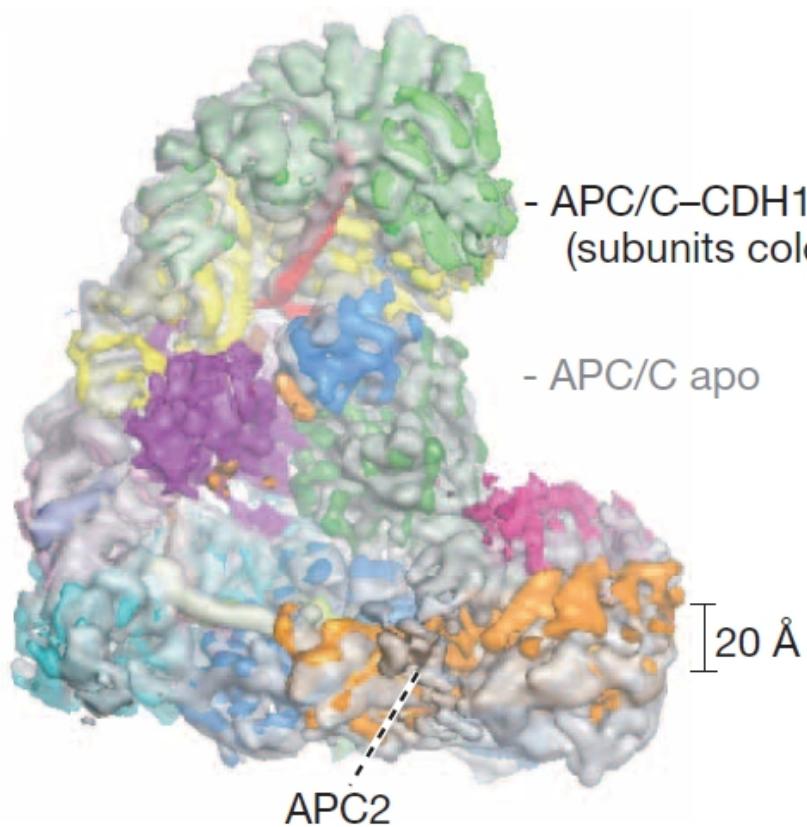
- Recognized sequences:
 - destruction box (D-box), RXXLXXXXN
 - KEN-box, KENXXXN
 - non-canonical motifs recognition
- Cdc20 and Cdh1 are receptors for the substrates (2 coactivators)
- Cdh1 is bigger than Cdc20 => broader substrate specificity
- Apc10 participation

APC/C SUBSTRATE RECOGNITION

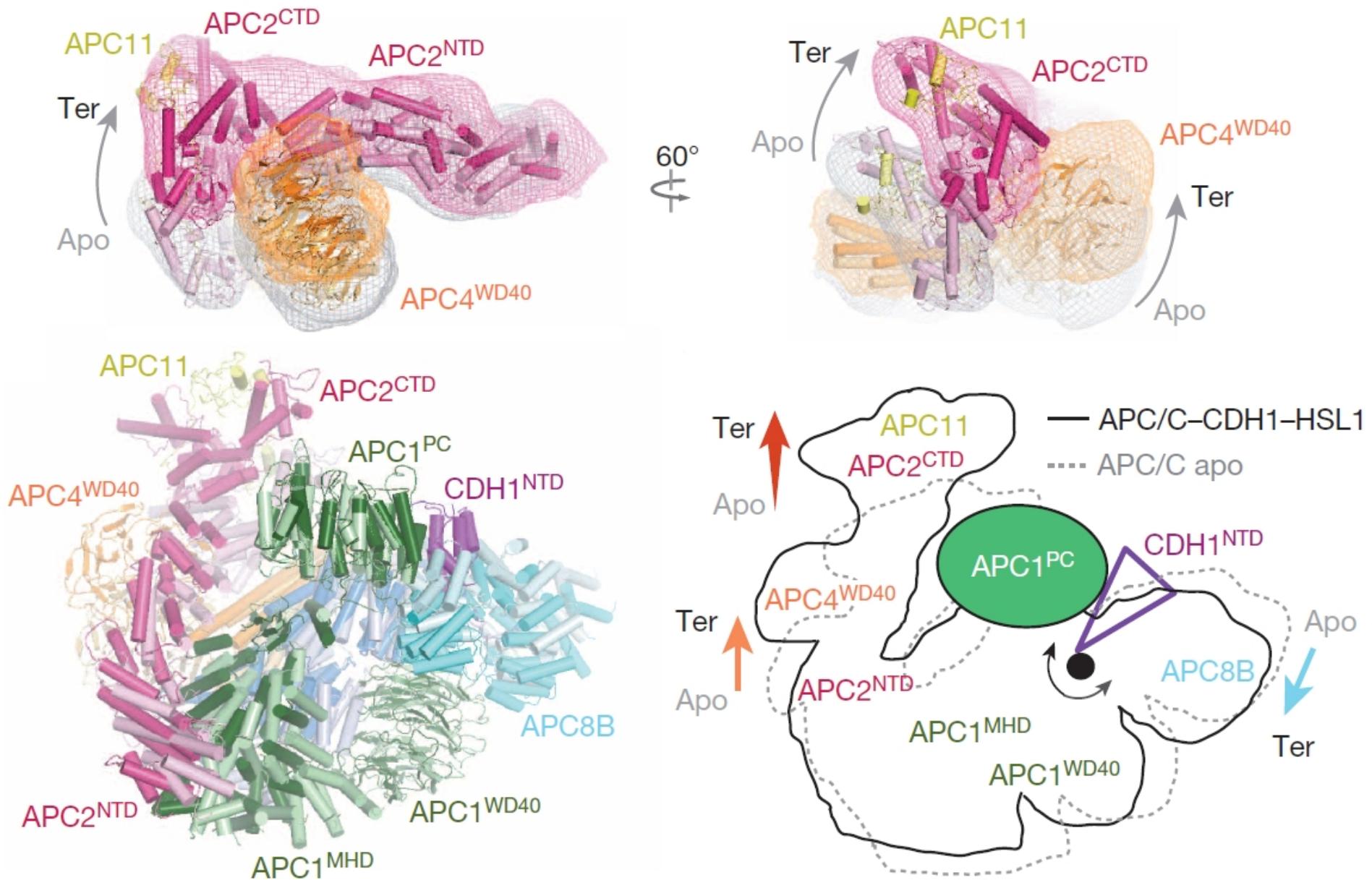


CDH1 ACTIVATION MECHANISM

- Enhancing the affinity of the APC/C for the E2
- Promoting the closed more reactive configuration of the E2 => more efficient ubiquitin attachment to the target lysines
- Inducing an allosteric transition of the E2

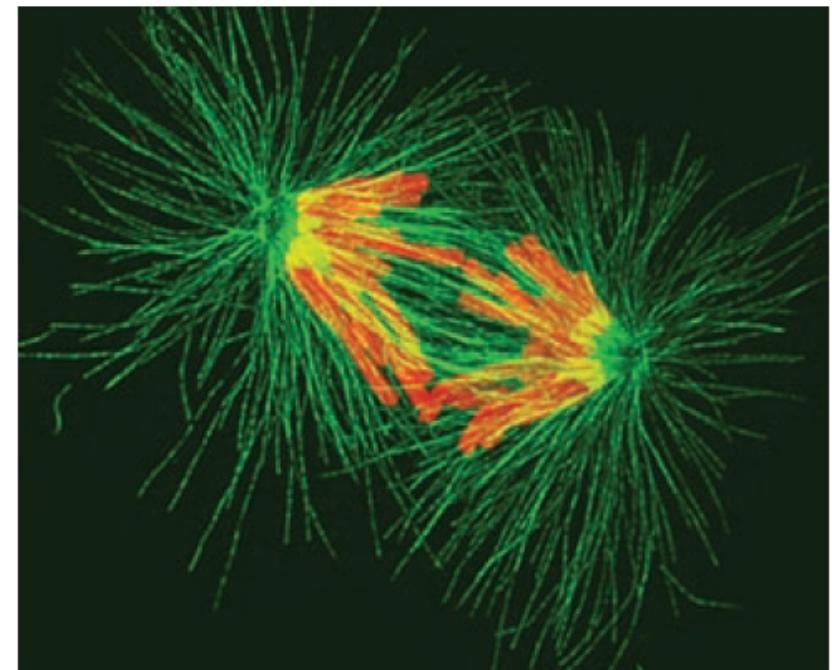
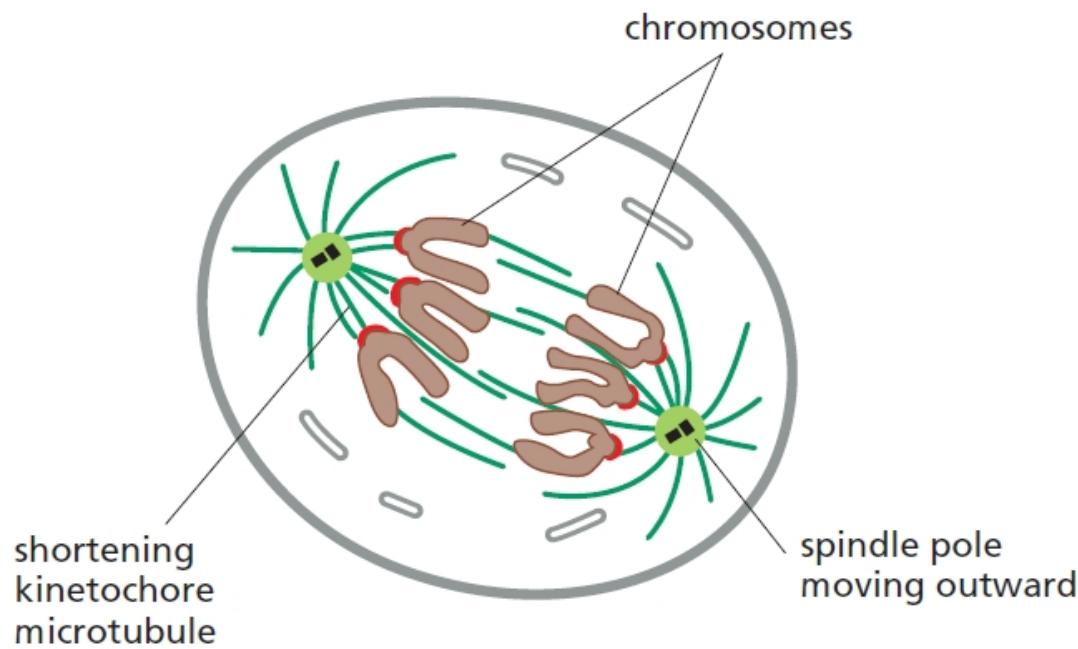


CDH1 ACTIVATION MECHANISM



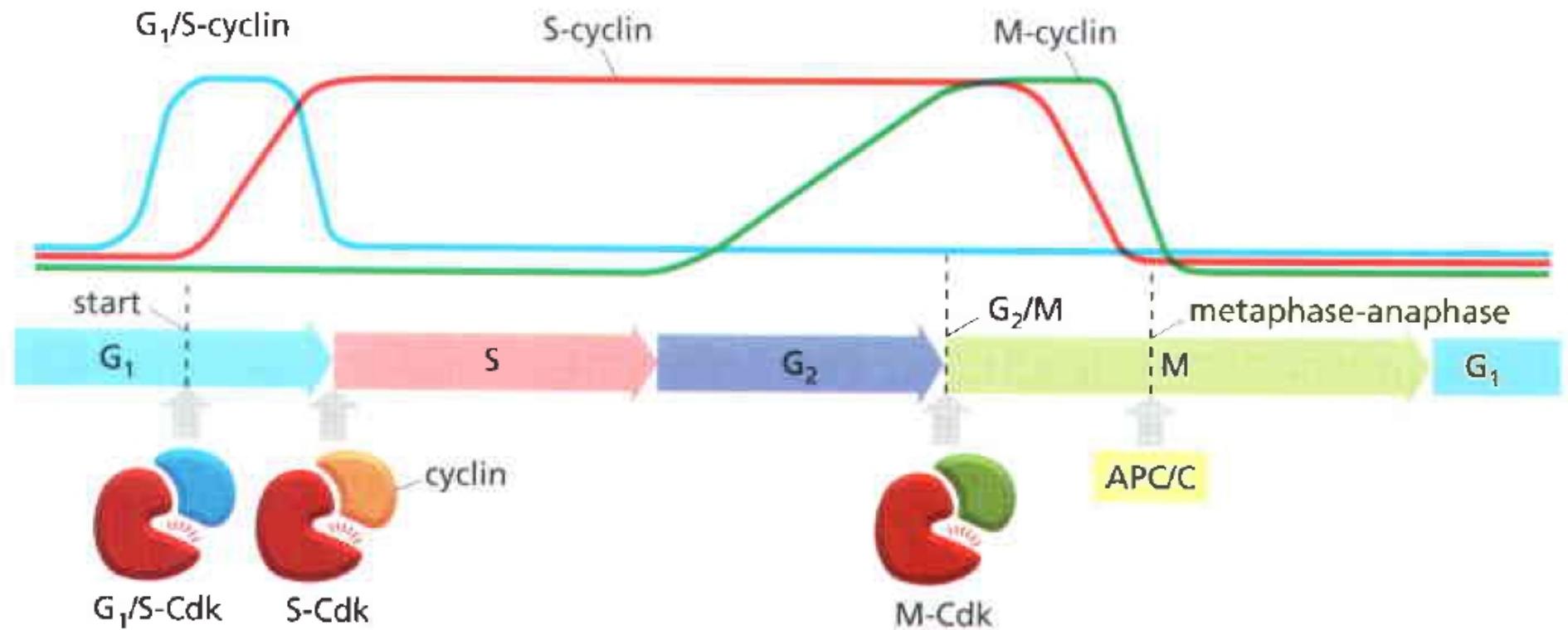
ANAPHASE

- The 4th step of metaphase
- Separation of sister chromatids

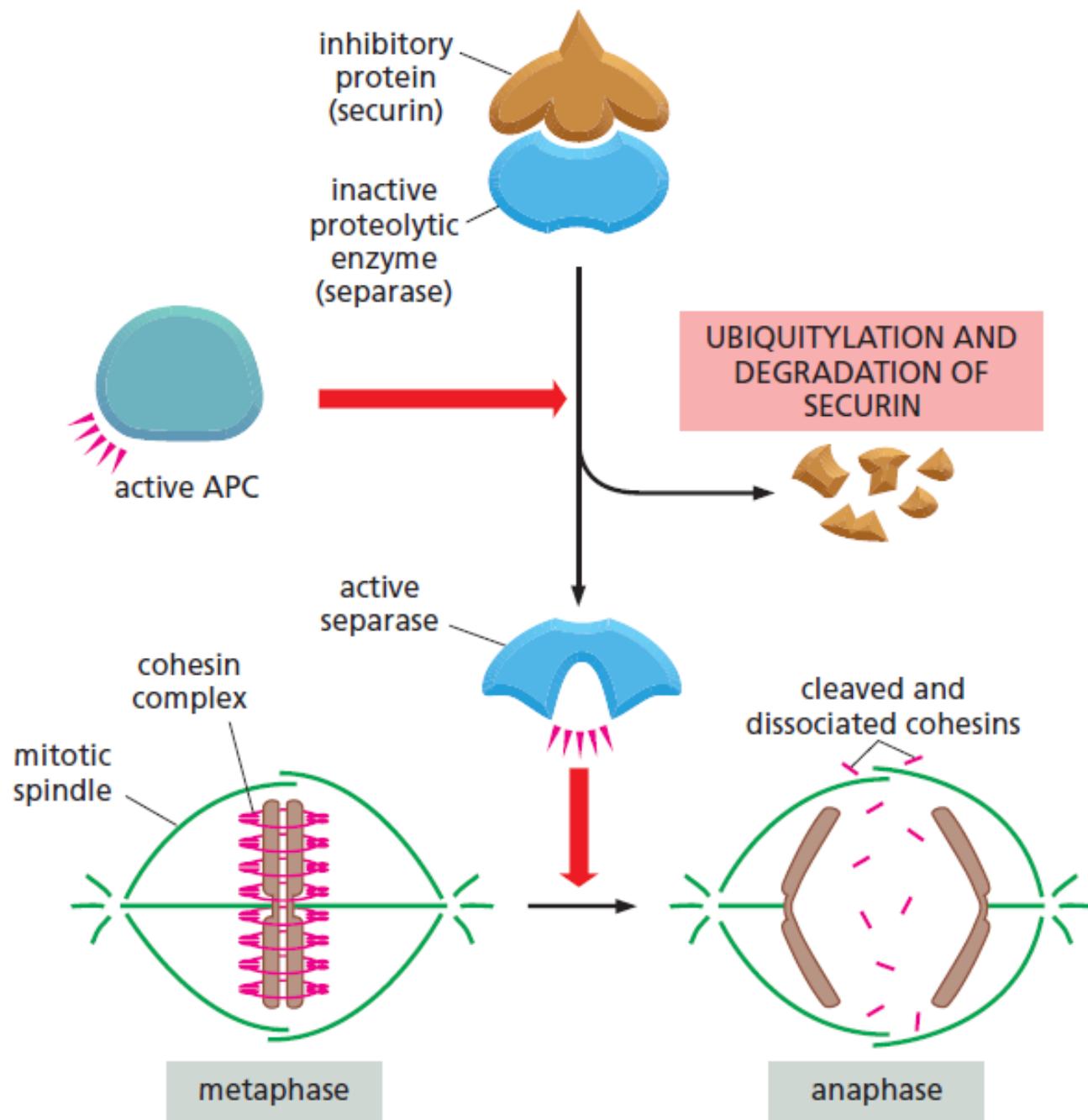


APC/C IN ANAPHASE

A protein complex that promotes the destruction of specific proteins, by catalyzing their ubiquitylation.



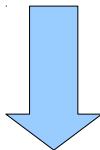
APC/C: GENERAL MECHANISM



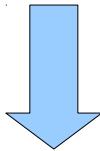
APC/C IN CYCLICAL PROTEOLYSIS

➤ Targets:

- securin => cohesin complex
- S- and M-cyclins => inactivation of Cdks



Dephosphorylation of many
Cdks targets

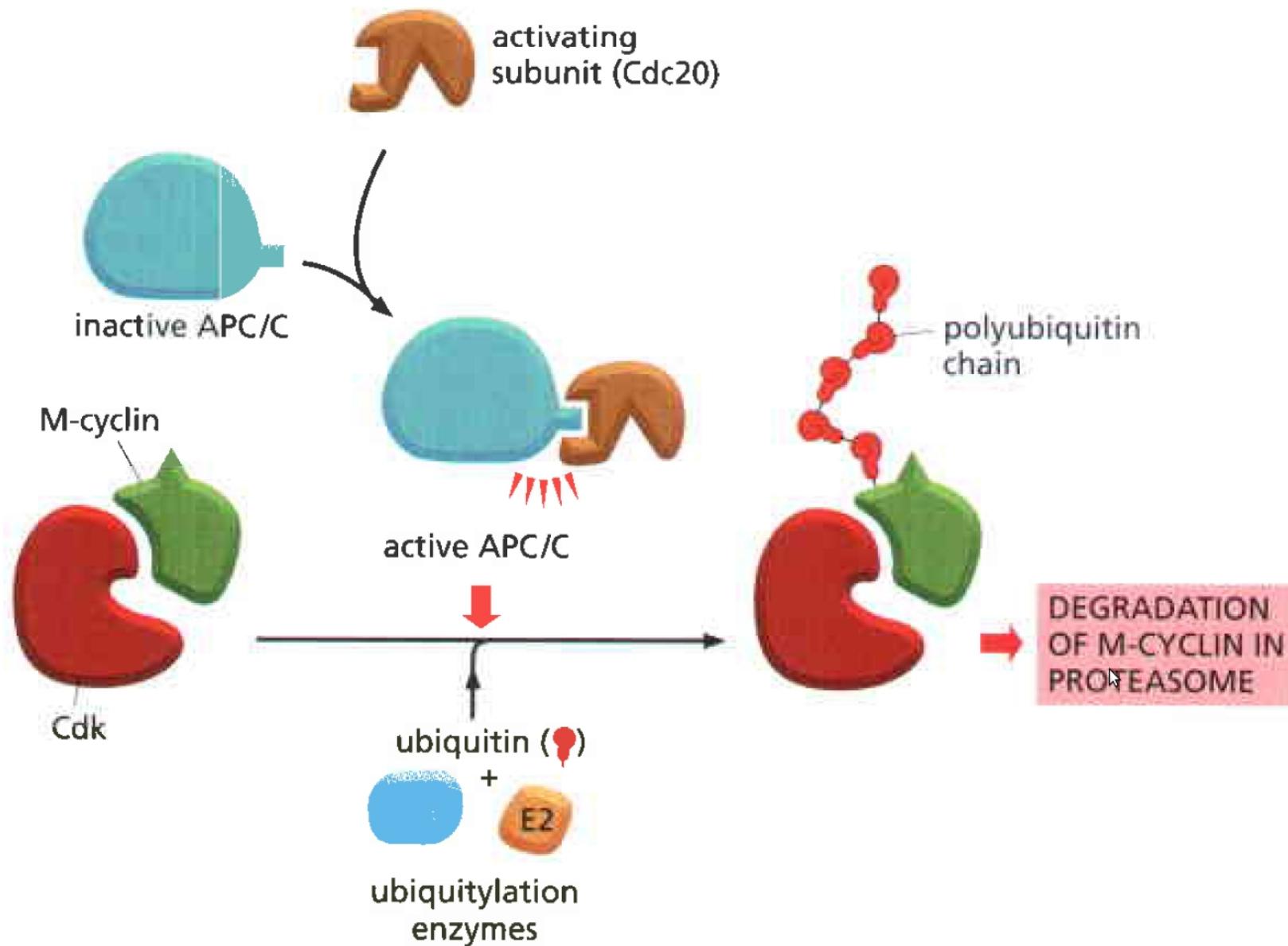


M phase completion

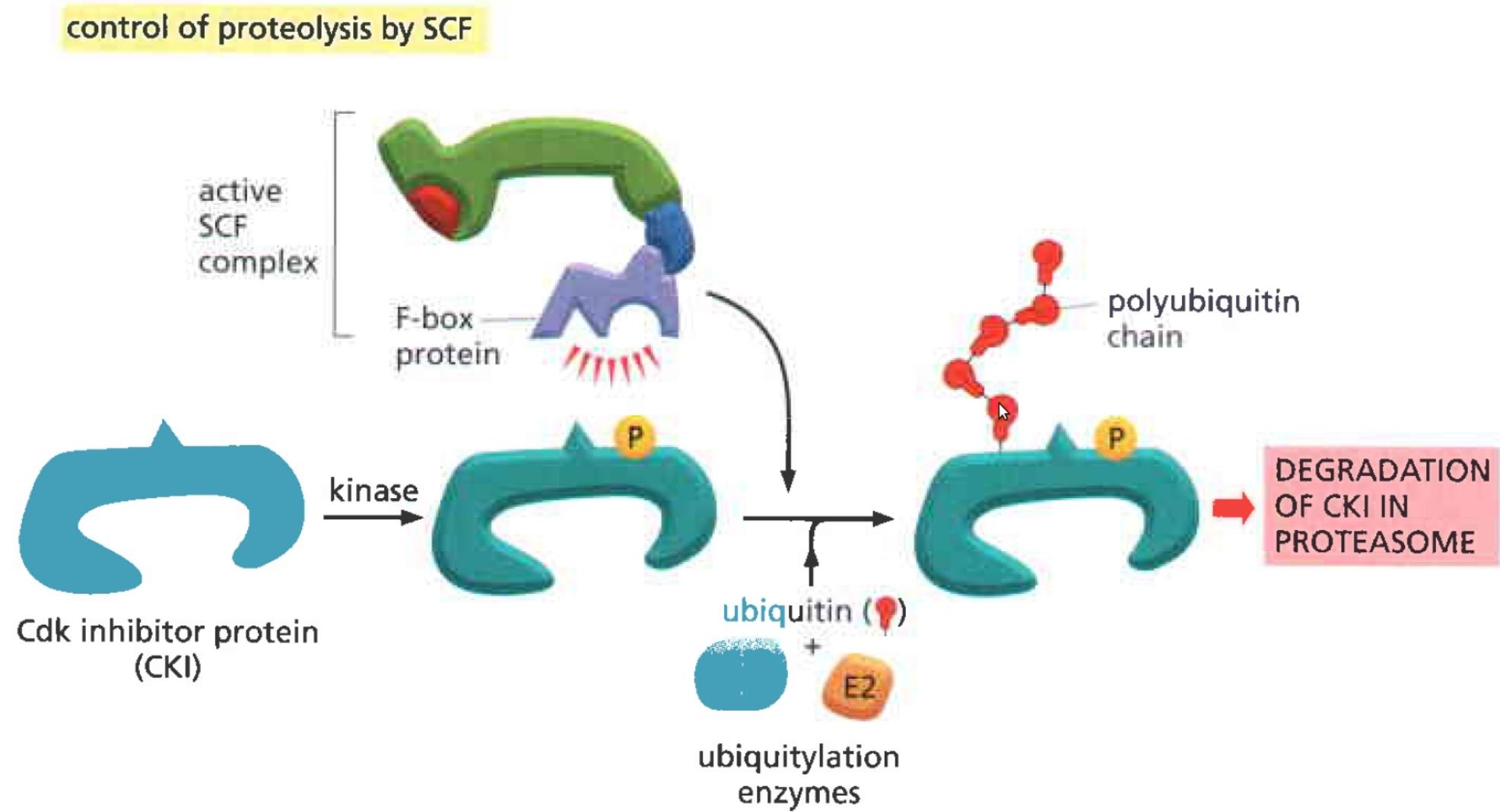
- Active until the end of G1 => Cdks inactivity
- Inactive from G1/S => new cyclin accumulation
- SCF- an another involved ubiquitin-ligase

