BHARATHIDASAN UNIVERSITY

Tiruchirappalli- 620024,
Tamil Nadu, India

Programme: M.Sc., Biomedical science

Course Title : Cancer Biology

Course Code : 18BMS59C16

Unit-III

TOPIC: Oncogenes, Bcr-Abl1 & ErbB2

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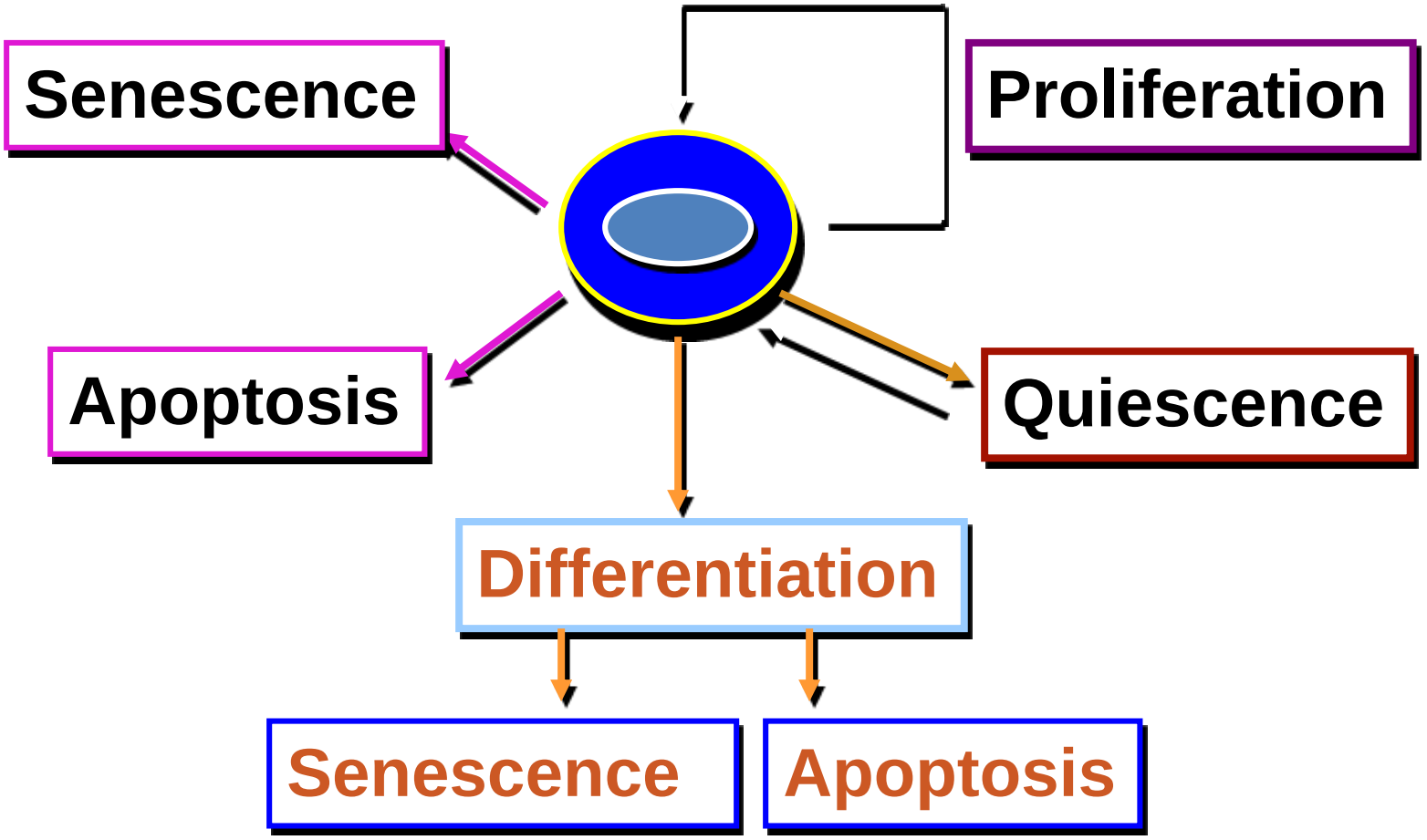
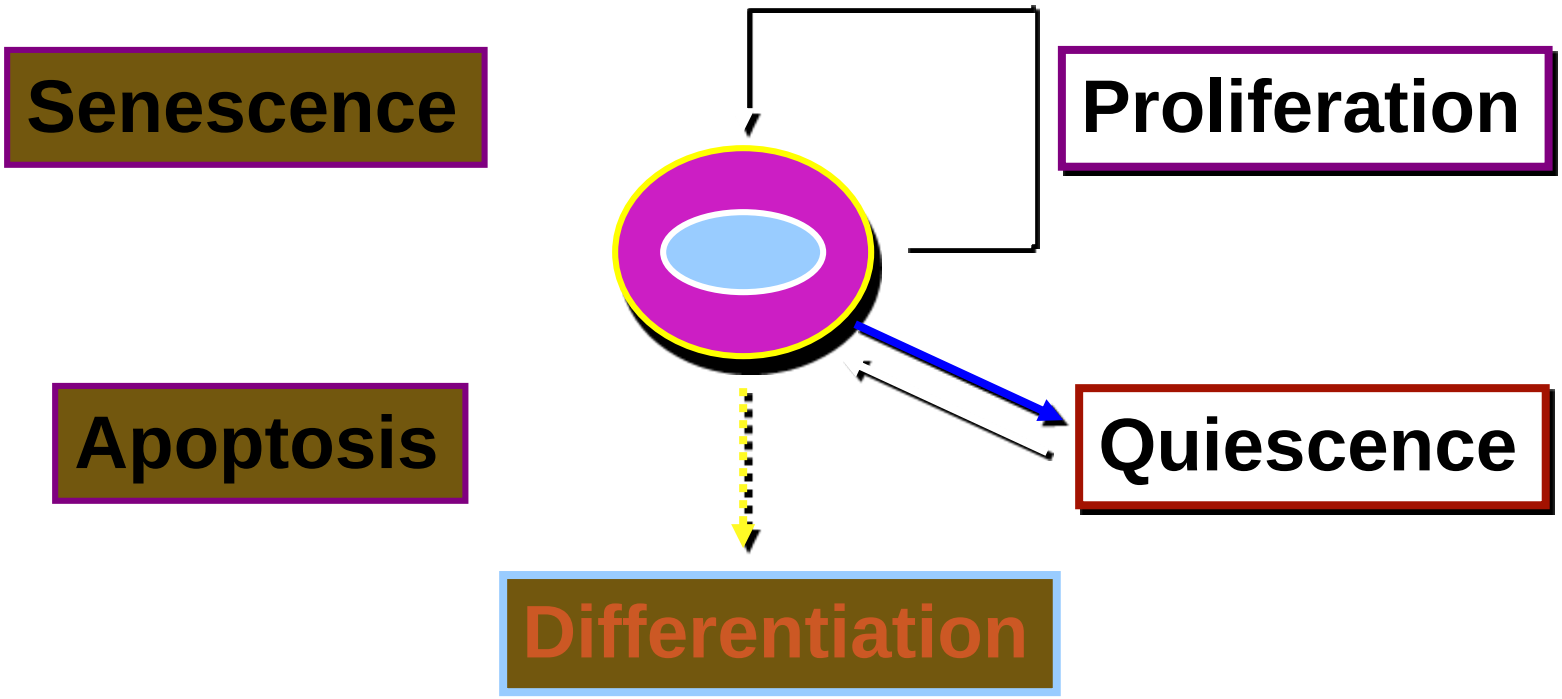
Normal Proliferation is Purposeful and Responsive to Stress



Abnormal Proliferation is Purposeless and Irresponsive to Stress



- Abnormal Proliferation Results from Genetic and Epigenetic Alterations

- In multiple Proto-Oncogenes &Tumor Suppressor Genes

Oncogenes

Stimulate Proliferation
Inhibit Differentiation
Inhibit Apoptosis

Tumor Suppressor Genes

Inhibit Proliferation
Promote Differentiation
Stimulate Apoptosis

What is proto-oncogene?

- A **proto-oncogene** is a normal gene that can become an oncogene due to mutations or increased expression. The resultant protein may be termed an **oncoprotein**.

- Proto-oncogenes code for proteins that help to regulate cell growth and Differentiation.

- **Abl is one of the first proto-oncogenes cloned (1981)**

- **Abl Oncogene fused to BCR in Philadelphia Chromosome of chronic myelogenous leukemia (CML).**

Retrovirus oncogene

- Two main types of oncogenes:
  - *Viral oncogene*: gene from the retrovirus itself
  - *Non-Viral oncogene (Cellular oncogene):* genes derived from the genes of the host cell that are in an inactive form usually. Occasionally if the gene incorporates with the viral genome will form a highly oncogenic virus.

How does a Proto-oncogene become an Oncogene?

