

Notes:

1. We will begin lecture 20 with a few slides left from Lecture 19; bring those on Thursday, March 17th
2. This package of 35 slides is labelled 20+, implying that we will not finish the topic in lecture 20 and therefore you will need to bring the slides for lecture 21.
3. Early next week I will post a new set of slides for lecture 21 (about 10 slides) to begin to introduce cell signaling, that we will cover after the lecture 20+ slides that completes the cancer topic.
4. There will be a tutorial this evening (Thursday, March 17th). As in the past, this will be a Q&A session to assist you with problematic topics.

Lecture 20+

Proto-oncogenes, oncogenes, and tumor suppressor genes

SPs: Figs 16-9, 10, 11, 12, 14, 16, 17; Fig 2-52

Vocabulary: tumor suppressor gene/proto-oncogene/
oncogene/retinoblastoma (Rb)/E2F/P53/apoptosis/
tumor virus.

Proto-oncogenes play critical roles in normal growth control.

Conversion to an **oncogene** induces carcinogenesis.

Mutation is usually dominant: only one copy leads to loss of growth control

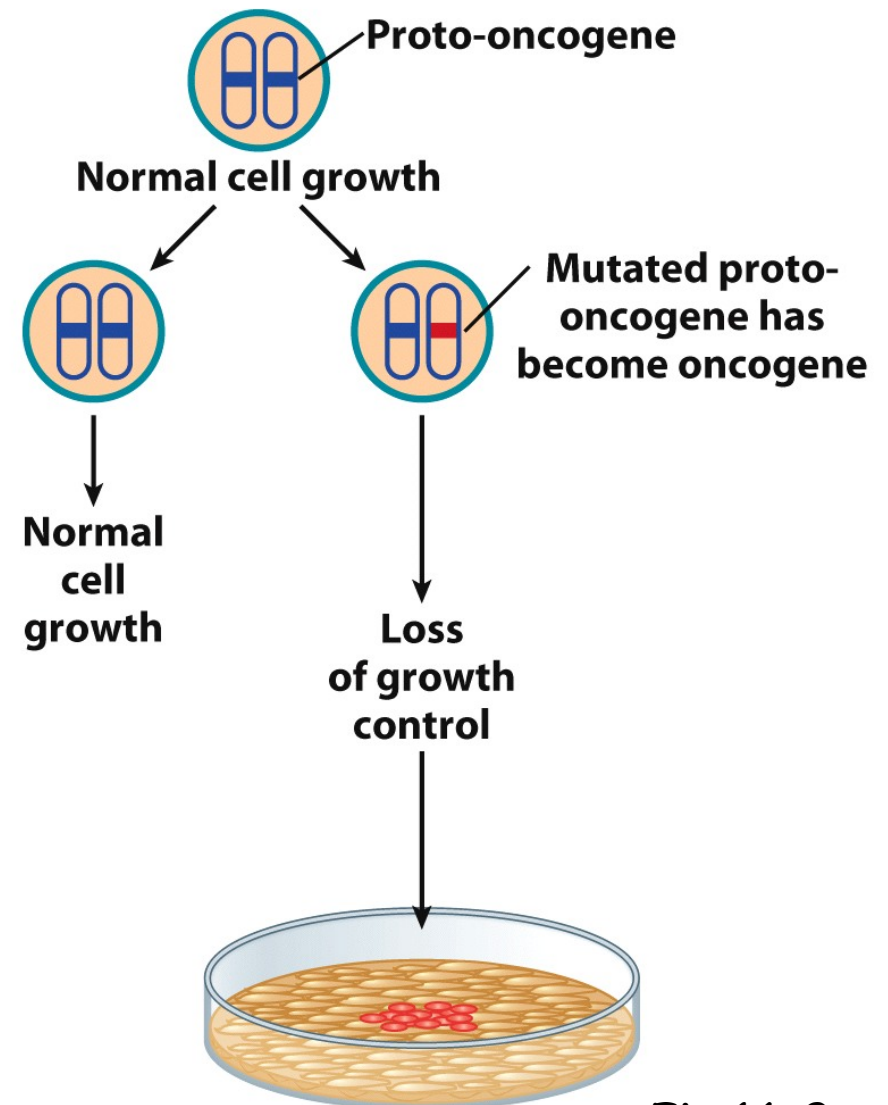


Fig 16-9

Many oncogenes are gain-of-function (GOF) mutants

Some examples:

Altered protein product affecting protein activity

- Mutation within the coding region to change protein

Inappropriate expression of normal protein
(e.g. in incorrect tissue/stage of development)

- Mutation in the promoter region
- Translocation to bring in new regulatory region

Proto-oncogene to oncogene conversion

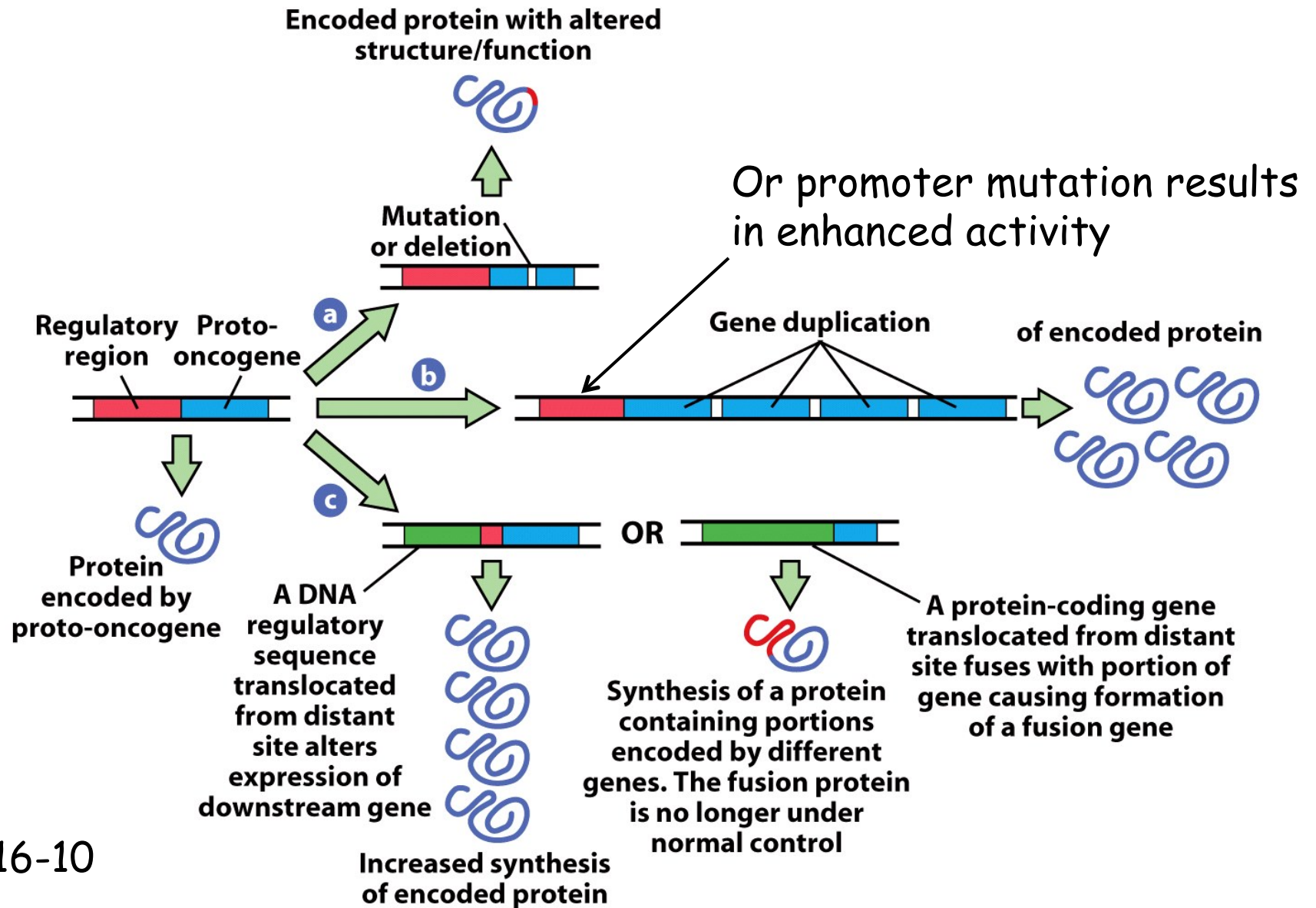
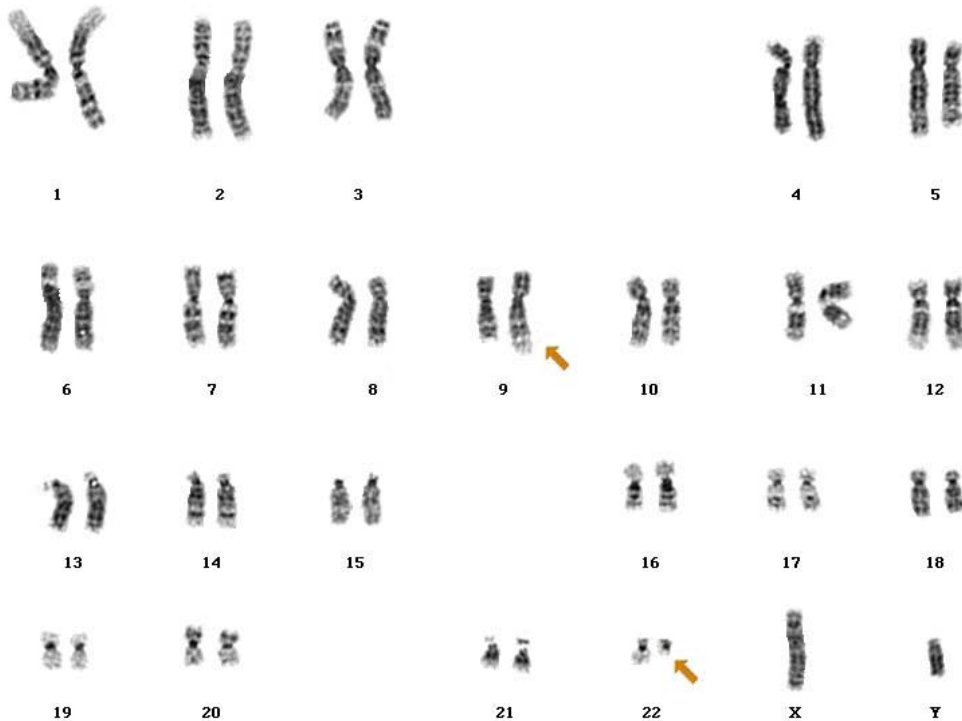