APC/C PROTEOLYSIS CONTROL

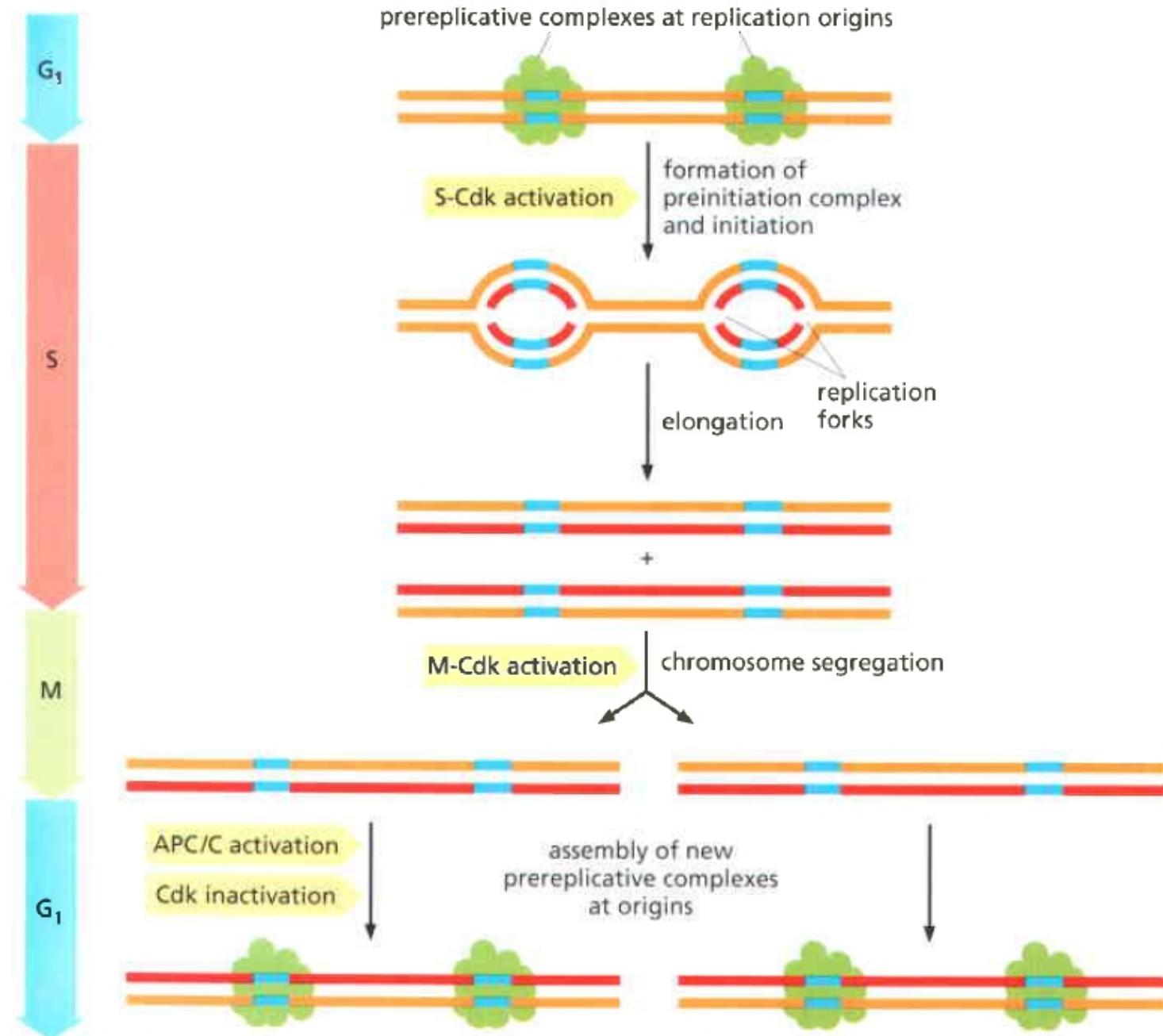
control of proteolysis by APC/C



APC/C PROTEOLYSIS CONTROL

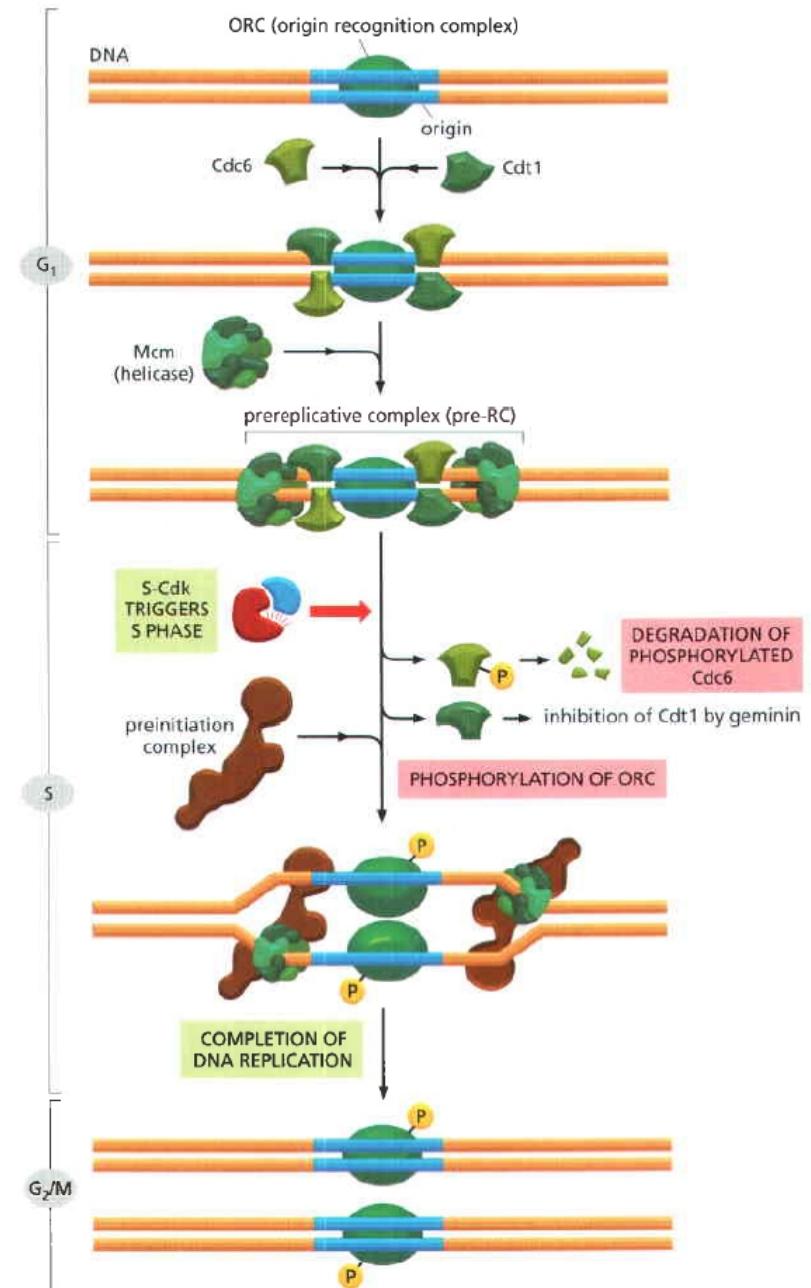
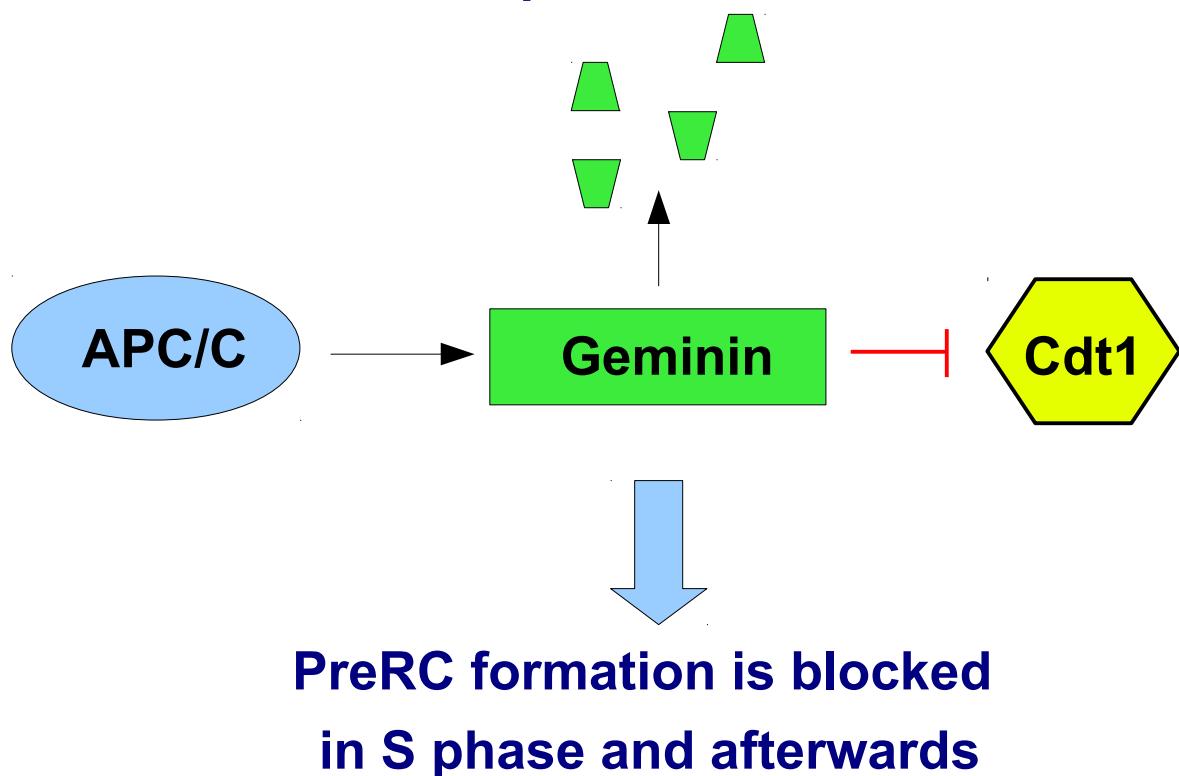


APC/C IN DNA REPLICATION PROCESSES



APC/C IN INITIATION OF DNA REPLICATION

- Original recognition complex (ORC): binding to replication origin
- + Cdc6 + Cdt1 + Mcm proteins
- S-Cdk triggers the start of replication
- APC/C:
 - active in G1 phase
 - inactive in S-M phases



APC/C IN REGULATION OF M-CYCLIN LEVEL

➤ APC/C Cdk destruction is a key

regulatory event in M phase:

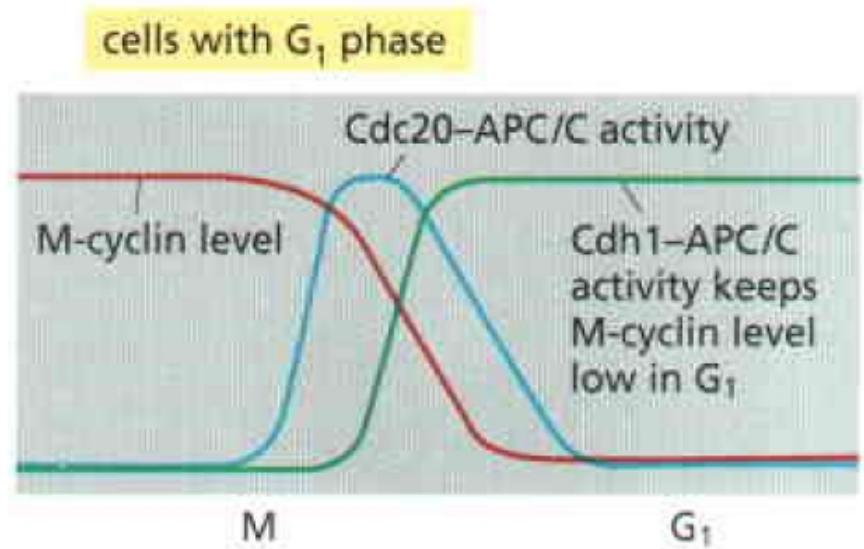
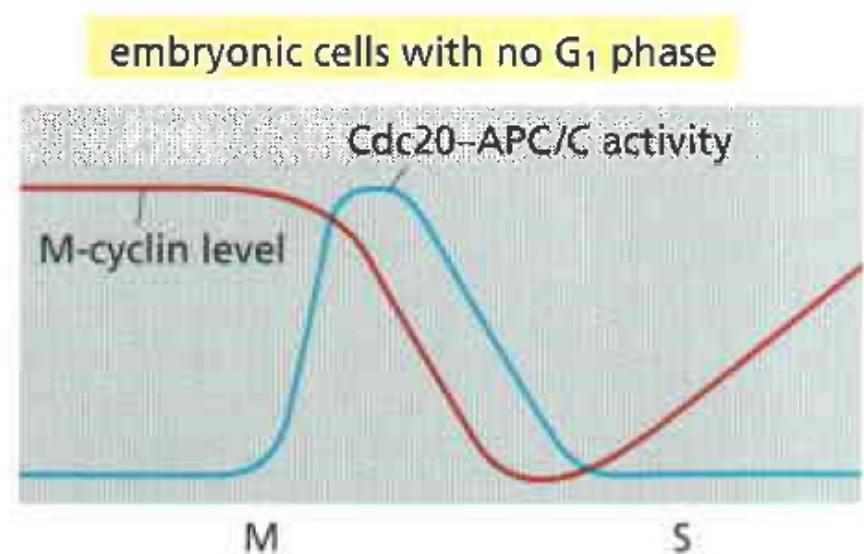
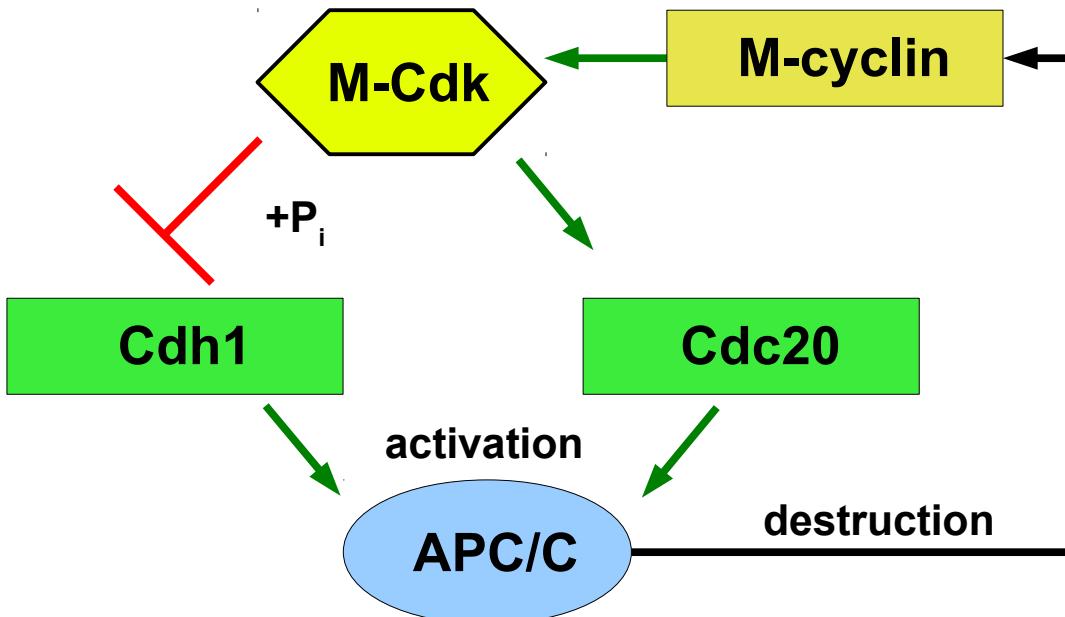
- end of mitosis

- promotion of cytokinesis

- synthesis of PreRC

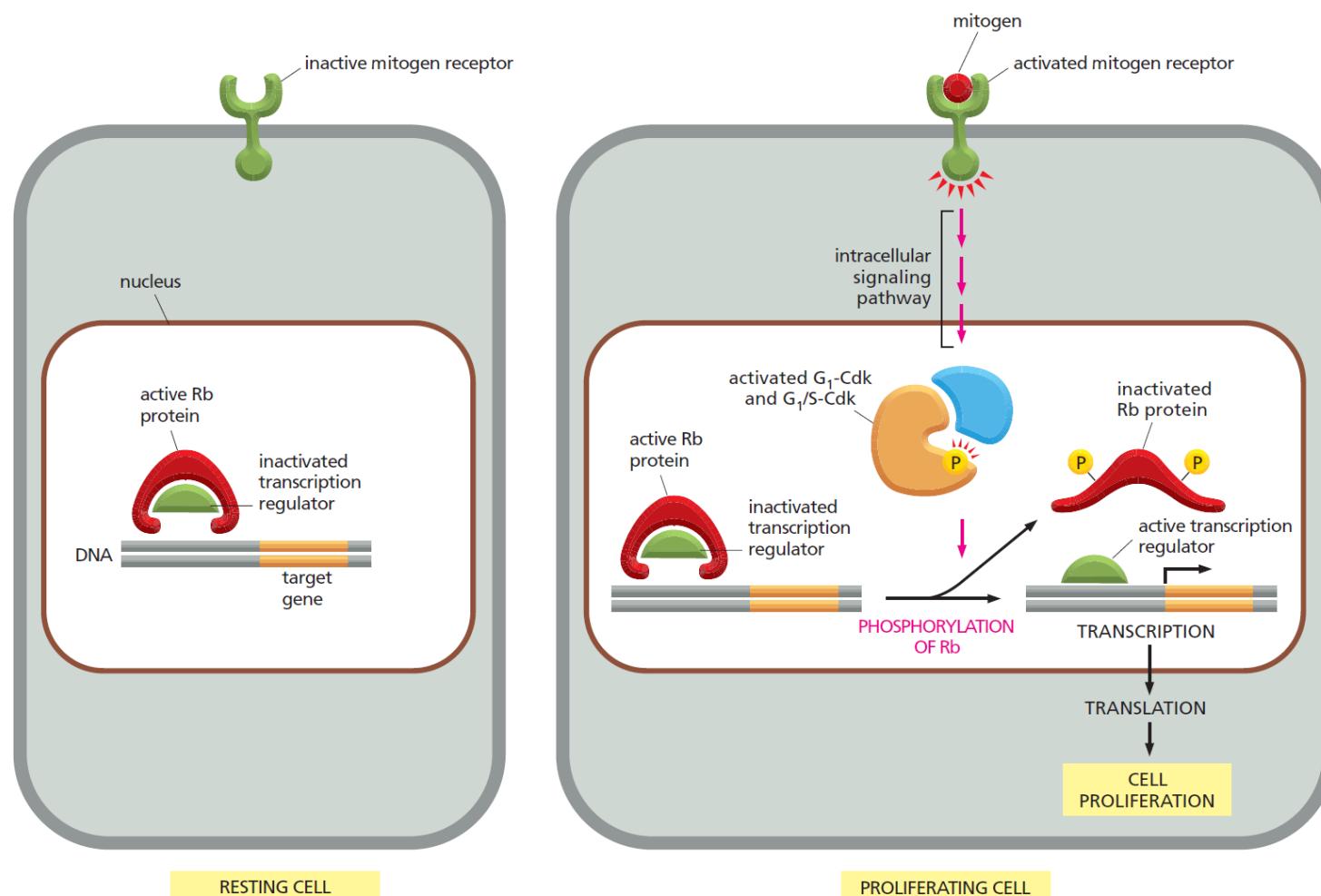
➤ G1 existence is guided by APC/C

activator Cdh1



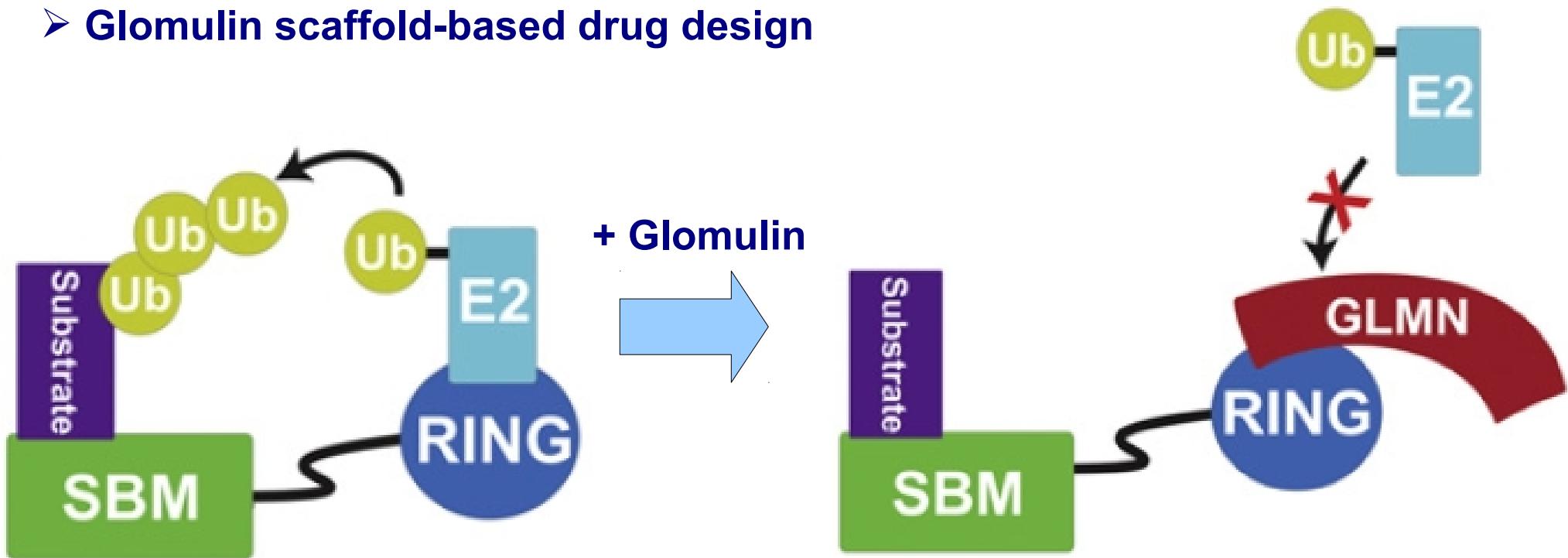
APC/C AND MITOGENS MECHANISM

- G₁-cyclins are resistant to Cdh1/APC/C => Rb phosphorylation => E2F gene expression
- S-cyclins are not resistant to Cdh1/APC/C
- G₁/S-Cdks inactivate Cdh1/APC/C



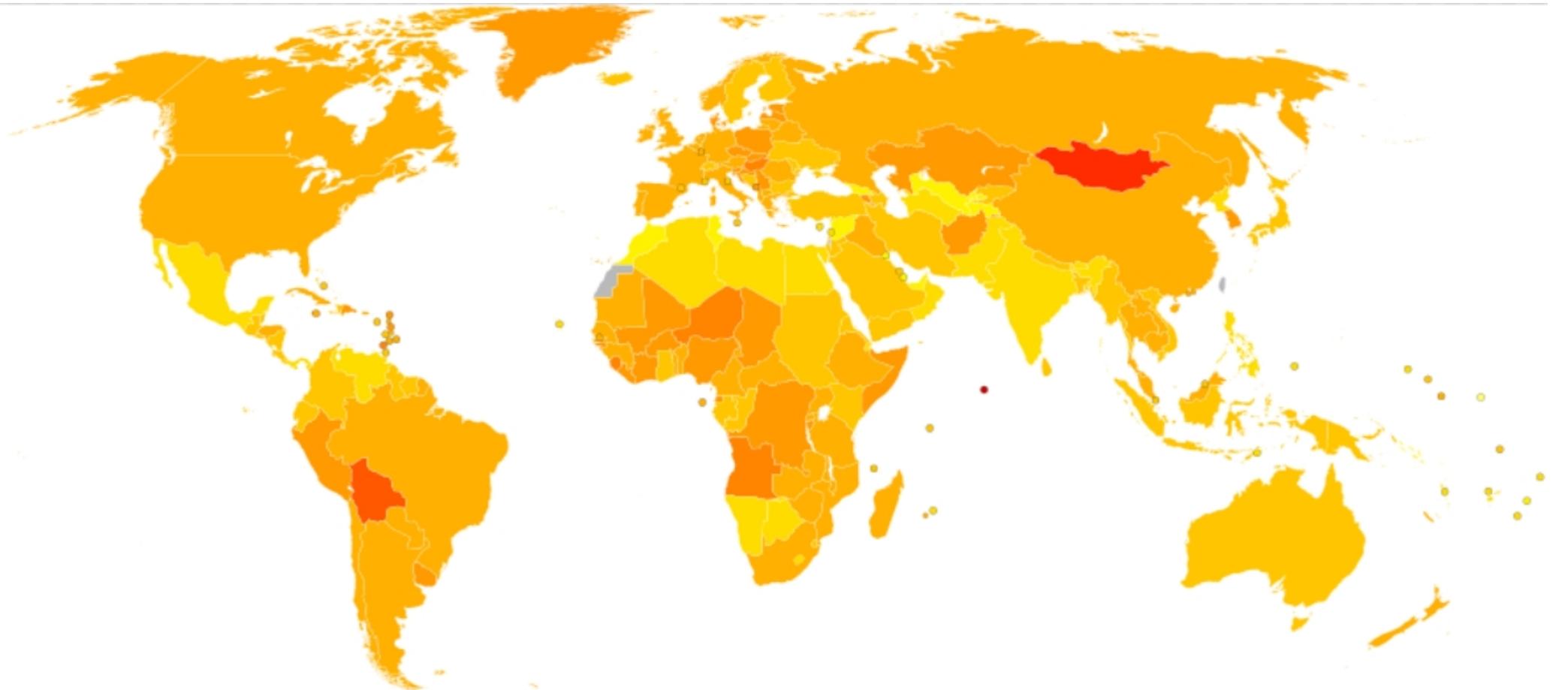
APC/C IN CANCER

- SBM: substrate binding module
- GLMN: glomulin
- Glomulin scaffold-based drug design