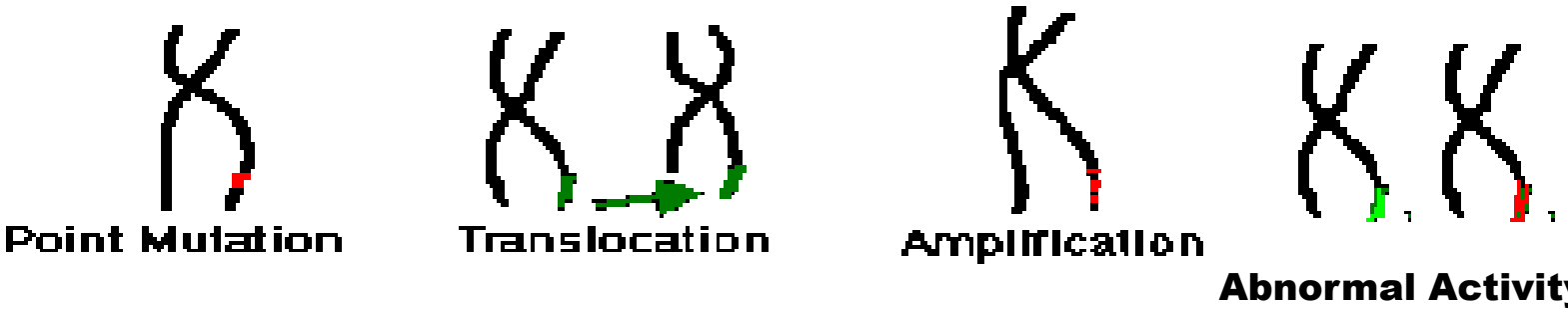
Proto-Oncogene → Oncogene

1.Mutation

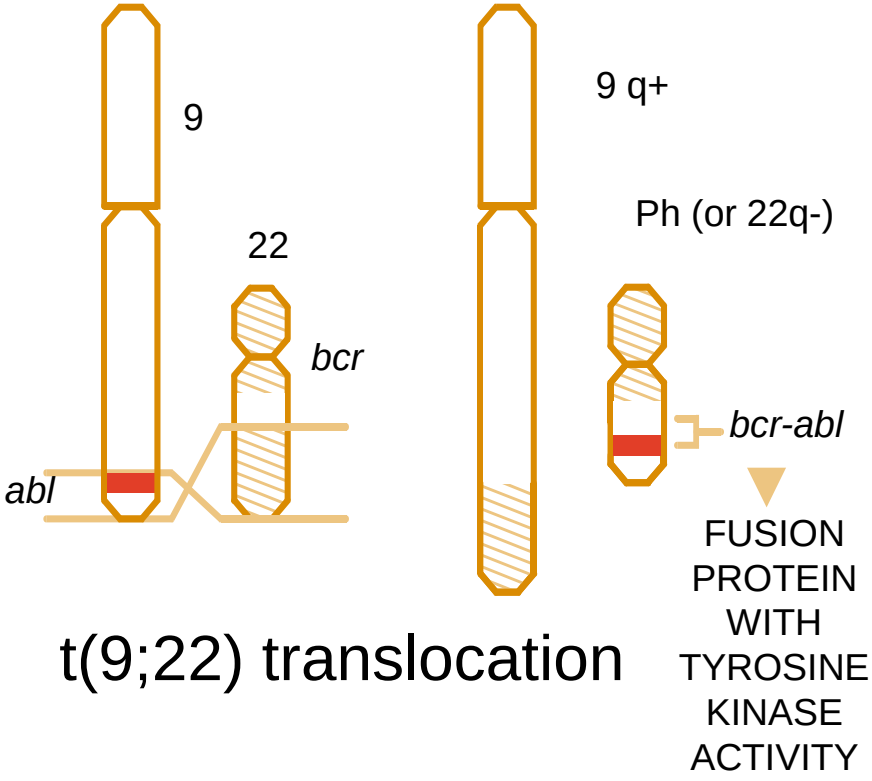
2. Abnormal Activity

3.Gene Translocation

4. Amplification

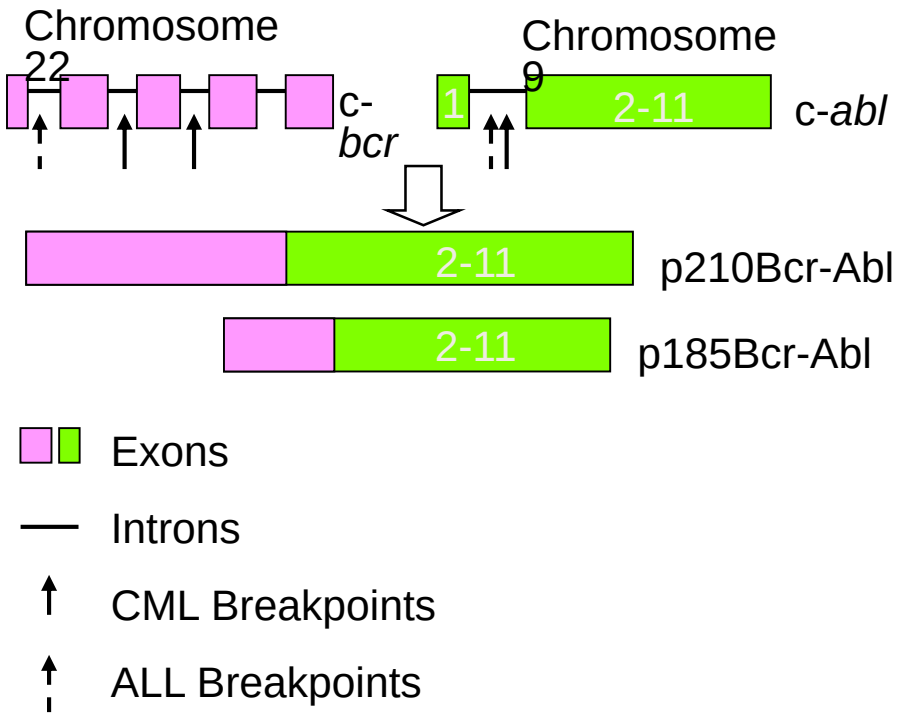


The Ph Chromosome and the bcr-abl Gene



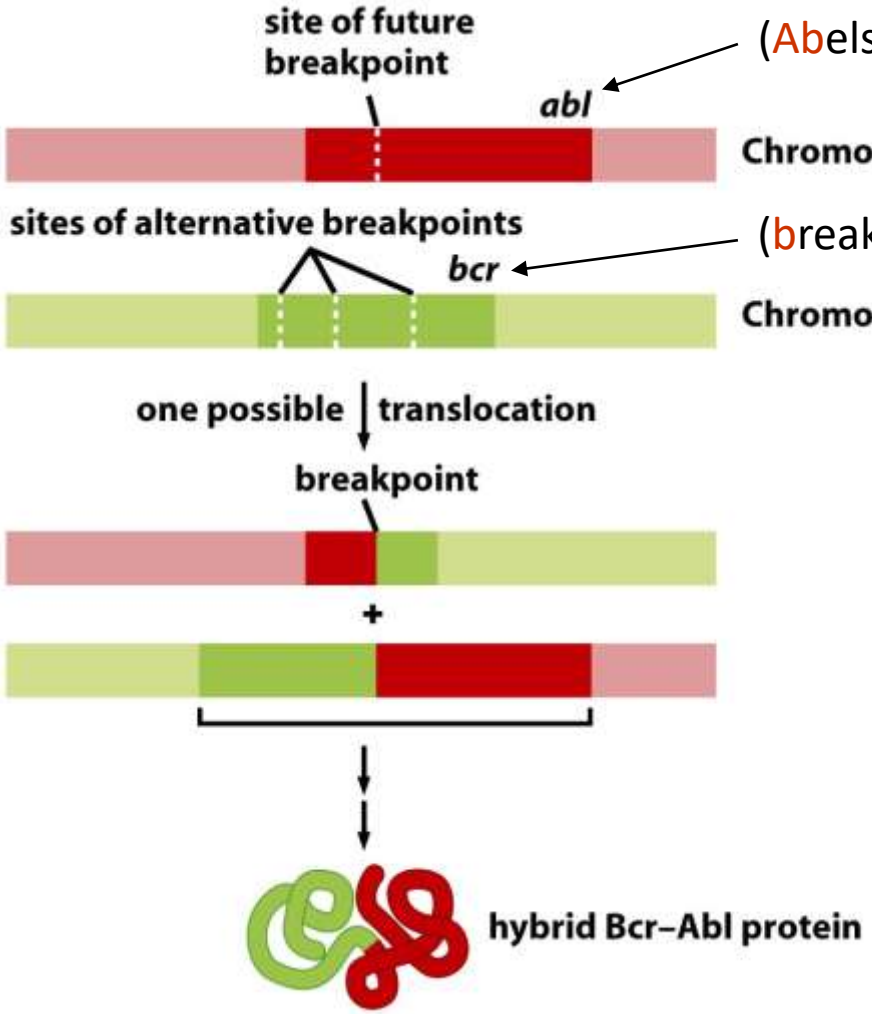
t(9;22) translocation

bcr-abl gene structure



- **The molecular weight of the protein can range from 185 to 210 kDa**
- Bcr-Abl codes for a receptor tyrosine kinase which is constitutively active, leading to uncontrolled cell proliferation.

