Growth Factors, Receptors, and Cancer

Cancer Biology I (PATH4500)

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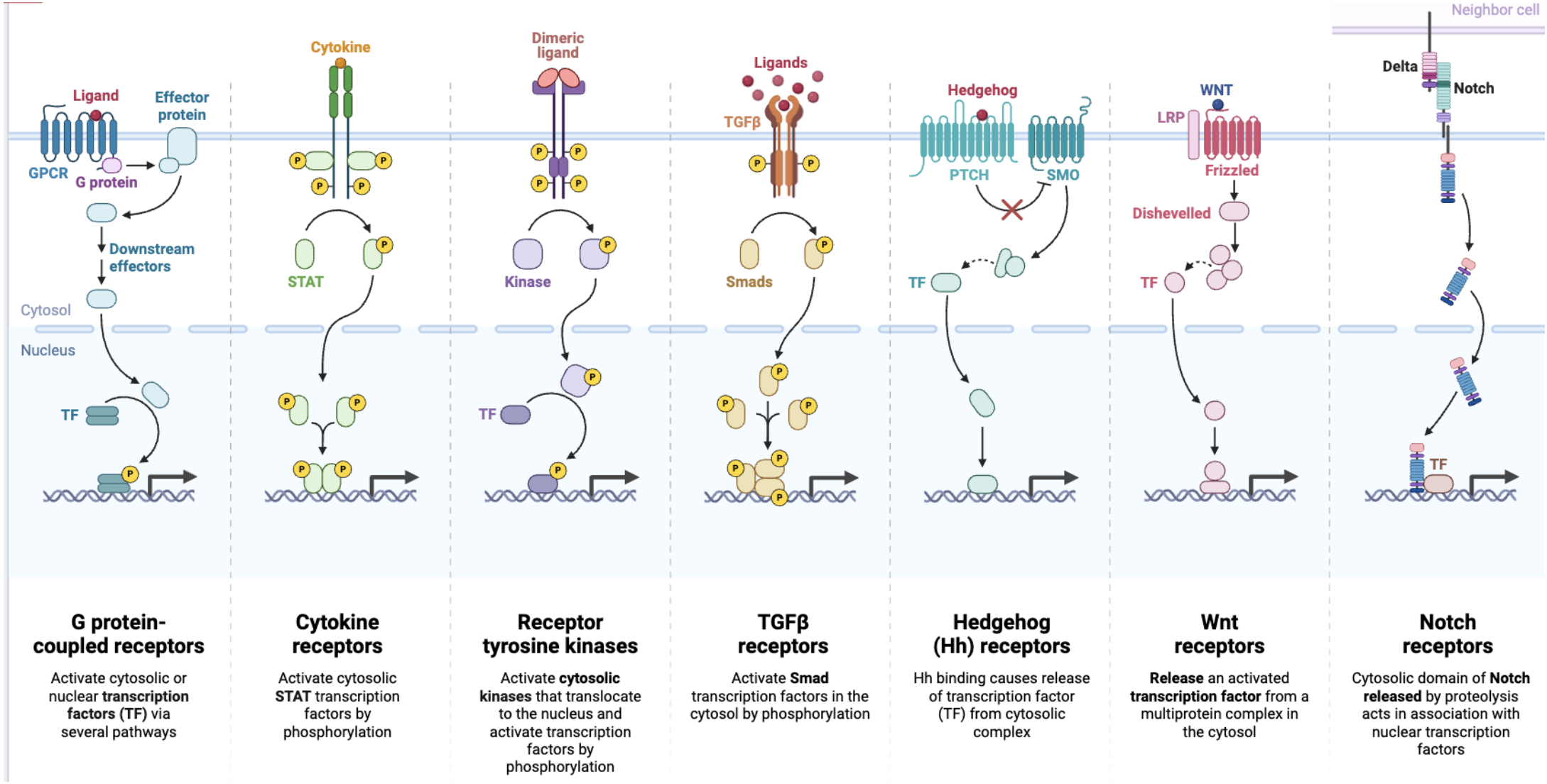
Growth Factors, Receptors and Cancer

Why do we need to study about growth factors, their receptors and associated signaling pathways?

- Deregulation of these signaling is central to the formation of cancer
- Antagonists of the receptors and mediators are used as **targeted therapies** for various cancers

Signaling pathways



G protein-coupled receptors

Activate cytosolic or nuclear **transcription factors (TF)** via several pathways

Cytokine receptors

Activate cytosolic **STAT** transcription factors by phosphorylation

Receptor tyrosine kinases

Activate **cytosolic kinases** that translocate to the nucleus and activate transcription factors by phosphorylation

TGFβ receptors

Activate **Smad** transcription factors in the cytosol by phosphorylation

Hedgehog (Hh) receptors

Hh binding causes release of transcription factor (TF) from cytosolic complex

Wnt receptors

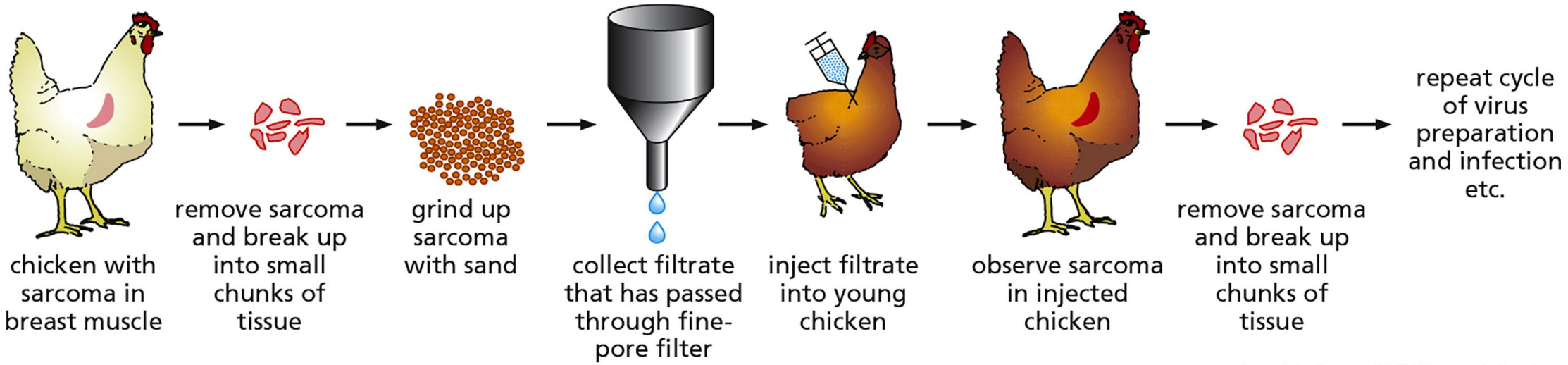
Release an activated **transcription factor** from a multiprotein complex in the cytosol

Notch receptors

Cytosolic domain of **Notch released** by proteolysis acts in association with nuclear transcription factors

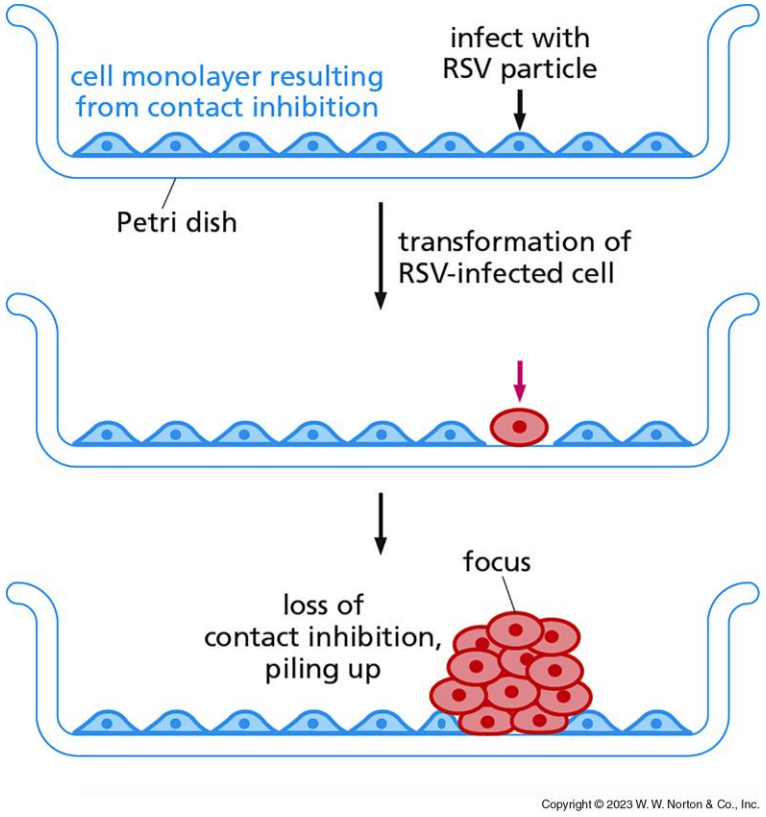
Biorender

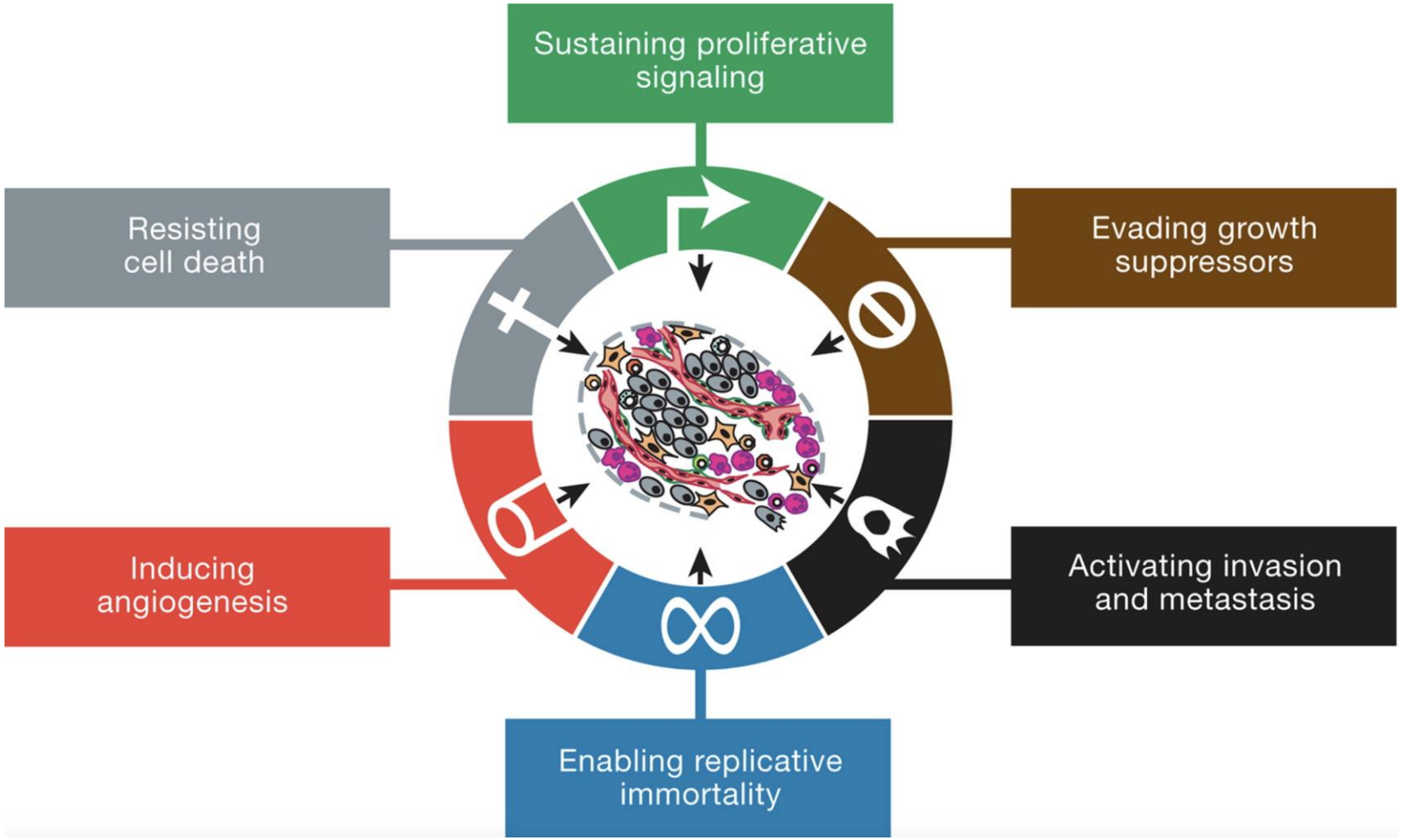
Rous's protocol to induce sarcoma in chickens



Rous Sarcoma Virus (RSV)

Hallmarks of cancer





Hanahan and Weinberg: Cell, 2011; 144(5): 646-74

How does an oncoprotein transform a normal cell into a cancer cell?

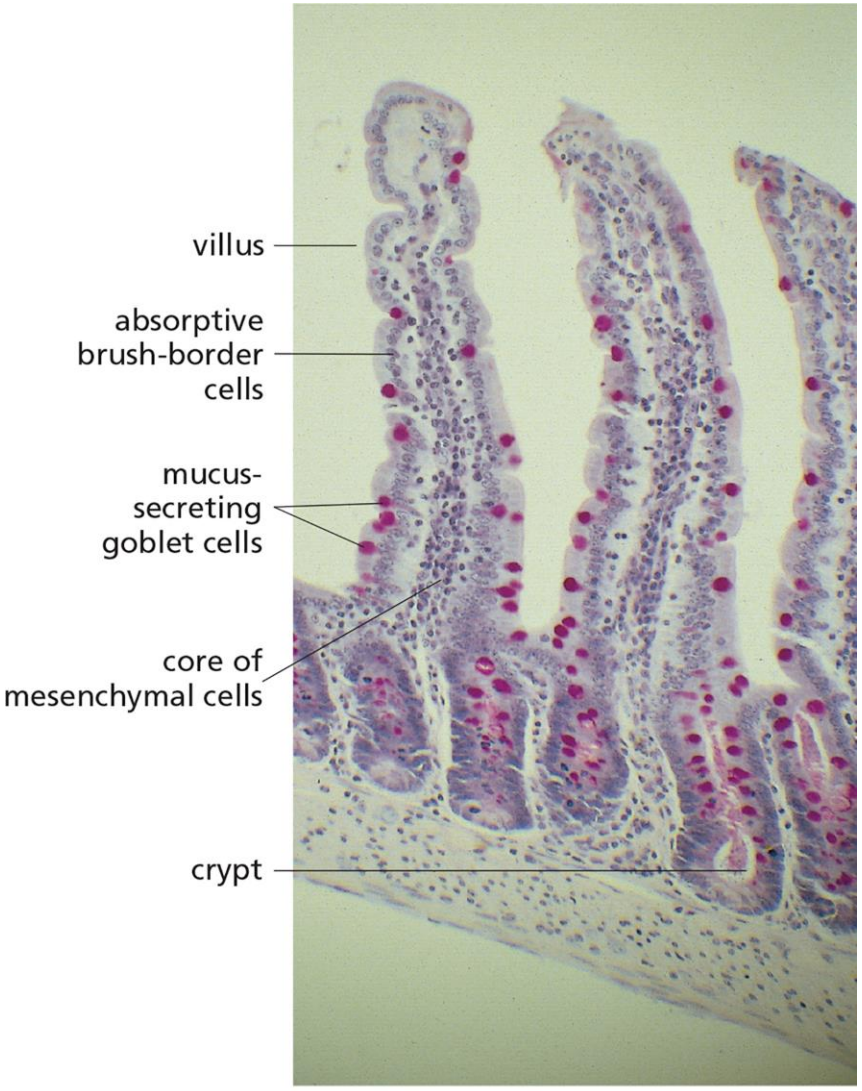
- How do oncoproteins perturb cell behavior so profoundly to transform a normal cell into a cancer cell?
- How could a single protein change so many regulatory pathways in the cell?

Studies of how normal cells regulate their growth and division

Normal cells receive growth-stimulatory signals from their surroundings. These signals are processed and integrated by complex circuits within the cell, which decide whether or not cell growth and division are appropriate.