CANCER

Group of diseases involving abnormal cell growth with the potential to
invade or spread to other parts of the body



55-300 per 100 000 per year adjusted by age
~ 13% of all deaths; ~ 25% in developed countries

CANCER



Dutch engraving, 1689

**Tissues
organization**

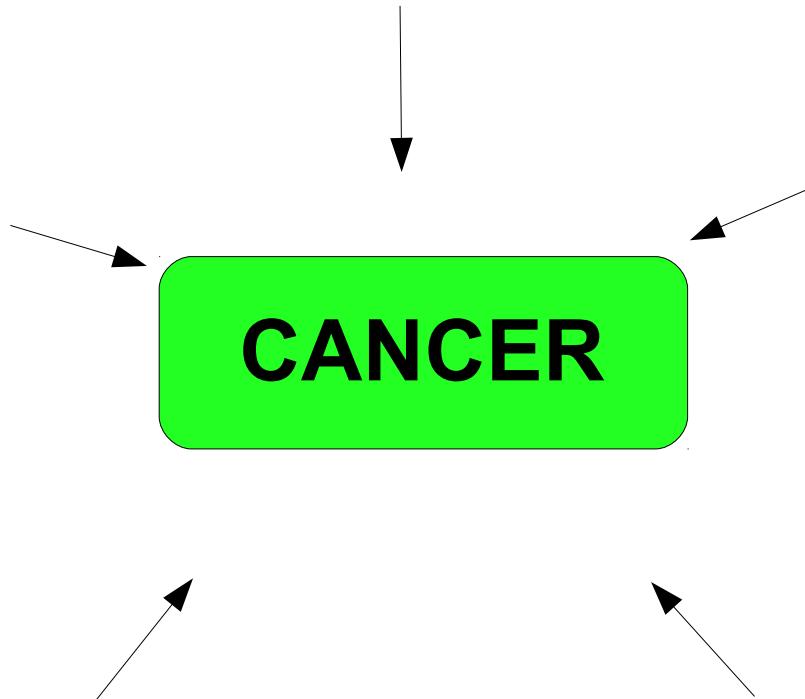
DNA repair

**Cell
signaling**

CANCER

Apoptosis

Cell cycle



CANCER

➤ **Social behaviour of the cells:**

- division
- growth
- rest
- differentiation
- death

➤ **Mutations**

➤ **Natural selections**

➤ **Microevolutionary process**



Metastases

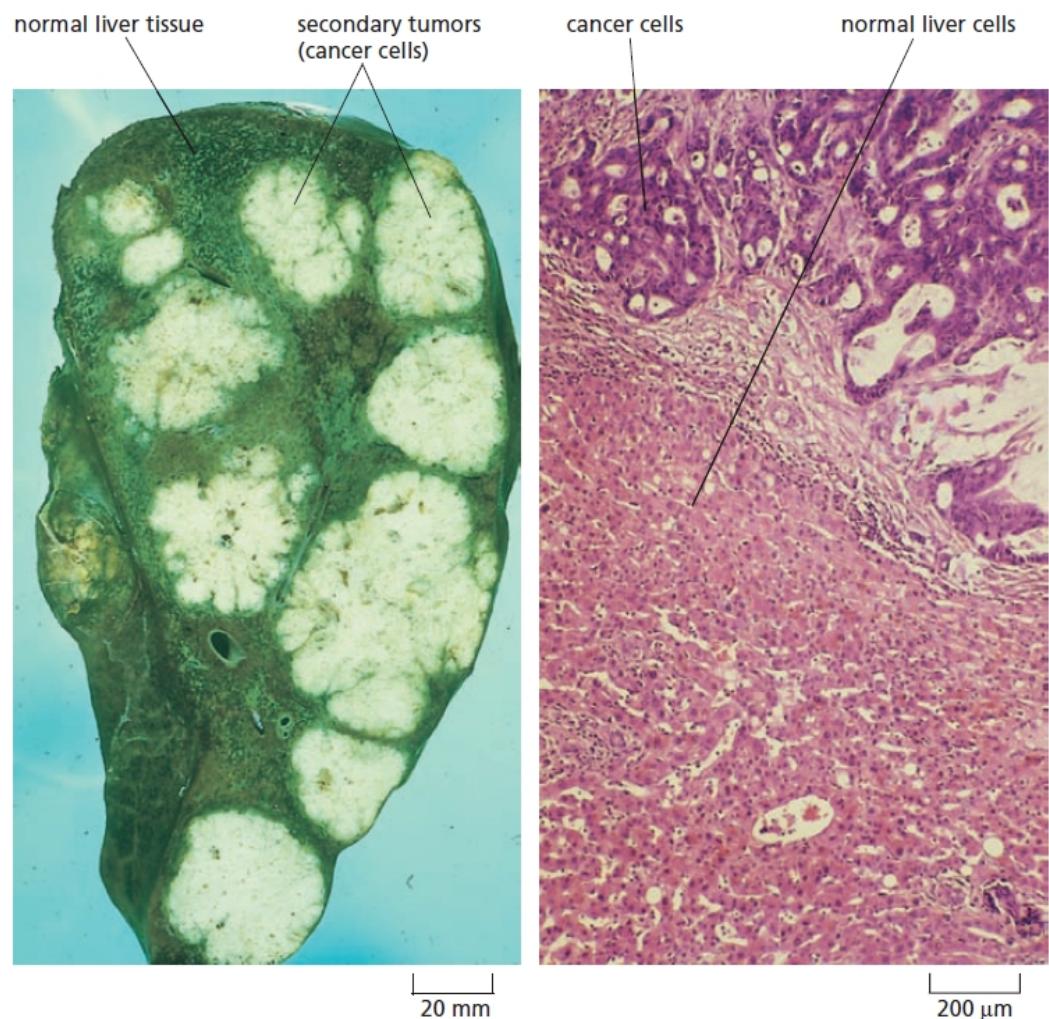
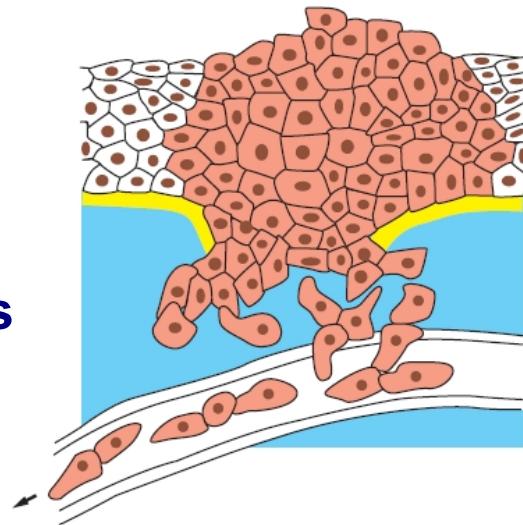
CANCER

➤ Causes:

- Proliferation in defiance of normal constraints (division/death balance)
- Invasion and colonization of other territories

➤ Cells:

- benign
- malignant
- metastases



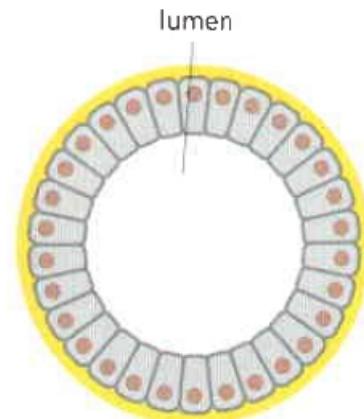
➤ Classifications:

- carcinoma (epithelium), ~ 80%
- sarcoma (organs, muscles)
- leukemia (blood)
- lymphoma (lymph)

- adenoma (benign)
- chondroma (malignant)

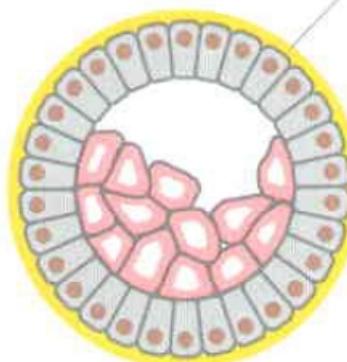
tumour TYPES

Not invasive



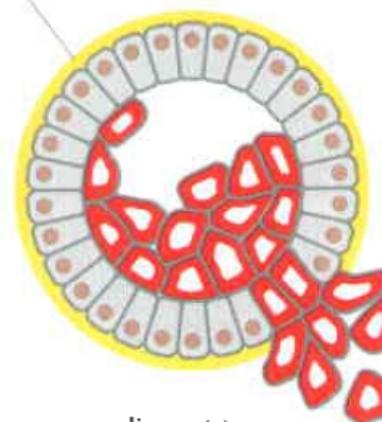
normal duct

basal lamina

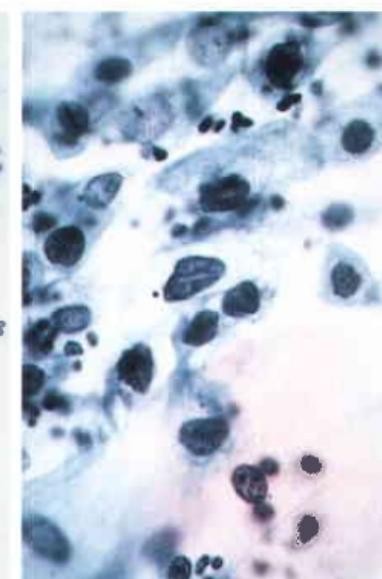
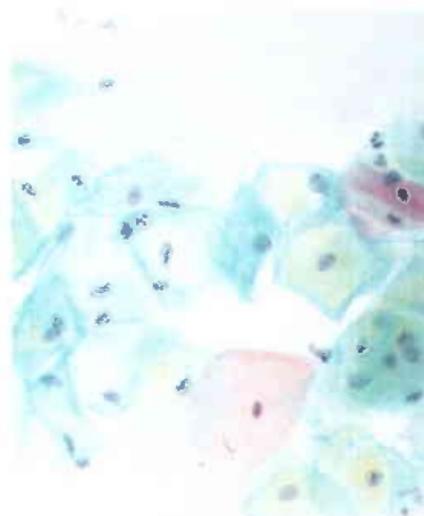


benign tumor

Invasive



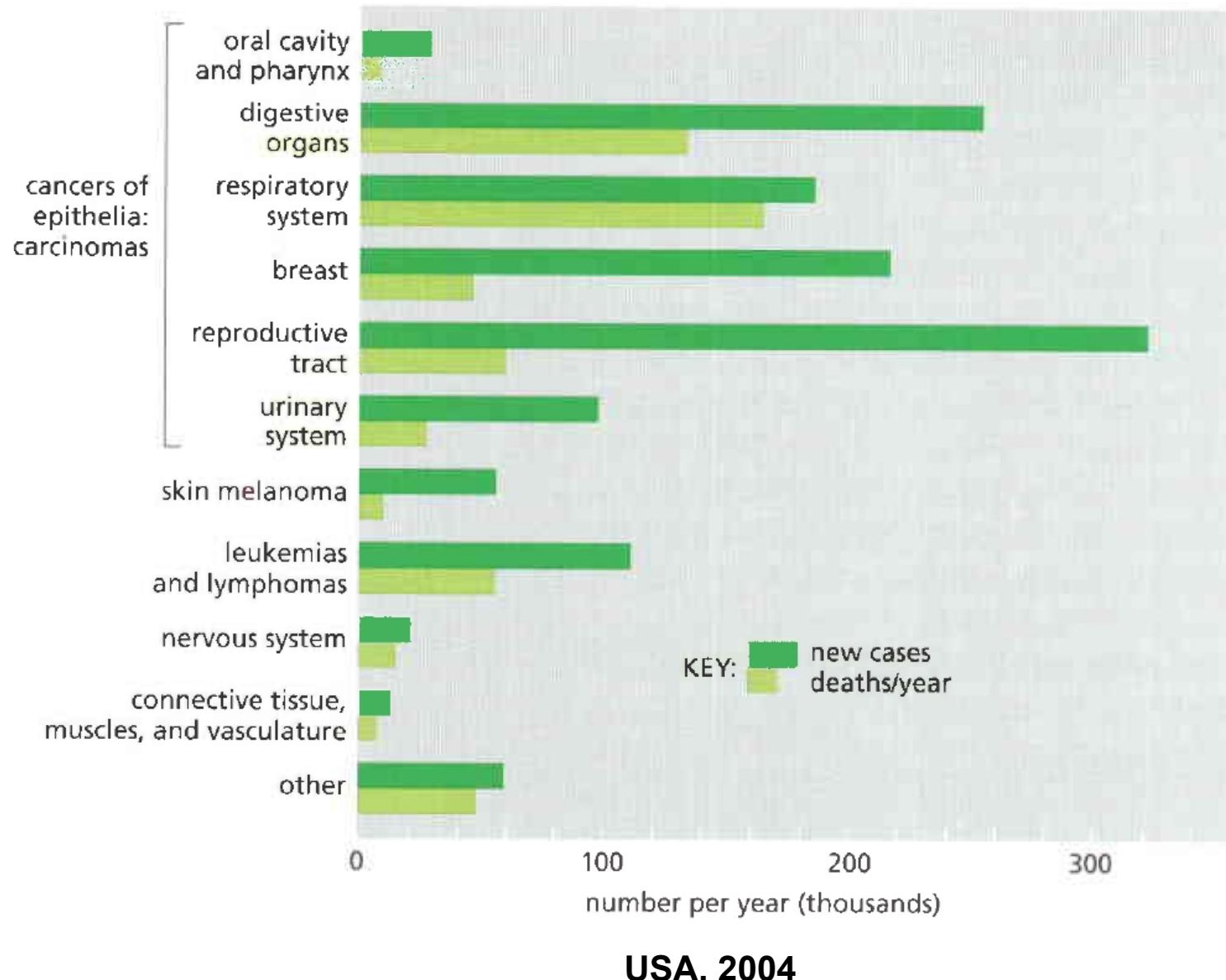
malignant tumor



Uterine cervix

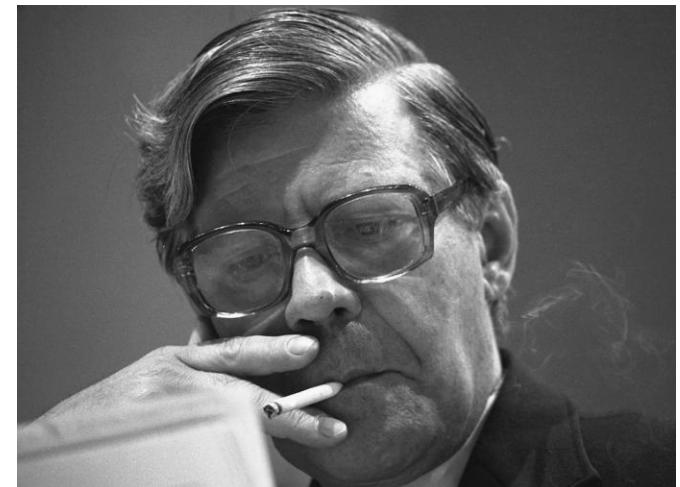
10 μm

CANCER TYPES AND MORTALITY

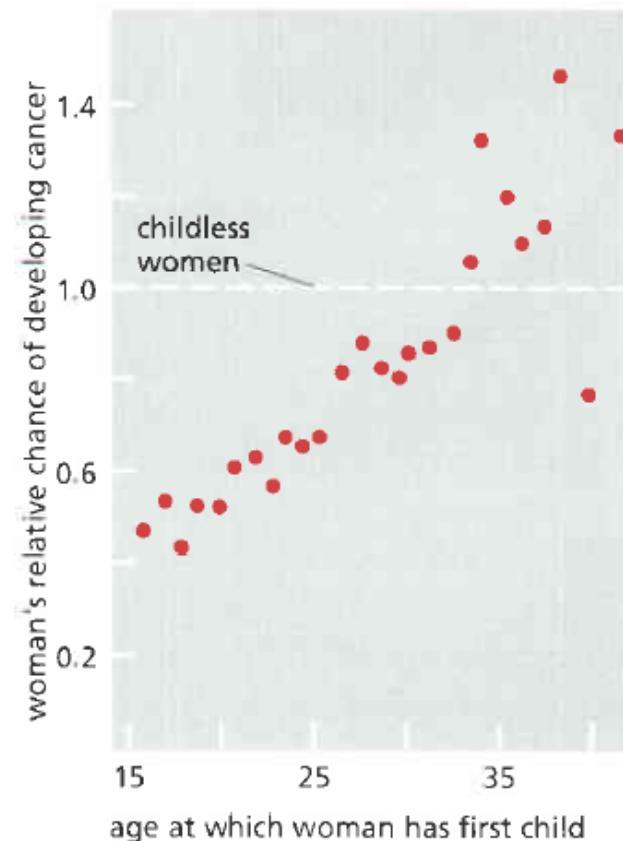


CANCER RISK FACTORS

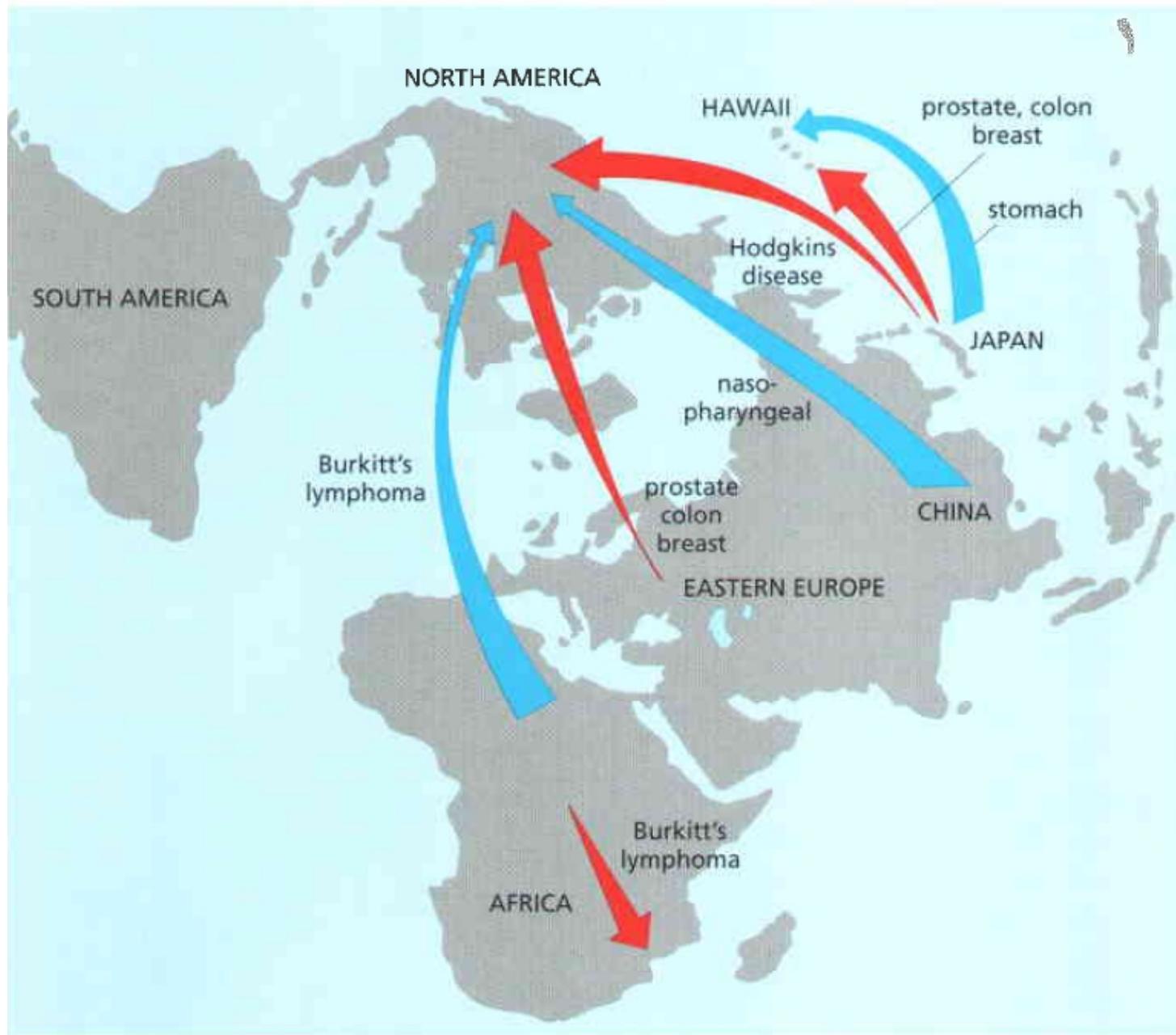
- Genetics < Environment
- Diet (obesity, alcohol, coffee, meat)
- Smoking
- Hormones
- 80-90% of cancers are avoidable



Helmut Schmidt (1918-2015)



CANCER RISK FACTORS



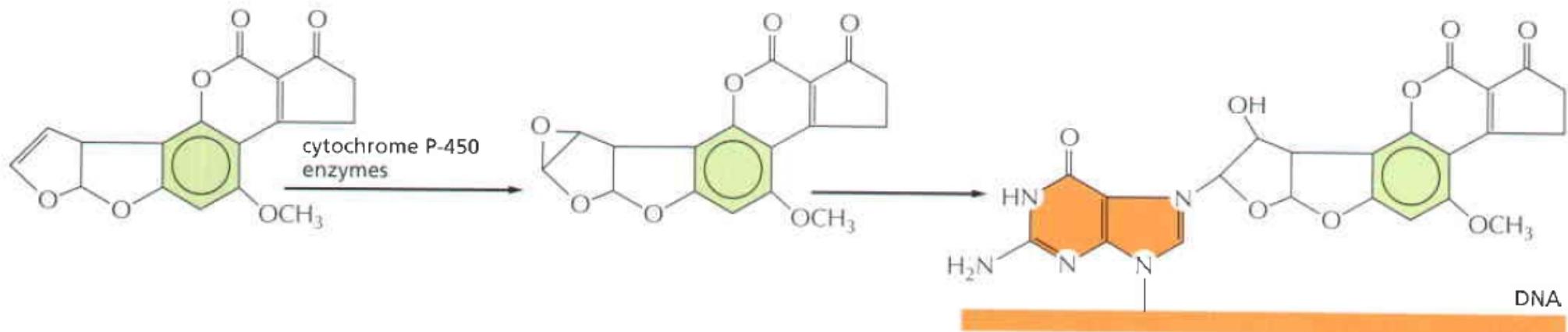
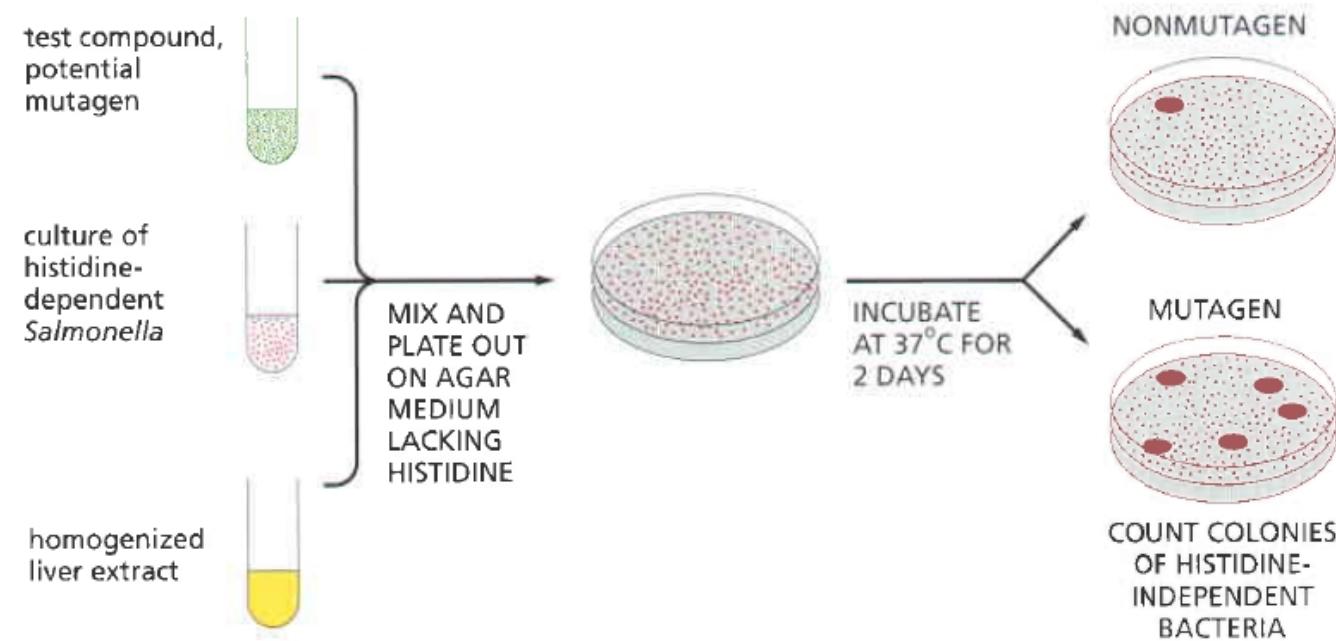
Increasing Decreasing

CANCER: DNA MOLECULAR BASIS

- Somatic DNA mutations
- Ionizing radiation and chemical carcinogens
- Probability:
 - error in replication: 10^{-6} per cell division per gene
 - 10^{16} in human lifetime
 - 10^9 errors
- Chromosomal abnormalities