Formation of the *bcr-abl* oncogene after t(9; 22) (q34; q11) translocation

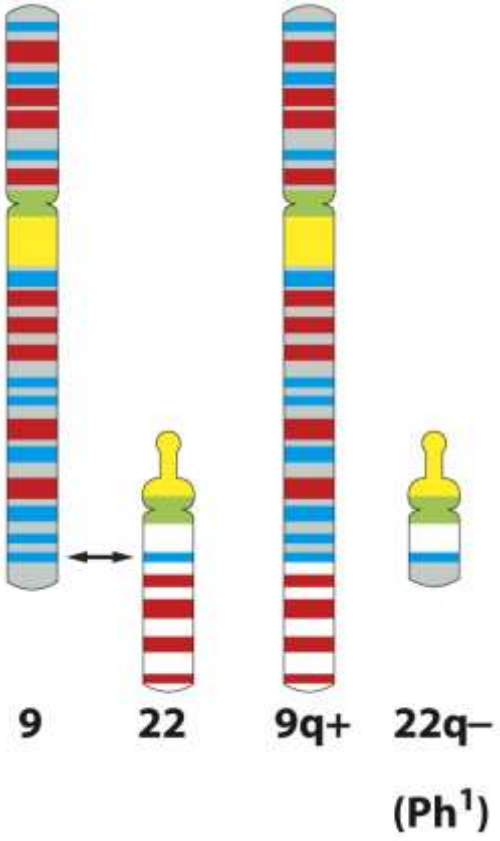


(Abelson murine leukemia virus)

(breakpoint cluster region)

Figure 4.15a *The Biology of Cancer* (© Garland Science 2007)

The great majority (> 95 %) of chronic myelogenous leukemia (CML) has t(9; 22) (q34; q11) translocation



Philadelphia chromosome (Ph^1)

Figure 2.23a *The Biology of Cancer* (© Garland Science 2007)

Different breakpoints in *bcr* results in different types of human leukemia

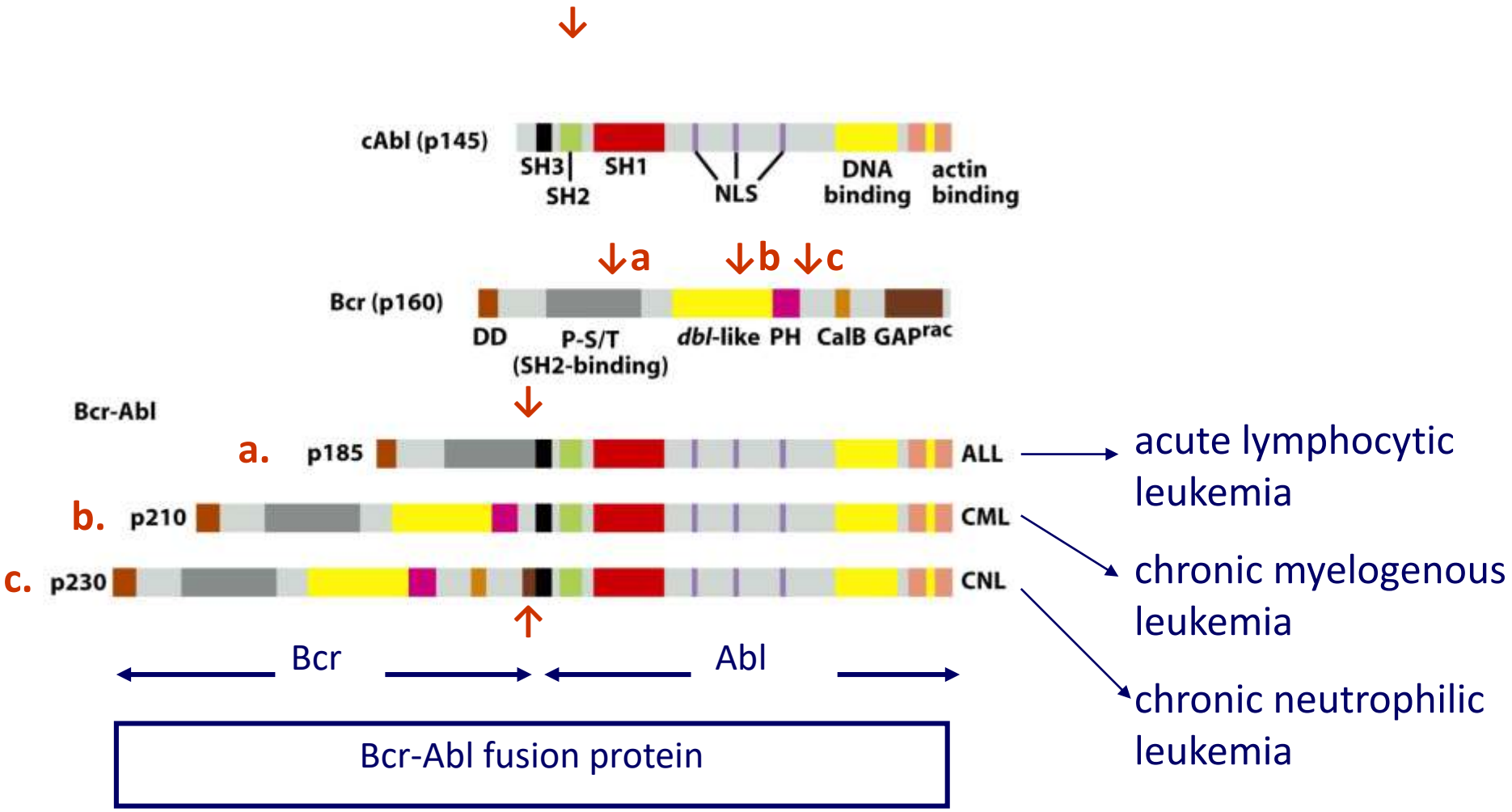


Figure 4.15b *The Biology of Cancer* (© Garland Science 2007)

Table 4.5 Translocations in human tumors that cause the formation of oncogenic fusion proteins of novel structure and function

Oncogene	Neoplasm
bcr/abl	chronic myelogenous leukemia; acute lymphocytic leukemia
dek/can	acute myeloid leukemia
E2A/pbx1	acute pre-B-cell leukemia
PML/RAR	acute promyelocytic leukemia
?/erg	myeloid leukemia
irel/urg	B-cell lymphoma
CBFβ/MYH11	acute myeloid leukemia
aml1/mtg8	acute myeloid leukemia
ews/fli	Ewing sarcoma
lyt-10/Cα1	B-cell lymphoma
hrx/enl	acute leukemias
hrx/af4	acute leukemias
NPM/ALK	large-cell lymphomas

Adapted from G.M. Cooper, Oncogenes, 2nd ed. Boston and London: Jones and Bartlett, 1995.

How To Make a Mammary-Gland Specific Transgenic Mouse

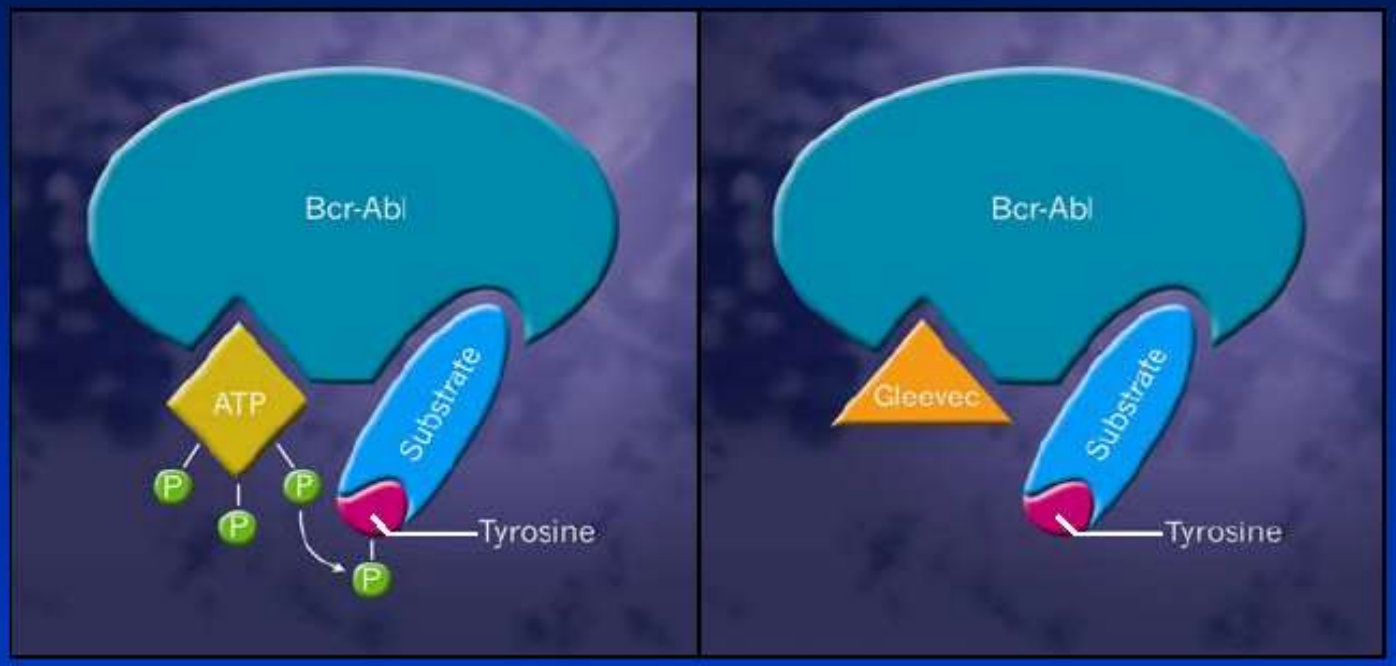
usually MMTV

mouse mammary tumor virus (MMTV)

