Cancer Biology I :

Topics covered

Week 5:
Lecture 5/Exercises-paper: Telomeres and cellular senescence
(Chapters 10 (Weinberg))

Weeks 6:
Lecture 6/Exercises-Q&A: Telomeres: length and cancer, aging, mouse models;
CDKs and G1/S control
(Chapter 8 (Weinberg book): pRb and control of the cell cycle clock)

1 week break

Week 7, Monday: Q & A session: discussion of your questions
(to be submitted via email to me **by October 24**!!!)
Wednesday October 29, 2025: exam (contrôle continu)

Telomerase is expressed in the germ line, during early embryogenesis, and in stem cells in the adult.

But: Most normal human somatic cells do not express telomerase.

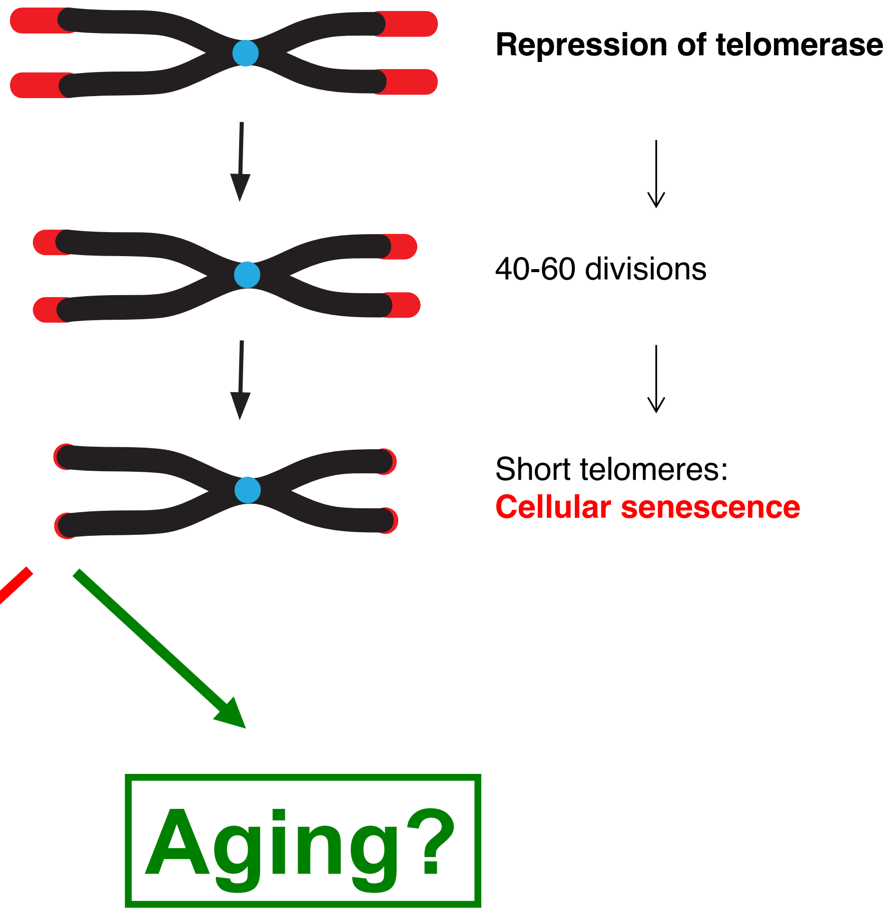
Telomere length and health: a 2- edged sword?

Telomere Shortening Suppresses Tumor Formation

Week 5:

- Telomerase activity is a hallmark of cancer (90%; 10% use ALT)
- Mutations in the hTERT promoter represent the most common noncoding mutations that occur in cancer, giving rise to monoallelic expression of TERT. Correcting these mutations in a brain tumor led to cancer regression.

→tremendous selective pressure during tumorigenesis to turn on hTERT.



Repression of telomerase

↓

40-60 divisions

↓

Short telomeres:
Cellular senescence

Cancer

Aging?

https://www.nature.com/articles/d41573-024-00102-7

- 14 June 2024

FDA approves first telomerase inhibitor

The US FDA has approved Geron’s imetelstat (Rytelo) for adults with low- to intermediate-risk **myelodysplastic syndromes (MDS).** The oligonucleotide-based drug is the first telomerase inhibitor to secure FDA approval.
In myelodysplastic syndromes dysfunctional malignant haematopoietic progenitor cells overgrow in the bone marrow. These cells often have high telomerase activity, helping them to achieve replicative immortality and clonal dominance. Most patients with myelodysplastic syndromes have anaemia, as well as an increased **risk** of developing **acute myeloid leukaemia**.
A telomerase inhibitor that can shorten telomeres of malignant cells, leading to their apoptosis, might help patients.

Long Telomeres Increase the Risk of Cancer Development

Three published examples:

Int J Epidemiol. 45(5):1634-1643 (2016).

Long telomeres and cancer risk among 95 568 individuals from the general population

Conclusions: Genetic determinants of long telomeres are associated with increased cancer risk, particularly melanoma and lung cancer. This genetic predisposition to enhanced telomere maintenance may represent a survival advantage for pre-cancerous cells, allowing for multiple cell divisions leading to cancer development.

eLife. 9:e61235 (2020).

***TINF2* is a haploinsufficient tumor suppressor that limits telomere length**

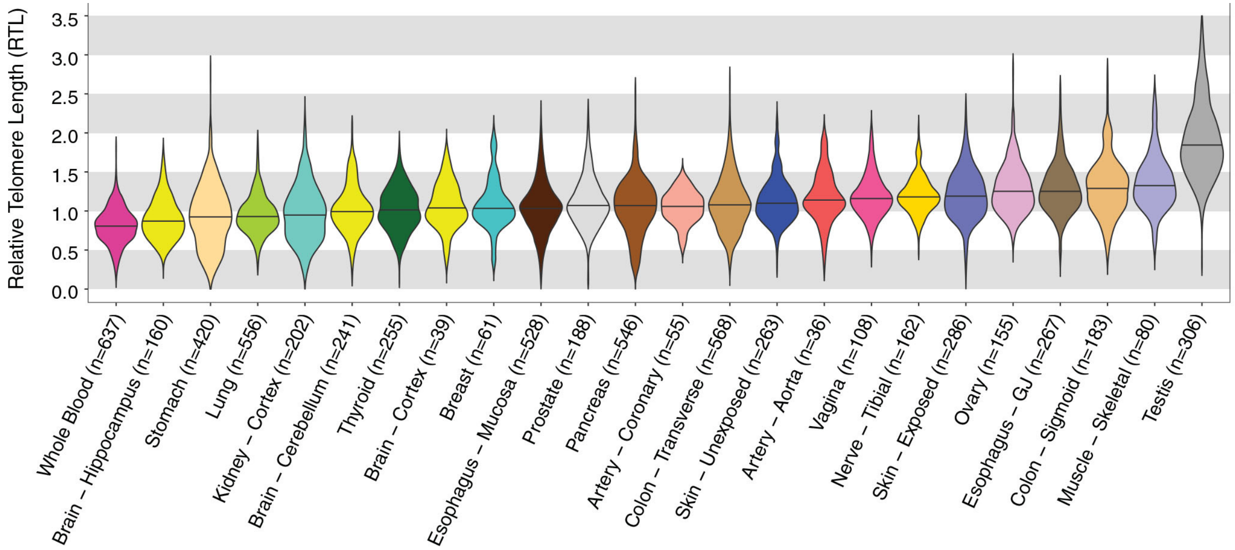
Conclusions: Cancer-prone families with truncated TIN2 (shelterin subunit) contain lymphocyte telomeres that are unusually long. *TINF2* truncations predispose to tumor syndromes. *TINF2* acts as a tumor suppressor that limits telomere length to ensure a timely Hayflick limit.

Science 379, 253–260 (2023)

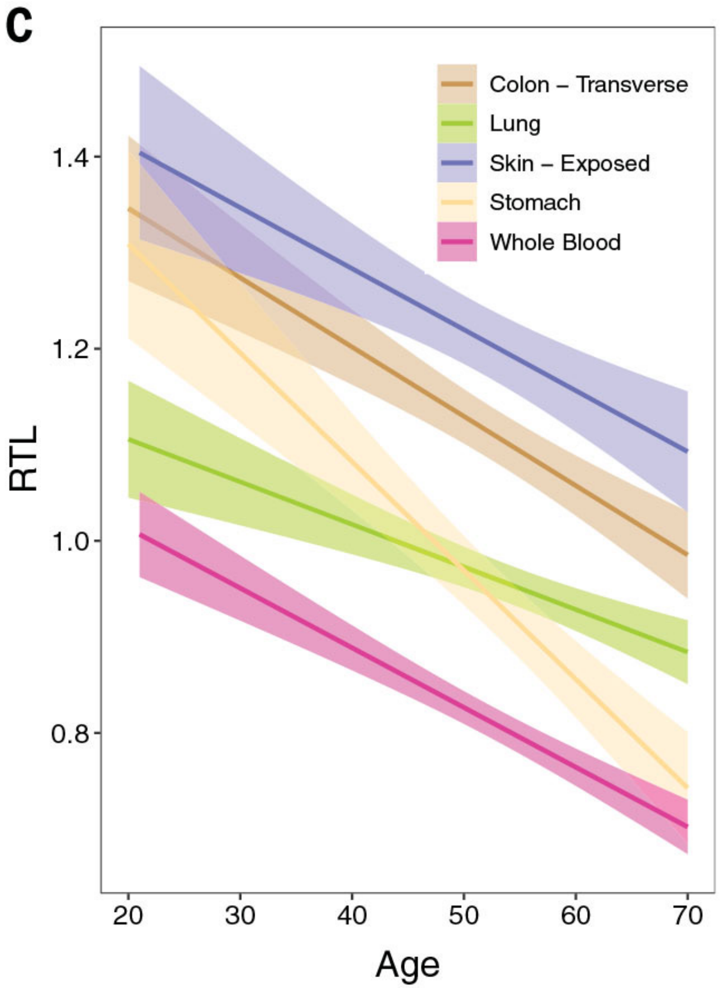
Heritable defects in telomere and mitotic function selectively predispose to sarcomas

Conclusions: Performed whole genome germline sequencing on 1644 sporadic cases and 3205 matched healthy elderly controls. Heritable defects in the shelterin complex link susceptibility to sarcoma, melanoma, and thyroid cancers. These mutations give rise to longer telomeres.

Relative telomere length in tissues and shortening with age



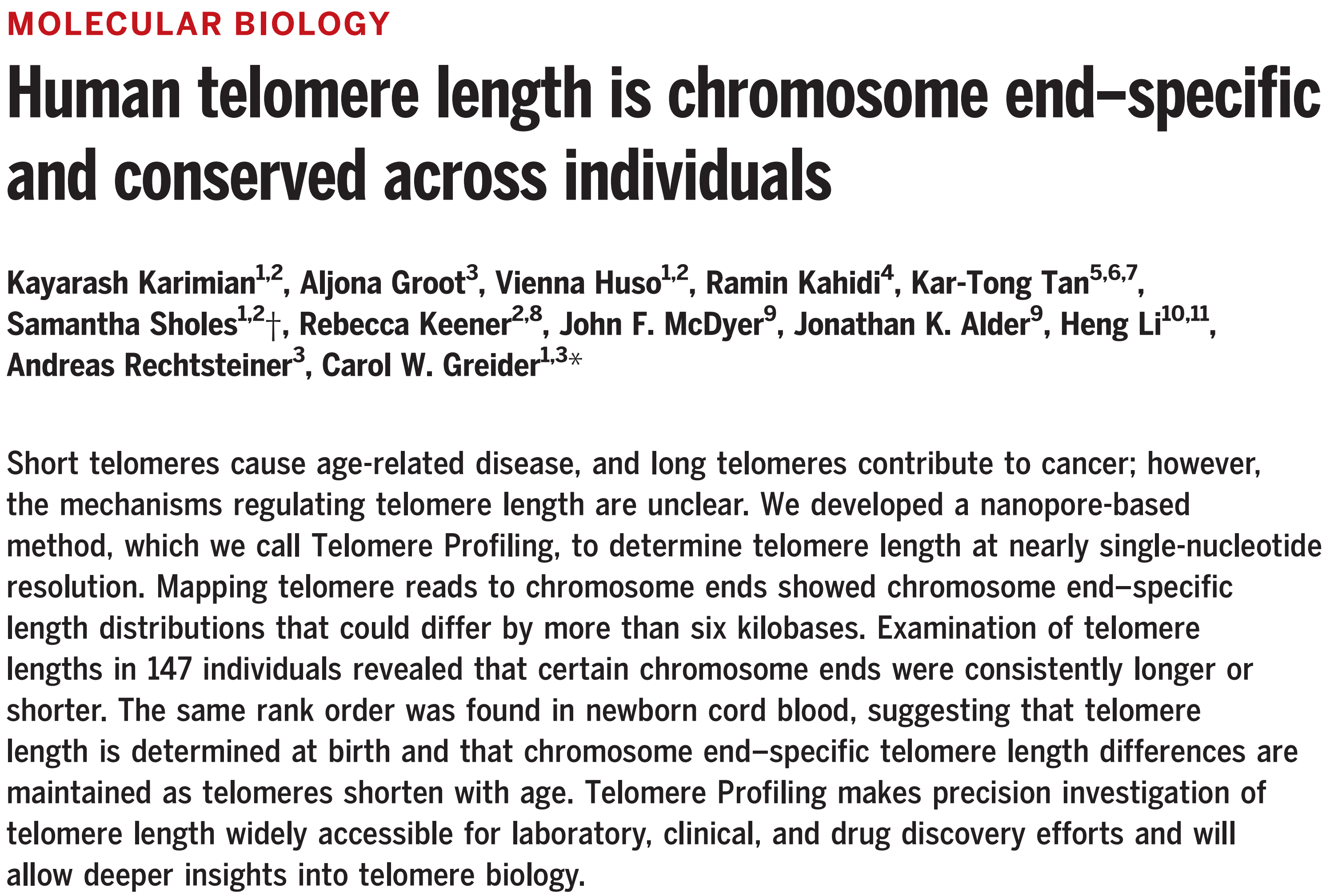
Measured relative telomere DNA repeat abundance in the samples



From: Science **369**, 1333 (2020)

Nanopore sequencing method developed for telomeres:

- →precise telomere length measurements at individual chromosome ends.
- →should have great impact to understand telomere length function during normal development, aging, multiple diseases including cancer.
- →diagnostic tool if implemented in the clinic.



MOLECULAR BIOLOGY

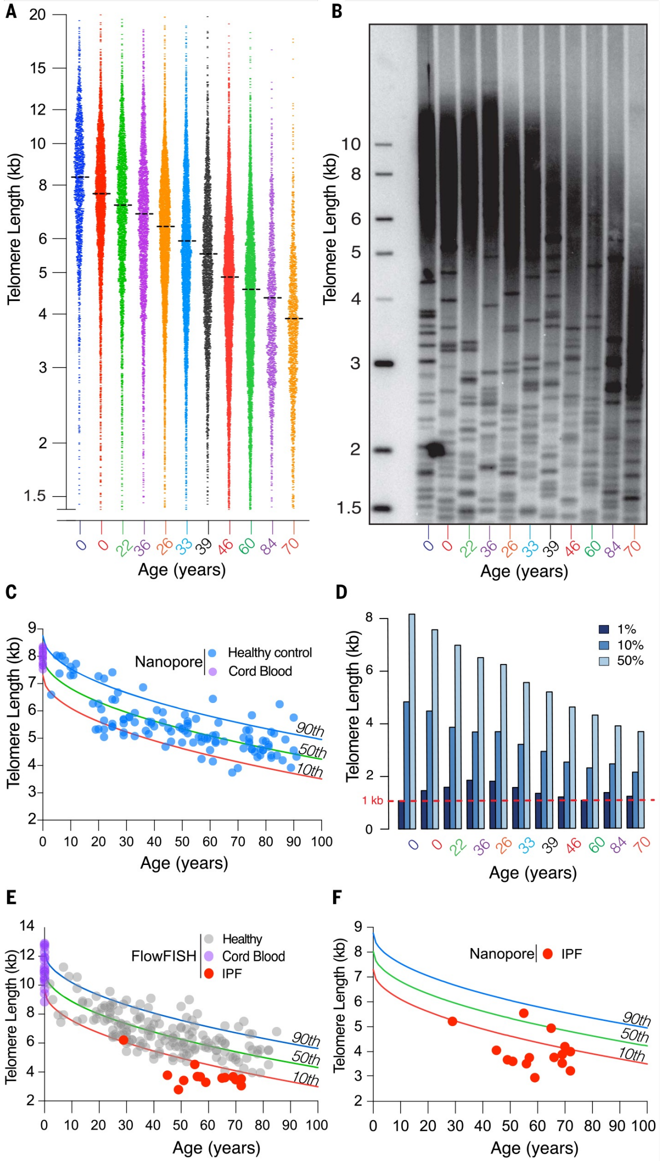
Human telomere length is chromosome end–specific and conserved across individuals

Kayarash Karimian[1,2], Aljona Groot[3], Vienna Huso[1,2], Ramin Kahidi[4], Kar-Tong Tan[5,6,7], Samantha Sholes[1,2]†, Rebecca Keener[2,8], John F. McDyer[9], Jonathan K. Alder[9], Heng Li[10,11], Andreas Rechtsteiner[3], Carol W. Greider[1,3]*

Short telomeres cause age-related disease, and long telomeres contribute to cancer; however, the mechanisms regulating telomere length are unclear. We developed a nanopore-based method, which we call Telomere Profiling, to determine telomere length at nearly single-nucleotide resolution. Mapping telomere reads to chromosome ends showed chromosome end–specific length distributions that could differ by more than six kilobases. Examination of telomere lengths in 147 individuals revealed that certain chromosome ends were consistently longer or shorter. The same rank order was found in newborn cord blood, suggesting that telomere length is determined at birth and that chromosome end–specific telomere length differences are maintained as telomeres shorten with age. Telomere Profiling makes precision investigation of telomere length widely accessible for laboratory, clinical, and drug discovery efforts and will allow deeper insights into telomere biology.

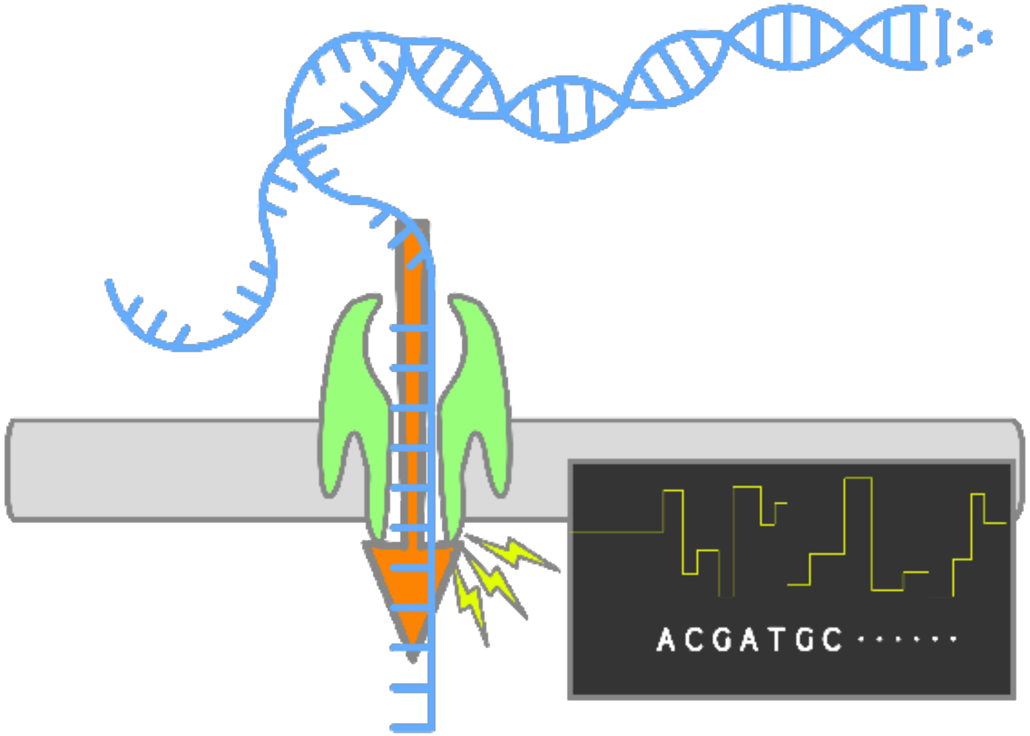
Karimian et al., Science 384, 533–539 (2024)

Fig. 2. Telomere Profiling measures telomere shortening with age and detects individuals with short telomere syndromes. (**A**) Telomere length profiles for 11 samples selected (age is noted at bottom). Each point is an individual read (total reads = 47,624). (**B**) Prospective Southern blot of same samples as (A). Age of individual noted at bottom. (**C**) The mean elomere length was determined for 132 individuals aged 0 to 91 (blue dots). Cord blood lengths are shown in purple. The population distribution and confidence intervals for the 90th (blue line), 50th (green line), and 10th percentiles (red line) for telomere length in this population are shown using parameters established for FlowFISH (*16*). (**D**) For each individual in (A), we examined the reads that fell into the 50th, 10th, and 1st percentiles for telomere lengths. The red dashed line indicates 1-kb telomere length. (**E**) Lymphocyte telomere length from FlowFISH data from (*16*) (gray dots); cord blood length is shown with purple dots. The lengths for 15 individuals with IPF were determined by FlowFISH (red dots). One point represents two individuals who have nearly identical length and are indistinguishable. (**F**) Nanopore telomere length profiles from the same 15 individuals with IPF are shown plotted against population distribution from (C) (total reads = 32,457).