CANCEROGENS



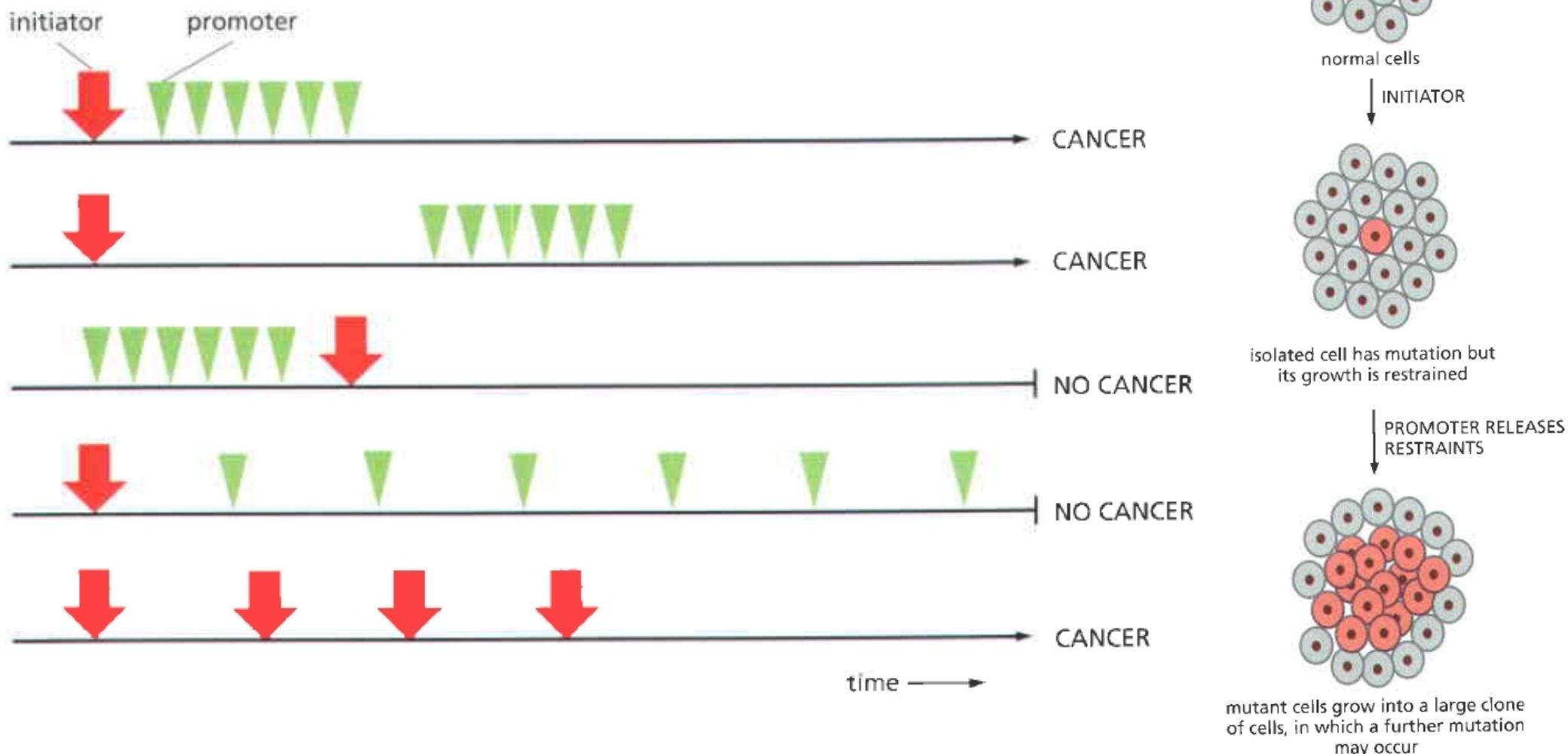
AFLATOXIN

AFLATOXIN-2,3-EPOXIDE

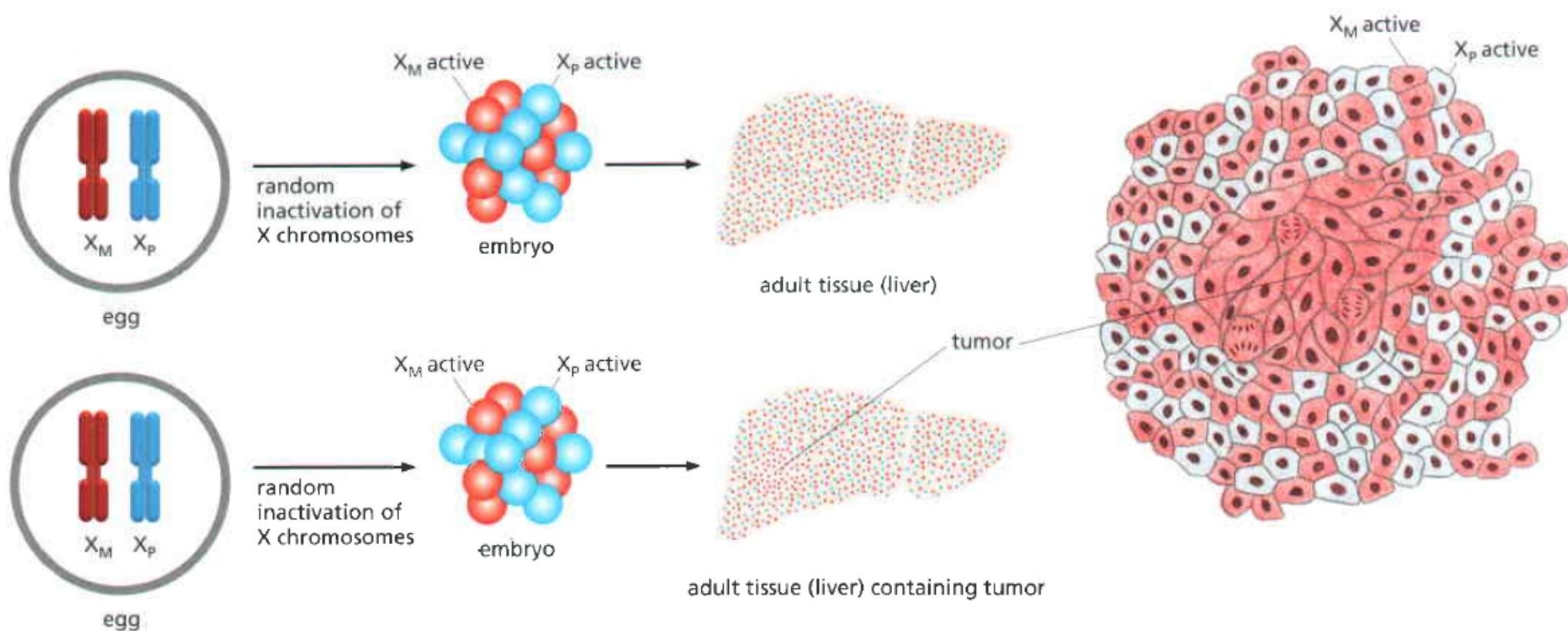
CARCINOGEN BOUND TO GUANINE IN DNA

tumour INITIATORS AND PROMOTORS

- Initiator: mutagen => increases the risk of cancer, latent
- Promotor: not a mutagen, promotes the cancer in risky cells (through growth factors, proteases etc.)
- Viruses

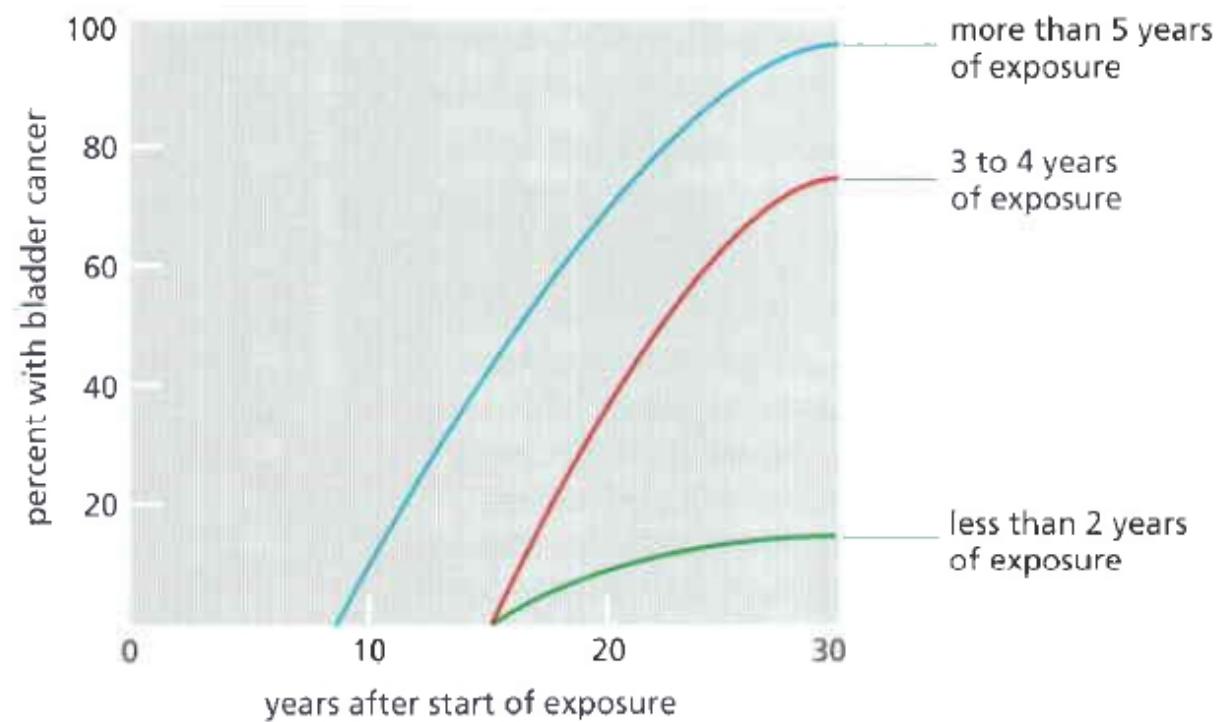
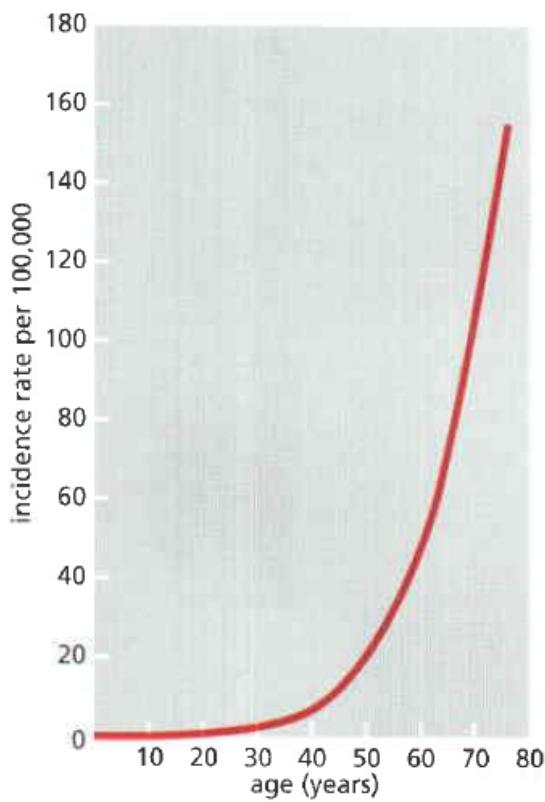


CANCER: DEVELOPMENT FROM A SINGLE CELL



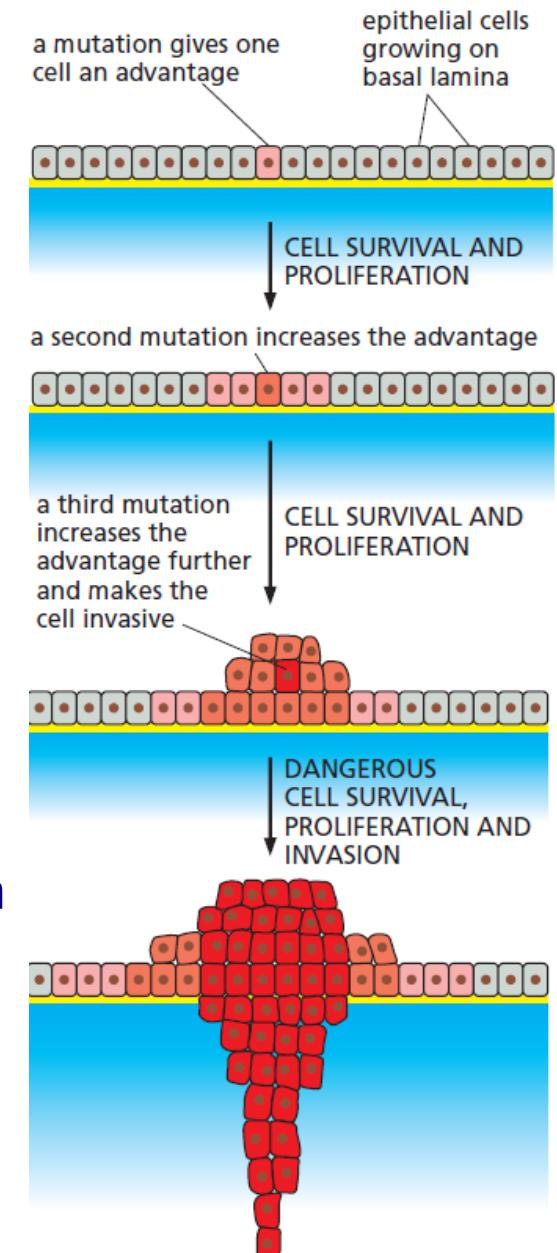
CANCER EVOLUTION IN THE BODY

- Gradual and slow development
- Mutations in at least 5 genes => tumour progression



CANCER CELLS ARE HIGHLY COMPETITIVE

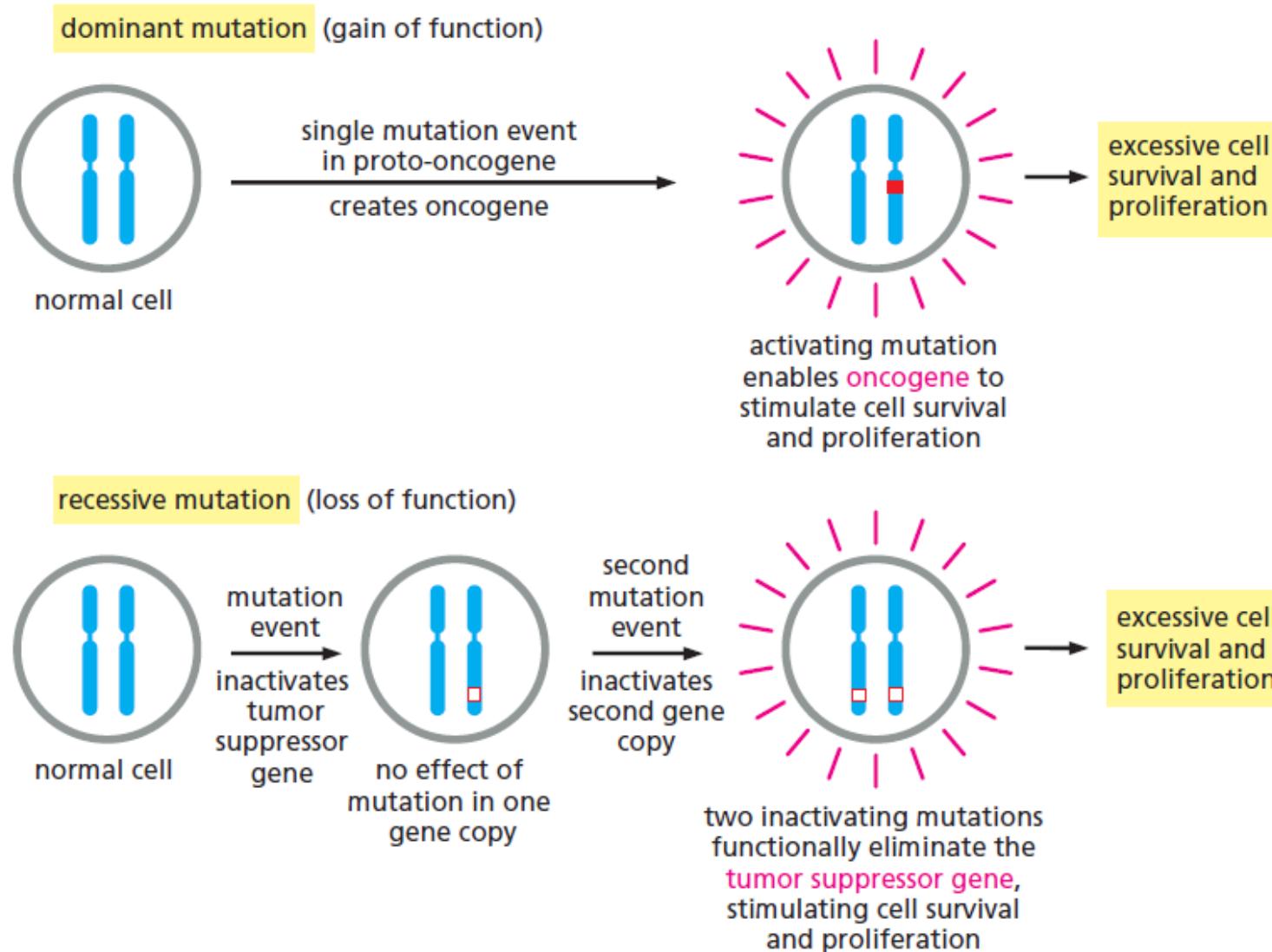
- In general, cancer cells are 'stronger' in division and proliferation
- Mutations are spread in a fast manner
- Key features of cancer cells:
 - reduced dependence on the signals from other cells
 - disrupted apoptosis (50% of cancers => p53 defect)
 - indefinite proliferation
 - unstable in terms of higher mutations rate
 - highly invasive
 - survival in metastases
- Evolution rate dependence:
 - the mutation rate
 - the number of reproducing individuals in the population
 - the rate of reproduction
 - the selective advantage



ONCOGENES

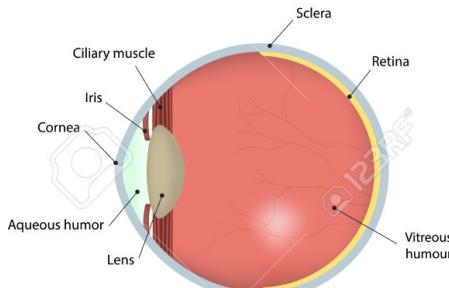
Oncogene: gene with a potential to cause cancer

➤ Dominant and recessive (tumour suppressor gene) mechanisms

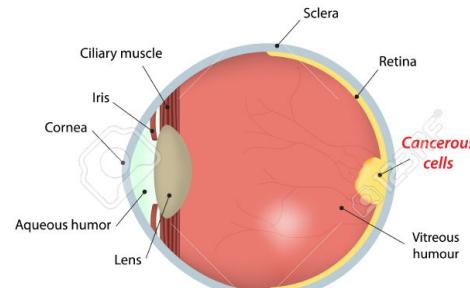


INACTIVATION OF tumour suppressOR GENES

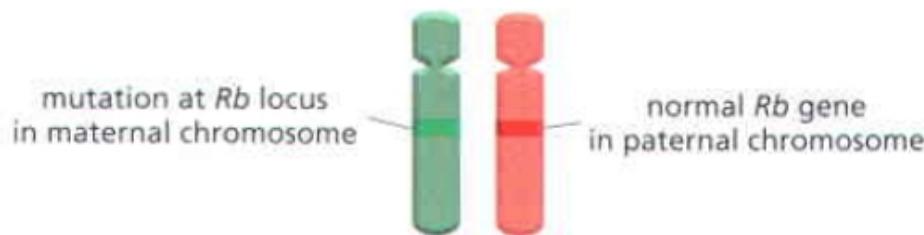
Healthy Eye



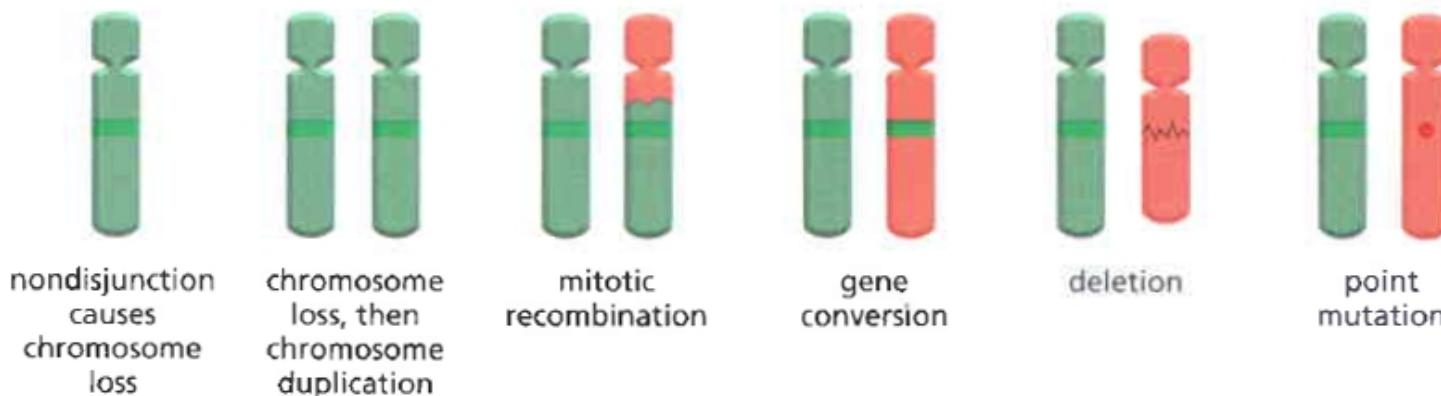
Retinoblastoma



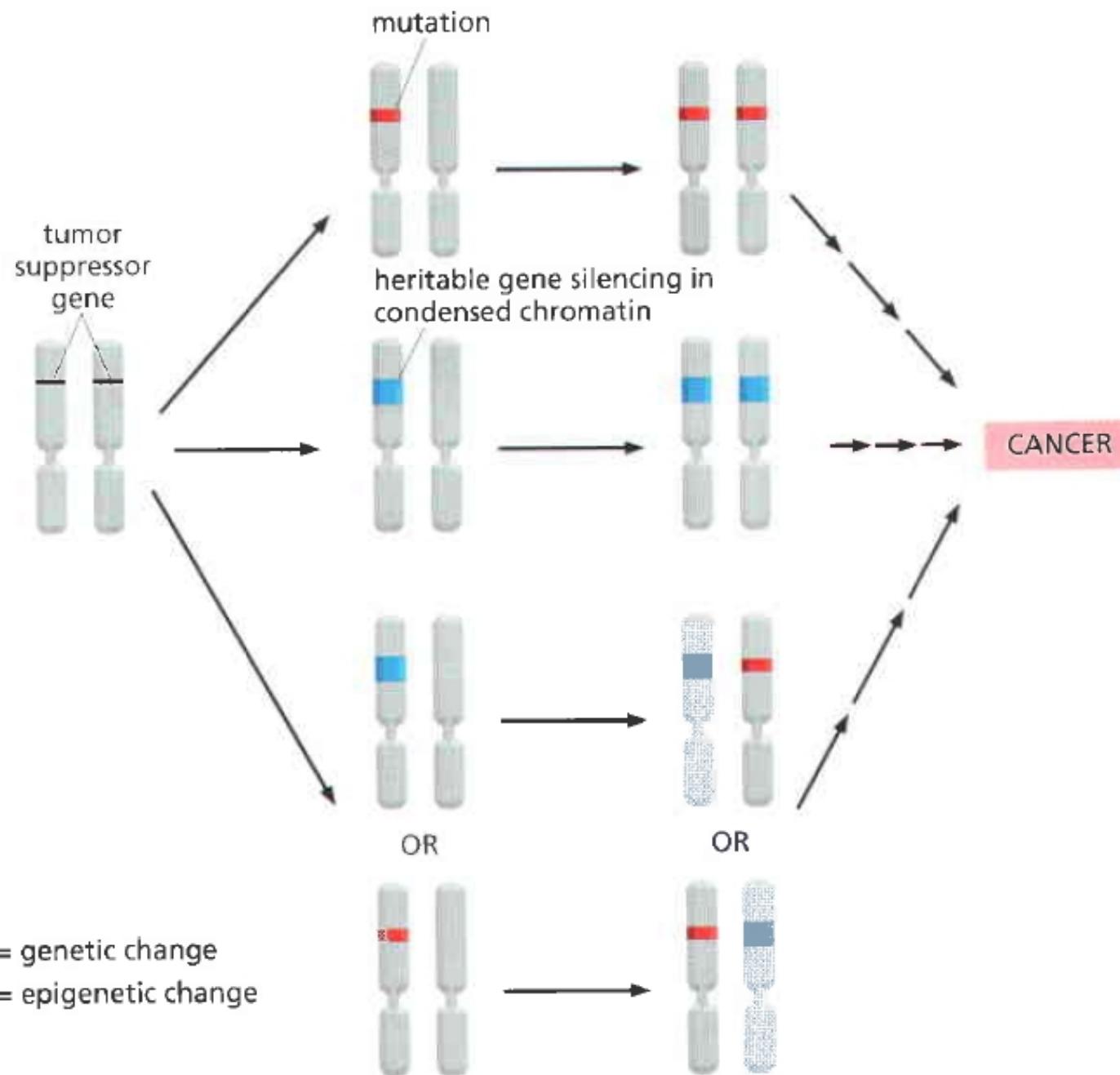
HEALTHY CELL WITH ONLY ONE NORMAL *Rb* GENE COPY