Inject into isolated mouse nucleus

Check mammary gland of female progeny for increased expression of your gene

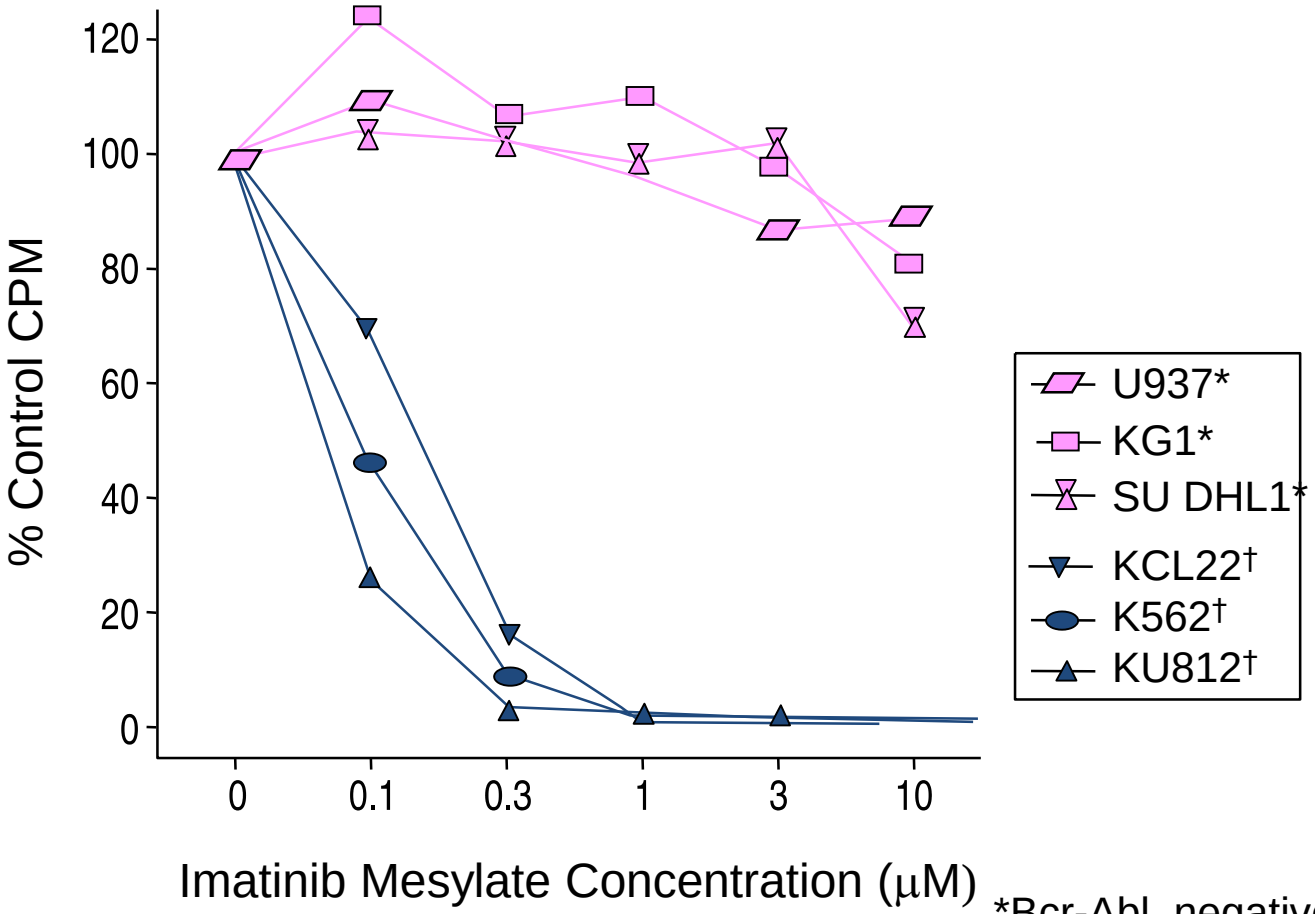
Gleevec® Targets the Cause of CML



- Gleevec—a specific inhibitor of a small family of tyrosine kinases, including Bcr-Abl, Kit, and PDGF receptor

STI571 (Gleevec, Novartis) is a 2-phenylamino pyrimidine derivate that competitively targets the adenosine 5′-triphosphate (ATP)–binding site of the kinase domain of *ABL* with high specificity

Imatinib Mesylate Inhibits the Growth of Bcr-Abl–Positive Cells



*Bcr-Abl–negative cell lines.

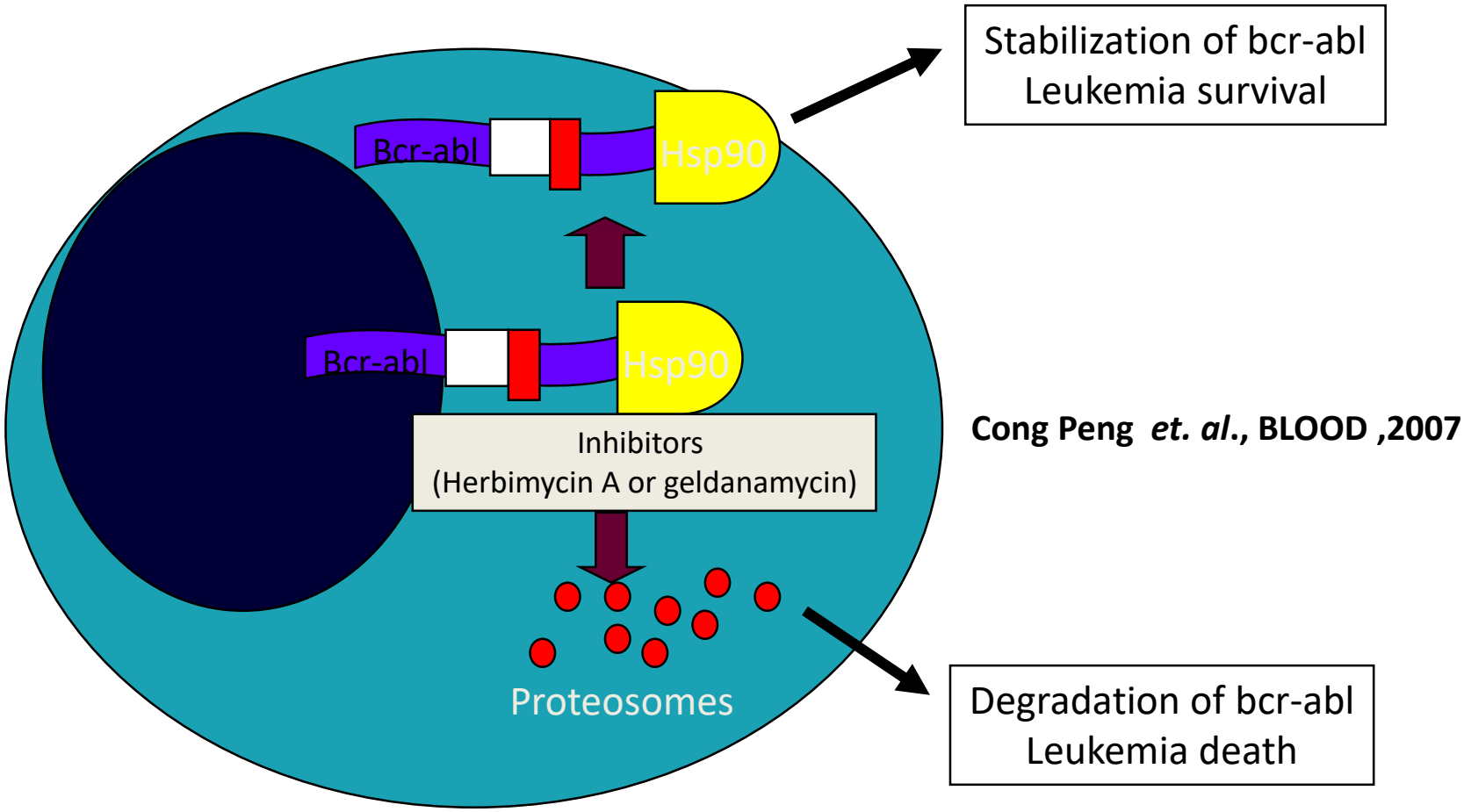
†Bcr-Abl–positive cell lines.

Gambacorti-Passerini C et al. *Blood Cells Mol Dis*. 1997;23:380-394.