Normal metazoan cells control each other's lives

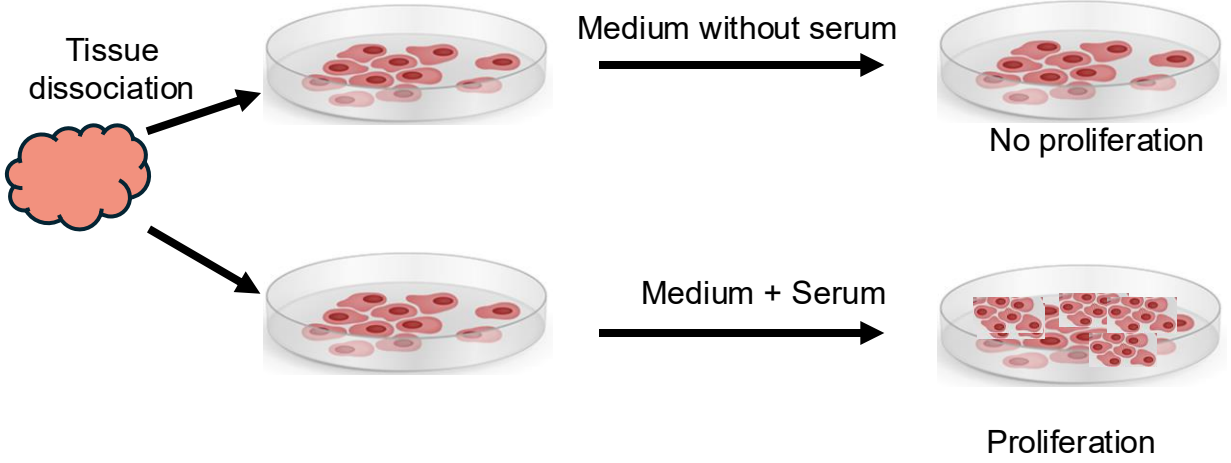
Several distinct cell types coexist in the epithelial lining of small intestine



(From B. Alberts et al., Molecular Biology of the Cell, 6th ed. New York: W.W. Norton, 2015.)

The relative positions and numbers of each cell type must be tightly regulated

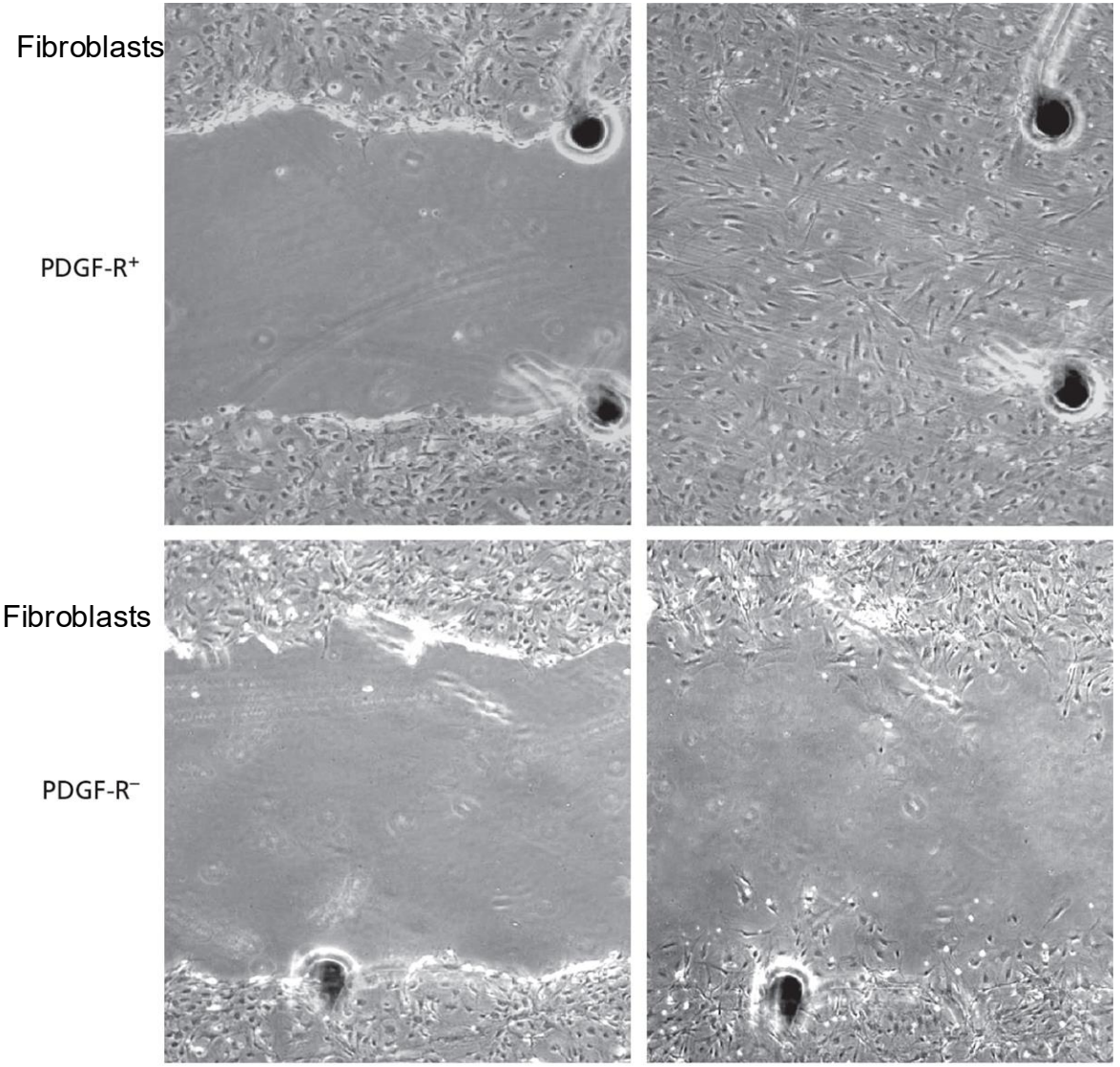
Effect of Growth Factors on cells



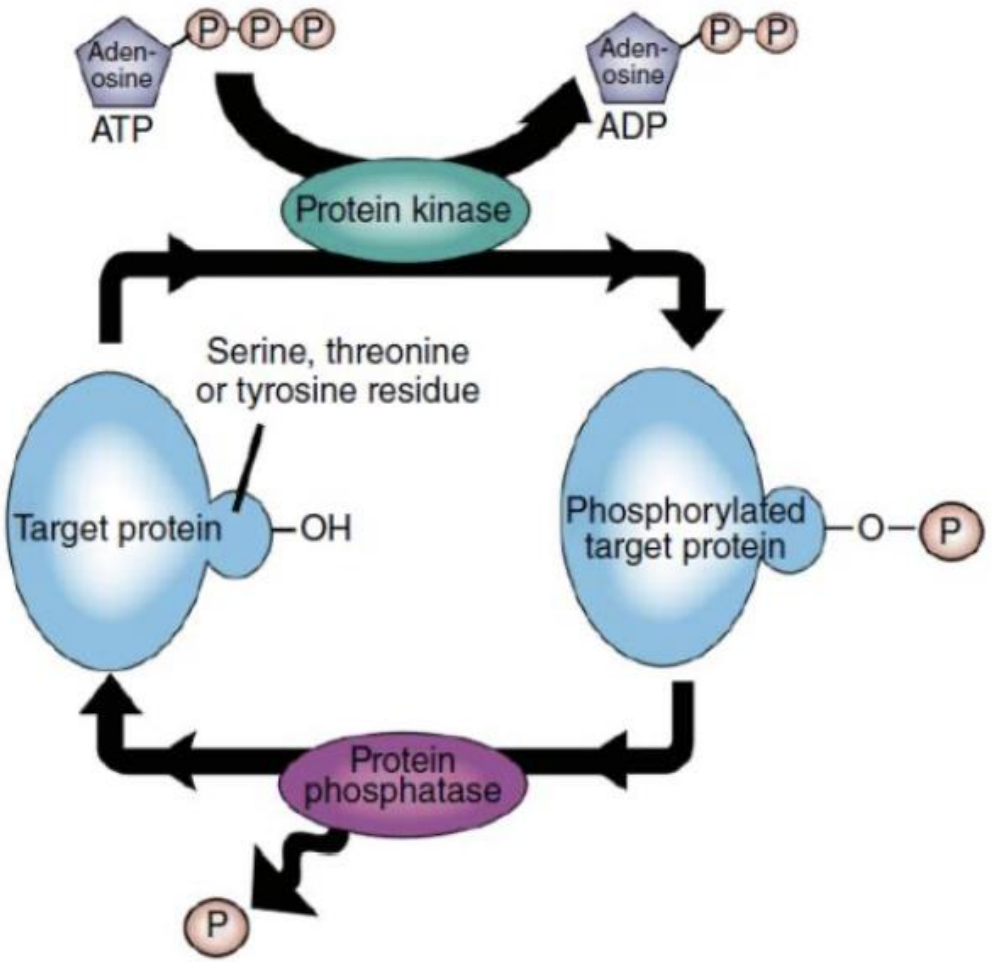
Serum contains growth factors:
PDGF, EGF, FGF2, VEGF, IGF, TGF-β1

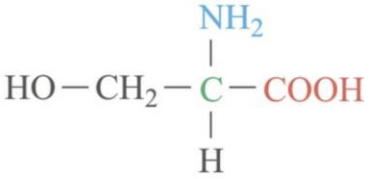
Mitogen : Stimulates cell division

Dependence of fibroblasts on growth-stimulatory signals released by a second cell type (in this case platelets)

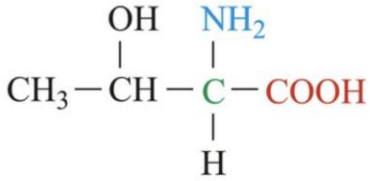


Protein kinase

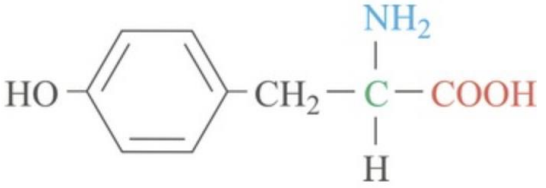




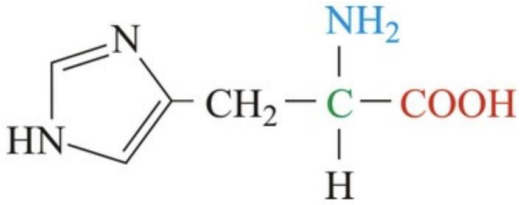
Serine (S)



Threonine (T)

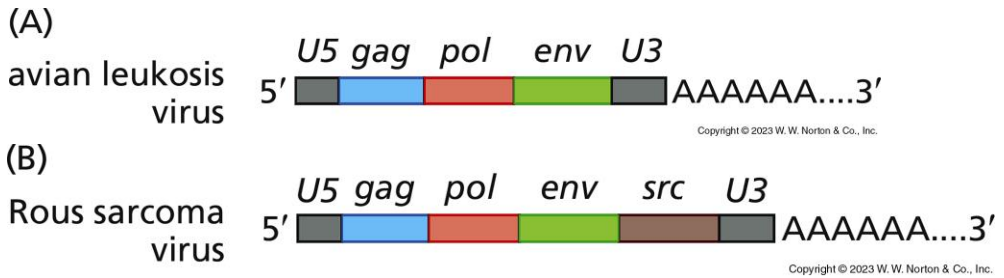


Tyrosine (Y)



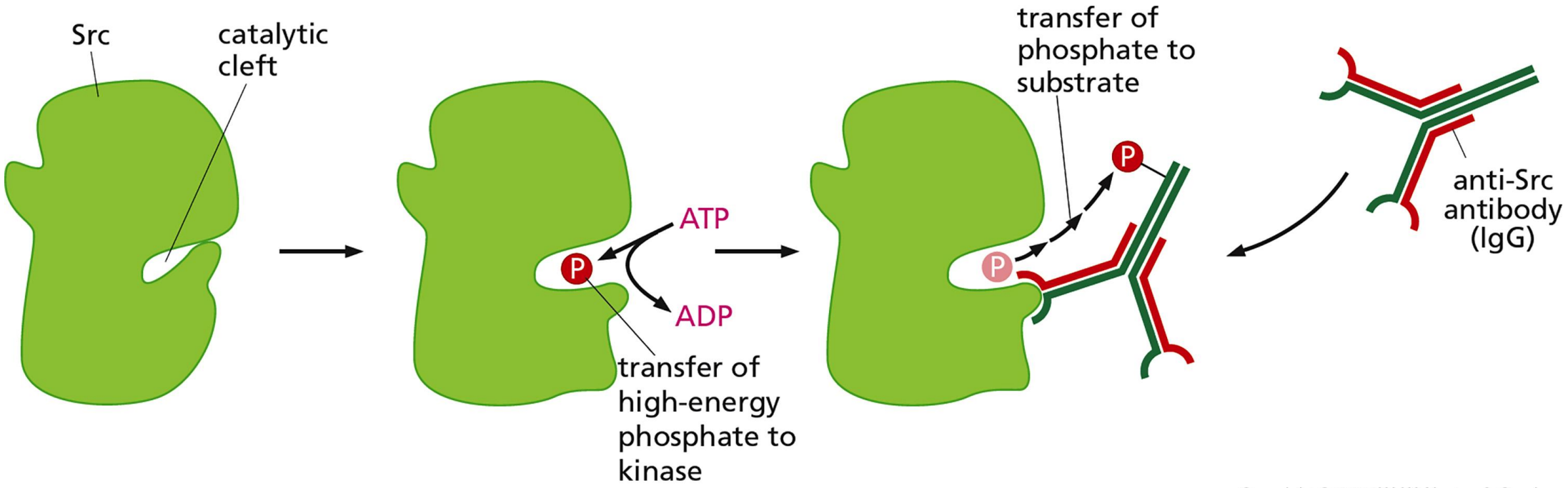
Histidine(H)

https://www.creative-diagnostics.com/intracellular-kinases-and-signal-transduction.htm

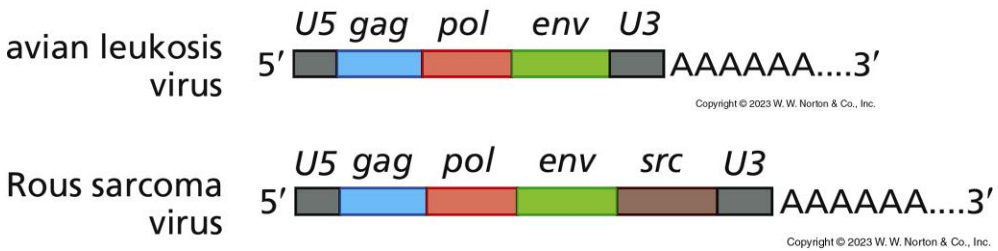


How does Src oncoprotein transform a normal cell into a cancer cell?

