Cancer Biology I :

Topics covered

Week 1:
Lecture 1: **Hallmarks of cancer – an overview; Oncogenes and tumor suppressor genes**
(Chapters 2, 4, 7 (Weinberg book))

Week 2:
Lecture 2 (Monday 14:15-16:00: room **AAC132**):
p53, genome instability and DNA repair of DNA double strand breaks; Synthetic lethality
Exercises: Wednesday 13:15-16:00: room **CE1103**

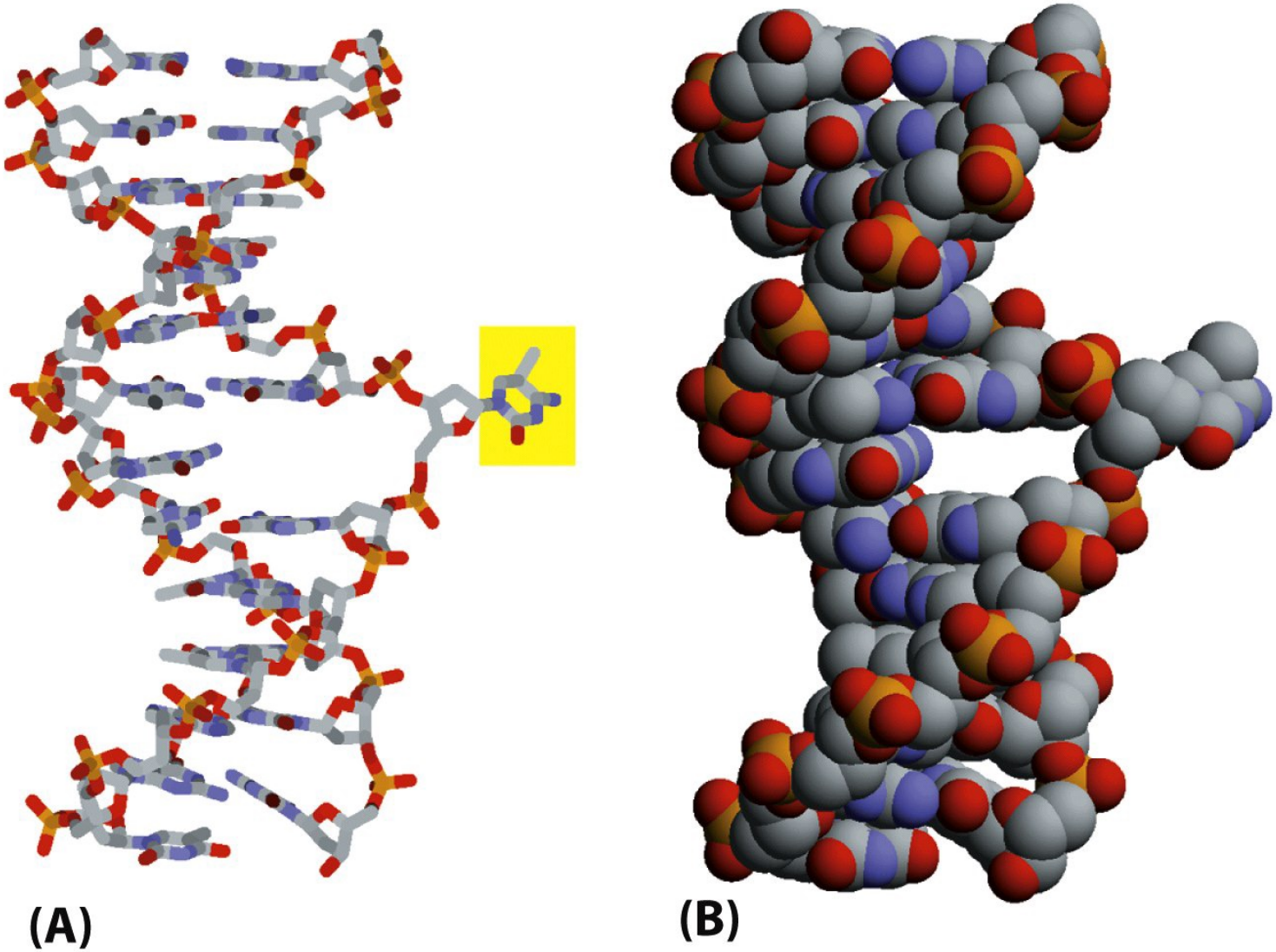
Week 3:
Lecture 3/Exercises: **Synthetic lethality continued; chromatin at double strand breaks; DNA repair: NER; the DNA damage response**

Week 4:
Lecture 4/Exercises: **BER; the DNA damage response; p53 and apoptosis**
(Chapters 9 (Weinberg))

Base Excision Repair (BER)

Several DNA Glycosylases do Exist:

They Recognize Different Specific Base-Modifications and Remove them

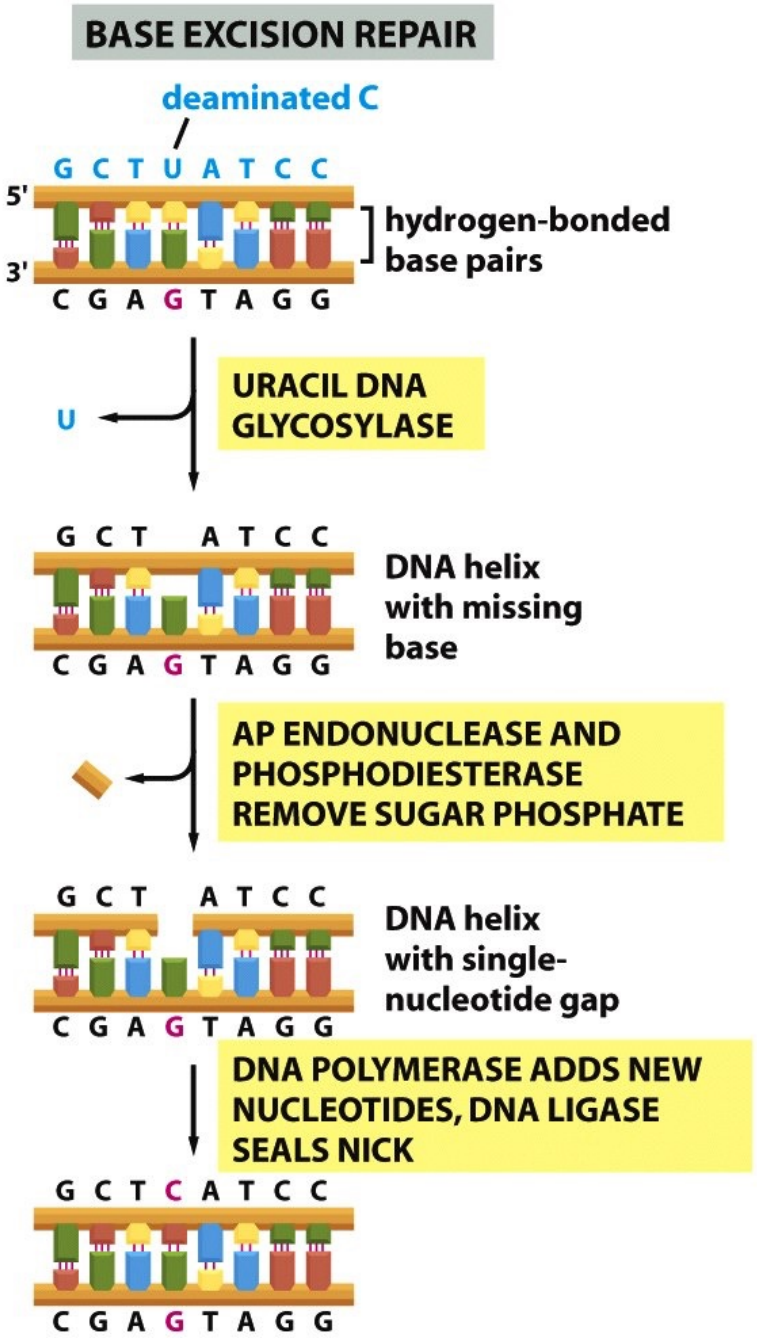


Base-Excision Repair

→ Removal of aberrant bases

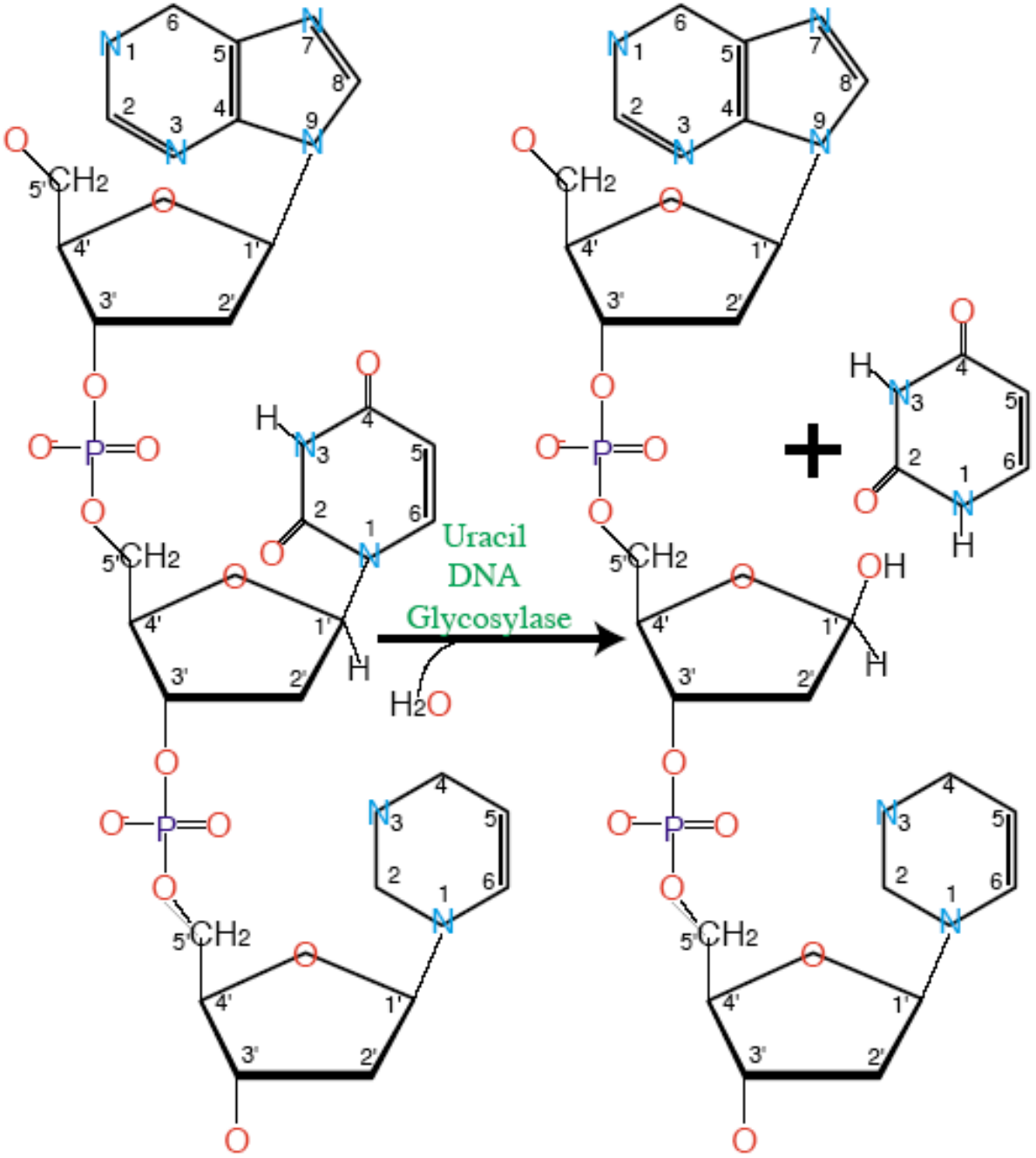
Apurinic/Apyrimidinic (AP) endonuclease

DNA polymerase involved: Pol Beta
mutations are frequently
found in cancer
But: BER is essential



PARP1-/- extracts: BER efficiency is strongly impaired

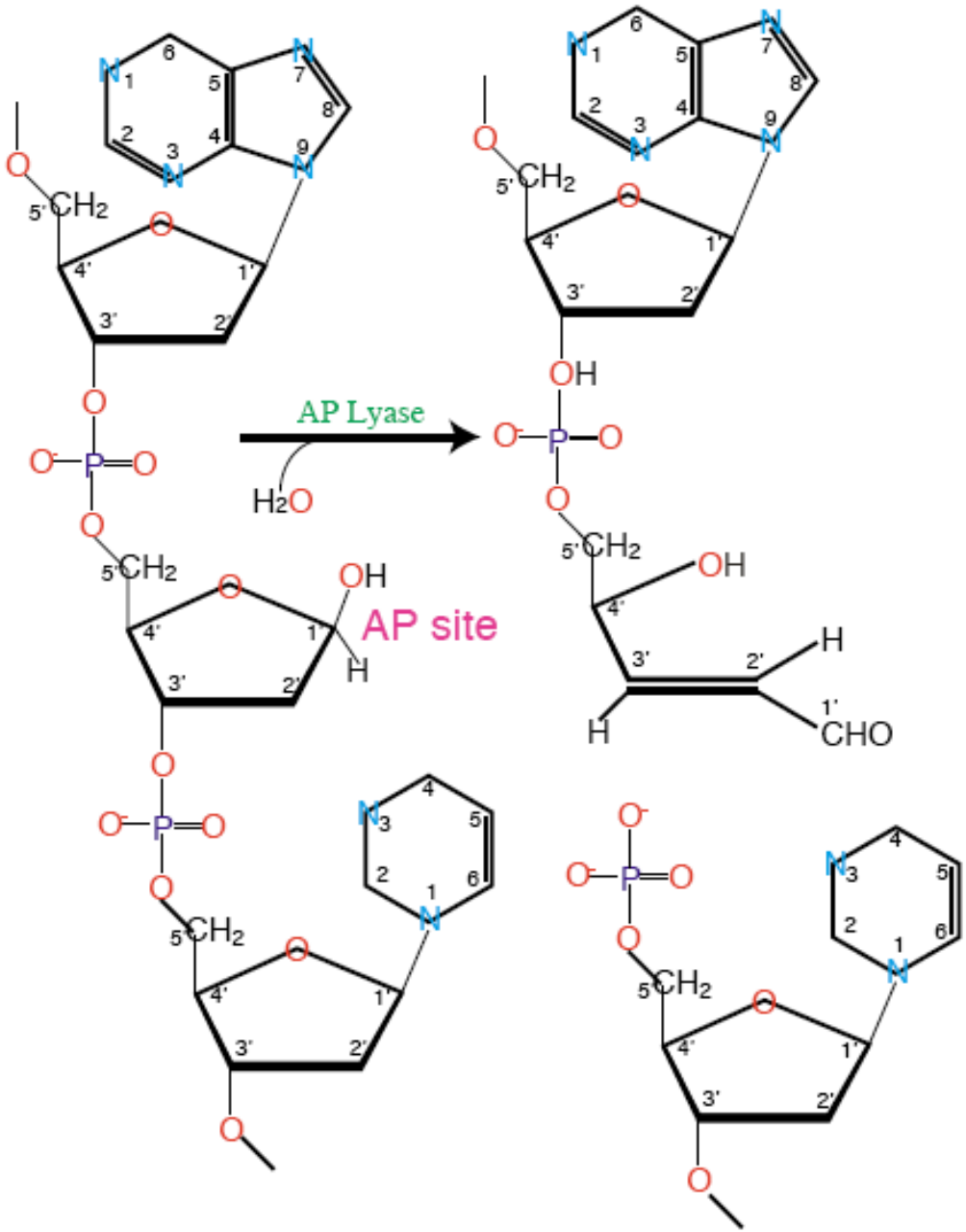
Glycosylases



BER: Specificity of DNA Glycosylases

Enzyme	Substrate
UNG	U in DNA ss/ds
SMUG1	U in DNA ss/ds; 5-hydroxymethyluracil (hmU) (Science 2021)
TDG	U:G or T:G
MBD4	U:G or T:G or ethenoC:G
OGG1	8-oxoG:C
MYH	A:8-oxoG (after replication)
NTH1	oxydated pyrimidines (ex: glycols of thymine)
NEI1	oxydated pyrimidines (ex: glycols of thymine)
MPG	alkylated purines (3-mA, 7-mA, N^6-ethenoadenine, hypoxantine)

Some Glycosylases have AP (apurinic/apyrimidinic) Lyase Activity



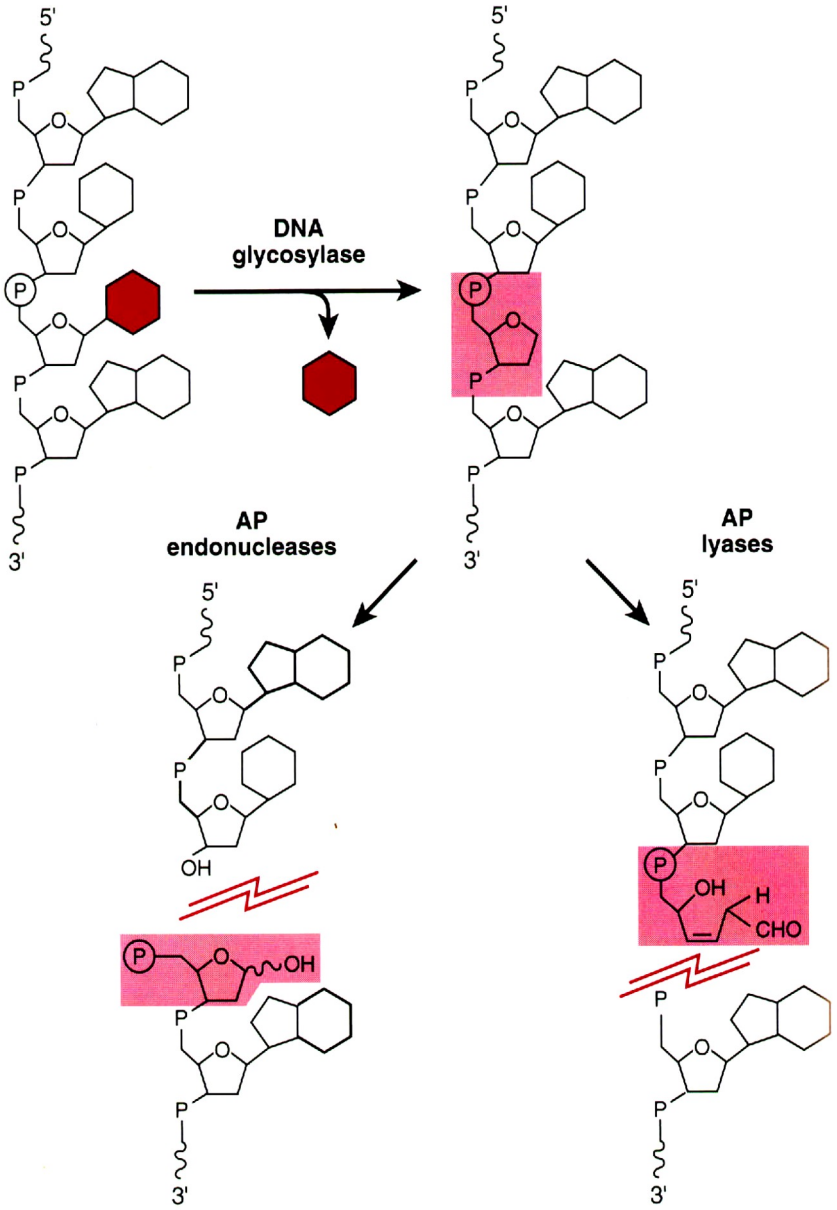
BER: Removal of a Damaged Base

DNA glycosylases:
UNG, SMUG1, TDG, MBD4, MYH,MPG

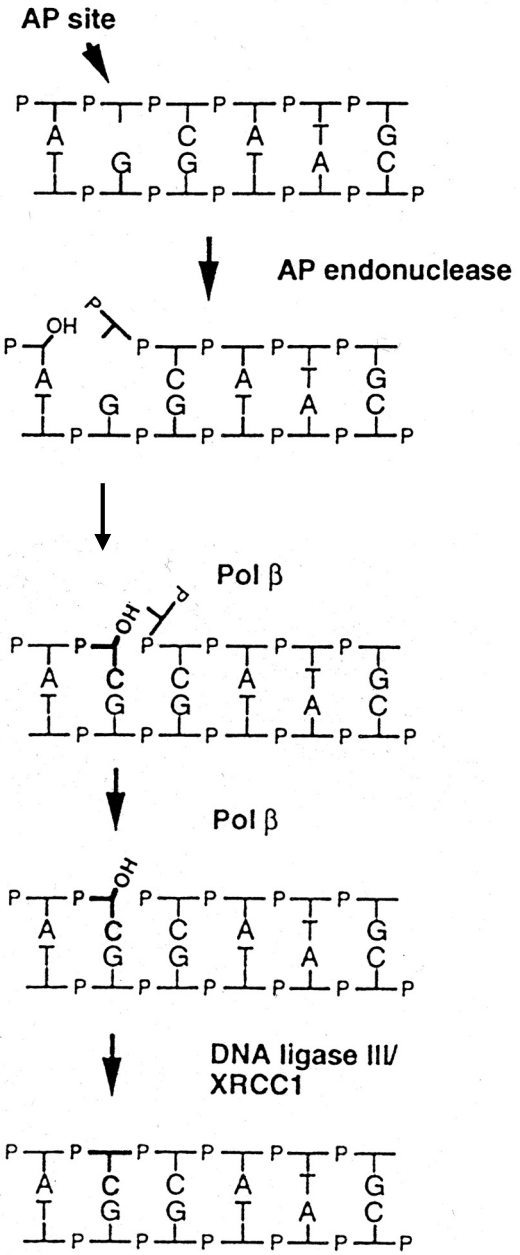
DNA glycosylases/AP lyases:
OGG1, NTH1, NEI1

AP-endonuclease (hydrolysis at 5 '):
APE1

AP lyase (cleavage at 3'):
Polymerase β



Simplified Representation of BER



NER:~30 factors involved. Repair of lesions that induce **helix distortion** (e.g. induced by UV). Repair involves DNA unwinding by TFIIH, dual incisions that flank the damaged location (XPF-ERCC1, XPG), excision and DNA repair synthesis
-->Xeroderma Pigmentosum

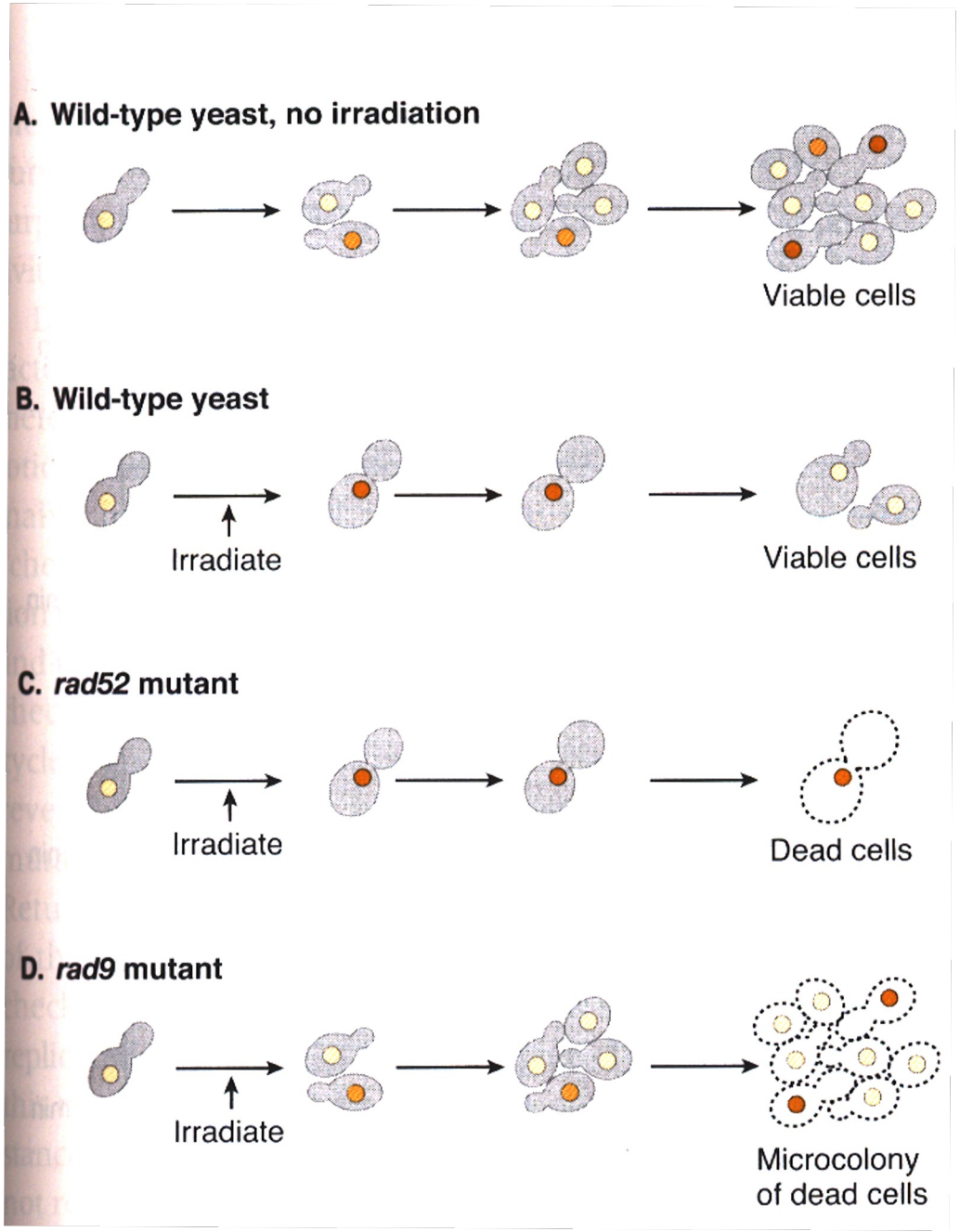
Not covered in lectures:
TCR-NER: Transcription coupled repair. Recognizes stalled RNA polymerase at helix-distorting lesions. NER-factors (but not XPC-hHR23B) plus CSA and CSB
-->Cockayne syndrome

BER (base excision repair): glycosylases (→AP(apurinic/apyrimidinic)-sites, AP lyases (hydrolysis at 3') , AP-endonuclease (hydrolysis at 5'), pol ß, DNA ligase III. Removal of uracil, 8-oxoguanine and other **modified bases**; repair of abasic sites.
-->Embryonic lethality, XRCC1 and pol ß mutations are associated with various cancers.

Not covered in lectures:
MMR (mismatch repair): MutS and MutL homologs assemble together with PCNA; RFC and RPA into a repairosome. MutL introduces nicks in the daughter strand. ExoI is loaded and its 5'-3' exonuclease activity will remove the **mismatch**. Conventional DNA replication enzymes (pol δ, PCNA, RFC) will fill the gap and DNA ligase 1 will seal the nick.
→Hereditary nonpolyposis colorectal cancer (HNPCC)

Cellular Responses to Replicative Stress and DNA Double Strand Breaks

Ted Weinert and Lee Hartwell 1988



Thought Question

- What mechanism may be defective in *rad52* cells?
- and what other mechanism may be defective in *rad9* mutant cells?