New Approaches

1. Increasing Imatinib 800 mg/600 mg qd
2. Imatinib and PEG-INF –CGR 66%
3. Velcade(PROTEASE INHIBITOR) + Imatinib
4. Histone deacetylase inhibitor (SAHA) + Imatinib
5. Zolendronate +Imatinib

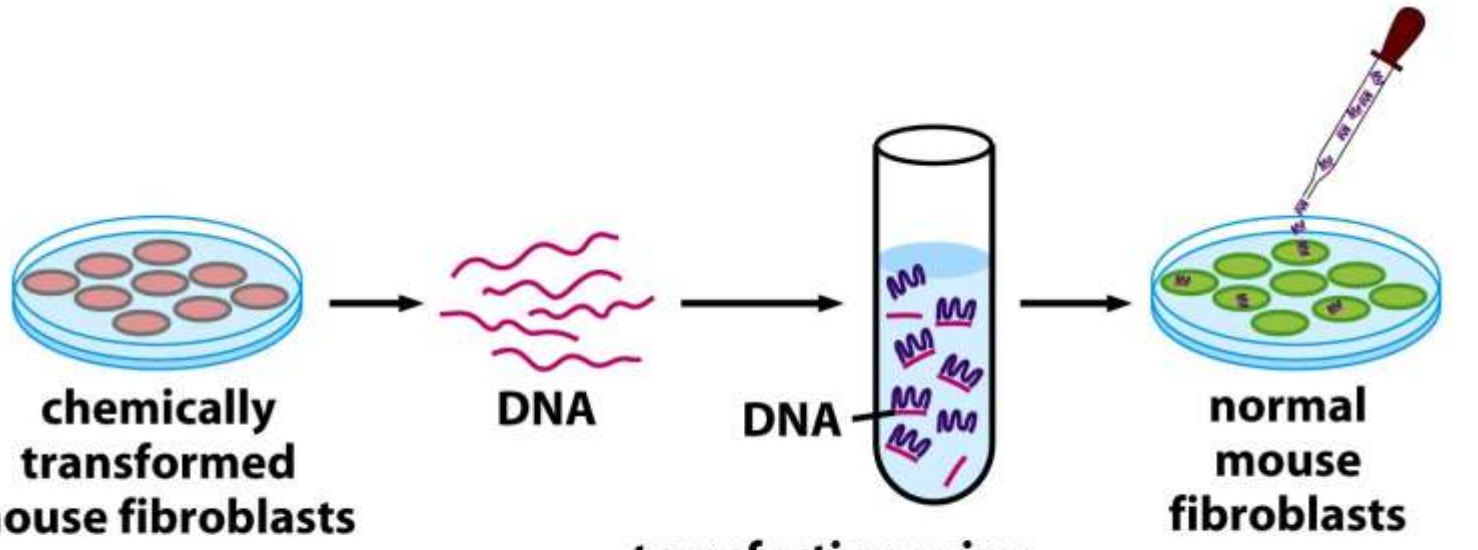
Selective apoptosis of CML by Hsp90 inhibitors



Cong Peng *et. al.*, BLOOD ,2007

Philadelphia chromosome–positive chronic myelogenous leukemia (CML) where all available kinase inhibitors in clinic are ineffective against the BCR-ABL mutant, T315I.
As an alternative approach to kinase inhibition, an orally administered heat shock protein 90 (Hsp90) inhibitor**, IPI-504**, was evaluated in a murine model of CML.

4.2 Transfection of DNA provides a strategy for detecting nonviral oncogenes

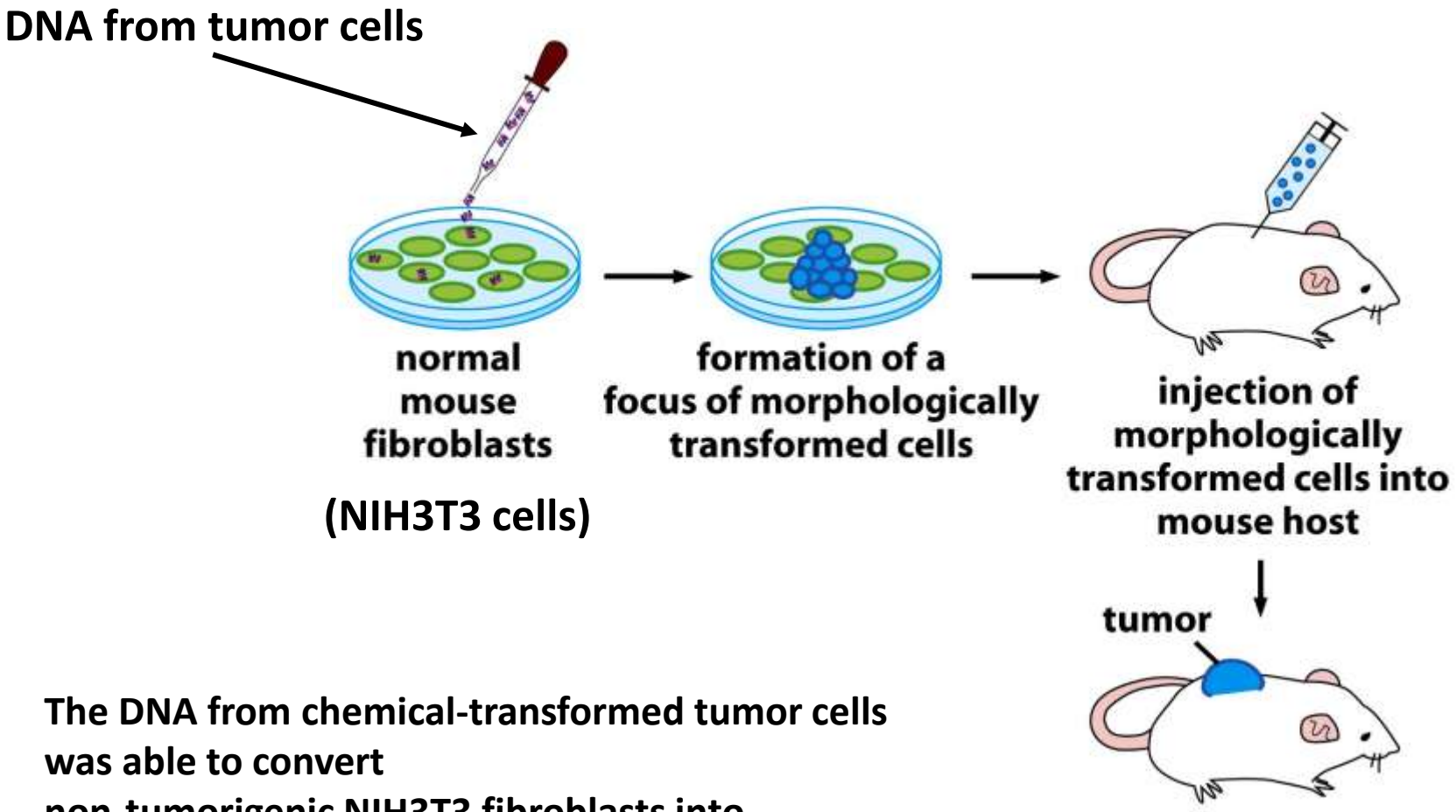


transformed by 3-methycholanthrene

Sidebar 4.2

Figure 4.2 (part 1 of 2) *The Biology of Cancer* (© Garland Science 2007)

Oncogenes discovered in human tumor cell lines are related to those carried by transforming retroviruses

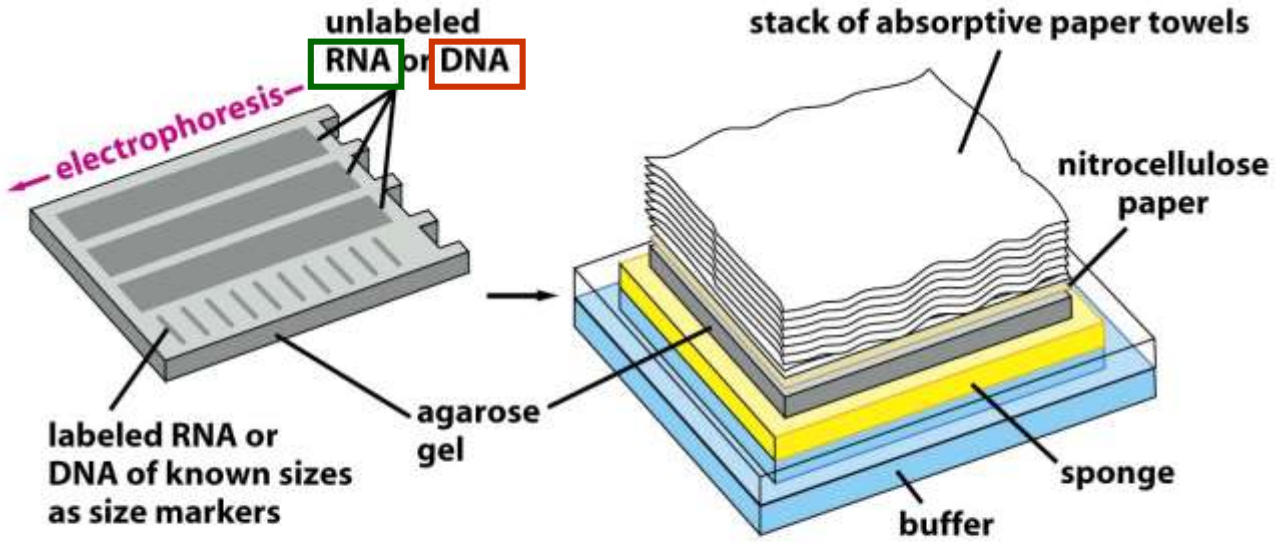


The DNA from chemical-transformed tumor cells was able to convert non-tumorigenic NIH3T3 fibroblasts into tumorigenic cells.