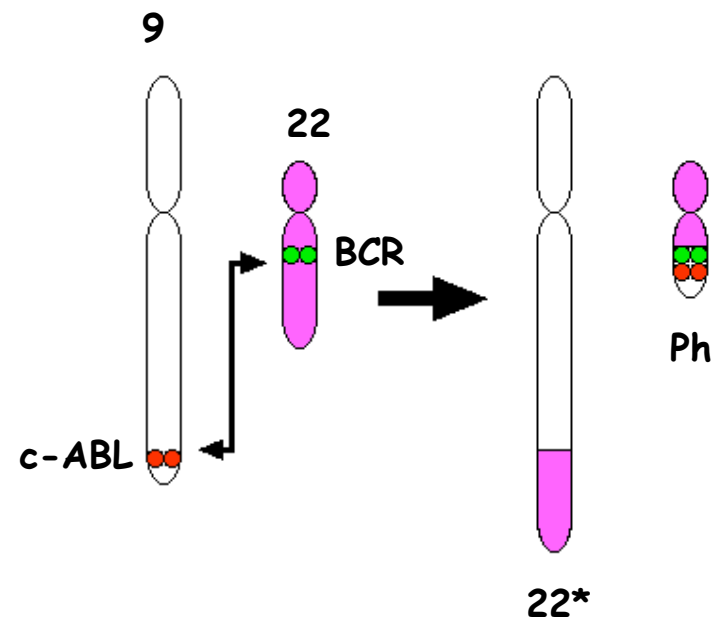
Fig 16-10

CML: chronic myelogenous leukemia

Molecular defect: reciprocal translocation between chromosome 9 and chromosome 22 (creates the “Philadelphia” chromosome).

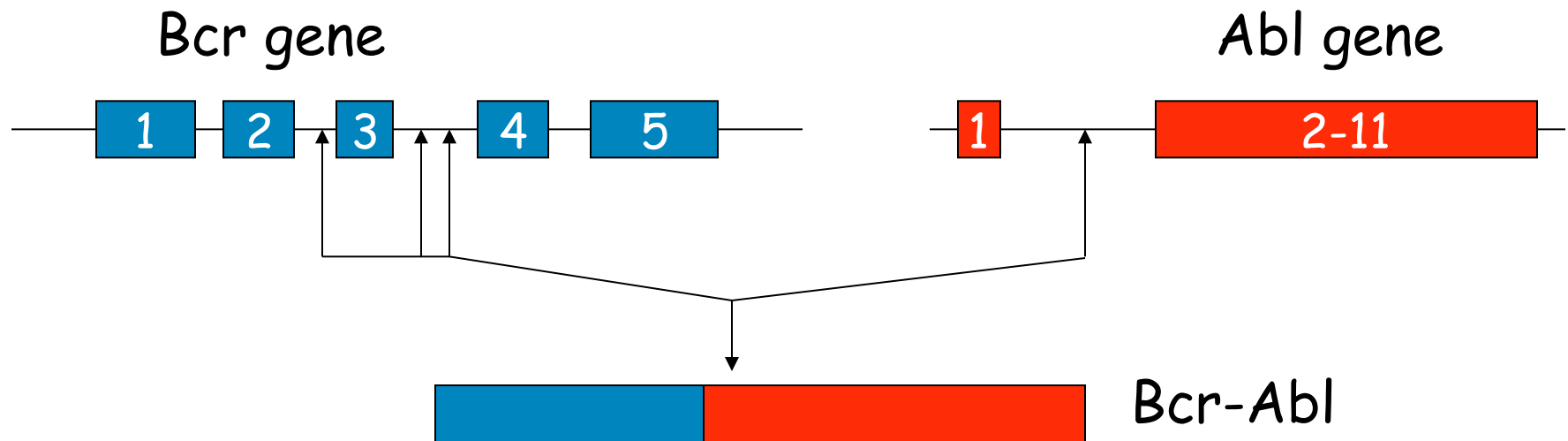


CML karyotype



Ph contains BCR/ABL fusion gene

The Bcr/Abl fusion gene



Abl encodes a tyrosine kinase that phosphorylates several proteins involved in cell proliferation

- Normally the kinase domain can be inactivated

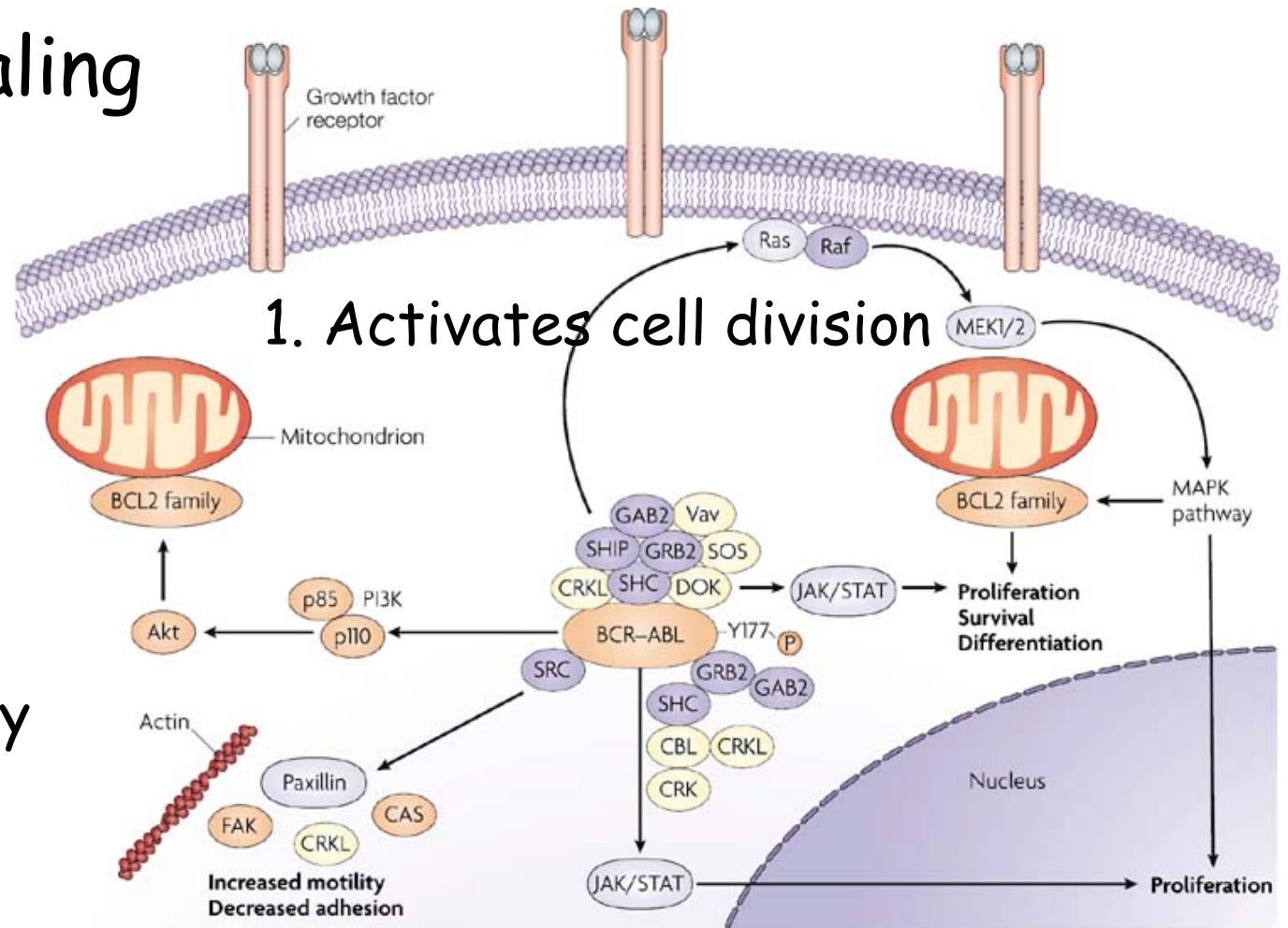
Bcr (breakpoint cluster region); also encodes a protein kinase

The Bcr/Abl fusion protein is constitutively active

Bcr/Abl signaling

2. Inhibits apoptosis

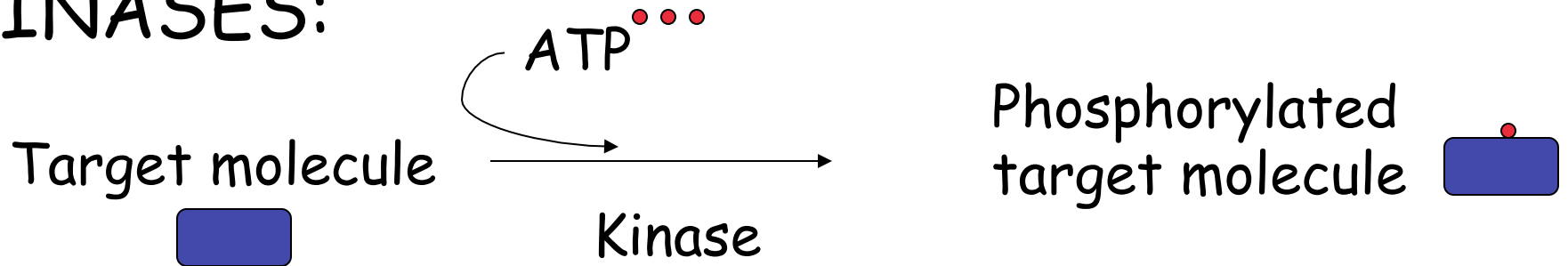
3. Alters mobility and adhesion



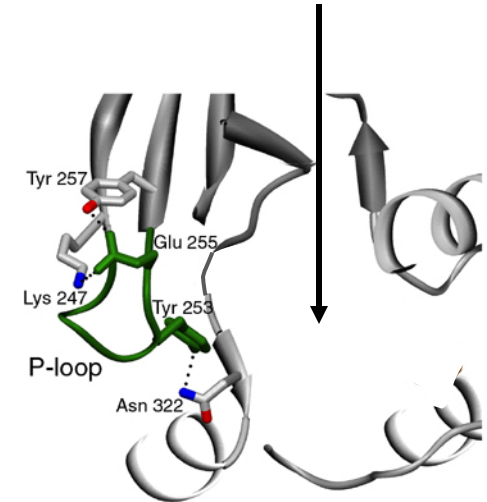
From: Nature Reviews Cancer 7:345-356 (2007)

Results in malignant phenotype

KINASES:



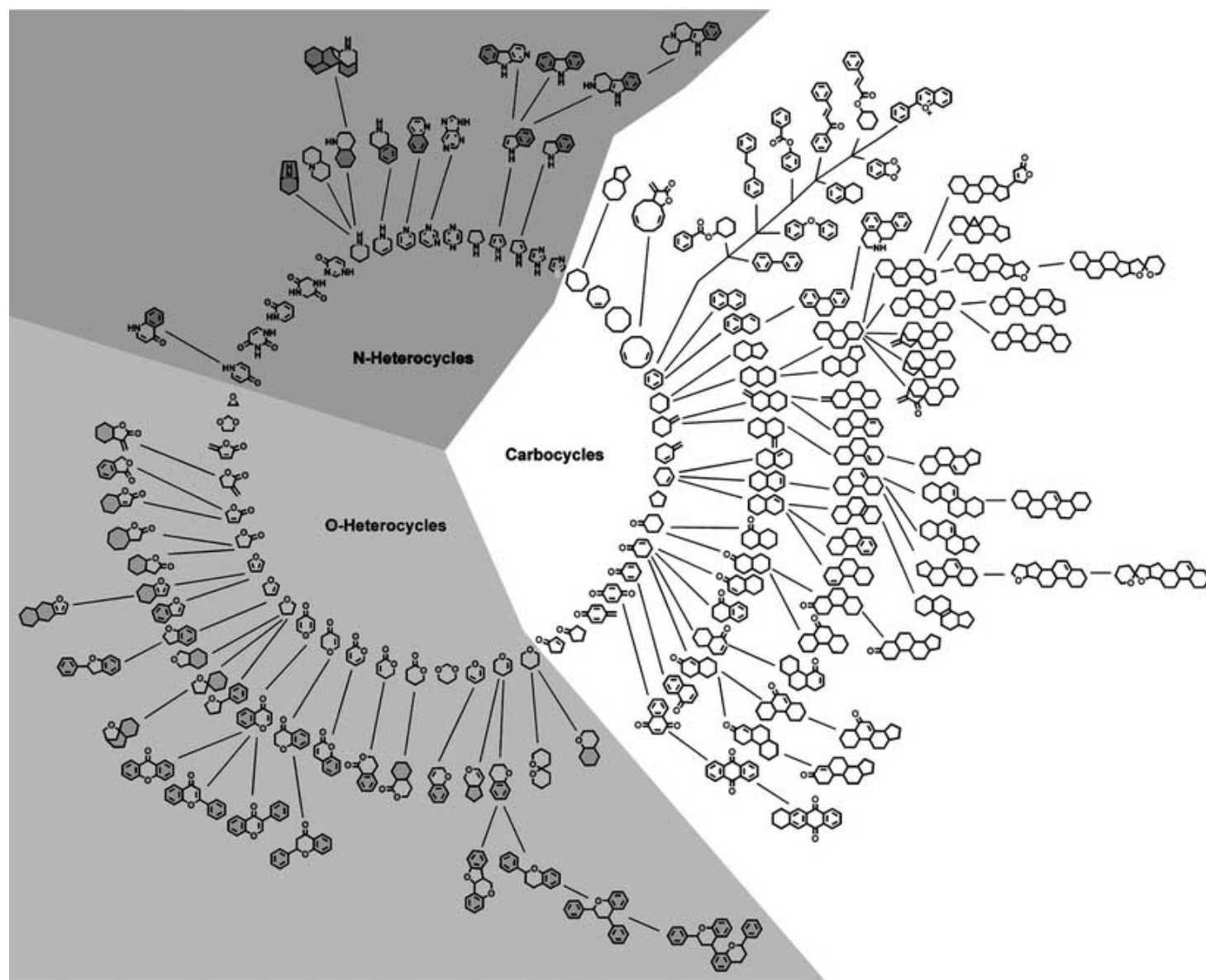
Structural studies of Abl kinase identify ATP binding pocket



Designer Drugs?

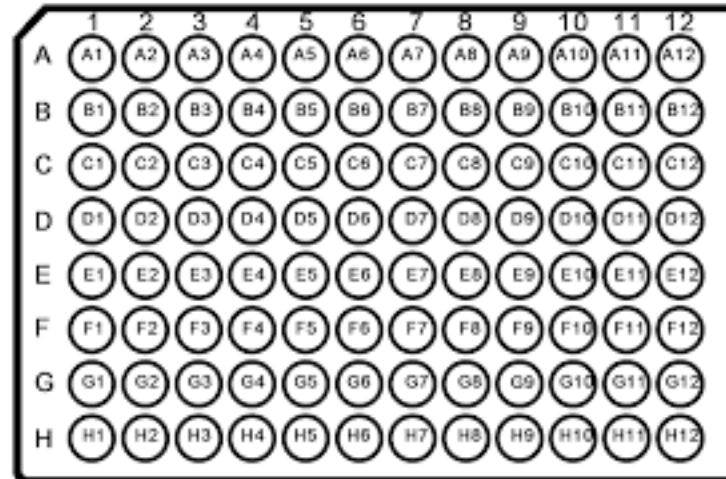
Rational based drug design-what will fit in the pocket, competitively inhibiting ATP binding and thus activity?

Chemical genetics: high-throughput screening of a large number of chemical compounds to see if they are effective



Step 1:
Create
chemical
'library'

Step 2: Place individual chemicals in wells of microplate



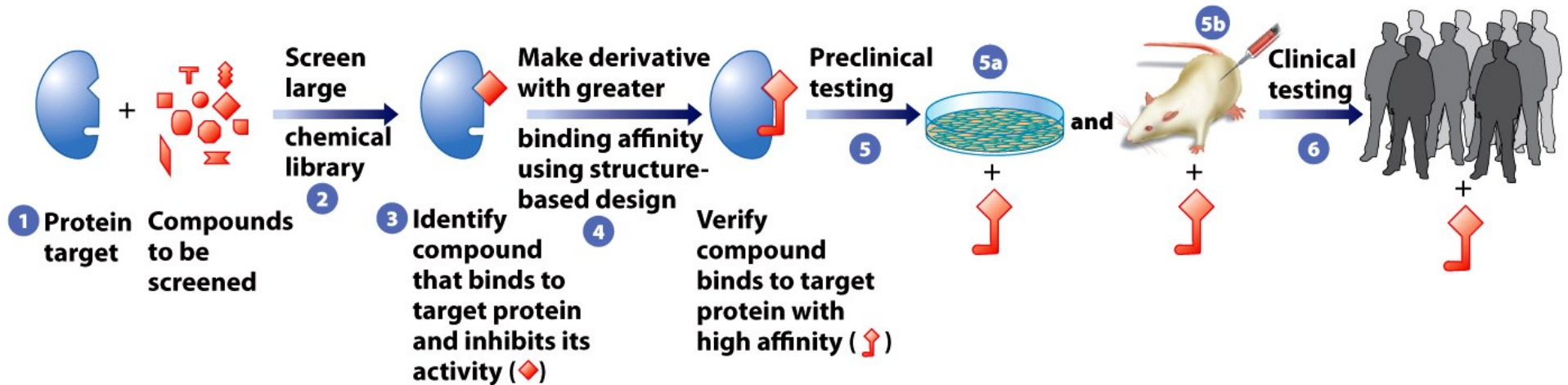
Step 3: Add sample: could be enzyme reaction or perhaps cells

Step 4: Is there any evidence that a specific compound works?

Simplest example: Drug discovery. Add cancer cells and observe: do any of the chemicals affect (e.g. kill) them?

(without killing normal cells)

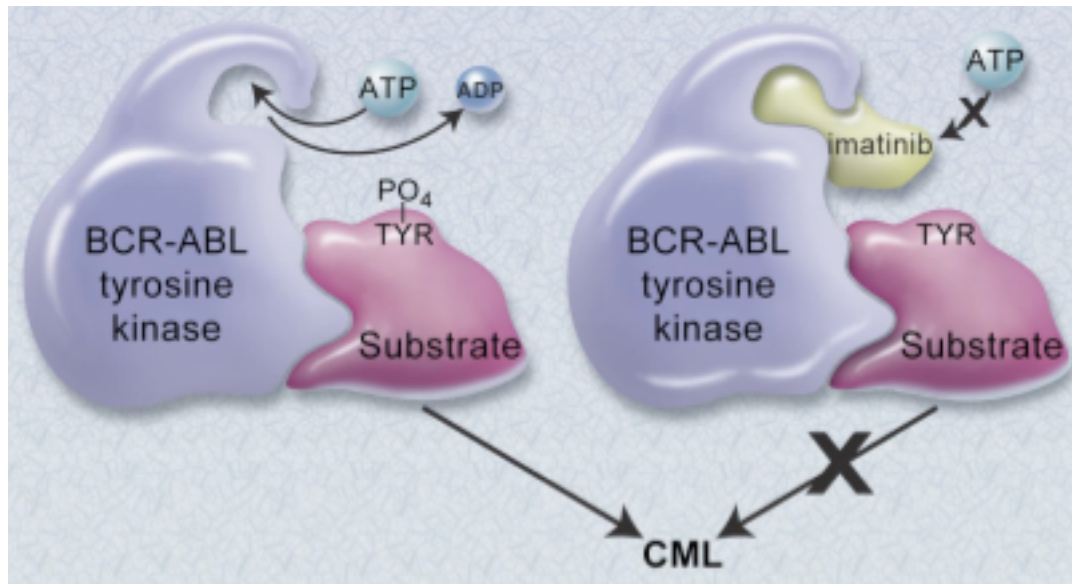
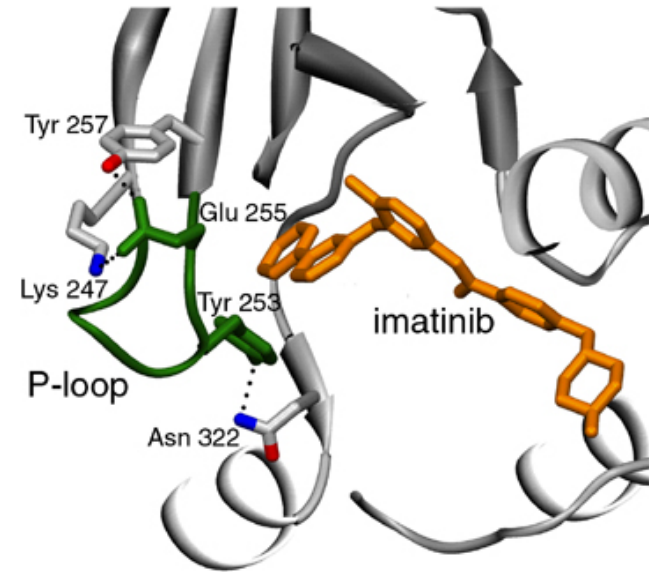
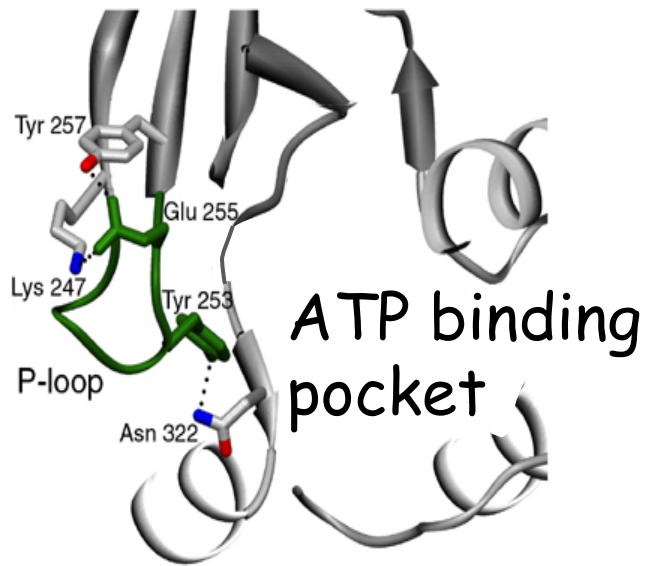
Development of a protein-targeting drug: Gleevec