Ted Weinert and Lee Hartwell 1988

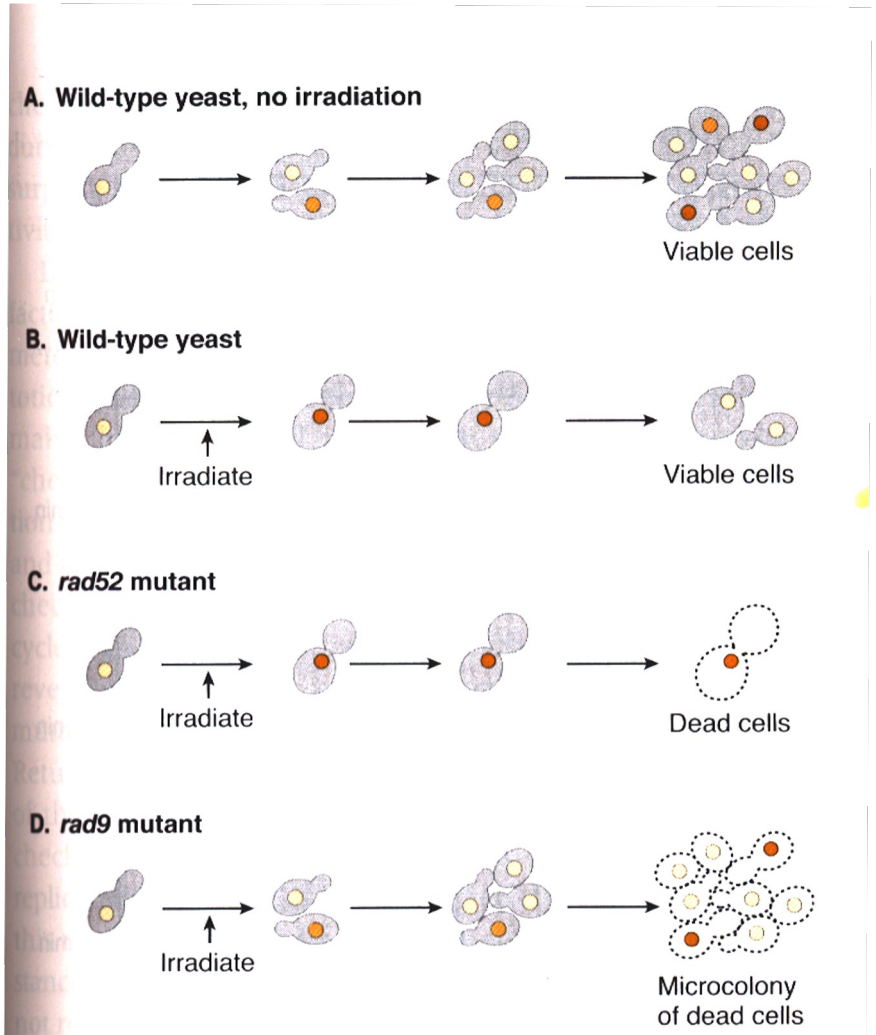
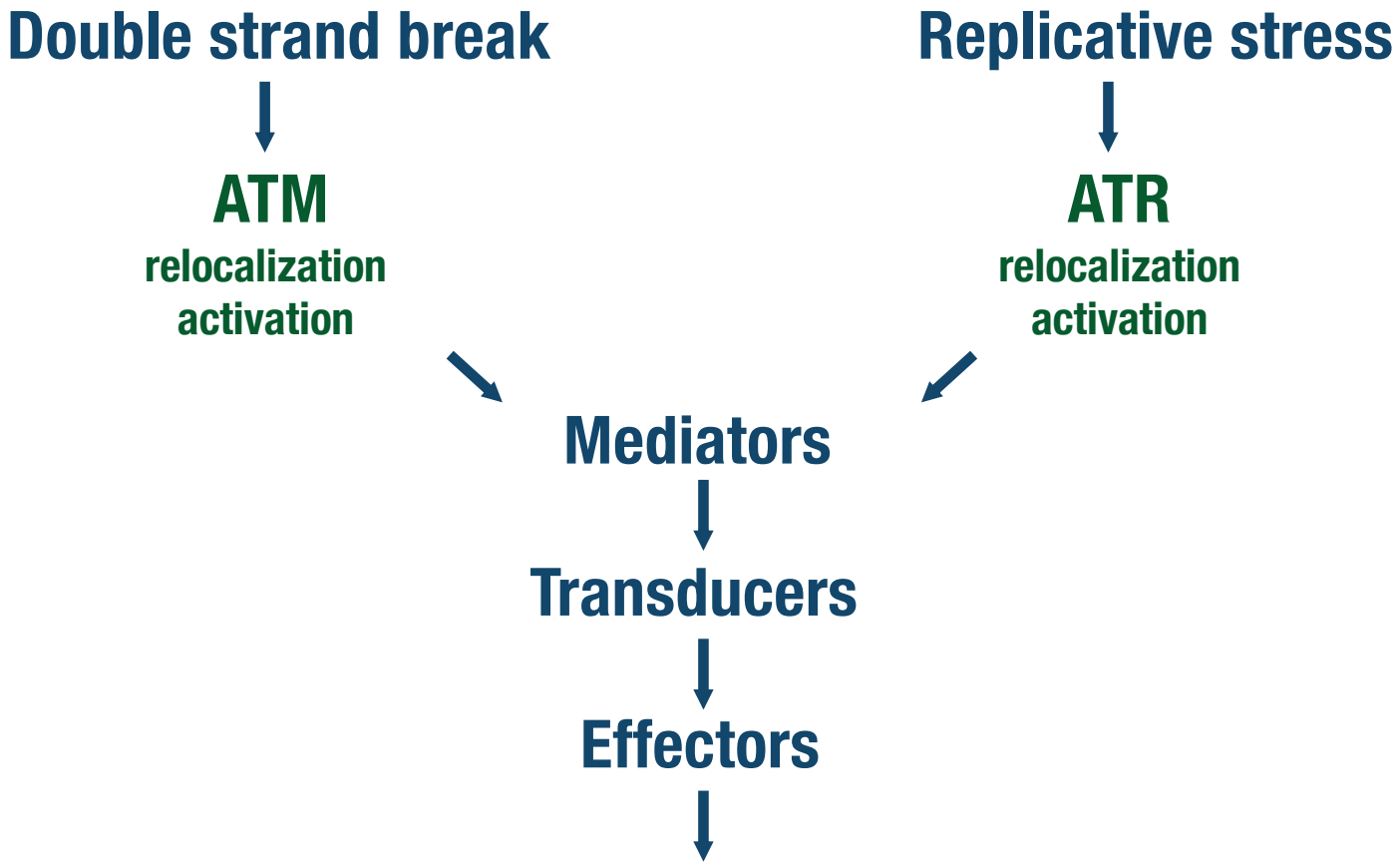


Figure 20–3 The basic observations of checkpoint arrest in budding yeast. (A and B) When irradiated with X rays, wild-type *S. cerevisiae* cells arrest as a large-budded cell in G_2/M, presumably to allow time for DNA repair before resuming cell cycle progression. (C) A repair-deficient mutant such as a *rad52* mutant stays arrested in G_2/M and cannot resume cell division. (D) An arrest-deficient mutant such as a *rad9* mutant does not arrest in G_2/M but continues cell cycle progression in the presence of unrepaired DNA damage, resulting in the formation of microcolonies of dead cells. (Adapted from reference 142.)

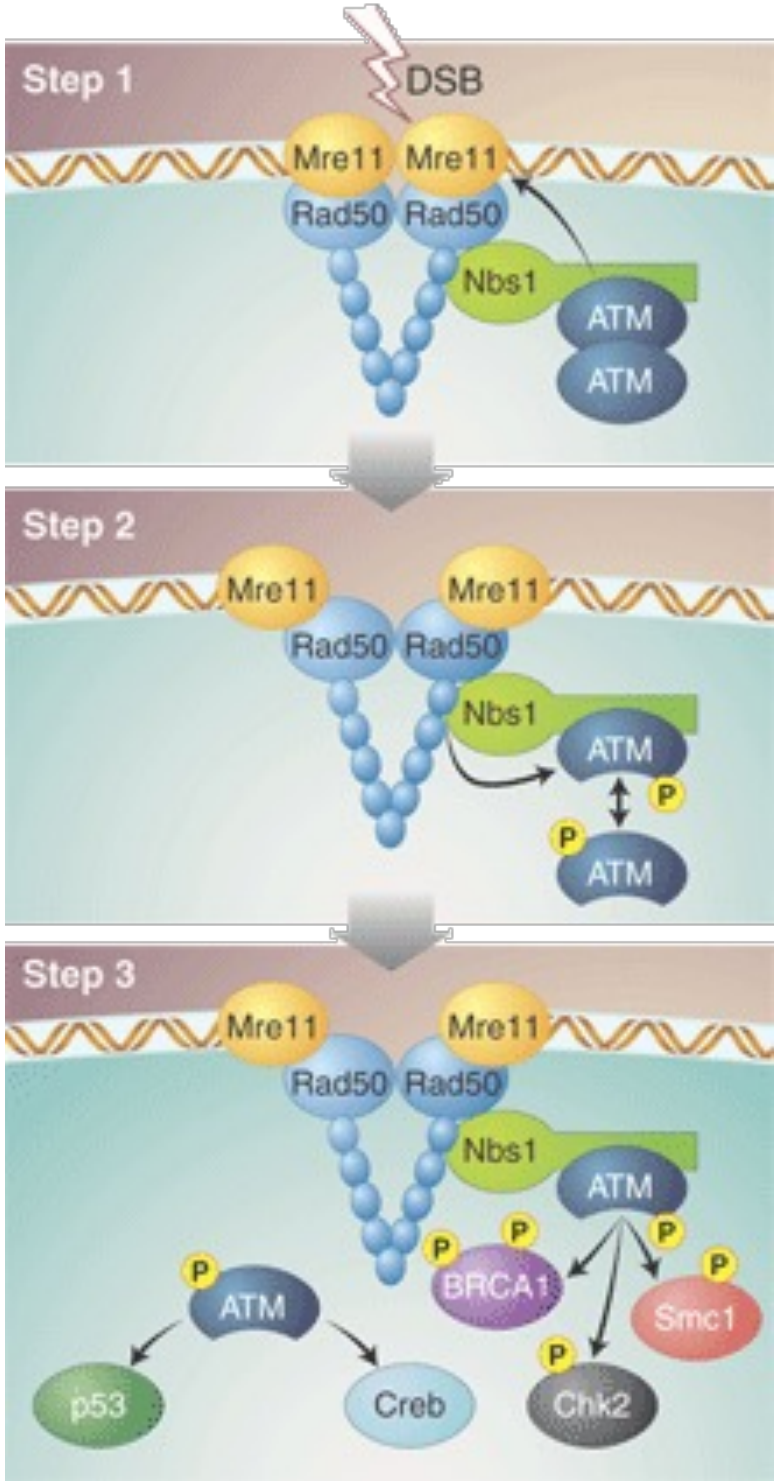
Checkpoint Activation by DNA Lesions

PI(3)K (phosphatidylinositol-3-OH kinase)-like kinases (PIKK): ATM & ATR Ser/Thr kinases



Activation of ATM

The MRN complex and ATM activation. (Step 1) The induction of **DSBs** in DNA leads to prompt **recruitment** of **MRN** complexes. These complexes form a bridge between free DNA ends via the coiled-coil arms of Rad50 dimers. Inactive ATM dimers are recruited to the DSBs through interaction with the carboxyl terminus of Nbs1. (Step 2) Activating signals are delivered to ATM dimers, possibly through a conformational change in Nbs1. **ATM** undergoes **phosphorylation at Ser^{1981}** accompanied by its conversion from a **dimer to** a **monomer**. The MR complex may also trigger a conformational change in ATM that stimulates substrate recruitment. (Step 3) Activated ATM monomers either remain in the vicinity of the DSB, where they phosphorylate colocalized substrates, or diffuse away from the DSB sites to phosphorylate nuclear substrates, such as p53.



Activation of ATR

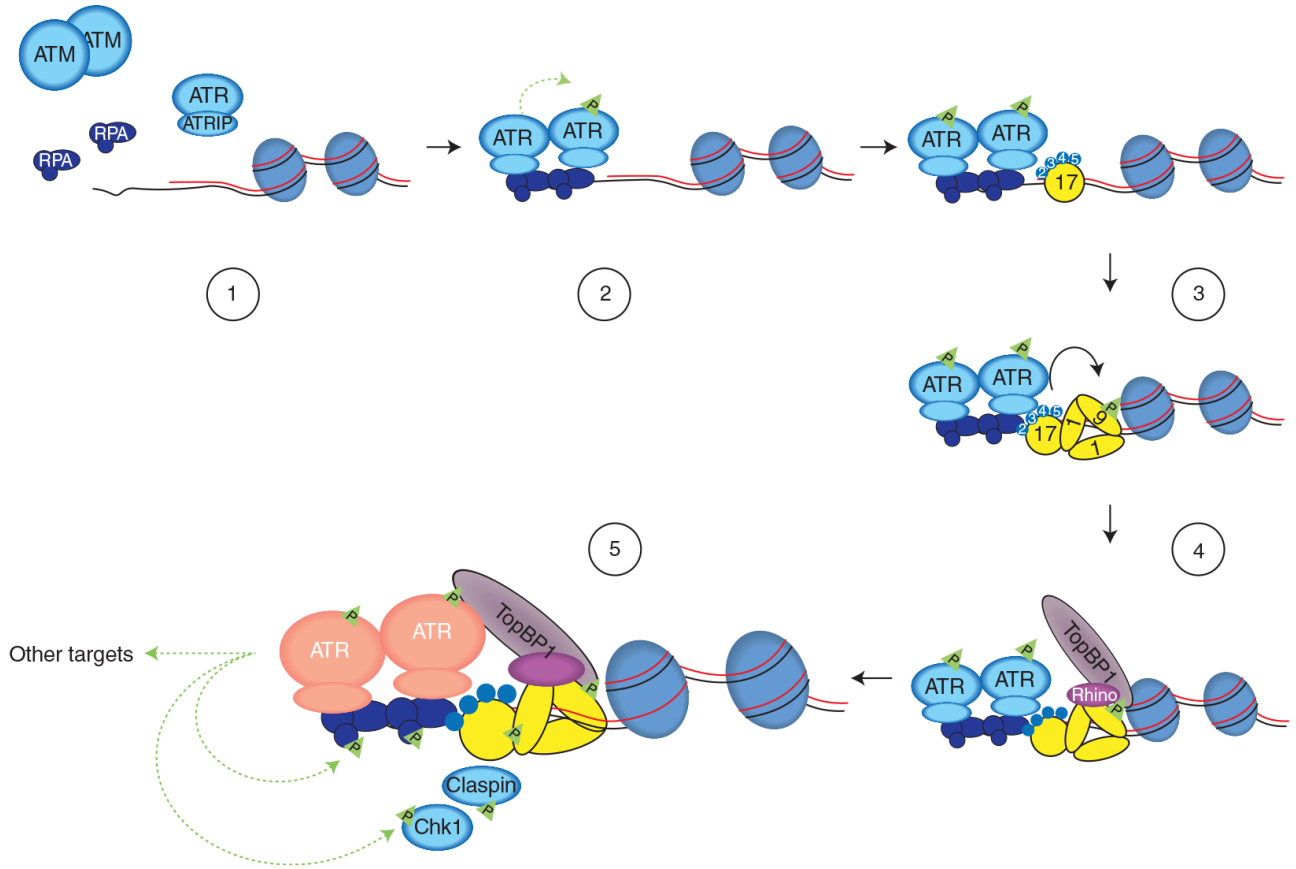


Figure 4. A fail-safe, multistep mechanism for ATR activation. Increased amounts of ssDNA are generated by resection of DNA ends or by uncoordinated DNA unwinding and synthesis at replication forks. Extensively resected DNA ends are no longer recognized by ATM efficiently. Once coated by RPA, ssDNA recruits the ATR-ATRIP complex (1), and promotes ATR *trans*-autophosphorylation (2). RPA-ssDNA also promotes the recruitment of the Rad17-Rfc2-5 clamp loader to junctions between ssDNA and dsDNA, and the loading of Rad9-Rad1-Hus1 (9-1-1) checkpoint clamps onto dsDNA (3). TopBP1 interacts with phosphorylated Rad9 and with Rhino, which associates with 9-1-1 (4). The TopBP1 recruited to dsDNA by 9-1-1 and Rhino engages the ATR-ATRIP complex on RPA-ssDNA through the ATR autophosphorylation site T1989. This process enables TopBP1 to stimulate ATR-ATRIP to its full capacity (pink) on ssDNA (5). TopBP1 may also function as a scaffold to facilitate ATR substrate recognition. This multistep process for ATR activation ensures that ATR is only activated when both ssDNA and ssDNA/dsDNA junctions are present at sites of DNA damage and are recognized by DNA damage sensors, providing a fail-safe but versatile mechanism to signal DNA damage. The dashed green lines represent phosphorylation events, and the solid black line represents the loading of 9-1-1 by the Rad17-RFC2-5 complex.

From: Cold Spring Harbor Perspect Biol 2013;5:a01276

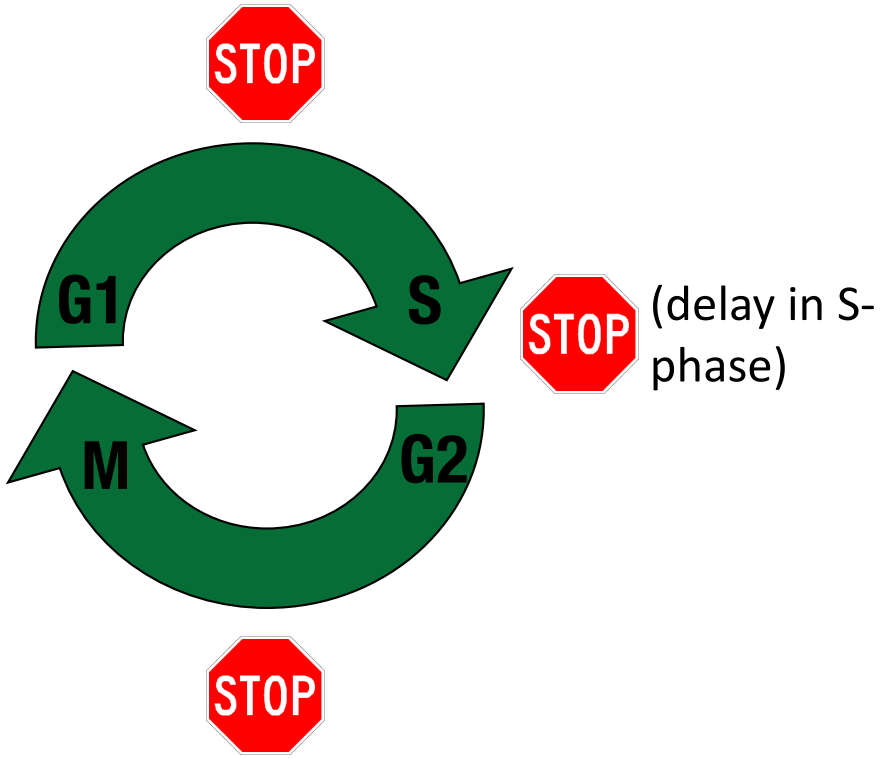
Signal Transducing Kinases

Checkpoint kinases-1 &-2:

ATM → CHK2

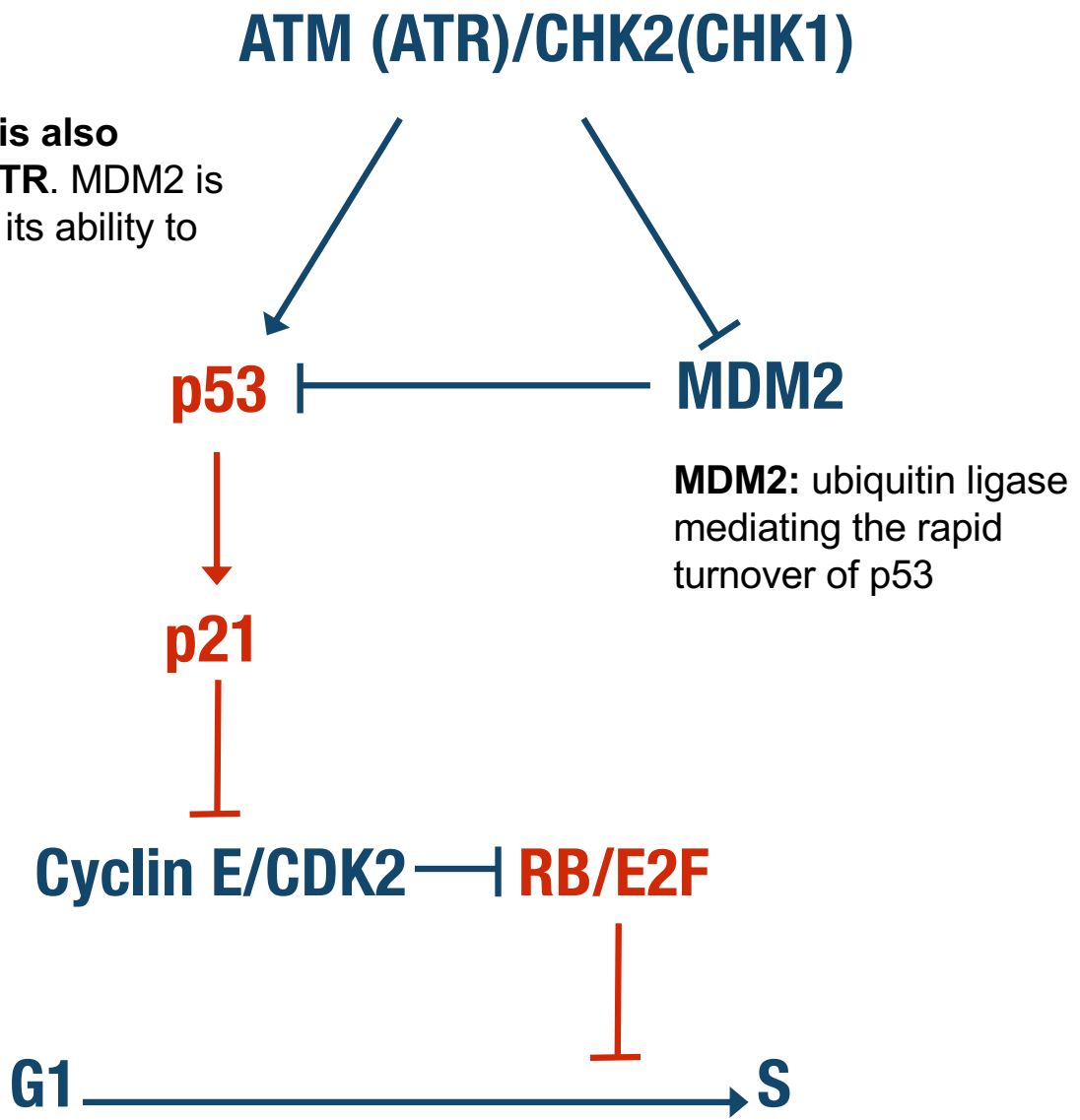
ATR → CHK1

Response to genotoxic stress :



Arrest in G1

ATM phosphorylates directly p53. p53 is also phosphorylated by CHK1, CHK2 and ATR. MDM2 is also phosphorylated by ATM, inactivating its ability to bind p53.