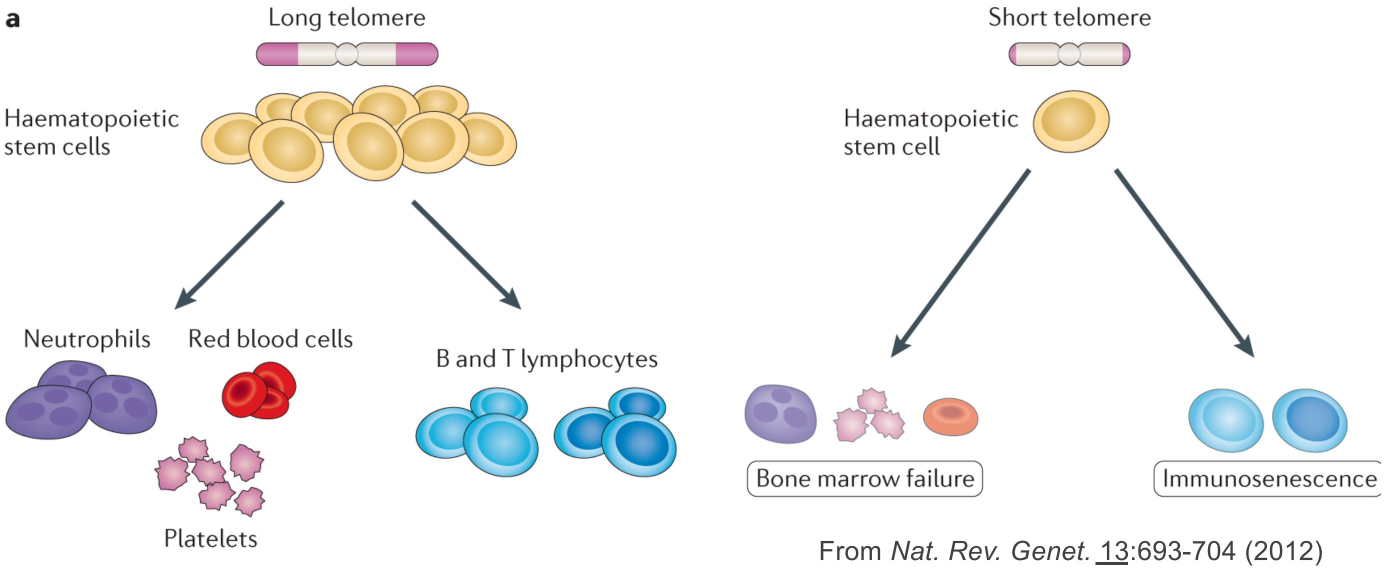
Idiopathic pulmonary fibrosis (IPF) is a condition in which the lungs become scarred and breathing becomes increasingly difficult.

For Aficionados: https://en.wikipedia.org/wiki/Nanopore_sequencing



The magnitude of the electric current density across a nanopore surface depends on the nanopore's dimensions and the composition of DNA or RNA that is occupying the nanopore. Sequencing was made possible because, passing through the channel of the nanopore, the samples cause characteristic changes in the density of the electric current flowing through the nanopore.

Telomere Syndromes: Humans with Defects in Telomerase Genes; Telomerase activity is important during embryogenesis and possibly in stem cells

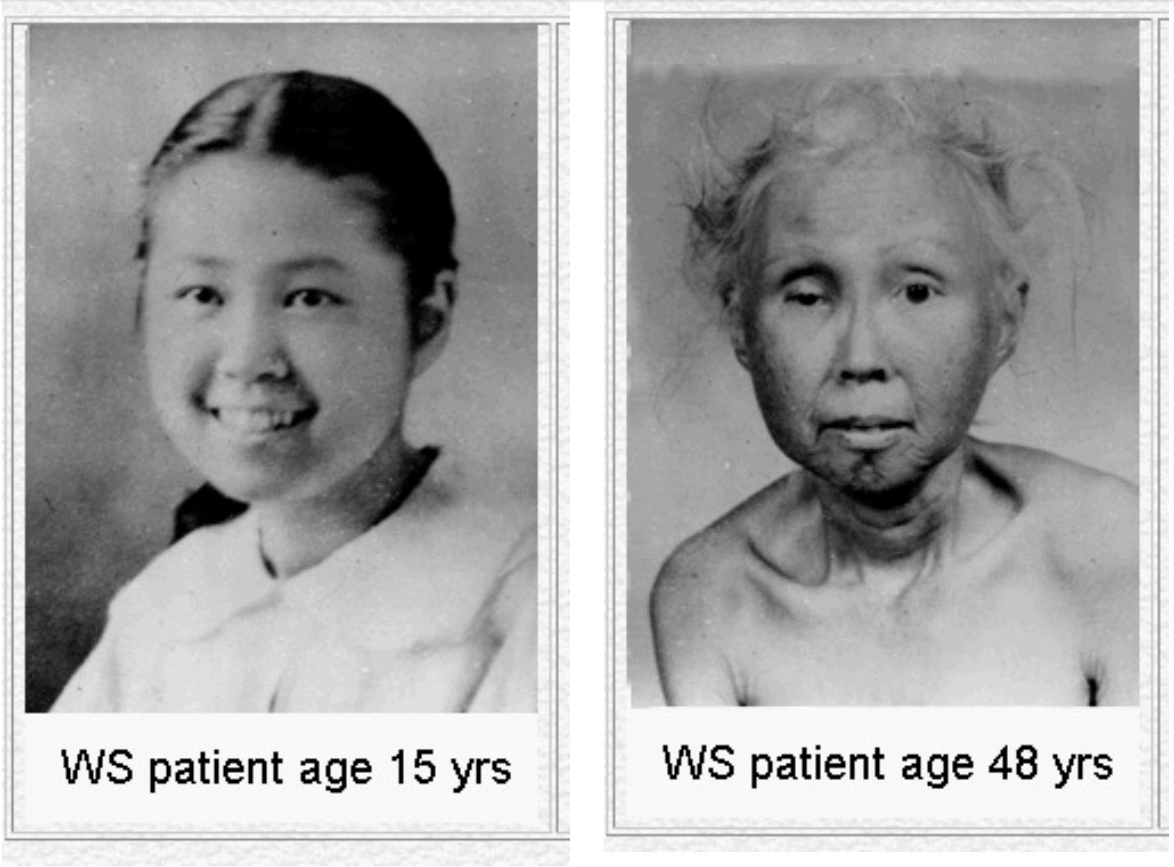


From *Nat. Rev. Genet.* 13:693-704 (2012)

Often: Premature death because of bone marrow failure or pulmonary fibrosis

(Telomere syndromes: Dyskeratosis congenita, aplastic anemia, Coats plus, Hoyeraal-Hreidarsson syndrome)

Werner Patient: Premature Aging Syndrome



WS patient age 15 yrs

WS patient age 48 yrs

A Japanese-American Werner patient as a teenager (left), and at age 48 (Case #1 Epstein *et al*, 1966, *Medicine* 45:177). At 48, she had hair loss and greying, thin extremities, chronic ulcerations of the ankles, atrophy of the skin and her right eye had been enucleated several years earlier due to acute glaucoma at the age of 27. She lived longer than many Werner patients, dying at 57

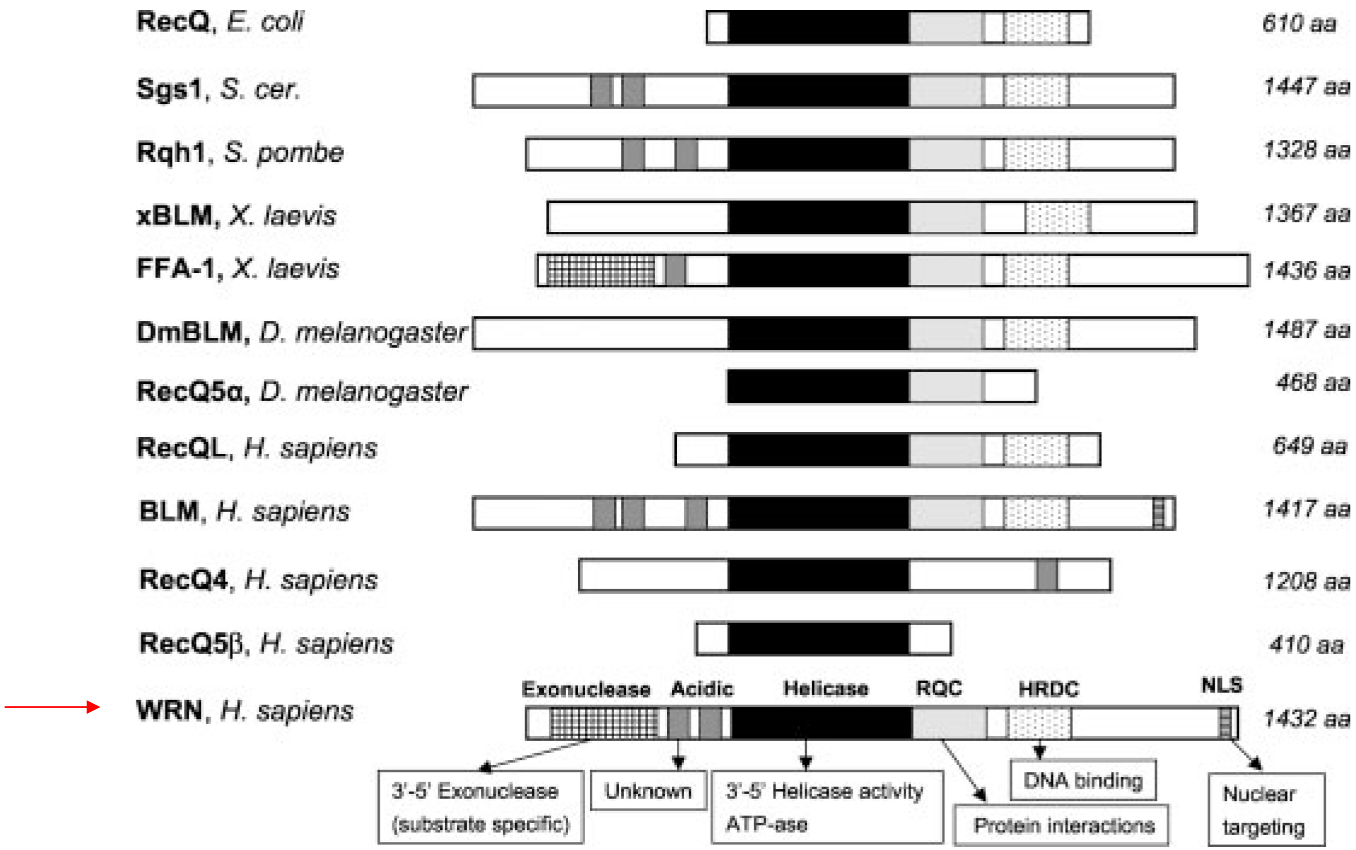
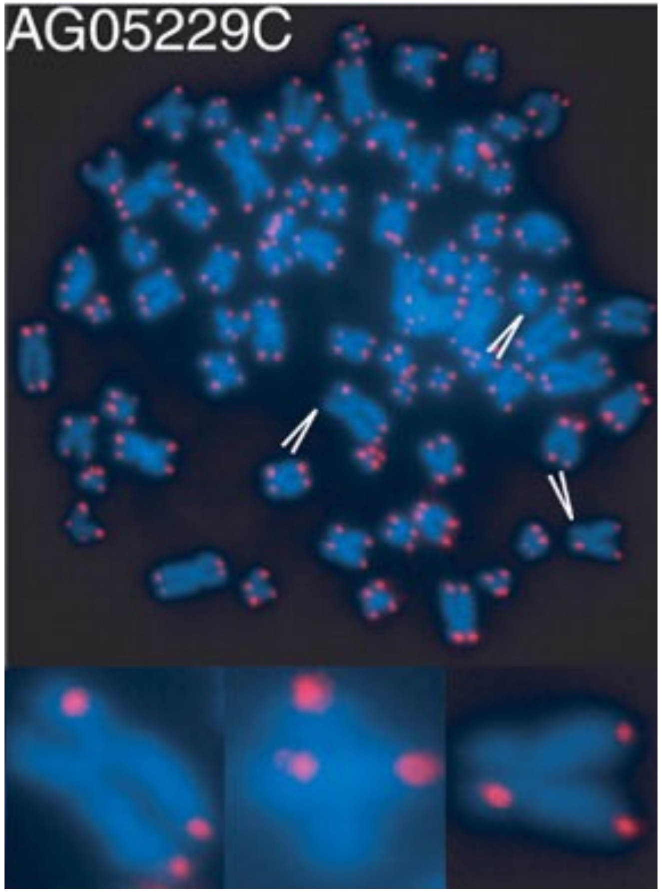


FIG. 1. **Family of RecQ helicases.** Schematic representation of selected members of the RecQ family is shown. *RQC*, RecQ conserved C-terminal domain; *HRDC*, helicase and RNase D C-terminal domain; *NLS*, nuclear localization signal. The proposed primary roles for each domain are indicated. *aa*, amino acids.

Crabbe L, Verdun RE, Haggblom CI, Karlseder J. **Defective telomere lagging strand synthesis in cells lacking WRN helicase activity.** Science. 2004 Dec 10;306(5703):1951-3. doi: 10.1126/science.1103619. PMID: 15591207.

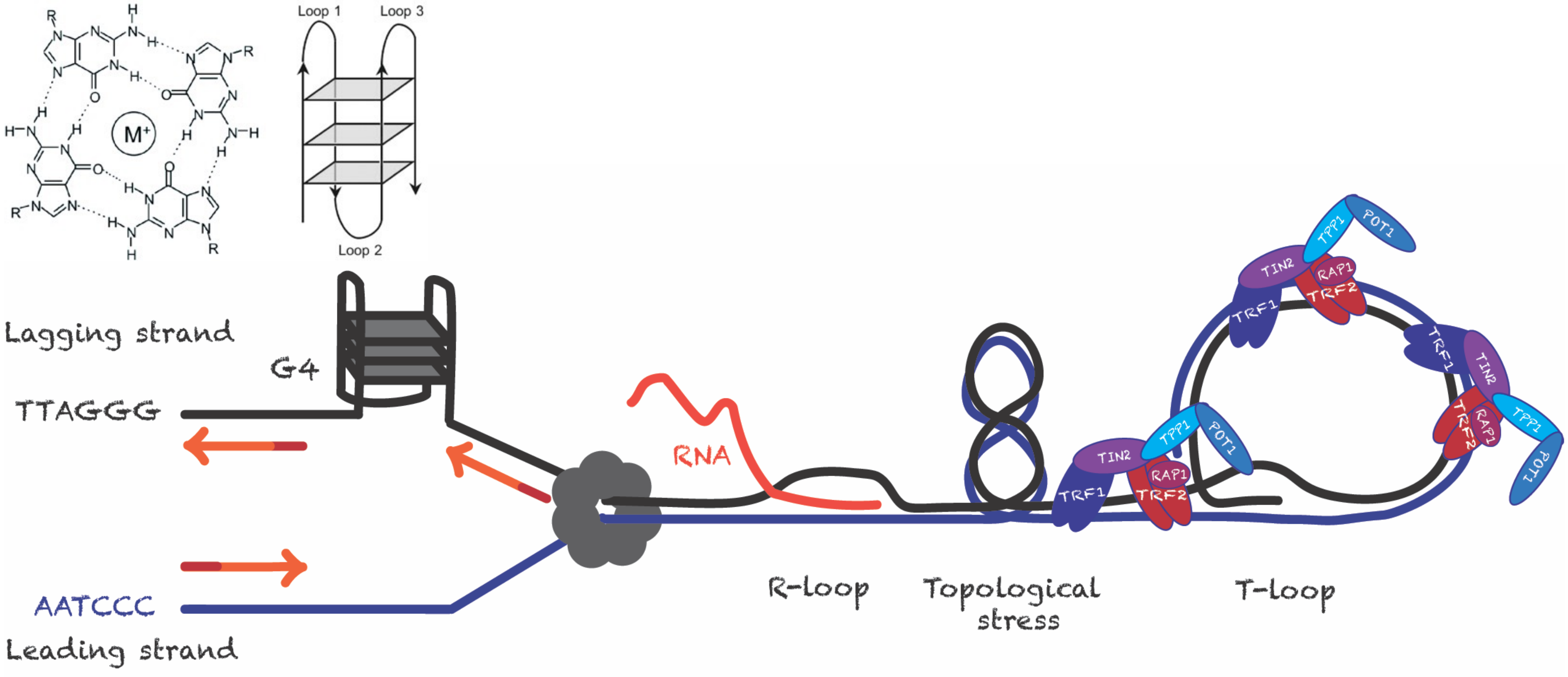


Frequent telomere loss events of telomeres replicated by discontinuous synthesis (lagging strand telomeres) in fibroblasts (telomerase negative) from WRN patients:

These telomere loss events can be counteracted by telomerase in the cells that do express it
→more dramatic consequences of WRN-loss in differentiated cells as opposed to stem cells (?).

The Bulk Telomere Replication Problem

At lagging strand telomeres, G-quadruplexes may form during replication, which need to be unwound by helicases. WRN is thought to contribute to G-quadruplex unwinding.



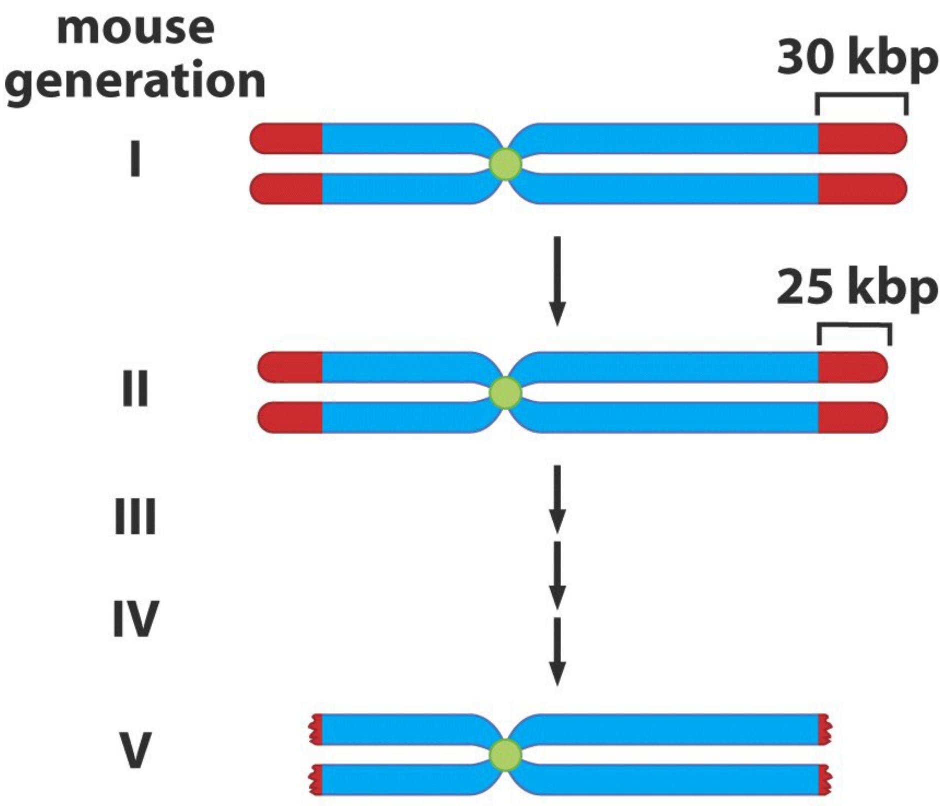
Mouse Models: Lack of the telomere shortening barrier during tumorigenesis

Some crucial differences in telomere biology between mice and human beings:

- **Telomerase** is **not** tightly **repressed** in somatic cells of mice. Telomeres of lab mouse strains are **much longer** than human **telomeres**.
- Mouse cells can be **immortalized readily** following extension in culture (spontaneous immortalization).
- Human cells require introduction of e.g. SV40 LT repressing p53 and pRb function (to avoid senescence) and the hTERT gene (to avoid crisis).

…Humans, whose cells pass through 10^{16} mitosis during lifetime should have a much greater risk than mice, which experience only 10^{11} cell divisions, to develop cancer; but 46% of *mus musculus* develop tumors during their short life time

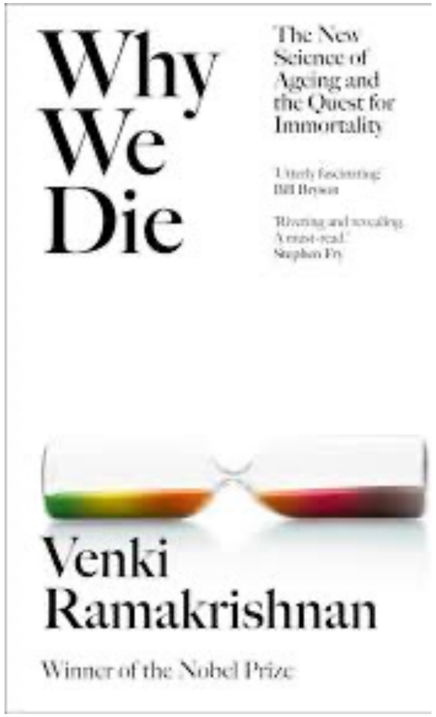
Mouse Models (mTR-/- mice: lacking the telomerase RNA gene)



G5: Diminished wound-healing, reduced fertility, atrophy in highly proliferative tissues

In case you want to know more about aging:

Listen to podcast with Venki Ramakrishnan:
https://think.kera.org/2024/04/16/how-the-science-of-dying-can-help-us-live-longer/



The Cell Cycle

The **action of most oncogenes and many tumor suppressor genes must be explained in terms of their effects on the cell cycle clocks**.