POSSIBLE WAYS OF ELIMINATING NORMAL *Rb* GENE

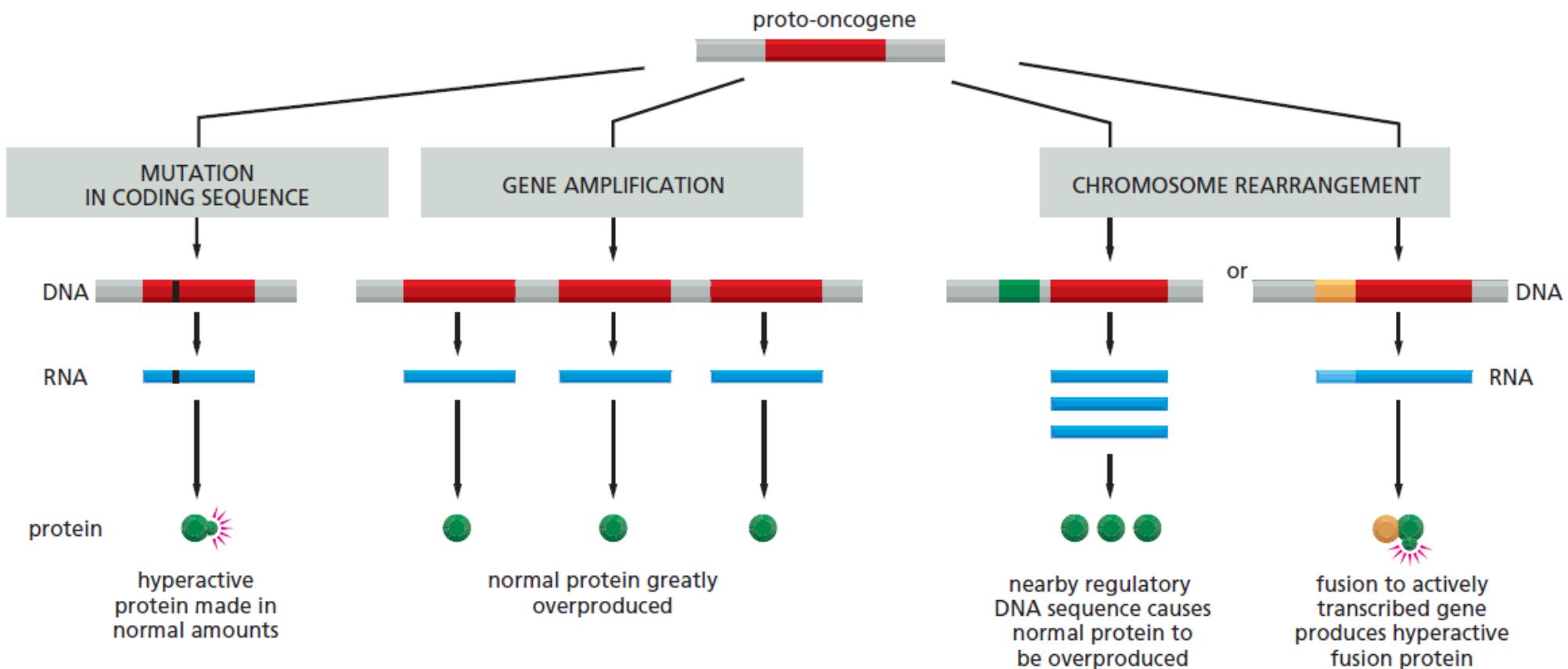


tumour suppressOR GENE FUNCTION



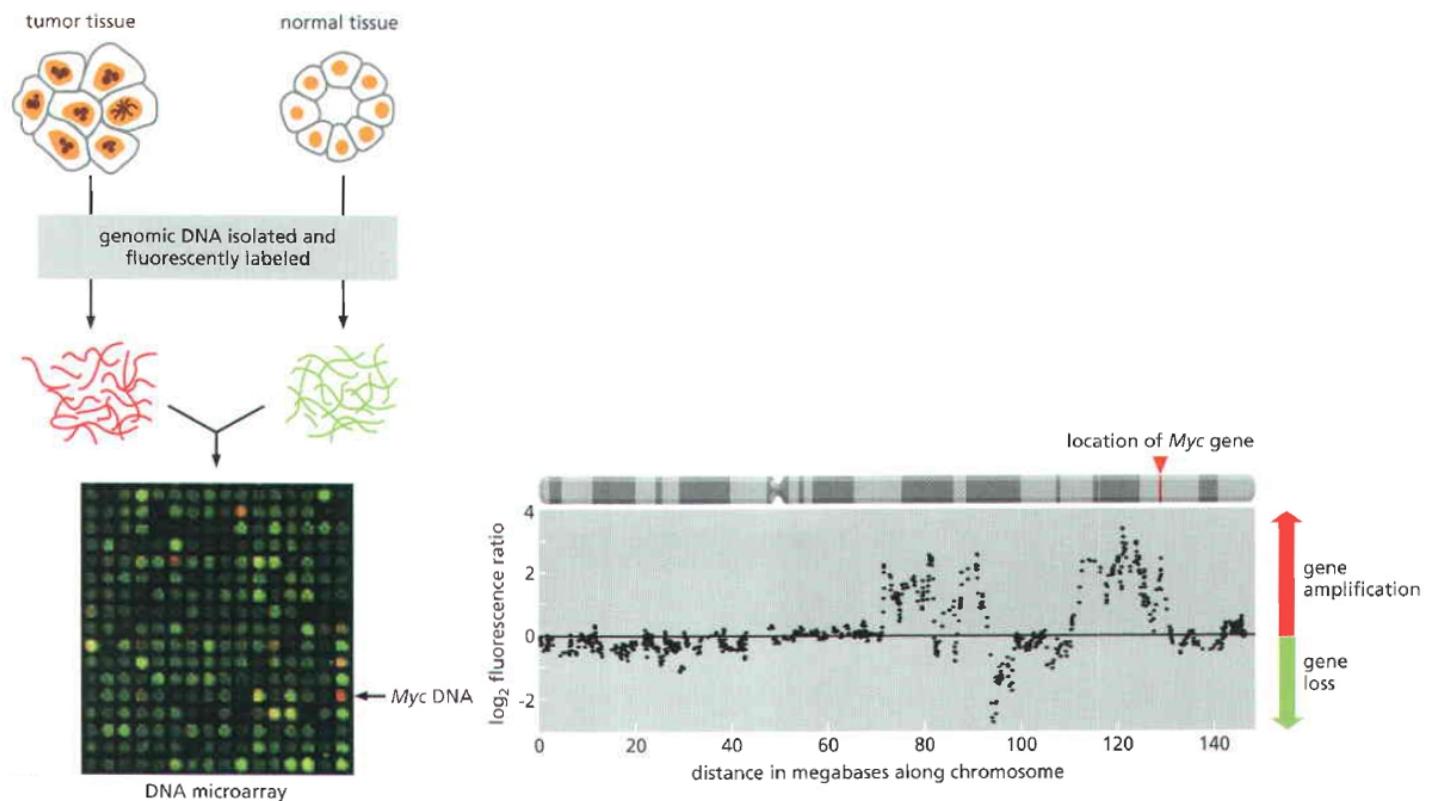
PROTOONCOGENE => ONCOGENE

TYPES OF GENETIC CHANGES



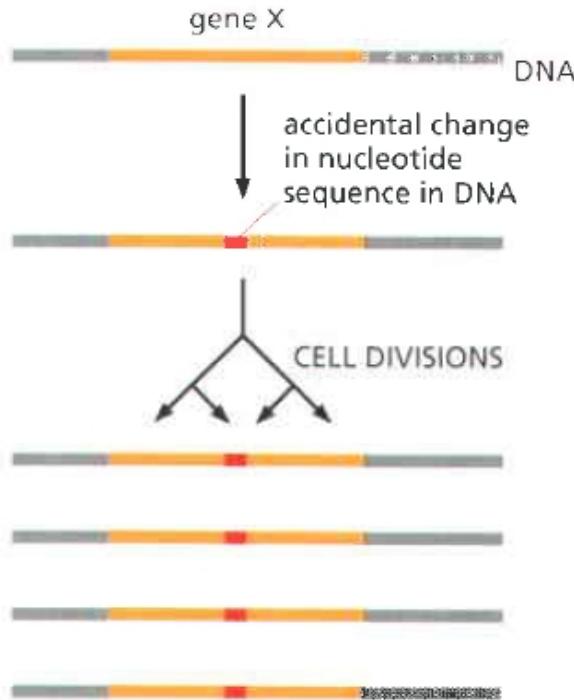
IDENTIFICATION OF ONCOGENES

- DNA sequencing
- Comparative genomic hybridization
- DNA microarrays
- RNA interference

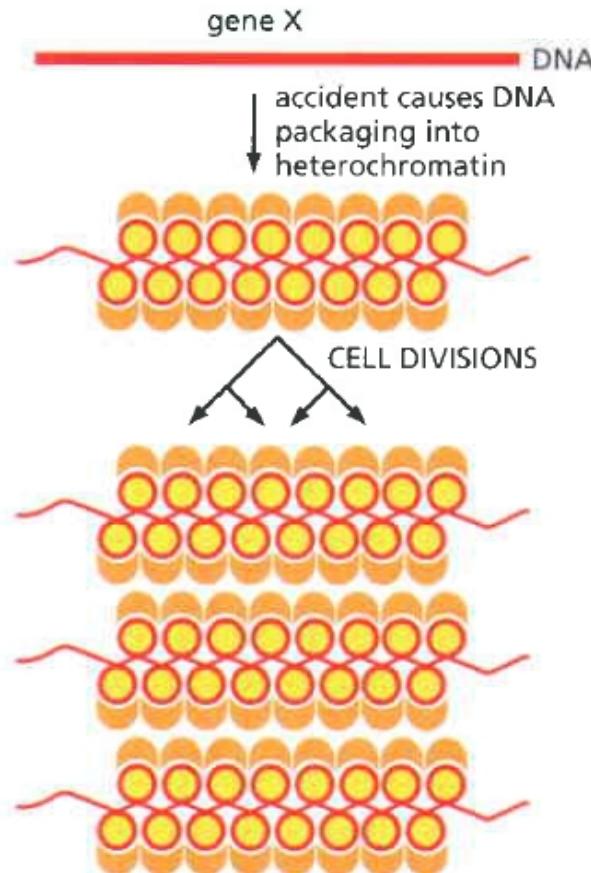


GENETICS VS. EPIGENETICS IN tumourS

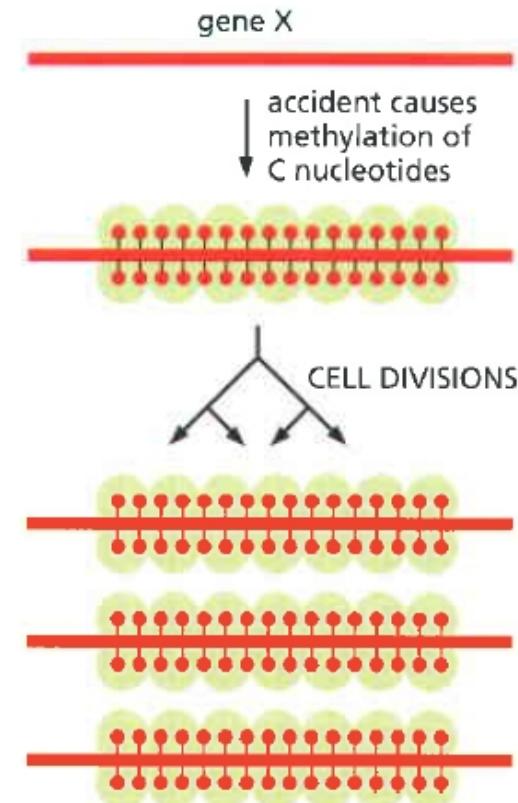
genetic gene inactivation



epigenetic gene inactivation



epigenetic gene inactivation

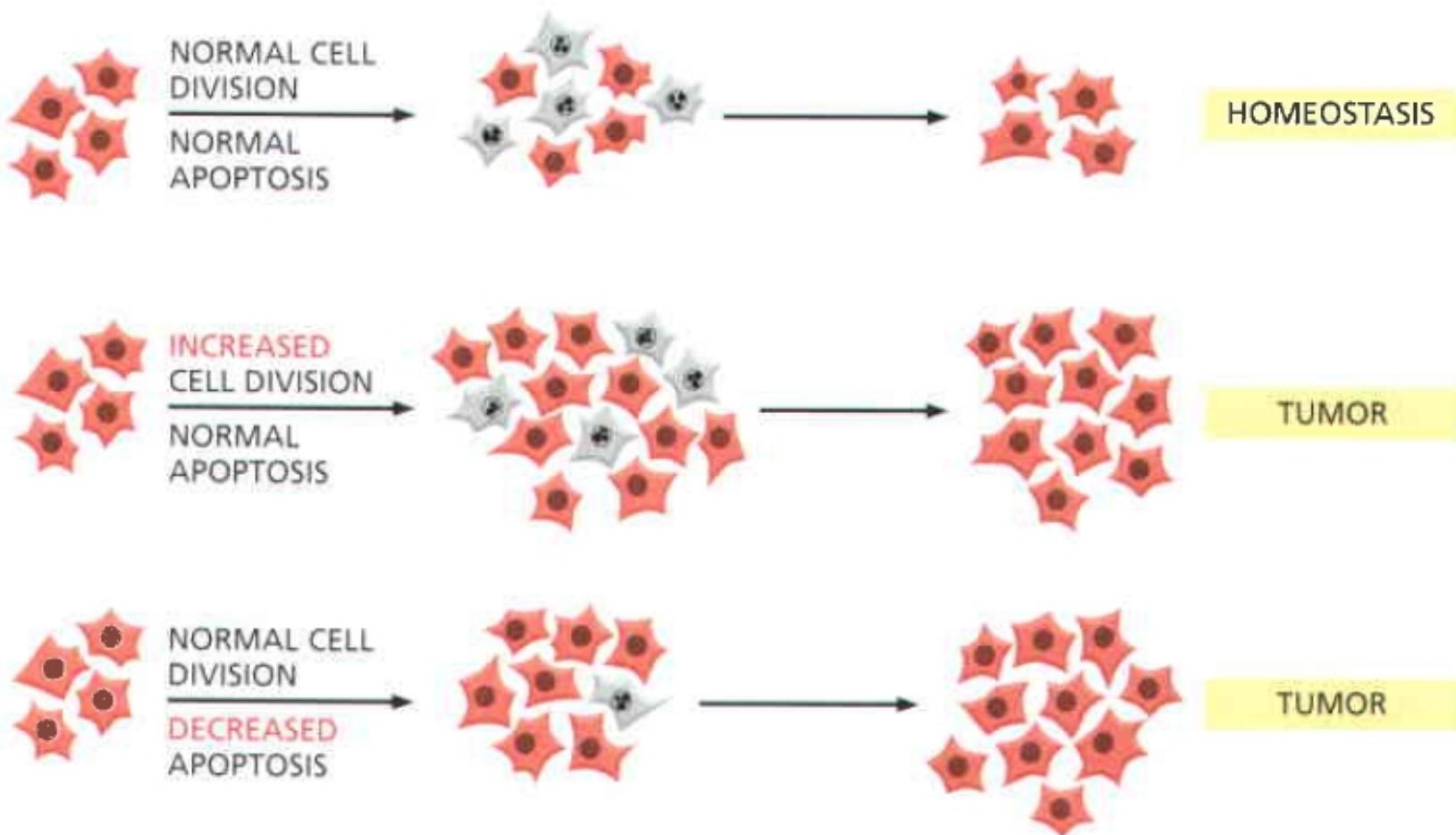


MANY CELLS WITH
INACTIVATED GENE X

MANY CELLS WITH
INACTIVATED GENE X

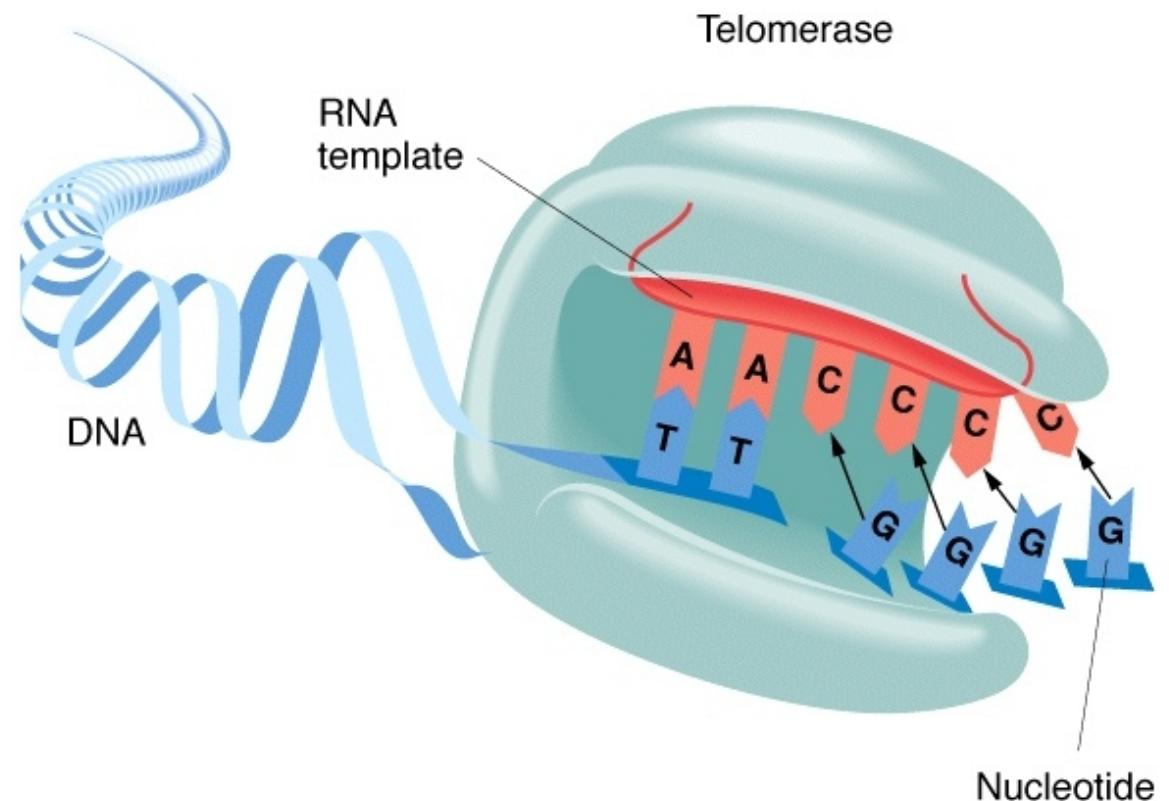
MANY CELLS WITH
INACTIVATED GENE X

CONNECTION TO CELL DIVISION, APOPTOSIS



REPLICATIVE CELL SENESCENCE

- Each normal cell divides a limited number of times (fibroblasts: 25-50 times)
- Telomerase – DNA caps mechanism
- Cancer cells:
 - hyperactive telomerase
 - lose of checkpoints controls



CANCER STEM CELLS

➤ Very small portion of cancer cells

➤ Properties:

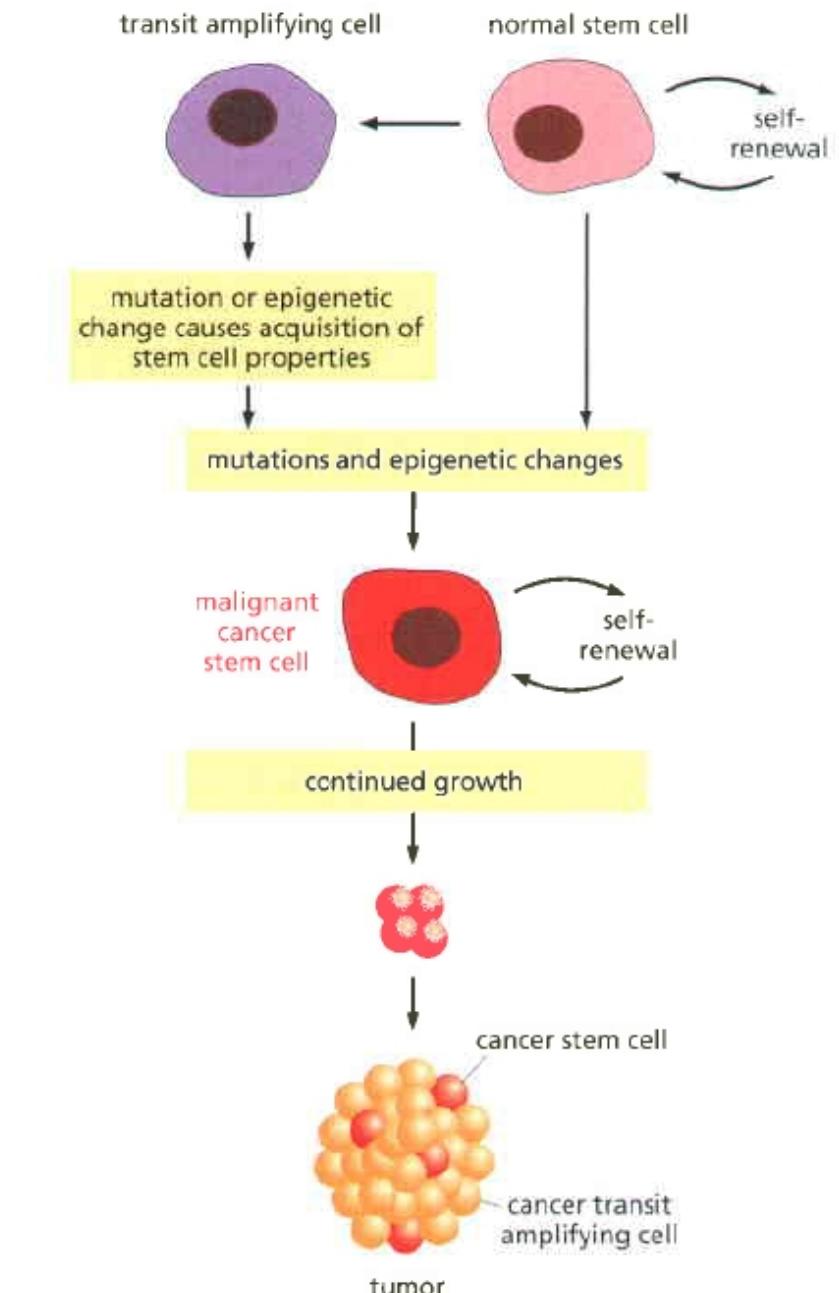
- prolonged self-renewal

- ability to retain in the body

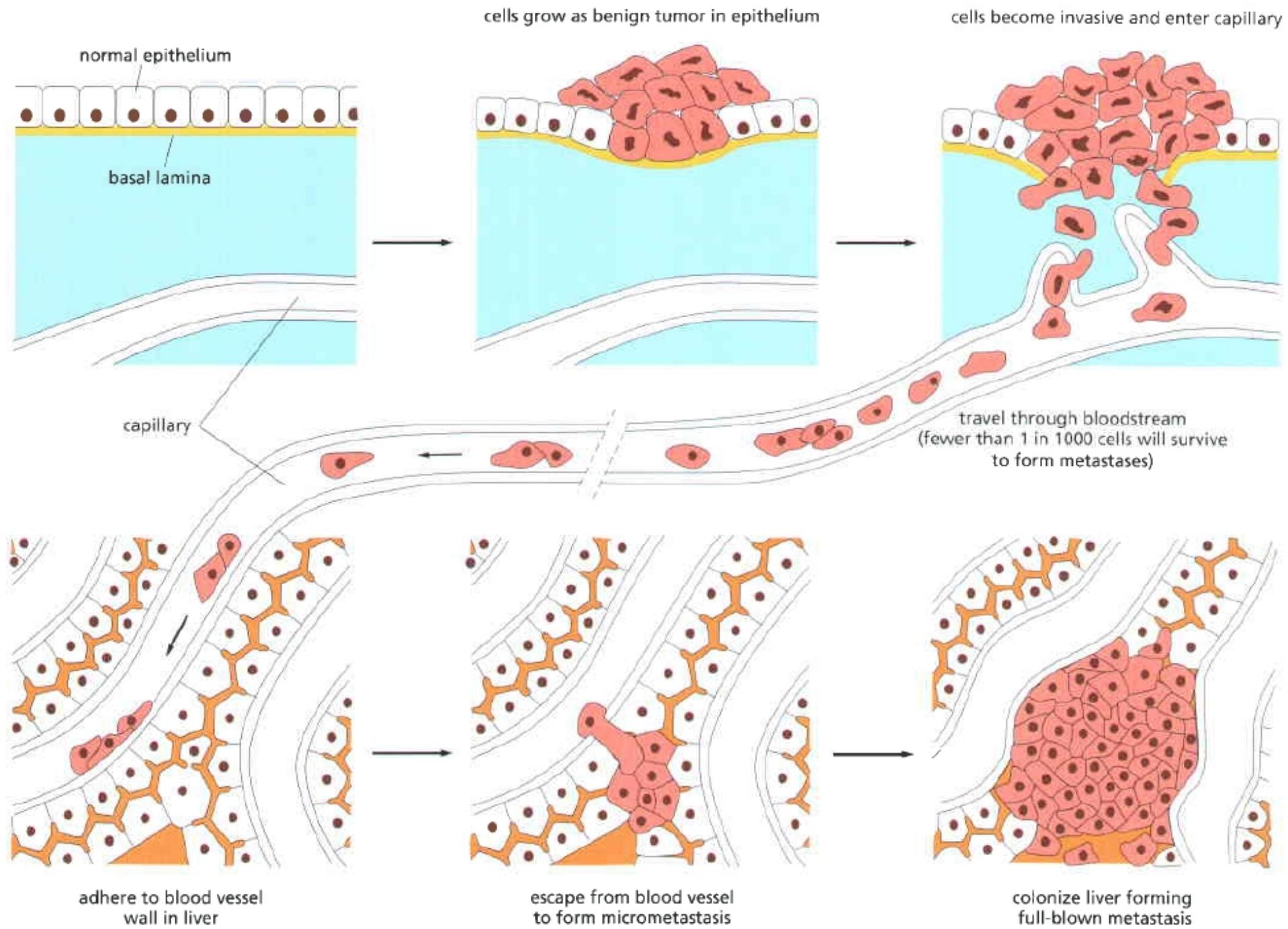
➤ Source:

- stem cells (epigenetic disruptions)

- transit amplifying cells

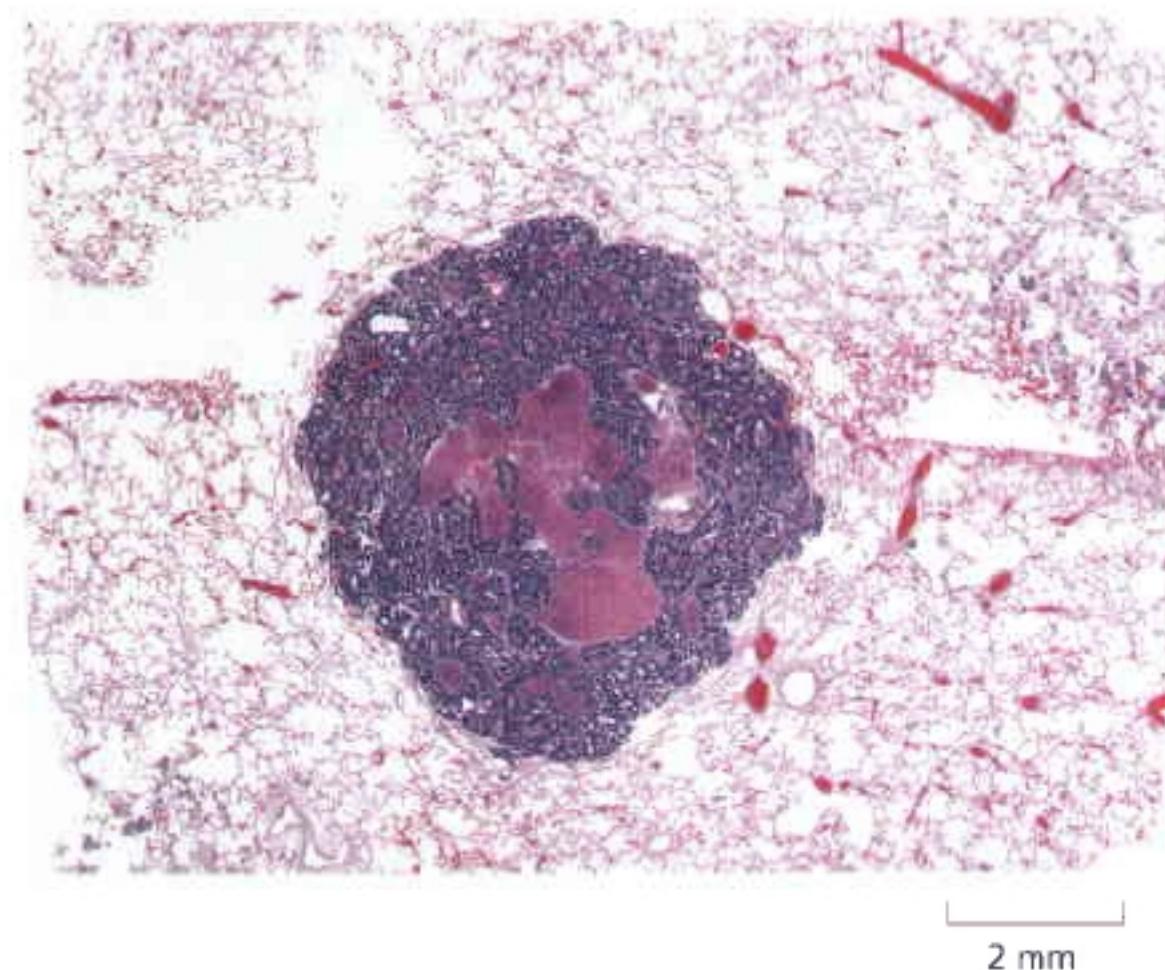


METASTASIS STAGES



tumourS AND ANGIOGENESIS

- Cancer cells: high need for nutrients supply
- Hypoxia Inducible Factor 1 α (HIF-1 α) => VEGF



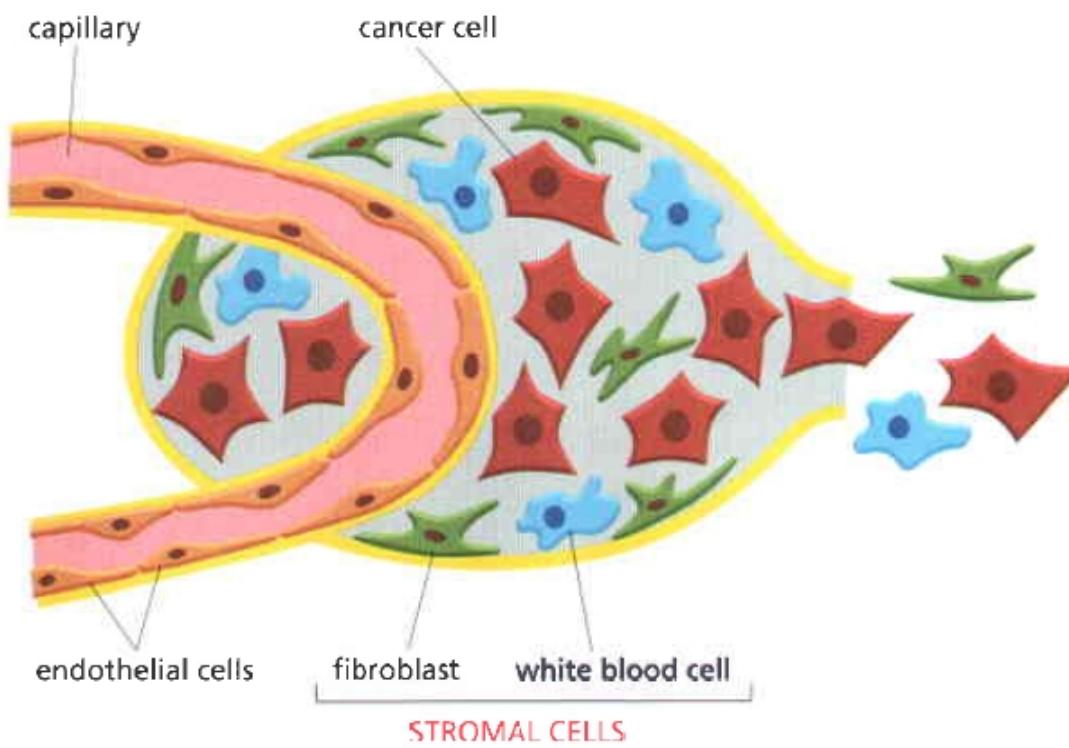
tumourS MICROENVIRONMENT

➤ Stroma cells:

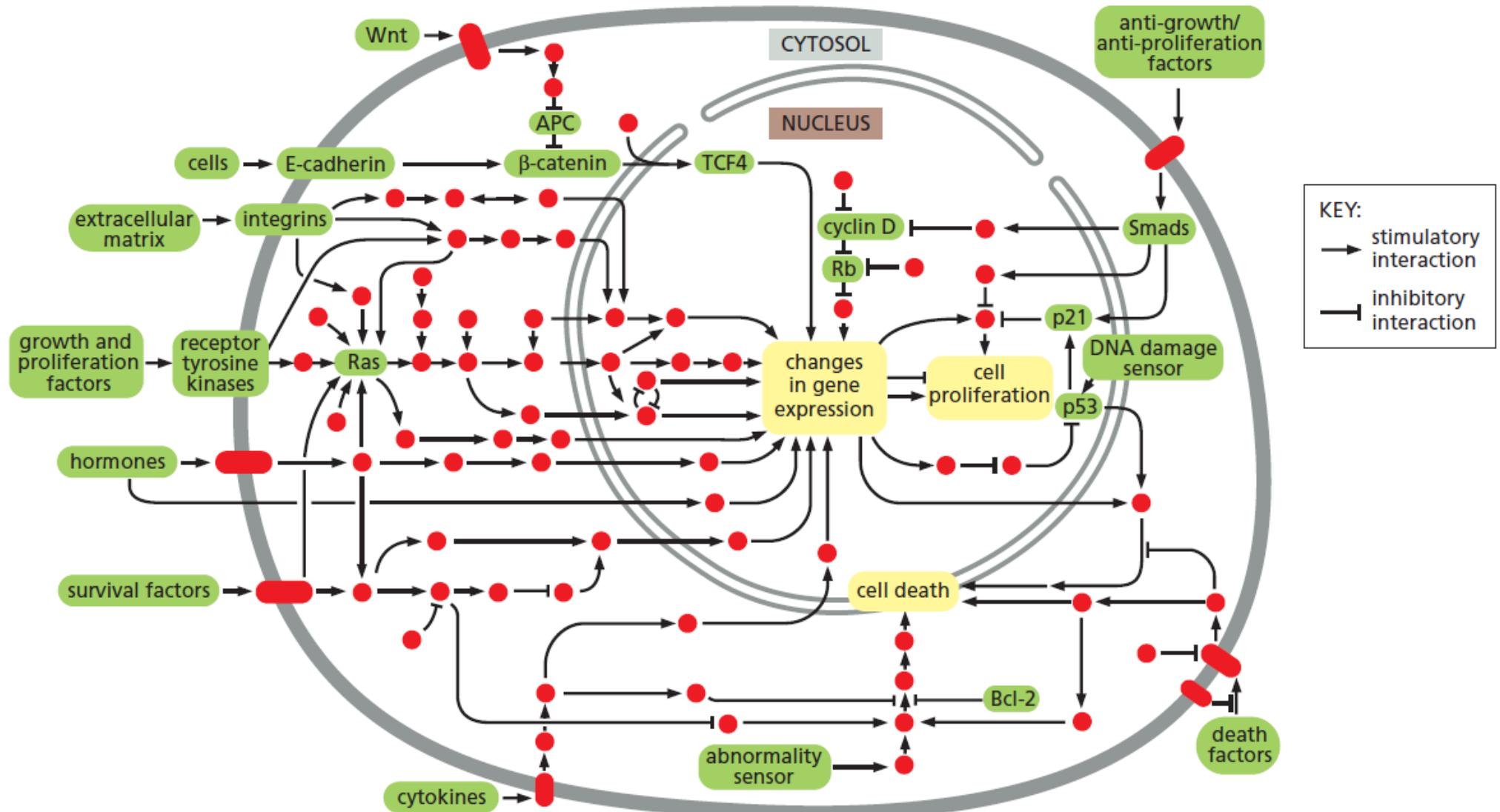
- fibroblasts
- white blood cells
- normal endothelial cells
- smooth muscle cells

➤ Mutual influence:

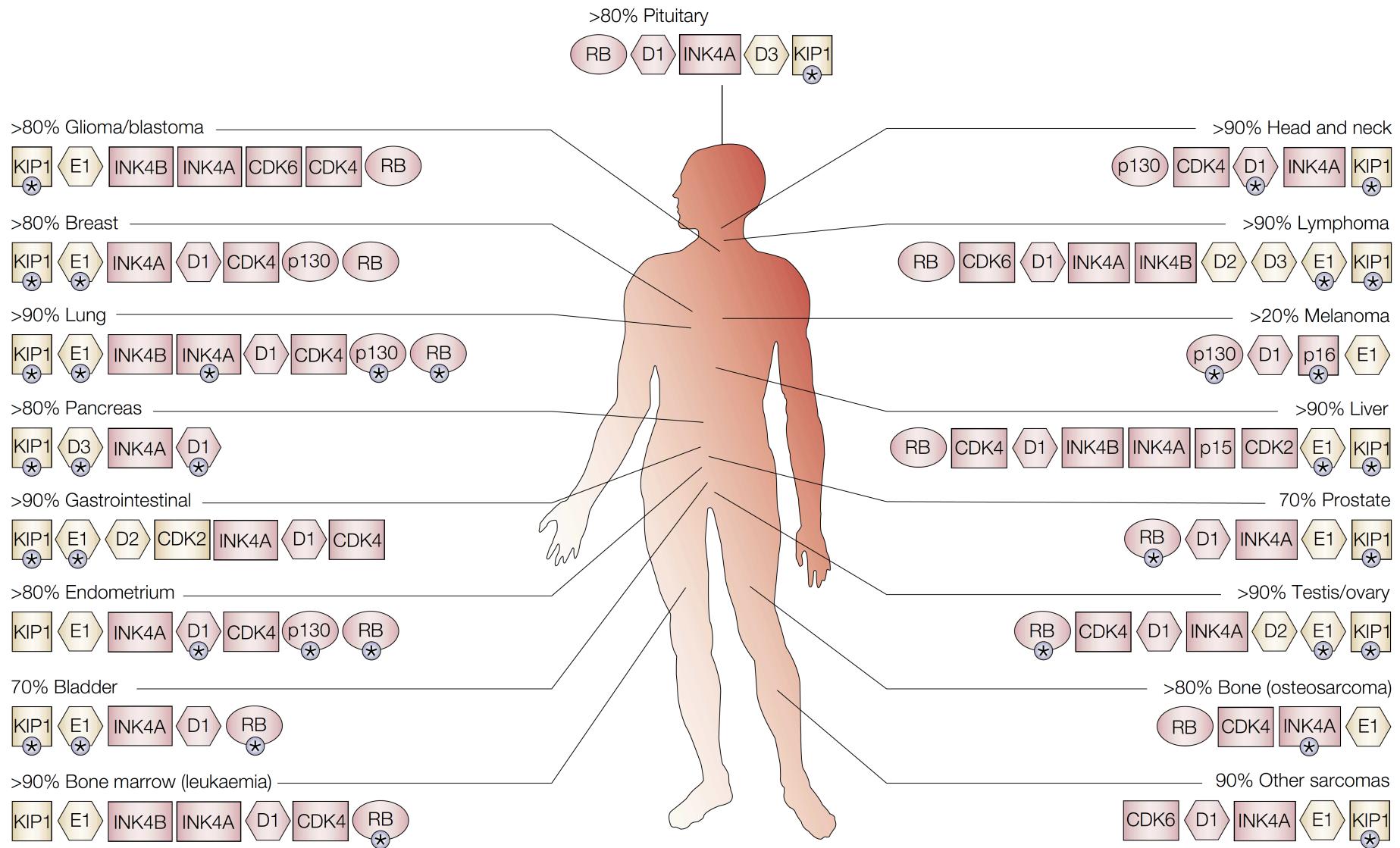
- signaling
- changes in ECM



CANCER: DIVERSITY OF GENES



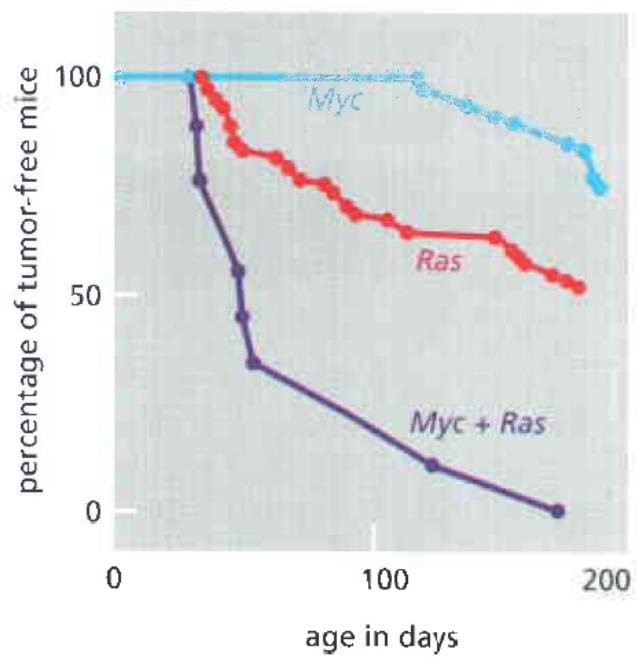
CANCER: KNOWN MUTATIONS FOR G1/S PHASES



Genetic/epigenetic regulation, no known mechanism, statistically significant *

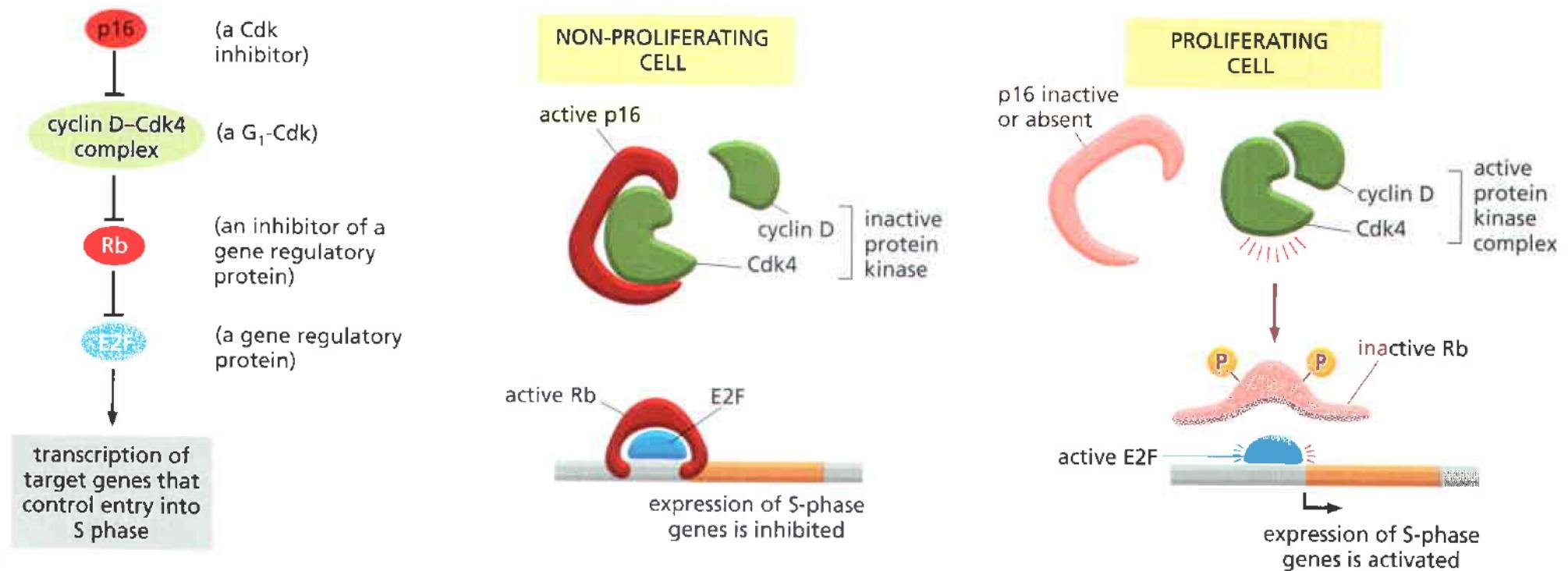
UNCOVERING THE ROLE OF ONCOGENS

- Direct cloning and investigation of particular oncogens (Ras, Rb etc.)
- Homology to already known oncogens
- Participation in
 - cell communication
 - cell division
 - DNA replication
 - etc.
- Model organisms:
 - *C. elegans*
 - *D. melanogaster*
 - transgenic mice
- Combination of several oncogens

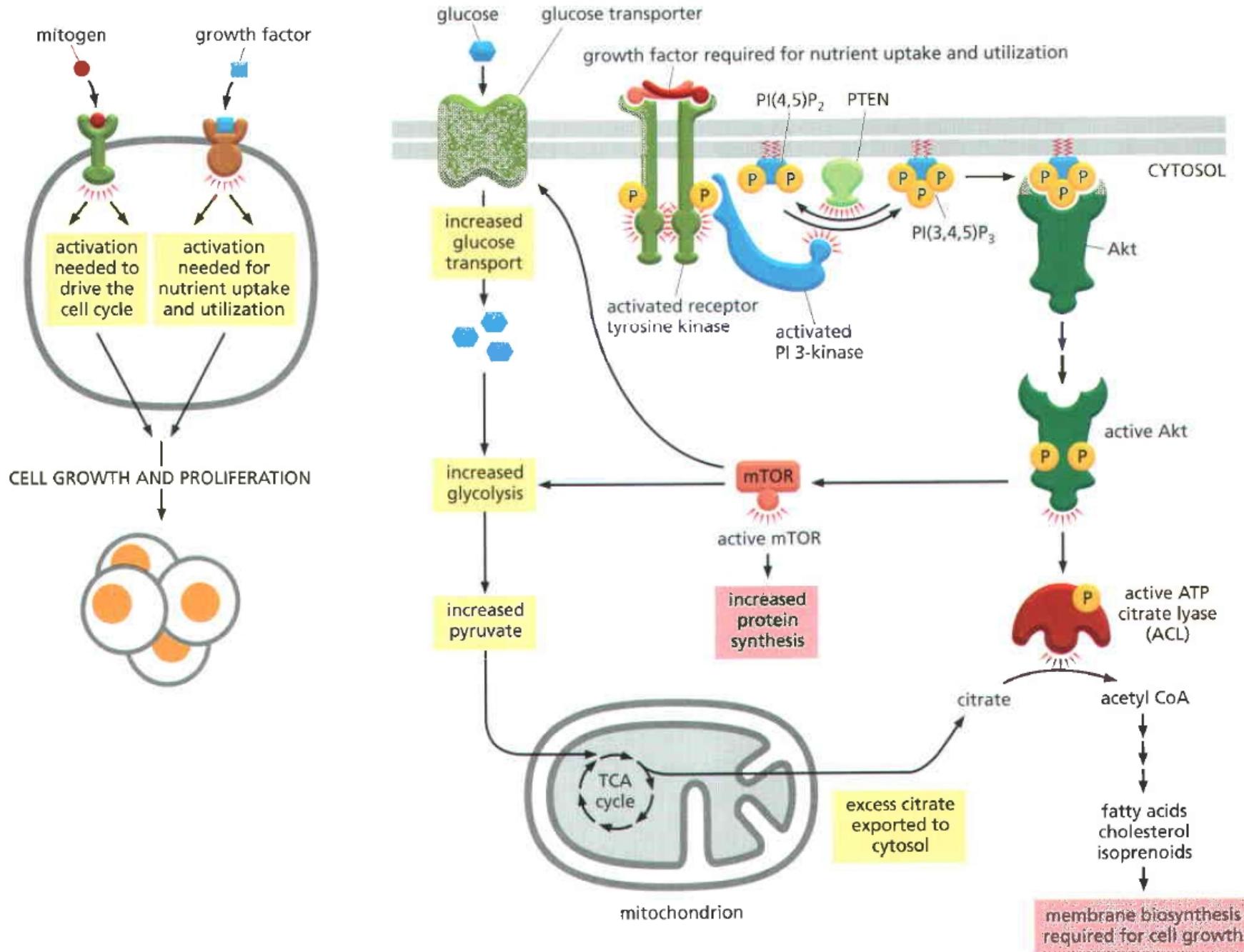


EXAMPLE: RB MECHANISM

- Cdk4 is key for phosphorylation and function of Rb
- p16 is produced under stress
- Inactivation of p16:
 - mutations
 - epigenetic mechanism