MDM2: ubiquitin ligase mediating the rapid turnover of p53

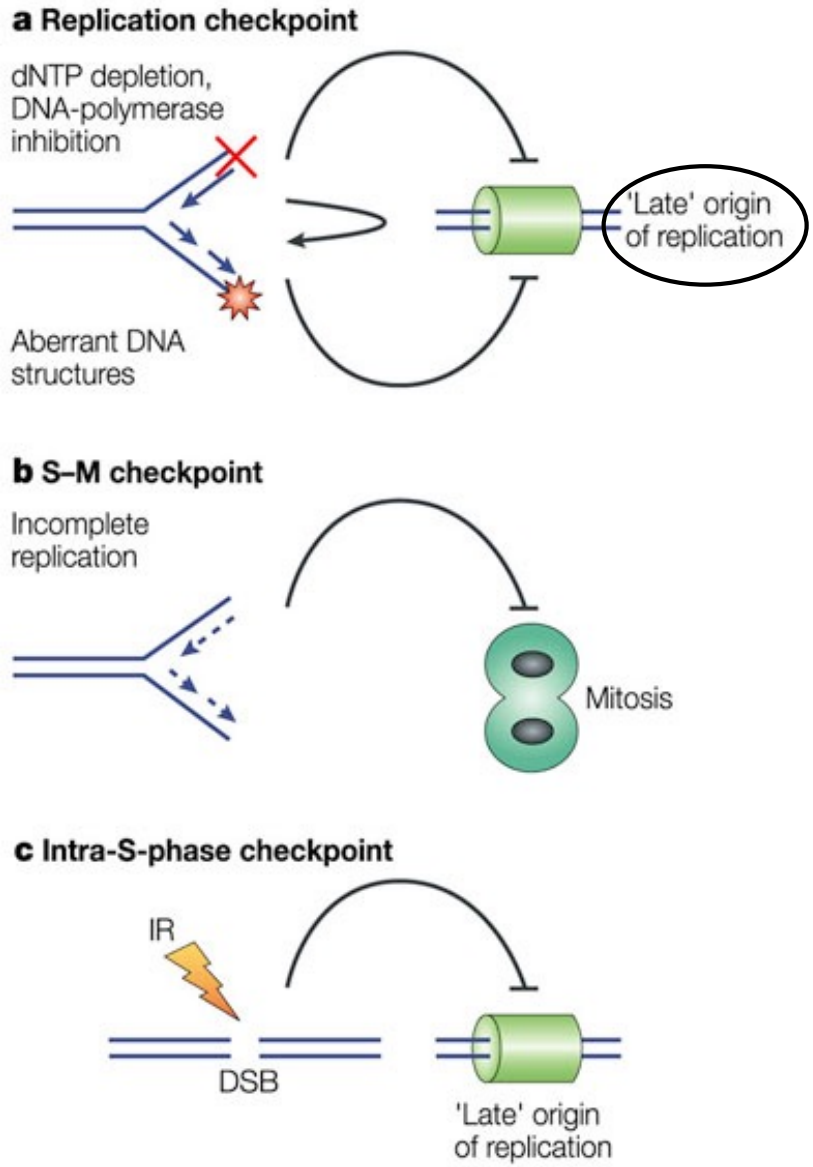
Three S Phase Checkpoints

Functions of the replication checkpoint

Reduction of DNA synthesis

Stabilization of blocked forks

Coordination of repair



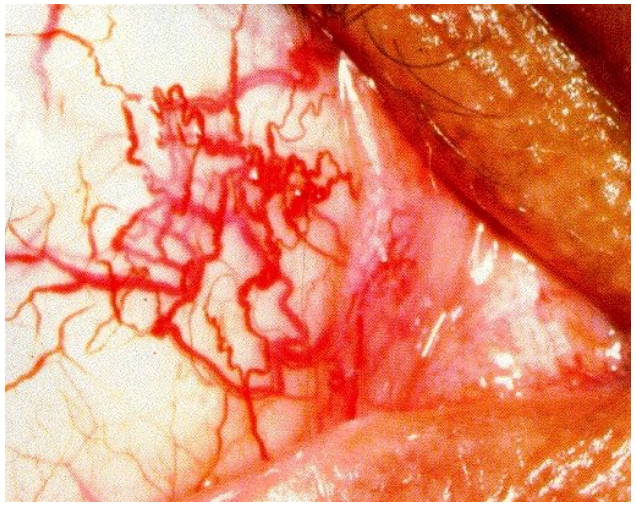
Nature Reviews | Molecular Cell Biology

Ataxia Telangiectasia

ATM: Ataxia telangiectasia mutated (autosomal recessive disorder)

Clinical symptoms:

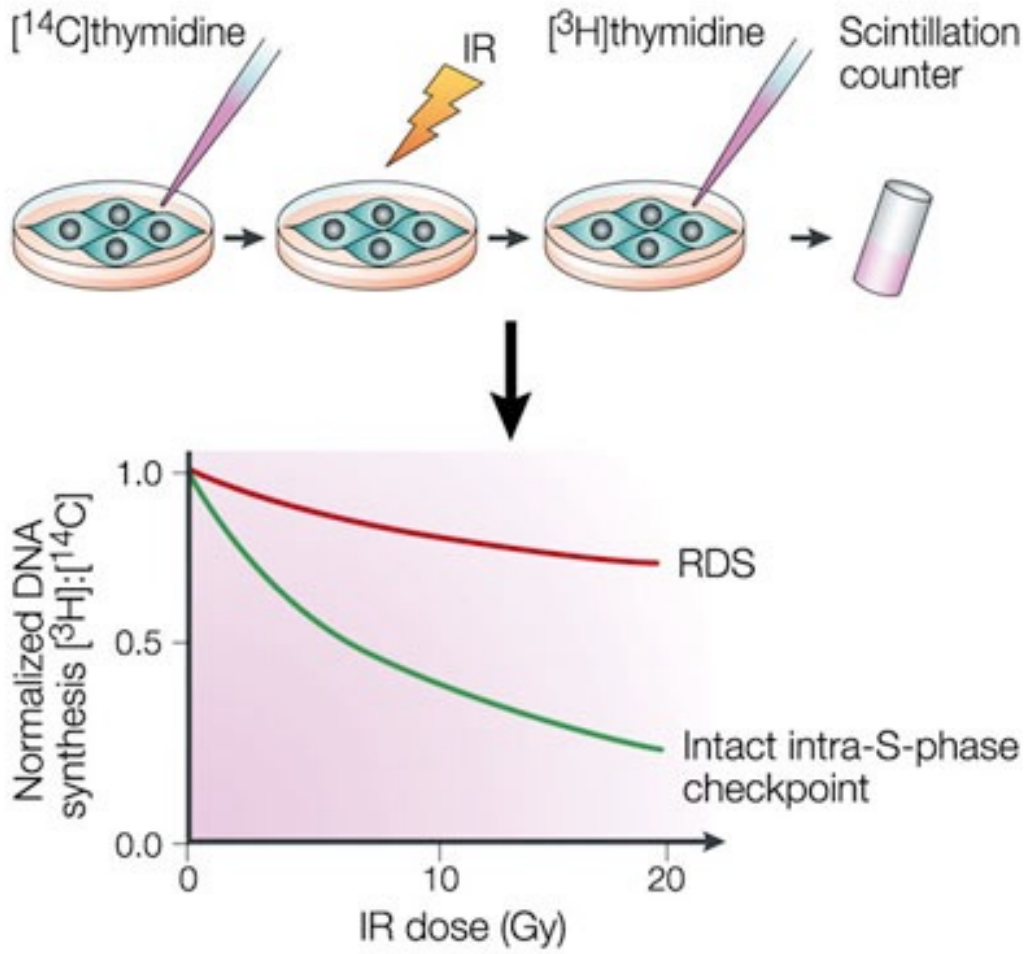
Severe immonodeficiency
Progressive Ataxia due to degeneration of cerebellum
(ataxia: lack of coordination of muscles)
Telangiectasia
Lymphomas
Carcinomas


Telangiectasia

Cellular phenotype:

Sensitivity to ionizing radiation
Spontaneous chromosome breaks, other chromosome abnormalities

Radioresistant DNA Synthesis in AT cells



Nature Reviews | Molecular Cell Biology

Seckel Syndrome

ATR: ATM- and RAD3- related

Hypomorphic alleles

ATR is an essential gene

Clinical symptoms:

Microcephaly (small circumference of the head)
Dwarfism
Large eyes, low ears, small chin
Severe mental retardation

Hematological abnormalities and chromosome breaks

But no increased cancer risk has been observed in ATR-Seckel mice…

ATR as a Drug Target

Replication stress is high in cancer because oncogene activation involves deregulation of physiologic DNA replication. Therefore, cancer cells heavily rely on ATR.

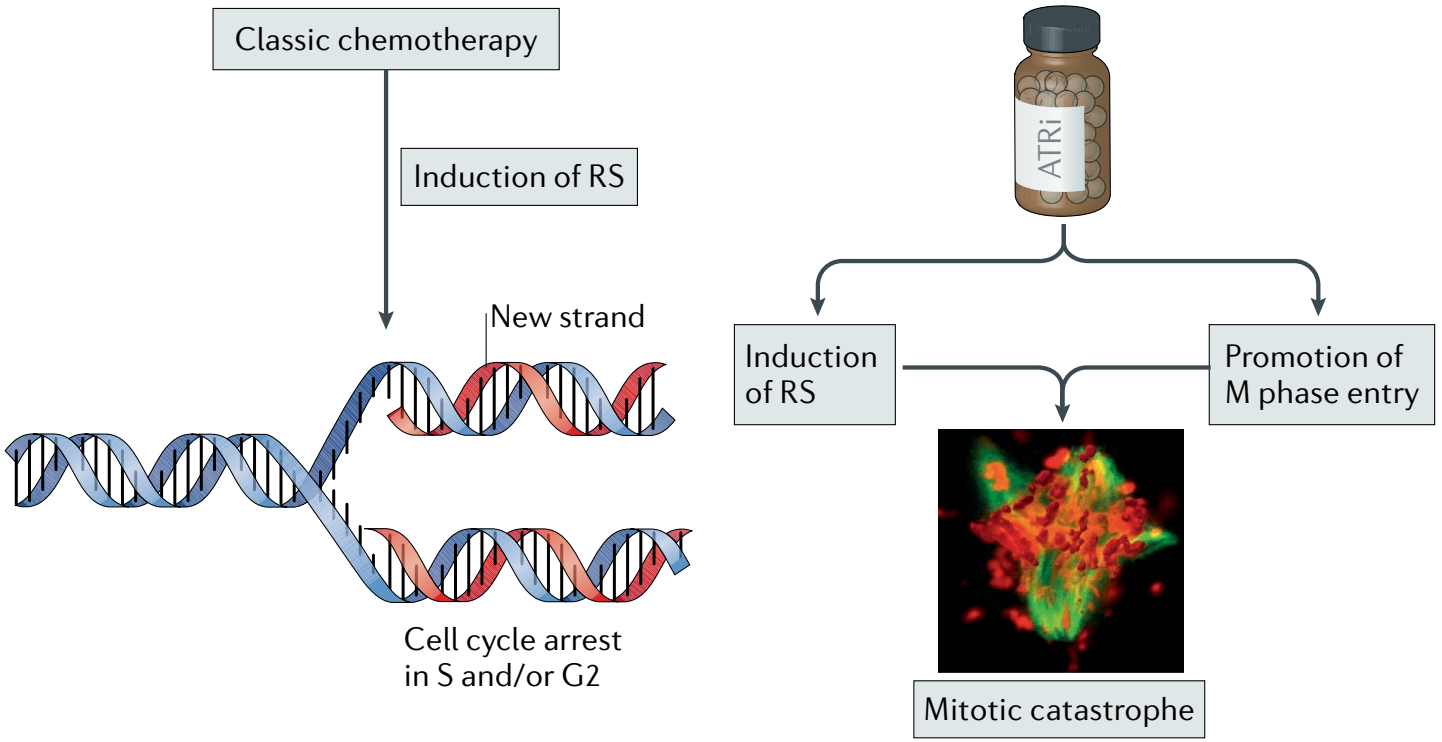


Fig. 2 | **Mechanism of action of ATR inhibitors.** Many of the currently used genotoxic chemotherapies (including nucleotide analogues, alkylating agents and topoisomerase inhibitors) are potent inducers of replication stress (RS). However, the cell cycle checkpoints triggered by these chemicals often lead to cell cycle arrest rather than cell death and thereby do not eliminate the tumour cells. The key advantage of ataxia telangiectasia and Rad3-related protein inhibitors (ATRi) is that, while these chemicals also induce high loads of RS, they additionally force premature mitotic entry. The combination of these two activities forces cells with unreplicated genomes into mitosis, leading to mitotic catastrophe and p53-independent (non-apoptotic) cell death.

From: Nature Reviews in Cancer 18, 586 (2018)

Oncogene-induced replication stress:
Fundamental step of tumorigenesis associated with many oncogenes.

Attributed to aberrant origin firing, replication-transcription collisions, defective nucleotide metabolism and others.

DDR Inhibitors in Clinical Use or Trials

Target	Inhibitor	Manufacturer	Reference
PARP1 or PARP1/2	Olaparib	AstraZeneca	Fong et al. 2009
	Rucaparib	Clovis Oncology	Coleman et al. 2017
	Niraparib	Tesaro	González-Martín et al. 2019
	Talazoparib	Medivation	Litton et al. 2018
	Veliparib	AbbVie	Coleman et al. 2019
	AZD5305	AstraZeneca	Johannes et al. 2021
ATR	Berzosertib (M6620, VX-970)	Merck KGaA	Yap et al. 2020
	Ceralasertib (AZD6738)	AstraZeneca	Yap et al. 2021a
	BAY 1895344	Bayer	Yap et al. 2021b
	RP-3500	Repare	Roulston et al. 2022
	ART0380	Artios	Unpublished
	ATRN-119	Artin	Unpublished
CHK1	Prexasertib (LY2606368)	Eli Lily	Lee et al. 2018
	SRA737	Sareum	Rogers et al. 2020
WEE1	Adavosertib (AZD1775)	AstraZeneca	Leijen et al. 2016
ATM	AZD1390	AstraZeneca	Jin and Oh 2019
	AZD0156	AstraZeneca	Jin and Oh 2019
DNA-PK	AZD7648	AstraZeneca	Fok et al. 2019
	Peposertib (M3814)	Merck KGaA	van Bussel et al. 2021
	BAY-8400	Bayer	Berger et al. 2021
ATM/DNA-PK	XRD-0394	XRad	Unpublished
DNA-PK/mTOR	CC-115	Celgene	Munster et al. 2019
POLθ	ART4215	Atrios	Zatreanu et al. 2021
	Novobiocin (VP-006)	Varsity Pharma	Zhou et al. 2021
RAD51	CYT-0851	Cyteir	Ratiu et al. 2017
USP1	KSQ-4279	KSQ	Unpublished
PKMYT1	RP-6306	Repare	Gallo et al. 2021
PRMT5	GSK3326595	GSK	Gerhart et al. 2018
	JNJ-64619178	Janssen	Brehmer et al. 2021
	TNG908	Tango	Unpublished
	PRT 811	Prelude	Unpublished

From: doi:10.1101/gad.349431.122
Genes Dev. 2022, 36: 278-293

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Week 4:
Lecture 4/Exercises: **BER; the DNA damage response; p53 and apoptosis**
(Chapters 9 (Weinberg))

From week 1:

p53: Missense Mutations: 95% Affect the DNA-binding Domain

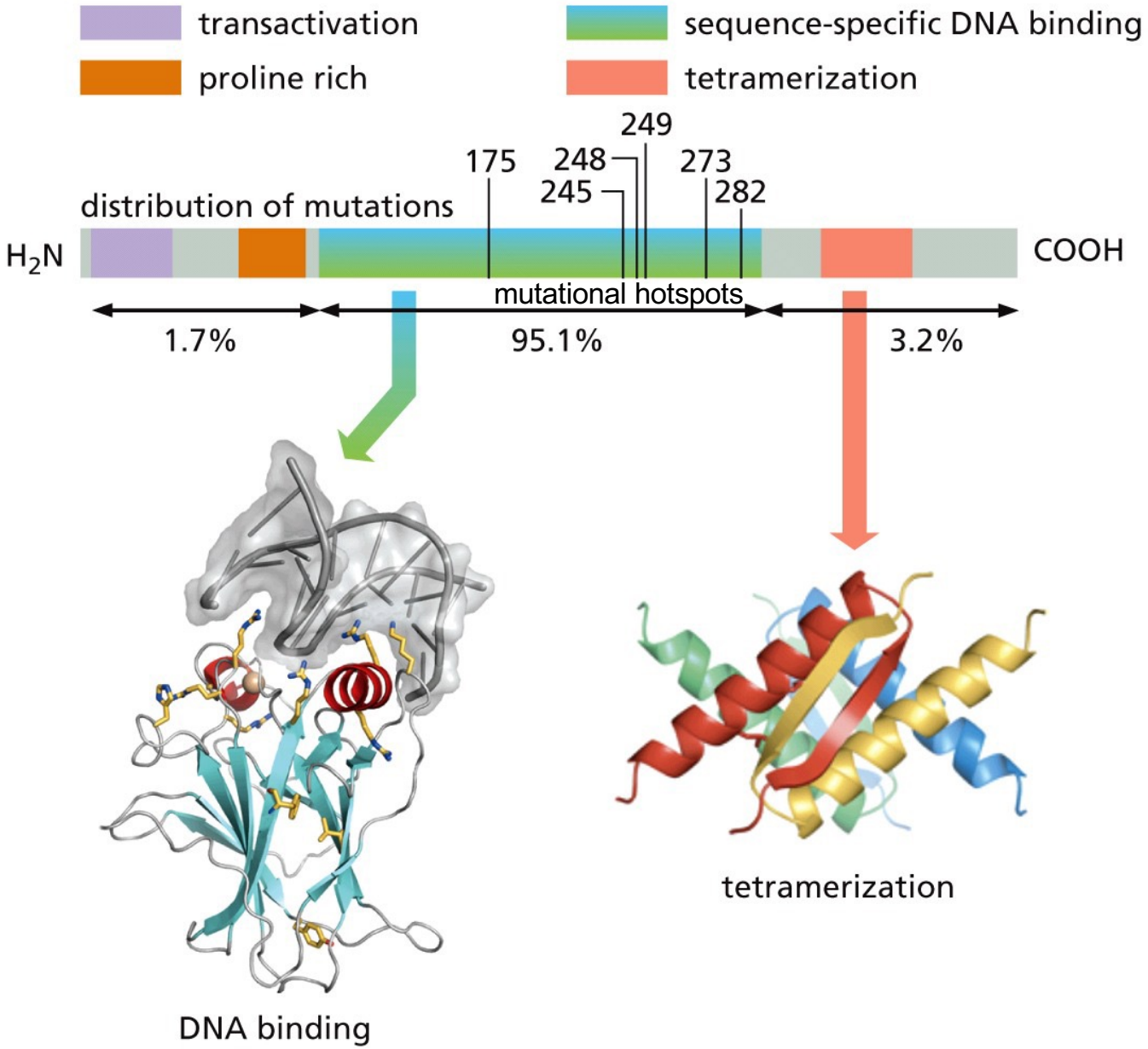


Figure 9.6b The Biology of Cancer (© Garland Science 2014)

From week 1:

p53: Why do Missense Mutations Prevail?

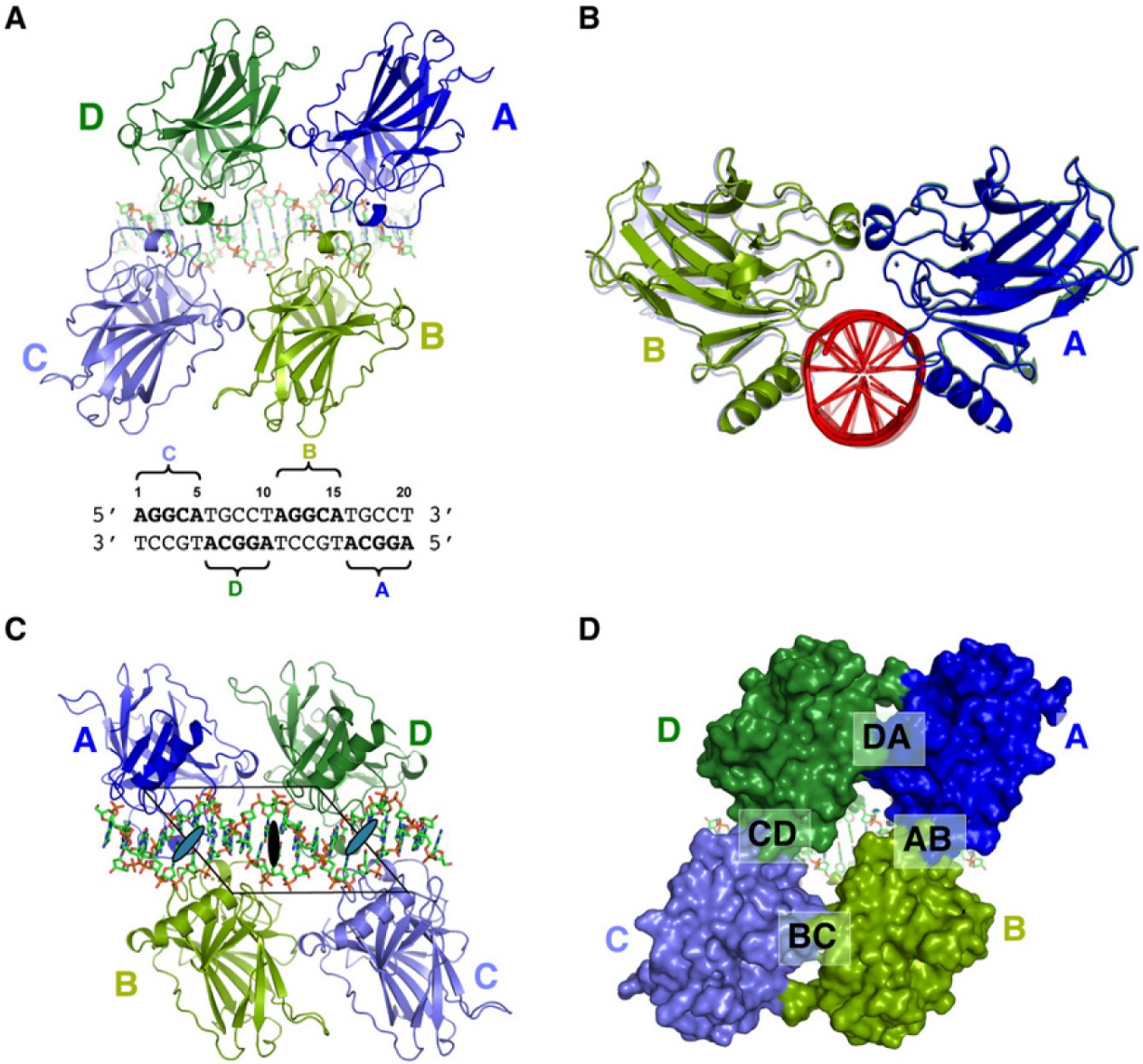


Figure 1. Overall Structure of the p53 Core Domain Bound to DNA as a Tetramer

(A) The tetramer viewed from the protein side. The four monomers are colored in blue (A), light green (B), light blue (C), and green (D). The same color scheme is used throughout the illustration unless indicated otherwise. The DNA is in stick model with its sequence shown below. The four pentameric motifs (quarter site) and their corresponding monomers are indicated in the sequence.

(B) A view of the tetramer along the DNA axis. This view shows that the tetramer has a planer structure wherein the A-B dimer (front) and C-D dimer align almost perfectly along the DNA axis.

(C) The tetramer viewed from the DNA side. The parallelogram is shown together with the global two-fold axis (dark oval) and the two local dyad aces (gray ovals).

(D) A surface model of the tetramer view in the same orientation as (A). The four protein-protein interfaces are indicated.

From Chen et al. Structure 18, 246–256, 2010

From week 1:

p53: Proposed Mechanism of Dominant-Negative Mutations

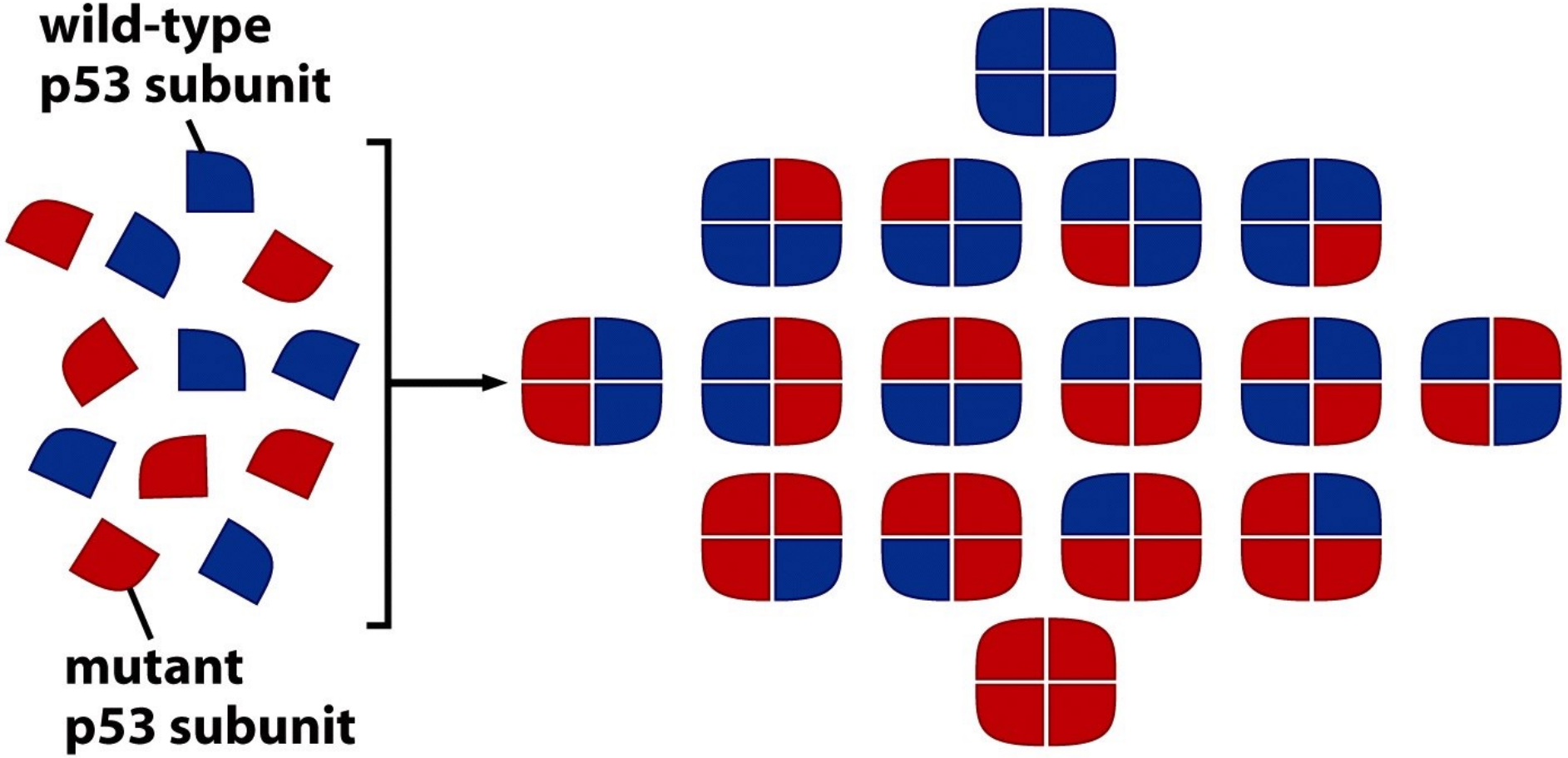


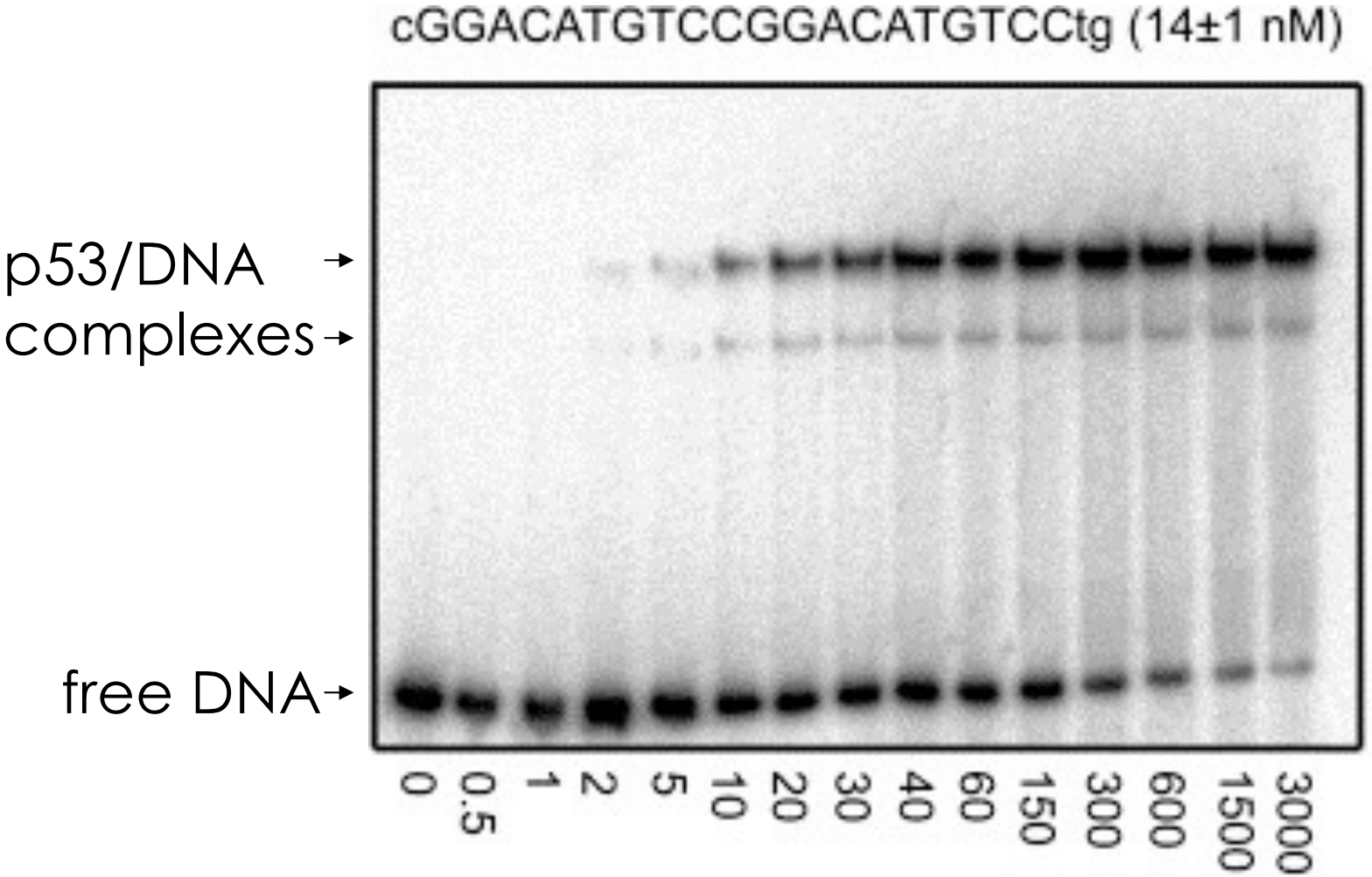
Figure 9.5 *The Biology of Cancer*

To be discussed during the exercises:

Illustration of dominant negative effect of p53 missense mutations in myeloid malignancies:
Boettcher et al., Science **2019** 365: 599-604

How would you measure DNA binding of p53 in vitro?