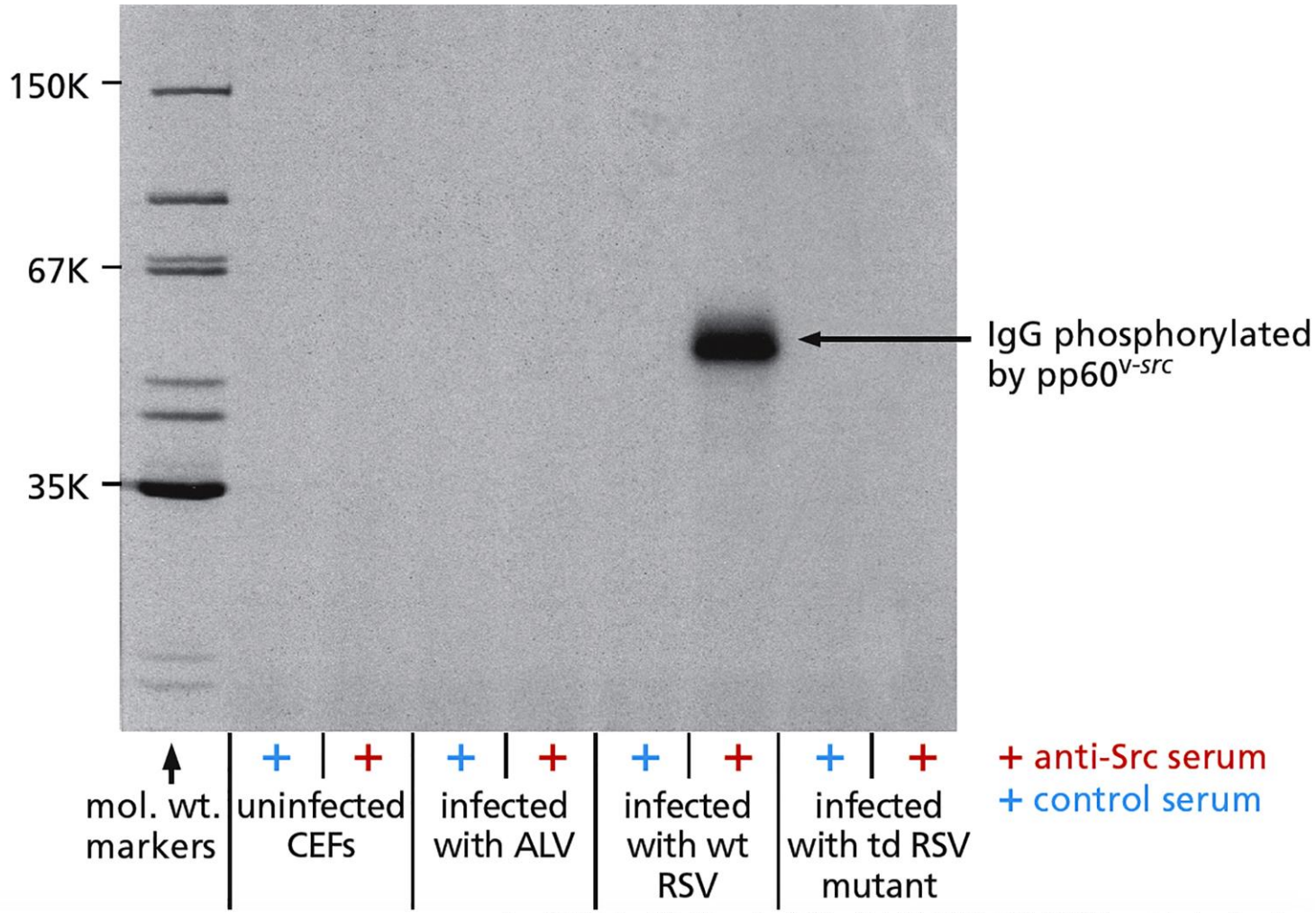
Src and protein kinase



(A)

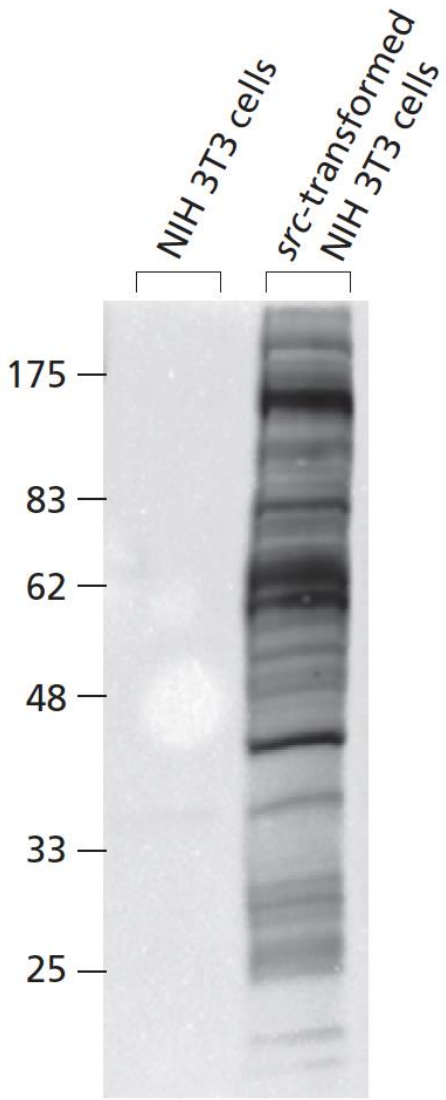


(B)



From M.S. Collett and R.L. Erikson, *Proc. Natl. Acad. Sci.* USA 75:2021–2024, 1978. With permission from the authors.

Protein tyrosine phosphorylation by Src transformation



Western blot: Using Anti-phospho-Tyrosine antibody

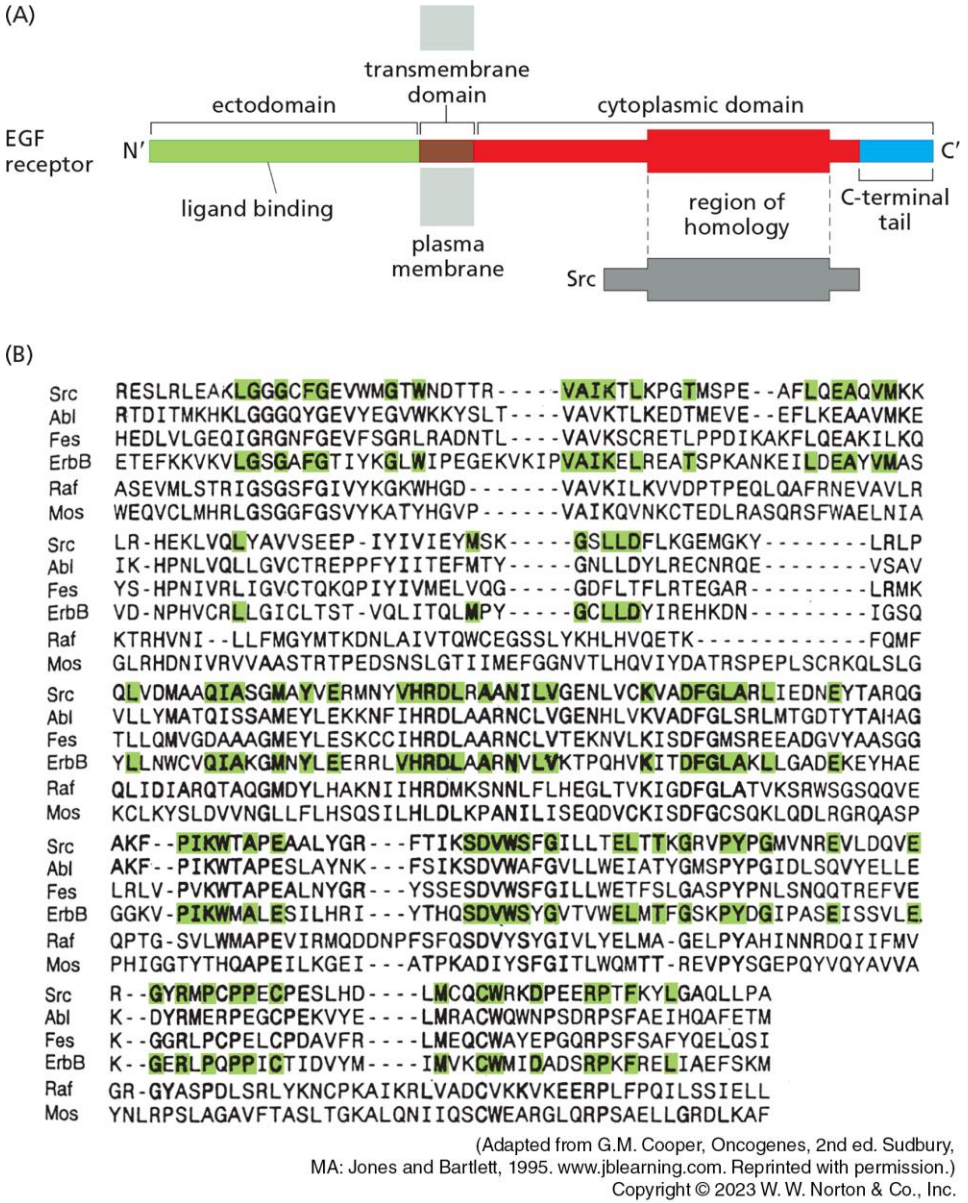
Src: PP60^{src}

- Src oncoprotein functions as a protein kinase (by its ability to phosphorylate protein substrates within cells)
- Src is itself a phosphoprotein
- Either Src autophosphorylates itself or a substrate of another protein kinase
- More than 50 substrates of Src have been enumerated
- Once phosphorylated, each of these substrate proteins may alter the functions of its own sets of downstream targets
- Explains how Src pleiotropically perturbs multiple cell phenotypes

Structural homology of EGF-receptor and Src and comparison of their amino acid sequence

- Epidermal growth factor (EGF) is the first to be discovered
- EGF was known for its ability to provoke premature eye opening in newborn mice
- EGF was found to have mitogenic effect when applied to a variety of epithelial cells
- EGF was able to bind to surface of cells whose growth it stimulated
- Cells to which EGF was unable to bind were unresponsive to its mitogenic effects

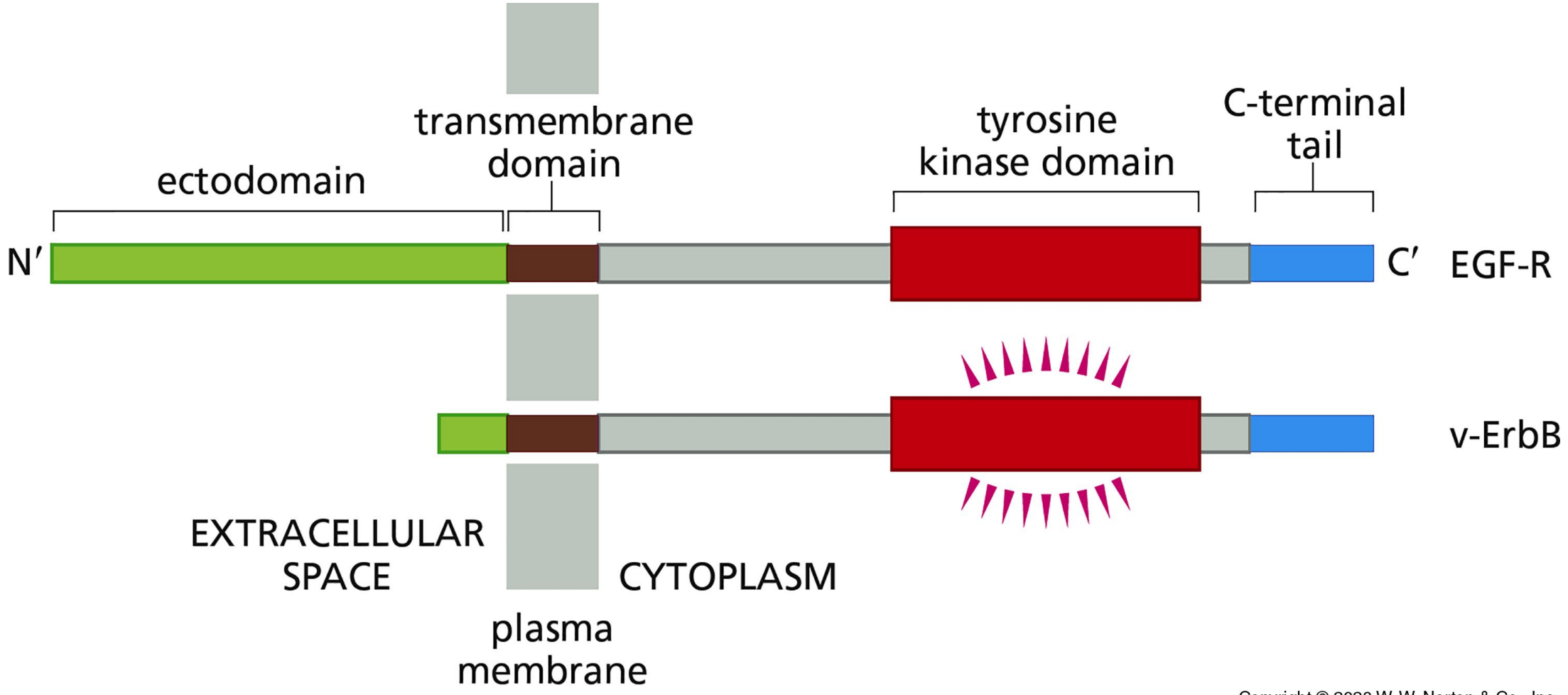
EGF receptor was purified and sequenced



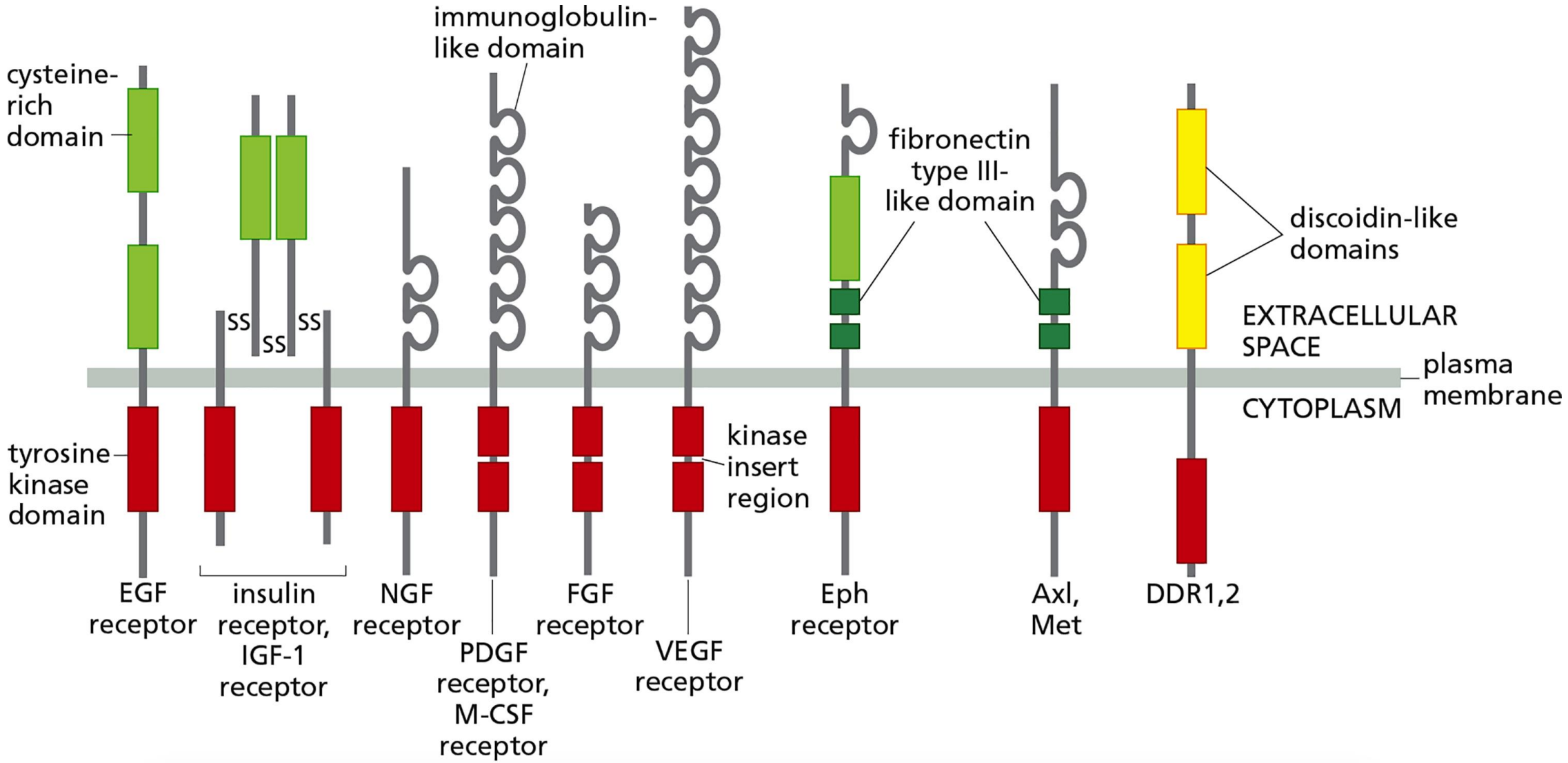
(Adapted from G.M. Cooper, Oncogenes, 2nd ed. Sudbury, MA: Jones and Bartlett, 1995. www.jblearning.com. Reprinted with permission.)

Altered growth factor receptor can function as an oncoprotein

In 1984: *erbB* oncogene in avian erythroblastosis virus (AEV) genome closely related to EGF receptor



Structure of tyrosine kinase receptors



(From B. Alberts et al., Molecular Biology of the Cell, 6th ed. New York: W.W. Norton, 2015.)