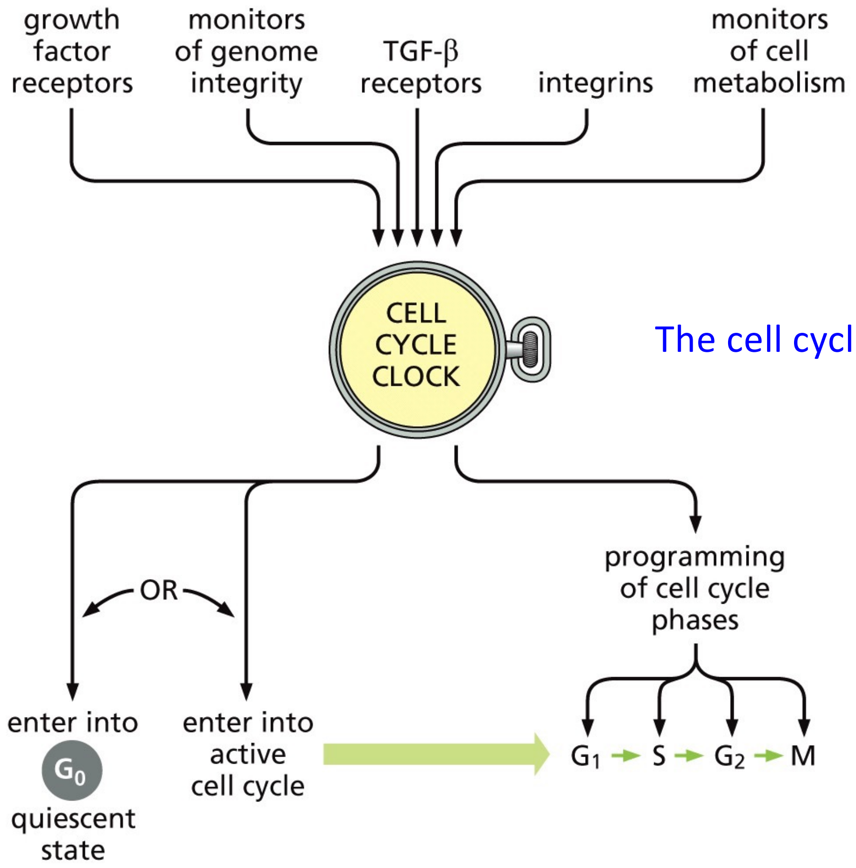

growth factor receptors
monitors of genome integrity
TGF-β receptors
integrins
monitors of cell metabolism
CELL CYCLE CLOCK
OR
enter into G_0 quiescent state
enter into active cell cycle
programming of cell cycle phases
$G_1 \rightarrow S \rightarrow G_2 \rightarrow M$

Figure 8.1. Weinberg, The Biology of Cancer

The cell cycle clock acts in the nucleus

Withdrawal from cell cycle (G_0): growth inhibitory factors such as transforming growth factor-ß (TGF-ß), lack of mitogenic growth factors.
If permanent: ”post-mitotic” cells (e.g. neurons)

The Cell Cycle

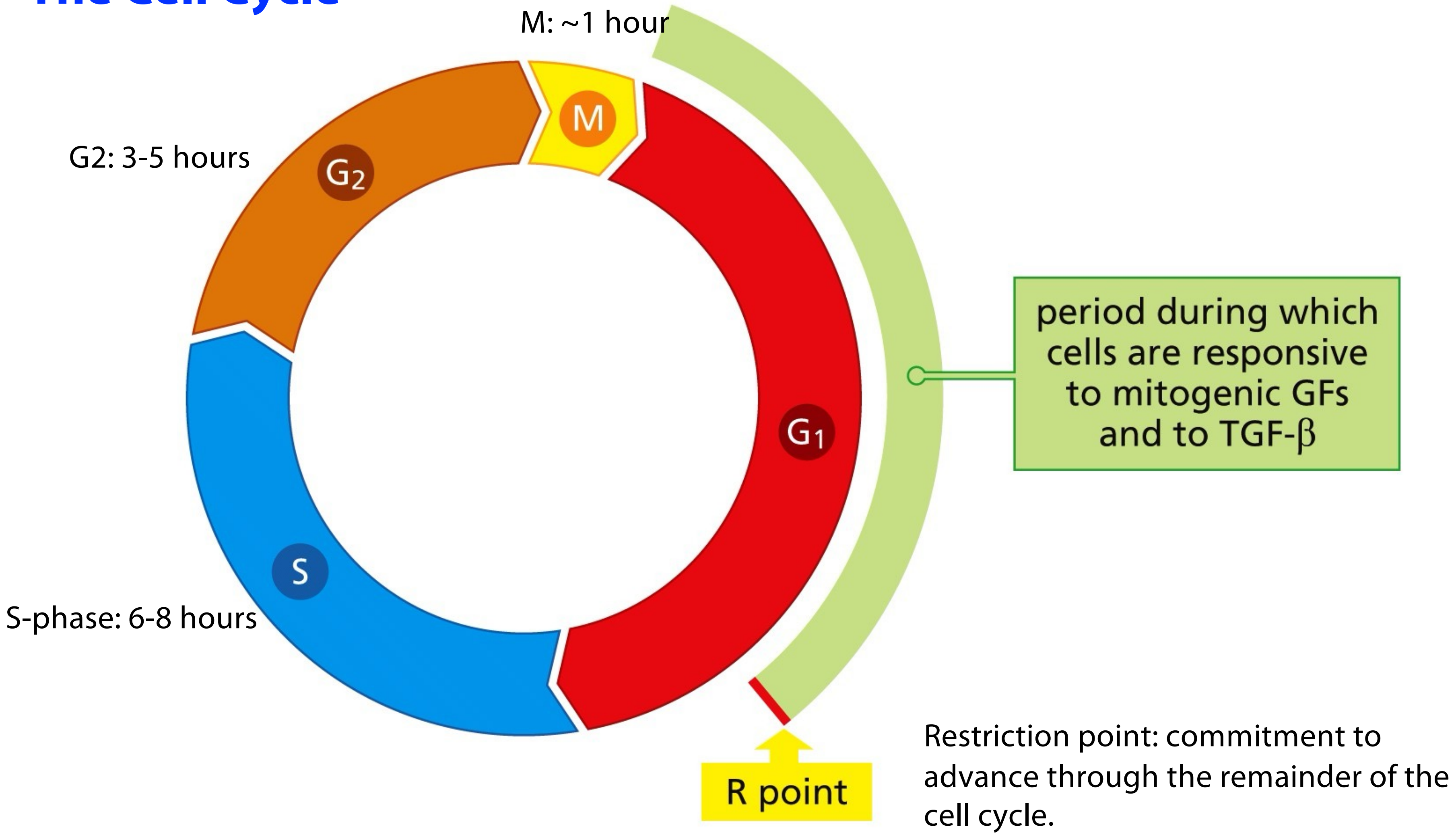
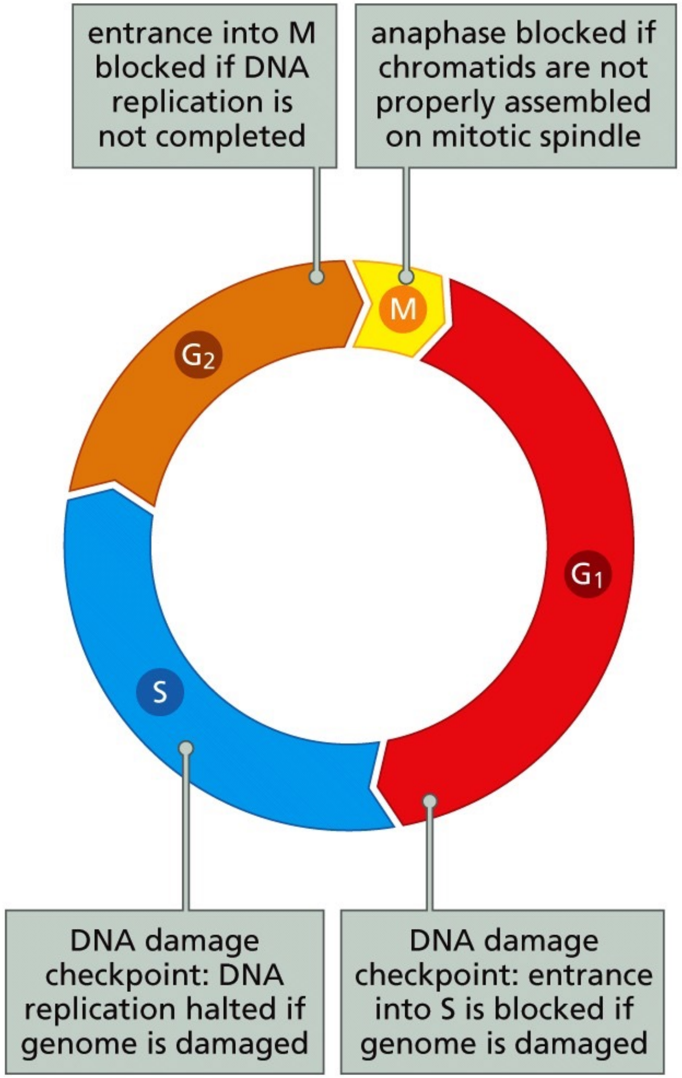


Figure 8.5. Weinberg, The Biology of Cancer

Restriction point: commitment to advance through the remainder of the cell cycle.
Cancer: deregulation of the R-point decision-making machinery

Examples of checkpoints



entrance into M blocked if DNA replication is not completed

anaphase blocked if chromatids are not properly assembled on mitotic spindle

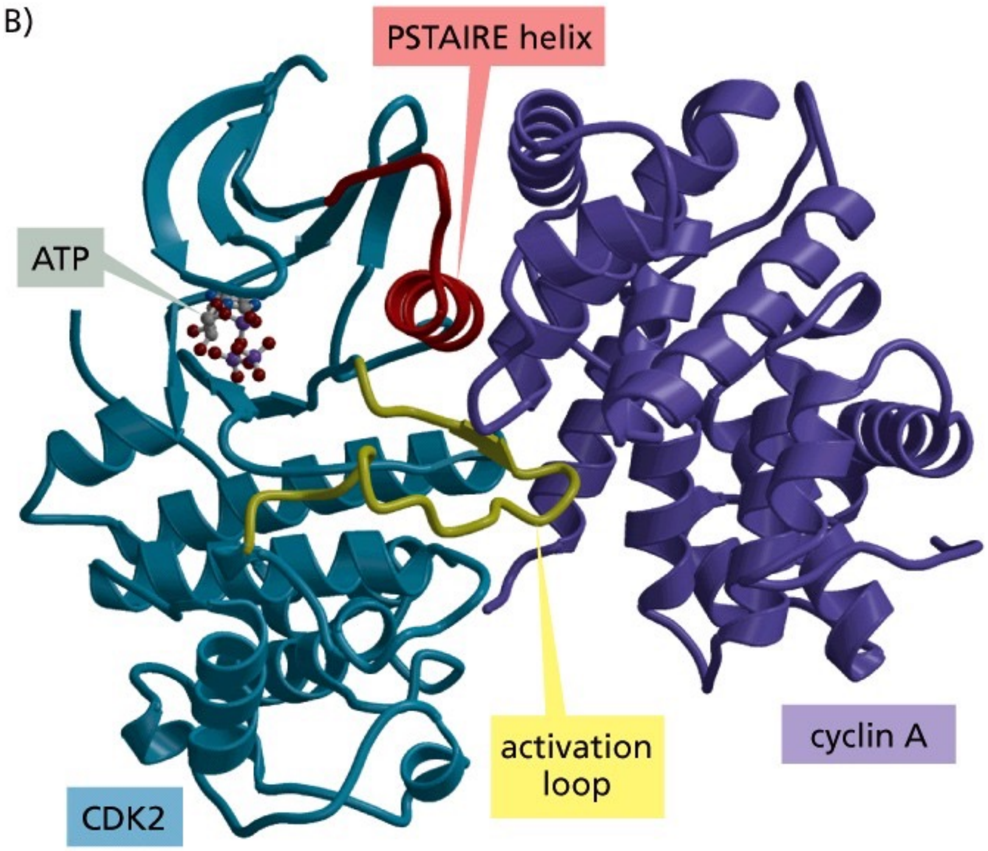
DNA damage checkpoint: DNA replication halted if genome is damaged

DNA damage checkpoint: entrance into S is blocked if genome is damaged

Not discussed:
spindle assembly checkpoint

Figure 8.4. Weinberg, The Biology of Cancer

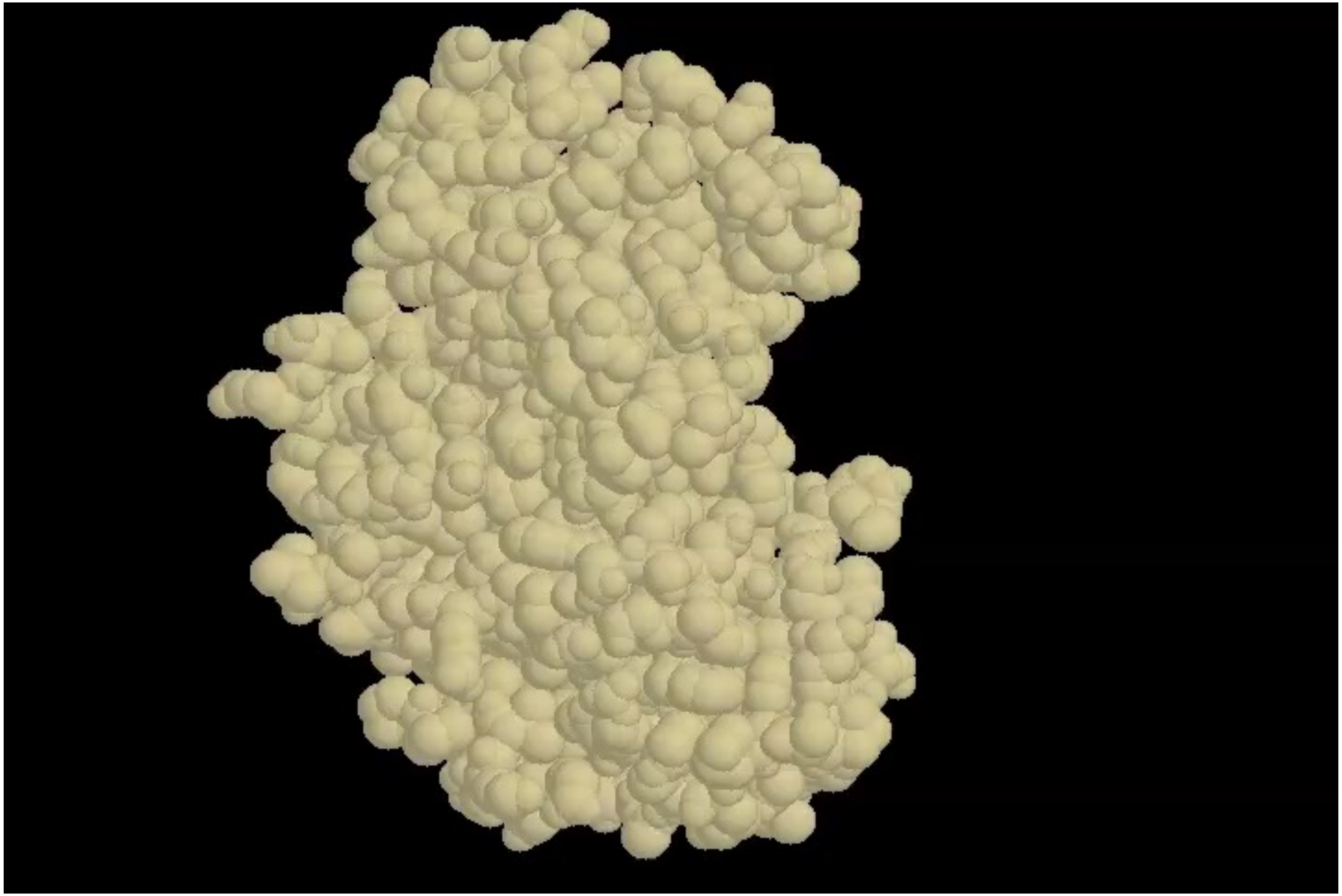
Cyclin-dependent Kinases: Core Components of the Cell Cycle Clock



PSTAIRE α-helix: cyclin binding

Activation-loop (also called **T-loop**): Phosphorylated on Thr by a CDK-activating enzyme (**CAK**).

- CDKs are Ser/Thr kinases
- Cyclins activate the catalytic activity (e.g. cyclin A activates CDK2 400,000x)
- Cyclins also promote correct protein substrate recognition
- Full activation requires phosphorylation by CAK



Pairing of Cyclins with Cyclin-Dependent Kinases

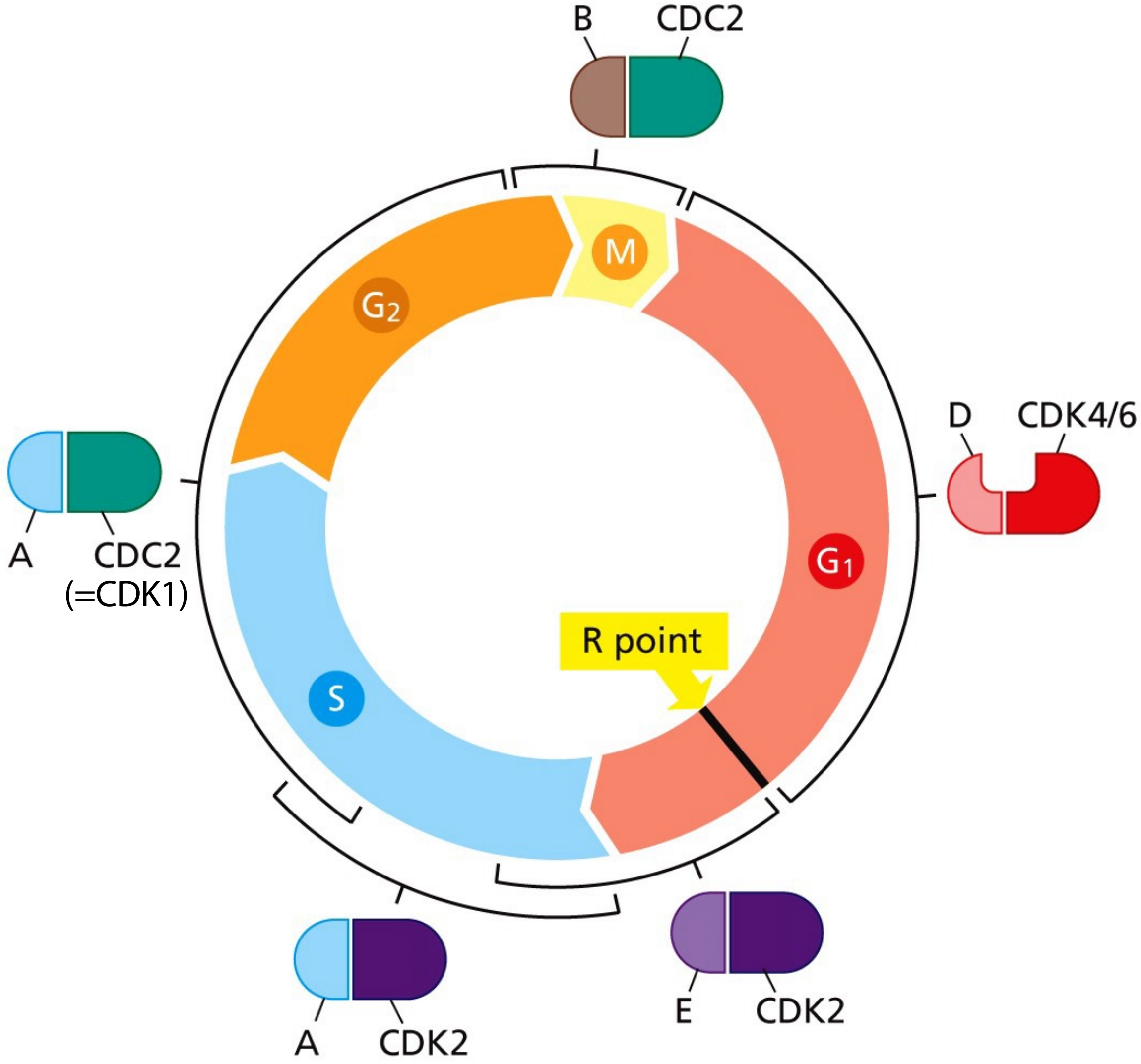


Figure 8.6. Weinberg, The Biology of Cancer

Cyclins fluctuate during the cell cycle

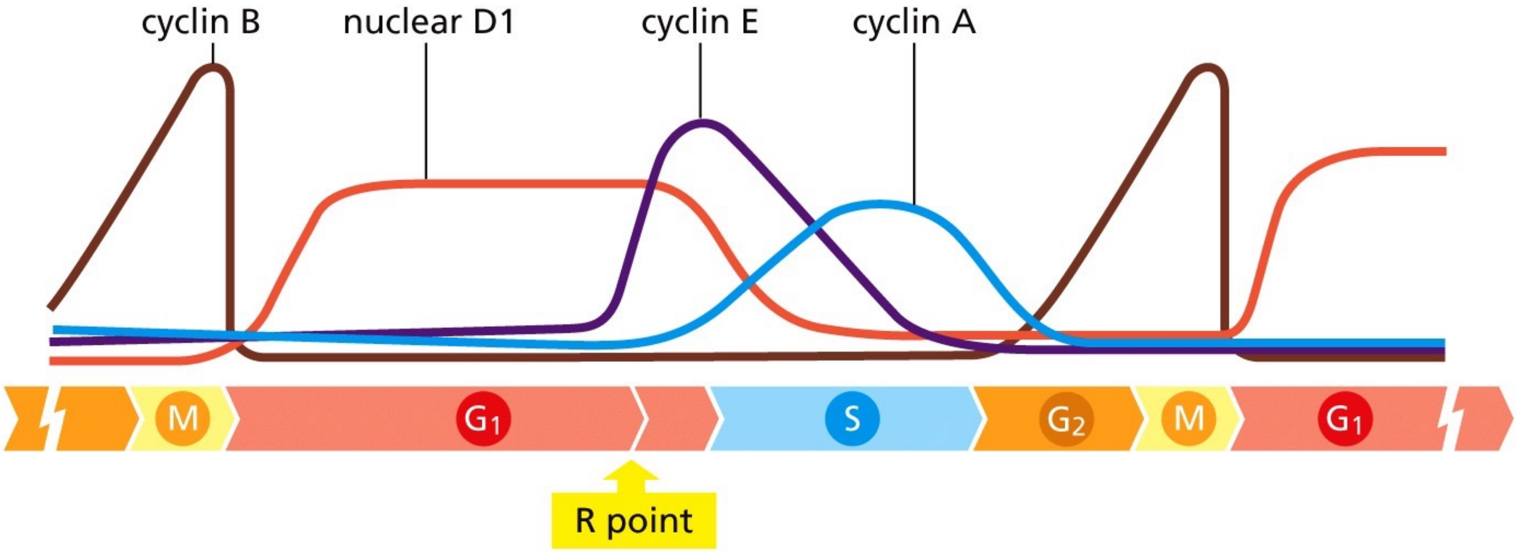


Figure 8.8. Weinberg, The Biology of Cancer

- Collapse of various cyclins is triggered by ubiquitin ligases →polyubiquitination→degradation by the proteasome

…cell cycle clock can only move in one direction

Cyclin D: levels are controlled by extracellular signals
Other cyclins: control by intracellular signaling

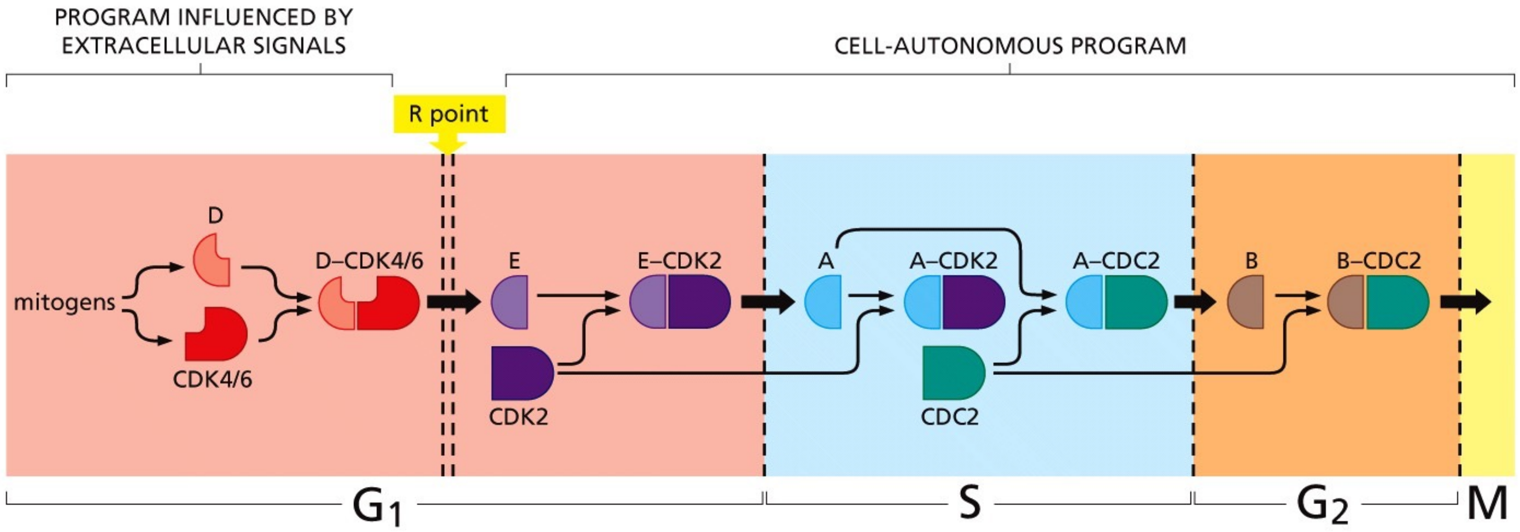


Figure 8.10. Weinberg, The Biology of Cancer

- Cyclin-CDK complexes in one phase of the cell cycle are responsible for activating those in the subsequent phase; and for shutting down those that were active in the previous phase (mechanism not well understood).