EMSA: Electromobility Shift Assay



Binding affinity measurements of p53DBD/DNA complexes. Radiolabeled and gel-purified hairpin duplexes (50 pM) and increasing amounts of p53DBD were incubated on ice for 3 hours in a buffer containing 50 mM Tris·HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM ATP, 25 U/ml BSA, 10% glycerol, 10 mM DTT and 200 mM KCl. Complexes were resolved from free DNA by electrophoresis on native gels (6%, 37.5:1 acrylamide:bisacrylamide ratio). The gels were run at 550 V and 4ºC in a running buffer containing 1 X TG (25 mM Tris·HCl (pH 8.3), 190 mM glycine) **(From Mol. Cell, 22, 441 (2006))**

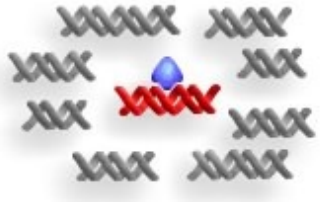
How Would You Measure DNA Binding of p53 in vivo?

Genome-wide Mapping of Protein Binding Sites

Most widely used method: ChIP-Seq = Chromatin immunoprecipitation followed by sequencing

1. Cross-linking of DNA binding protein to DNA

2. Chromatin shearing by sonication



3. Incubation with an antibody specific for the DNA-binding protein

4. Isolation of bound fragments and sequencing

Modified from http://www.bio.brandeis.edu/haberlab/jehsite/chIP.html

Formaldehyde Crosslinking:

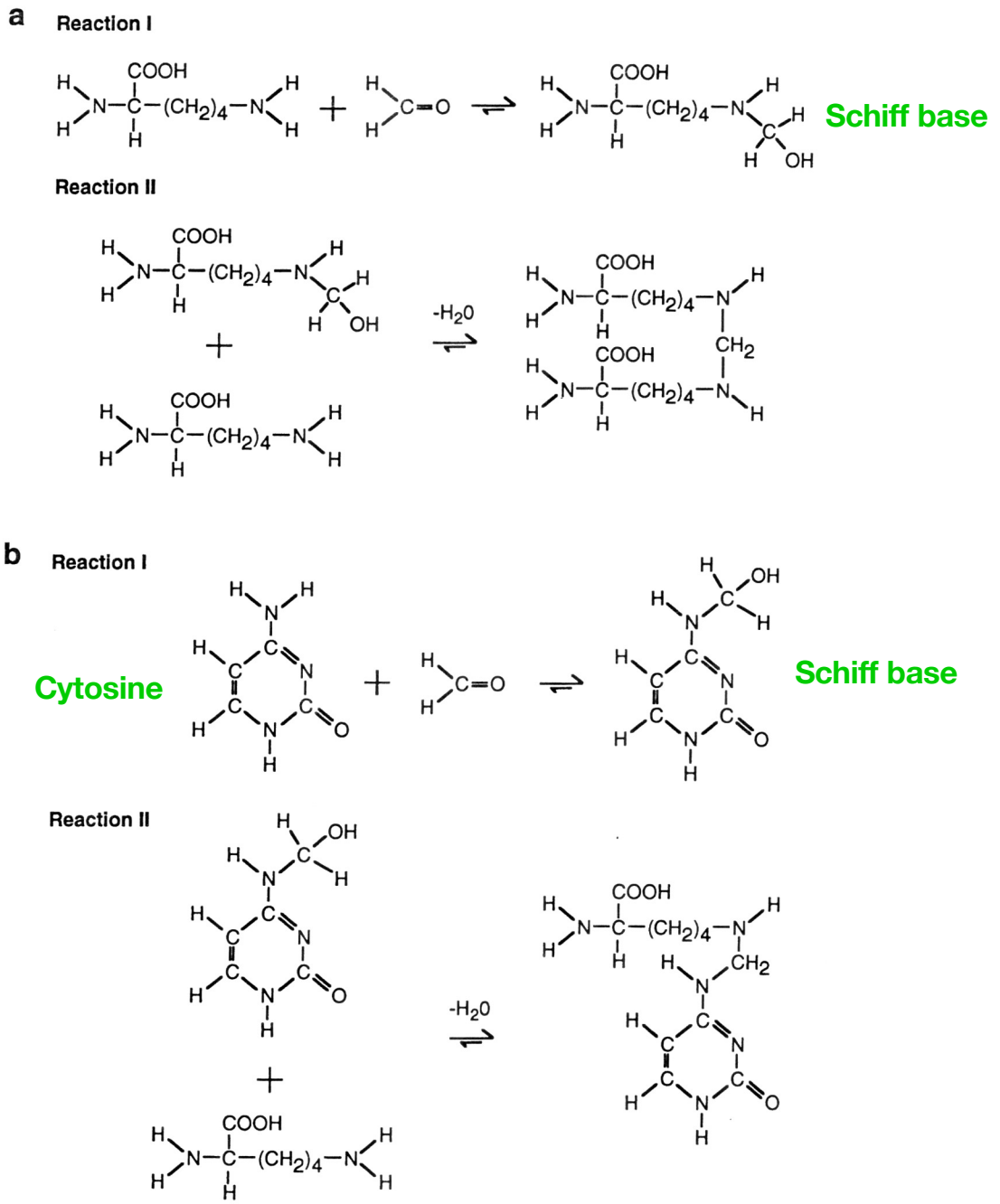


FIG. 2. Chemical cross-linking of DNA and proteins by formaldehyde. Formaldehyde (HCHO) is a very reactive dipolar compound in which the carbon atom is the nucleophilic center. Amino and imino groups of proteins (e.g., the side chains of lysine and arginine) and of nucleic acids (e.g., cytosine) react with formaldehyde, leading to the formation of a Schiff base (reaction I). This intermediate can react with a second amino group (reaction II) and condenses (19, 20). Cross-links may be reversed by heating in Tris–HCl-containing buffers. This leads to a drop in pH and protonation of amino groups, thus forcing the equilibrium in the reverse direction. (a) Formaldehyde-mediated cross-linking between the side chains of two lysines. (b) Cross-linking between cytosine and lysine.

Consensus DNA Sequence Bound by p53

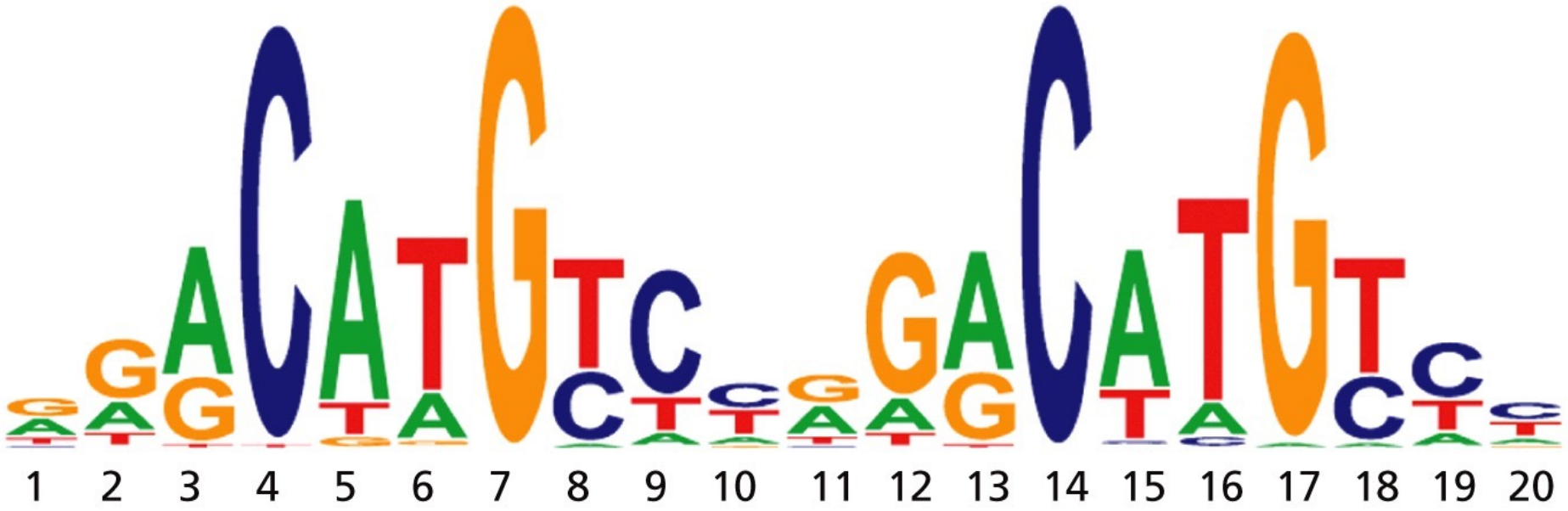


Figure 9.15. Weinberg, The Biology of Cancer

Analyzed 1546 sites; consensus sequence: relative size of letter indicates frequency of DNA base at the position

p53 Regulation

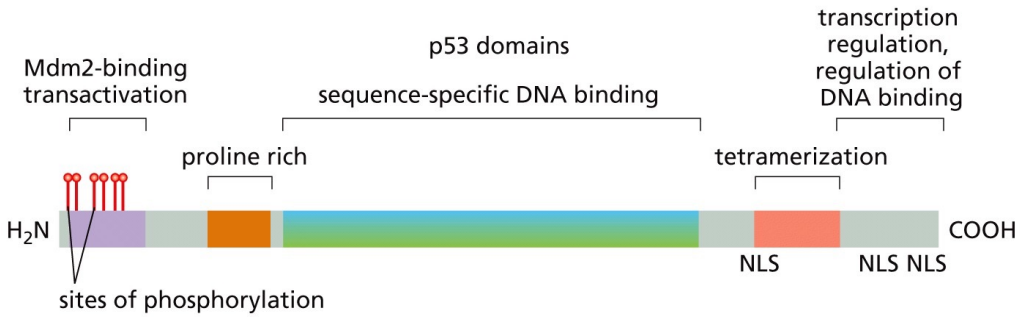


Figure 9.12b The Biology of Cancer (© Garland Science 2014)

p300/CBP: recruited to promoters by p53 via Taz2 domain of p300: p300 relaxes the chromatin structure adding acetyl groups to histones. It also recuits pol II to promoters

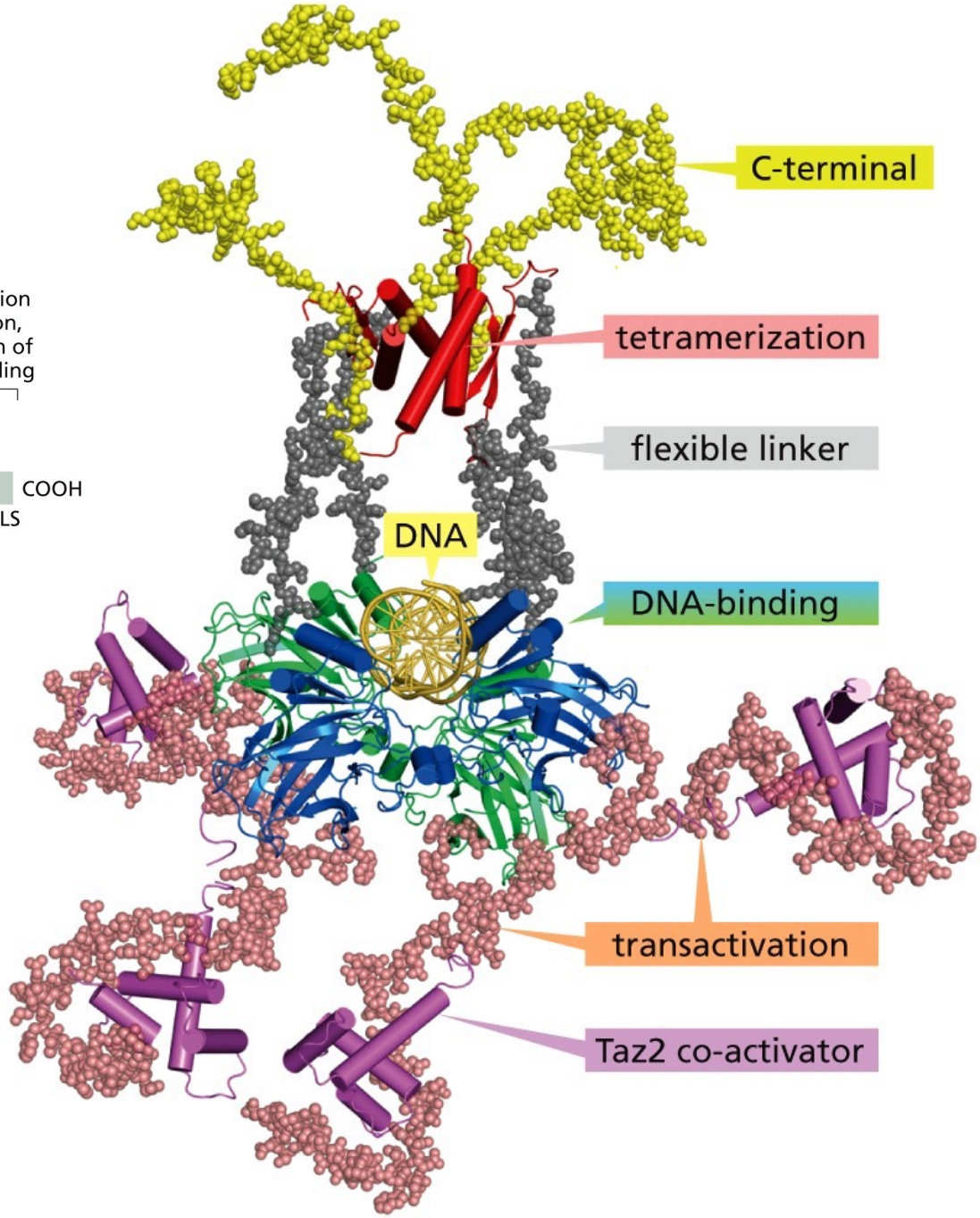


Figure 9.6c The Biology of Cancer (© Garland Science 2014)

How is p53 Regulated?

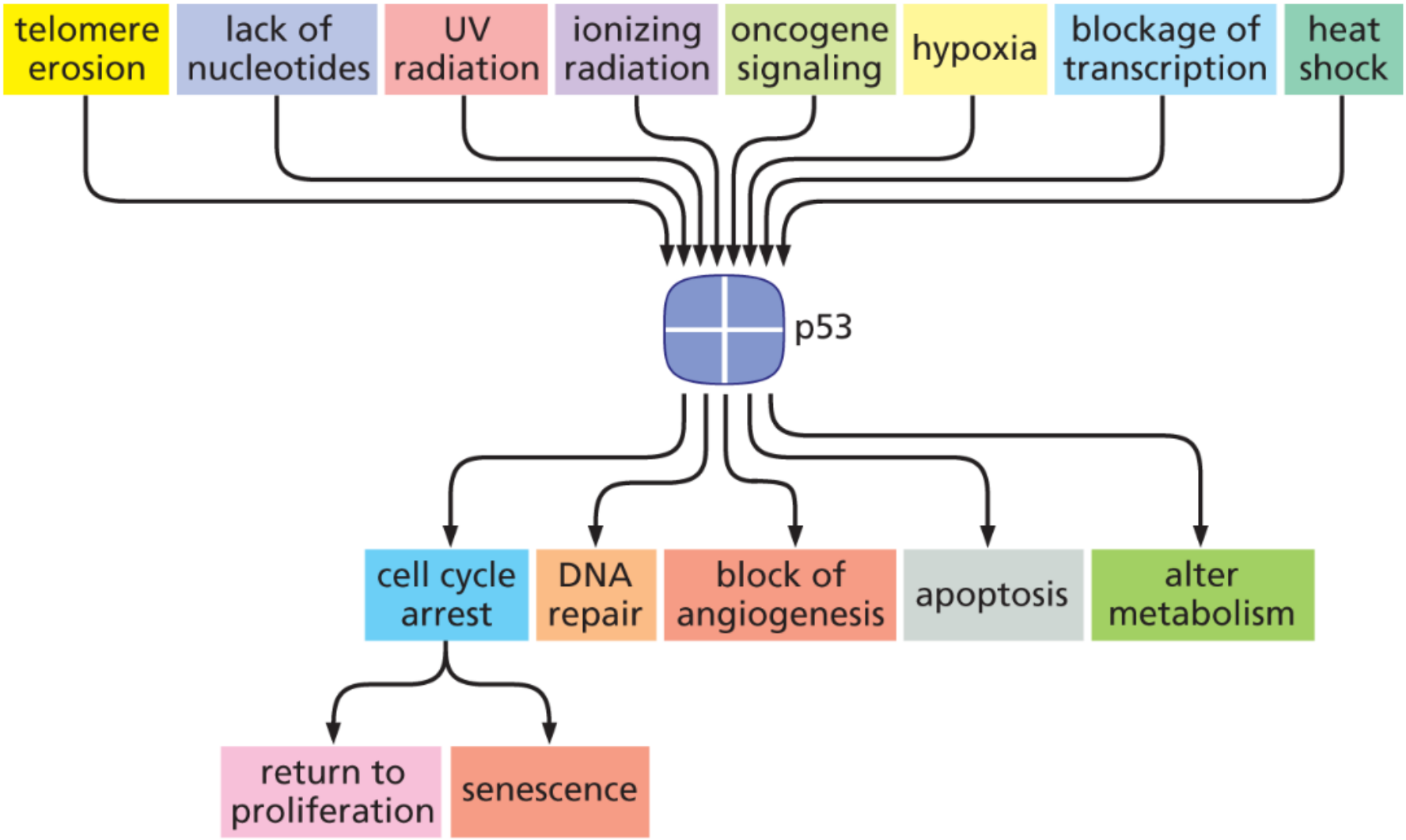
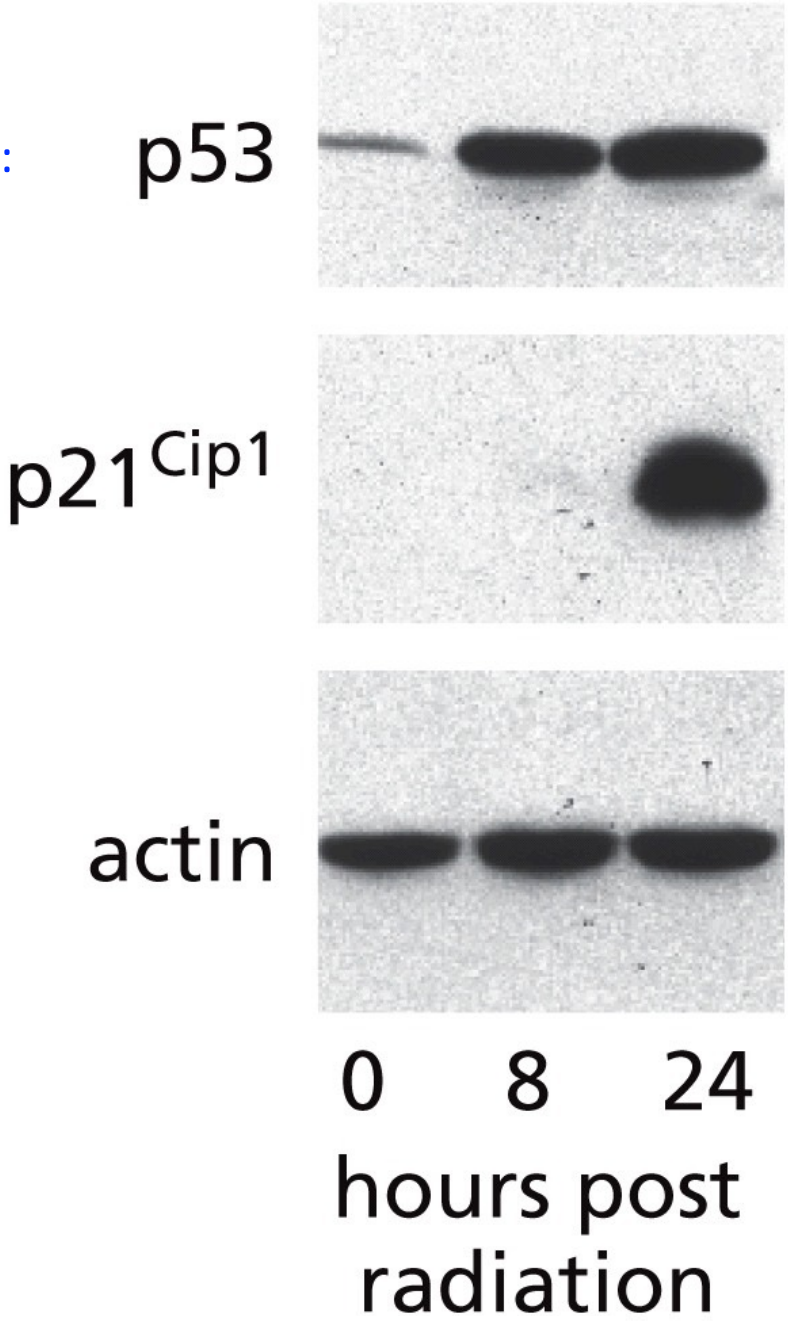


Figure 9.8. Weinberg, The Biology of Cancer

Example of p53 Induction: X-ray Exposure

Western Blots:



…CDK inhibition by p21 and cell cycle arrest

Figure 9.7. Weinberg, The Biology of Cancer

X-ray Irradiation: p53-Dependent Apoptosis of Thymocytes in Mice

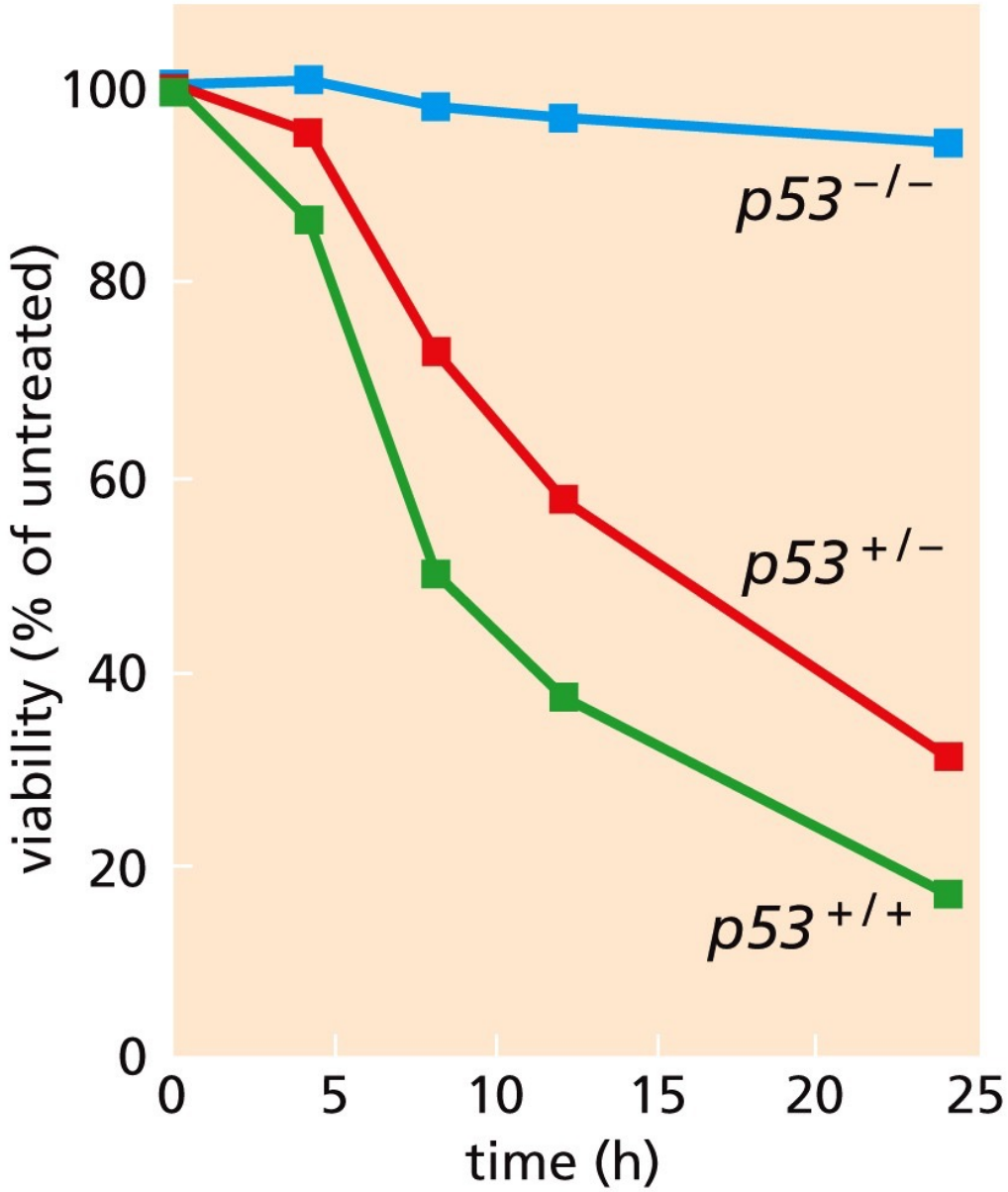


Figure 9.7. Weinberg, The Biology of Cancer

How is p53 Regulated? Control by Mdm2/HDM2 E3 Ubiquitin Protein Ligase

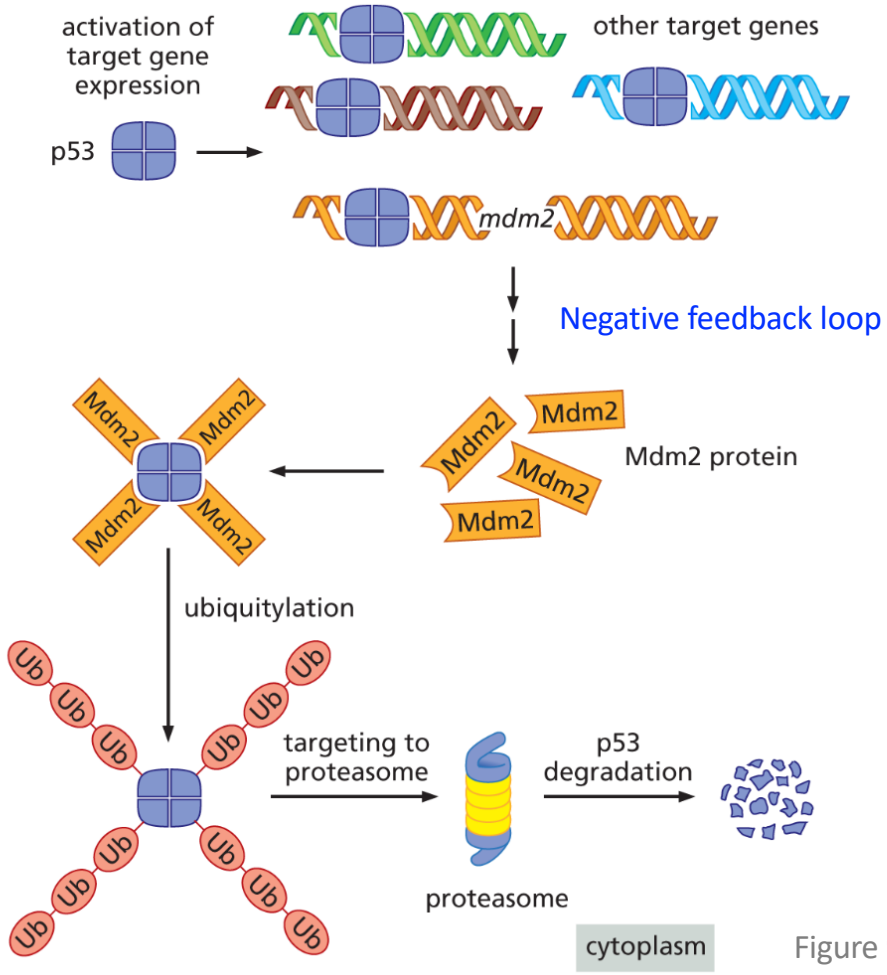


Figure 9.10. Weinberg, The Biology of Cancer

Half life (p53; unstressed cells): **9** min

Mdm2/HDM2 is frequently amplified in tumors.