Name of GF	Name of receptor	Cells responding to GF
PDGF[a]	PDGF-R	endothelial, VSMCs, fibroblasts, other mesenchymal cells, glial cells
EGF[b]	EGF-R[c]	many types of epithelial cells, some mesenchymal cells
NGF	Trk	neurons
FGF[d]	FGF-R[e]	endothelial, fibroblasts, other mesenchymal cells, VSMCs, neuroectodermal cells
HGF/SF	Met	various epithelial cells
VEGF[f]	VEGF-R[g]	endothelial cells in capillaries, lymph ducts
IGF[h]	IGF-R1	wide variety of cell types
GDNF	Ret	neuroectodermal cells
SCF	Kit	hematopoietic, mesenchymal cells

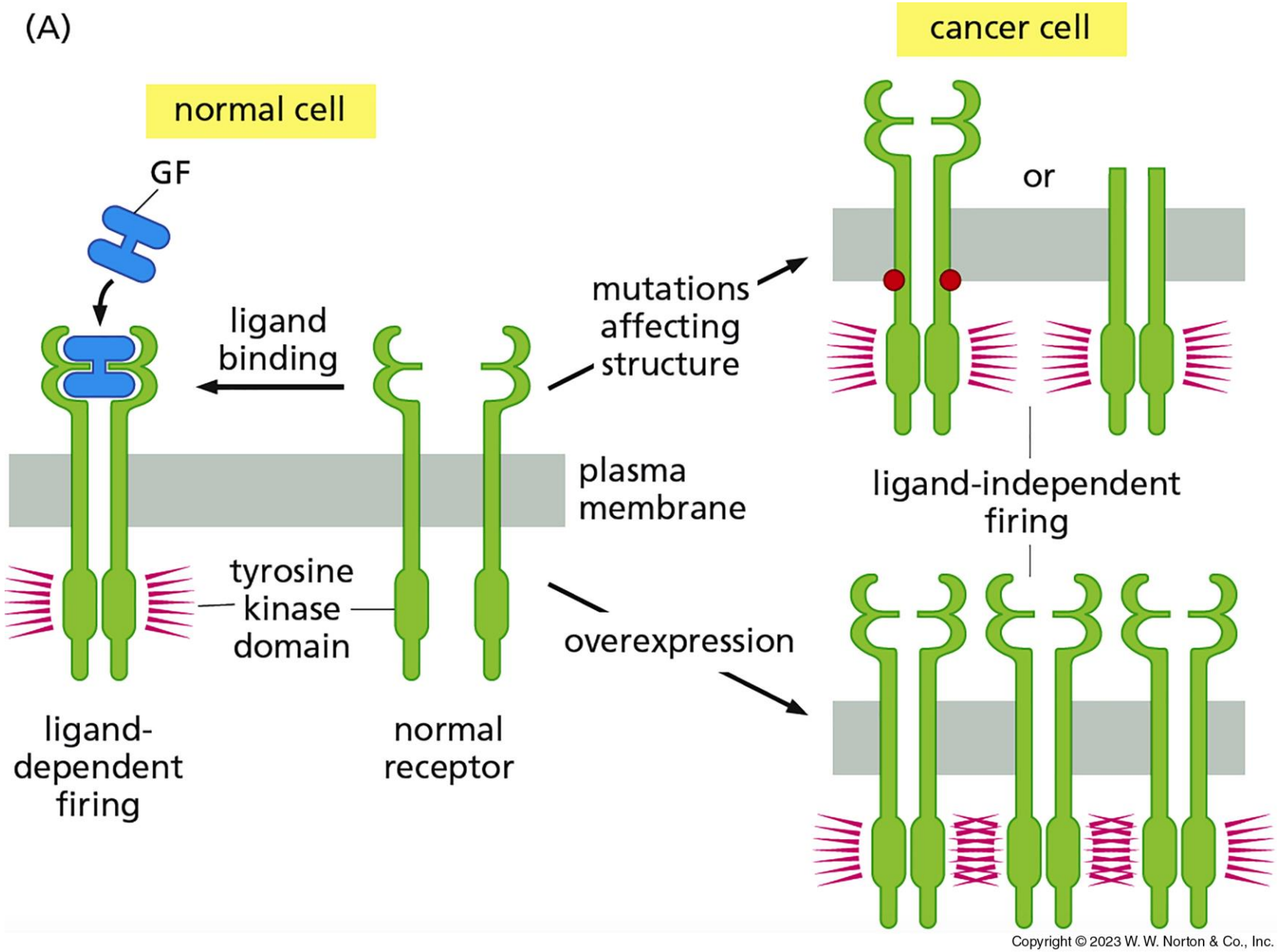
[a]PDGF is represented by four distinct polypeptides, PDGF-A, -B, -C, and -D. The PDGF-Rs consist of at least two distinct species, α and β, that can homodimerize or heterodimerize and associate with these ligands in different ways.
[b]The EGF family of ligands, all of which bind to the EGF-R (ErbB1) and/or heterodimers of erbB1 and one of its related receptors (footnote c), includes—in addition to EGF—TGF-α, HB-EGF, amphiregulin, betacellulin, and epiregulin. In addition, other related ligands bind to heterodimers of ErbB2 and ErbB3 or ErbB4; these include epigen and a variety of proteins generated by alternatively spliced neuregulin (NRG) mRNAs, including heregulin (HRG), glial growth factor (GGF), and less well-studied factors such as sensory and motor neuron–derived factor (SMDF).
[c]The EGF-R family of receptors consists of four distinct proteins, ErbB1 (EGF-R), ErbB2 (HER2, Neu), ErbB3 (HER3), and ErbB4 (HER4). They often bind ligands as heterodimeric receptors, for example, ErbB1 + ErbB3, ErbB1 + ErbB2, or ErbB2 + ErbB4; ErbB3 is devoid of kinase activity and is phosphorylated by ErbB2 when the two form heterodimers. ErbB2 has no ligand of its own but does have strong tyrosine kinase activity. ErbB3 and ErbB4 bind neuregulins, a family of more than 15 ligands that are generated by alternative splicing.
[d]FGFs constitute a large family of GFs. The prototypes are acidic FGF (aFGF) and basic FGF (bFGF); in addition there are other known members of this family.
[e]There are four well-characterized FGF-Rs.
[f]There are four known VEGFs. VEGF-A and -B are involved in angiogenesis, while VEGF-C and -D are involved predominantly in lymphangiogenesis.
[g]There are three known VEGF-Rs: VEGF-R1 (also known as Flt-1) and VEGF-R2 (also known as Flk-1/KDR), involved in angiogenesis; and VEGF-R3, involved in lymphangiogenesis.
[h]The two known IGFs, IGF-1 and IGF-2, both related in structure to insulin, stimulate cell growth (i.e., increase in size) and survival; they also appear to be weakly mitogenic.
Abbreviation: VSMC, vascular smooth muscle cell.
Adapted in part from B. Alberts et al., Molecular Biology of the Cell, 6th ed. New York: W.W. Norton, 2015.

A growth factor gene can become an oncogene

In 1983: Platelet derived growth factor (PDGF) B-chain is related in sequence to v-*sis* oncogene of simian sarcoma virus

- PDGF is unrelated in structure to EGF
- PDGF stimulates the proliferation different sets of cells than EGF
- PDGF stimulates mesenchymal cells (such as fibroblast, adipocytes, smooth muscle cells)
- EGF stimulates epithelial cells
- The specificity of action : PDGF-R is expressed on mesenchymal cells and not on epithelial cells
- Cells transformed with simian sarcoma virus : *sis* oncogene causes release of PDGF-like Sis protein and activates PDGF-R mediated cell proliferation

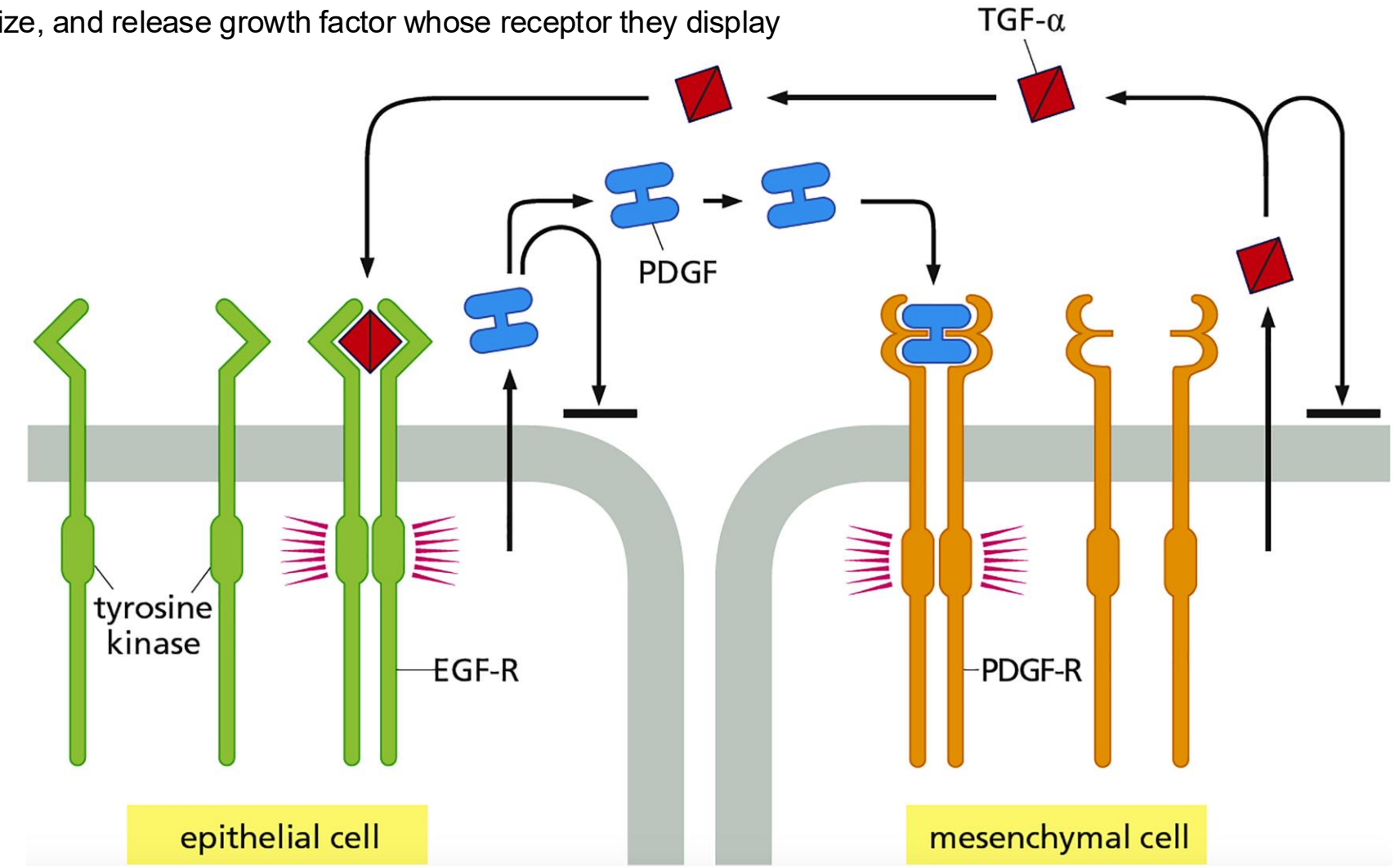
Regulation of growth factor receptor signaling



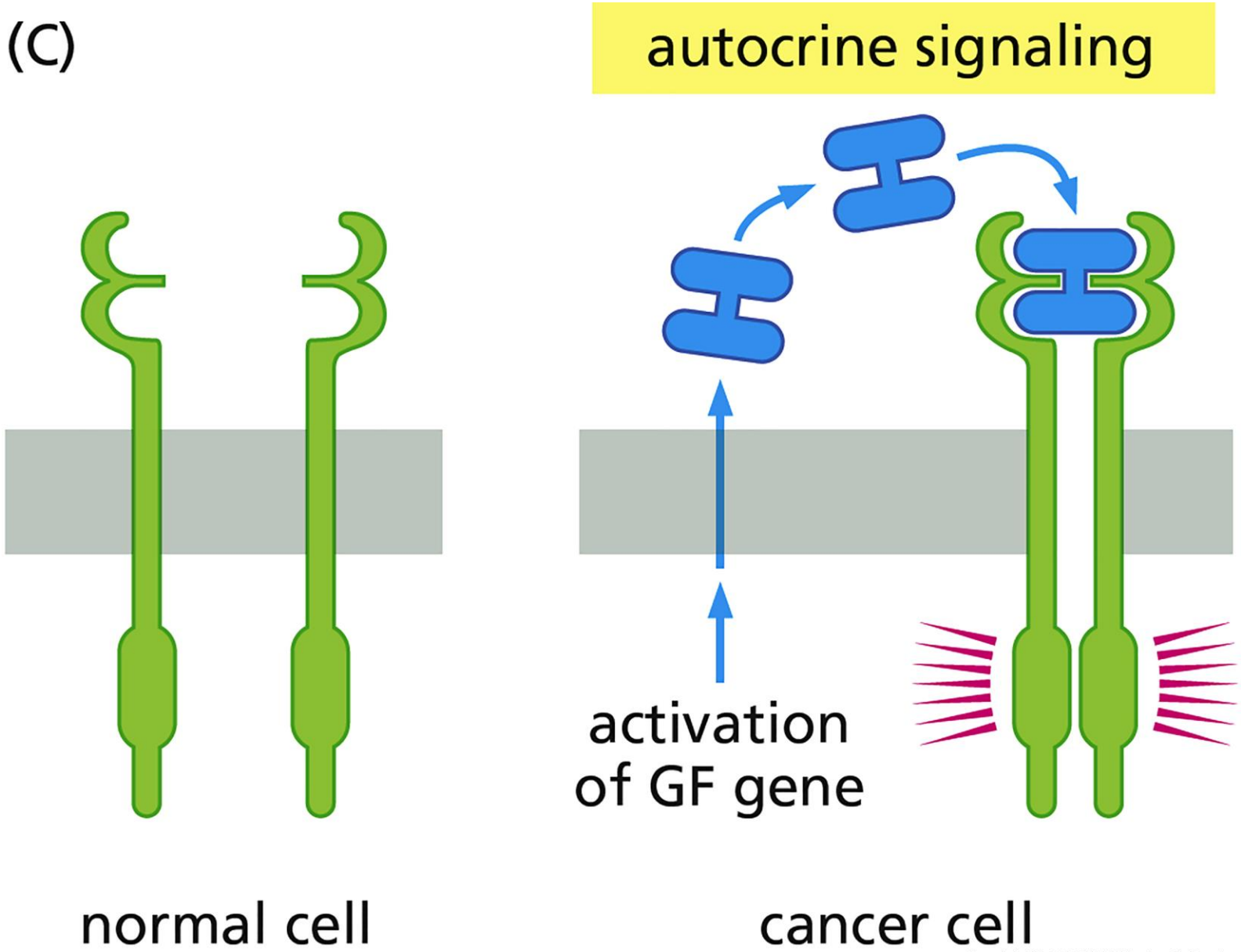
Paracrine signaling

(B) normal paracrine signaling

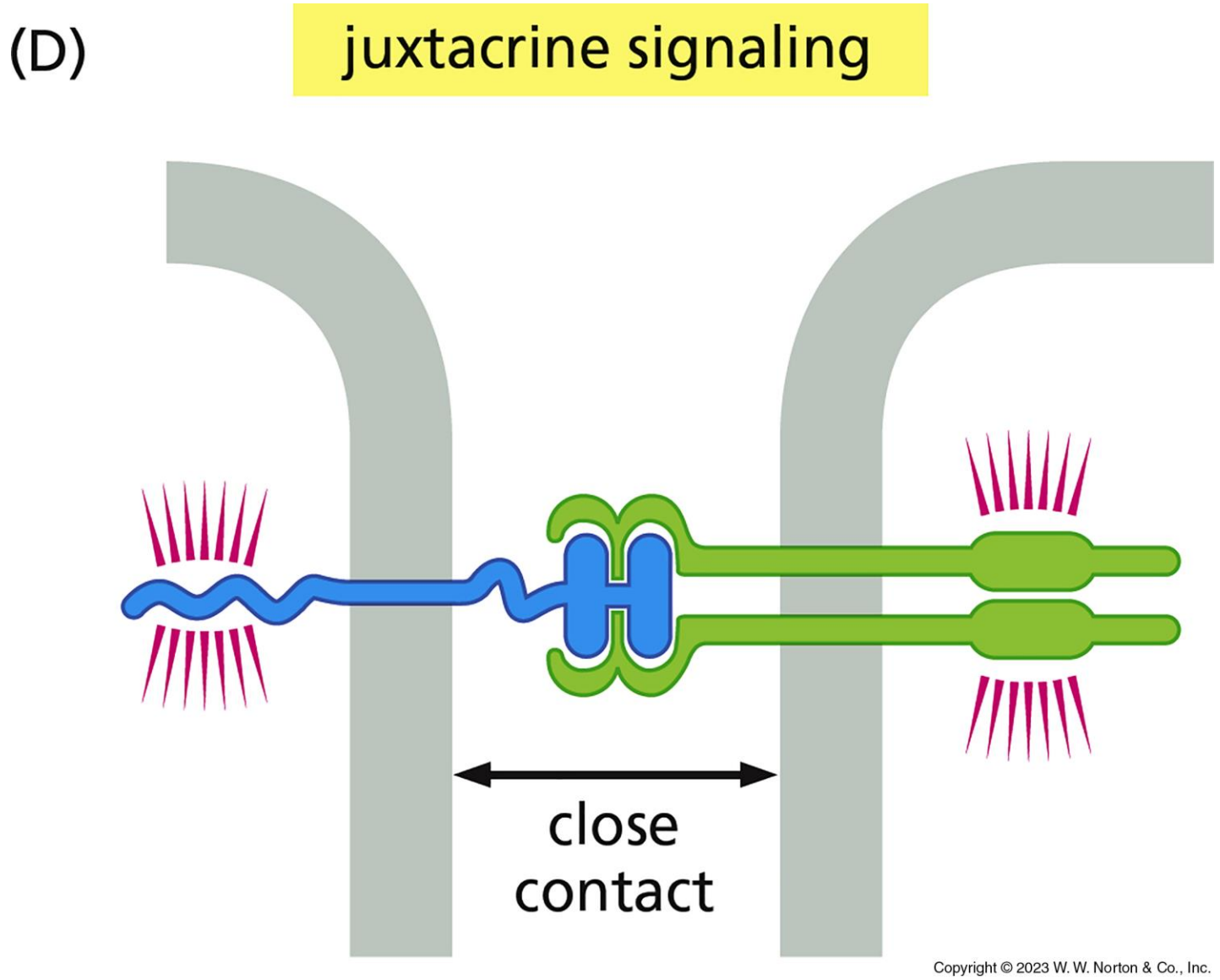
Normal cells do not synthesize, and release growth factor whose receptor they display