Figure 4.2 (part 2 of 2) *The Biology of Cancer* (© Garland Science 2007)

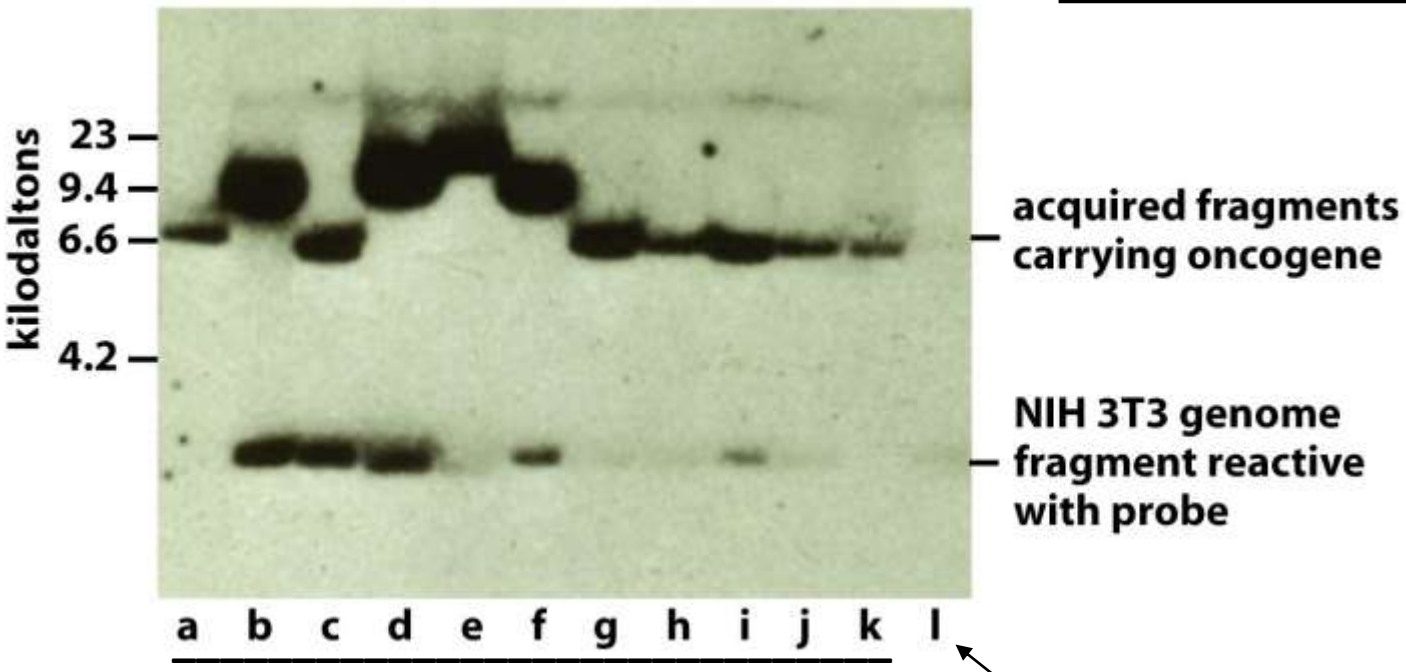
4.3 Oncogenes discovered in human tumor cell lines are related to those carried by transforming retroviruses

Southern blotting (DNA)
Northern blotting (RNA)



Homology between transfected oncogenes and retroviral oncogenes

probe used: H-ras oncogene present in Harvey rat sarcoma virus



11 (a ~ k) NIH3T3 cell lines transfected with DNA extracted from a **human** bladder carcinoma cell line

Figure 4.5 *The Biology of Cancer* (© Garland Science 2007)

Table 4.1 Examples of retrovirus-associated oncogenes that have been discovered in altered form in human cancers

Name of virus	Species	Oncogene	Type of oncoprotein	Homologous oncogene found in human tumors
Rous sarcoma	chicken	*src*	receptor TK	colon carcinoma[a]
Abelson leukemia	mouse	*abl*	nonreceptor TK	CML
Avian erythroblastosis	mouse	*erbB*	receptor TK	gastric, lung, breast[b]
McDonough feline sarcoma	cat	*fms*	receptor TK	AML[c]
H-Z feline	cat	*kit*	receptor TK[d]	gastrointestinal stromal
Murine sarcoma 3611	mouse	*raf*	Ser/Thr kinase[e]	bladder carcinoma
Simian sarcoma	monkey	*sis*	growth factor (PDGF)	many types[f]
Harvey sarcoma	mouse/rat	H-*ras*[g]	small G protein	bladder carcinoma
Kirsten sarcoma	mouse/rat	K-*ras*[g]	small G protein	many types
Avian erythroblastosis	chicken	*erbA*	nuclear receptor[h]	liver, kidney, pituitary
Avian myeloblastosis E26	chicken	*ets*	transcription factor	leukemia[i]
Avian myelocytoma	chicken	*myc*[j]	transcription factor	many types
Reticuloendotheliosis	turkey	*rel*[k]	transcription factor	lymphoma

[a]Mutant forms found in a small number of these tumors.
[b]Receptor for EGF; the related erbB2/HER2/Neu protein is overexpressed in 30% of breast cancers.
[c]Fms, the receptor for colony-stimulating factor (CSF-1), is found in mutant form in a small number of AMLs; the related Flt3 (Fms-like tyrosine kinase-3) protein is frequently found in mutant form in these leukemias.
[d]Receptor for stem cell factor.
[e]The closely related B-Raf protein is mutant in the majority of melanomas.
[f]Protein is overexpressed in many types of tumors.
[g]The related N-*ras* gene is found in mutant form in a variety of human tumors.
[h]Receptor for thyroid hormone.
[i]27 distinct members of the Ets family of transcription factors are encoded in the human genome. Ets-1 is overexpressed in many types of tumors; others are involved in chromosomal translocations in AML and in Ewing sarcomas.
[j]The related N-*myc* gene is overexpressed in pediatric neuroblastomas and small-cell lung carcinomas.
[k]Rel is a member of a family of proteins that constitute the NF-κB transcription factor, which is constitutively activated in a wide range of human tumors.

Adapted in part from J. Butel, *Carcinogenesis* 21:405–426, 2000; and G.M. Cooper, Oncogenes, 2nd ed. Boston and London: Jones and Bartlett, 1995.

TK : tyrosine kinase

Table 4.1 *The Biology of Cancer* (© Garland Science 2007)

ErbB- Gene symbol

- The gene symbol, ErbB, is derived from the name of a viral oncogene to which these receptors are homologous: Erythroblastic Leukemia Viral Oncogene.

- v-ErbBs are homologous to EGFR, but lack sequences within the ligand binding ectodomain.

- Insufficient ErbB signaling in humans is associated with the development of neurodegenerative diseases, such as multiple sclerosis and Alzheimer's Disease.

Receptor Tyrosine Kinases

- The Receptor Tyrosine Kinases (RTKs) are membrane receptors.