How is p53 Regulated? Phosphorylation by ATM, ATR, CHK1, CHK2 at the N-terminus Prevents Mdm2 binding

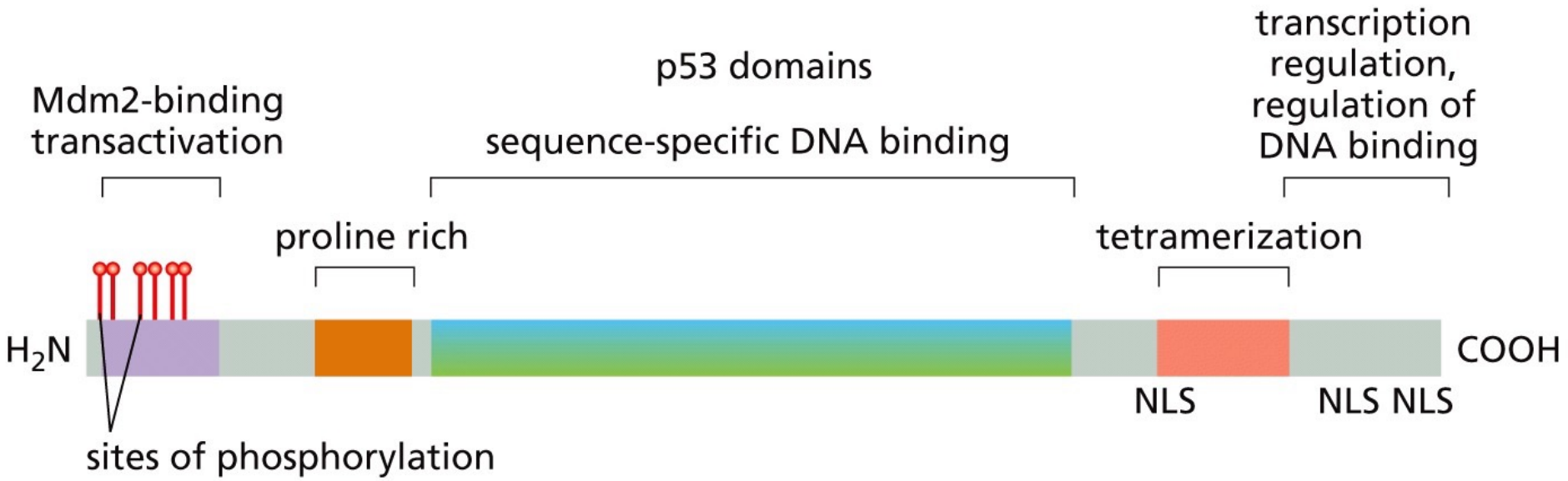


Figure 9.11. Weinberg, The Biology of Cancer

Accumulation of p53 (blackened nuclei) in Ovarian Carcinoma: Why???

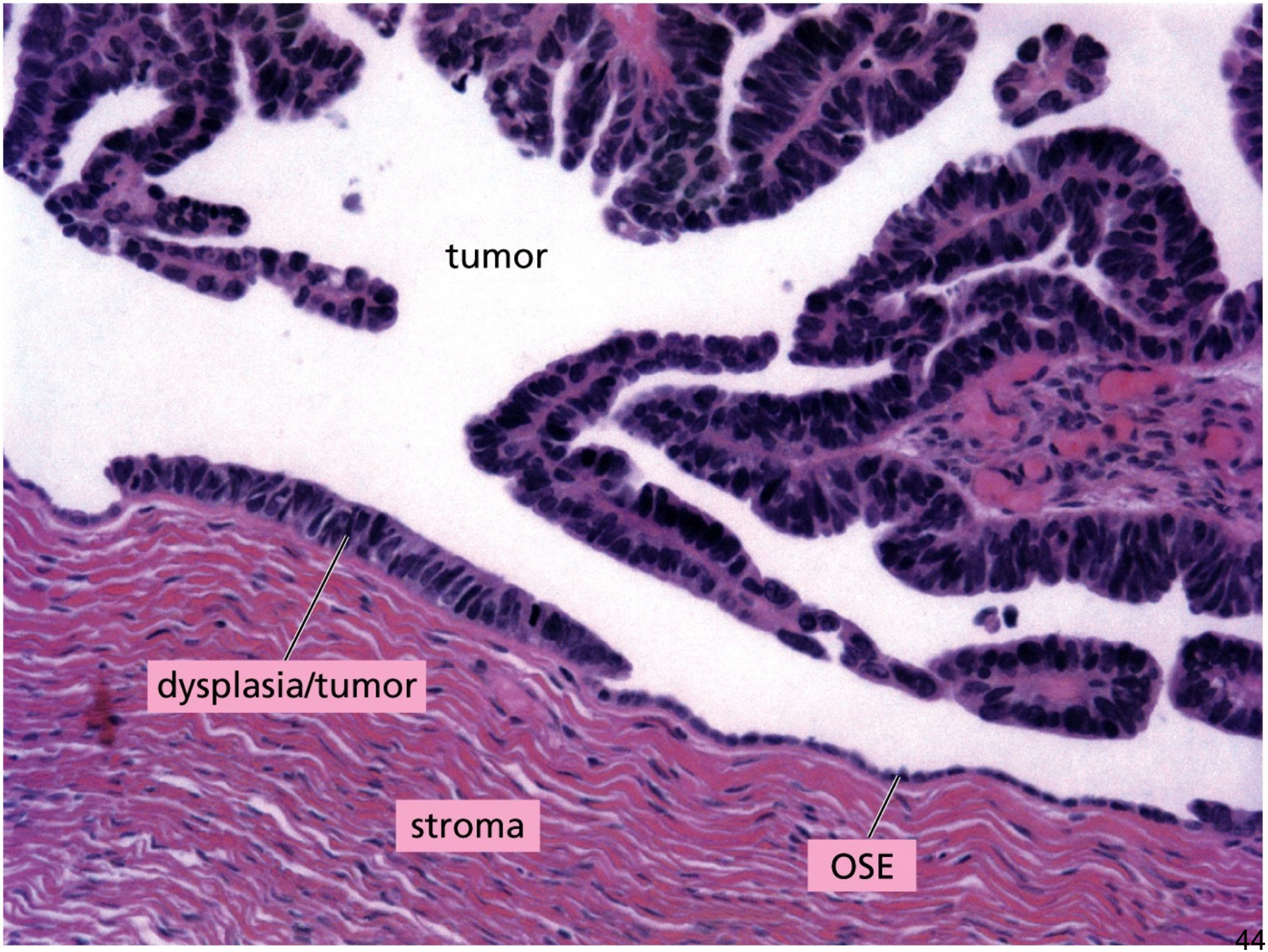


Figure 9.16. Weinberg, The Biology of Cancer

Unusual Gene Structure of the Mdm2 antagonist $p14^{ARF}$

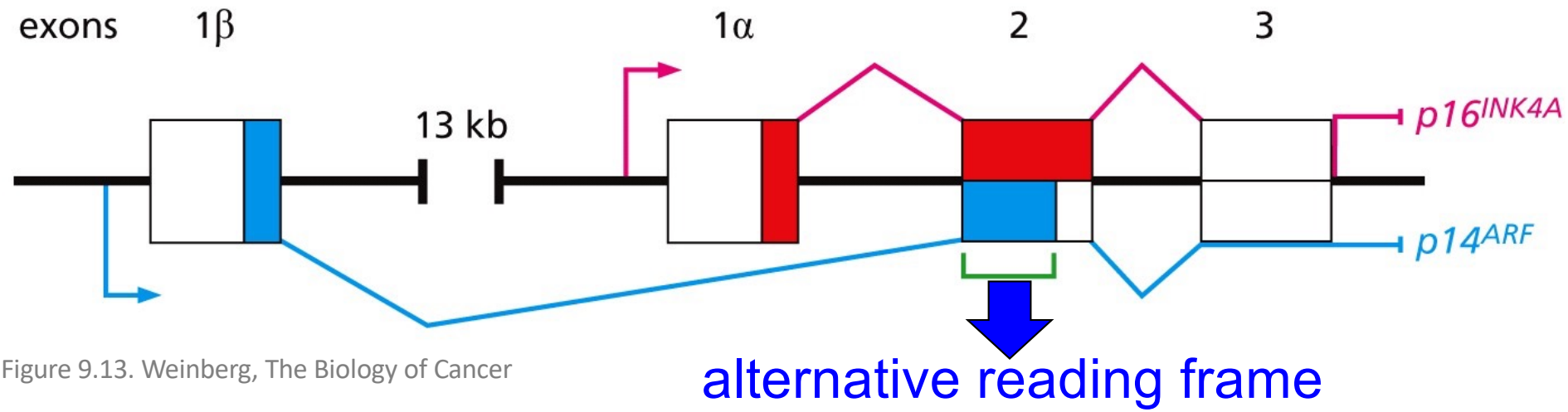
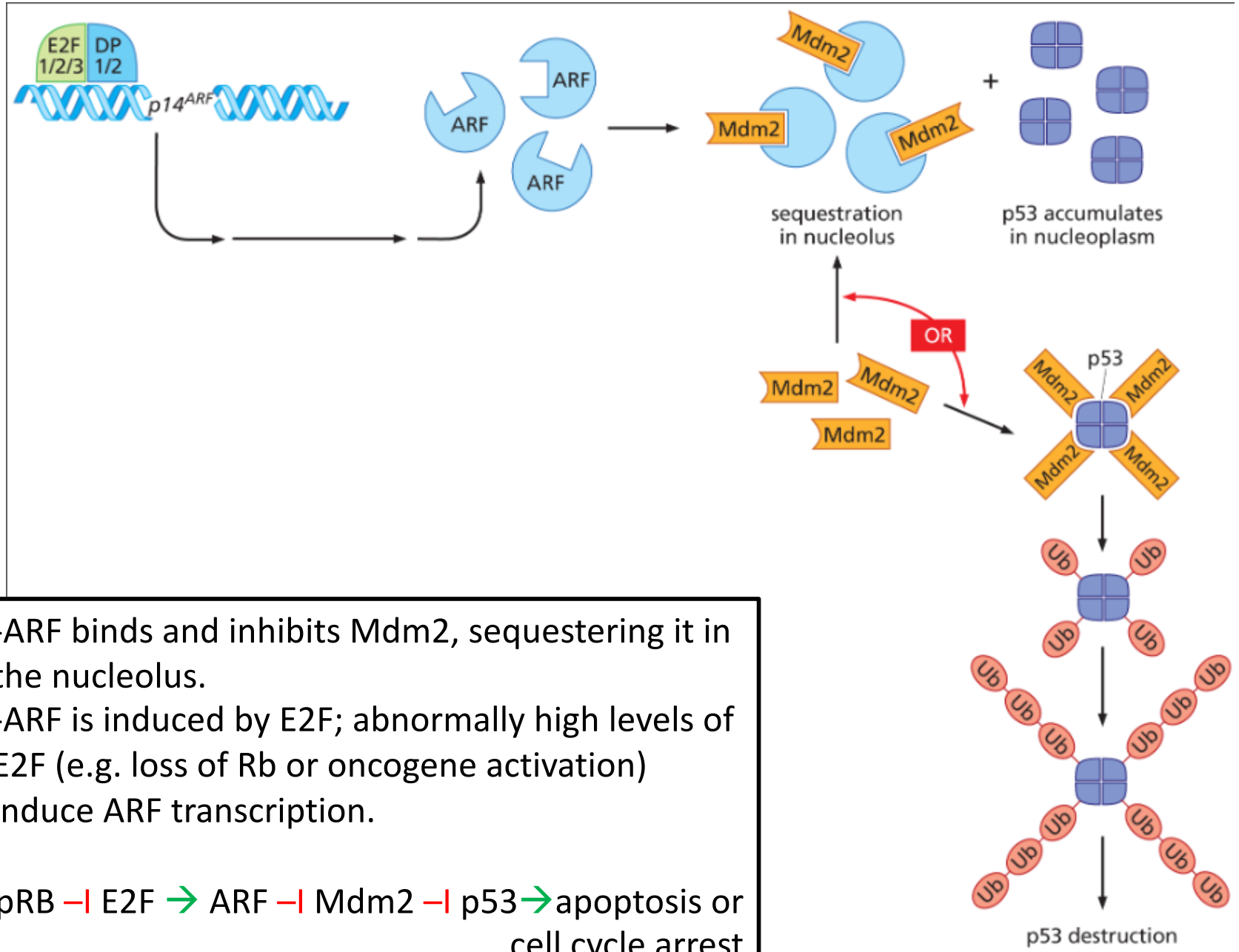


Figure 9.13. Weinberg, The Biology of Cancer

p16INK4A is an important inhibitor of CDK4 and CDK6 which phosphorylate Rb (week 6). $p14^{ARF}$ (p19 in mice) shares its 2nd exon with p16 but contains an alternative reading frame! The boxes indicate exons, while the filled areas indicate reading frames.

Control of Apoptosis by ARF

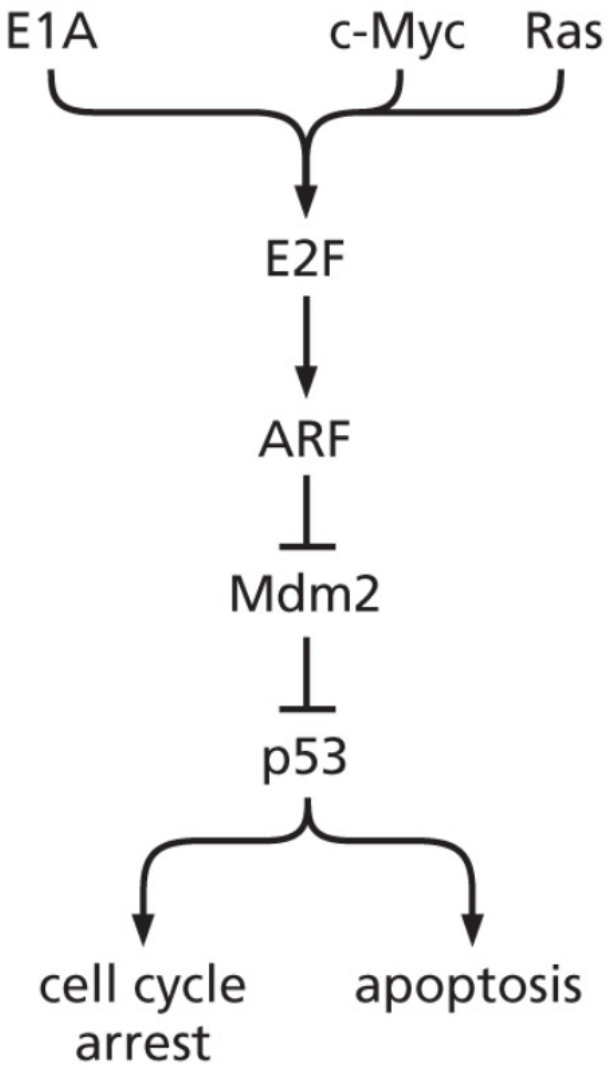


-ARF binds and inhibits Mdm2, sequestering it in the nucleolus.
-ARF is induced by E2F; abnormally high levels of E2F (e.g. loss of Rb or oncogene activation) induce ARF transcription.

pRB –| E2F → ARF –| Mdm2 –| p53 → apoptosis or cell cycle arrest

Figure 9.14. Weinberg, The Biology of Cancer

Control of Apoptosis by ARF



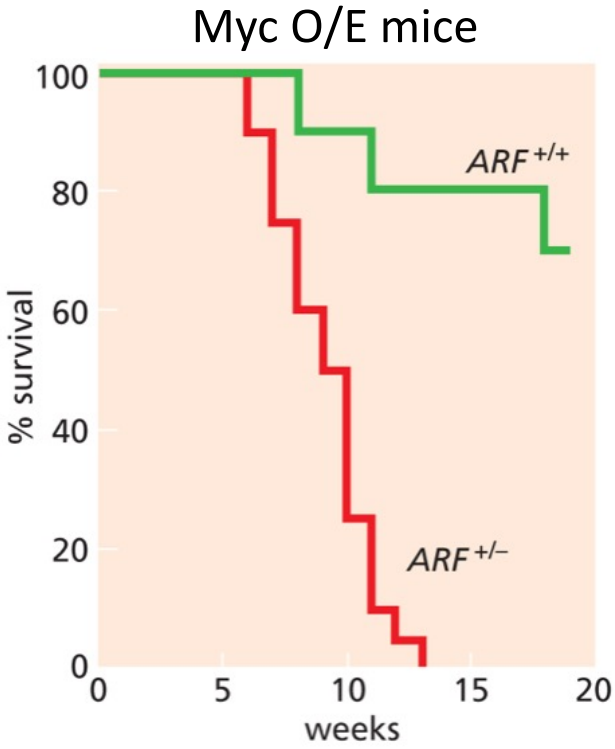


Figure 9.14. Weinberg, The Biology of Cancer

Signaling pathway eliminates cells with hyperactive E2F, which is induced by oncoproteins

p53 Activates Transcription of Multiple Genes: >100 Direct Targets

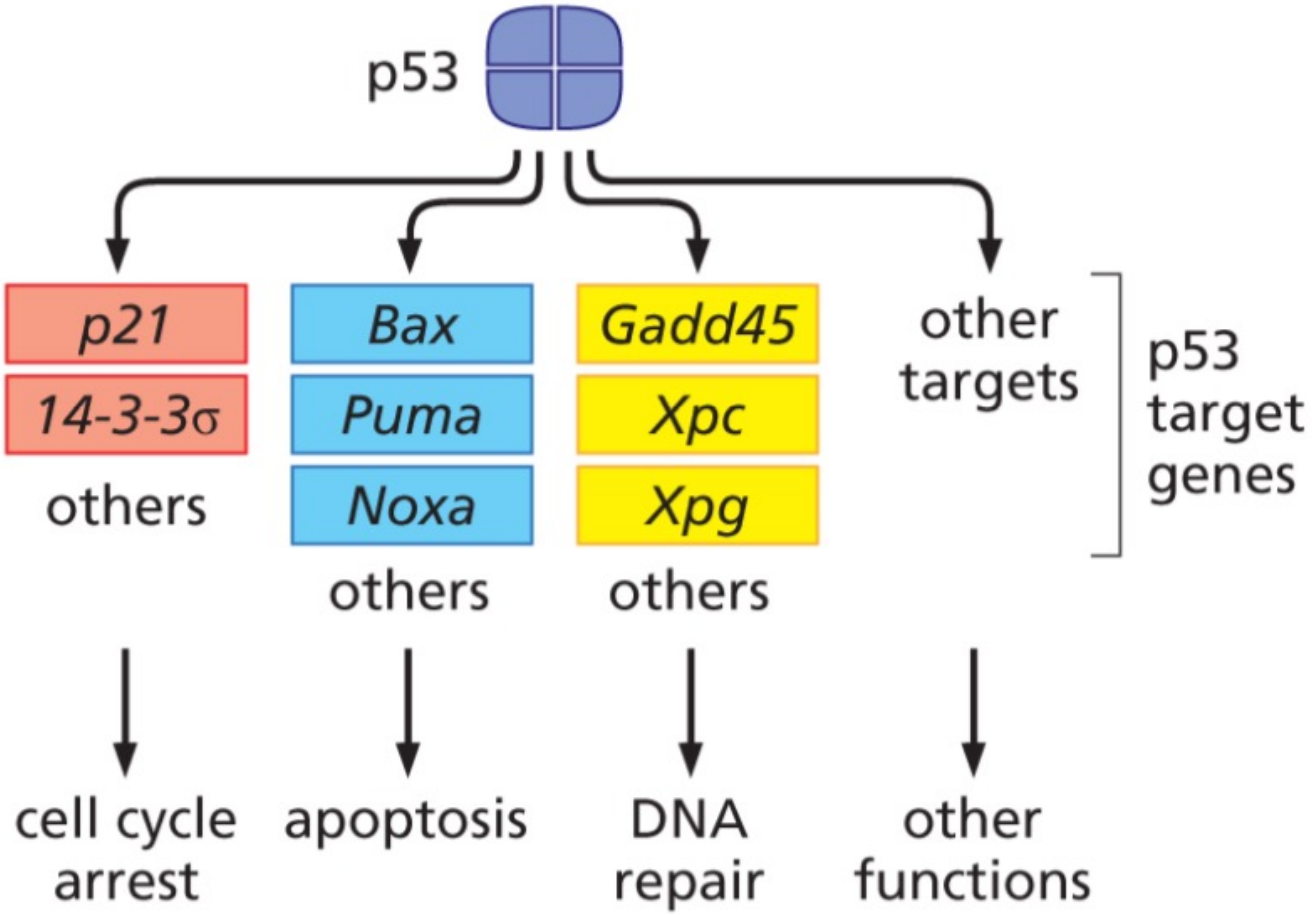
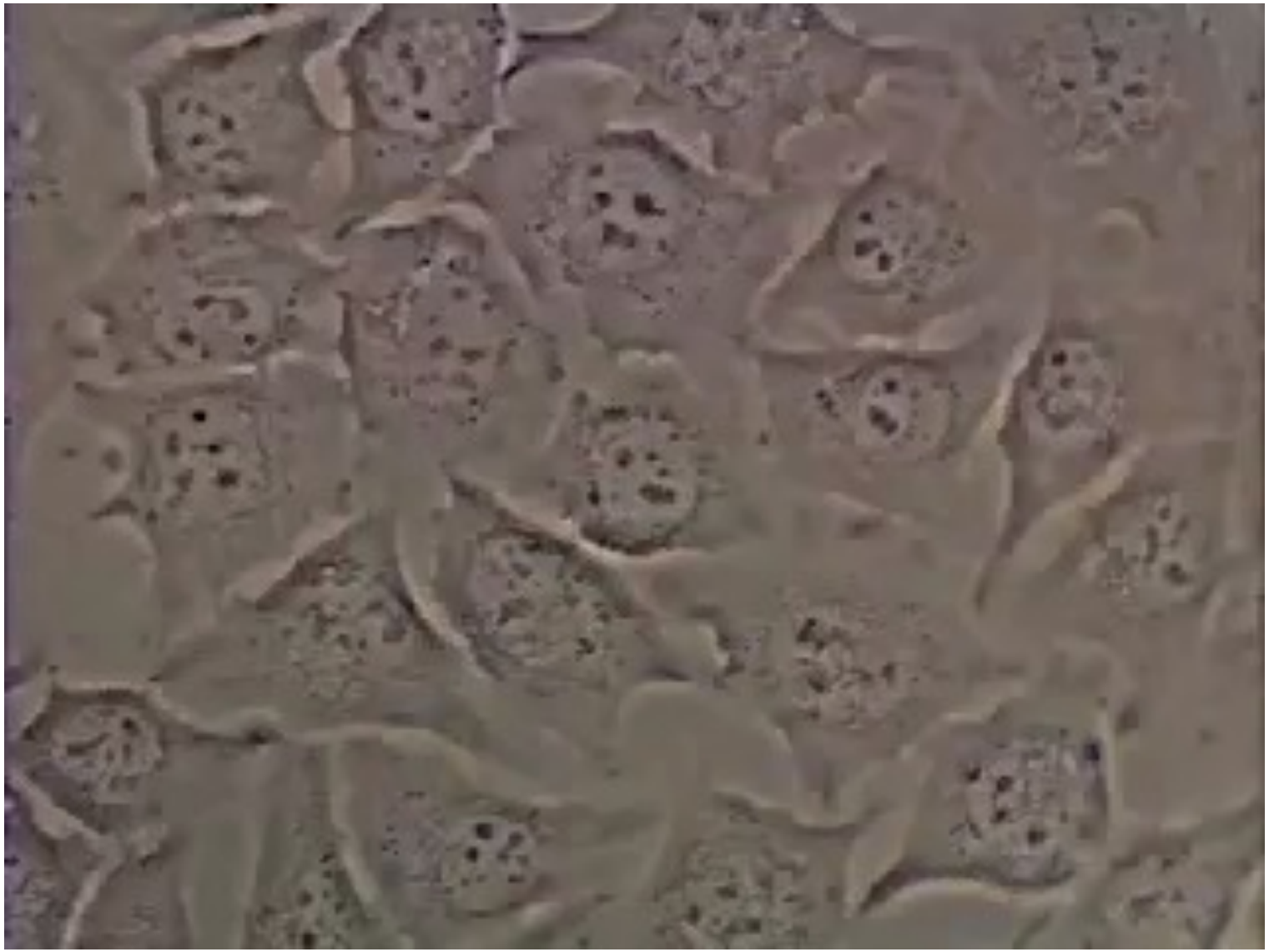


Figure 9.15. Weinberg, The Biology of Cancer

Movie: the Apoptotic Death Program

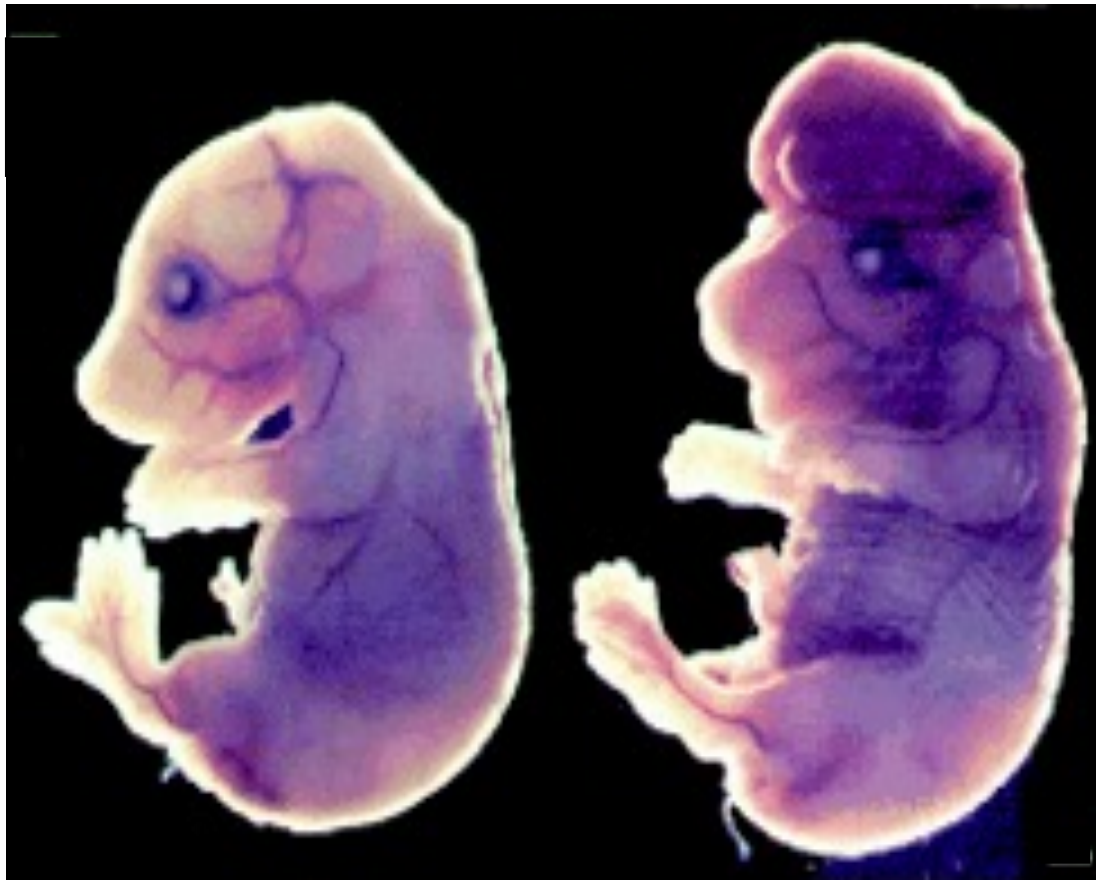


Programmed Cell Death in Normal Development

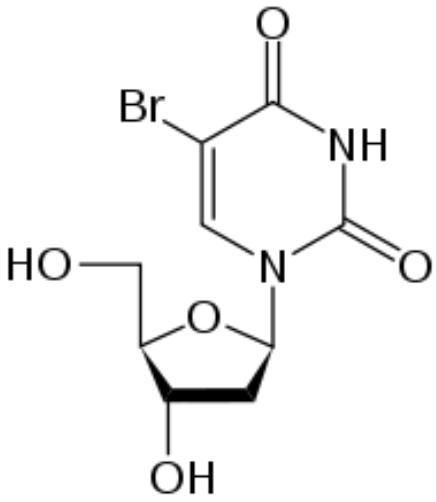
Example*: Caenorhabditis elegans*: In this organism 1090 somatic cells are generated in the formation of the adult worm; 131 of these cells undergo apoptosis.