Exception: Cyclin D: levels are controlled by extracellular signals

Northern blot:

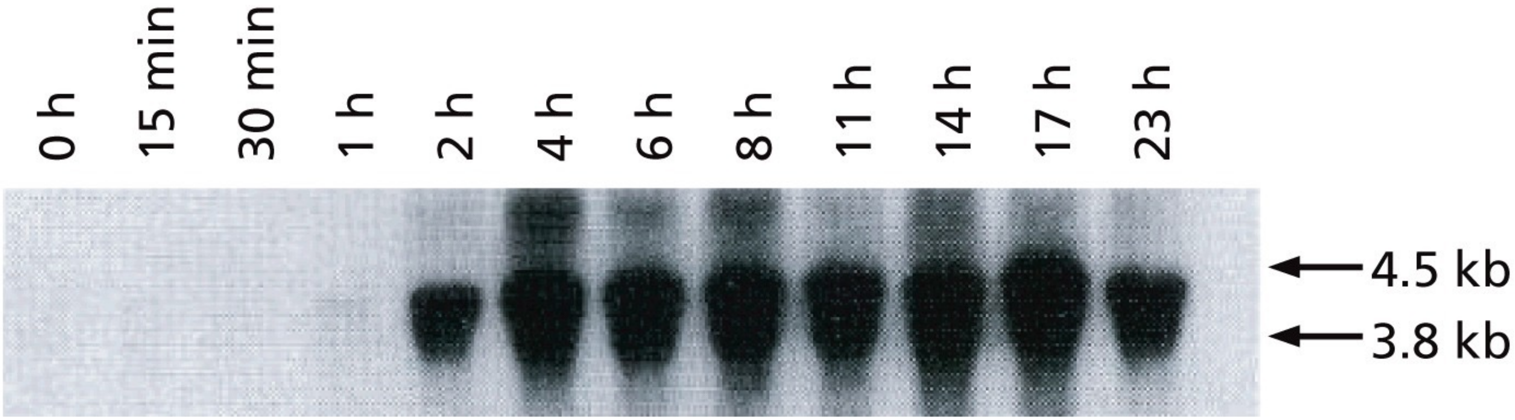


Figure 8.9. Weinberg, The Biology of Cancer

Starved macrophages were exposed to the mitogen CSF-1 (colony-stimulating factor 1)

Various signaling cascades induce the cyclin D promoters

- D-type cyclins: convey signals from the extracellular environment.
- D-type cyclins assemble with CDK4 and CDK6 both of which have similar enzymatic activities.
- Cyclin D1, D2, D3: induced by different transcription factors.

Estrogen receptors are overexpressed in 70% of breast cancer.
>70% of tumors overexpress MYC

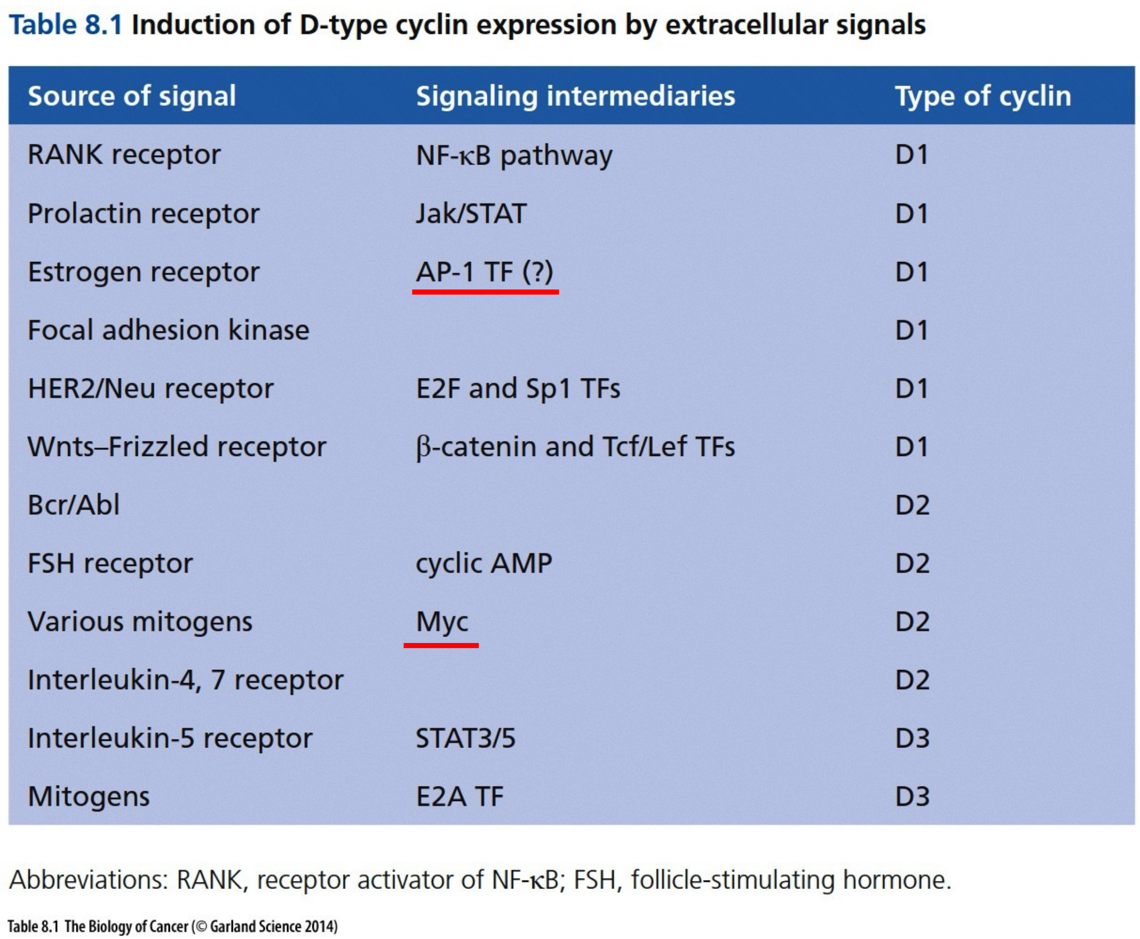


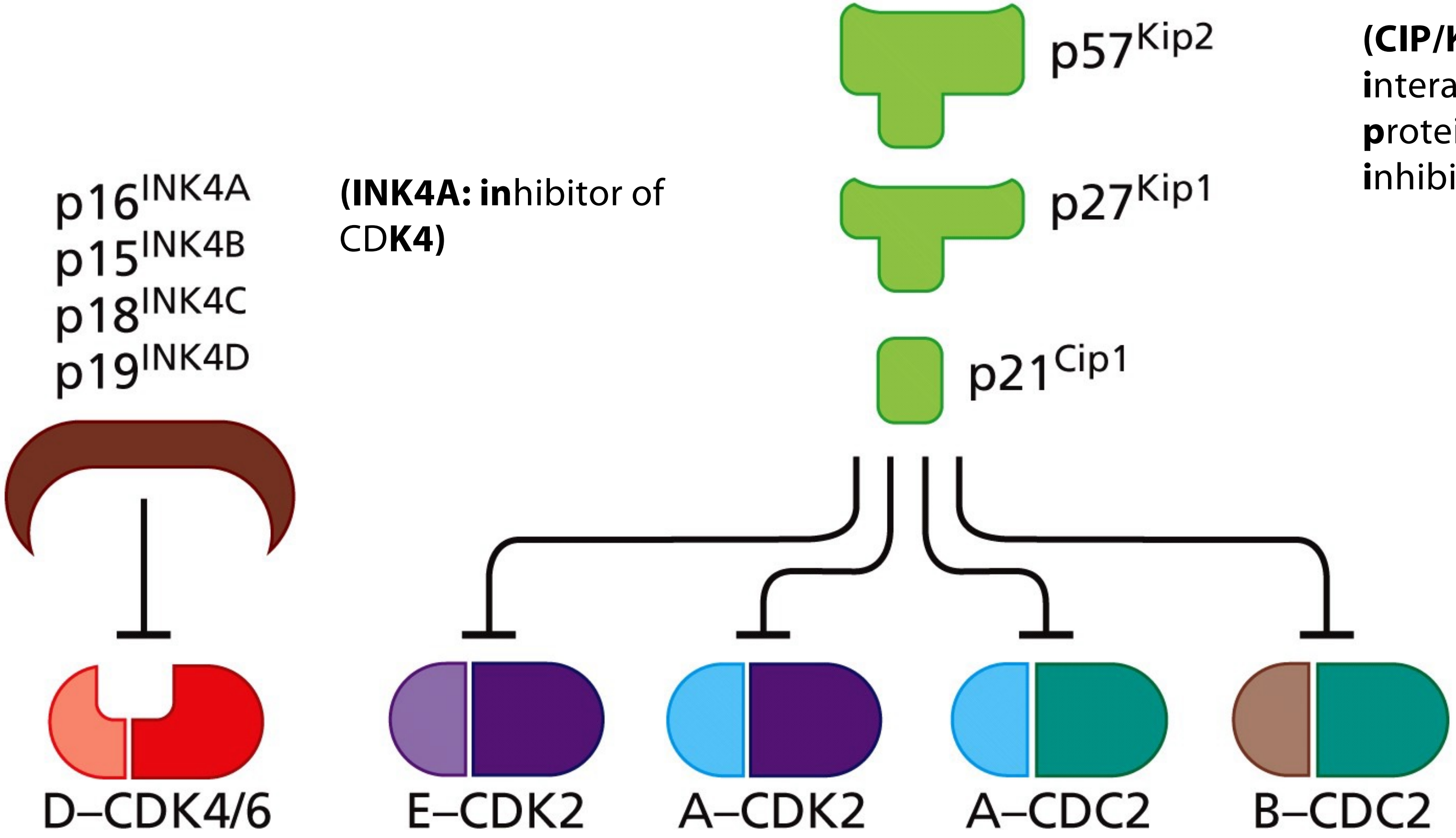
Table 8.1 Induction of D-type cyclin expression by extracellular signals

Source of signal	Signaling intermediaries	Type of cyclin
RANK receptor	NF-κB pathway	D1
Prolactin receptor	Jak/STAT	D1
Estrogen receptor	AP-1 TF (?)	D1
Focal adhesion kinase		D1
HER2/Neu receptor	E2F and Sp1 TFs	D1
Wnts–Frizzled receptor	β-catenin and Tcf/Lef TFs	D1
Bcr/Abl		D2
FSH receptor	cyclic AMP	D2
Various mitogens	Myc	D2
Interleukin-4, 7 receptor		D2
Interleukin-5 receptor	STAT3/5	D3
Mitogens	E2A TF	D3

Abbreviations: RANK, receptor activator of NF-κB; FSH, follicle-stimulating hormone.

Table 8.1 The Biology of Cancer (© Garland Science 2014)

Cyclin-CDK complexes: regulation by 7 CDK-inhibitors (CKIs)



(**INK4A: in**hibitor of CD**K4**)

(**CIP/KIP: C**DK **i**nteracting **p**rotein/**K**inase **i**nhibitory **p**rotein)

Figure 8.11. Weinberg, The Biology of Cancer

p27Kip1 obstructs the ATP-binding site of CDK2

($p16^{INK4A}$ distorts the cyclin binding site of CDK6)

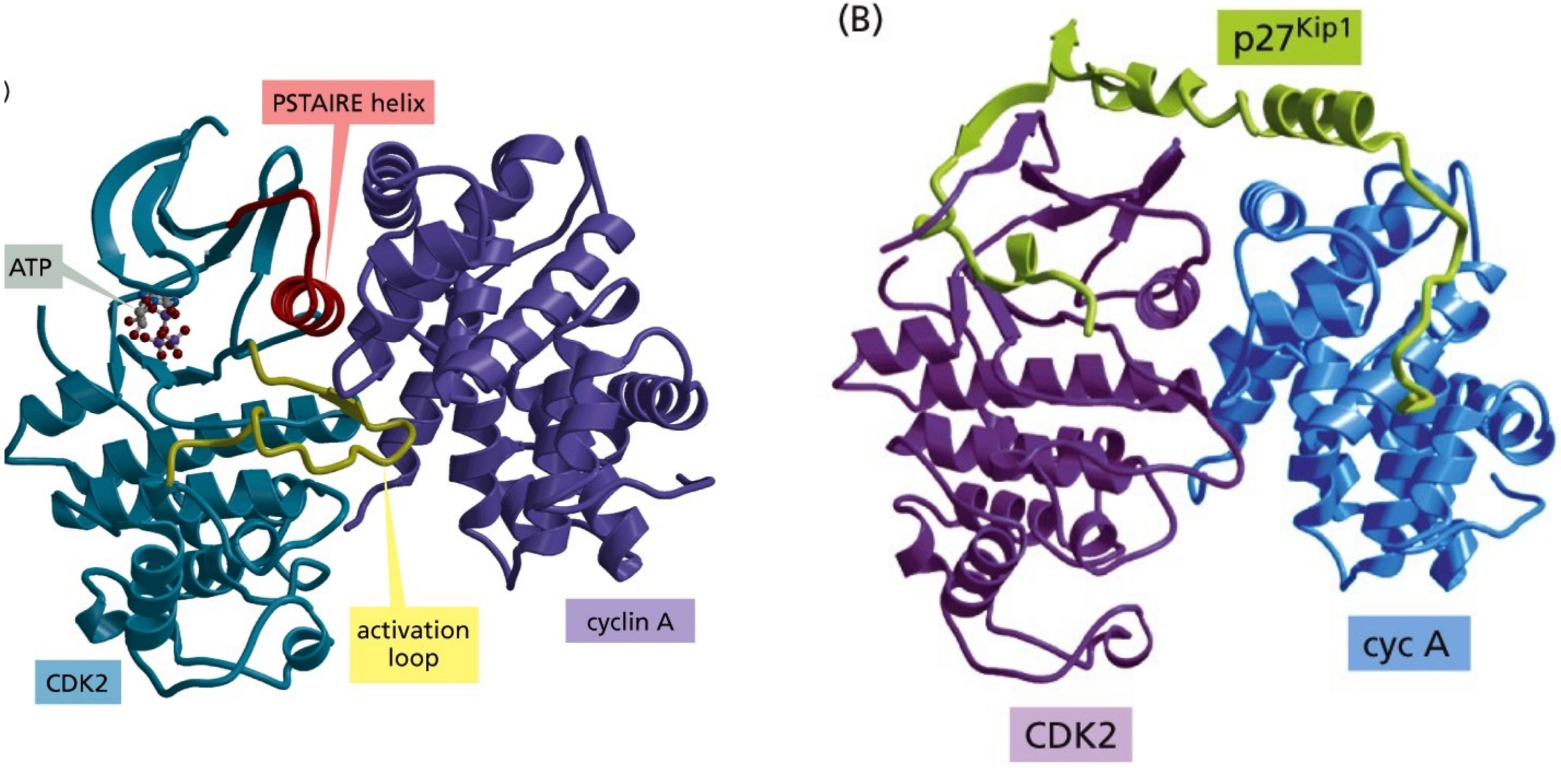


Figure 8.11. Weinberg, The Biology of Cancer

Extracellular signals acting via CKIs

- TGF-ß induces expression of p15^{INK4B} (and weakly p21; which is stongly induced by p53)
- In opposing fashion, mitogens activate the phosphatidylinositol 3-kinase (PI3K) pathway activating Akt/PKB; AKT/PKB phophorylates p21 and p27 causing their export into the cytoplasm.

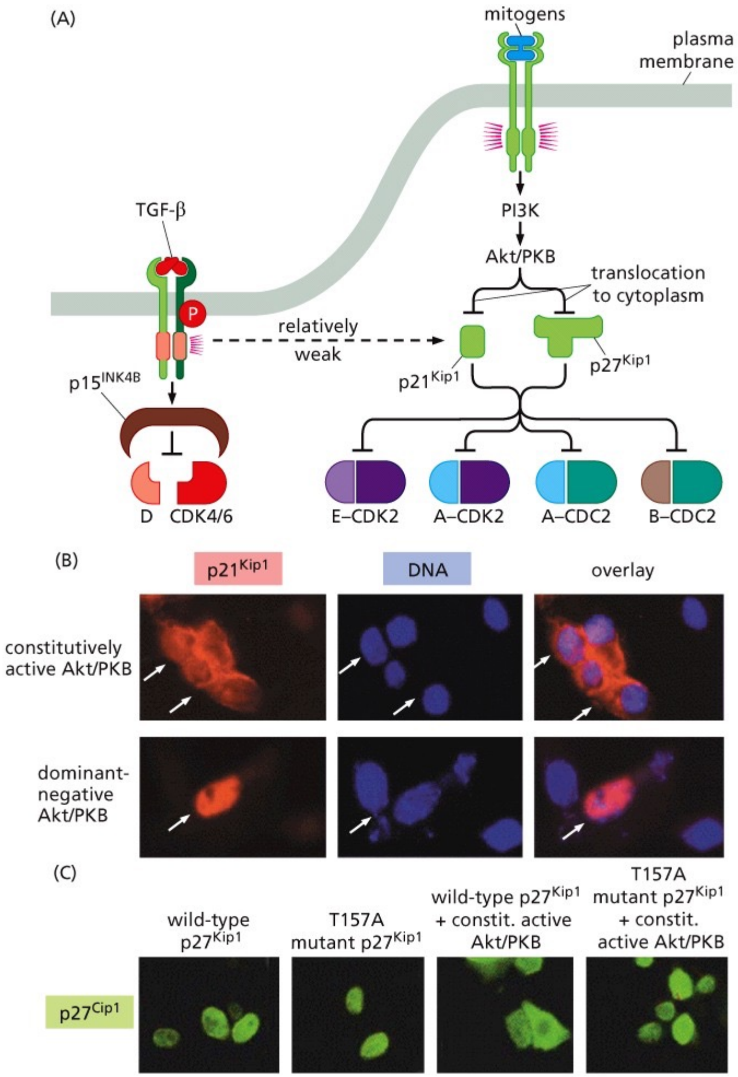


Figure 8.13. Weinberg, The Biology of Cancer

- T157A mutant of p27: not phosphorylated by Akt/PKB

While inhibiting CDK2 and CDC2, p21 and p27 stimulate assembly of cyclin D with CDK4/6

Growth factors
→ cyclinD-CDK4/6 accumulate
→ p27/p21 is captured by cyclinD-CDK4/6
→ cyclinE-CDK2 is liberated from p27/p21
→ Advancement through R-point

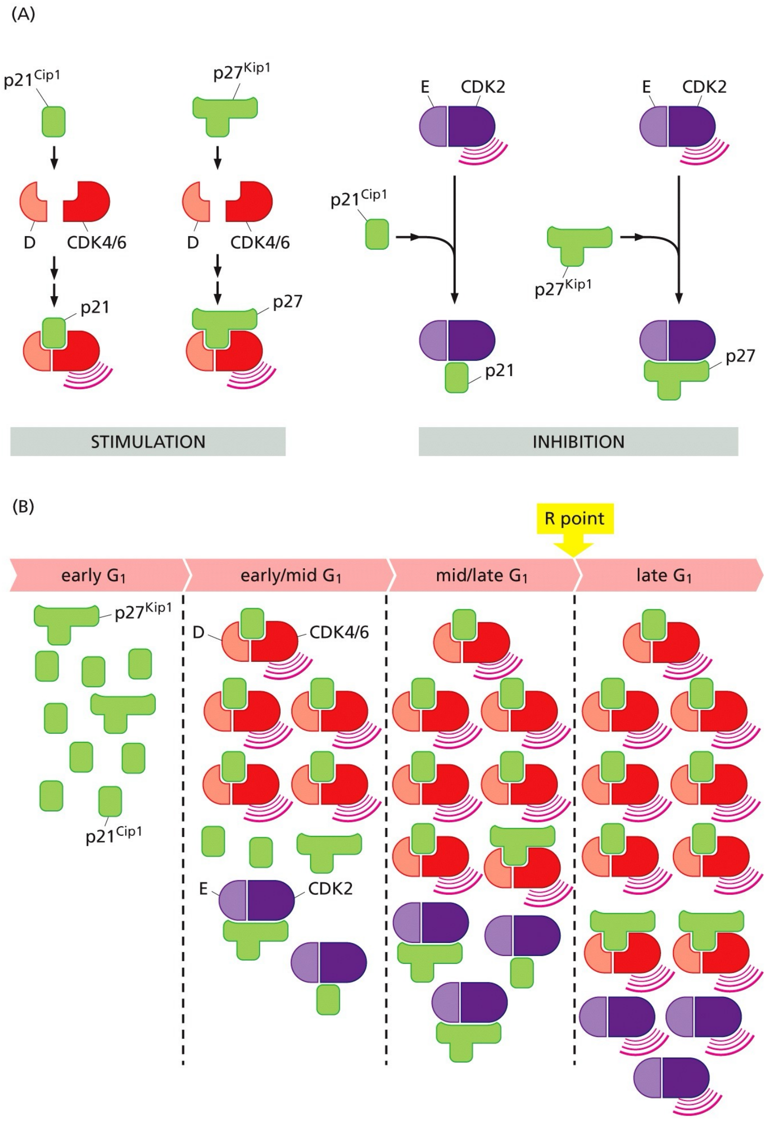


Figure 8.14. Weinberg, The Biology of Cancer

Execution of R-point? Phosphorylation of the Rb tumor suppressor during the cell cycle

- pRB: unphosphorylated in G_0.
- Weakly phosphorylated (hypophosphorylated at Ser807 and Ser 811) after enty into G1 (by cyclin D-CDK4/6).
- Hyperphosphorylated (at 12 or more sites) on Ser and Thr residues with advancement through R.
- Protein phosphatase 1 (PP1) strips off the phosphate groups after mitosis.