Autocrine signaling in cancer cells

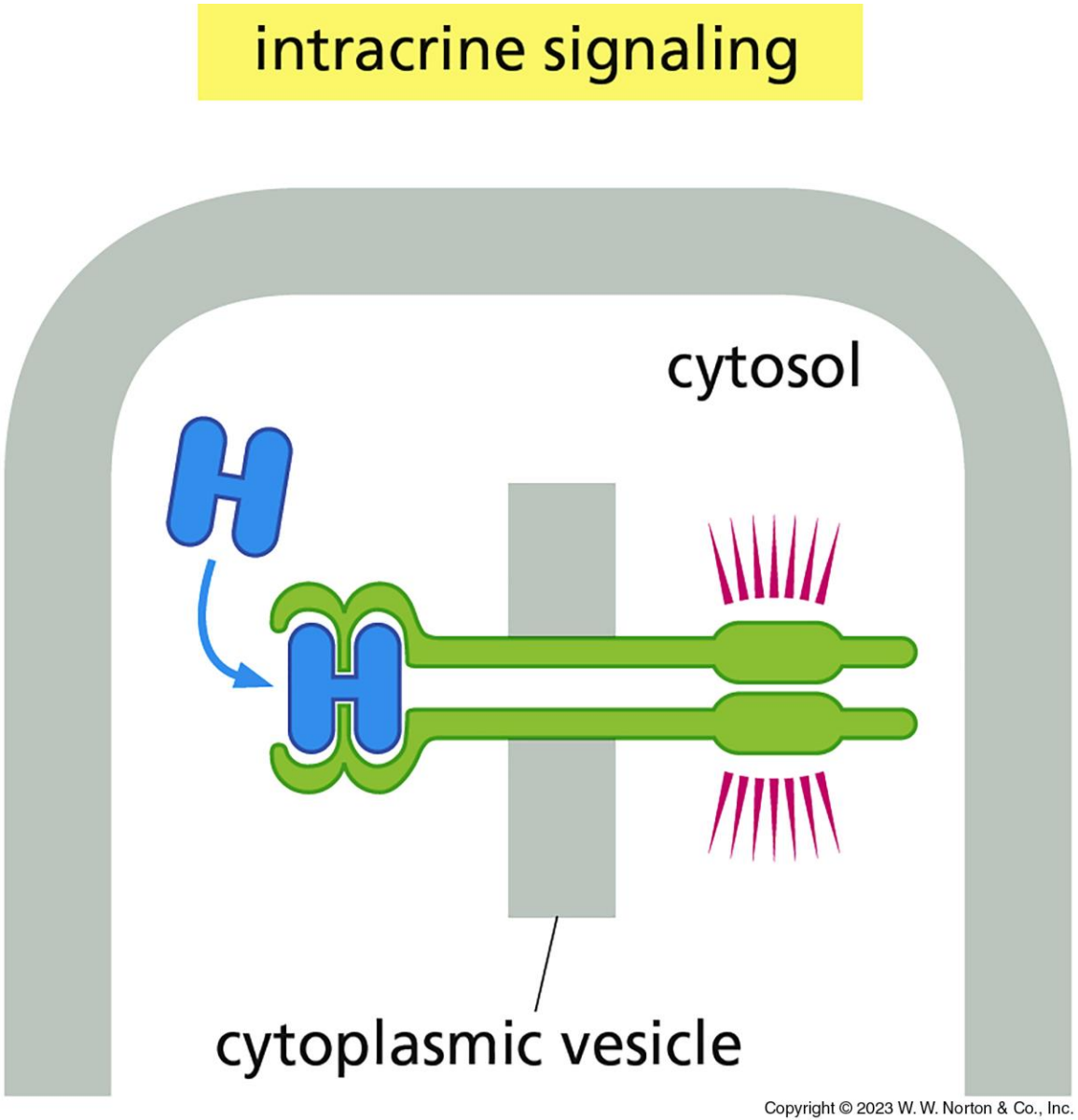


Juxtacrine signaling



Intracrine signaling

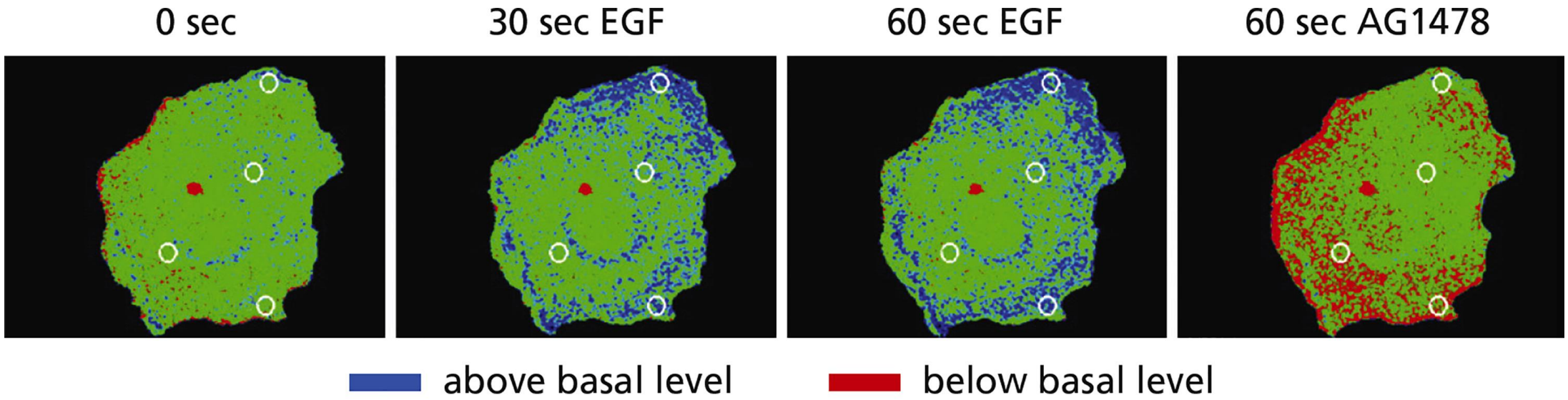
- After synthesis ligand and receptor bind one another without moving to the cell surface
- Ligand-receptor binding on cell surface, thereafter internalized



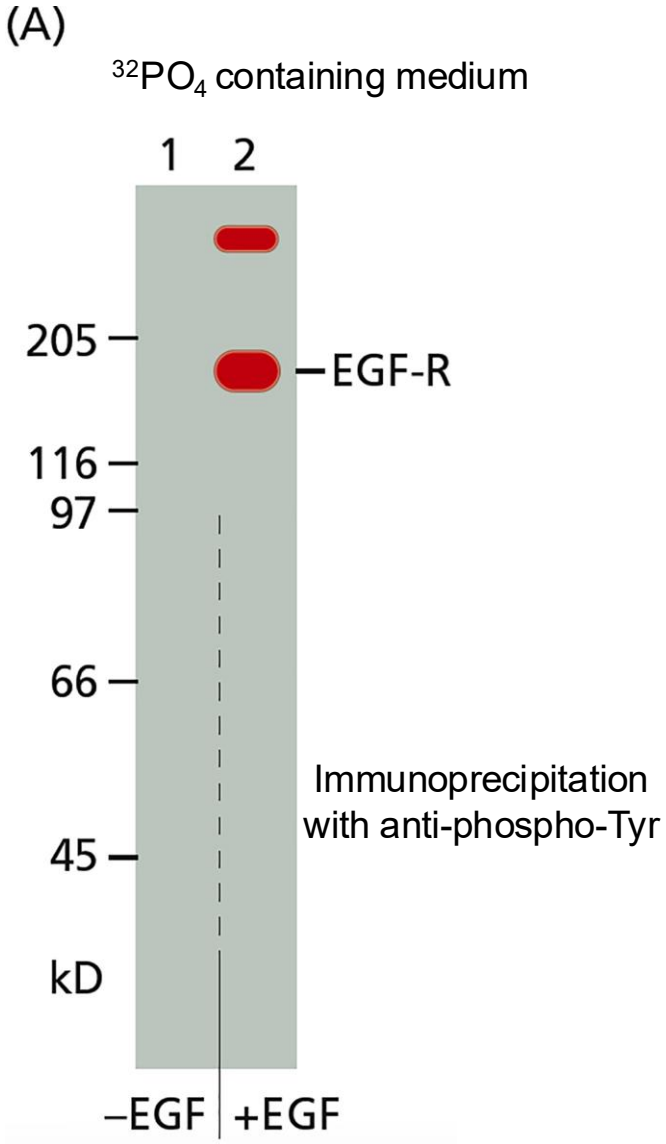
Formation of phospho-tyrosine on EGF-R following ligand (EGF) addition

Fluorescent reagent that binds specifically to phospho-tyrosine residues on EGF-R

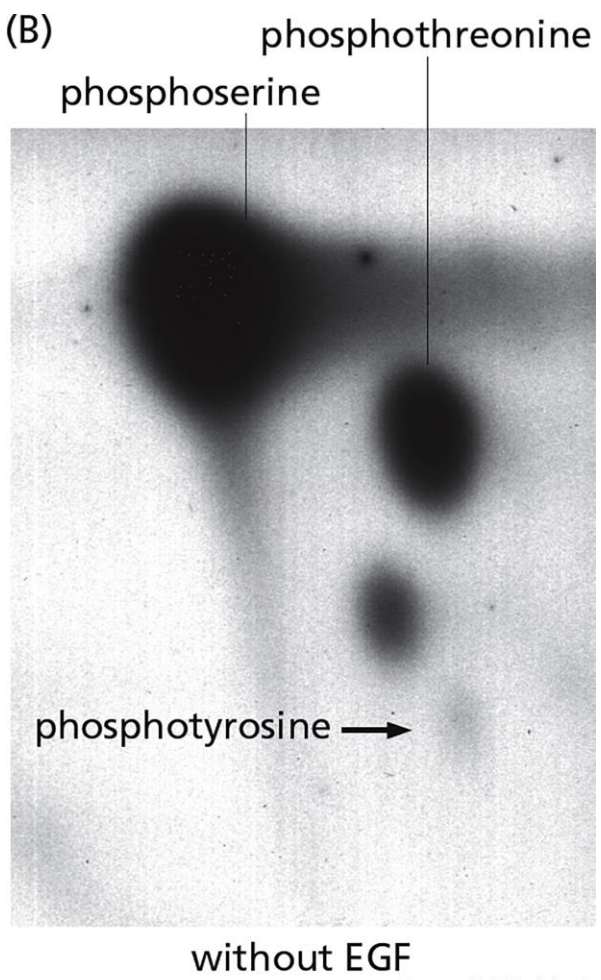
AG1478: Inhibitor of EGF-R kinase

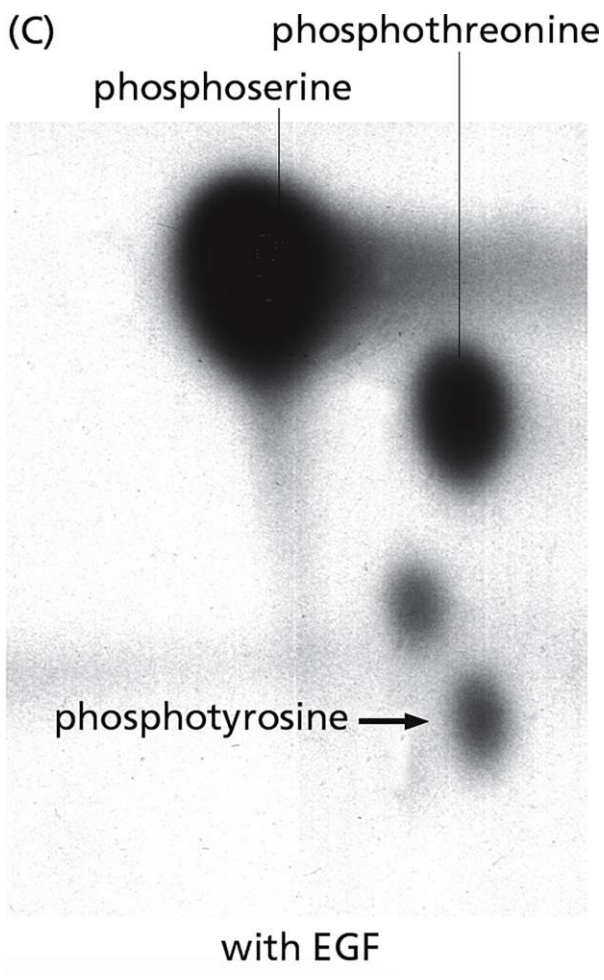


EGF stimulated Tyrosine phosphorylation of EGF-R in Human A431 epidermoid carcinoma cells

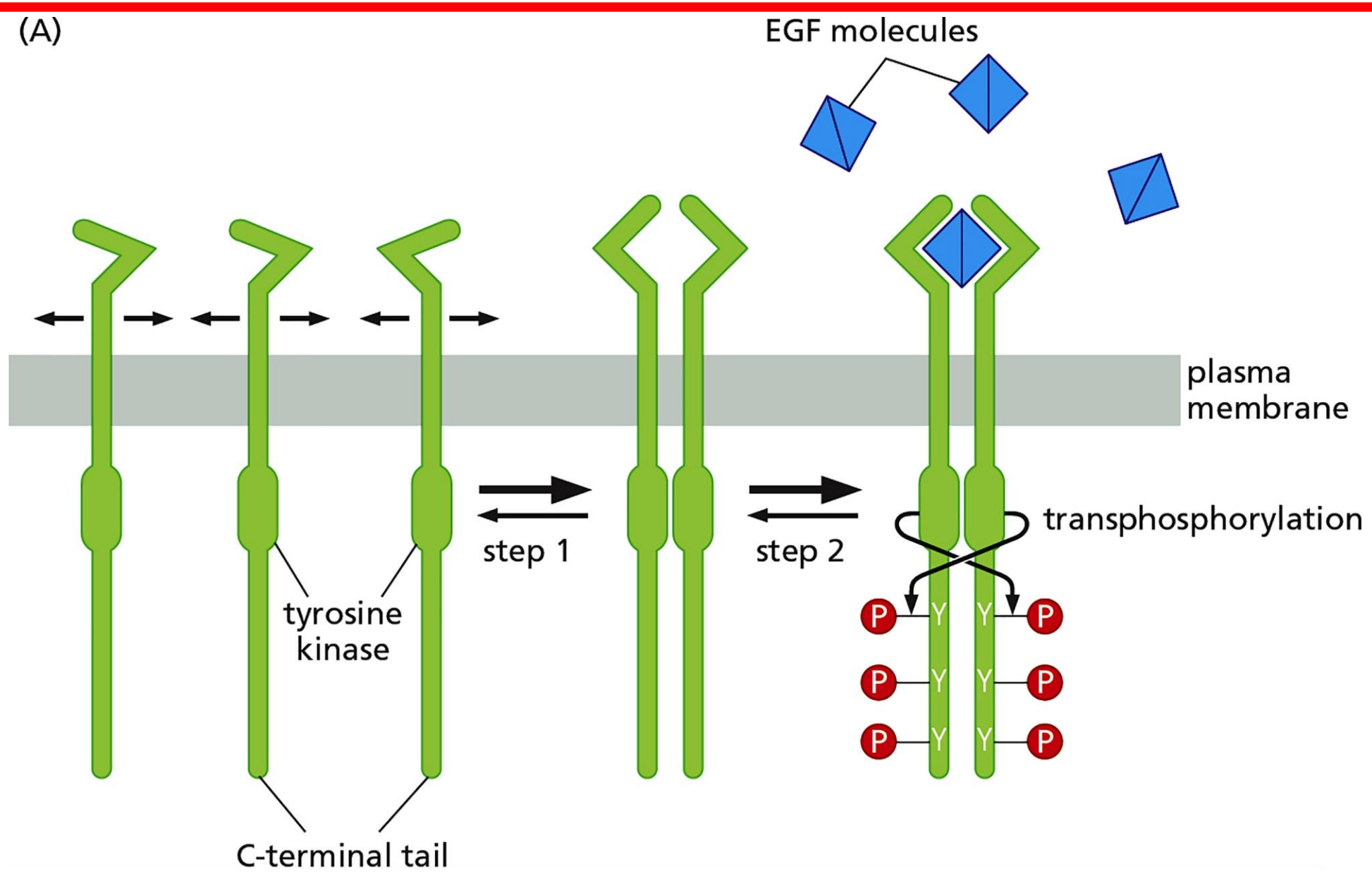


From A.B. Sorokin et al., *FEBS Lett.* 268:121–124, 1990.
With permission from John Wiley and Sons.