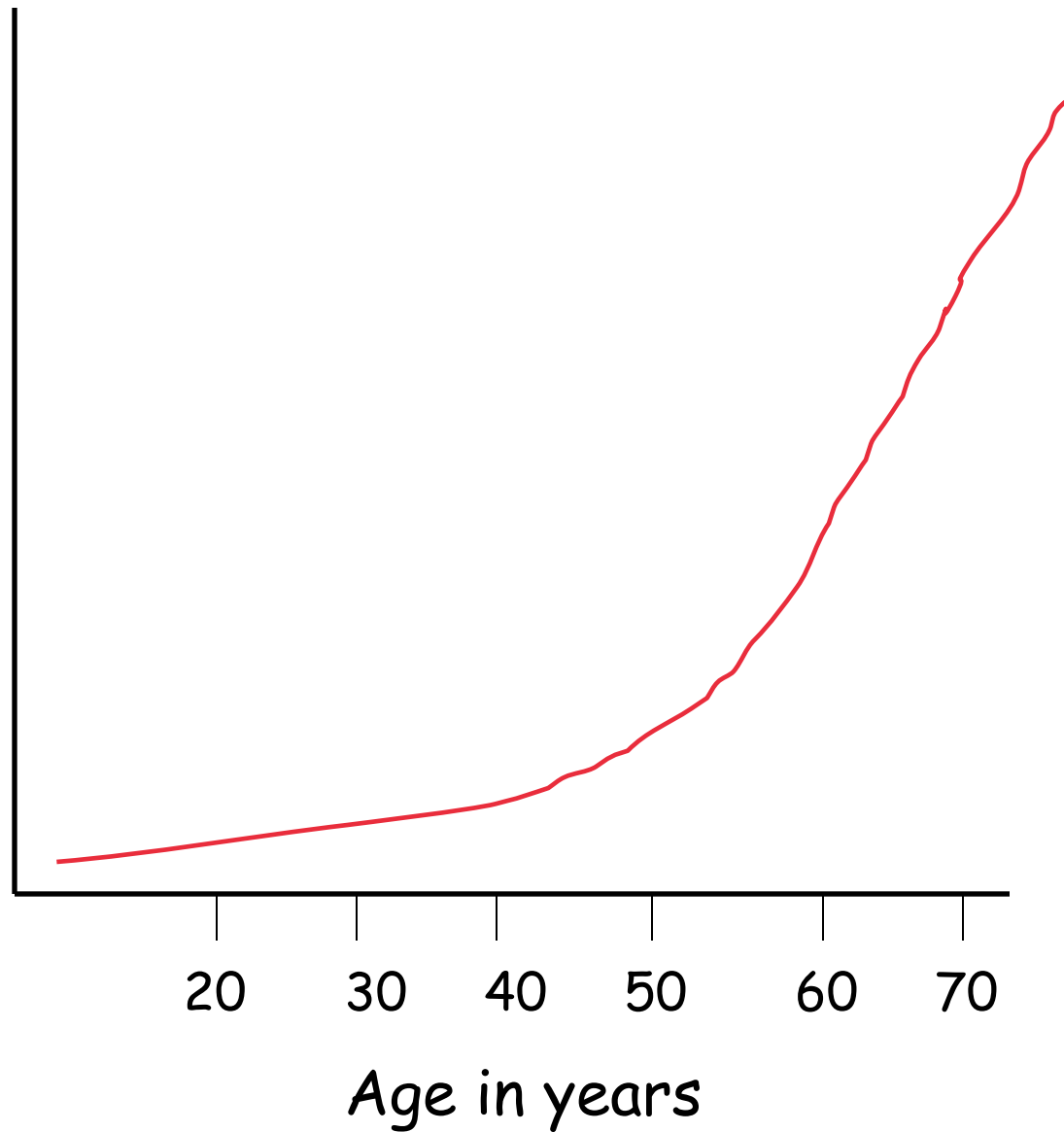
ABL 2PAP (Original molecule) → 'Gleevec' (Analog with 'stronger' effect) Safety/efficacy tests

Fig 2-52

Discovery of Imatinib (Gleevec): 'cures' CML



Cancers
per
100,000
people



Mutations are cumulative: older individuals at greater risk
Proto-oncogene to oncogene conversion but what about TSGs?

Tumor suppressor genes:

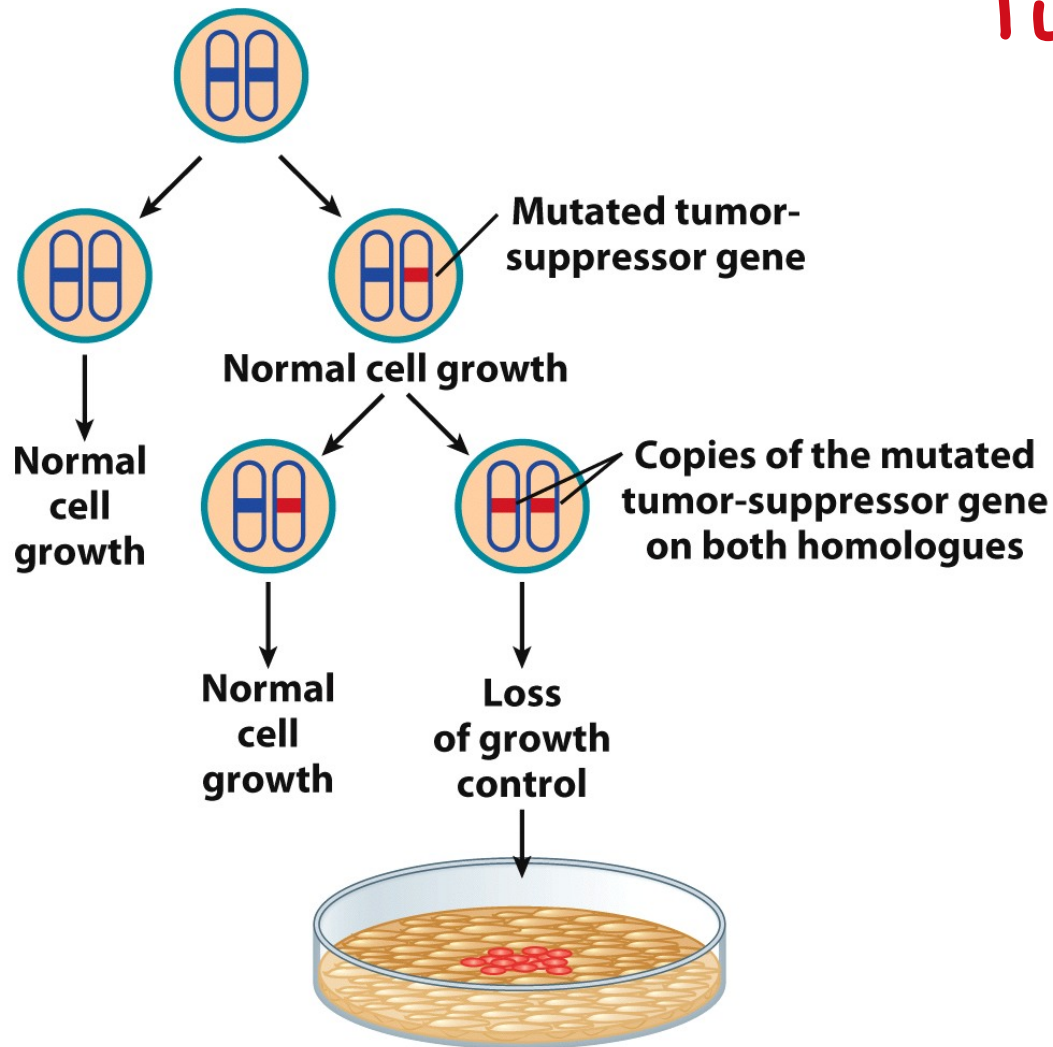


Fig 16-9

Suppressor protein controls cell cycle progression

TSGs are dominant, and mutations in them act recessively

Both copies must be mutated in order for loss of growth control

Cell fusion experiments suggest that TSGs exist

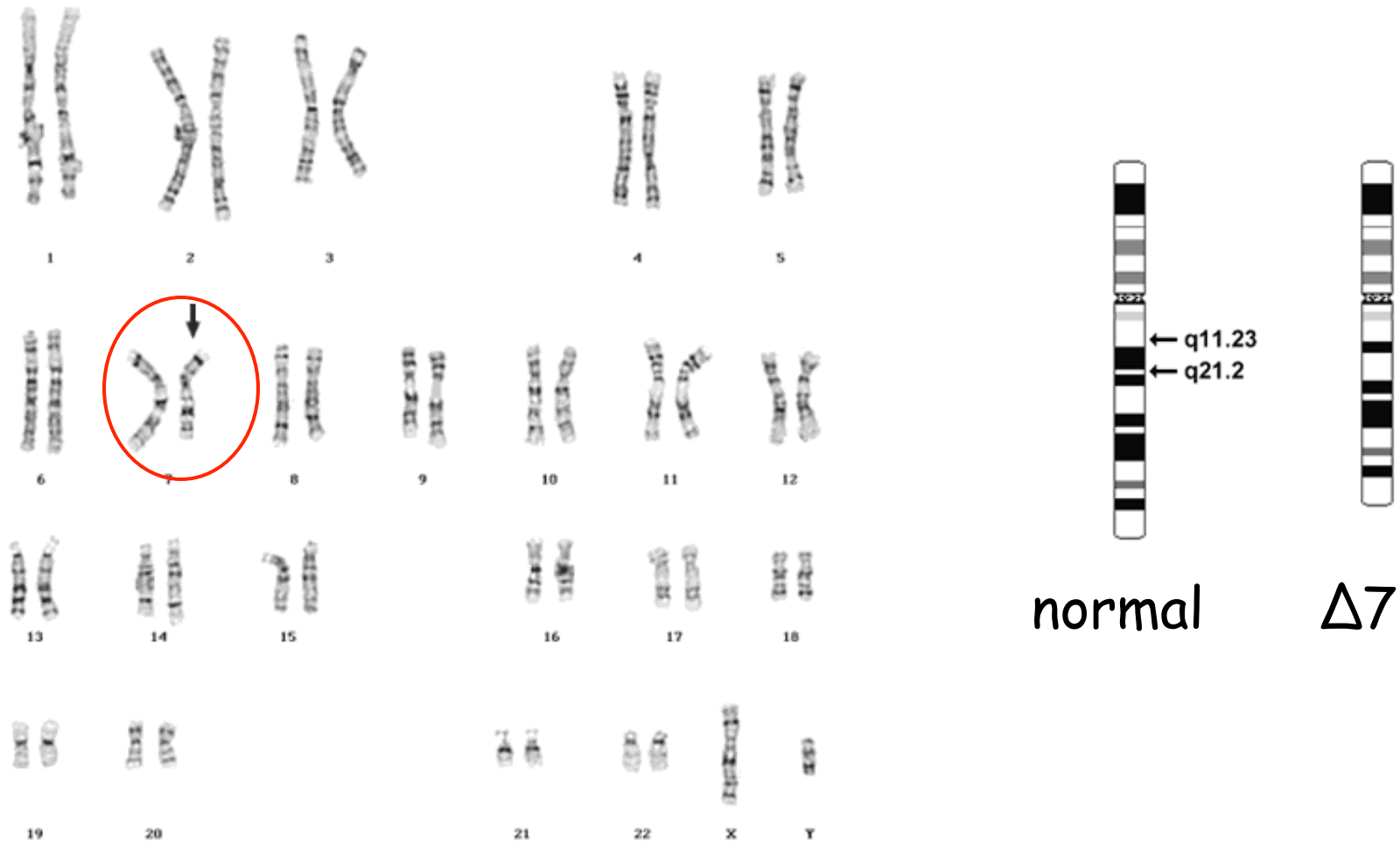
Normal x malignant \longrightarrow Normal hybrid

Normal cell contains 'dominant' factor(s) that suppresses malignant characteristics.

Karyotype analysis reveals chromosome defects:

Chromosome banding with dyes provides a means to examine gross features of the chromosomes



Karyotype: chromosome 7 deletion



Many cancers: specific deletions and/or translocations are observed: suggests 'protective' genes lost/mutated.

Over 20 known Tumor Suppressor Genes

Table 16.1 Tumor-Suppressor Genes

Gene	Primary tumor	Proposed function	Inherited syndrome
<i>APC</i>	Colorectal	Binds β -catenin acting as transcription factor	Familial adenomatous polyposis
<i>BRCA1</i>	Breast	DNA repair	Familial breast cancer
<i>MSH2, MLH1</i>	Colorectal	Mismatch repair	HNPCC
<i>E-Cadherin</i>	Breast, colon, etc.	Cell adhesion molecule	Familial gastric cancer
<i>INK4a</i>	Melanoma, pancreatic	p16: Cdk inhibitor ARF: stabilizes p53	Familial melanoma
<i>NF1</i>	Neurofibromas	Activates GTPase of Ras	Neurofibromatosis type 1
<i>NF2</i>	Meningiomas	Links membrane to cytoskeleton	Neurofibromatosis type 2
 <i>TP53</i>	Sarcomas, lymphomas, etc.	Transcription factor (cell cycle and apoptosis)	Li-Fraumeni syndrome
<i>PTEN</i>	Breast, thyroid	PIP ₃ phosphatase	Cowden disease
 <i>RB</i>	Retinal	Binds E2F (cell cycle transcription regulation)	Retinoblastoma
<i>VHL</i>	Kidney	Protein ubiquitination and degradation	von Hippel-Lindau syndrome
<i>WT1</i>	Wilms tumor of kidney	Transcription factor	Wilms tumor

P53: Fig 16:14-16

Retinoblastoma: RB: Fig 16:11, 12

Tumor Suppressor Gene: Rb

Rb=retinoblastoma

1st discovered in children who developed blastoma of the retina

Cytological examination: deletion in chromosome 13

Early onset: Usually one mutant Rb gene inherited from each parent.

Late onset: Received one mutant gene from one parent. The other gene suffers a mutation at some point of development.

Retinoblastoma occurs only when both Rb genes mutated

Suppose you inherit a mutant Rb gene from one parent

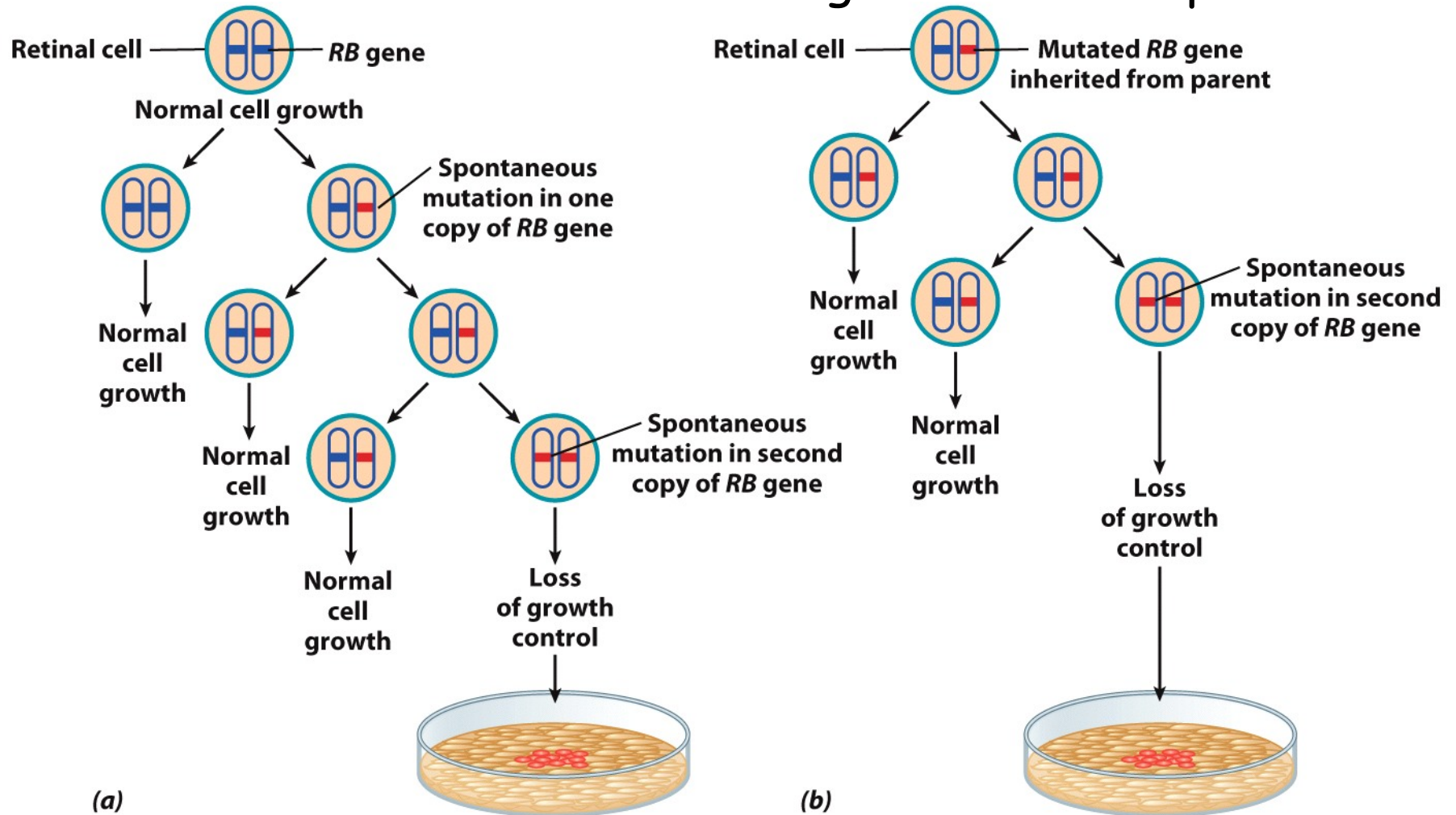


Fig 16-11

What is Rb and what does it do?

Rb encodes a protein that negatively regulates cell cycle

HOW? Controls E2F activity.

E2F=transcription factor required for
entry of cells into S phase

If Rb/E2F interact, E2F cannot activate S-phase genes

Activity of Rb depends on its phosphorylation state,
which is controlled by cyclin-dependent kinases.