Programmed Cell Death in Normal Development

Caspase-9 -/-



Apaf -/-



BrdU

Supplementary Sidebar 9.2 The TUNEL assay The TUNEL assay provides a highly specific test of whether a cell is in the apoptotic state. It is in frequent use because it is reproducible and relatively simple to execute experimentally (Figure S9.2).

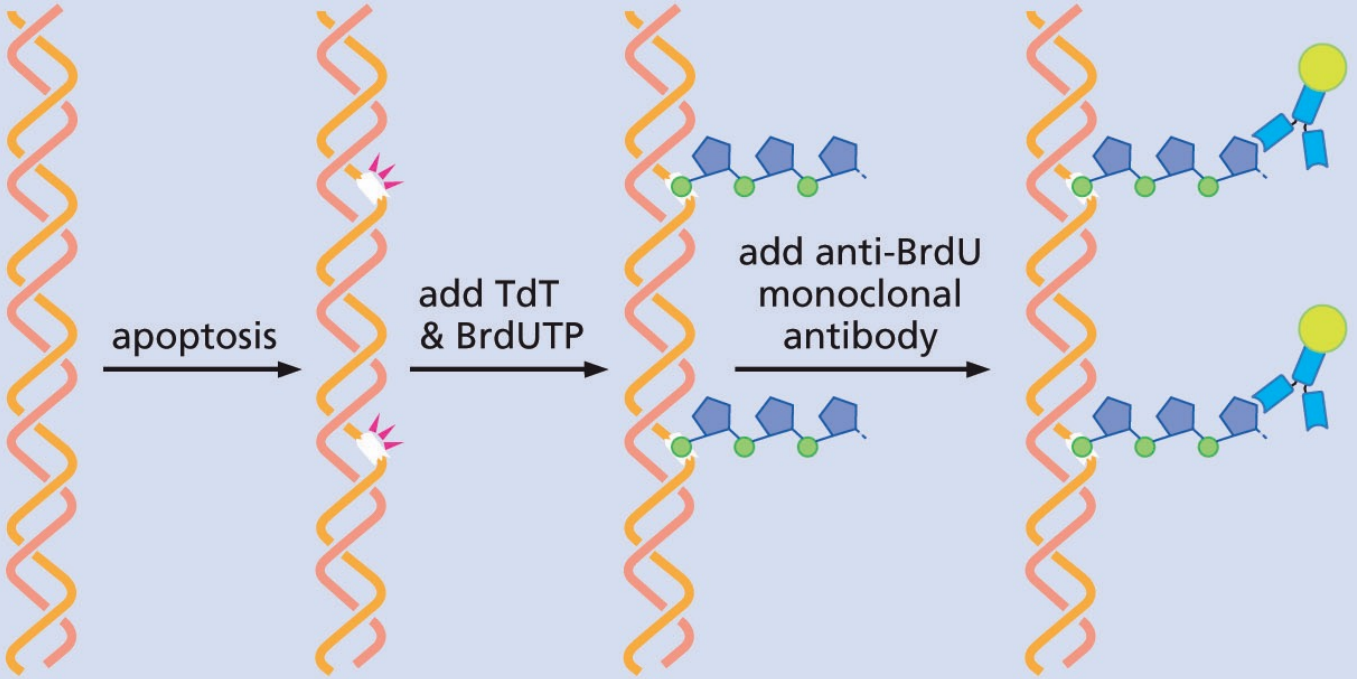


Figure S9.2 The TUNEL procedure Apoptotic cells can be detected because their chromosomal DNA has become fragmented (see Figure 9.18C), exposing 3′-OH DNA ends. The latter can be extended by the terminal deoxyribonucleotide transferase (TdT) enzyme, which acts processively to generate long tails from these ends, in this case doing so using bromodeoxyuridine triphosphate (BrdUTP) as substrate. The resulting BrdU-incorporated oligonucleotide tails can be detected with an anti-BrdU monoclonal antibody that has been coupled to a dye molecule (*yellow green*).

Programmed Cell Death in Normal Development: TUNEL Staining of Mouse Paw

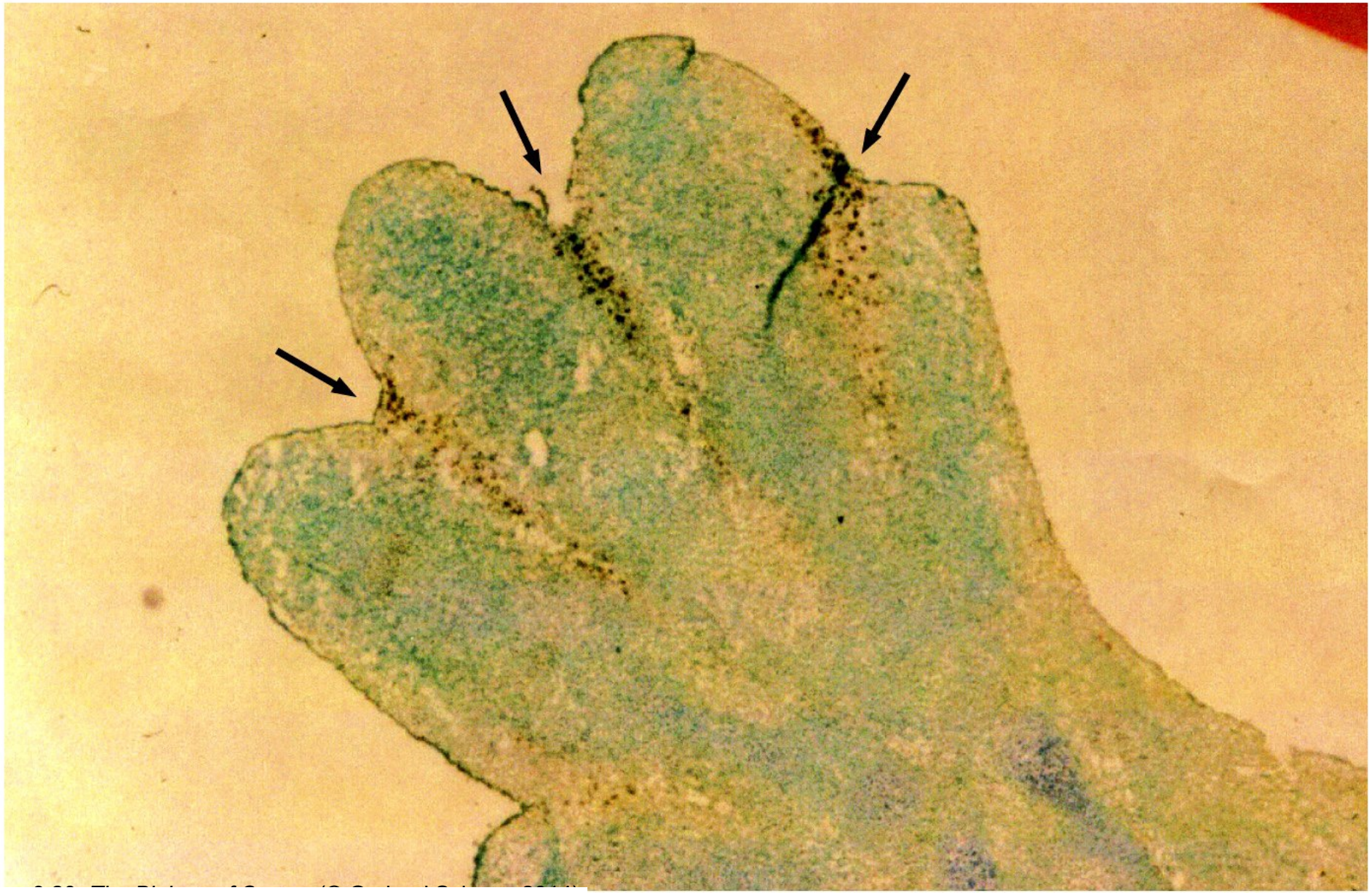
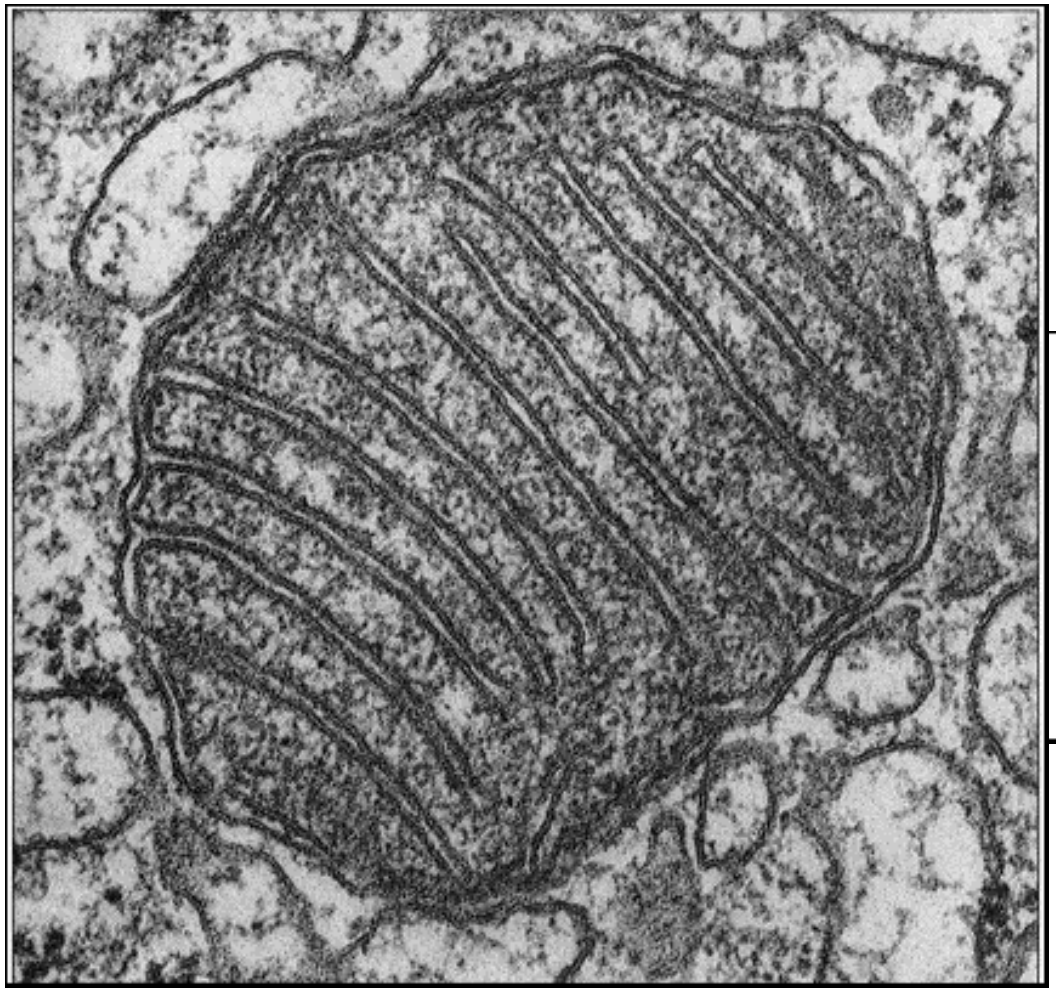


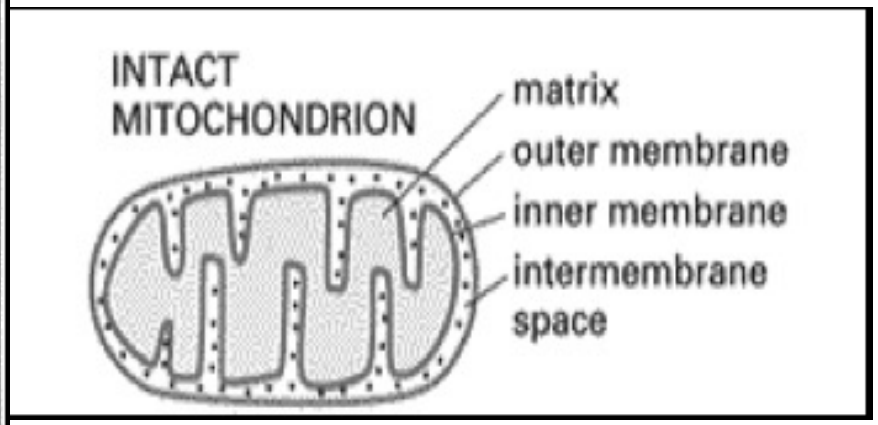
Figure 9.18. Weinberg, The Biology of Cancer

How do mitochondria control apoptosis?

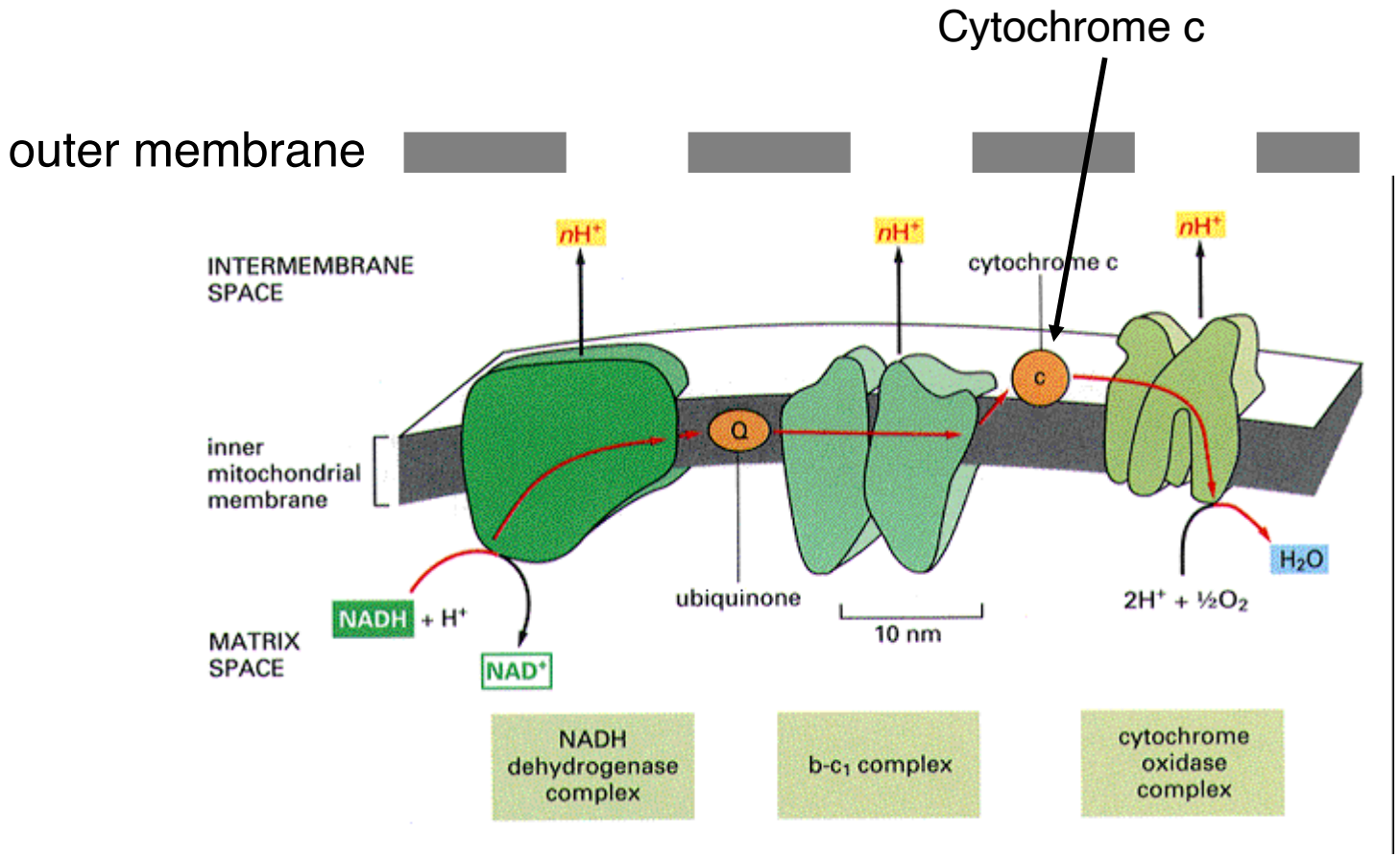
Mitochondria



The mitochondria contains an outer and inner membrane that create two internal compartments



Mitochondria, Cytochrome C



Cytochrome c functions to transfer electrons as part of oxidative phosphorylation

Release of Cytochrome C into the Cytosol

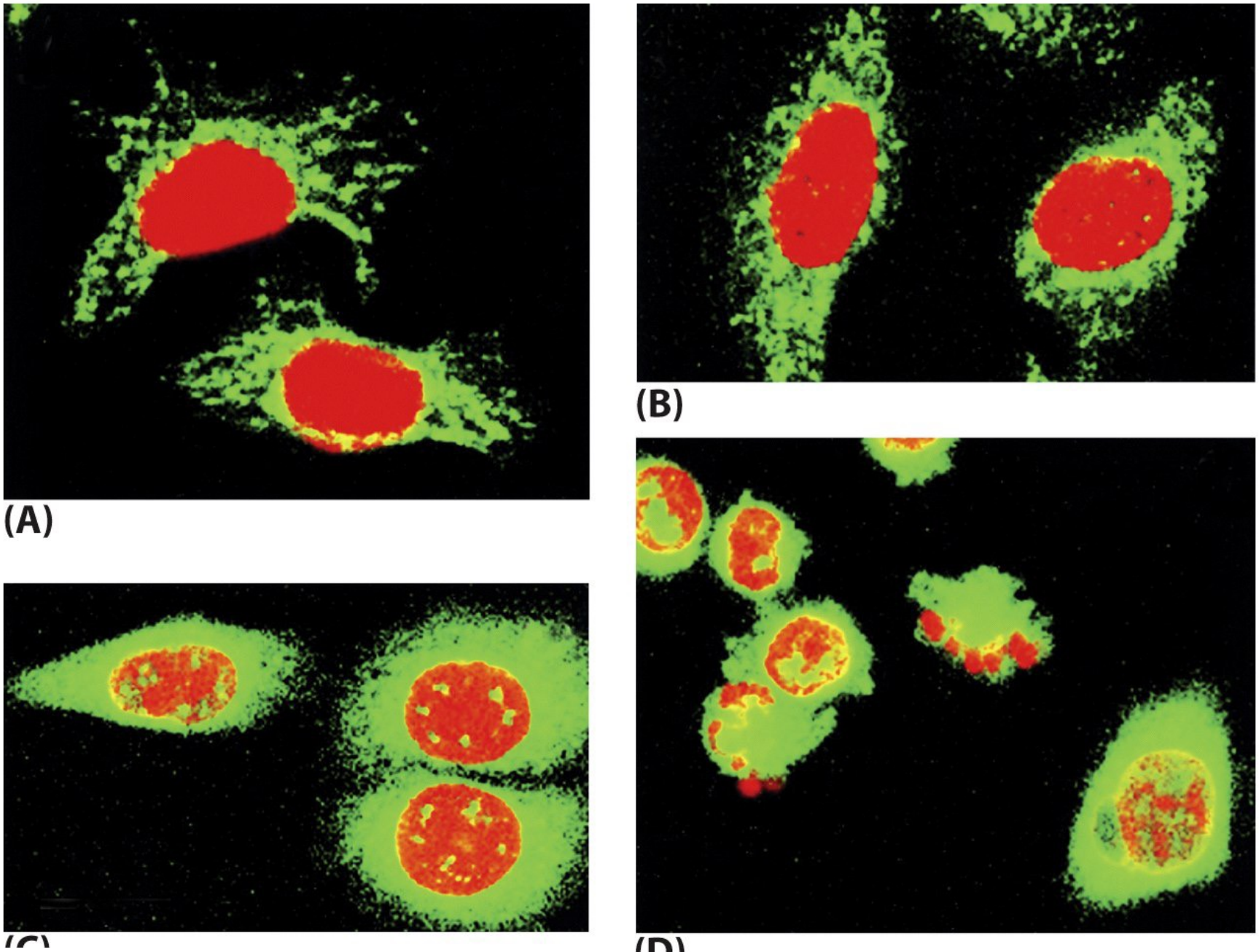
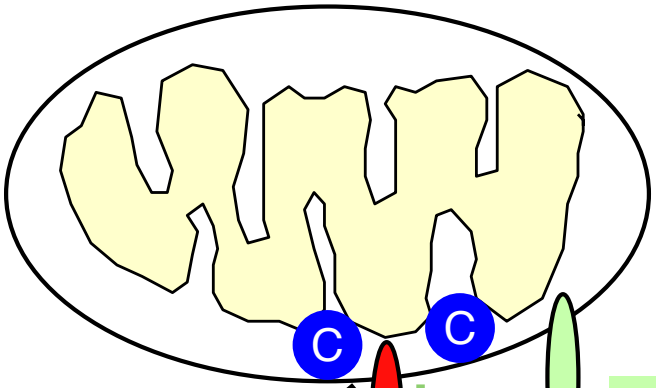


Figure 9.22. Weinberg, The Biology of Cancer

Cytochrome C Release



Cytochrome c released
= something is wrong!!!

Bcl-2 anti-apoptotic

Bcl-2 related pro-apoptotic

C

Apaf-1 =**Apoptotic protease activating factor 1;**
cytoplasmic cytochrome c receptor

Caspase 9

Apoptosis

- anti-apototic bcl-2 members inhibit cytochrome c release
- pro-apoptotic bcl-2 members induce cytochrome c release

The Bcl-2 Family: Three Classes

BH: Bcl-2 Homology Domain (protein-protein interaction)

Human genome: 24 Bcl-2-related proteins: 6 anti-apoptotic and 18 pro-apoptotic proteins.