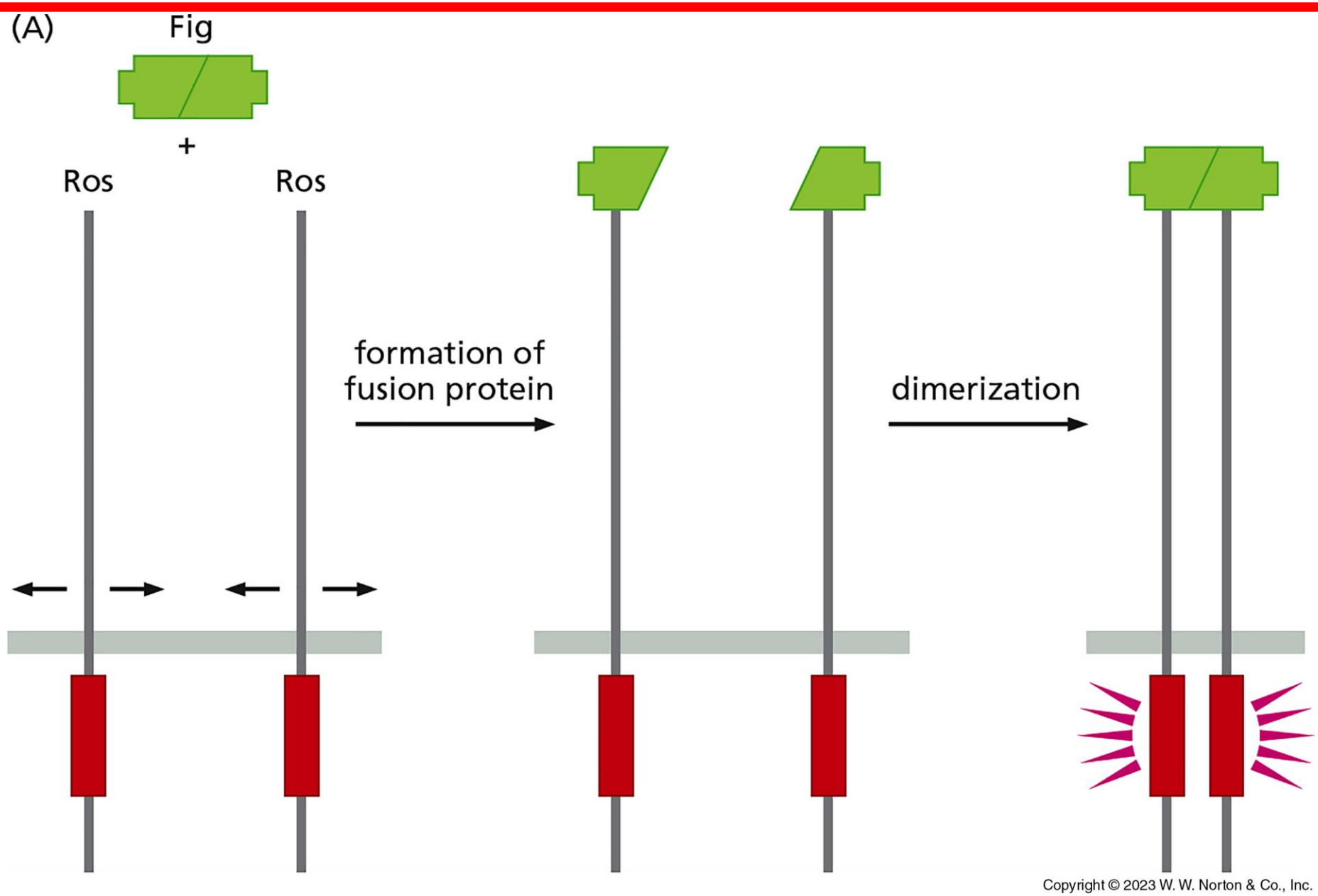


Receptor dimerization, ligand binding, transphosphorylation

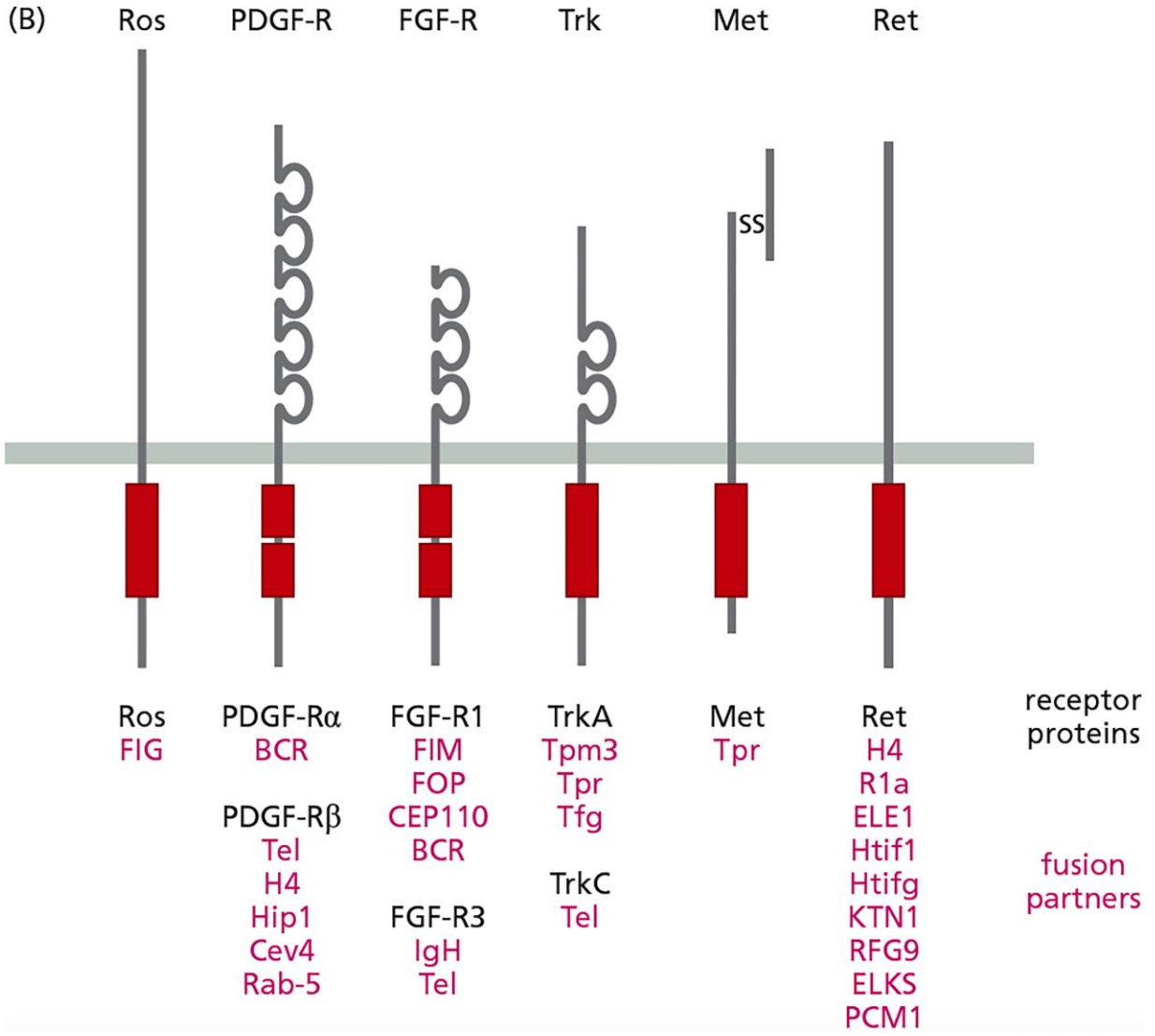


Gene fusion causing constitutively dimerized receptors

Glioblastoma

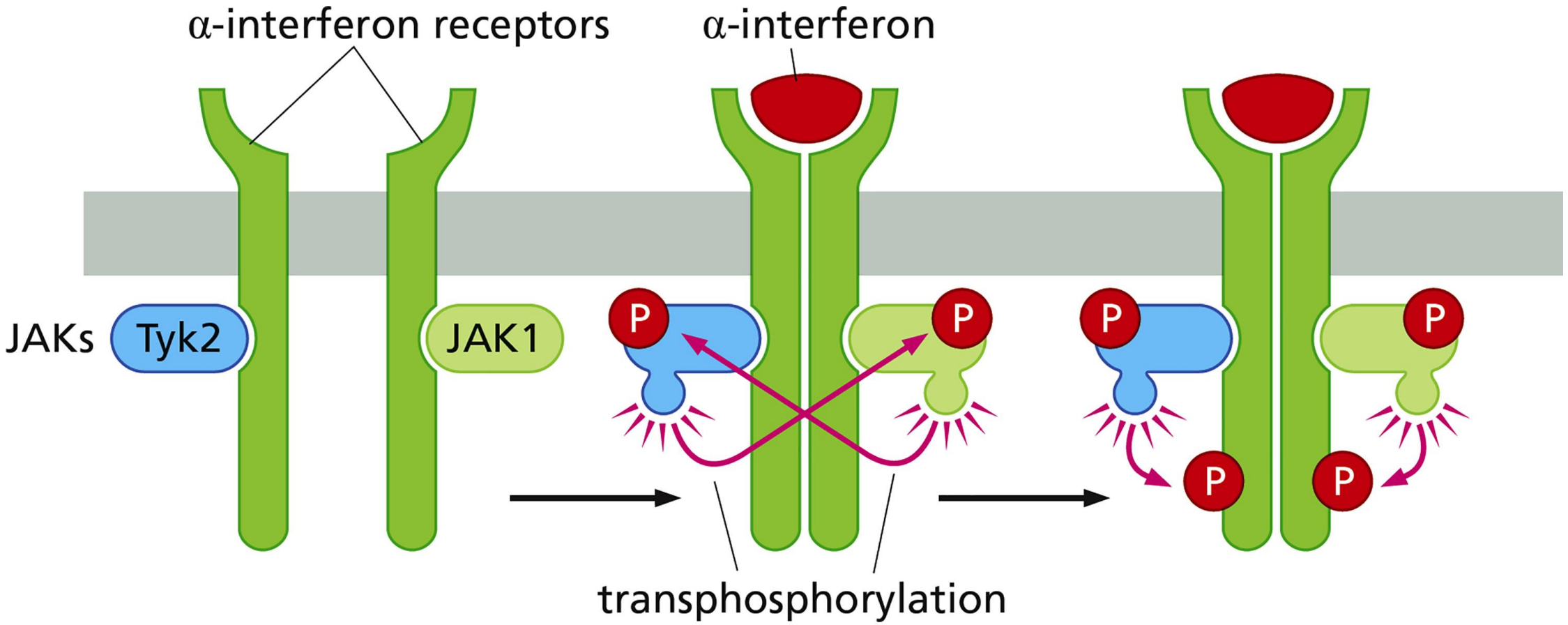


Tumors: Receptor dimerization through fusion of growth factor receptors with unrelated genes whose protein products naturally dimerize

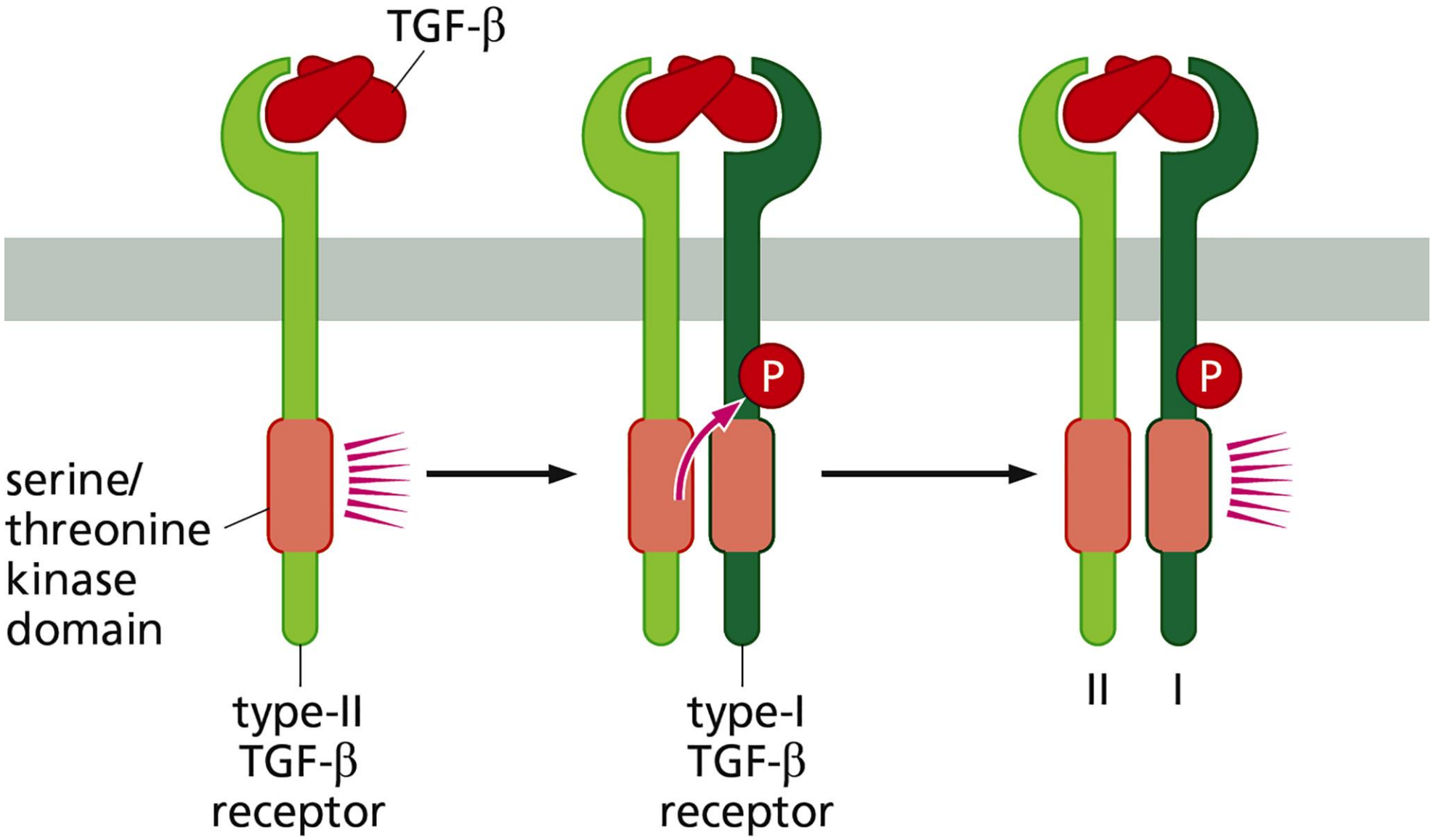


Courtesy of A. Charest.