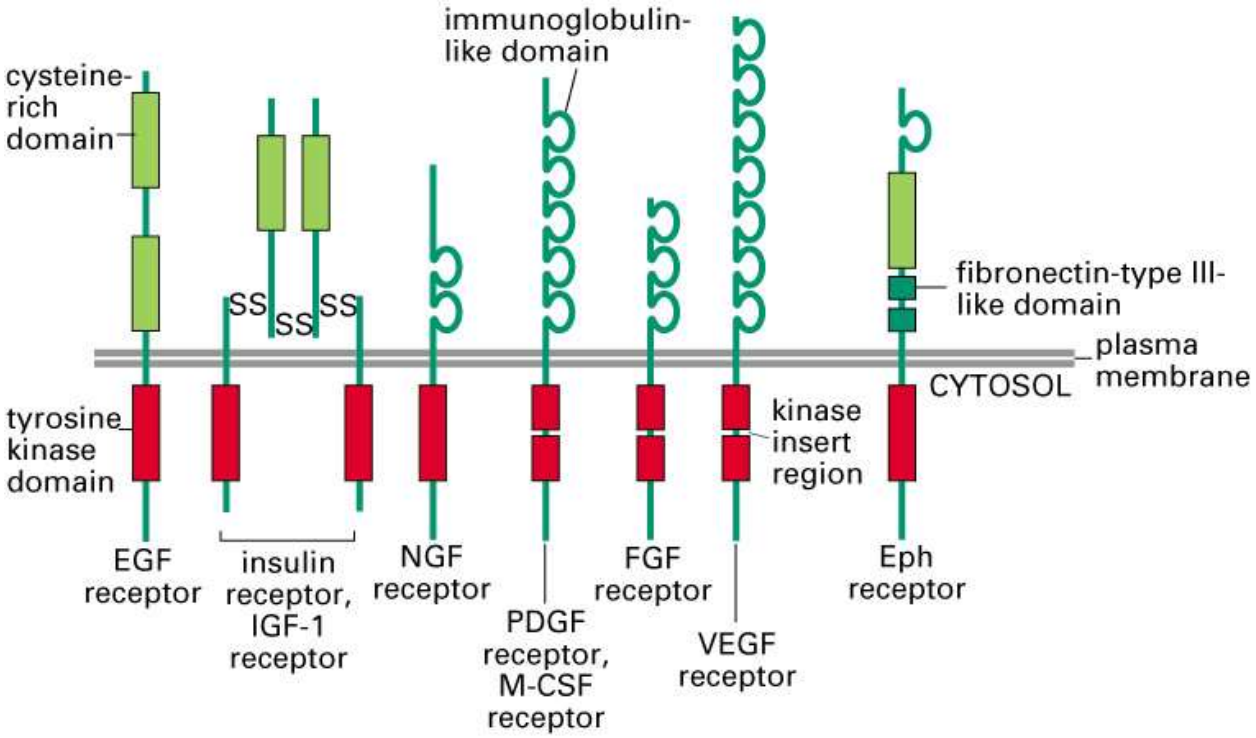
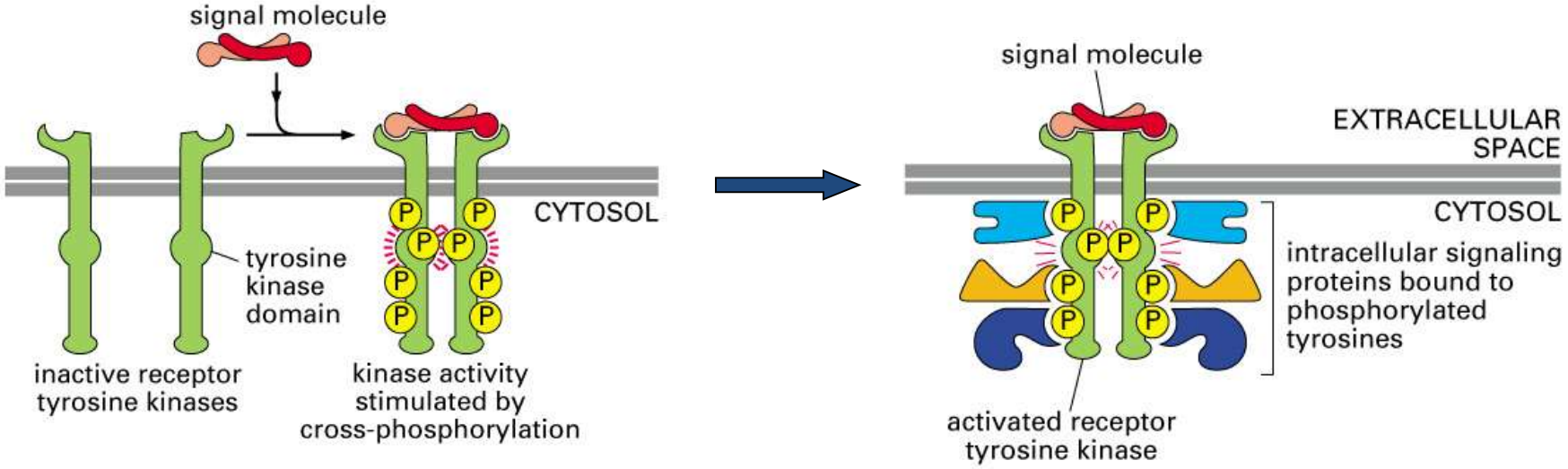


Figure 15–49. Molecular Biology of the Cell, 4th Edition.

Receptor Tyrosine Kinases

- Most of these recepors are activated by ligand-induced dimerization, resulting in increased kinase activity of the TK domain.

- This results in trans-autophosphorylation of the cytoplasmic domains.

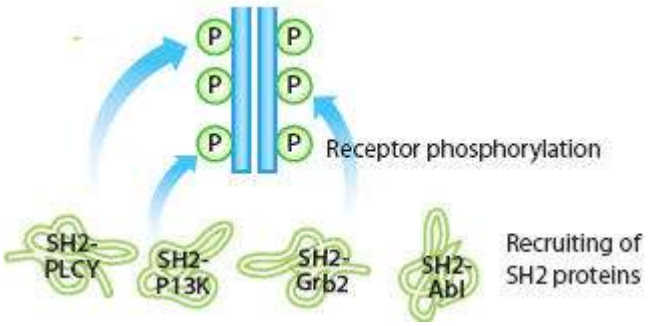


Receptor Tyrosine Kinases

- Most of these recepors are activated by ligand-induced dimerization, resulting in increased kinase activity of the TK domain.

- This results in trans-autophosphorylation of the cytoplasmic domains.

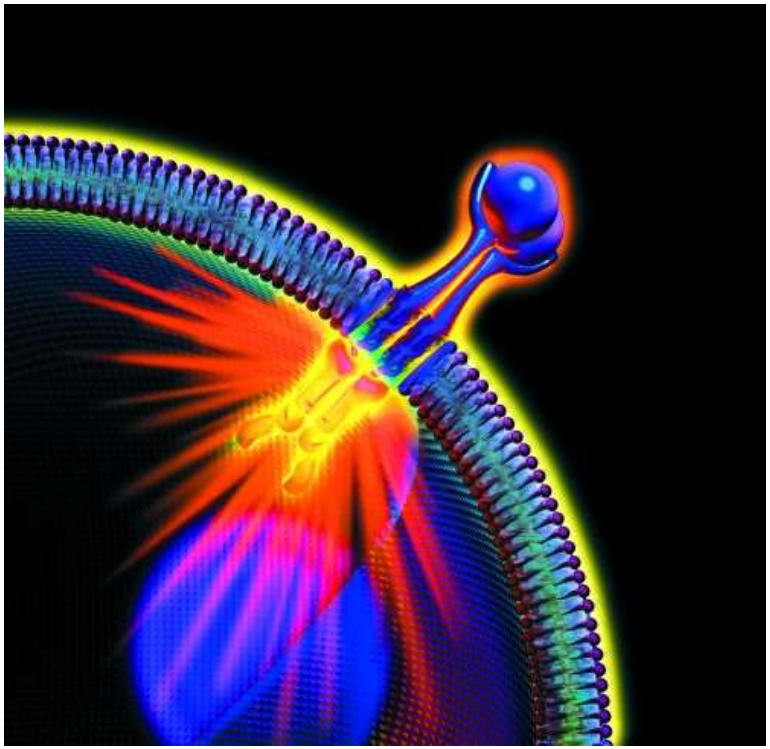
- The phospho-tyrosines can then recruit SH2 domains of different signaling proteins.



EGFR

- EGFR is an RTK protein. It was the first receptor that was linked directly to cancer.

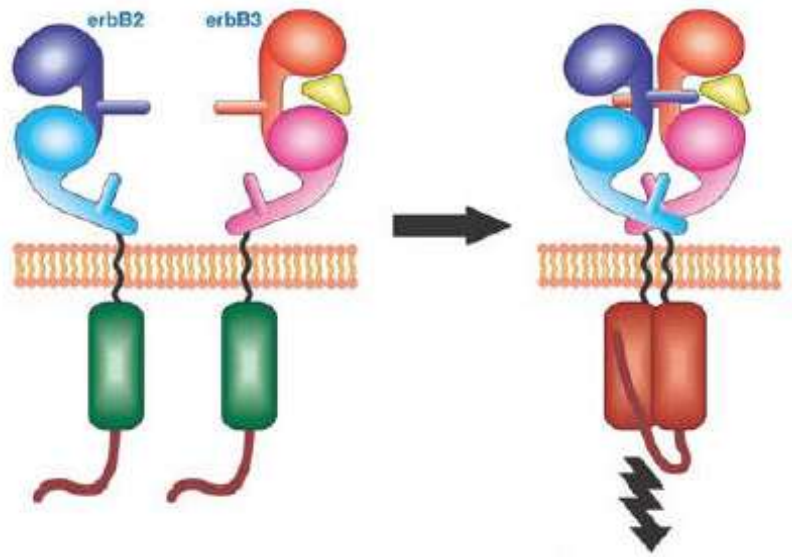
- Mutations in EGFR which result in overactivity are associated with several types of cancers.



The ErbB family

- EGFR belongs to the ErbB family which contains four RTKs.

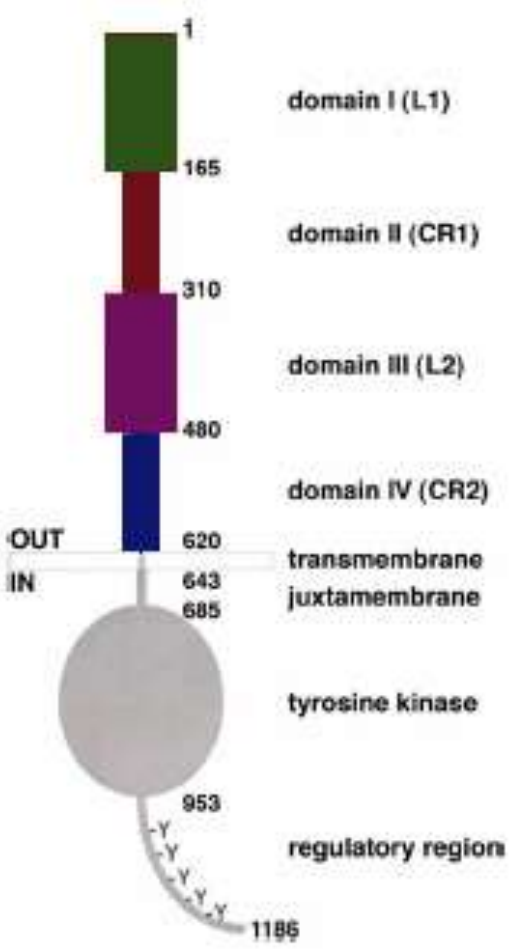
- They are capable of forming homo- or heterodimers and possibly higher-order oligomers, following activation by their ligands.