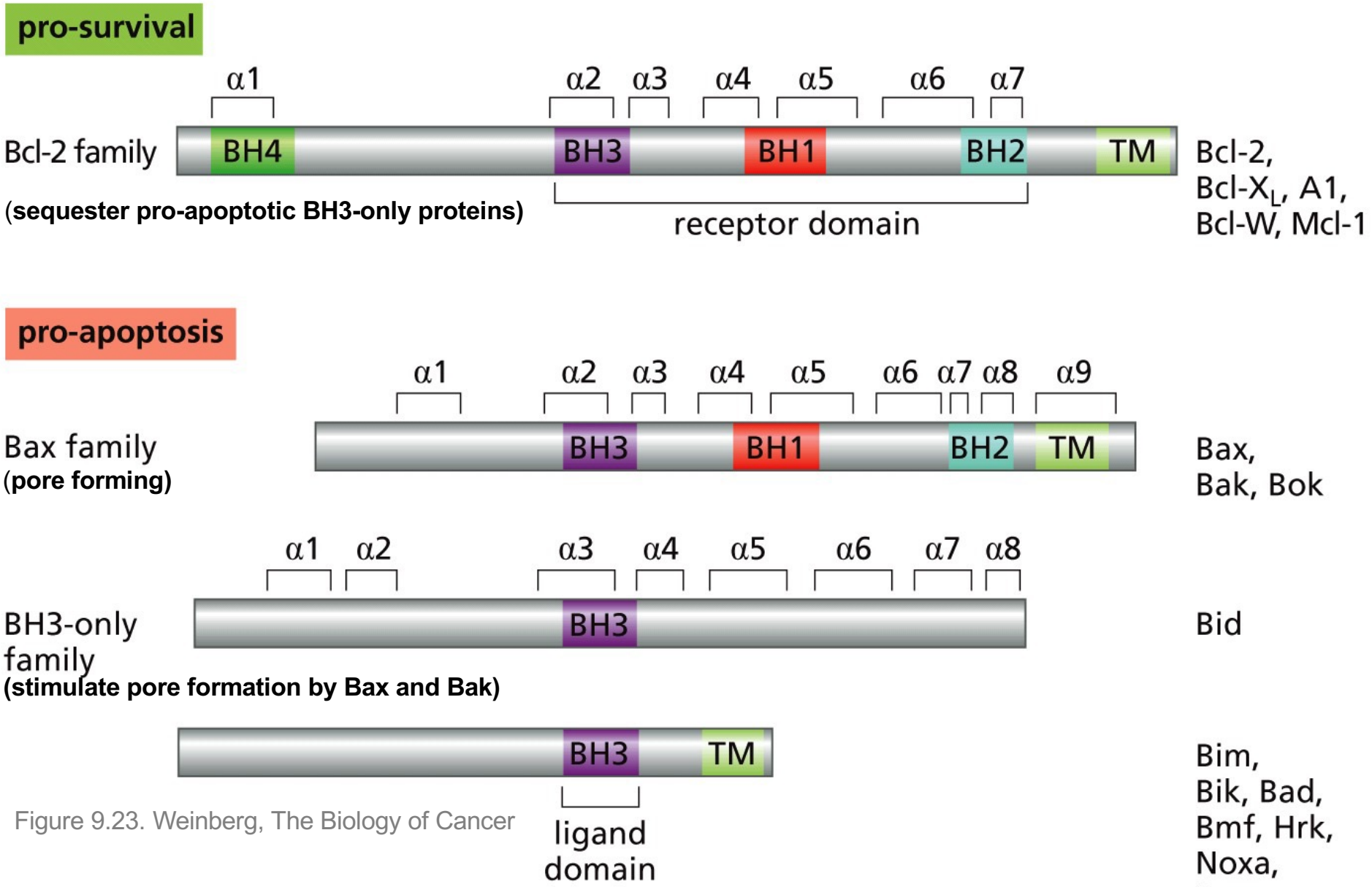
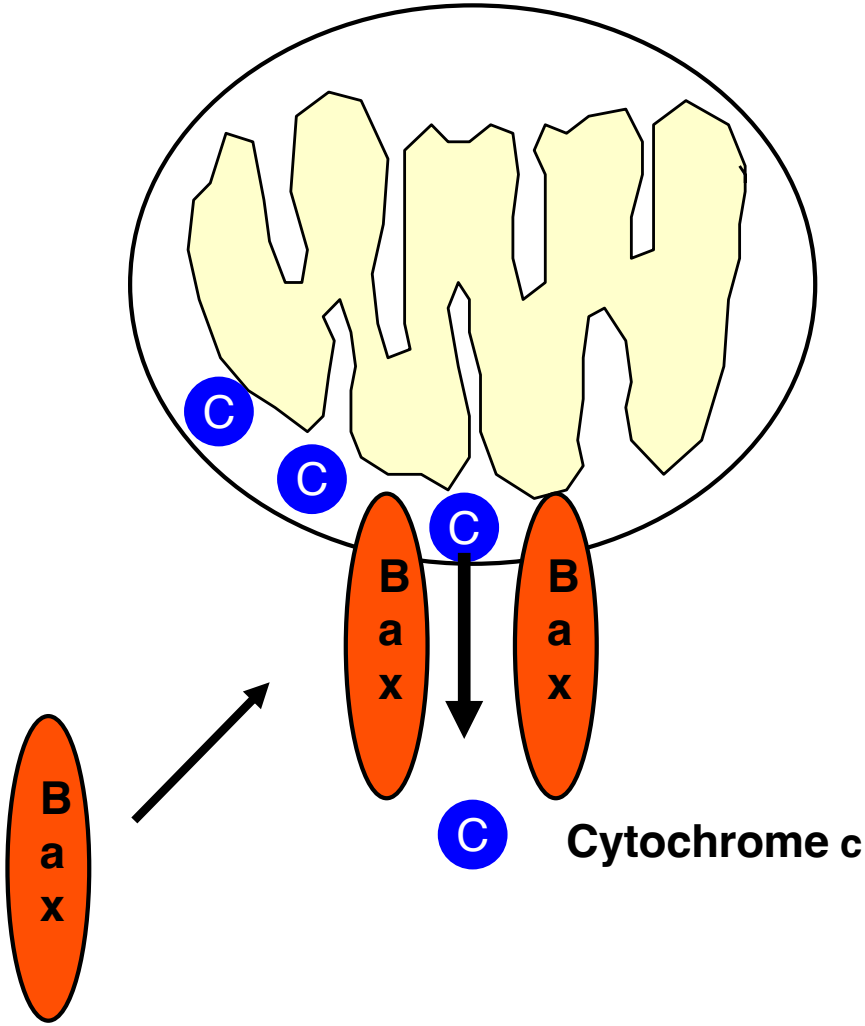


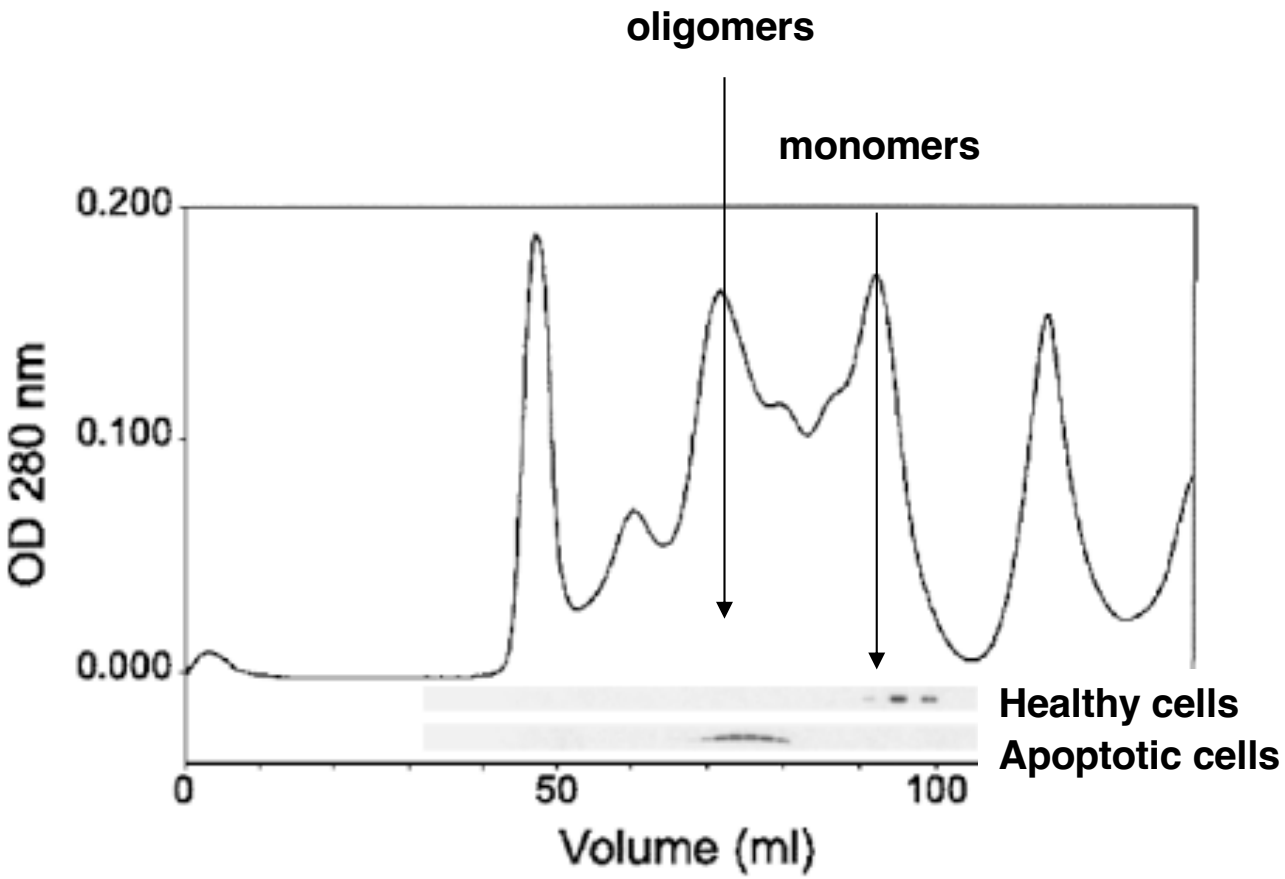
Figure 9.23. Weinberg, The Biology of Cancer

BCL2-family proteins: control of mitochondrial outer membrane pores

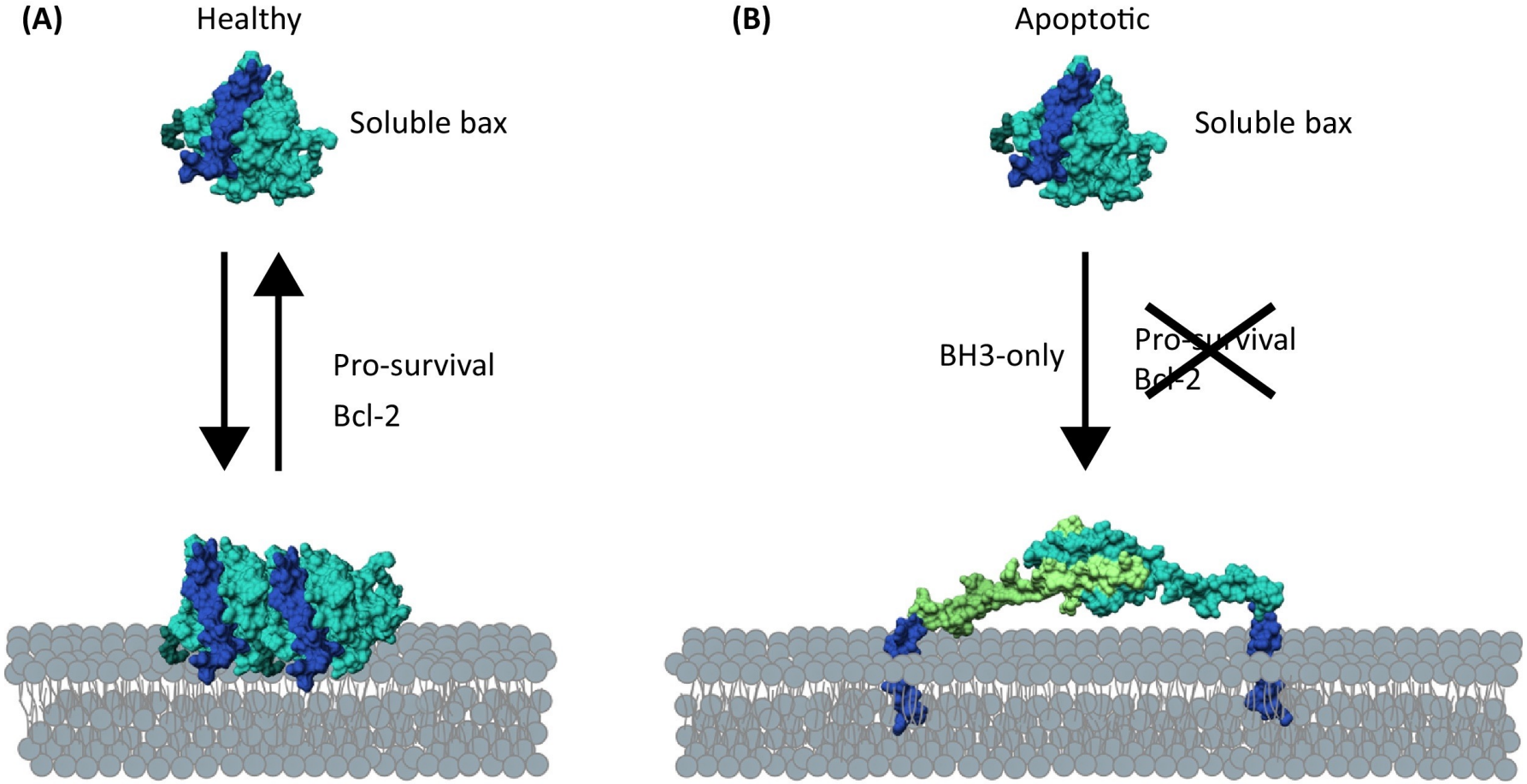
Channel Model



Bax Oligomerizes During Apoptosis



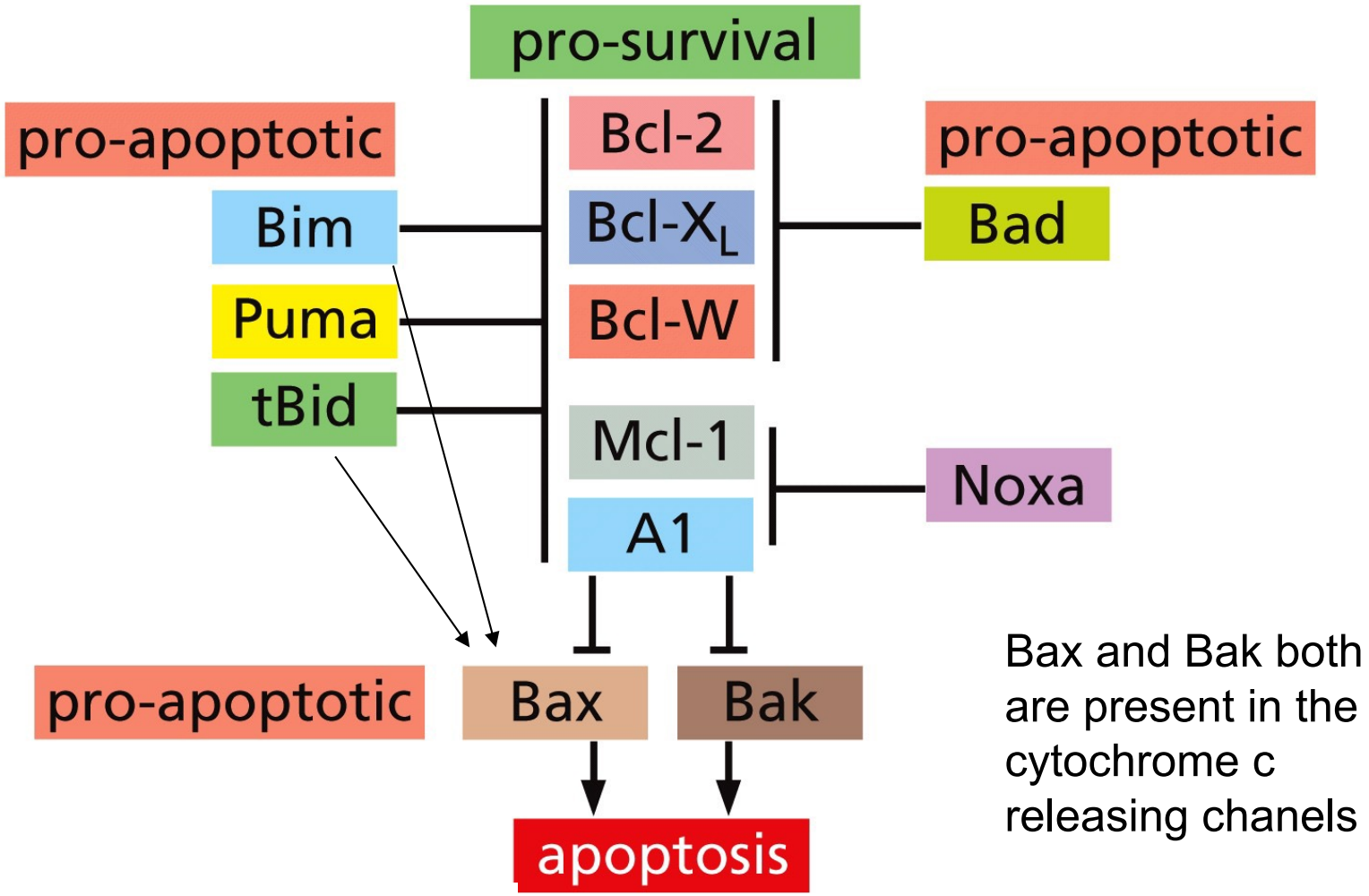
Bax Assembly and Mitochondrial Permeabilization



Trends in Cell Biology

Regulation of Bax Activity by Other Bcl-2 Proteins. (A) Under healthy conditions Bax (cyan) associates with the mitochondrial outer membrane (MOM) (gray) but is continuously retrotranslocated to the cytosol by prosurvival Bcl-2 proteins. (B) During apoptosis **Bcl-2 homology (BH)3-only proteins** break this steady state by **inhibiting the action of prosurvival Bcl-2 proteins** and **activating Bax**. Insertion of α9 (blue) in the membrane and further conformational changes allow Bax oligomerization and induce mitochondrial outer membrane permeabilization.

From: TIC 27, 266 (2017)



Bax and Bak both are present in the cytochrome c releasing chanels

Figure 9.25. Weinberg, The Biology of Cancer

The affinities and relative abundance of the BCL-2 family proteins dictate the interactions between anti- and pro-apoptotic BLC-2 proteins that regulate mitochondrial outer membrance permeabilization.

(This complex and poorly understood network is reviewed in:
Cell Death and Differentiation 25, 65-80 (2018))

Various Stresses Can Activate the Intrinsic Apoptotic Program

Death Receptors: → extrinsic apoptosis or receptor-activated apoptotic pathway

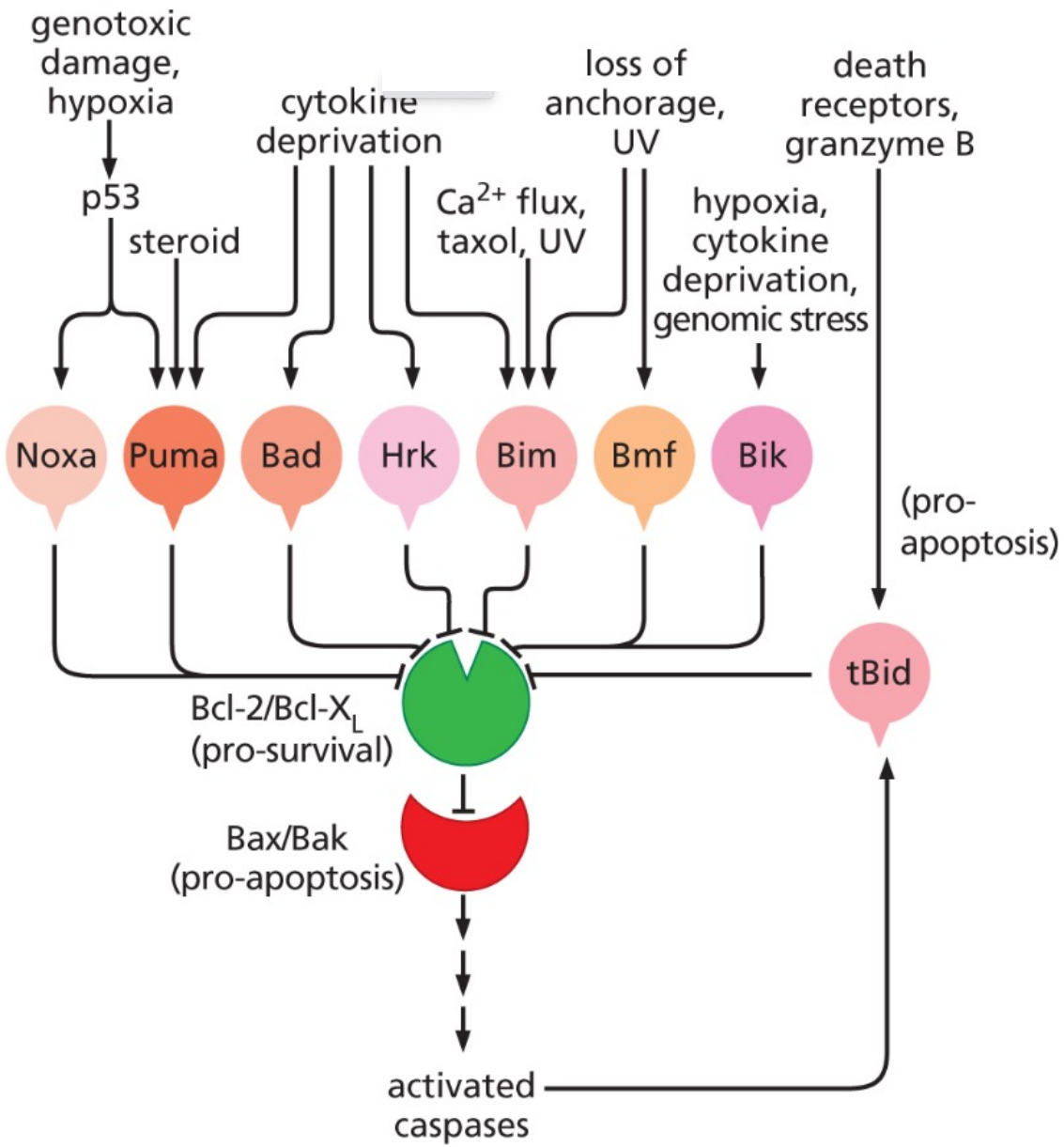
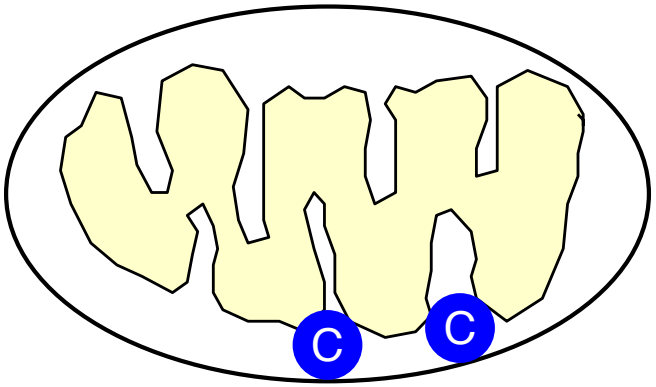


Figure 9.25. Weinberg, The Biology of Cancer

Cytochrome c



Cytochrome c released
= something is wrong!!!

C **Apaf-1** =**Apoptotic protease activating factor 1;**
cytoplasmic cytochrome c receptor

Caspase 9

Apoptosis

The Apoptosome-Wheel of Death

The apoptosome = heptameric Apaf-1/dATP/cytochrome c complex

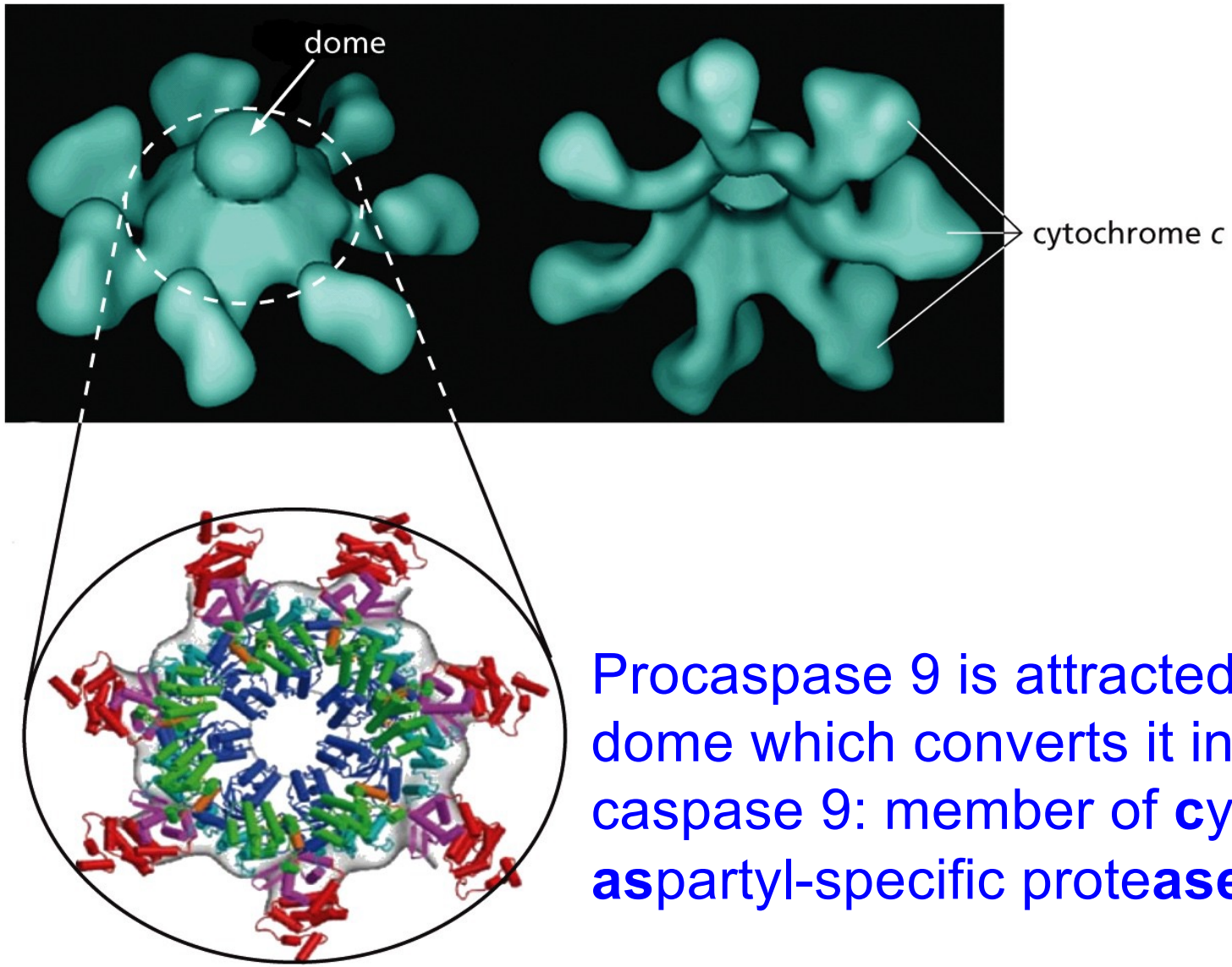


Figure 9.26. Weinberg, The Biology of Cancer

Procaspase 9 is attracted to the dome which converts it into active caspase 9: member of **c**ysteine **as**partyl-specific prote**ases.**

The Apoptotic Caspase Cascade

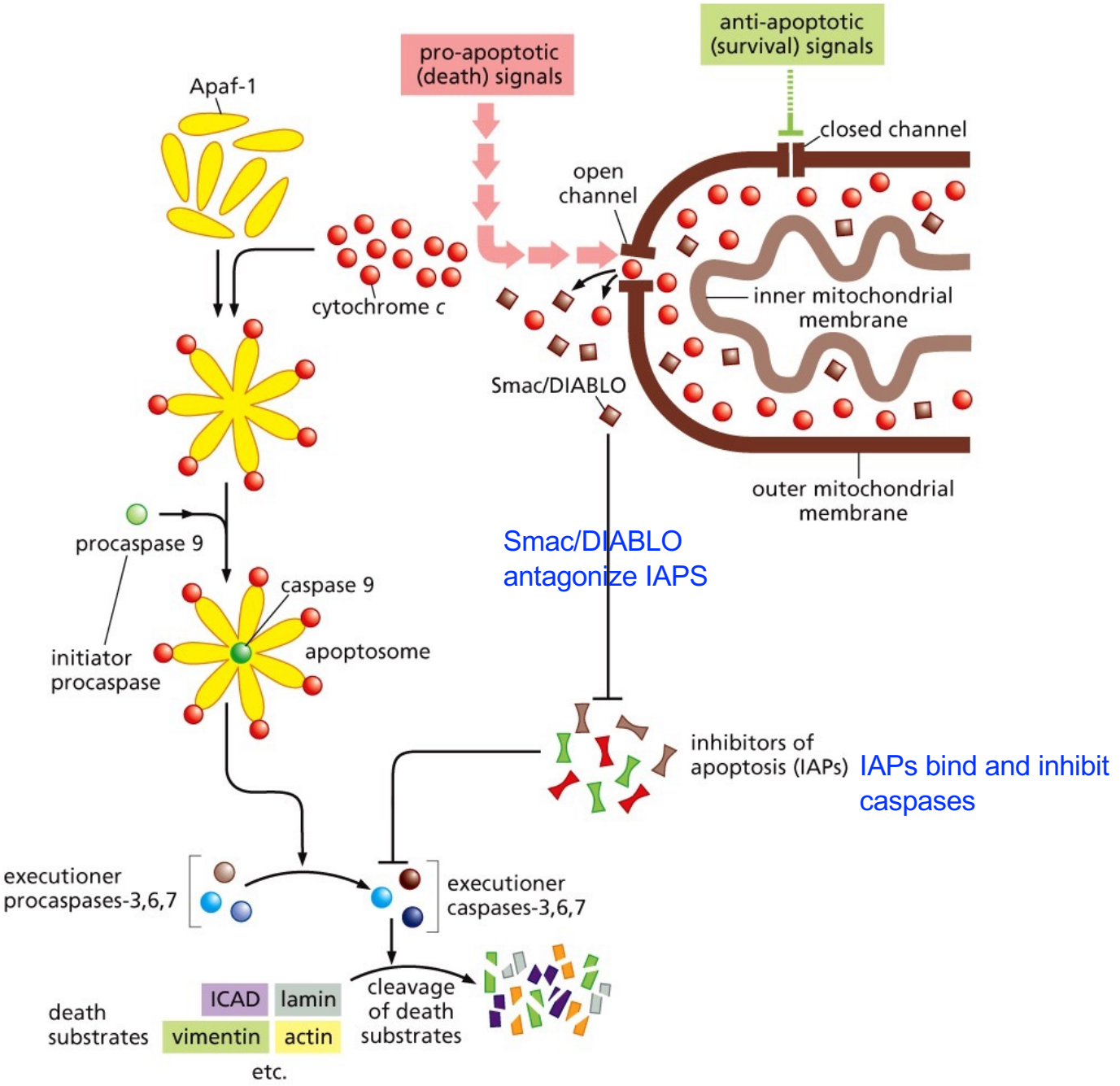


Figure 9.27. Weinberg, The Biology of Cancer

Substrates of Caspases

(**c**ysteine-dependent **asp**artate-directed prote**ases**)