Rb acts to negatively regulate the cell cycle by controlling E2F function

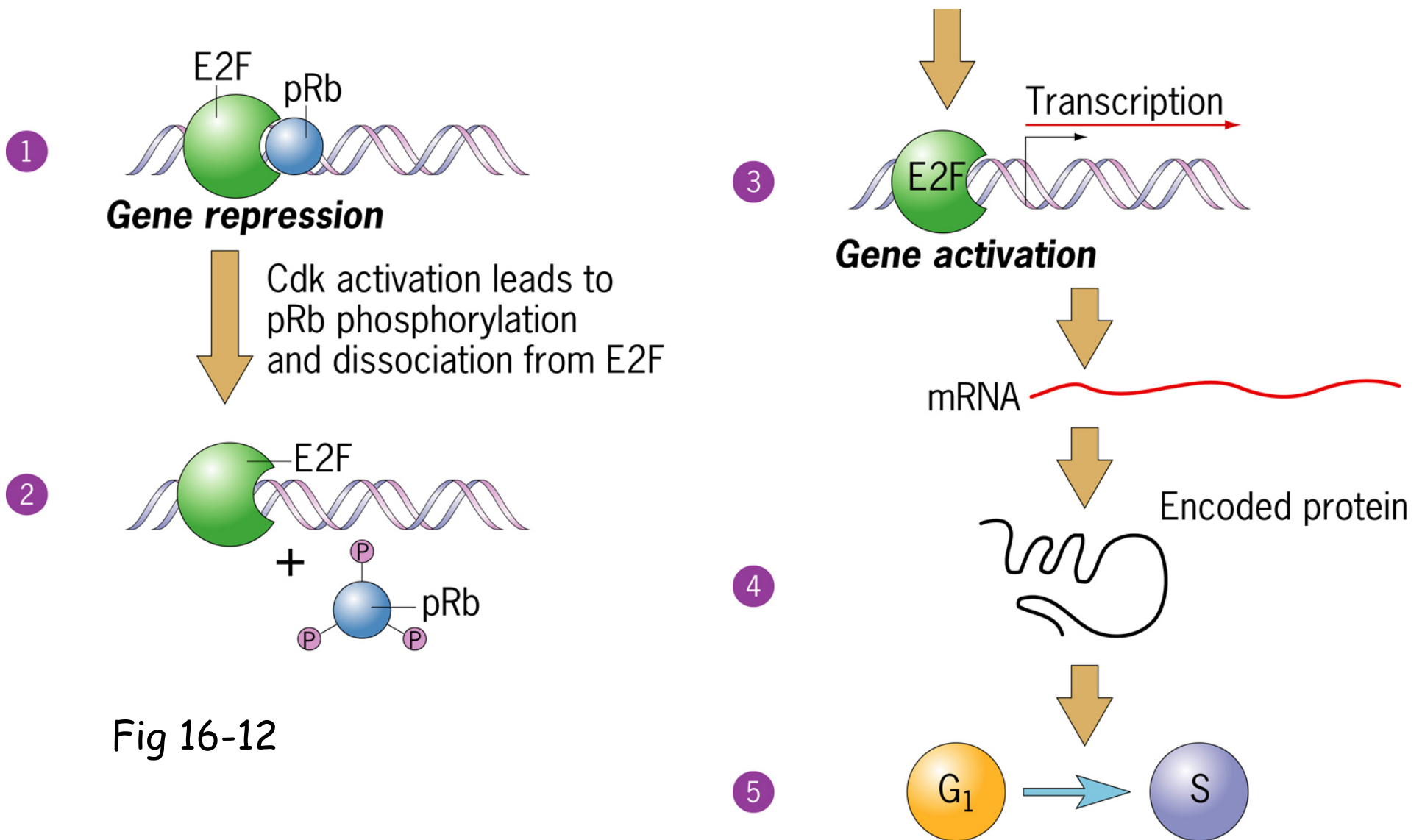


Fig 16-12

Multiple Regulatory Pathways?

When Rb is mutant, E2F acts continuously to sponsor uncontrolled cell cycle progression.

Rb binds to over 40 cellular proteins, so it may control a variety of cellular processes.

Rb contains 16 different sites at which it can be phosphorylated by various CDKs

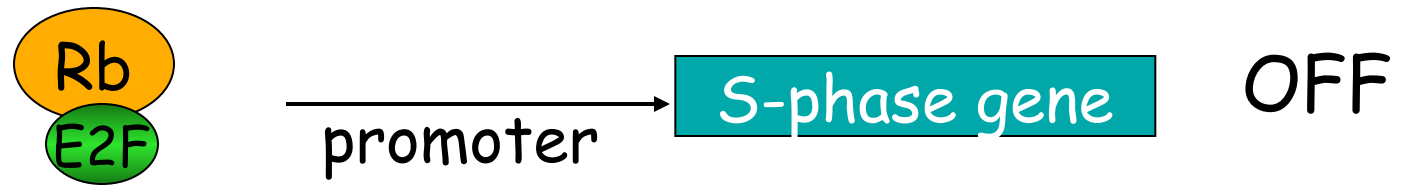
Phosphorylation of various combinations of sites may control different downstream targets

Some viruses cause cancer

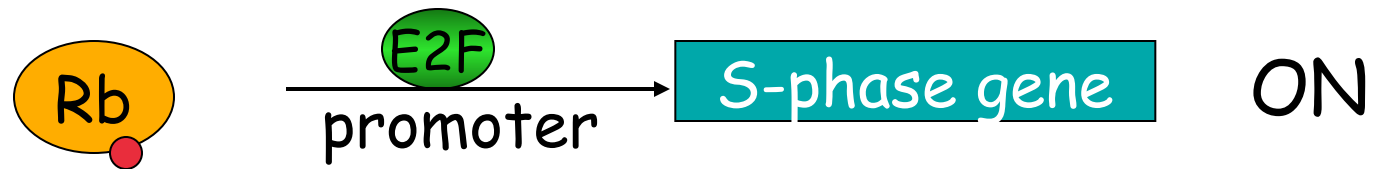
DNA tumor viruses like Human papilloma virus (HPV)

HPVs viral proteins such as E7 can bind and inactivate important tumour suppressor proteins such as Rb.

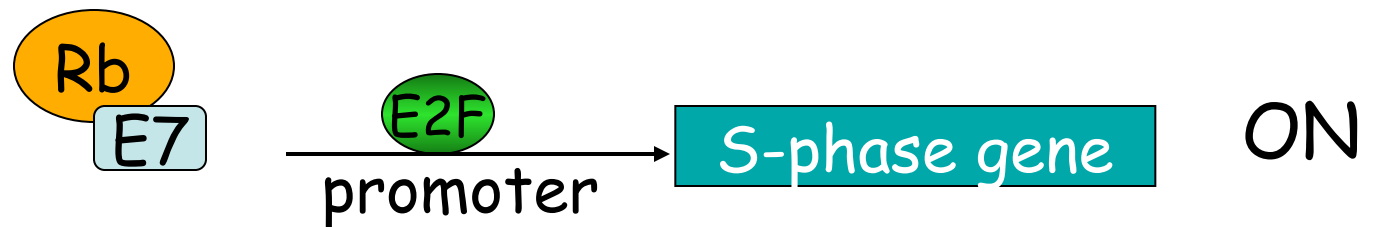
Normally in G1 cells:



When CDK phosphorylates Rb: signal to initiate S-phase:



When cell infected by HPV and expresses E7 protein:



- Constitutively on, cell cycle deregulated

Cancer genetics in the media: Gardasil

Cervical cancer is the fifth leading cause of death from cancer in women world-wide



Vaccine contains a self-assembling coat protein similar to that of the live virus-stimulates immune system to produce antibodies against it; antibodies prevent infection

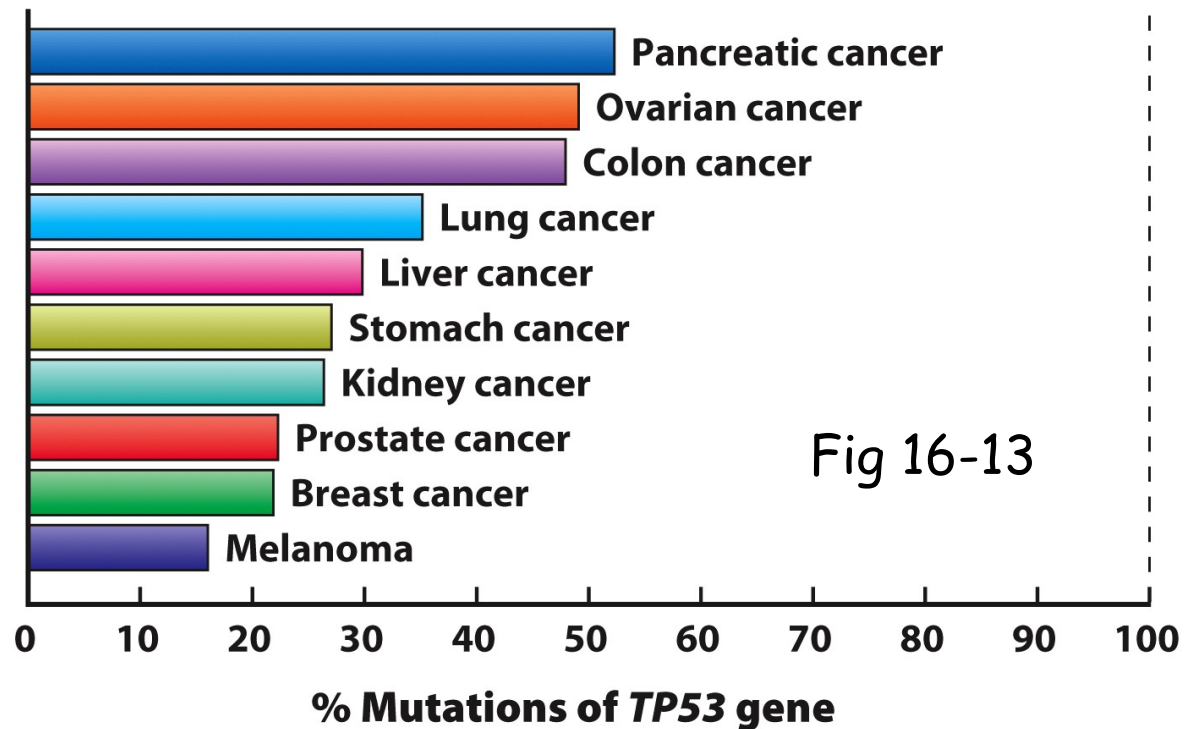
Want more info?: see Blackboard for Health Canada link

Tumor Suppressor Gene: P53

Loss of P53 function associated with many different types of cancers.

Many human cancers are associated with mutations or deletions in both copies of P53.

“Guardian of the Genome”



P53 expression and action influenced by DNA damage.