The ErbB protein family consists of 4 members

- **Epidermal growth factor receptor (EGFR) family**
- ErbB-1, also named epidermal growth factor receptor (EGFR)
- ErbB-2 stands for "Human Epidermal growth factor Receptor 2" and is a protein giving higher aggressiveness in breast cancers.
- Also named , **HER2/neu** (HER2 in humans and neu in rodents)
- ErbB-3, also named HER3
- ErbB-4, also named HER4

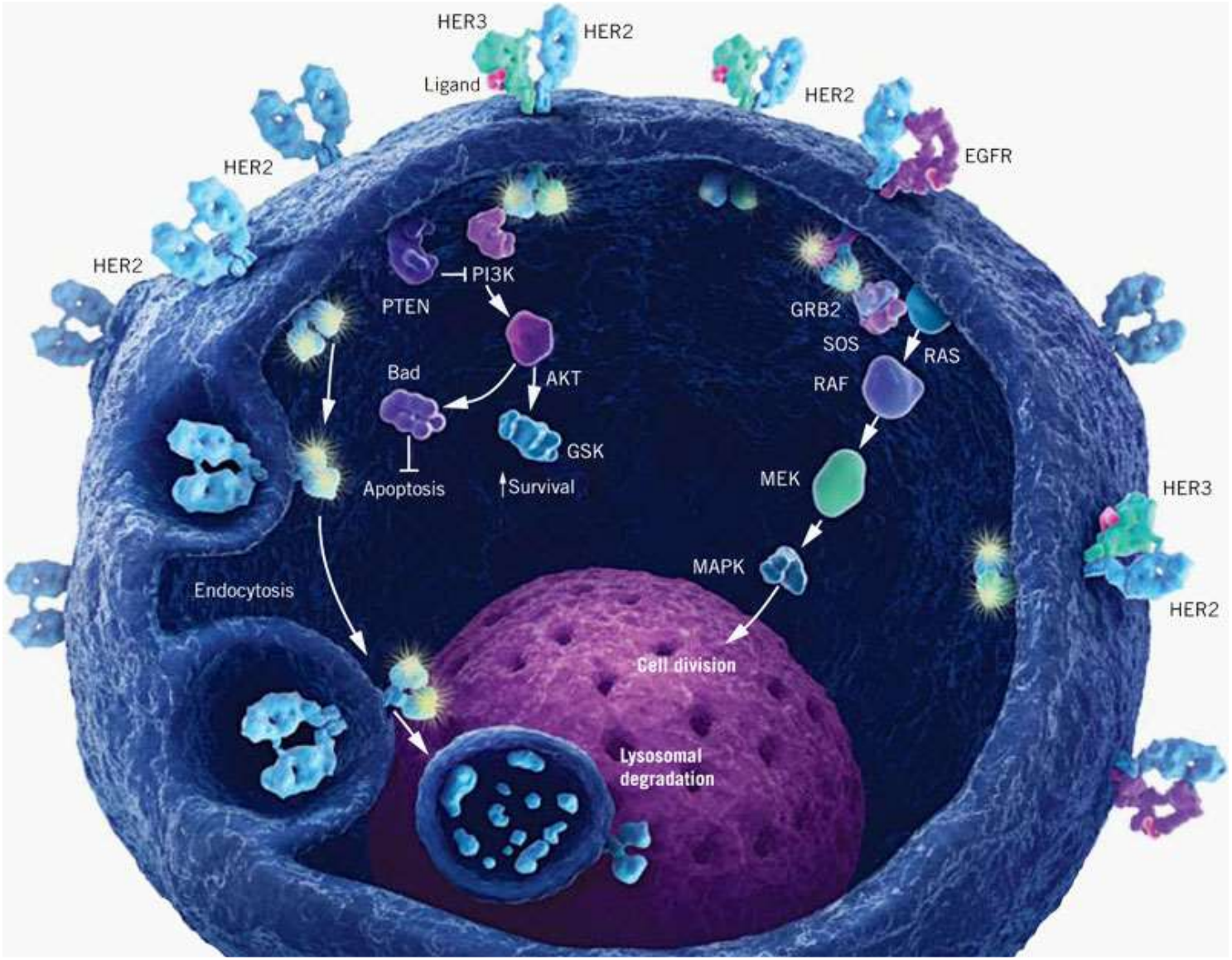
Domain organization



The four erbB receptors are closely related glycoproteins. They consist of:

1) An extracellular ligand binding region (**620 residues**)
2) A single transmembrane domain (**23 residues**)
3) Juxtamembrane (**40 residues**)
4) An intracellular tyrosine kinase domain (**260 residues**)
5) C-terminal regulatory region (**232 residues**)

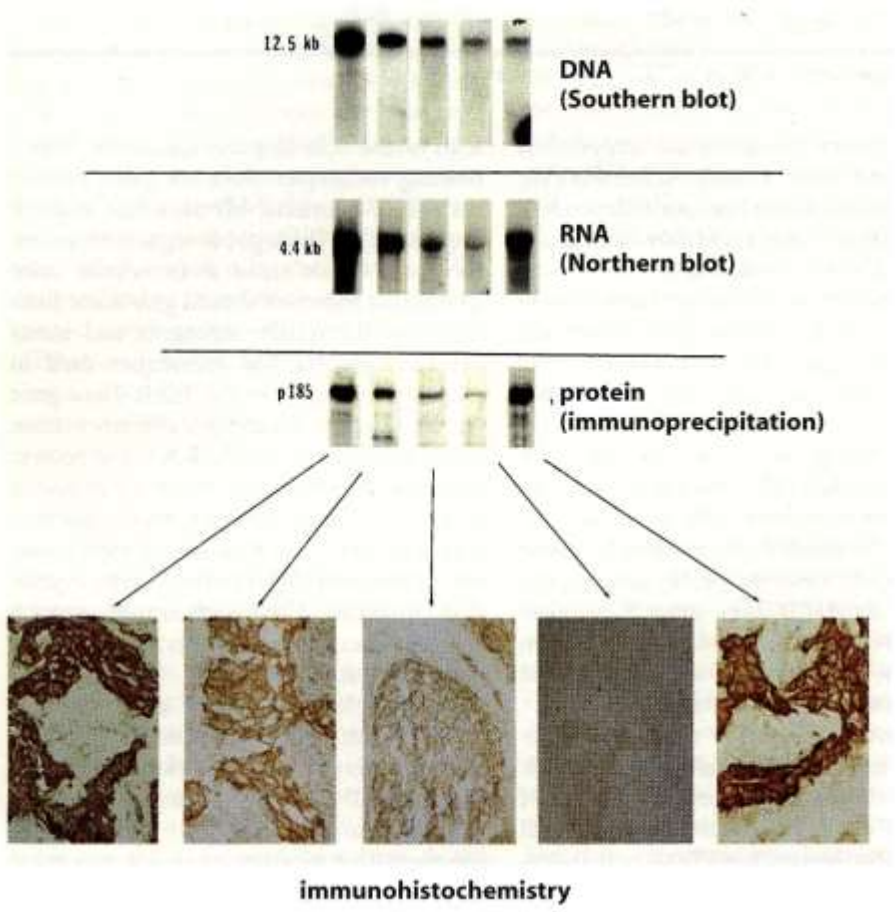
ErbB2/ErbB3 in cancer



ErbB

- ErbB-1 is overexpressed in many cancers.
- Drugs such as panitumumab, cetuximab, gefitinib, erlotinib are used to inhibit it.
- ErbB-2 (HER-2) is often overexpressed in breast cancer.
- It is revealed that patients with ER+/HER2+ breast cancers may actually benefit more from drugs that inhibit the PI3K/AKT molecular pathway

erbB2/HER2/neu oncogene can be amplified or overexpressed in human breast carcinoma cells



erbB2/neu oncogene is amplified

erbB2/neu mRNA is overexpressed

increased level of *erbB2/neu*-encoded protein

Figure 4.6c *The Biology of Cancer* (© Garland Science 2007)

Dr. Dennis Slamon and the book *HER-2: The Making of Herceptin, a Revolutionary Treatment for Breast Cancer* by Robert Bazell.

- Breast Cancer drug Herceptin, over the course of 8 years from 1988 to 1996.
- Dr. Slamon is a research doctor at UCLA Medical Center (Los Angeles), where he has developed the experimental drug Herceptin, which he believes will become a treatment for breast cancer.

Thanks for your attention

Acknowledgement

- The presentation is being used for educational and non-commercial purposes.
- Thanks are due to all the original contributors and entities whose pictures were used in the creation of this presentation.