Structure of Cytokine receptor



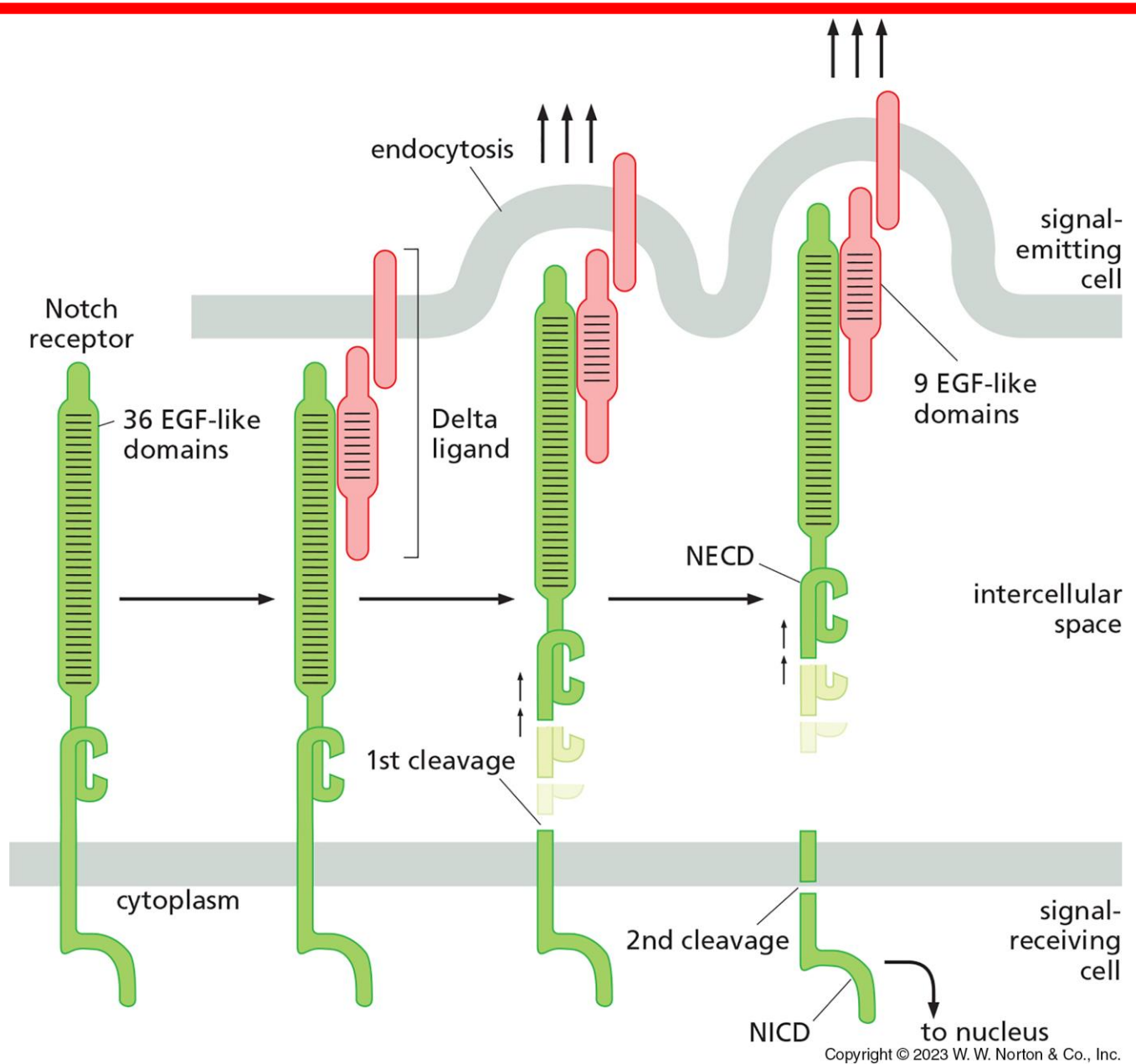
Structure of TGF-β receptor: Serine/threonine kinase



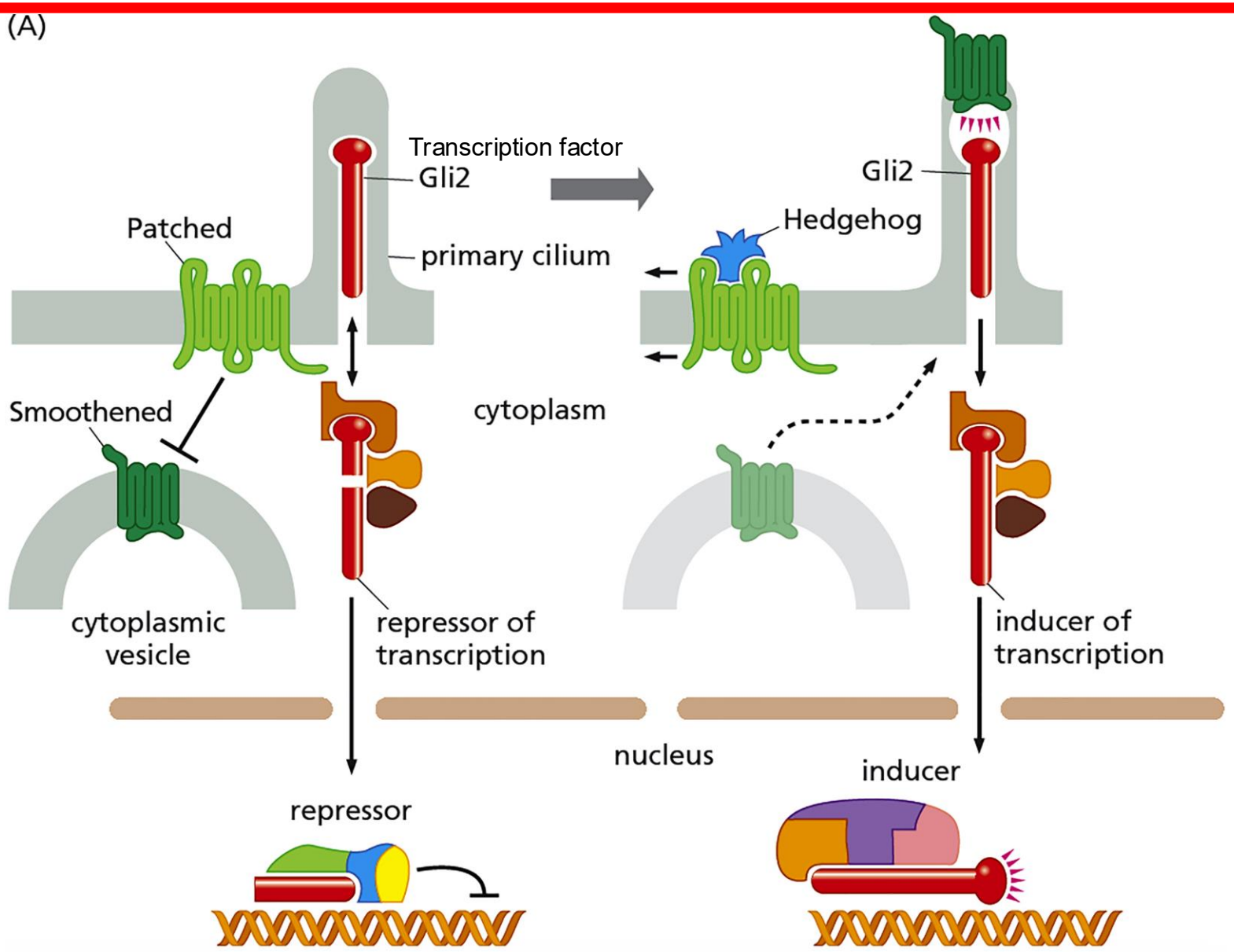
Structure of Notch receptor

Mammals have 5 distinct Notch ligands and 4 Notch receptors

Mutant Notch found in half of adult T-cell leukemia

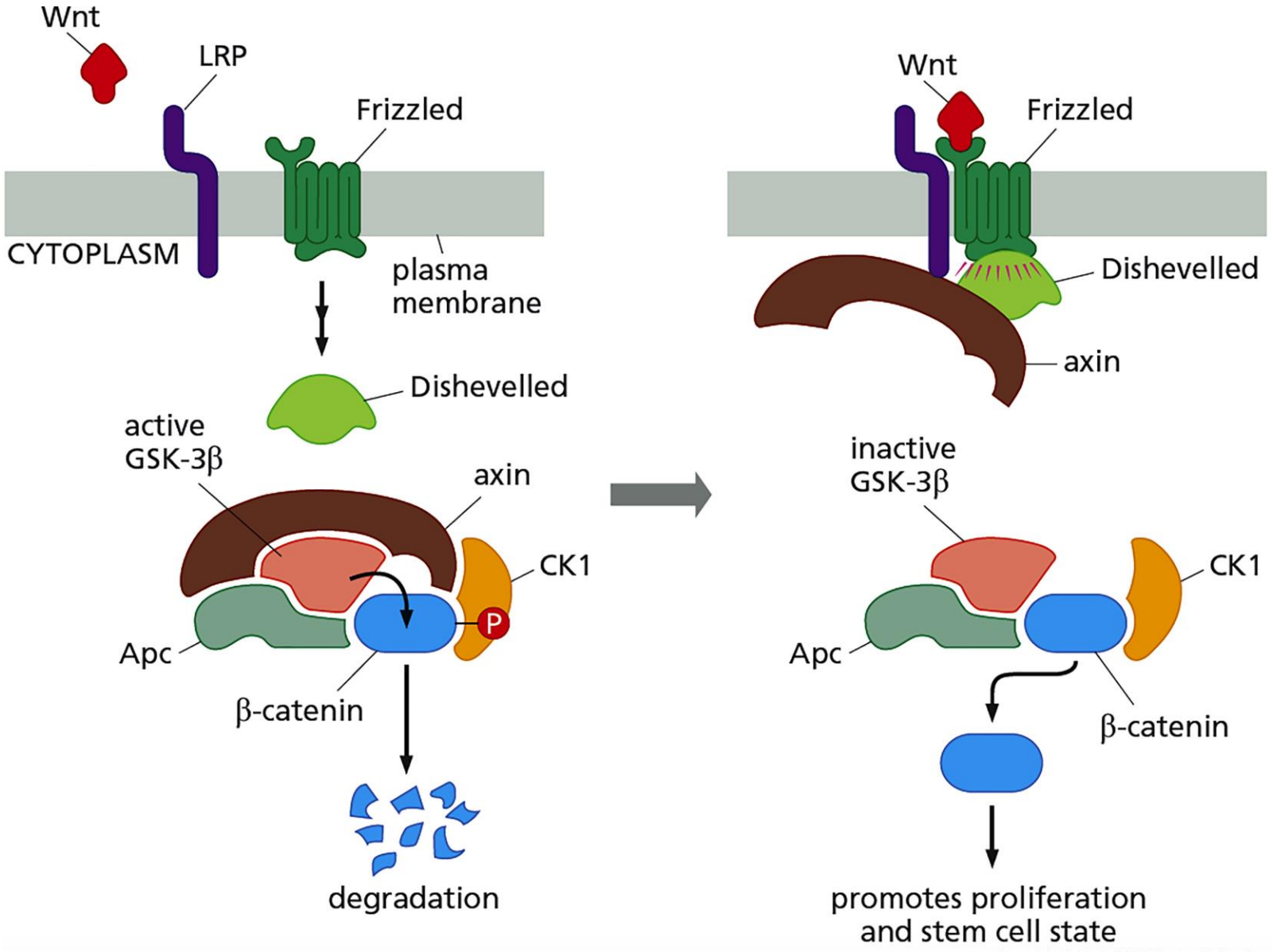


Patched-Smoothened signaling

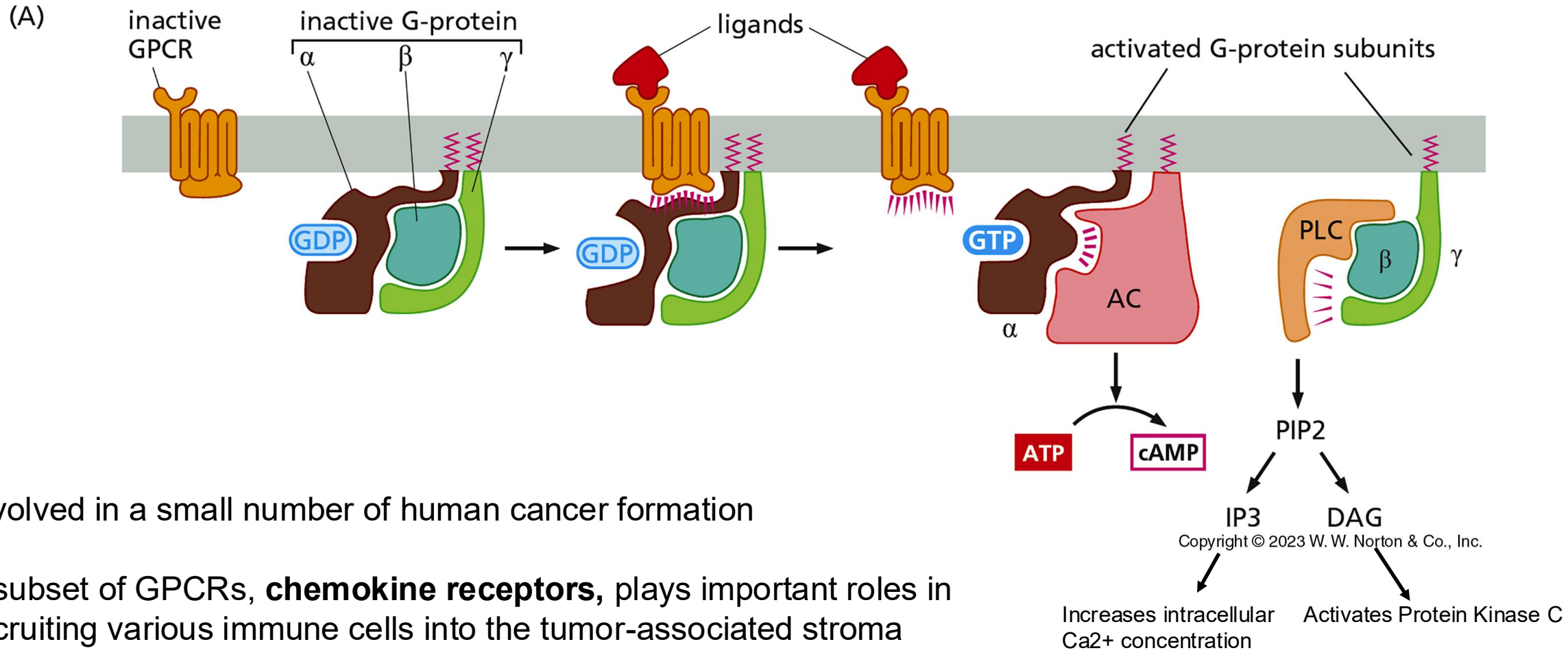


Courtesy of D. Thomas and adapted from S. Goetz and K.V. Anderson, *Nat. Rev. Genet.* 11:331–344, 2010.