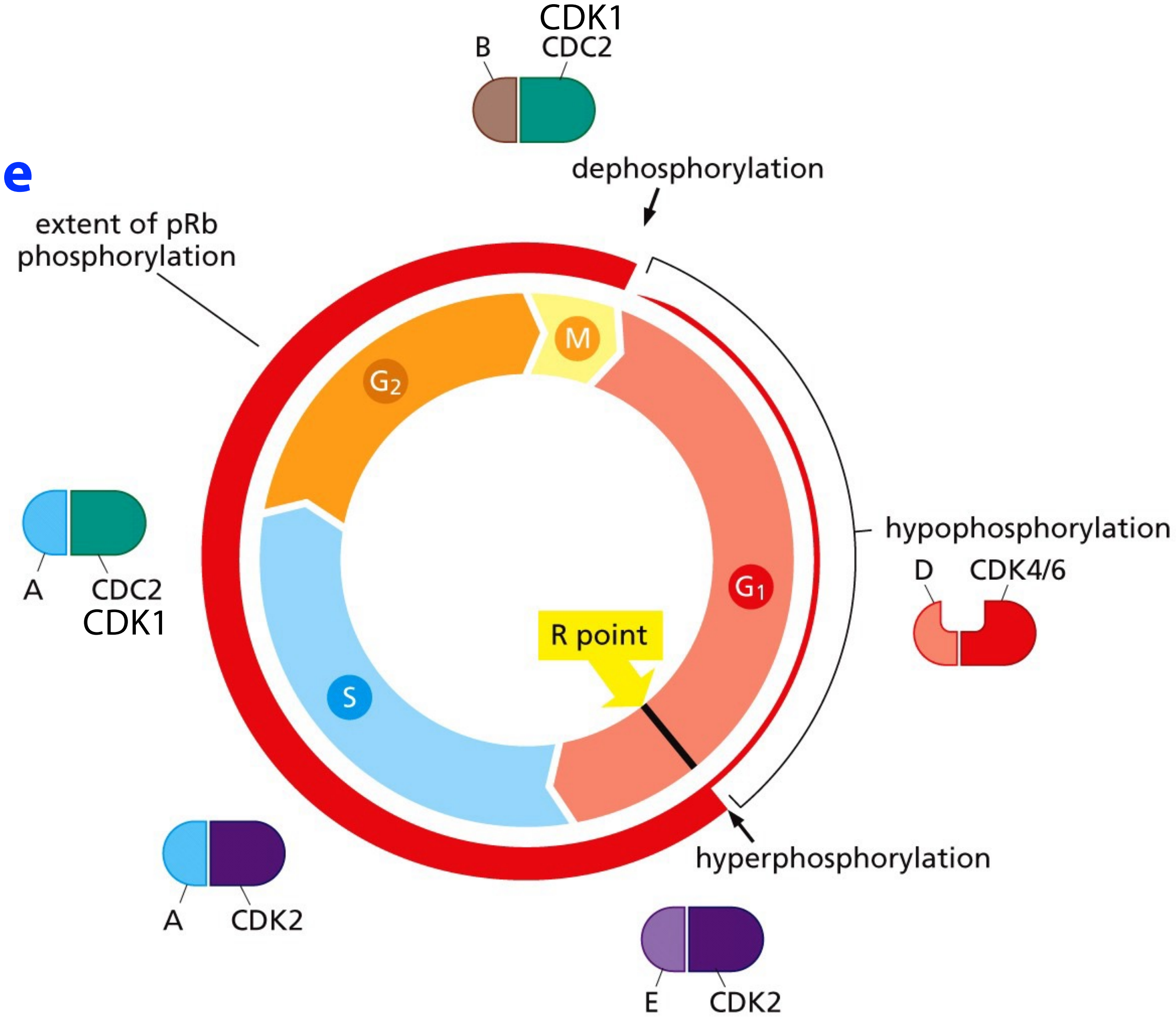


Figure 8.15. Weinberg, The Biology of Cancer

Tumor viruses sequester pRb

E1A oncoprotein of adenovirus, SV40 large T oncoprotein, and E7 of human papillomavirus (HPV) bind and inactivate pRB.

…necessary for optimal viral replication in infected cells. The virus exploits the cellular DNA replication machinery.

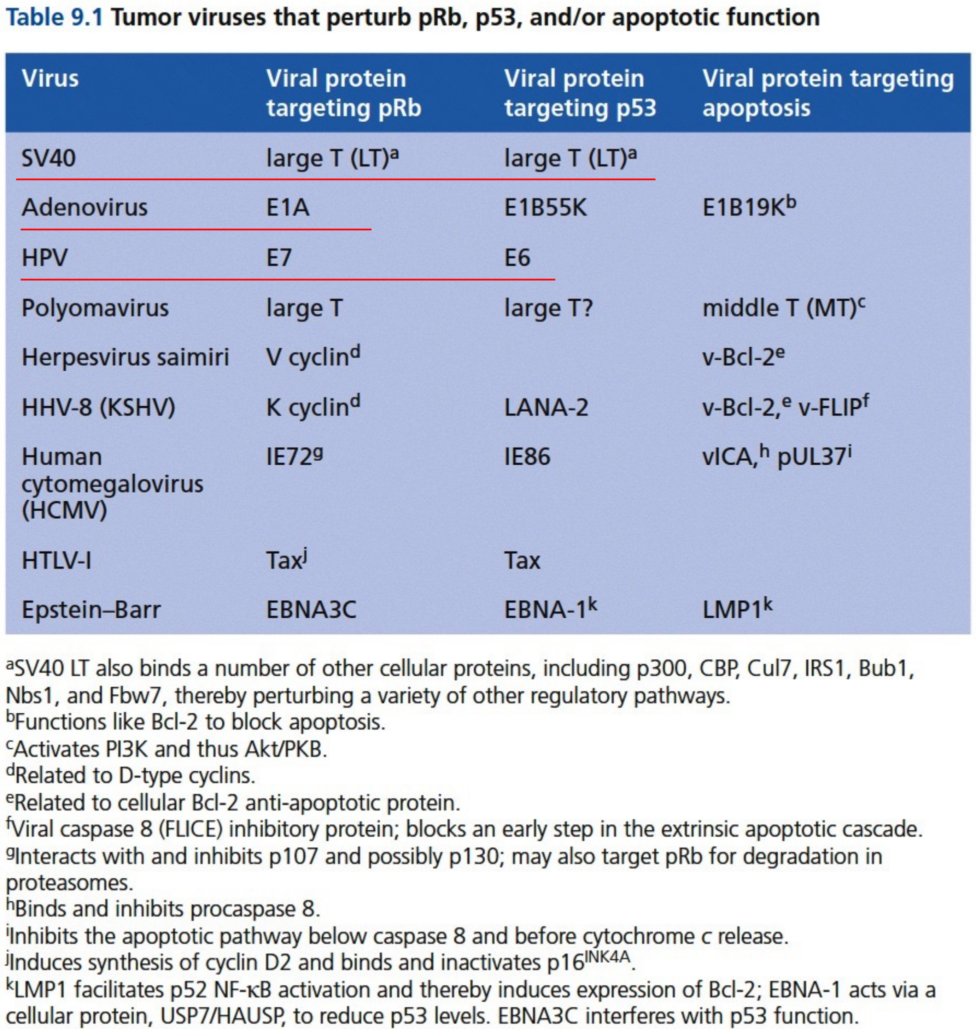


Table 9.1 Tumor viruses that perturb pRb, p53, and/or apoptotic function

Virus	Viral protein targeting pRb	Viral protein targeting p53	Viral protein targeting apoptosis
SV40	large T (LT)[a]	large T (LT)[a]	
Adenovirus	E1A	E1B55K	E1B19K[b]
HPV	E7	E6	
Polyomavirus	large T	large T?	middle T (MT)[c]
Herpesvirus saimiri	V cyclin[d]		v-Bcl-2[e]
HHV-8 (KSHV)	K cyclin[d]	LANA-2	v-Bcl-2,[e] v-FLIP[f]
Human cytomegalovirus (HCMV)	IE72[g]	IE86	vICA,[h] pUL37[i]
HTLV-I	Tax[j]	Tax	
Epstein–Barr	EBNA3C	EBNA-1[k]	LMP1[k]

[a] SV40 LT also binds a number of other cellular proteins, including p300, CBP, Cul7, IRS1, Bub1, Nbs1, and Fbw7, thereby perturbing a variety of other regulatory pathways.
[b] Functions like Bcl-2 to block apoptosis.
[c] Activates PI3K and thus Akt/PKB.
[d] Related to D-type cyclins.
[e] Related to cellular Bcl-2 anti-apoptotic protein.
[f] Viral caspase 8 (FLICE) inhibitory protein; blocks an early step in the extrinsic apoptotic cascade.
[g] Interacts with and inhibits p107 and possibly p130; may also target pRb for degradation in proteasomes.
[h] Binds and inhibits procaspase 8.
[i] Inhibits the apoptotic pathway below caspase 8 and before cytochrome c release.
[j] Induces synthesis of cyclin D2 and binds and inactivates $p16^{INK4A}$.
[k] LMP1 facilitates p52 NF-κB activation and thereby induces expression of Bcl-2; EBNA-1 acts via a cellular protein, USP7/HAUSP, to reduce p53 levels. EBNA3C interferes with p53 function.

Pocket proteins

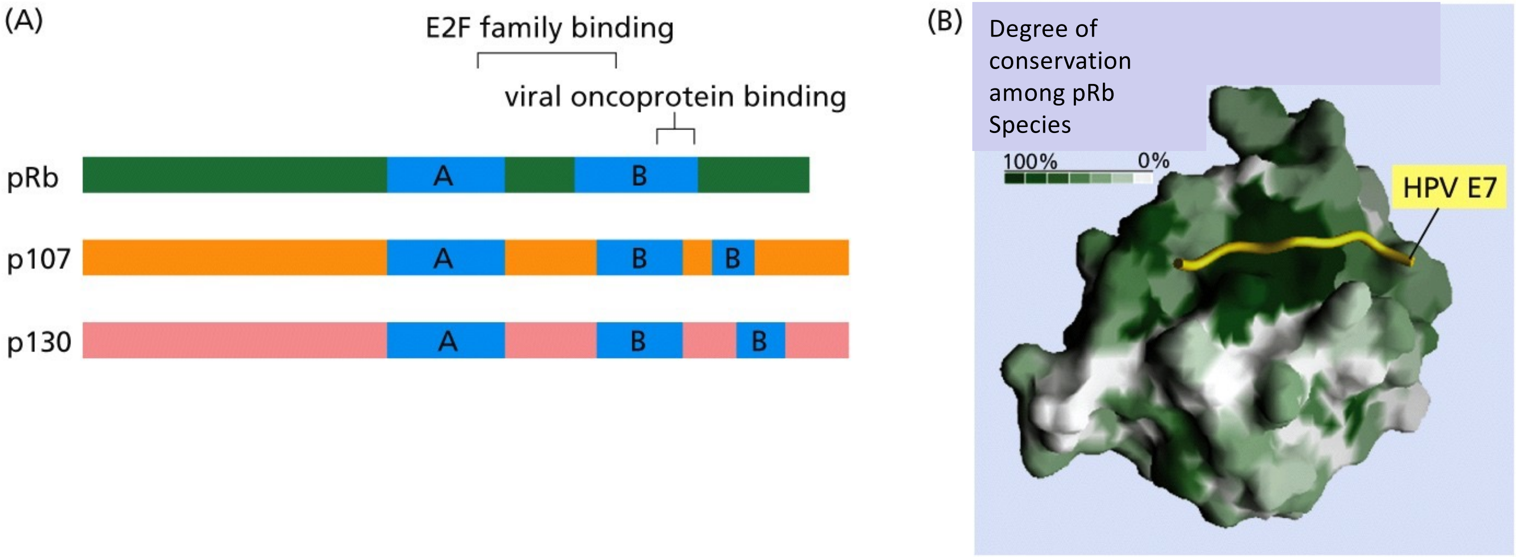
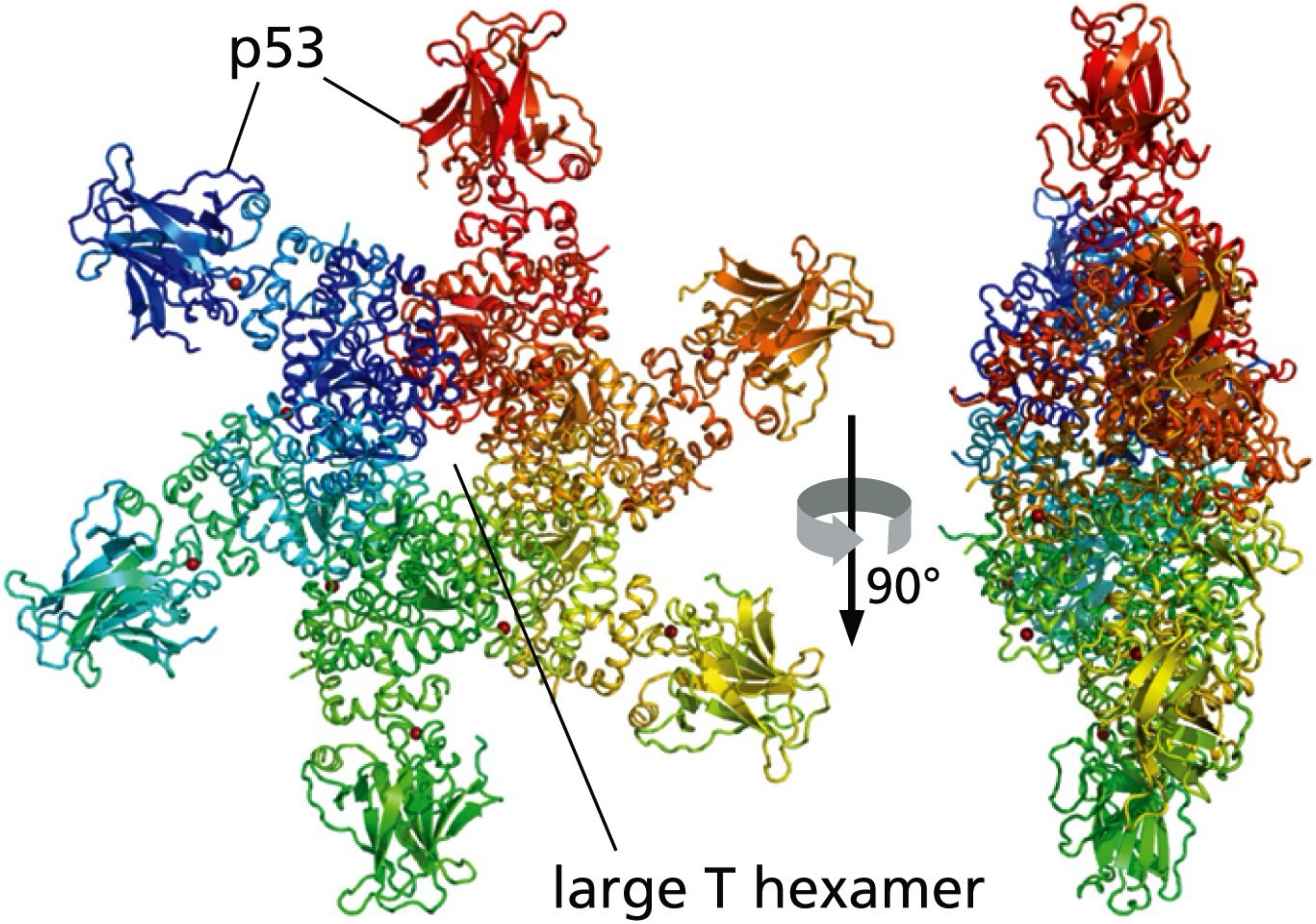


Figure 8.21 The Biology of Cancer (© Garland Science 2014)

pRb is one of three related proteins referred to as pocket proteins. All three are bound by adenovirus E1A, SV40 large T, and papillomavirus E7 (in their 'pocket').
The pocket interacts with E2F and the viral oncoproteins.

p53: Discovered through its Association with with SV40 Large T



Step-wise phosphorylation of pRb by cyclin D-CDK4/6 and cyclin E-CDK2

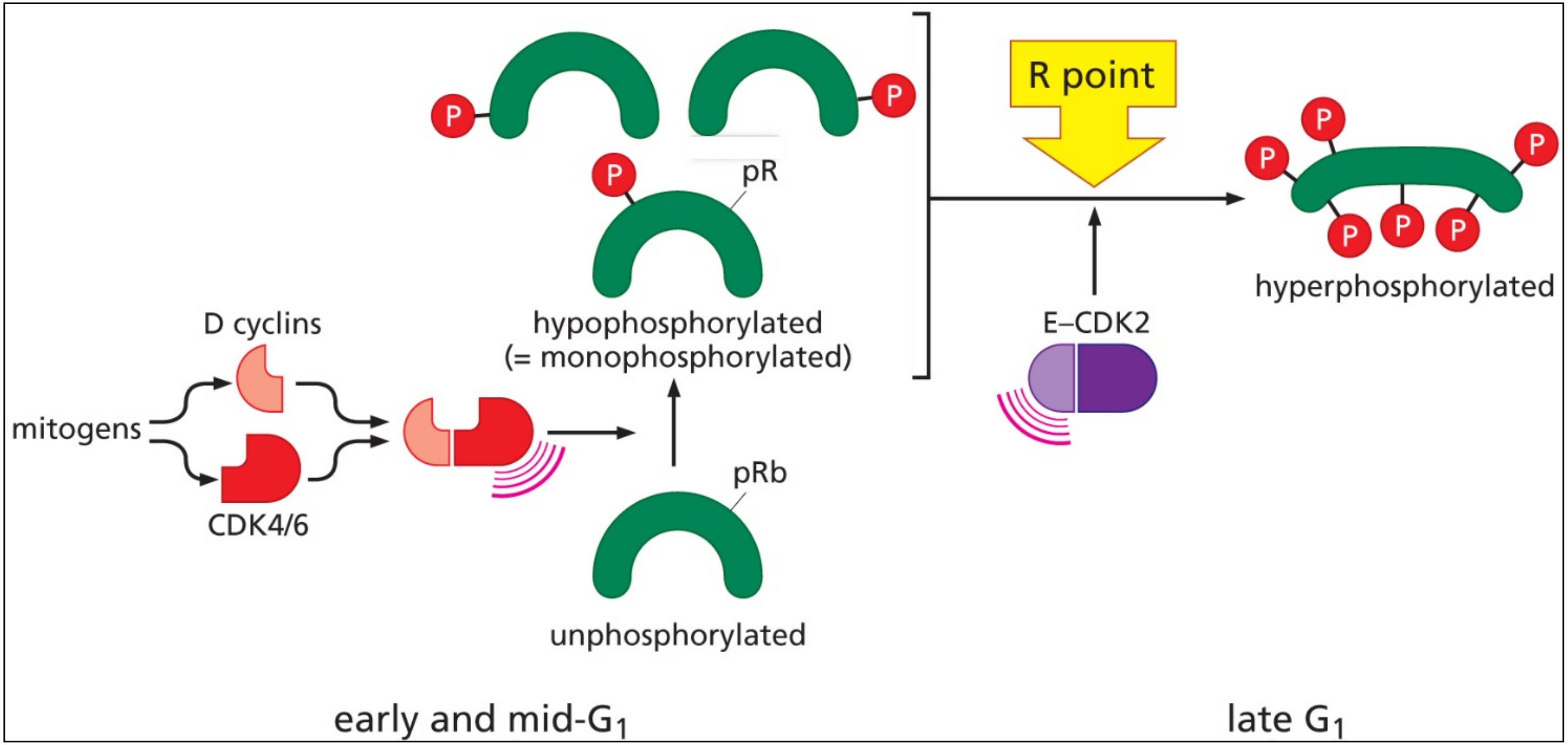


Figure 8.17. Weinberg, The Biology of Cancer

Liberation of E2F from Rb (and its cousins p107, p130)

E2Fs: a class of proteins with several members.

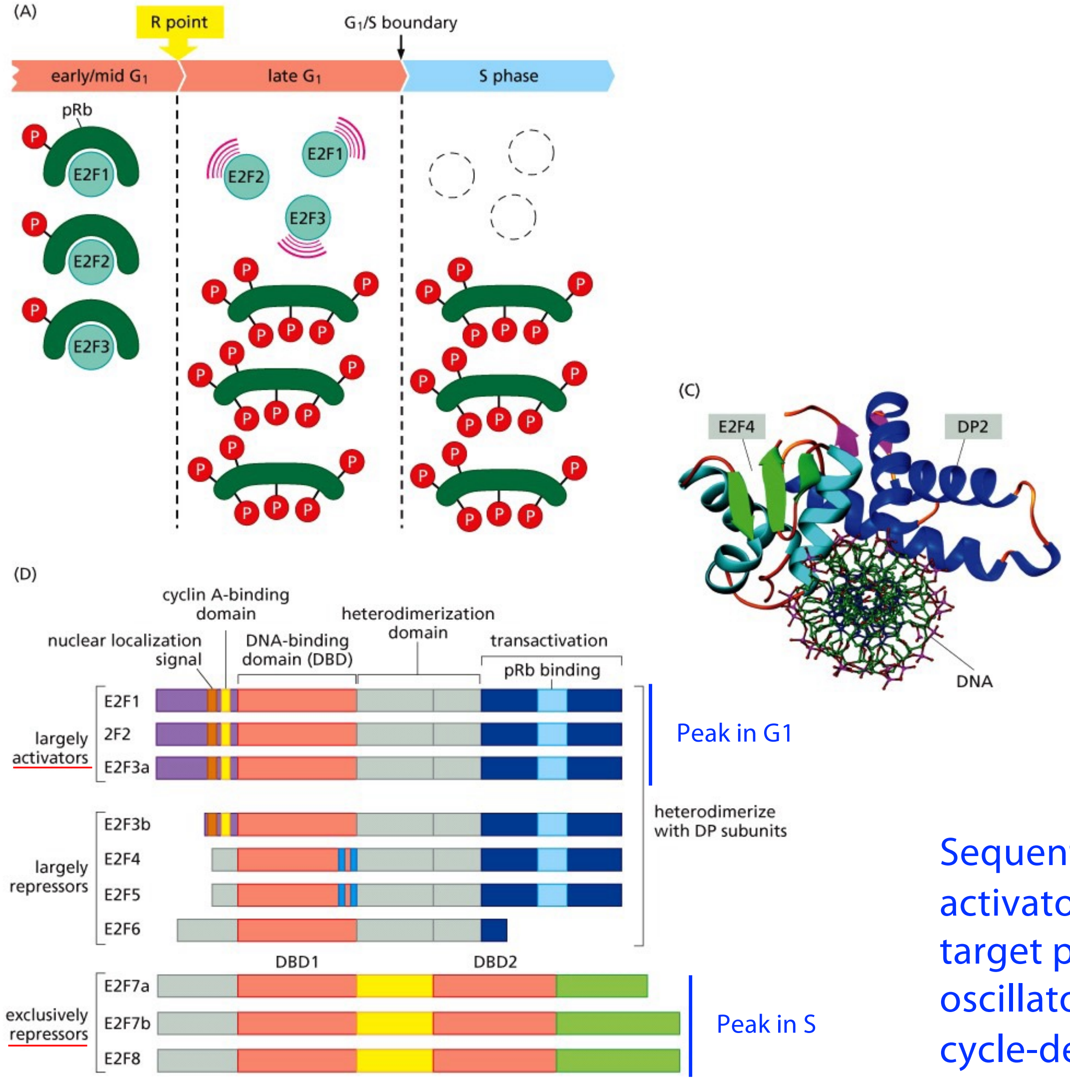


Figure 8.18. Weinberg, The Biology of Cancer

Negative feedback loop

Peak in G1

Peak in S

E2F1-6 bind DNA as a heterodimeric complex with DP (TTTCCCGC consensus); E2F7-8 bind on their own.