Model for P53 function

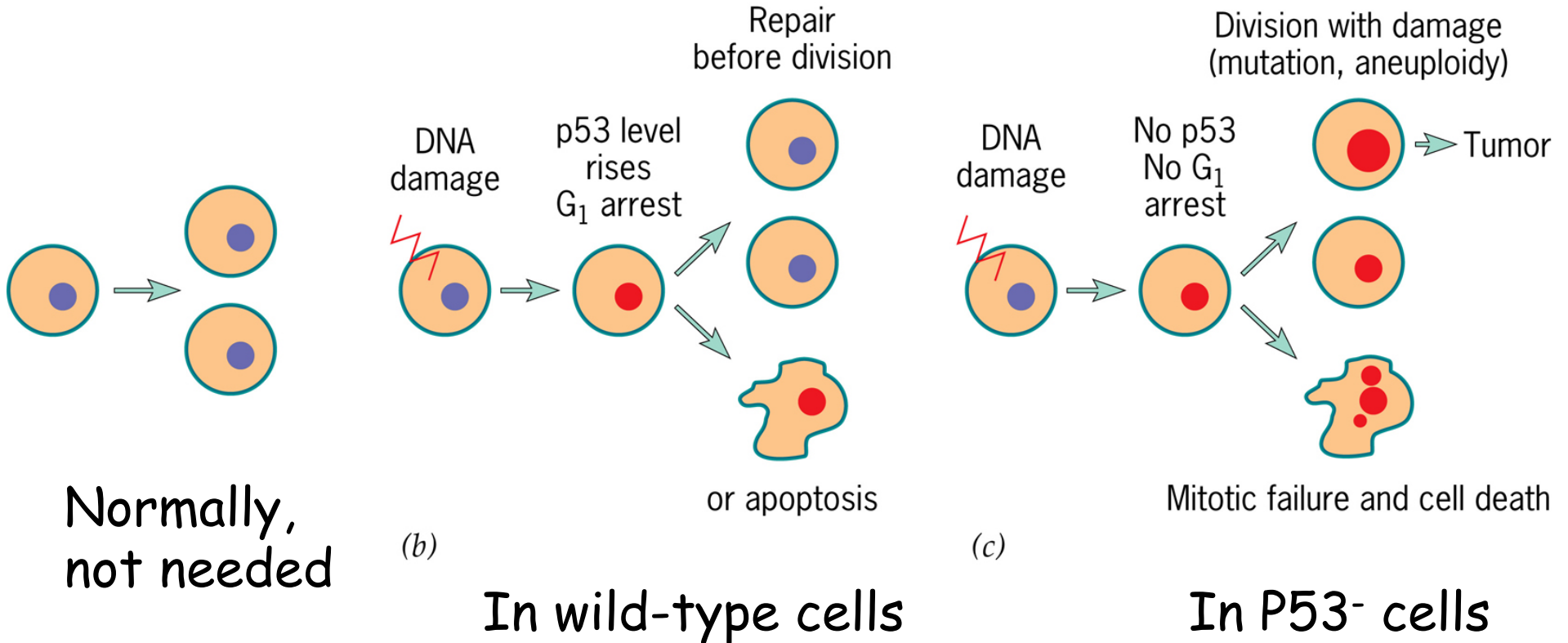


Fig 16-14

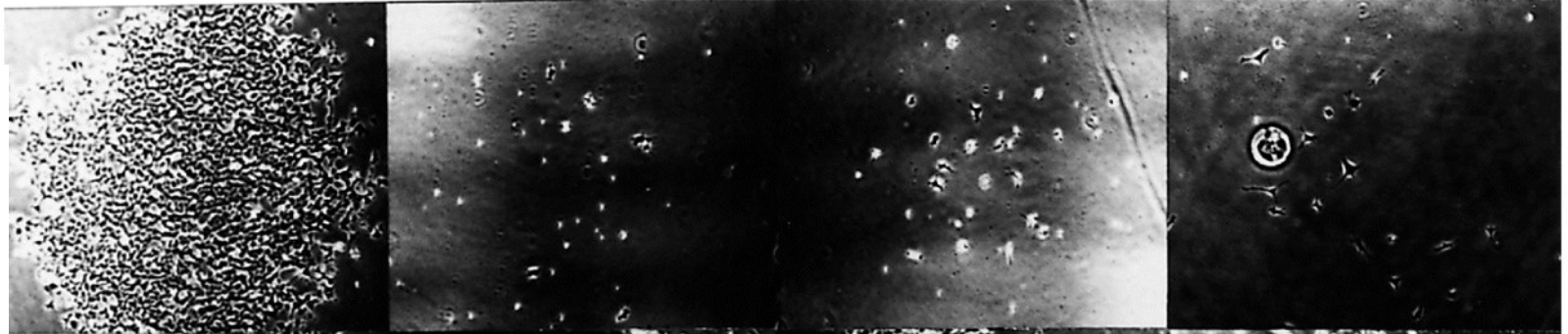
Apoptosis=programmed cell death

P53 induces apoptosis when genome damage is severe

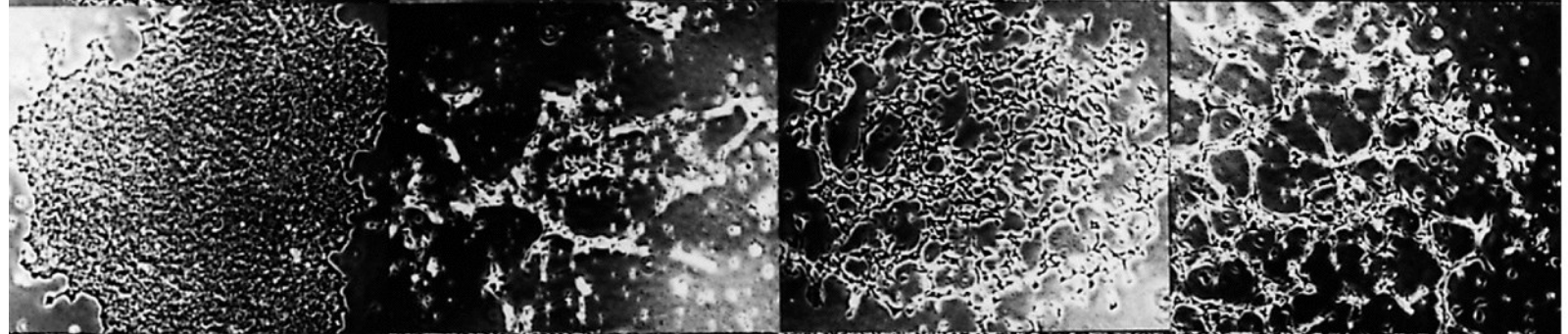
← Cytotoxic chemicals →

Untreated 5-Fluorouracil Etoposide Adriamycin

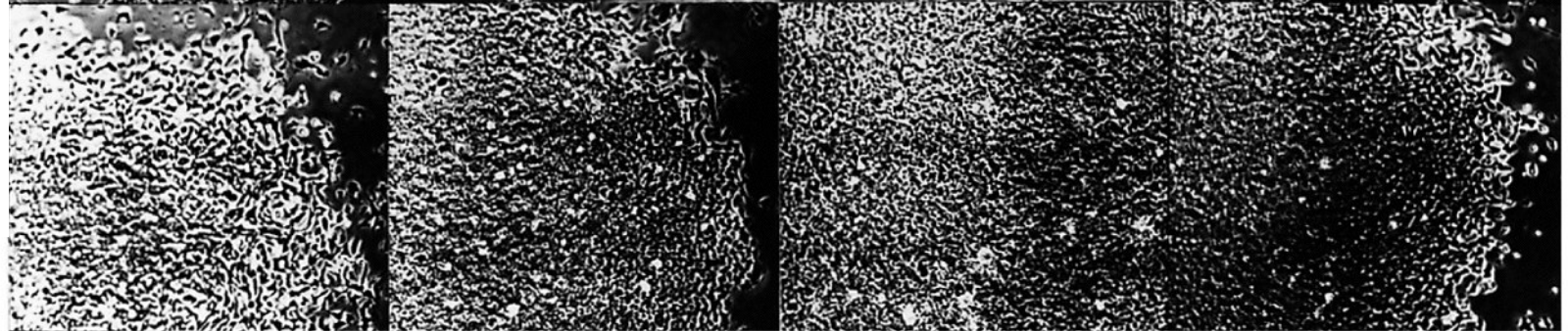
P53/P53



P53/-



-/-
(no P53)



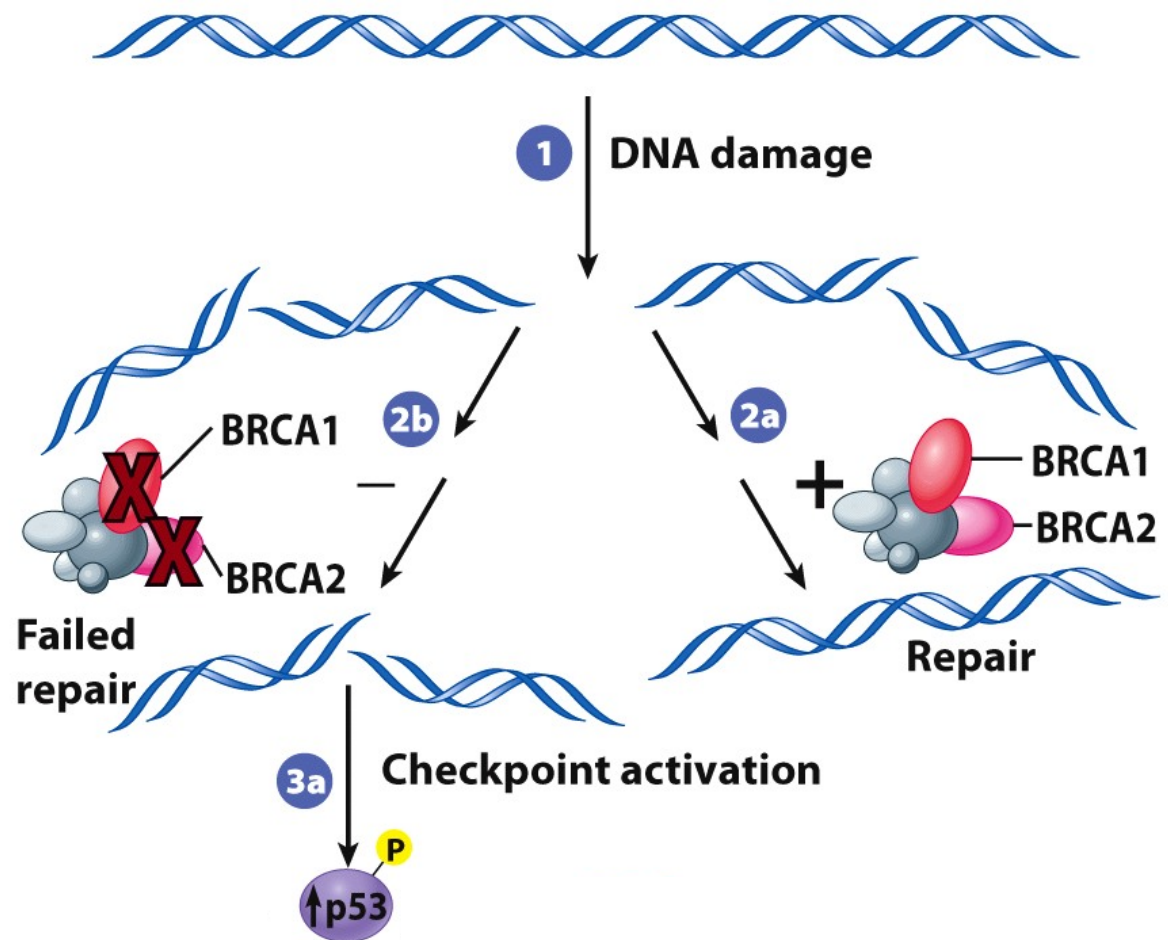
No P53: no cell cycle arrest/no apoptosis