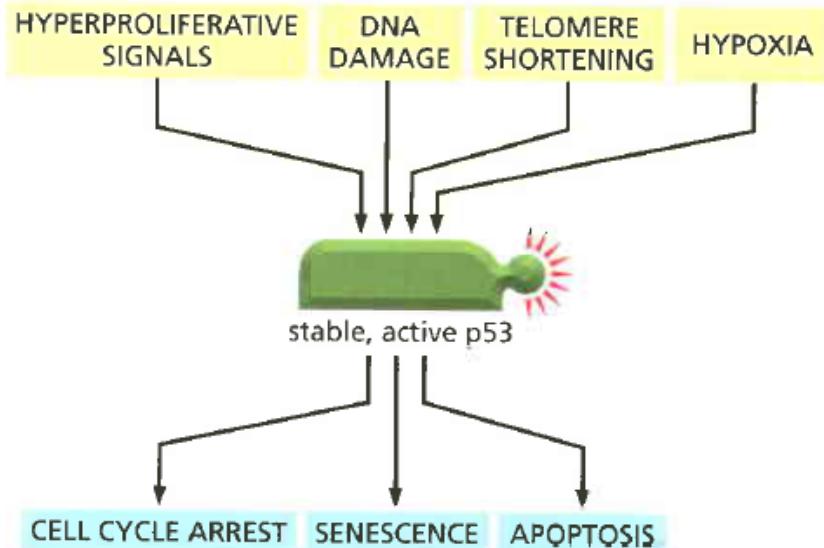


COMBINATION OF SEVERAL CANCEROGENIC PATHWAYS: AKT KINASE



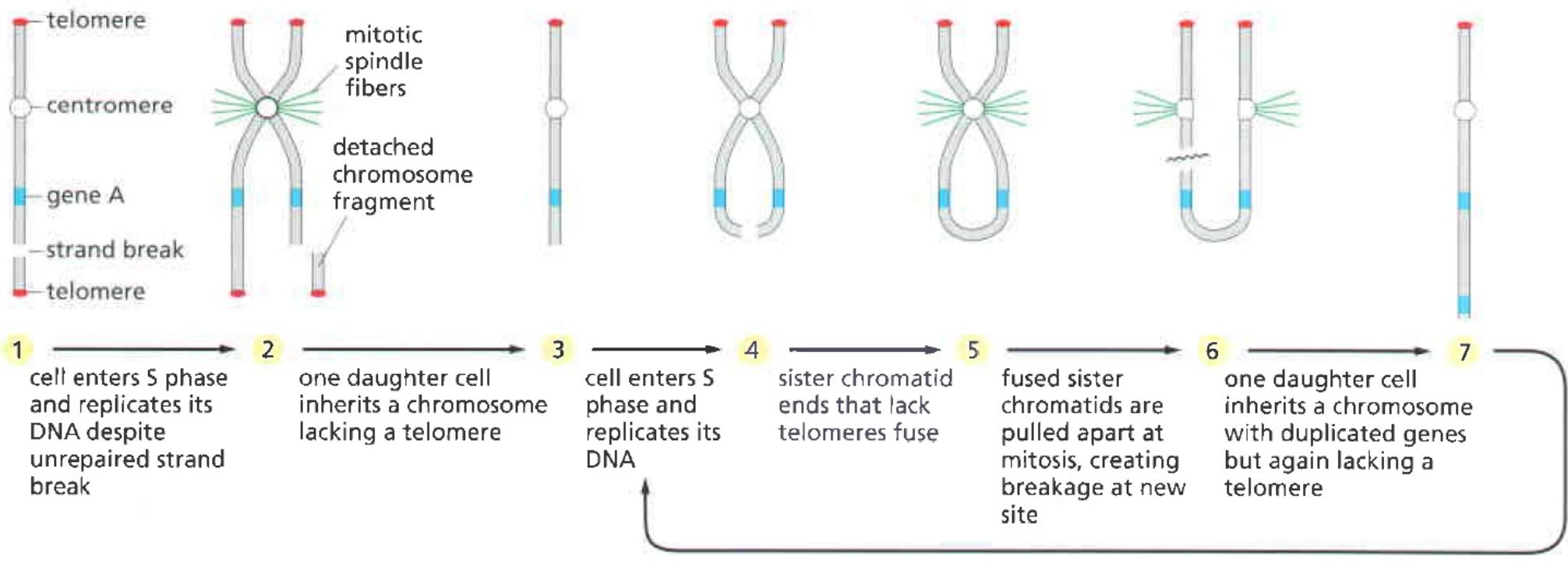
EXAMPLE: P53

- Low concentration
- Not required for normal development
- Stress sensor
- Most mutations: DNA-binding domain
- Mechanisms:
 - binding DNA => p21 synthesis => Cdks inhibition
 - binding Bcl2 => apoptosis
- Cancer associated with p53:
 - damaged DNA proceed through cell cycle
 - apoptosis is escaped
 - accumulation of cells with damaged chromosomes
 - resistance to anticancer drugs and irradiation



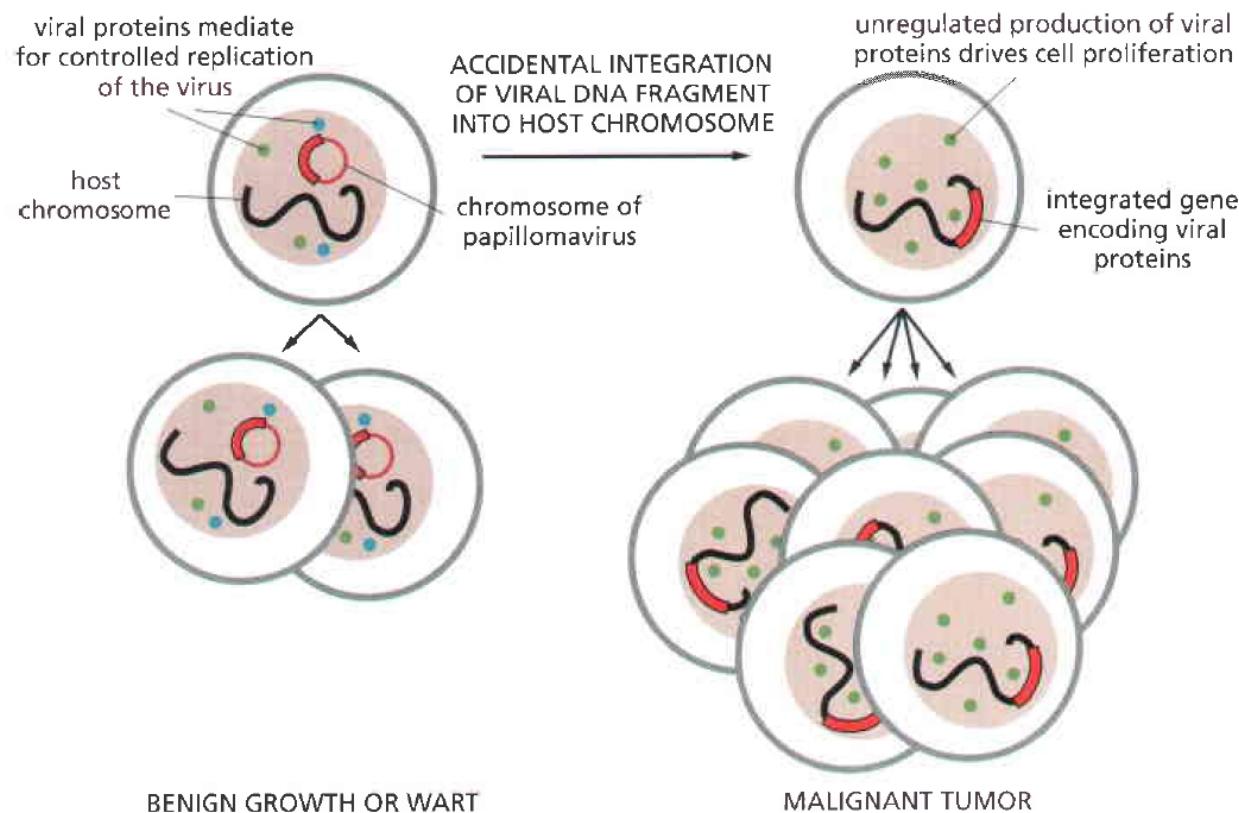
P53 AND REPLICATION OF DAMAGED DNA

- No p53 => no checkpoint for S phase
- Further replication of the damaged DNA
- Transfer of the “error” into a daughter cell



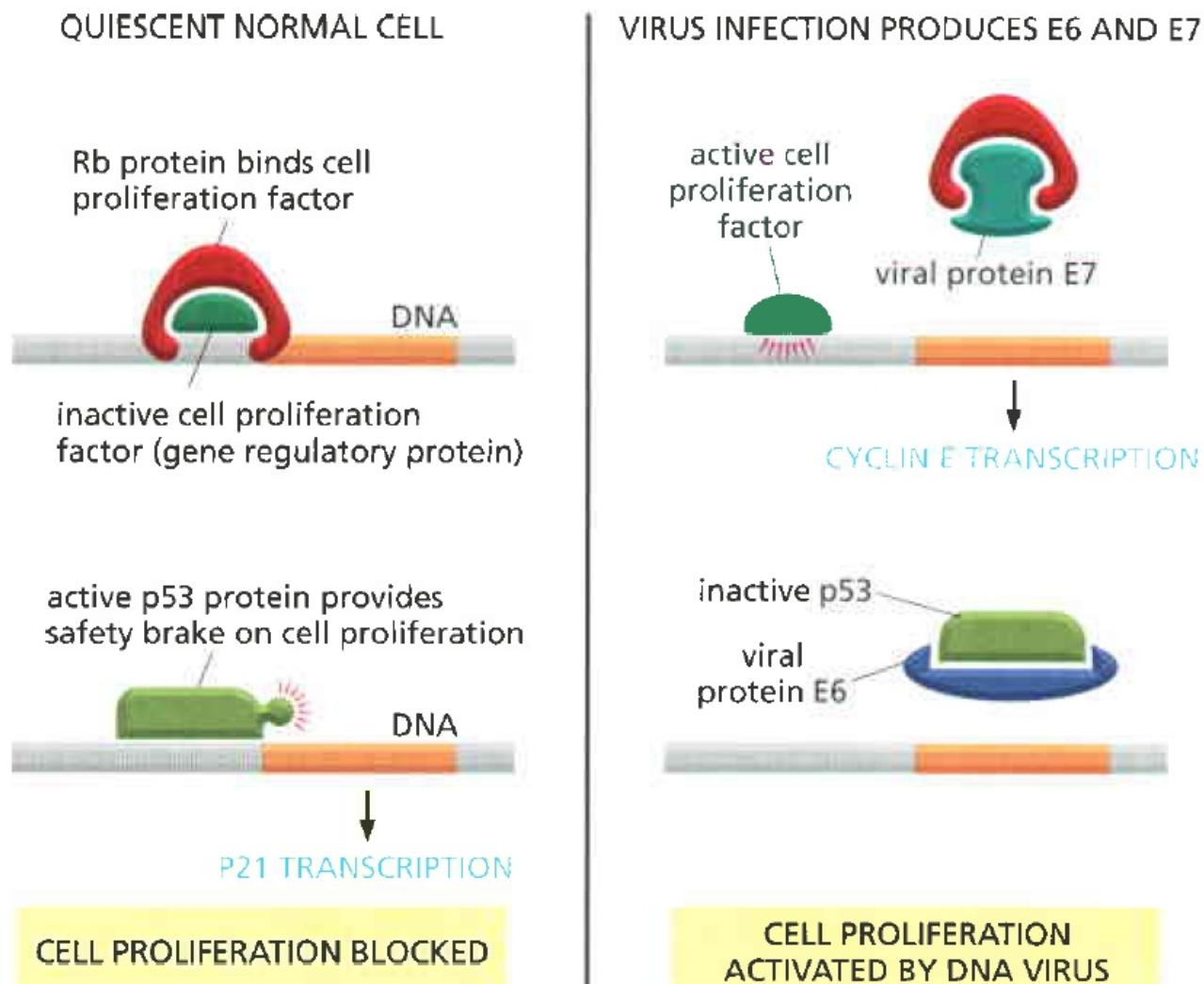
DNA tumour VIRUSES

- Use of host's replication machinery
- Latent and active states
- DNA tumour viruses (f.i. papillomaviruses => wart)



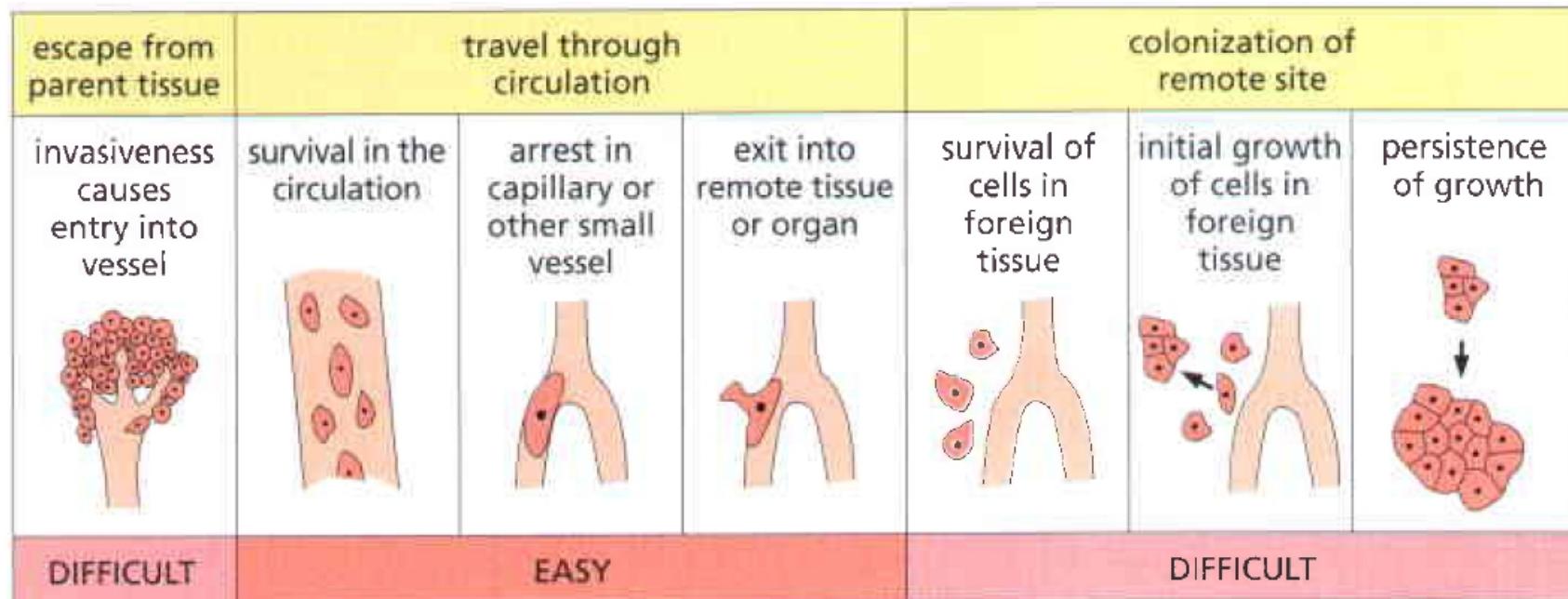
DNA tumour VIRUSES

- E6 and E7 viral proteins
- Binding suppressors (p53, Rb)
- Inducing uncontrolled DNA replication and division



MOLECULAR BASIS OF METASTASIS

- Overexpression of RhoC => actin-based cell motility
- Requirements for metastatic cells:
 - escaping from their original confines (epithelial-mesenchymal transition, E-cadherin, VEGF involvement)
 - targeting to the destination through blood/lymph



COLORECTAL CANCER

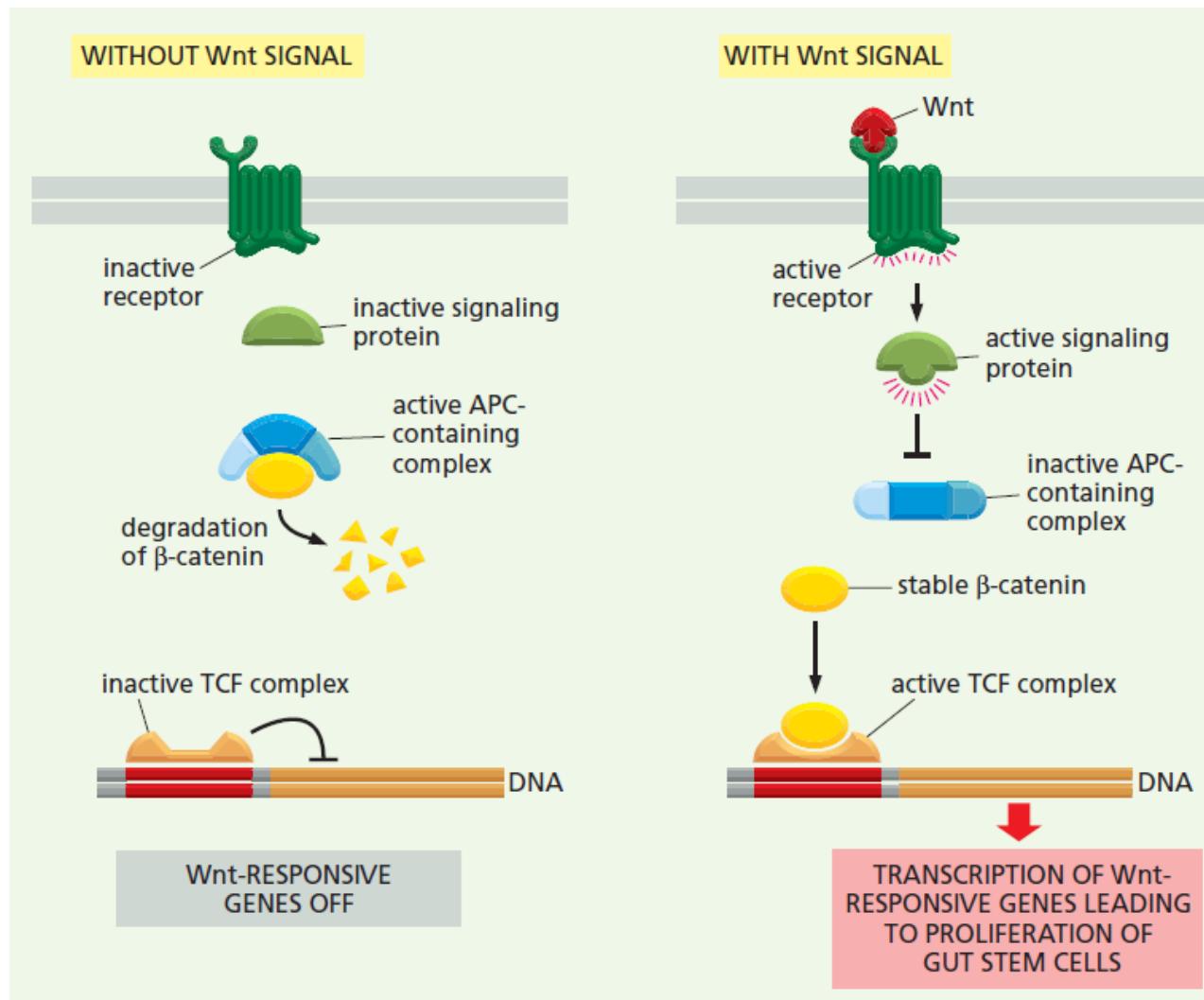
- Adenomatous Polyposis Coli gene (tumour suppressor): deletion/inactivation
- One “good” and one somatically mutated copy
- Further mutation can inactivate the “good” copy
- 60% of colorectal cancer => APC
- ~ 10% of deaths from cancer (USA)



1 mm

COLORECTAL CANCER: MECHANISMS

- APC was isolated with β -catenin
- Armadillo in *Drosophila* is similar to β -catenin
- TCF4 role in the discovery



COLORECTAL CANCER: GENES INVOLVED

GENE	CLASS	PATHWAY AFFECTED	HUMAN COLON CANCERS (%)
<i>K-Ras</i>	oncogene	receptor tyrosine-kinase signaling	40
<i>β-catenin</i> ¹	oncogene	Wnt signaling	5–10
<i>Apc</i> ¹	tumor suppressor	Wnt signaling	> 80
<i>p53</i>	tumor suppressor	response to stress and DNA damage	60
<i>TGFβ receptor II</i> ²	tumor suppressor	TGFβ signaling	10
<i>Smad4</i> ²	tumor suppressor	TGFβ signaling	30
<i>MLH1</i> and other DNA mismatch repair genes	tumor suppressor (genetic stability)	DNA mismatch repair	15 (often silenced by methylation)

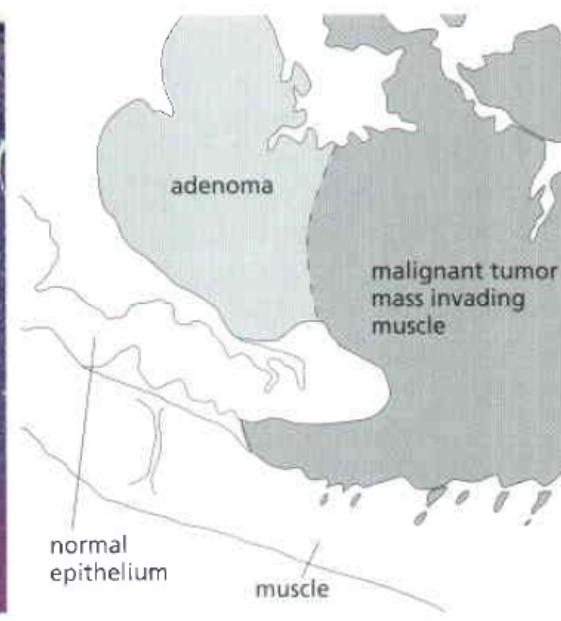
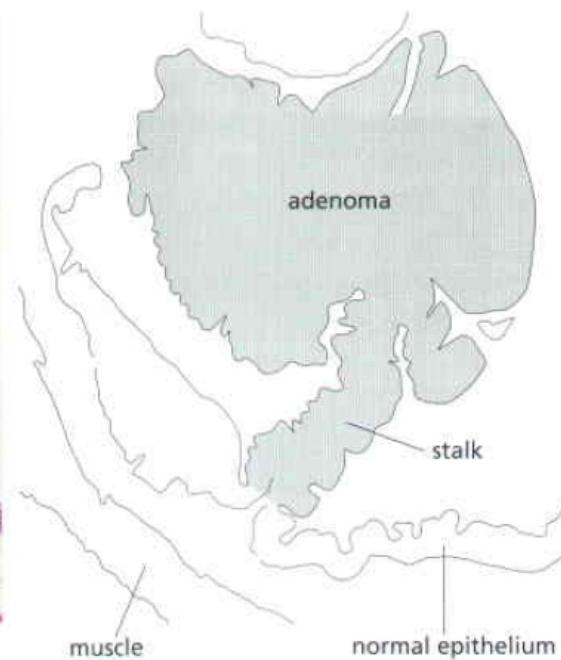


Healthy gut



Gut with colon cancer

COLORECTAL CANCER: DEVELOPMENT STAGES

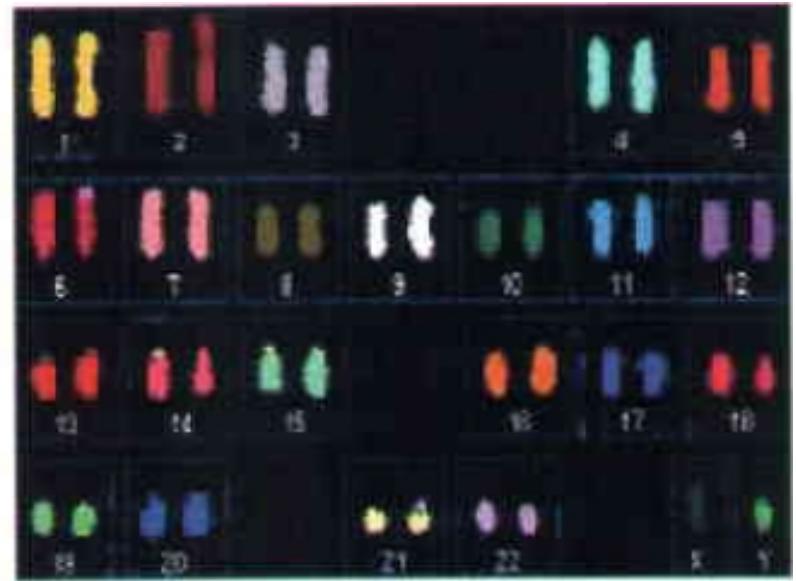


COLORECTAL CANCER: DNA MISMATCH REPAIR

- Non-polyposis colon cancer
- DNA mismatch repair system: MutS, MutL
- Mechanisms:
 - chromosome instability
 - DNA mismatch