Sequential binding of E2F activators and repressors to target promoters leads to oscillatory nature of cell cycle-dependent gene expression

Modification of chromatin by Rb and its cousins p107/130 and E2F

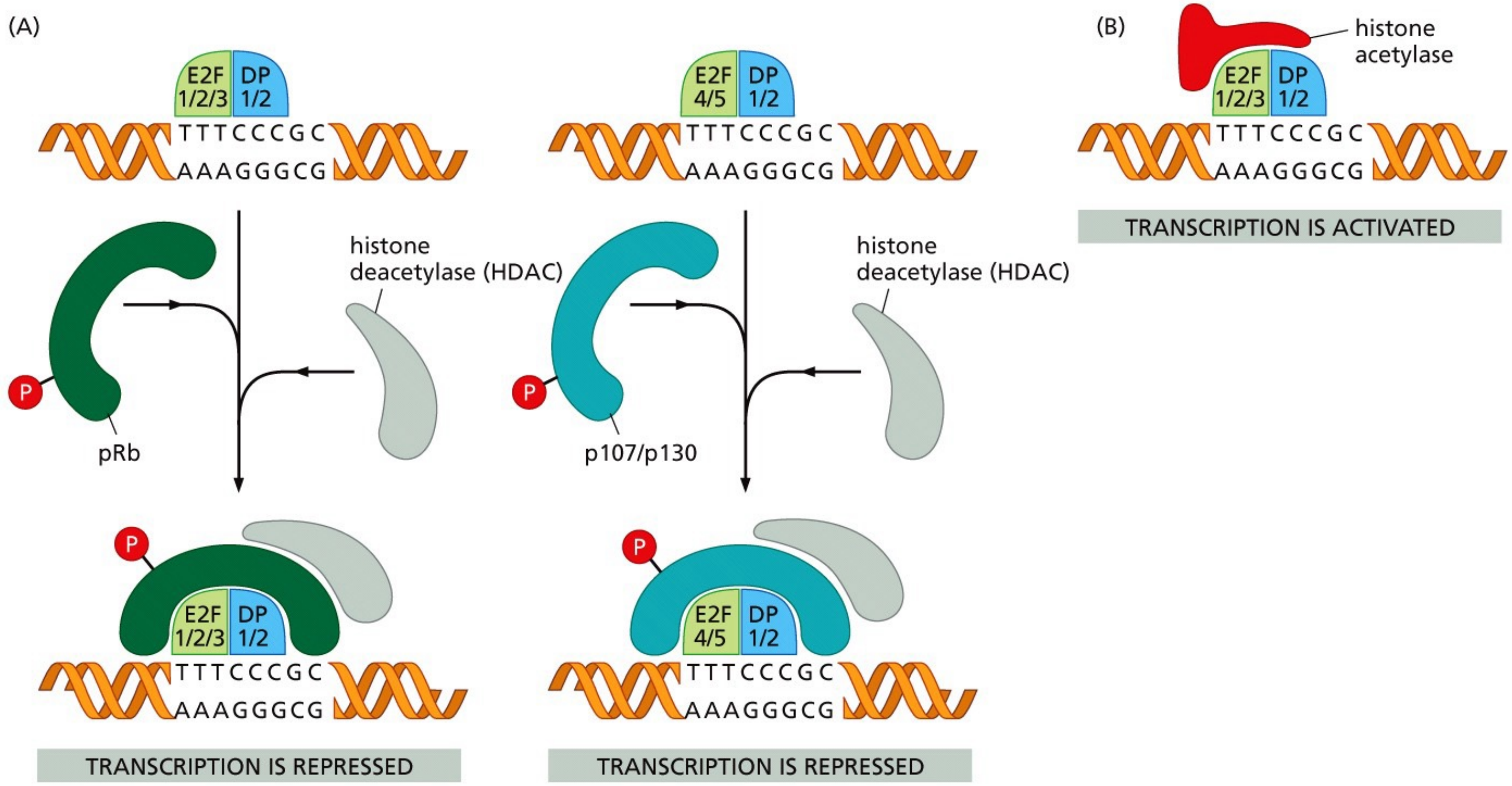
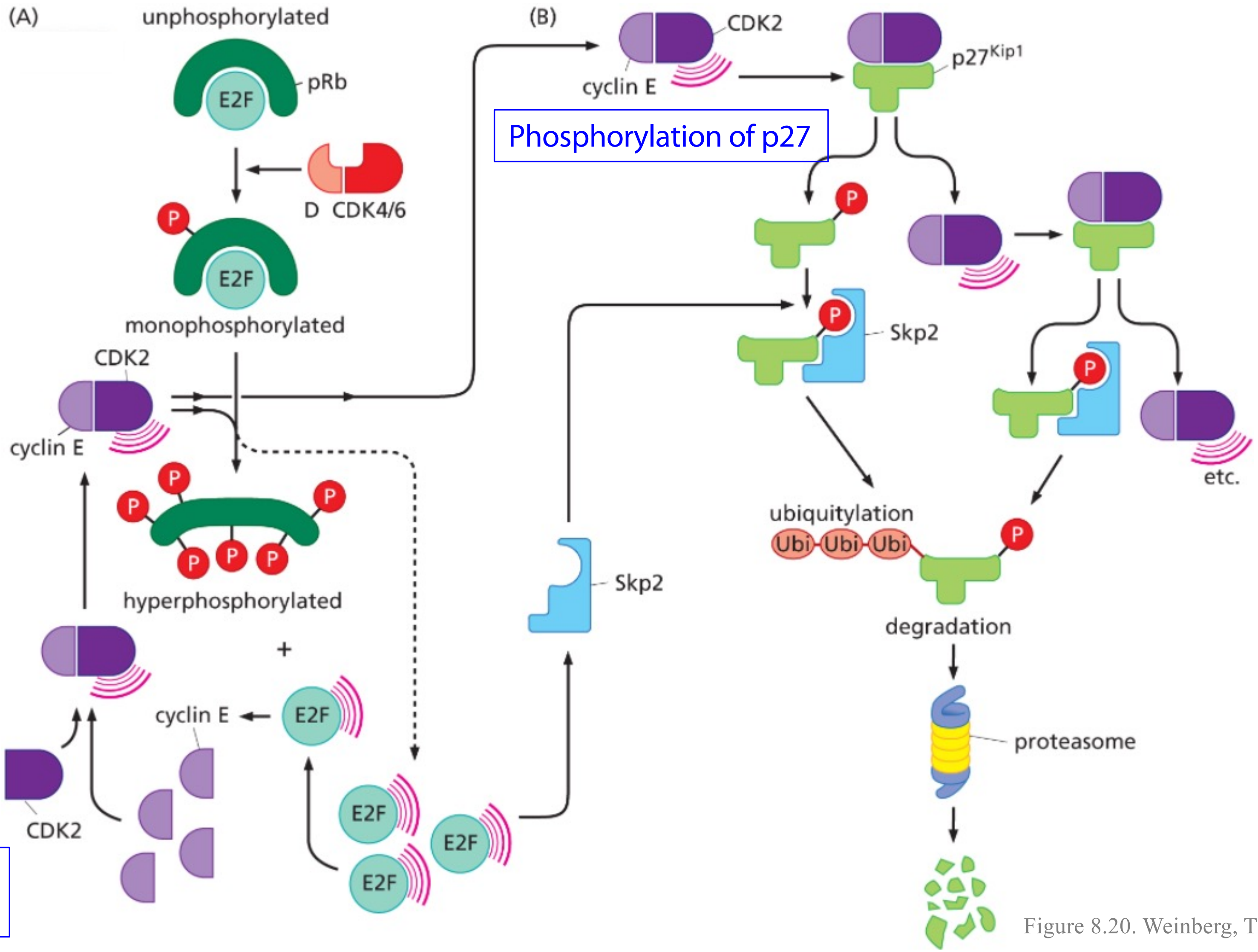


Figure 8.19. Weinberg, The Biology of Cancer

Hypophosphorylated Rb attracts histone deacetylases functioning as transcriptional repressor; E2Fs attract histone acetylases. ~500 genes are being induced (nucleotide biosynthesis, DNA replication proteins; cyclin E and E2F → positive feedback loop)

Positive-feedback loops drive the cell cycle forward



Phosphorylation of p27

E2F-activated genes:
Cyclin E, E2F1

Figure 8.20. Weinberg, The Biology of Cancer

S-phase: E2F becomes inactive: degradation

cyclin A-CDK2 are active: phosphorylate E2F and DP subunits
→ dissociation;
…ubiquitination and degradation of E2F

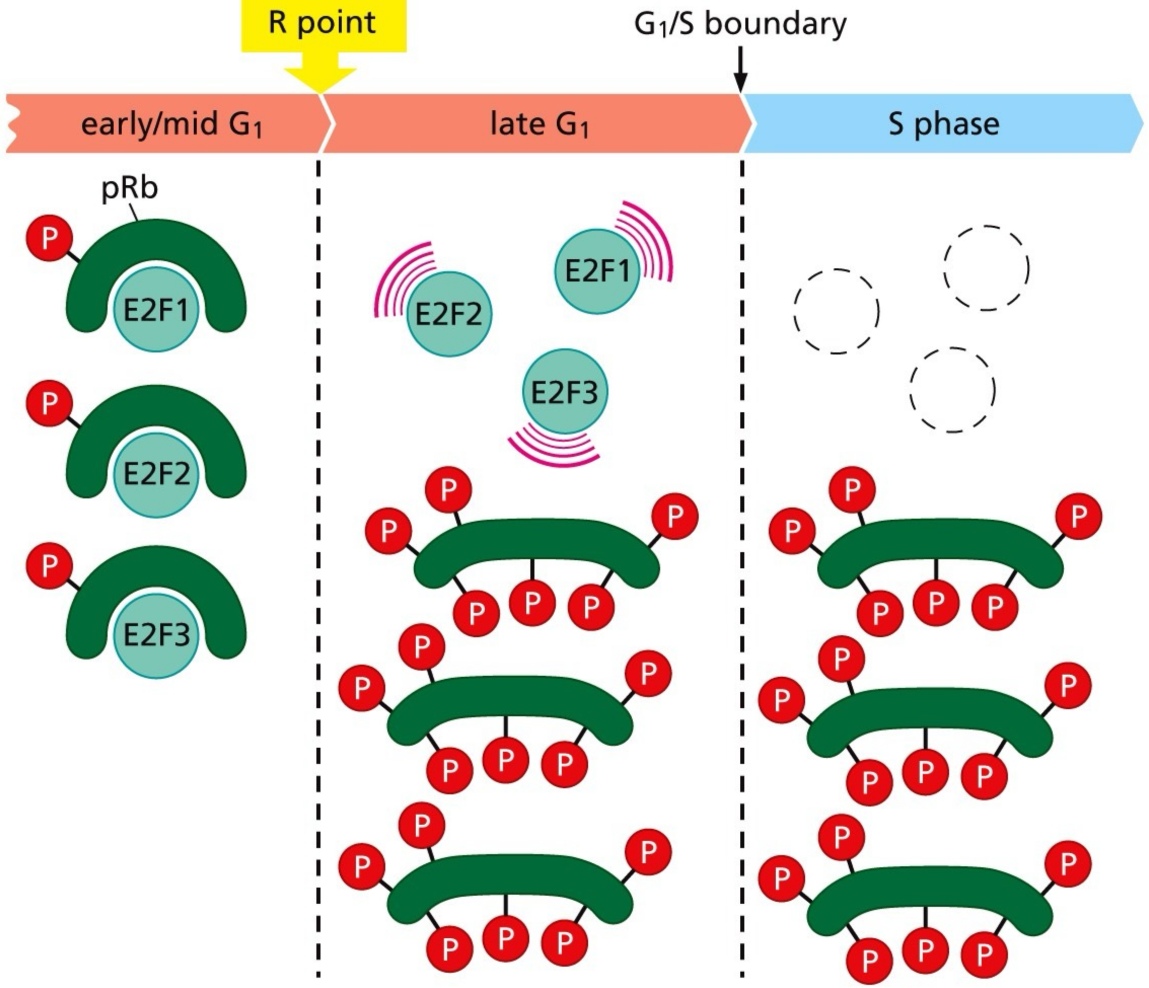
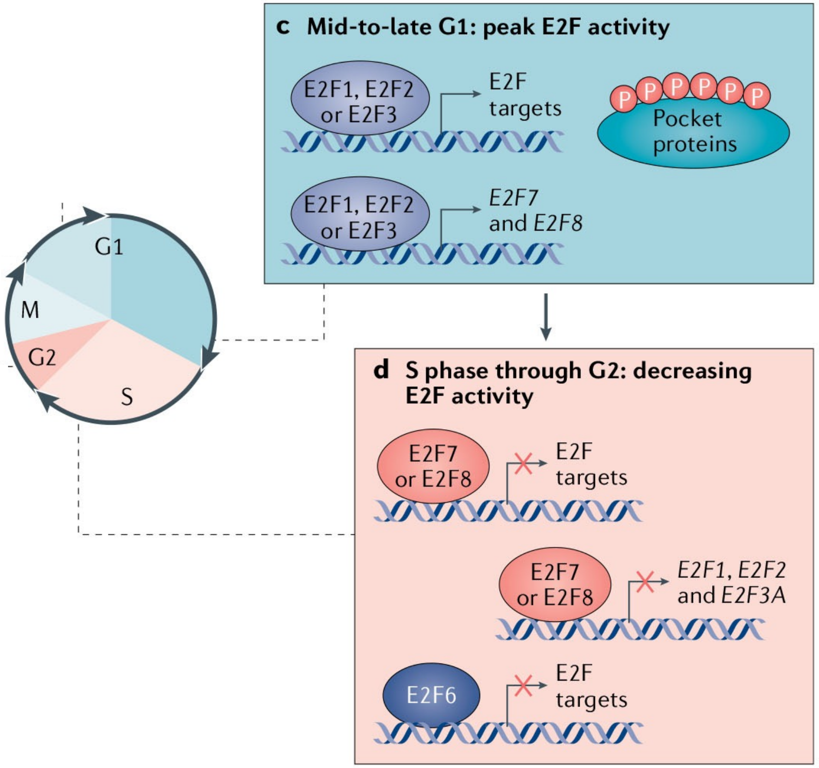


Figure 8.18. Weinberg, The Biology of Cancer

S-phase: E2F becomes inactive: transcriptional repression

E2F transcriptional activity peaks at mid-to-late G1/S inducing G1-S phase cell cycle genes, including E2F7 and E2F8. As S phase progresses, accumulation of E2F6, E2F7 and E2F8 on target promoters results in decreased expression of E2F1,2,3A activators and of E2F target genes.



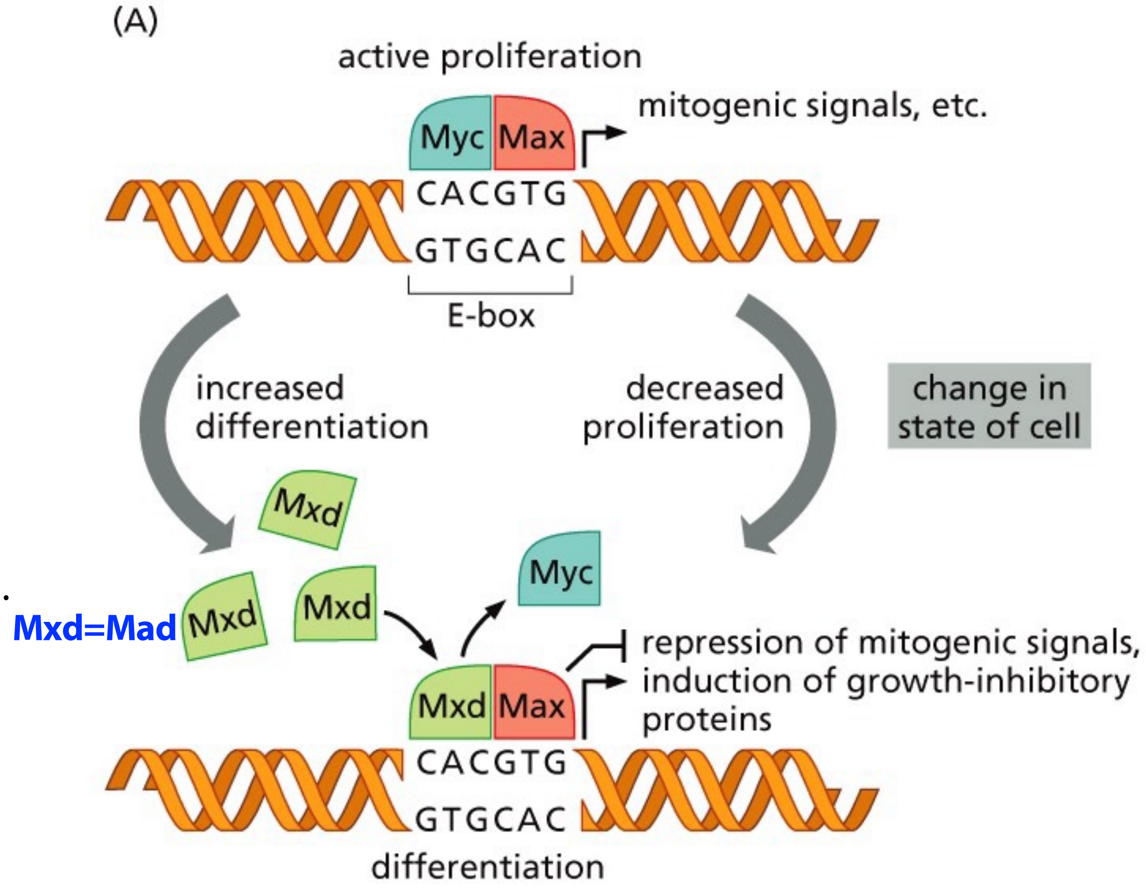
From: NatRevCancer **19**, 326 (2019)

How do oncoproteins elicit their effects on the cell cycle?

- MYC

Myc: bHLH (basic helix-loop-helix) Transcription factor

- >70% of tumors overexpress **c-Myc**, N-Myc or L-Myc.
- Binds to E-box (CACGTG) as heterodimer.
- **Myc-Max:** promotes transcription elongation releasing RNA pol II from pause sites located ~ 50 bp downstream of transcription start sites.
- **MxD-Max** prevent the release.
- The number of genes (thousands) found to bind Myc exceeds the absolute number of Myc molecules!
- Targets (out of many): cyclin D2, CDK4, E2F; Cul1 which drives ubiquitination and degradation of p27.



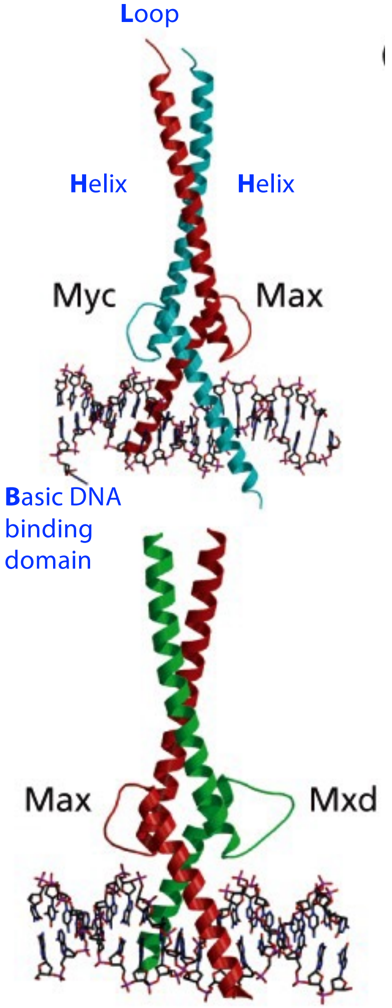


Figure 8.21. Weinberg, The Biology of Cancer

Myc and the cell cycle clock

- Targets (out of many): cyclin D2, CDK4, E2F; Cul1 which drives ubiquitination and degradation of p27.
- When associating with Miz-1, Myc <u>represses</u> expression of p15, p21 and p27.

- Myc also promotes cell growth (cell size) inducing e.g. genes of ribosomal proteins.