Fig 16-15

Fig 16-16

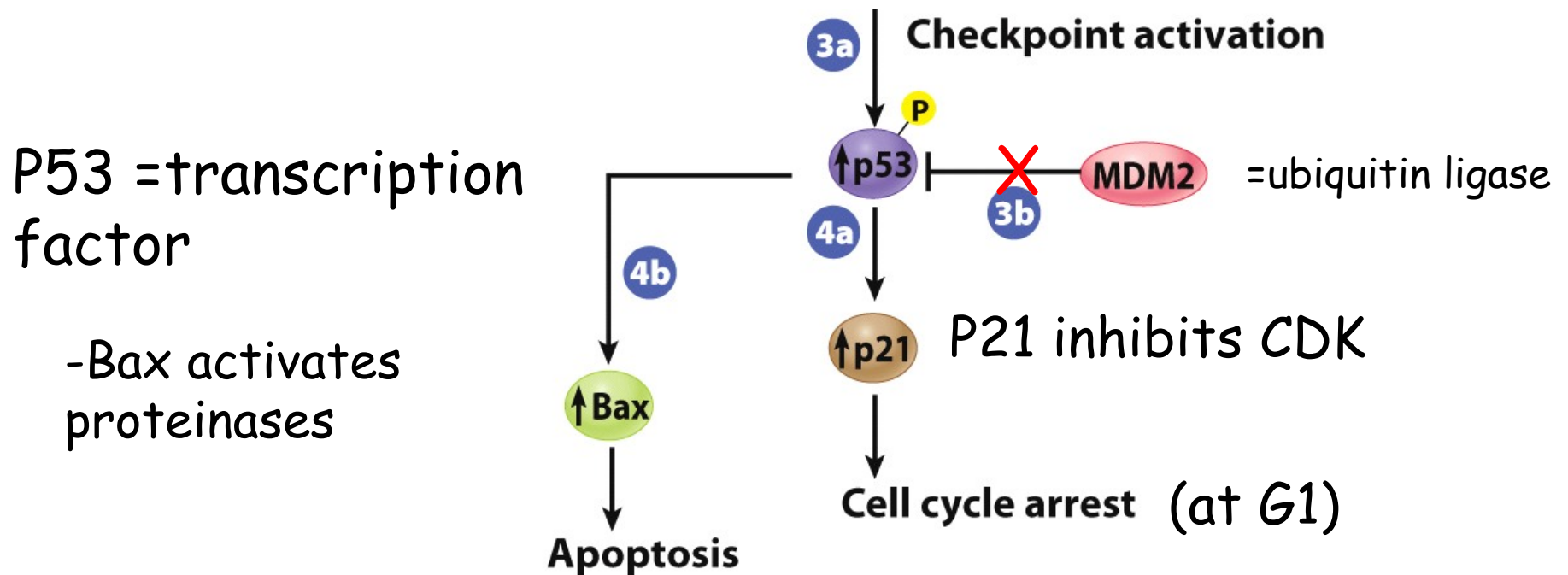
DNA damage
activates multiple
pathways

BRCA1/
BRCA2 repair



DNA damage activates multiple pathways

Fig 16-16



In normal cells:

MDM2: ubiquitin ligase that keeps P53 level low

If P53 phosphorylated, MDM2 can't work

Thus, P53 stabilized, can accumulate

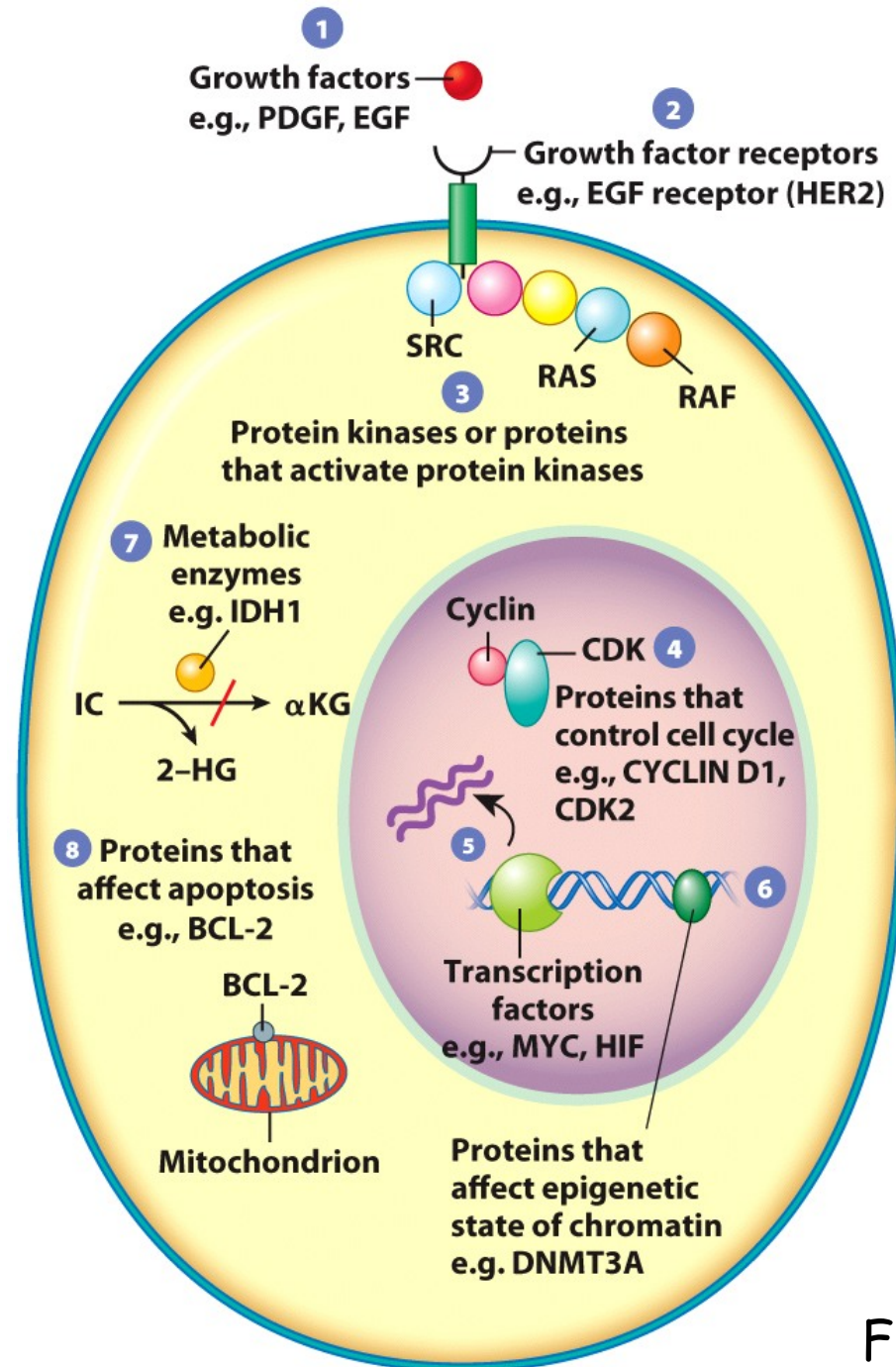
If there is no P53:

Cell cycle arrest at G1 cannot occur (no P21 produced)

Apoptosis not induced (no Bax activation)

Cell proceeds into S phase with damaged DNA

Types of proto-oncogene proteins

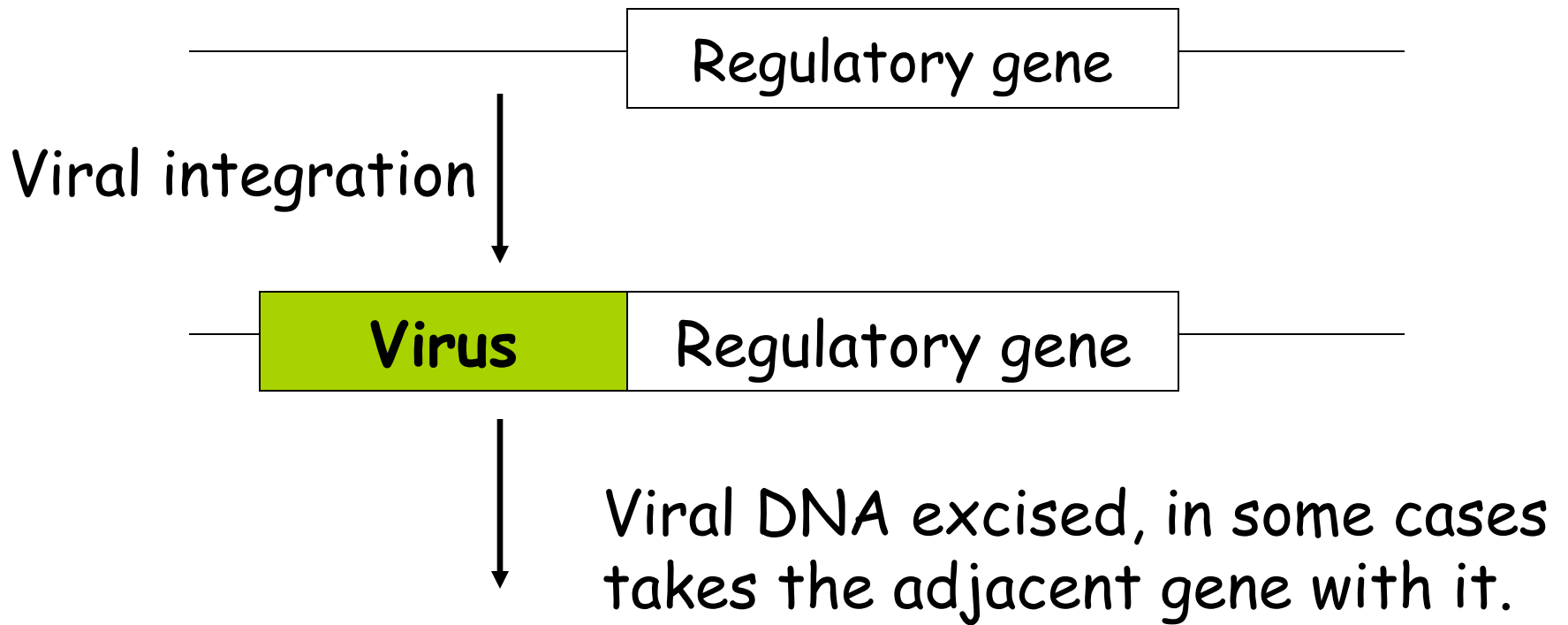


BAX: pro-apoptotic

Bcl2: anti-apoptotic

Fig 16-17

Some viruses 'pick up' regulatory genes and transform their next host with them



Upon infection of new host, they bring an additional copy of the regulatory gene, upsetting the balance of cell cycle control factors-can lead to oncogenic state.

Many genes involved in

- Replication/repair
- Transcription
- Cell cycle control
- Intracellular/intercellular signaling networks

Coordination of all components critical for homeostasis

Imbalances in any regulator may contribute to oncogenic potential