SiteO	Cleaved protein	Proposed role	Predicted consequence of cleavage
DEVD	PARP	DNA repair (stress)	Disable DNA repair
DEVD	DNAPKcs	DNA repair (ds)	Disable DNA repair
DGPD	UI-70K snRNP	pre-mRNA splicing	Reduce productive transcripts
DxxD	hnRNP-C	pre-mRNA splicing	Reduce productive transcripts
DEPD	SREBP	Sterol biosynthesis	Elevate sterols (engulfment)
DELD	D4-GDI	Sustain Rho-GTPase	Cytoskeletal disassembly
→ DETD	ICAD I (+)	DNA fragmentation	Dismantle genome
DAVD	ICAD site 11		
DEVD	DNA-RC C140	DNA replication	Halt DNA replication
DMQD	PKC 8 (+)	Cell-cycle progression	G2 M arrest
DSID	Rb	Cell cycle progression	loss of GI arrest
DVPD	HDM2/MDM2	p53 modulation	p53 stabilization
DQTD	FAK	Regulate cell adhesion	Cell detachment/migration
DSLD	NUMA	Nuclear structure integrity	Nuclear disassembly
xxxD	Pro-caspase (+)	Protease activation	
DMQD	Fodrin	Cortical cytoskeleton	Disassembly
SRVD	Gas2	Cytoskeletal microfilaments	Disassembly
ELPD	gActin	Cytoskeletal compound	Disassembly
VEID	Larnins (caspase-6)	Nuclear envelope mesh	Disassembly

→ caspase-activated deoxyribonuclease (CAD) is inhibited by (ICAD)

DxxD ↓ **caspase-3**

>150 proteins cleaved

Apoptosis induced by pro-apoptotic LATS2 protein in lanes 2 and 4

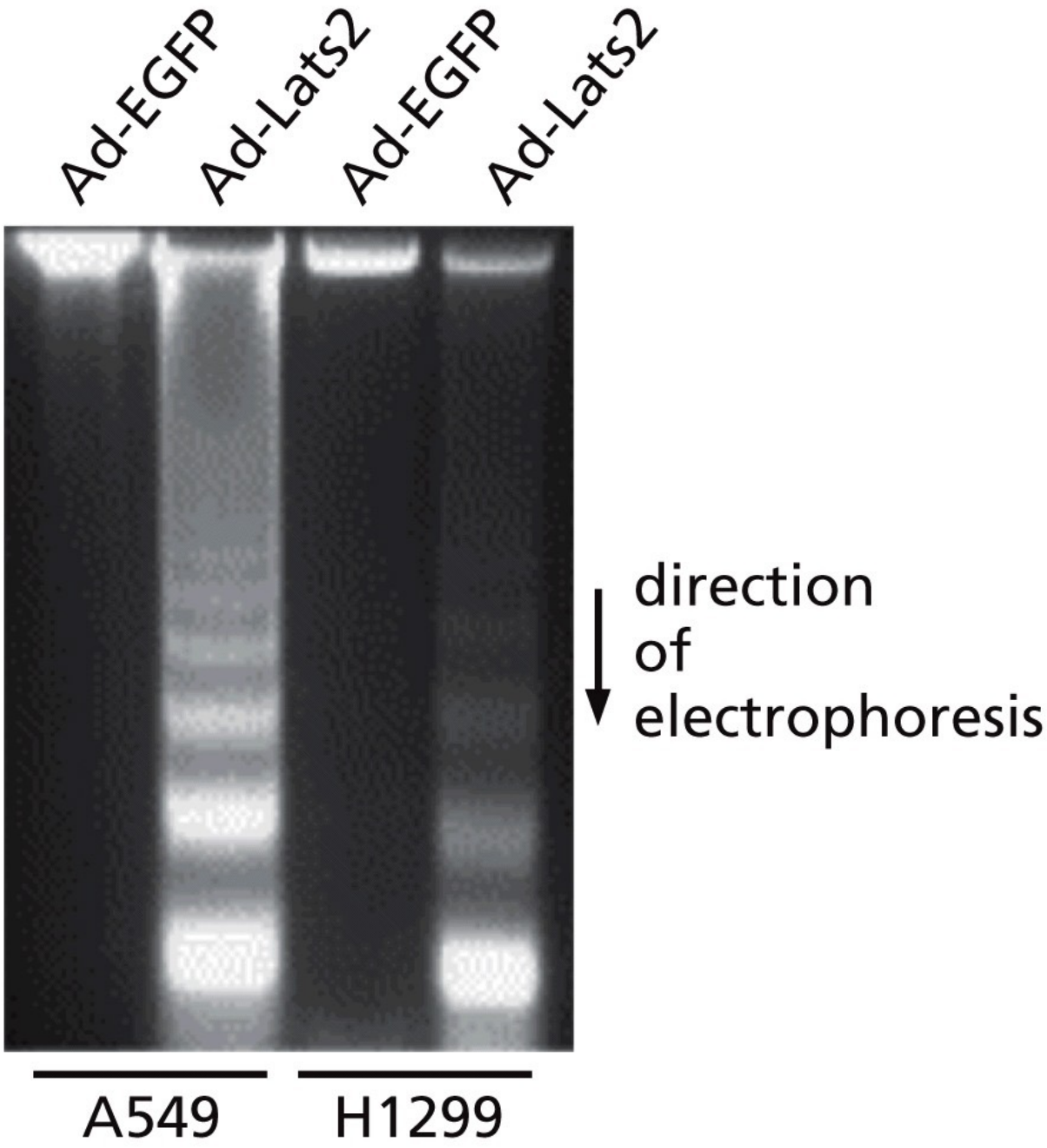


Figure 9.19. Weinberg, The Biology of Cancer

Examples of anti-apoptotic alterations found in human tumor cells

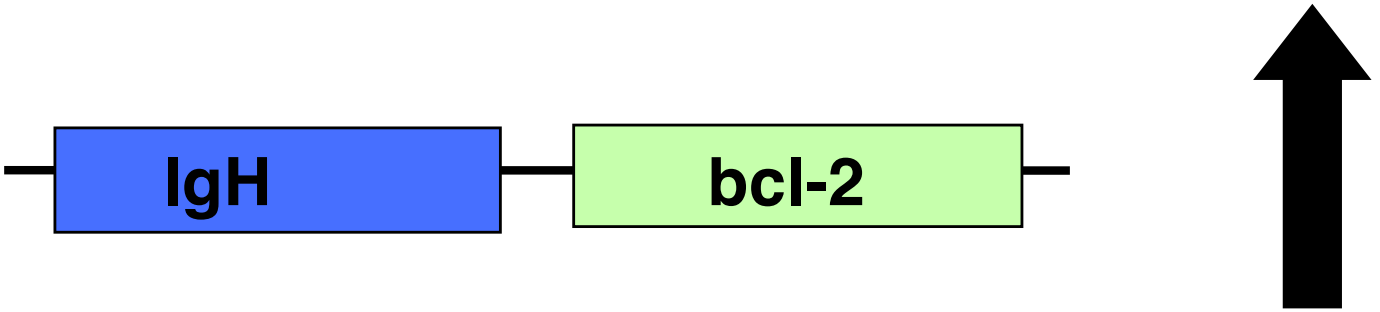
Alteration	Mechanism of anti-apoptotic action	Types of tumors
CASP8 promoter methylation	inactivation of extrinsic cascade	SCLC, pediatric tumors
CASP3 repression	inactivation of executioner caspase	breast carcinomas
Survivin overexpression[a] (IAP)	caspase inhibitor	mesotheliomas, many carcinomas
ERK activation	repression of caspase 8 expression	many types
ERK activation	protection of Bcl-2 from degradation	many types
Raf activation	sequestration of Bad by 14-3-3 proteins	many types
PI3K mutation/activation	activation of Akt/PKB	gastrointestinal
NF-κB constitutive activation[b]	induction of anti-apoptotic genes	many types
p53 mutation	loss of ability to induce pro-apoptotic genes	many types
$p14^{ARF}$ gene inactivation	suppression of p53 levels	many types
Mdm2 overexpression	suppression of p53 levels	sarcomas
IAP-1 gene amplification	antagonist of caspases 3 and 7	esophageal, cervical
APAF1 methylation	loss of caspase 9 activation by cytochrome *c*	melanomas
BAX mutation	loss of pro-apoptotic protein	colon carcinomas
Bcl-2 overexpression	closes mitochondrial channel	~½ of human tumors
PTEN inactivation	hyperactivity of Akt/PKB kinase	glioblastoma, prostate carcinoma, endometrial carcinoma

Table 9.4. Weinberg, The Biology of Cancer

Bcl-2

- Oncogene highly expressed in follicular B-cell lymphomas in humans
- Translocation breakpoint t(14:18)
- Overexpression:
  - inhibits apoptosis (to diverse stimuli)
  - detectable in several tumors

- transgenic mice ‘ polyclonal B-cell expansion ‘ lymphomas



Transgenic Mice that O/E *myc* and *bcl-2* from the IgG-Promoter

…expression in cells of the B lymphocyte lineage

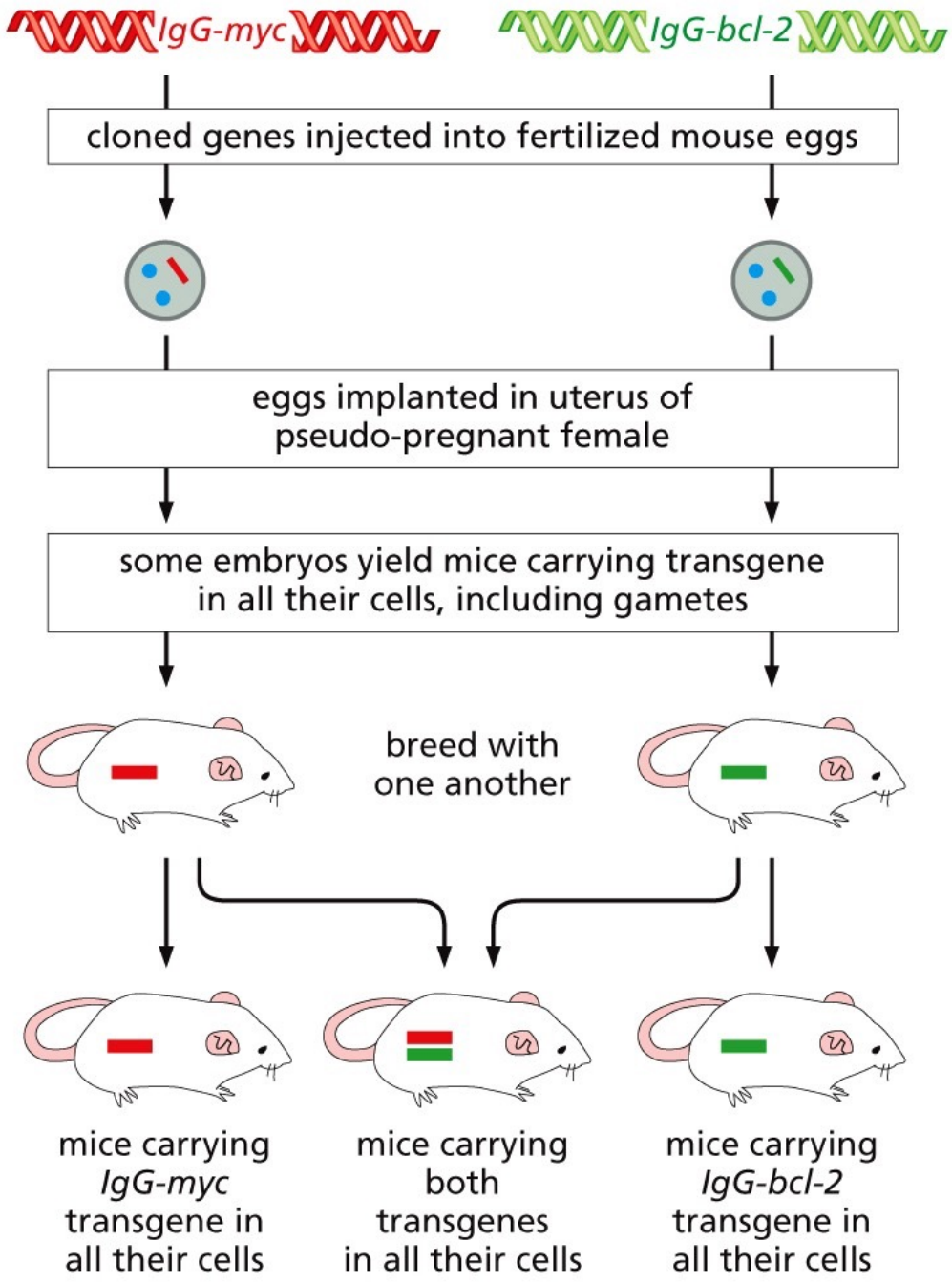


Figure 9.20. Weinberg, The Biology of Cancer

Death Due to Lymphomas

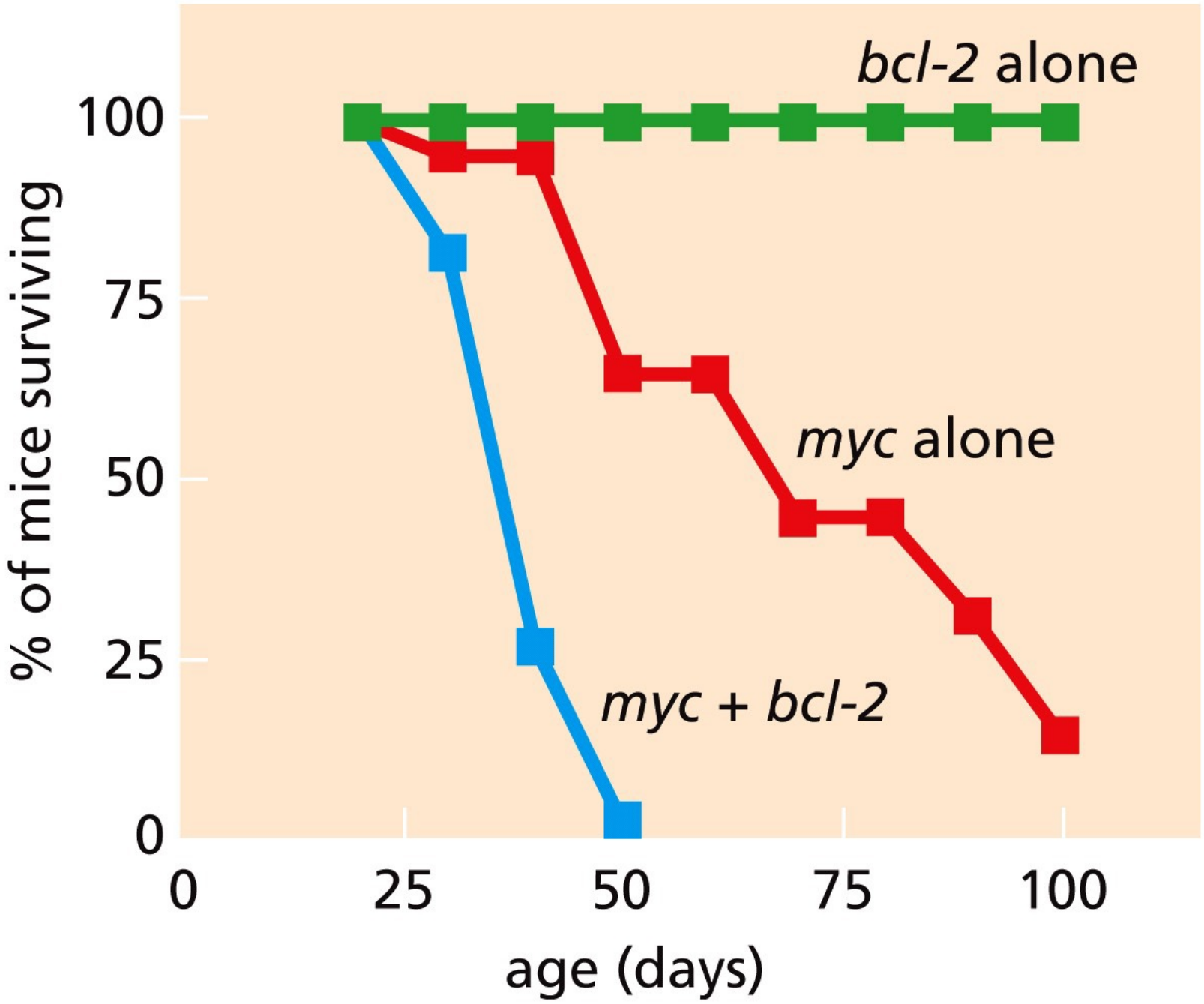


Figure 9.20. Weinberg, The Biology of Cancer

Would Reactivation of p53 in a Tumor Lead to Tumor Regression?

Reactivation of p53 in a Mouse Model for Liver Cancer

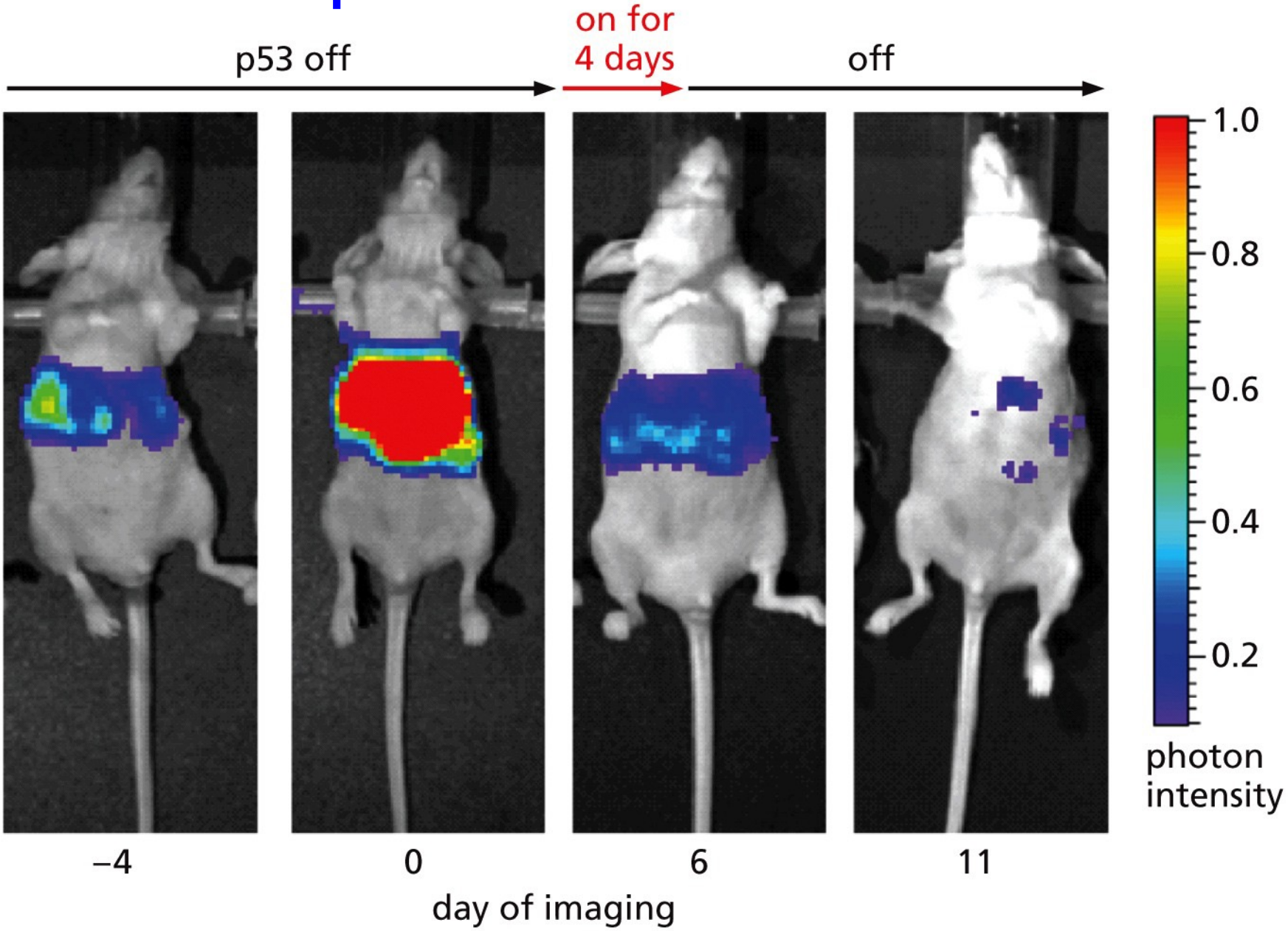


Figure 9.17. Weinberg, The Biology of Cancer

H-ras oncogene was introduced into cultured hepatoblasts (luciferase labeled).
p53 was suppressed by an inducible siRNA vector

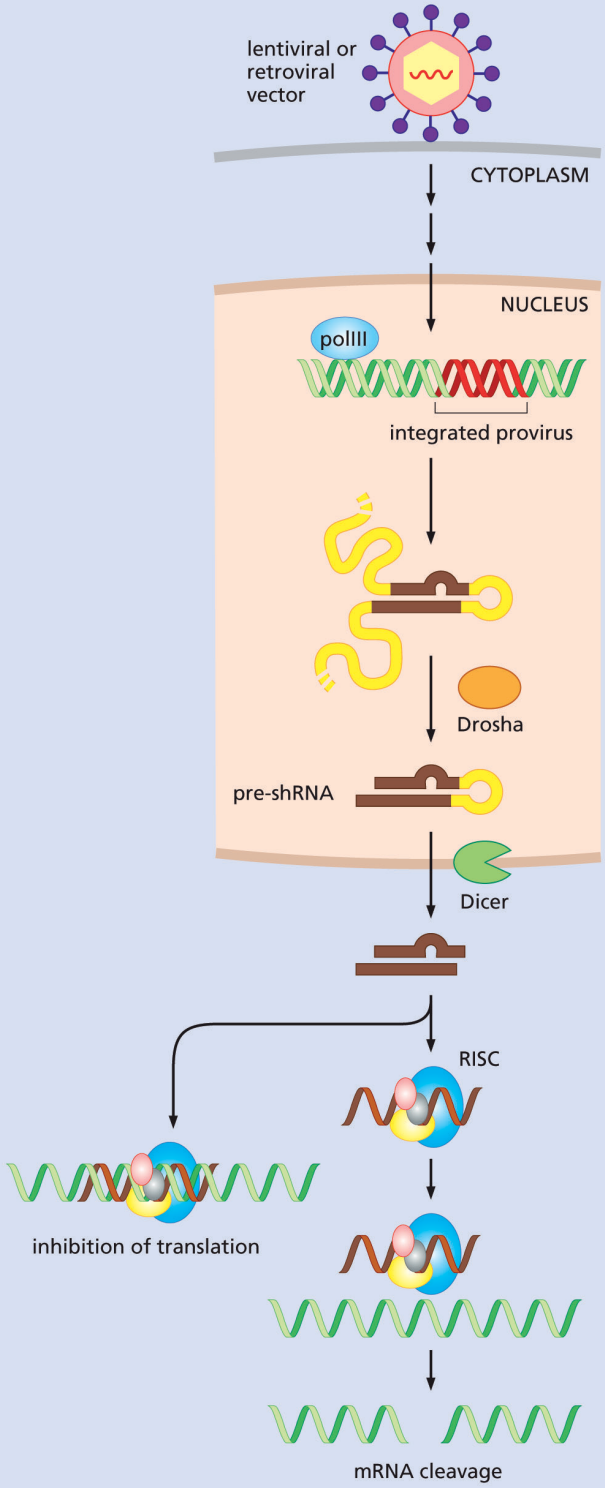


Figure S1.4 Suppression of RNA function by RNA interference The process of RNA interference (RNAi) operates normally in cells to produce microRNAs (miRNAs) that suppress mRNA function, as described in Section 1.10 and Figure 1.20. This process can be exploited experimentally to suppress targeted mRNAs. Two major strategies can be used. In the first, a retrovirus or lentivirus vector (see Supplementary Sidebar 3.3) is constructed that uses an RNA polymerase III promoter to drive expression of a transcript that serves as precursor of an shRNA (small hairpin RNA). The resulting pre-shRNA is processed by the Dicer enzyme into an siRNA (small interfering RNA) of 19–21 nucleotides, which is reduced to a single-stranded RNA (ssRNA). The ssRNA associates with the Argonaut protein (Ago2) and other proteins to form a RISC (RNA-induced silencing complex), which anneals to partially homologous target sequences that are usually located in the 3′ untranslated region (3′UTR) of certain mRNAs. This results either in the cleavage of the mRNA or inhibition of its translation, blocking in both cases expression of the mRNA and thus the gene encoding this mRNA. Because the retroviral or lentiviral provirus is integrated into the cell chromosome and is transmitted heritably to the progeny of an initially infected cell, the expression of a targeted mRNA can be suppressed stably over many successive cell generations.

As an alternative strategy, an siRNA of a particular configuration can be chemically synthesized and transfected into a cell. While this is easier than the procedure described above, the targeted mRNA will be suppressed only transiently, since there is no mechanism to ensure the continued synthesis of the siRNA in the descendants of the initially transfected cell.

Key Concepts

- p53 turnover is blocked during cell-physiologic stress or DNA damage
- p53 mutations that occur in cancer are often dominant-negative
- p53 can induce cell cycle arrest or apoptosis
- p53 levels are controlled among others by Mdm2 and ARF
- Activation of the apoptotic cascade can be triggered by opening of channels in the outer membrane of mitochondria, which release cytochrome c
- Membrane opening is determined by the relative levels of of Bcl-2-related anti-apoptotic and pro-apoptotic proteins
- Activation of a caspase cascade results in cell destruction