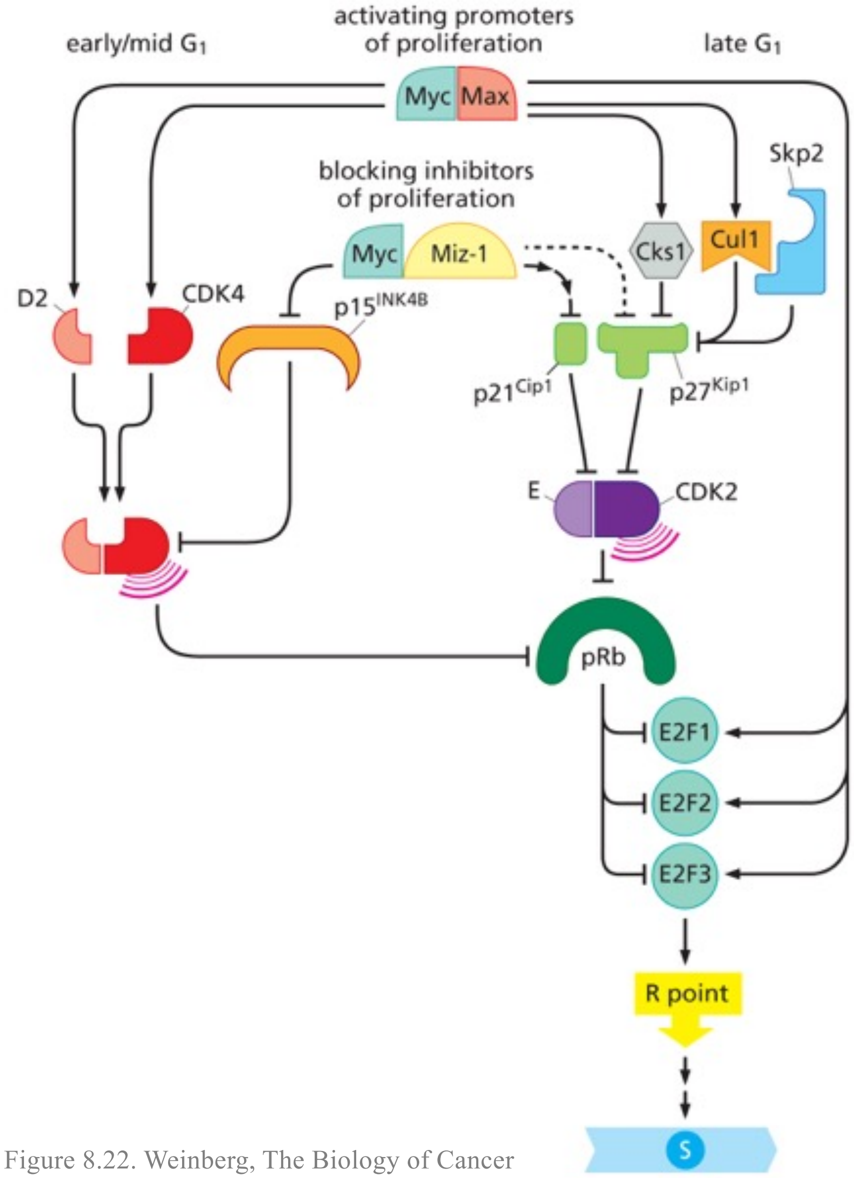
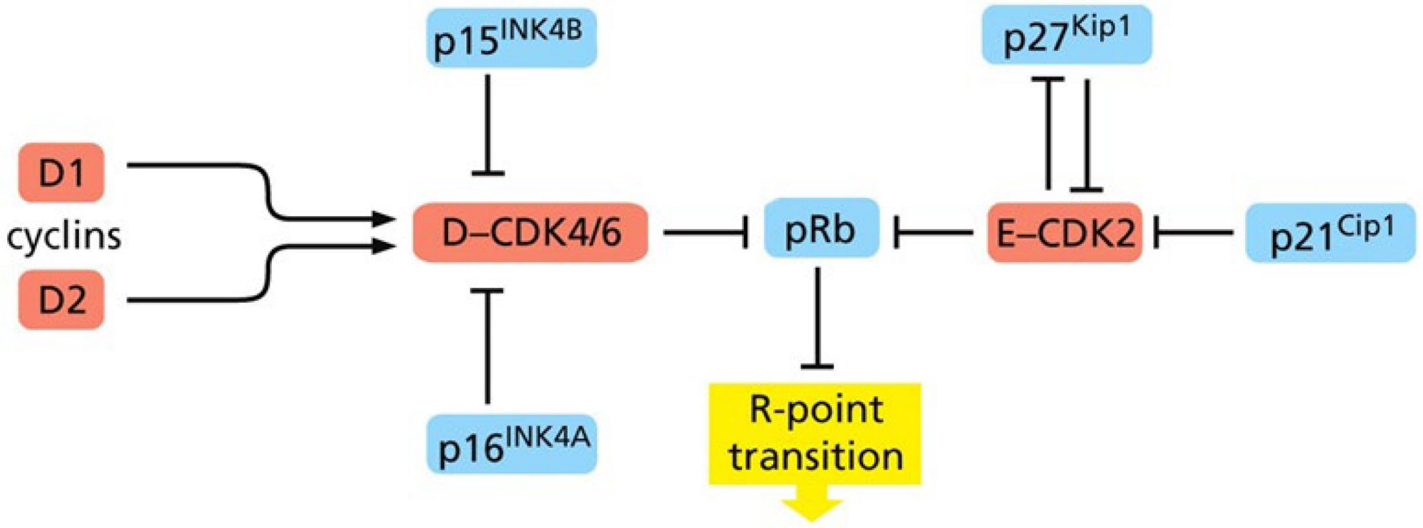


Figure 8.22. Weinberg, The Biology of Cancer

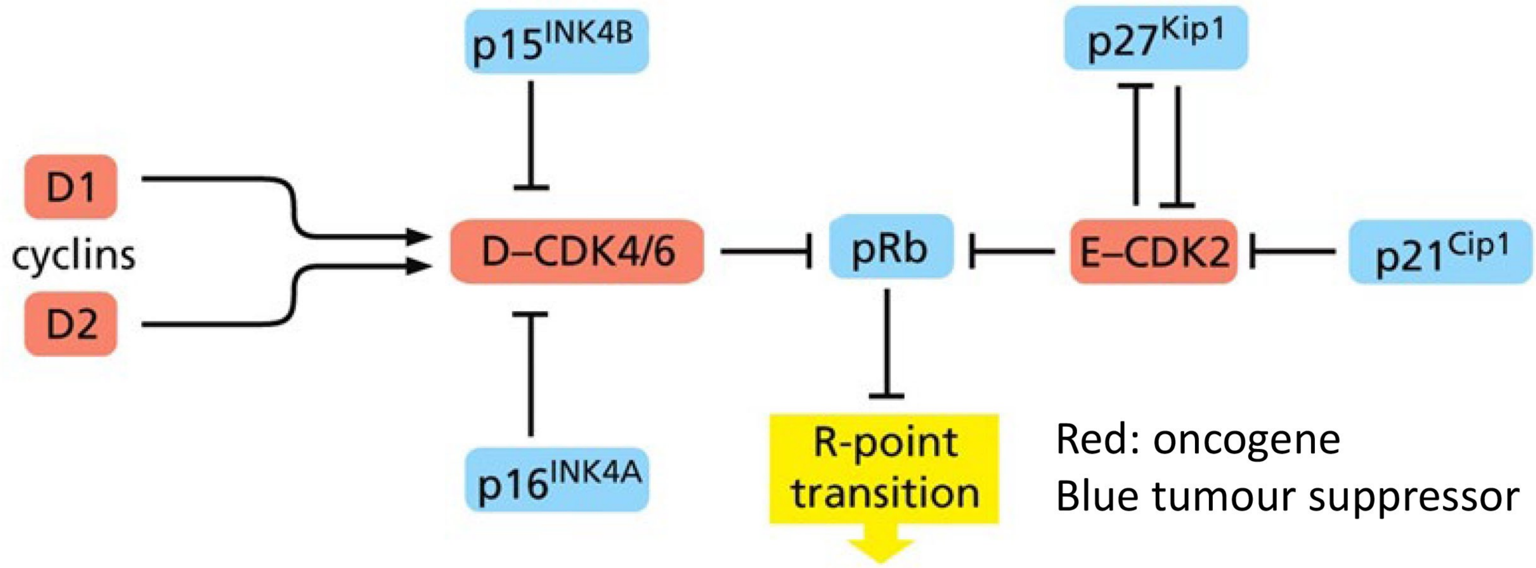
Control of pRb function is perturbed in most if not all human cancers

How is the R-point transition deregulated in Cancer?



Red: oncogene
Blue tumour suppressor

How is the R-point transition deregulated in Cancer?



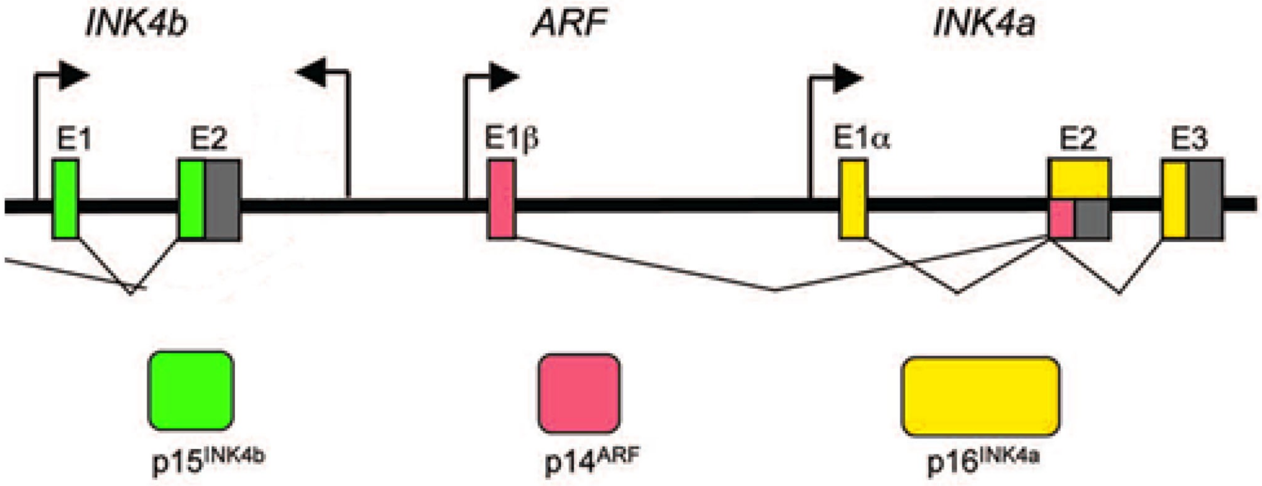
Red: oncogene
Blue tumour suppressor

RB inactivated by mutation, promoter methylation.
D and E cyclins overexpressed.
Mutation in CDK4 in melanoma that prevents p16 binding.
Deletion or epigenetic silencing of p15 or p16.
Decreased expression of p27

How is the R-point transition deregulated in Cancer?

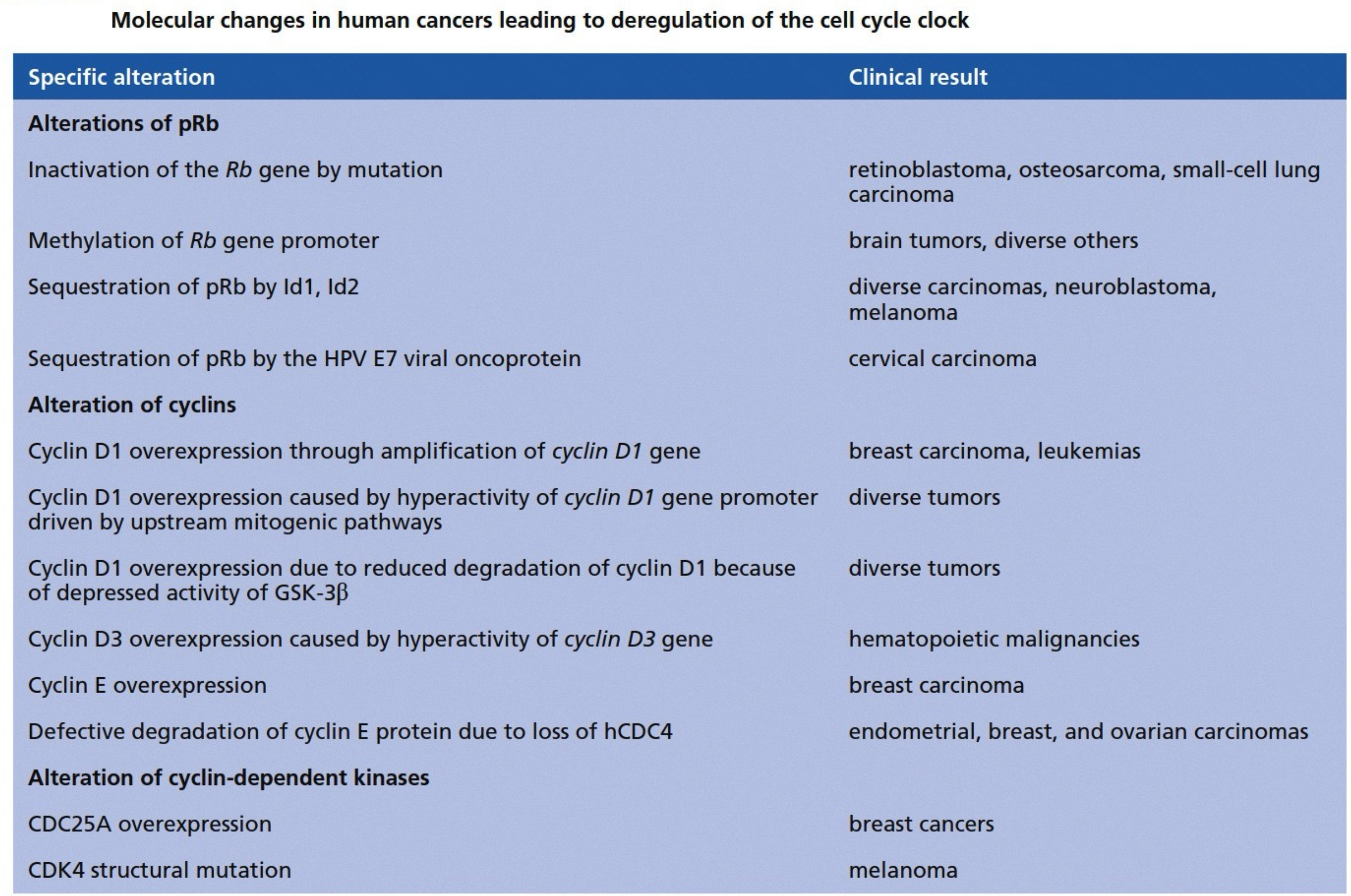
The CDKN2A and CDKN2B loci

CDKN2B CDKN2A



Chromosome arm 9p

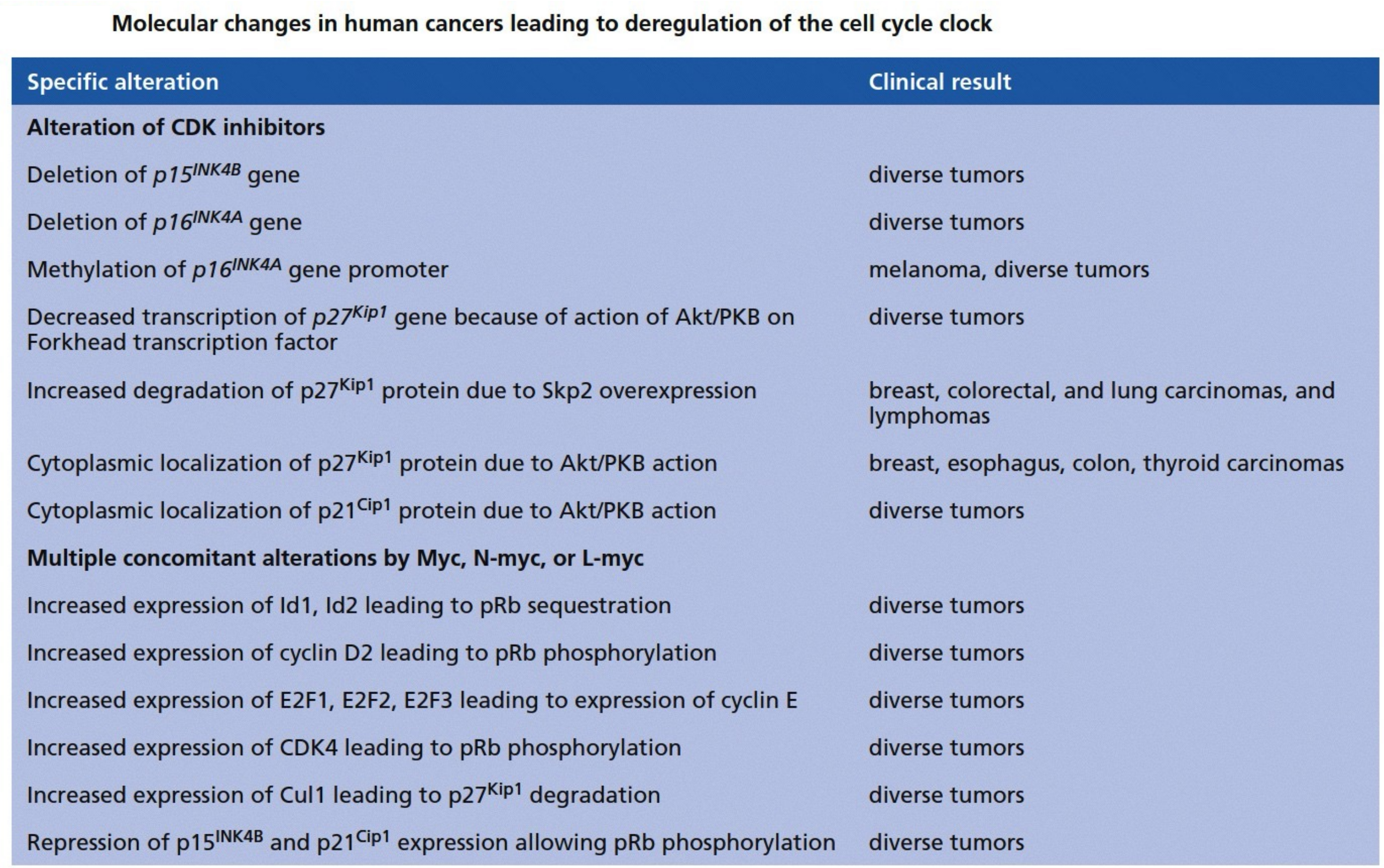
These loci encode two CKIs and a regulator of p53.
They are frequently mutated or epigenetically silenced in Cancer.



Molecular changes in human cancers leading to deregulation of the cell cycle clock

Specific alteration	Clinical result
Alterations of pRb	
Inactivation of the *Rb* gene by mutation	retinoblastoma, osteosarcoma, small-cell lung carcinoma
Methylation of *Rb* gene promoter	brain tumors, diverse others
Sequestration of pRb by Id1, Id2	diverse carcinomas, neuroblastoma, melanoma
Sequestration of pRb by the HPV E7 viral oncoprotein	cervical carcinoma
Alteration of cyclins	
Cyclin D1 overexpression through amplification of *cyclin D1* gene	breast carcinoma, leukemias
Cyclin D1 overexpression caused by hyperactivity of *cyclin D1* gene promoter driven by upstream mitogenic pathways	diverse tumors
Cyclin D1 overexpression due to reduced degradation of cyclin D1 because of depressed activity of GSK-3β	diverse tumors
Cyclin D3 overexpression caused by hyperactivity of *cyclin D3* gene	hematopoietic malignancies
Cyclin E overexpression	breast carcinoma
Defective degradation of cyclin E protein due to loss of hCDC4	endometrial, breast, and ovarian carcinomas
Alteration of cyclin-dependent kinases	
CDC25A overexpression	breast cancers
CDK4 structural mutation	melanoma

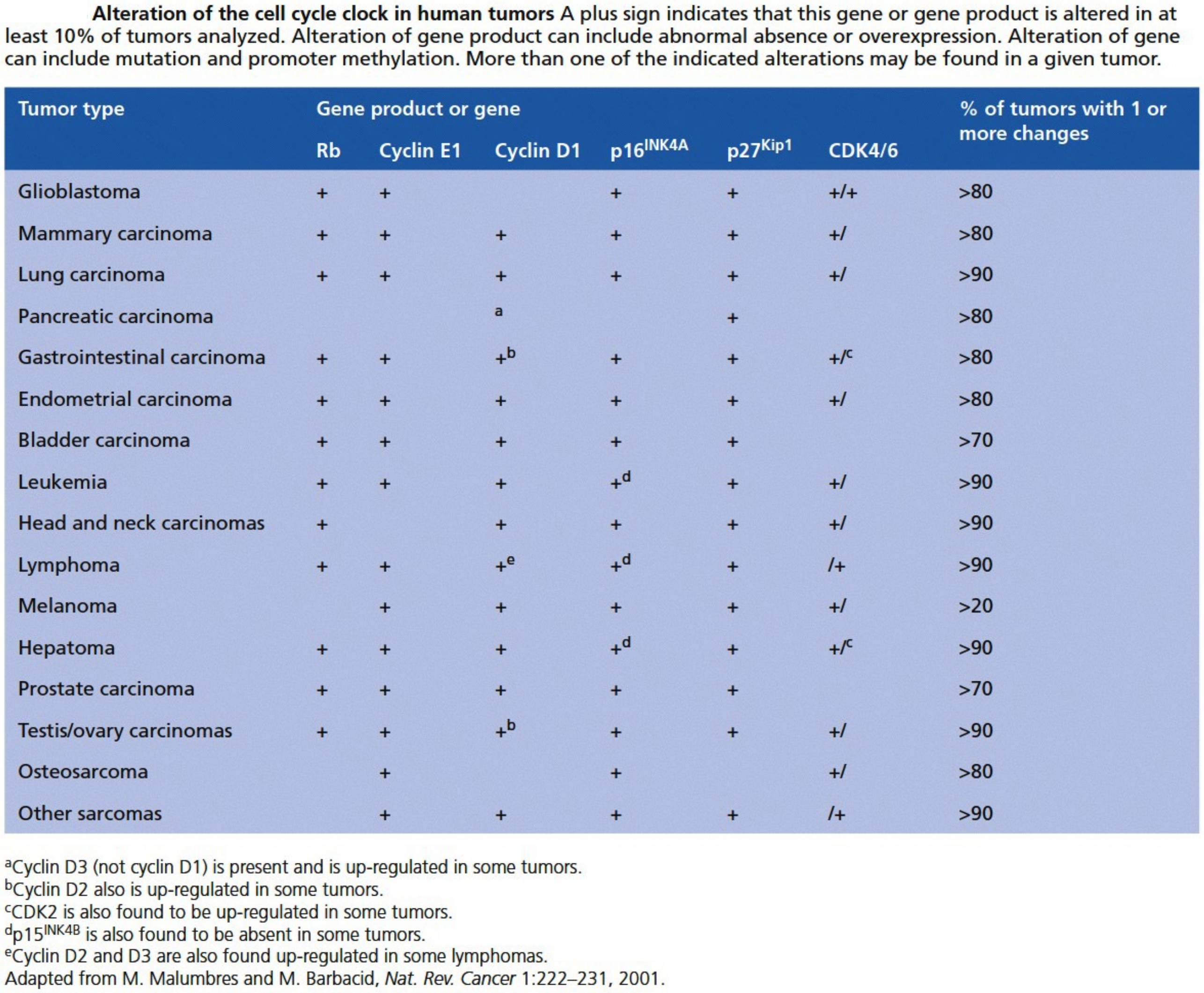
Table 8.2. Weinberg, The Biology of Cancer



Molecular changes in human cancers leading to deregulation of the cell cycle clock

Specific alteration	Clinical result
Alteration of CDK inhibitors	
Deletion of *p15*^{INK4B} gene	diverse tumors
Deletion of *p16*^{INK4A} gene	diverse tumors
Methylation of *p16*^{INK4A} gene promoter	melanoma, diverse tumors
Decreased transcription of *p27*^{Kip1} gene because of action of Akt/PKB on Forkhead transcription factor	diverse tumors
Increased degradation of p27$^{\mathrm{Kip1}}$ protein due to Skp2 overexpression	breast, colorectal, and lung carcinomas, and lymphomas
Cytoplasmic localization of p27$^{\mathrm{Kip1}}$ protein due to Akt/PKB action	breast, esophagus, colon, thyroid carcinomas
Cytoplasmic localization of p21$^{\mathrm{Cip1}}$ protein due to Akt/PKB action	diverse tumors
Multiple concomitant alterations by Myc, N-myc, or L-myc	
Increased expression of Id1, Id2 leading to pRb sequestration	diverse tumors
Increased expression of cyclin D2 leading to pRb phosphorylation	diverse tumors
Increased expression of E2F1, E2F2, E2F3 leading to expression of cyclin E	diverse tumors
Increased expression of CDK4 leading to pRb phosphorylation	diverse tumors
Increased expression of Cul1 leading to p27$^{\mathrm{Kip1}}$ degradation	diverse tumors
Repression of p15$^{\mathrm{INK4B}}$ and p21$^{\mathrm{Cip1}}$ expression allowing pRb phosphorylation	diverse tumors

Table 8.2. Weinberg, The Biology of Cancer



Alteration of the cell cycle clock in human tumors A plus sign indicates that this gene or gene product is altered in at least 10% of tumors analyzed. Alteration of gene product can include abnormal absence or overexpression. Alteration of gene can include mutation and promoter methylation. More than one of the indicated alterations may be found in a given tumor.