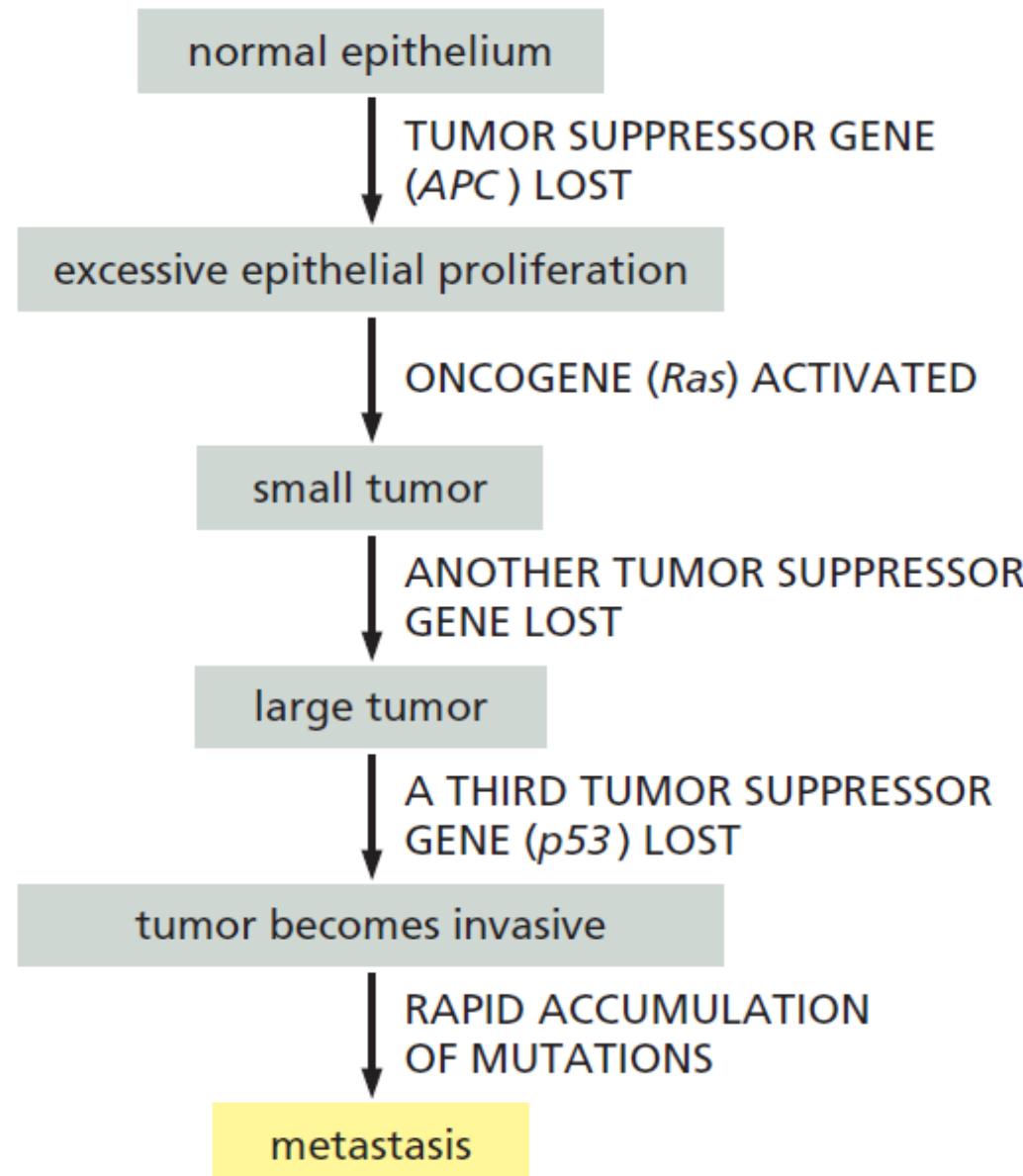


Chromosome instability



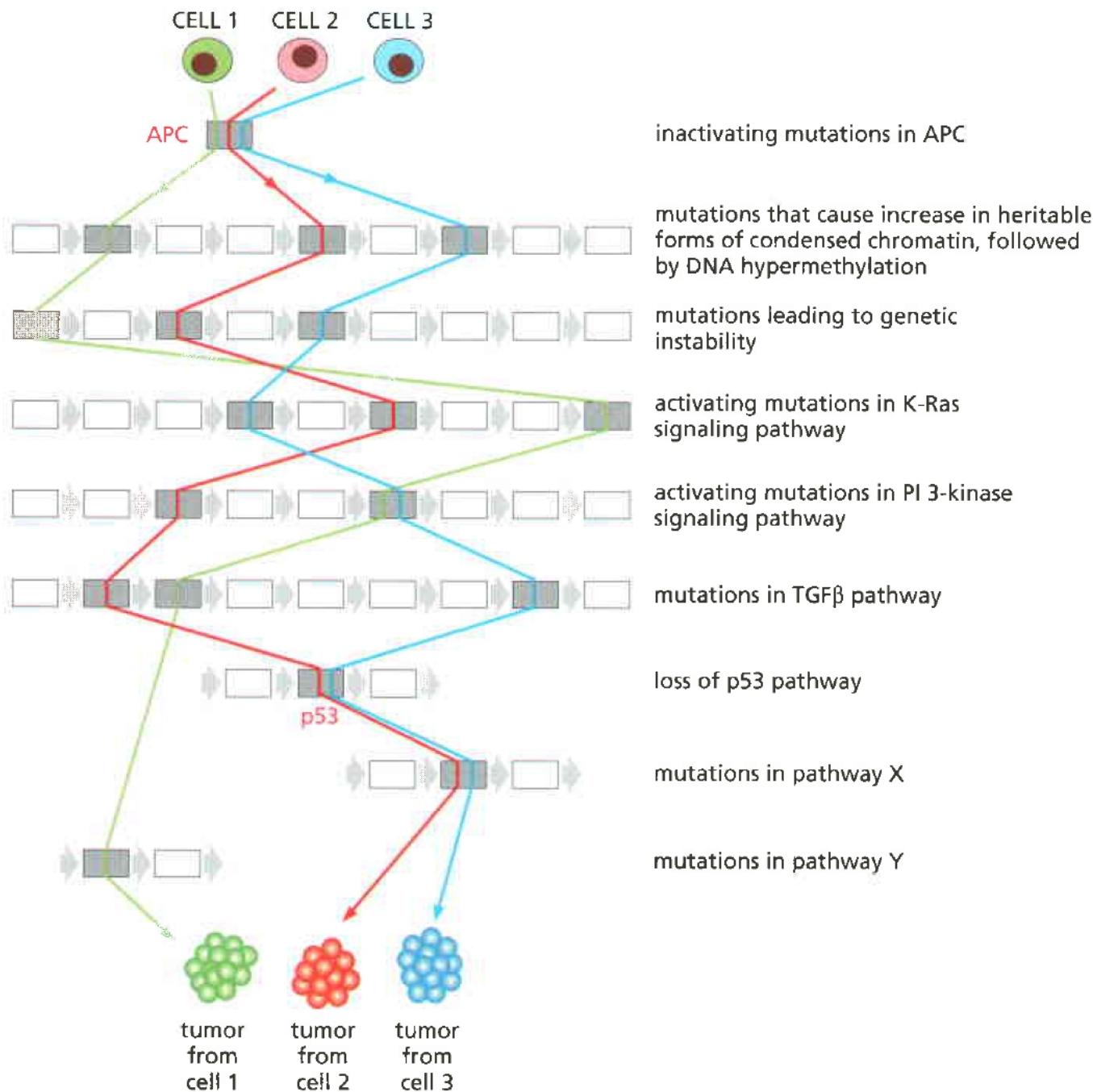
Mismatch repair

COLORECTAL CANCER => INVASIVE CANCER

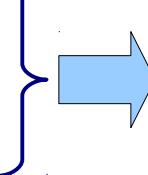


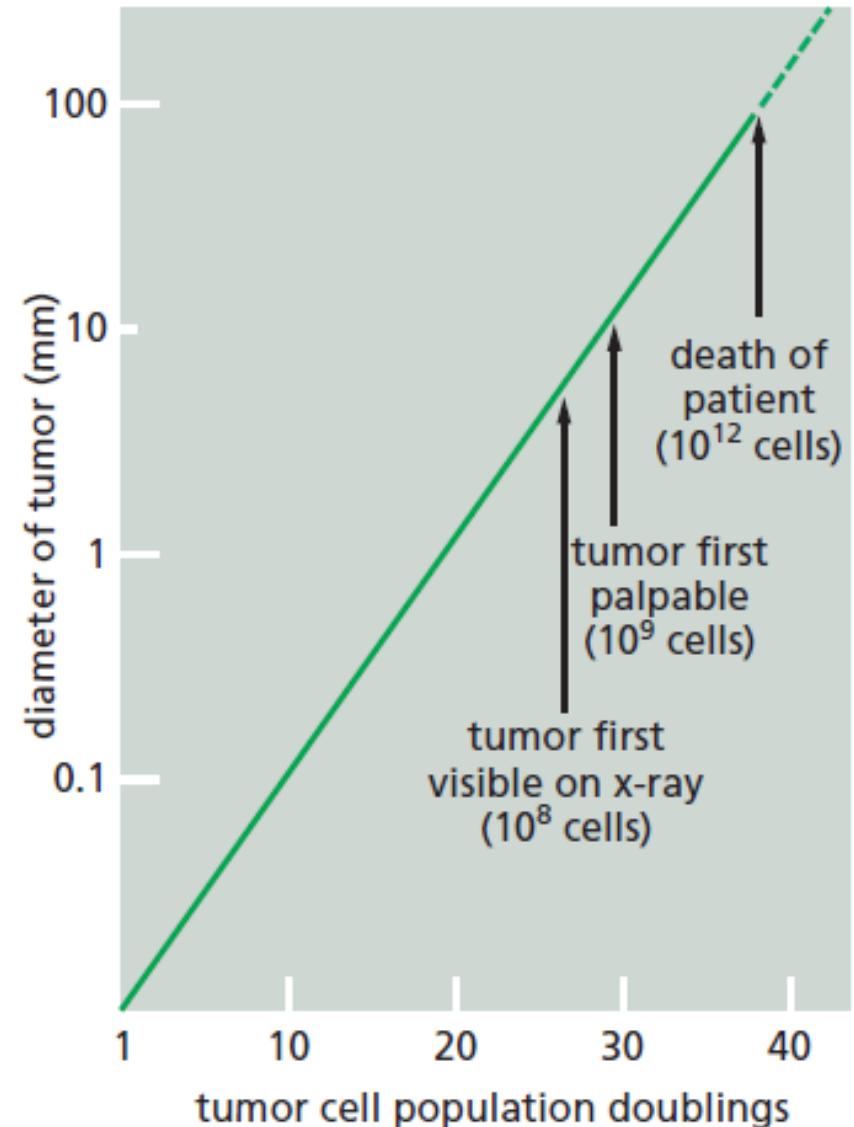
Time ~ 10-20 years

COLORECTAL CANCER: PATHWAYS



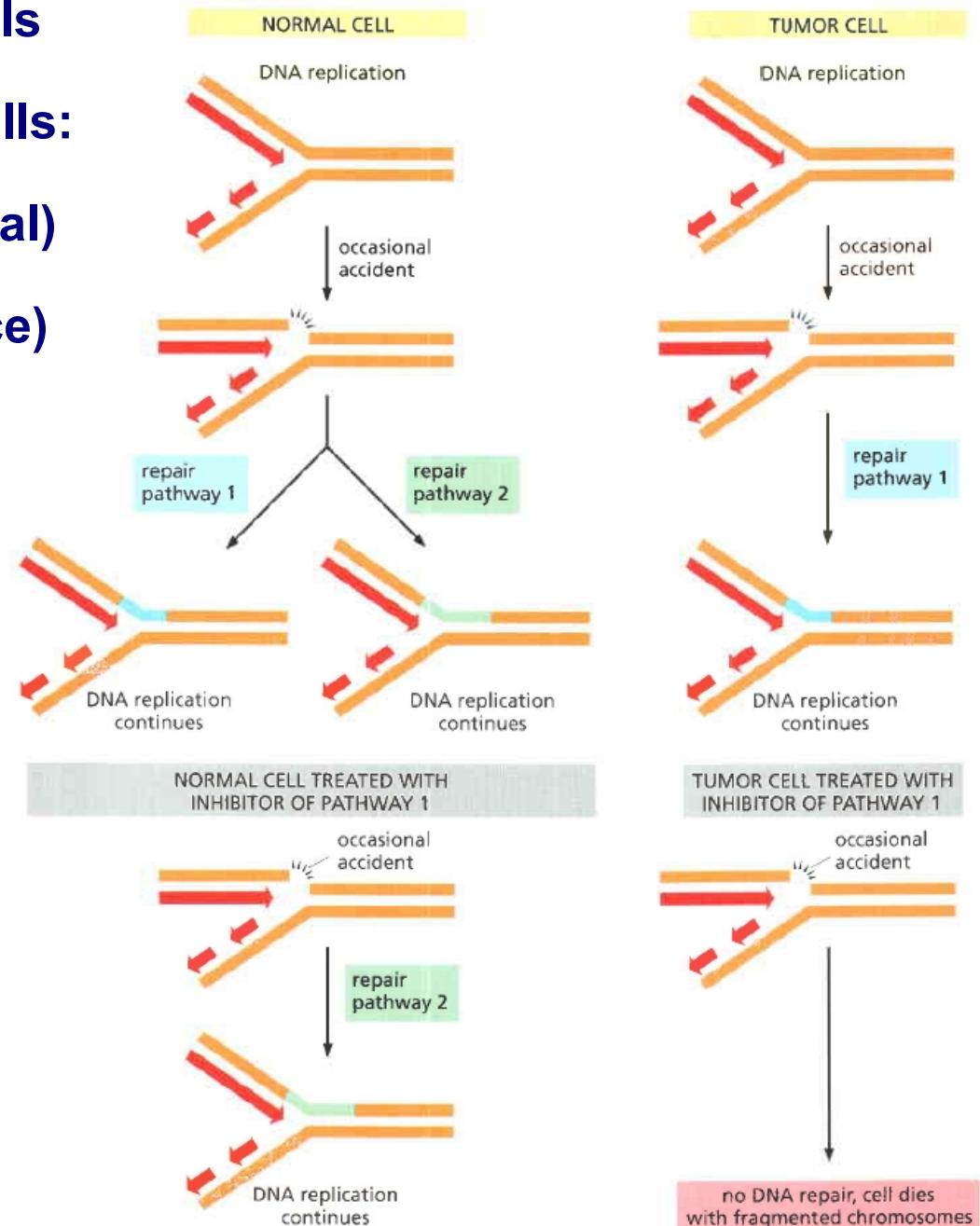
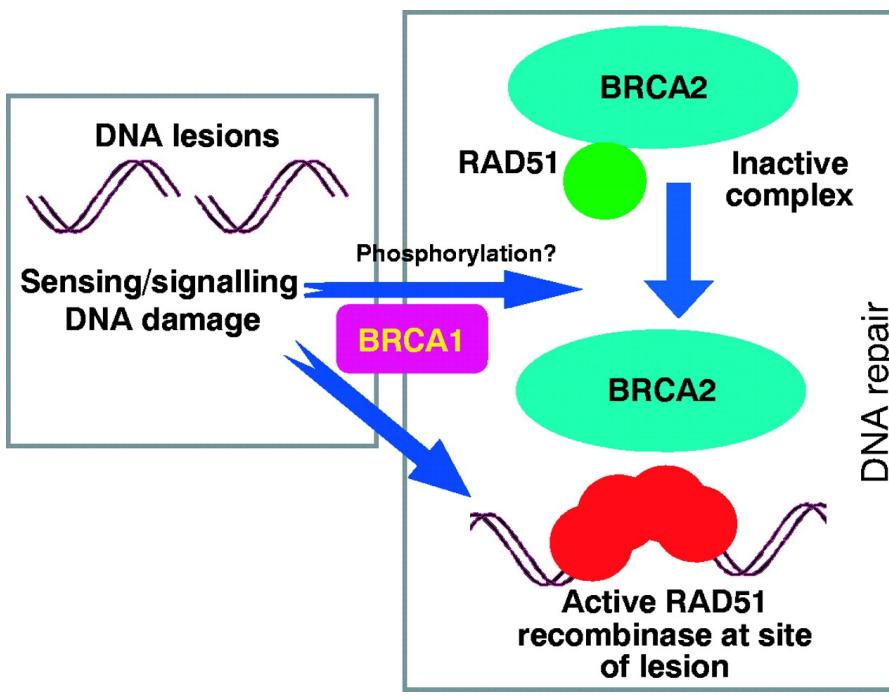
CANCER TREATMENT

- Three stages:
 - prevention
 - diagnosis (gene expression profile)
 - treatment
- No single treatment
- Surgery
- Radiotherapy
- Chemotherapy }  Damaged checkpoints
- Blocking blood supply
- Vaccination => immune response



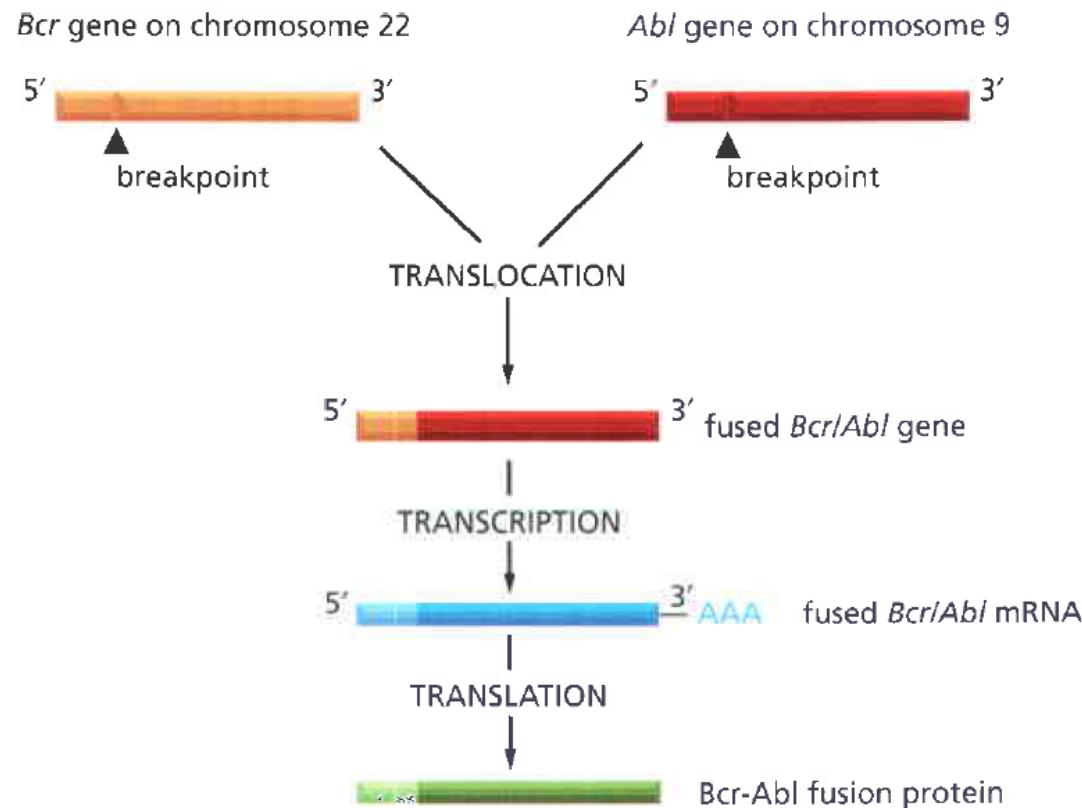
CANCER TREATMENT: ROLE OF tumour GENETIC INSTABILITY

- Therapies aim to kill only cancer cells
- General DNA instability in cancer cells:
 - advantages (differences from normal)
 - disadvantages (multidrug resistance)
- Example: BRCA1, BRCA2, PARP

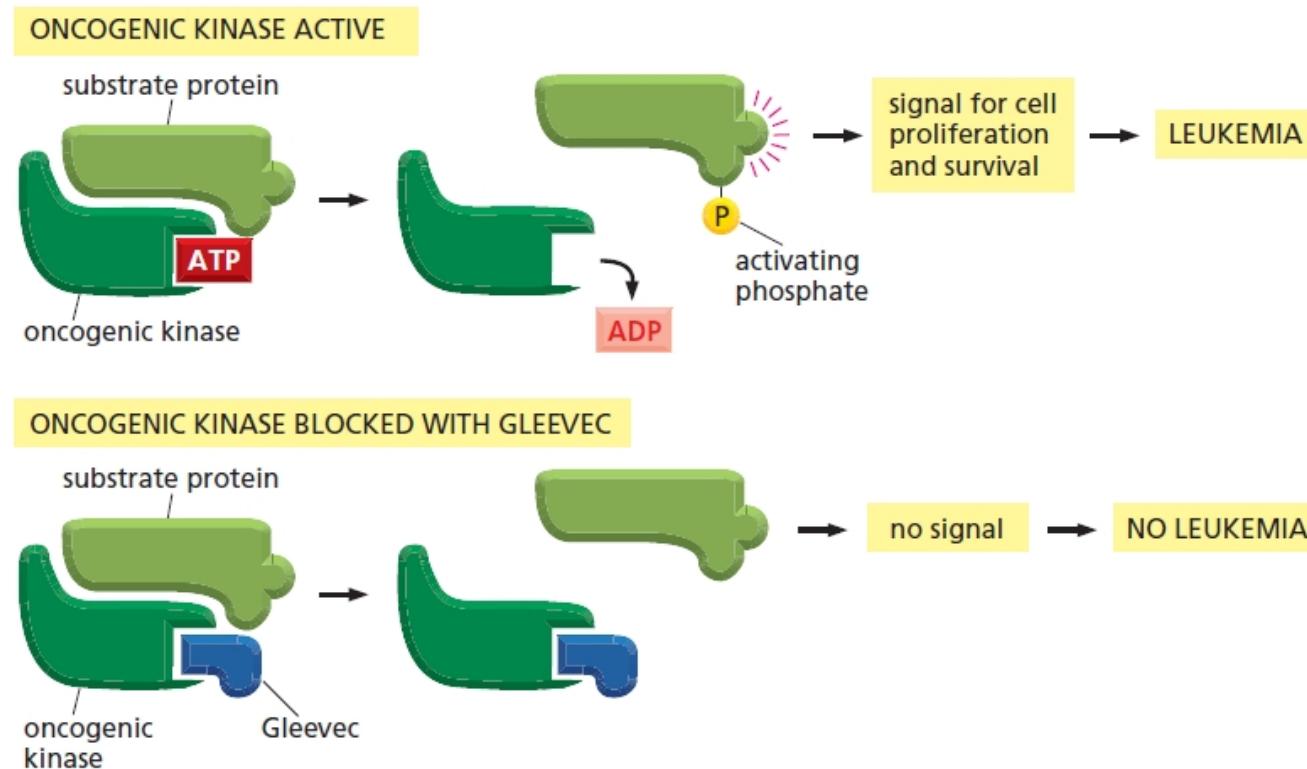


CANCER TREATMENT EXAMPLE: GLEEVEC IN CHRONIC MYELOID LEUKEMIA

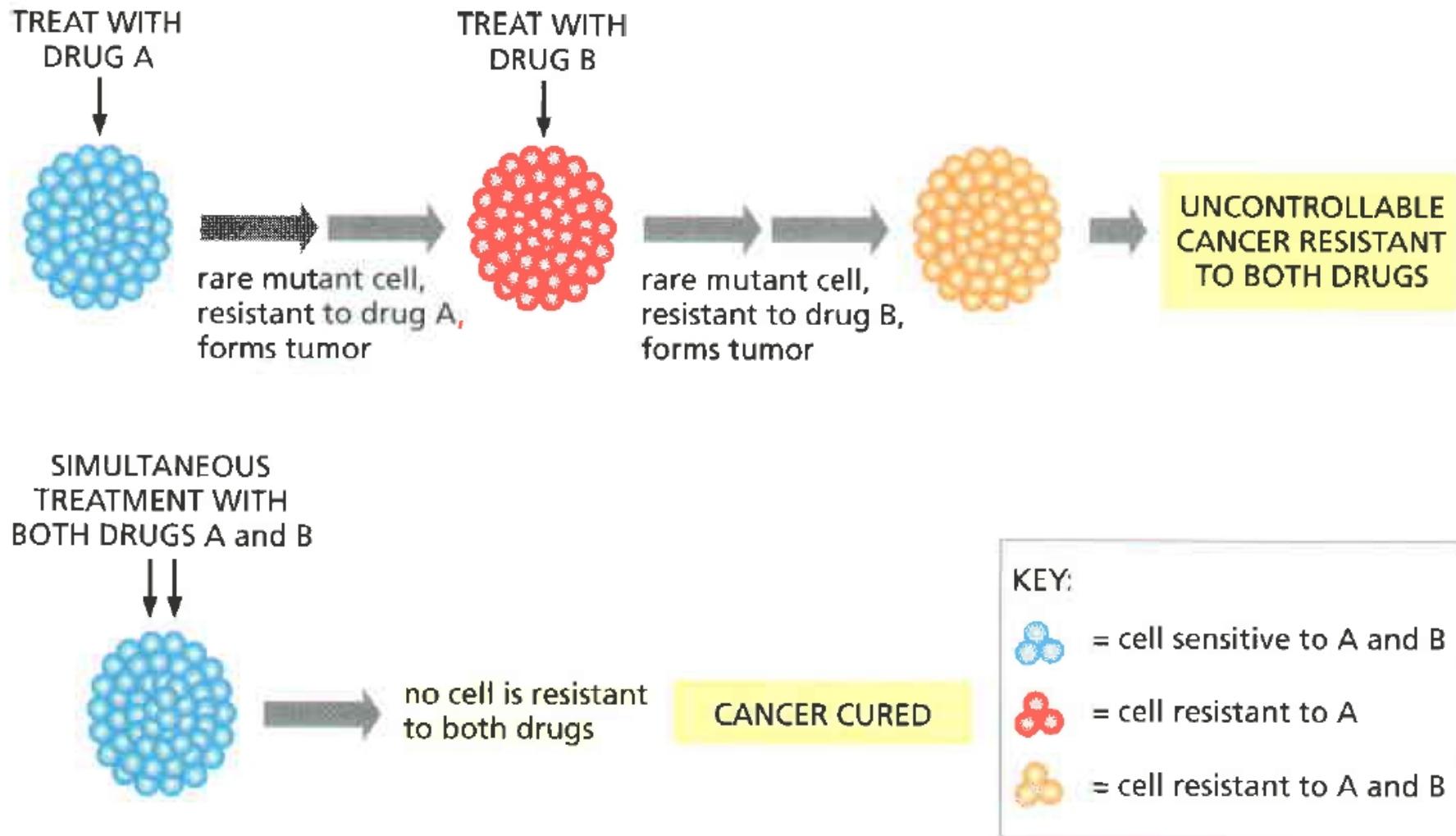
- Abl: Tyr-kinase involved in cell signaling
- Bcr-Abl fusion protein => Abl high activity => proliferation



CANCER TREATMENT EXAMPLE: GLEEVEC IN CHRONIC MYELOID LEUKEMIA



CANCER MULTIDRUG TREATMENT



CDK-INHIBITORS IN INDUSTRY

Compound	Primary target(s)	Clinical trials	Sponsor
AG-024322	CDK1 CDK2 CDK4	Phase I, advanced cancer: NCT00147485 Discontinued (2007)	Pfizer
AT-7519	CDK1 CDK2 CDK4 CDK5 GSK3 β	Phase I/II, advanced or metastatic tumours: NCT00390117	Astex
P276-00	CDK1 CDK4 CDK9	Phase I/II, advanced refractory neoplasms: NCT00407498	Piramal
P1446A-05	CDK4	Phase I, advanced refractory malignancies: NCT00772876	Piramal
PD-0332991	CDK4 CDK6	Phase I, advanced cancer: NCT00141297	Pfizer
R547 (also known as Ro-4584820)	CDK1 CDK2 CDK4 CDK7	Phase I, advanced solid tumours: NCT00400296	Hoffmann-LaRoche
Roscovitine (also known as seliciclib and CYC202)	CDK2 CDK7 CDK8 CDK9	Phase II, non-small cell lung cancer, nasopharyngeal cancer, haematological tumours: NCT00372073	Cyclacel
SNS-032 (also known as BMS-387032)	CDK1 CDK2 CDK4 CDK7 CDK9 GSK3 β	Phase I, B-lymphoid malignancies: NCT00446342 Phase I, solid tumours: NCT00292864	Sunesis

LECTURES 23-24: CELL CYCLE II

➤ Ubiquitylation and proteosome degradation

➤ APC/C in the cell cycle:

- molecular mechanisms

- structure-function relationship

➤ Cancer:

- microevolutionary process

- causes of cancer

- oncogens and molecular basis

- treatment