Tumor type	Gene product or gene						% of tumors with 1 or more changes
	Rb	Cyclin E1	Cyclin D1	p16^{INK4A}	p27^{Kip1}	CDK4/6	
Glioblastoma	+	+		+	+	+/+	>80
Mammary carcinoma	+	+	+	+	+	+/	>80
Lung carcinoma	+	+	+	+	+	+/	>90
Pancreatic carcinoma			[a]		+		>80
Gastrointestinal carcinoma	+	+	+[b]	+	+	+/[c]	>80
Endometrial carcinoma	+	+	+	+	+	+/	>80
Bladder carcinoma	+	+	+	+	+		>70
Leukemia	+	+	+	+[d]	+	+/	>90
Head and neck carcinomas	+		+	+	+	+/	>90
Lymphoma	+	+	+[e]	+[d]	+	/+	>90
Melanoma		+	+	+	+	+/	>20
Hepatoma	+	+	+	+[d]	+	+/[c]	>90
Prostate carcinoma	+	+	+	+	+		>70
Testis/ovary carcinomas	+	+	+[b]	+	+	+/	>90
Osteosarcoma		+		+		+/	>80
Other sarcomas		+	+	+	+	/+	>90

[a] Cyclin D3 (not cyclin D1) is present and is up-regulated in some tumors.
[b] Cyclin D2 also is up-regulated in some tumors.
[c] CDK2 is also found to be up-regulated in some tumors.
[d] p15^{INK4B} is also found to be absent in some tumors.
[e] Cyclin D2 and D3 are also found up-regulated in some lymphomas.
Adapted from M. Malumbres and M. Barbacid, *Nat. Rev. Cancer* 1:222–231, 2001.

Table 8.3. Weinberg, The Biology of Cancer

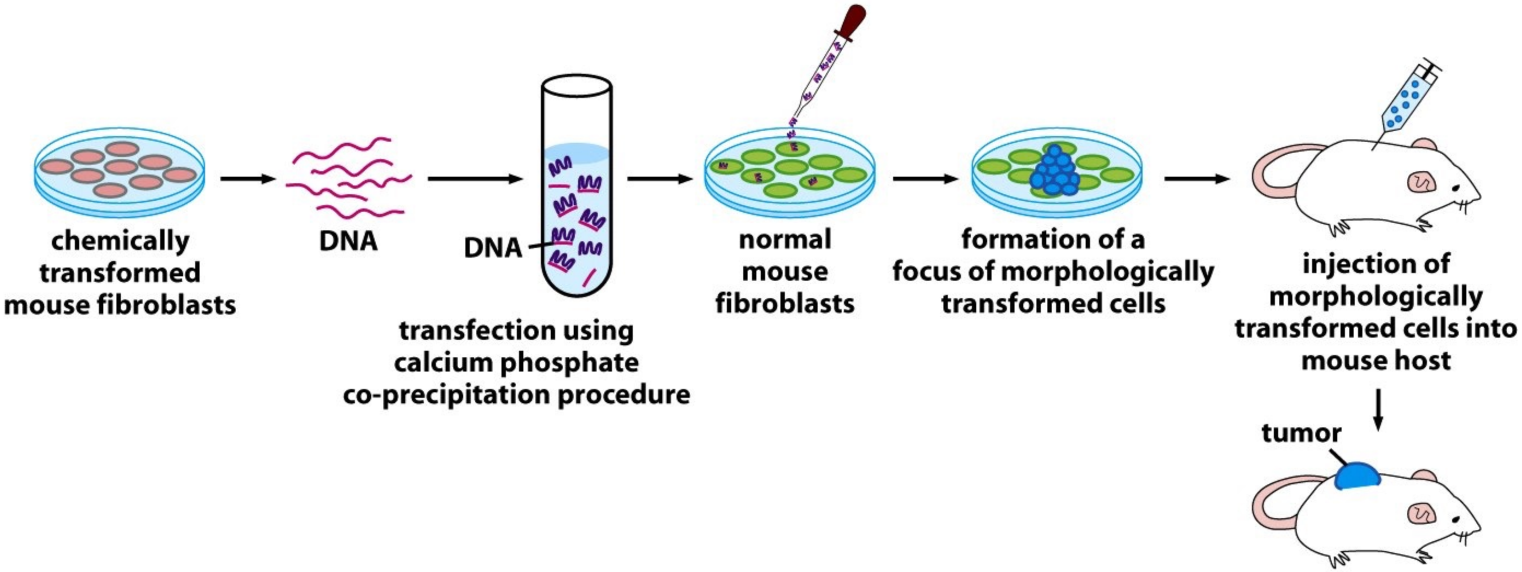
- Many oncogenes act by mimicking normal growth signals.

Example: Mutated RAS releases a flux of mitogenic signals into cells, without ongoing stimulation by their normal upstream regulators (→overexpression of cyclin D1,…)

(50% of human colon cancer bear mutant ras)

…Tendency to overgrow a confluent monolayer, formation of tumors when reintroduced into susceptible animals

From Week 1:
Identification of Oncogenes through Transfection



NIH3T3 cells: standard fibroblast line: not truly normal, adapted to grow in culture, immortal.
… genetically/epignetically altered!

Several Stresses can Trigger Cellular Senescence

- Inappropriate growth conditions (e.g. high oxygen)
- Telomere shortening
- **Some oncogenes!**

Cancer Cell **11**, 389 (2007)

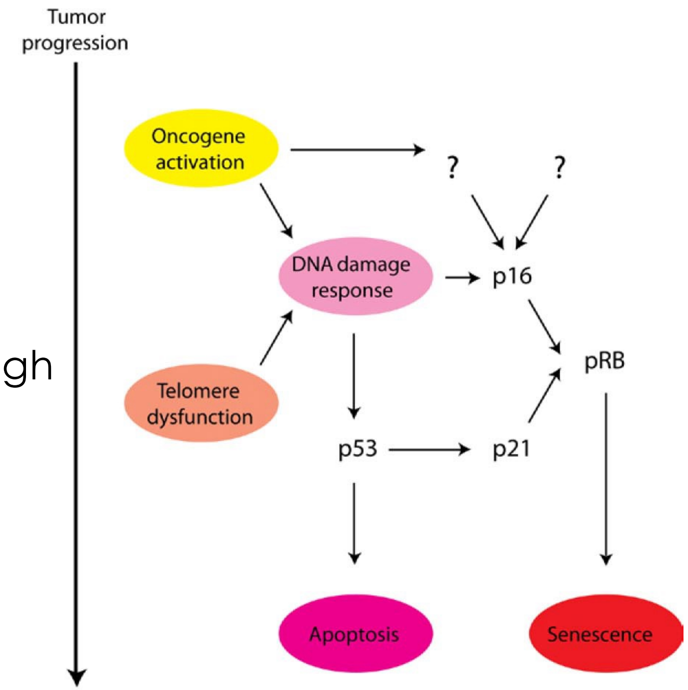


Figure 1. Cancer Suppression Signaling Pathways Leading to Senescence and Apoptosis

Inappropriate signaling elicited by activation of oncogenes, usually an early event during tumor progression, can elicit a DNA damage response resulting in the activation of p53. Oncogene activation often also upregulates p16, either as a consequence of the DNA damage response or through independent mechanisms. The p16 and p21 branches converge on pRB, whose activation strongly promotes senescence. Although the relative contributions of the p21 and p16 branches are not yet well understood, in many cases p16 is believed to play a crucial role. This is underscored by its frequent silencing during tumor progression, and evidence is accumulating that the key selective pressure is to relieve the senescence checkpoint. Telomere dysfunction, expected to occur at later stages of tumor progression, also causes a DNA damage response and strongly activates p53. The manner in which activation of p53 is channeled into either an apoptotic response or senescence is not well understood, but these choices appear to be strongly influenced by cell type and perhaps other contexts.

Oncogene-Mediated Induction of Cellular Senescence

Expression of Ras oncogene in normal fibroblast:

IMR90: human diploid fibroblasts
MEFs: mouse embryo fibroblasts
REF52: rat fibroblast cell line

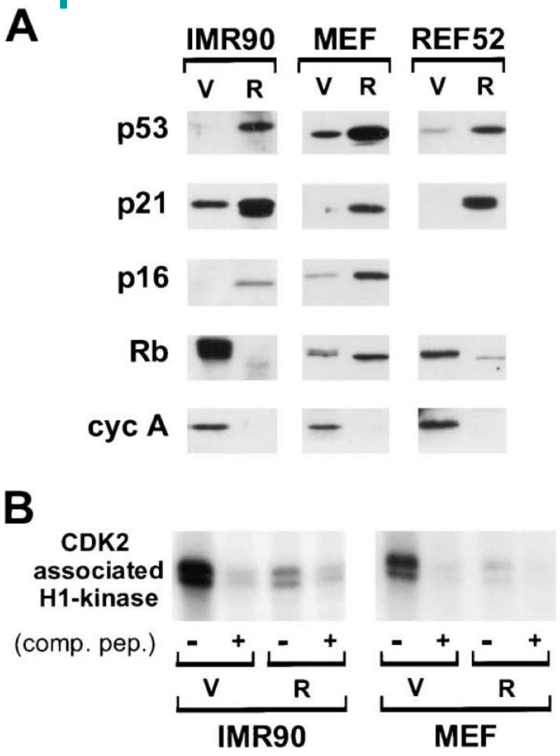
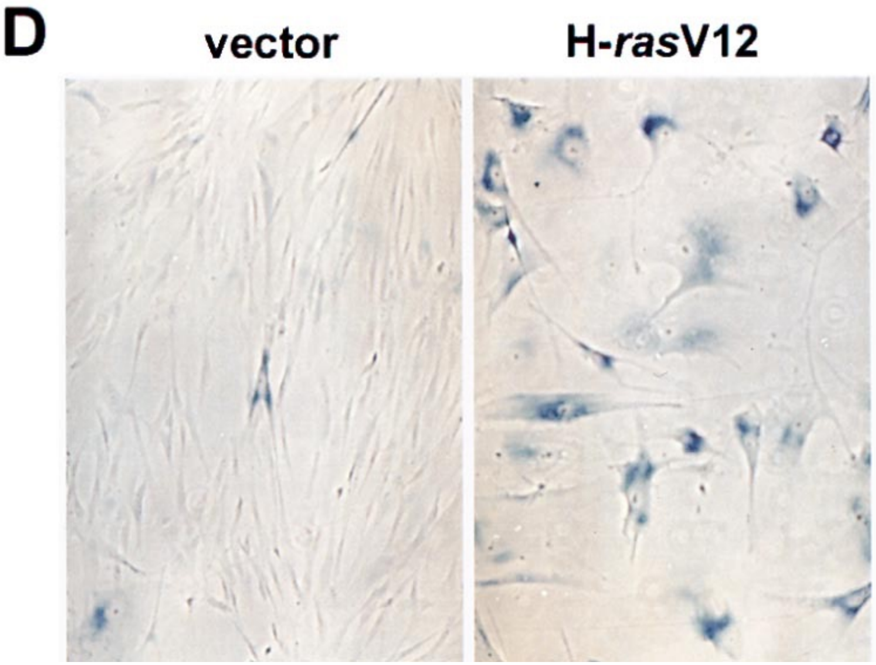


Figure 3. Effect of H-*ras*V12 on Cell-Cycle Regulatory Proteins

(A) Immunoblots of cellular lysates corresponding to cells transduced with empty vector (V) or with H-*ras*V12-expressing (R) retroviruses. Our anti-p16 antibodies did not crossreact with rat p16, so we could not examine p16 expression in REF52 cells.

(B) CDK2 kinase activity against histone H1 obtained after immunoprecipitation of the indicated cellular lysates, (V) or (R) as in (A), with anti-CDK2 in the absence (−) or presence (+) of competing antigenic peptide.



Beta-galactosidase expression is a marker of senescence; visualized as blue precipitate when incubated with the chromogenic substrate X-Gal.

IMR90 cells stained for Beta-Gal activity at day 6.

(from Serrano et al., Cell 88, 593 (1997))

… growth arrest and senescence

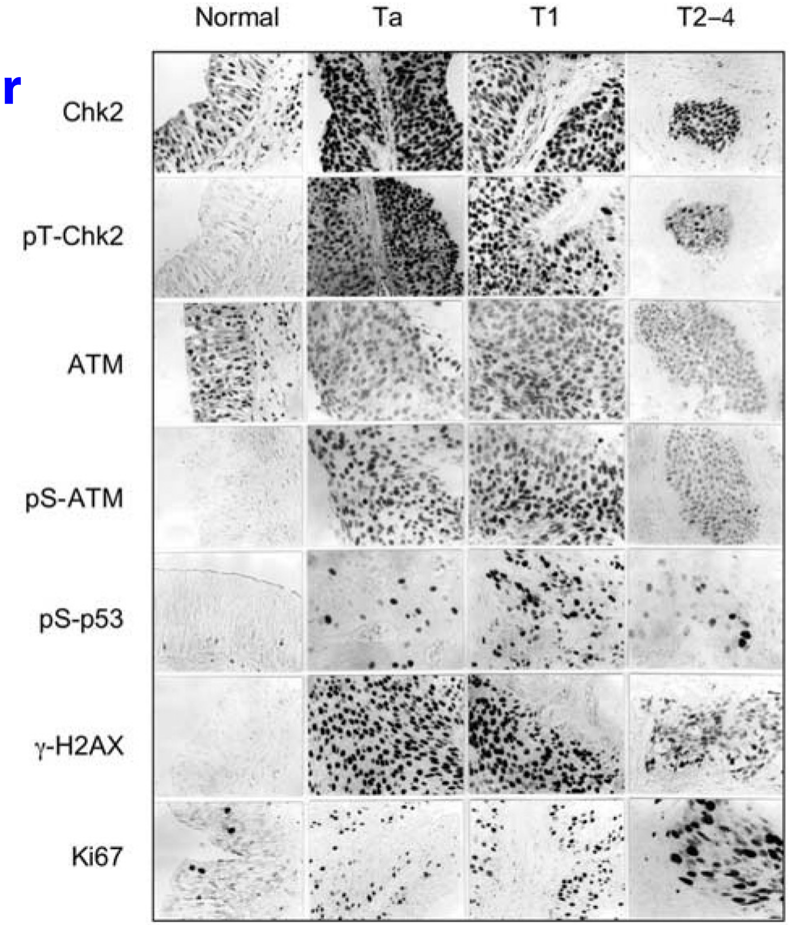
Does Oncogene-Mediated Induction of Cellular Senescence Occur In Vivo During Tumorigenesis ?

How Do Oncogenes Induce Cellular Senescence?

DNA Checkpoints and Cancer

- **Early tumors: activated damage checkpoints. Oncogene-driven cell-division cycles trigger DNA damage.**

- **Late tumors: mutations in tumor suppressor genes**



Bartkova et al., Nature 434, 864 (2005): **Figure 1** Constitutive activation of the ATM–Chk2–p53 pathway in human urinary bladder cancer. Immunohistochemistry of normal uroepithelium, early superficial lesions (Ta), earliest invasive (T1) and more advanced primary carcinomas (T2–4). Chk2 and ATM proteins are ubiquitously expressed, but Thr 68-phosphorylated Chk2 (pT-Chk2), Ser 1981-phosphorylated ATM (pS-ATM), Ser 15-phosphorylated p53 (pS-p53) and Ser 139-phosphorylated histone H2AX (γ-H2AX) are detectable only in tumour tissues. They are all present at the early stages of tumour development. Ki67 is a marker of proliferating cells. Original magnification, ×100.

Oncogene-induced cellular senescence

Oncogenes → Short G1 → Origin firing in transcribed genes → Fork collapse → Genomic instability

From: Macheret and Halazonetis, Nature 555, 1122 (2018)

Proposed Model:

- Oncogenes induce origin firing from within highly transcribed genes.
- Conflicts between transcription and replication leads to DNA double stranded breaks
- Ensuing checkpoint signaling may cause oncogene-induced senescence.

Side note:…other mechanisms may also contribute to the increased replication stress of cancer cells: unrepaired DNA lesions. Depletion of nucleotides upon treatment with chemotherapeutic agents. Loss of G1/S checkpoint leads to accumulation of DNA errors and early entry into S phase.
→increased sensitivity of cancer cells to ATRi, CHK1i, DNA damaging agents, DNA repair factor inhibitors etc (ATRi are currently tested in late phase clinical trials; CHK1i are in early stage trials)
(reviewed in Nature Cancer (2023) https://doi.org/10.1038/s41573-022-00558-5**)**

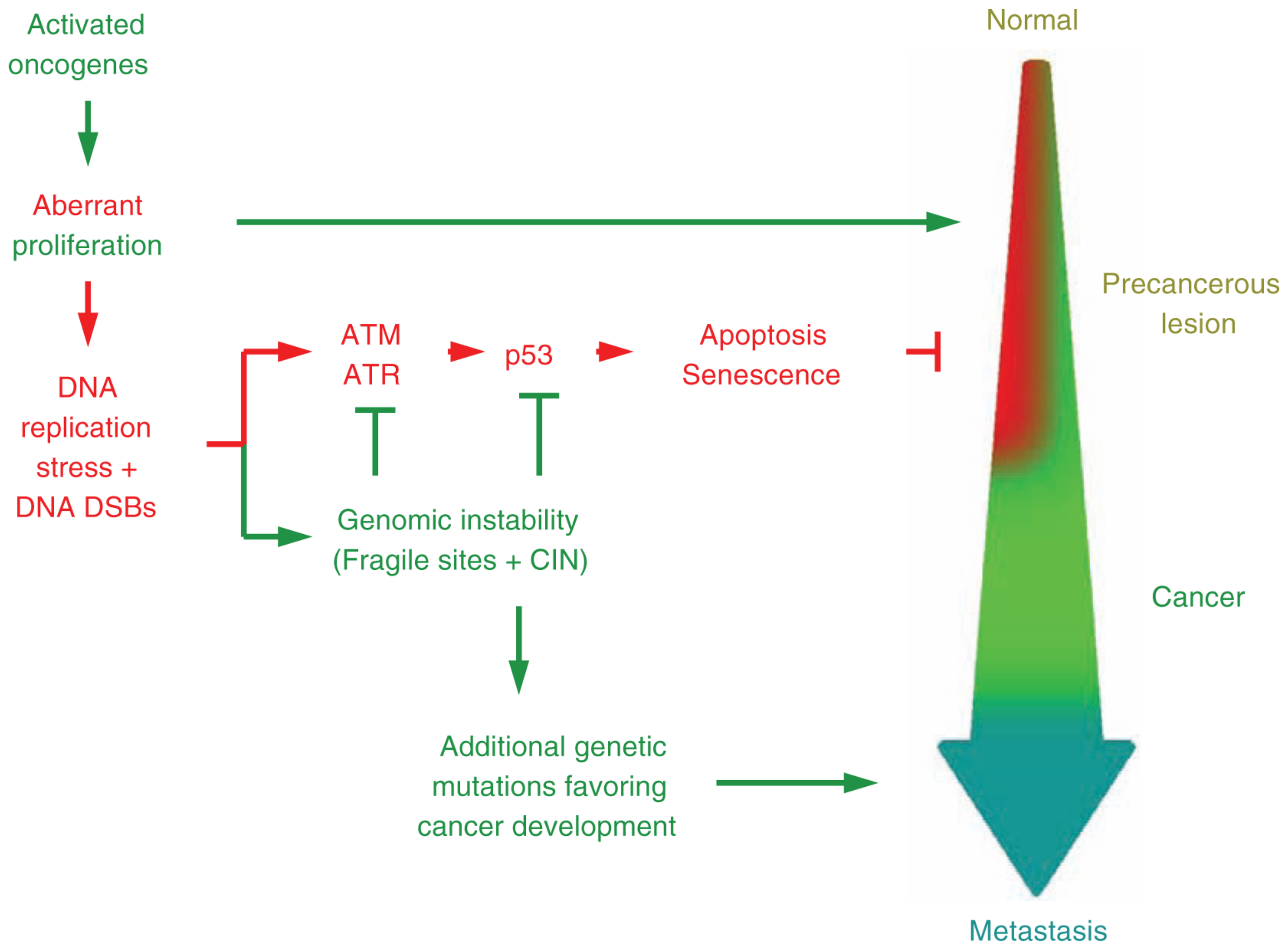


Fig. 2. Oncogene-induced DNA damage model for cancer development and progression. Genomic instability and tumor suppression are direct outcomes of oncogene-induced DNA replication stress and are both present from the beginning of cancer development, before the transition from precancerous lesion to cancer.

(from Halazonetis *et al.*, Science 319, 1352 (2008))

Key concepts

- Specific steps of the cell cycle are controlled by changing levels of cyclins, which acivate CDKs.
- CDK inhibitors antagonize cyclin-CDK complexes.
- Cyclin D levels are primarily controlled through extracellular signals.
- Other cyclins operated on a preordained schedule. They are degraded rapidly at defined stages ensuring the directionality of the cell cycle.
- The restriction point represents a point at which the cell is commited to complete the remainder of the cell cycle.
- Checkpoints ensure that a new step in the cell cycle is not undertaken before the preceding step is properly executed. Loss of checkpoint control in cancer contributes to genome instability.
- pRb inactivation (loss of R-point control) is important for cancer cells (inactivation of its cousins p107 and p130 is rare). → several mechanisms.
- Hypophosphorylated pRB blocks R point passage; hyperphosphorylated pRB permits the passage. pRB binds E2F releasing it when hyperphosphorylated.
- Oncogene-induced senescence: abnormal S phases, DNA damage, checkpoint activation.