Canonical Wnt signaling via Frizzled receptors



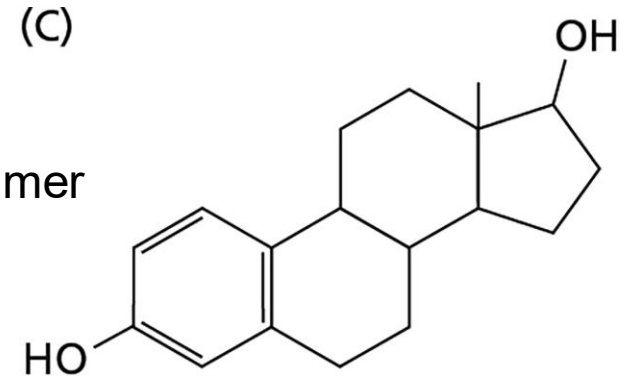
Signaling via G-protein coupled receptors (GPCRs)



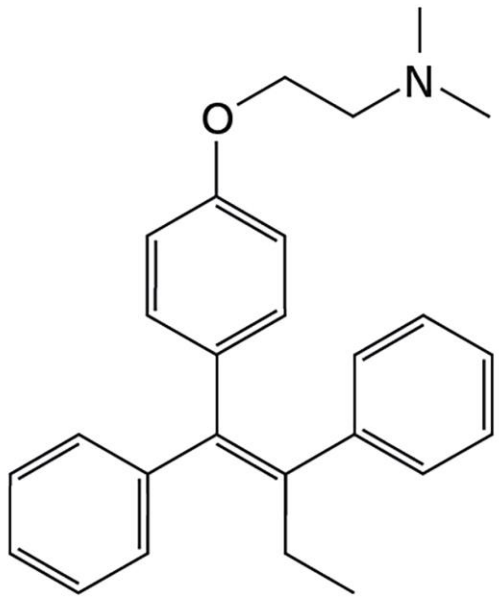
- Involved in a small number of human cancer formation
- A subset of GPCRs, **chemokine receptors,** plays important roles in recruiting various immune cells into the tumor-associated stroma

Nuclear receptors

Estrogen receptor (ER): Homodimer



E2/estrogen

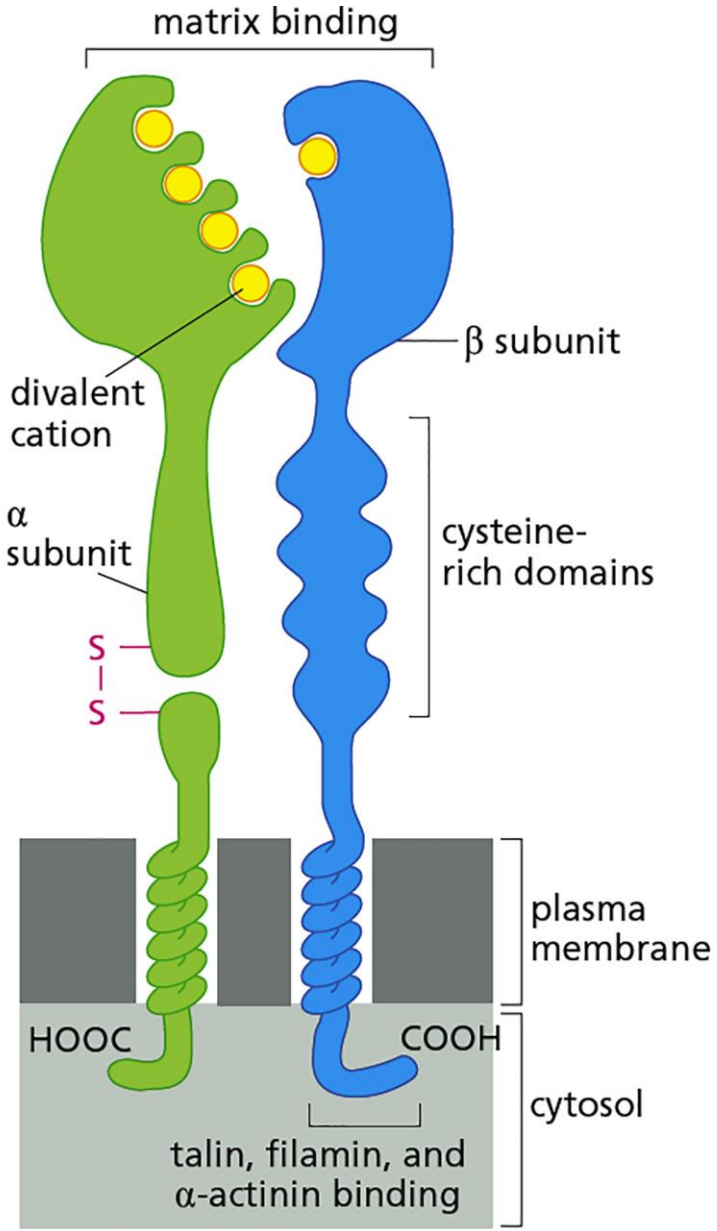


4-OHT/tamoxifen

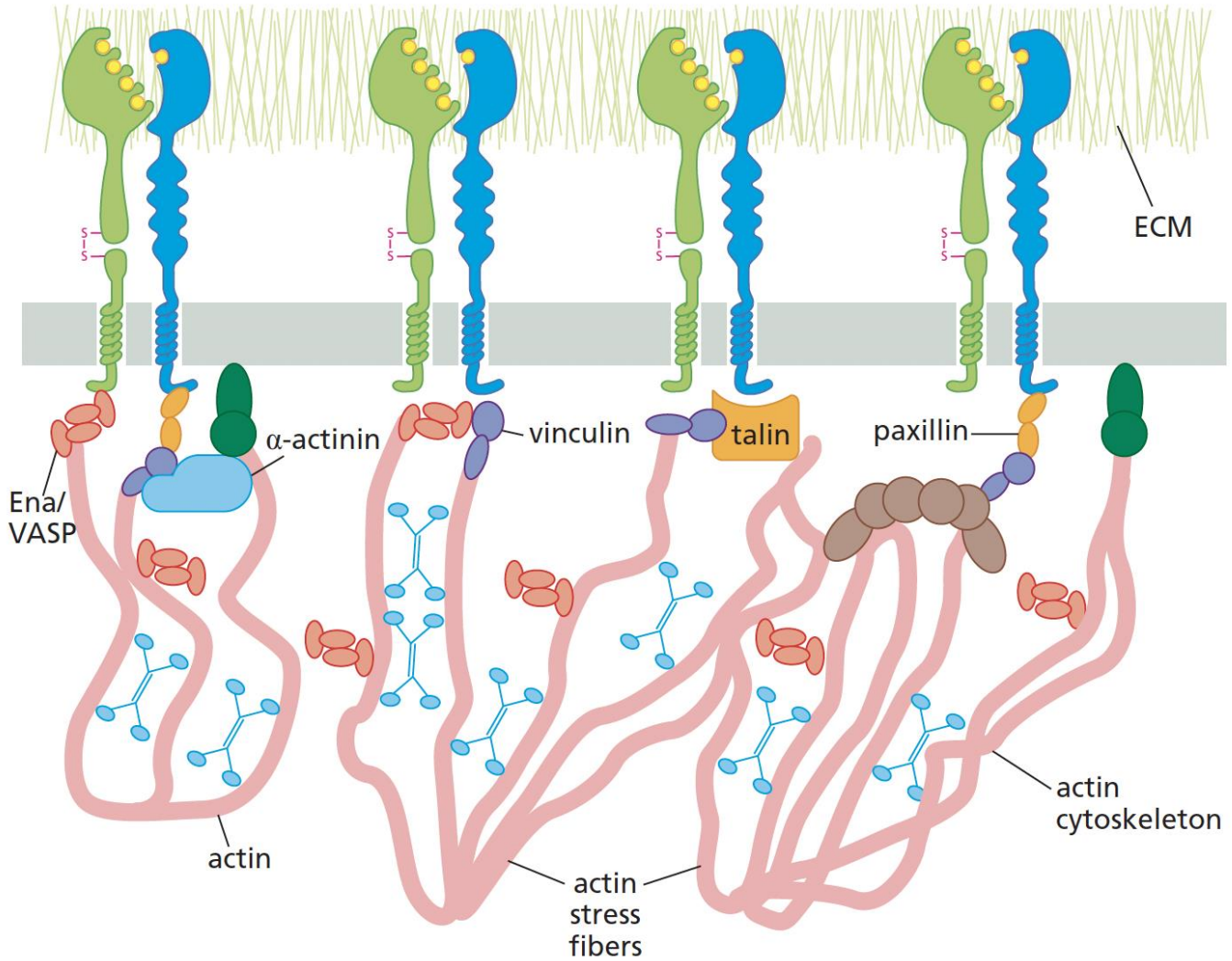
Tamoxifen causes ER to release co-activators and to recruit co-repressors :blocking of E2 signaling

Clinical benefit: Blocking E2 signaling by Tamoxifen treatment resulted twofold reduction in breast cancer relapse rate in postmenopausal women whose ER+ primary tumor had been removed

Structure of Integrins and signaling



Integrin tethering of ECM and cytoskeleton



Normal cell proliferation requires

1. Sufficient growth factor in the surroundings

2. adequate anchoring to specific components of the ECM

***ras* is an oncogene encoding Ras-oncoprotein that is attached to inner side of the cell membrane**

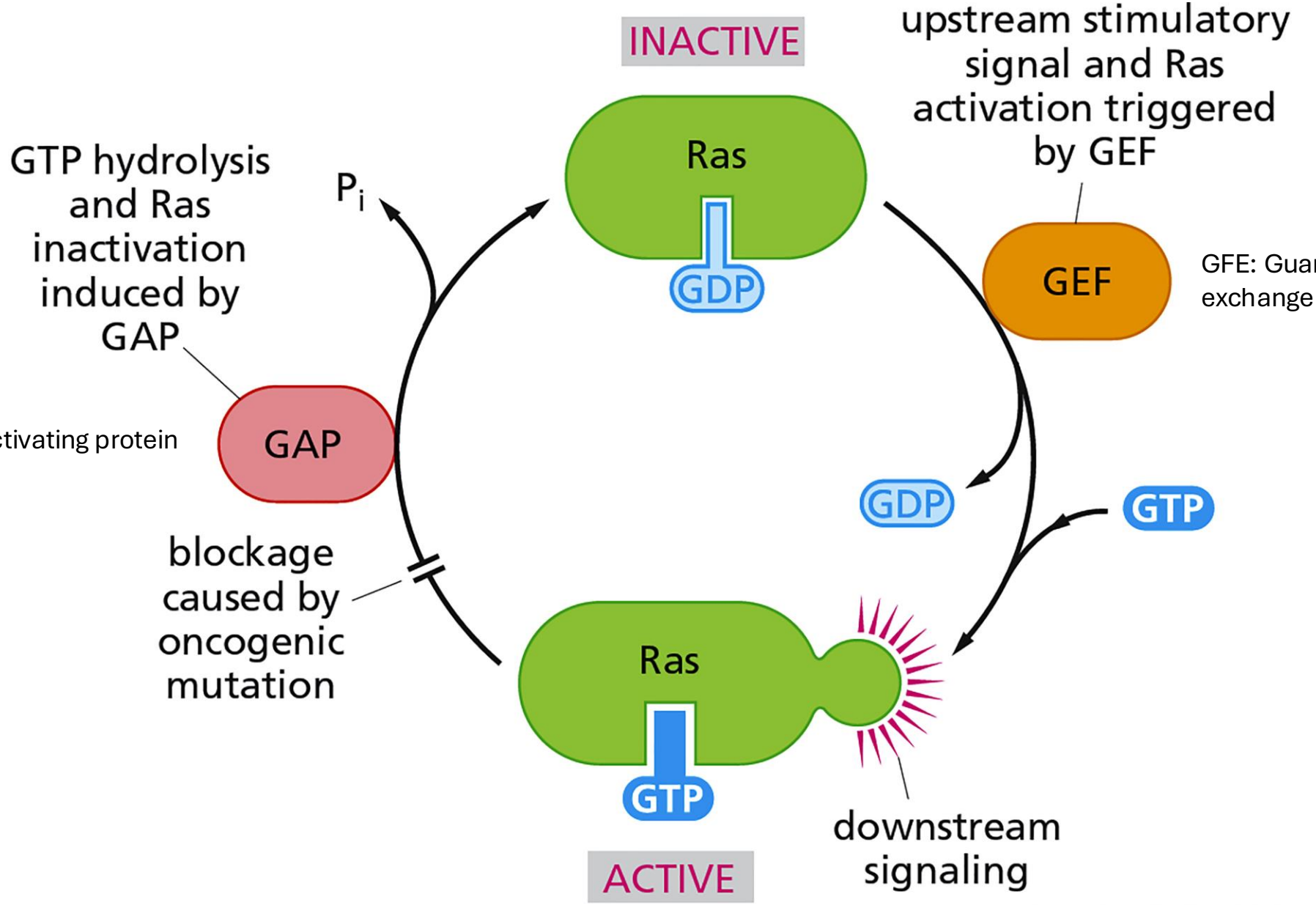
***ras* oncogene transformed cells abrogate both these requirements**

***ras*-transformed cells can grow in low concentrations of serum**

Many types of *ras*-transformed cells can grow in anchorage independent manner

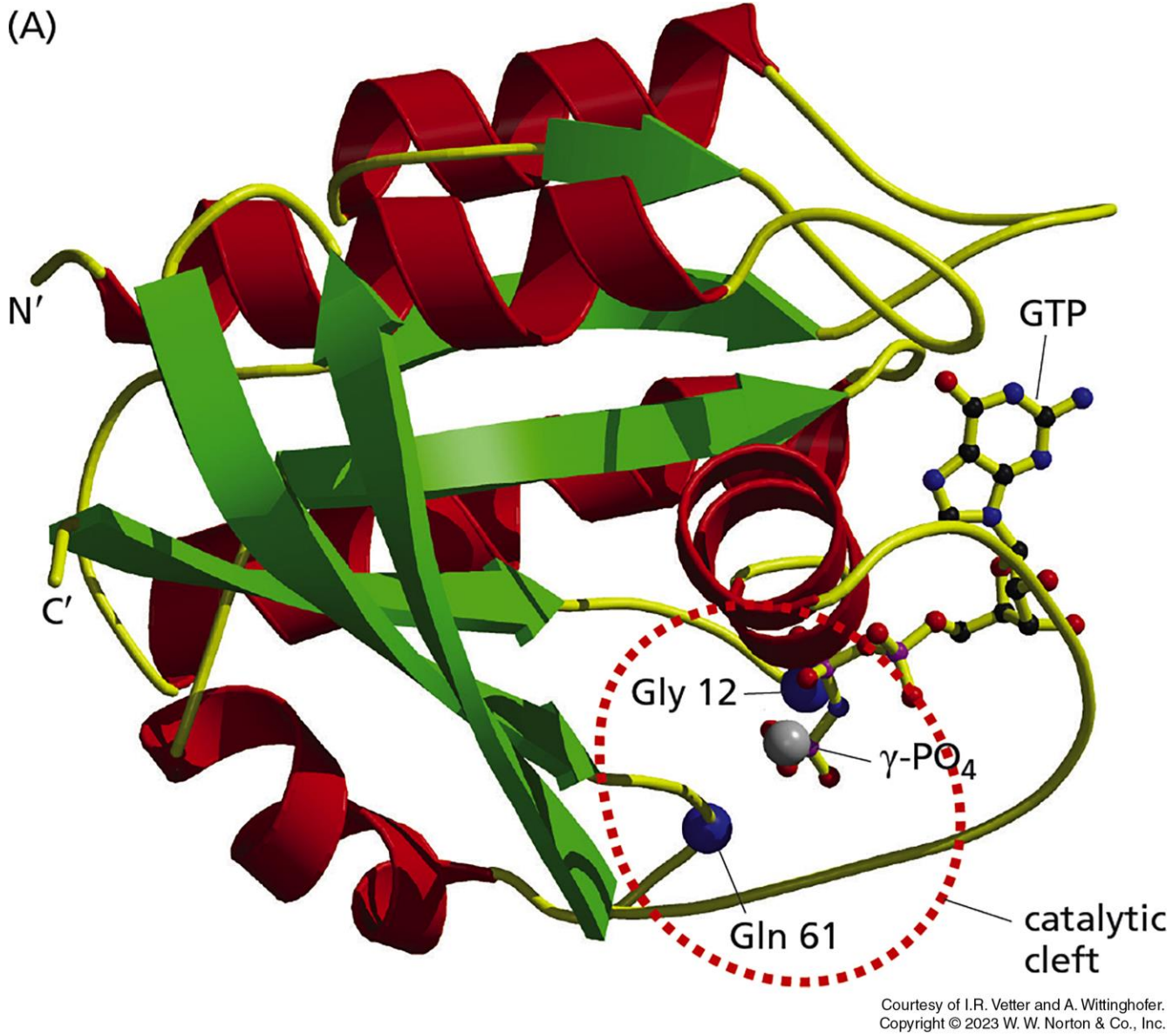
Ras oncogene triggered many of the same changes in cells that were seen when transformed with *src, erbB* or *sis*

Component of downstream signaling: Ras signaling cycle

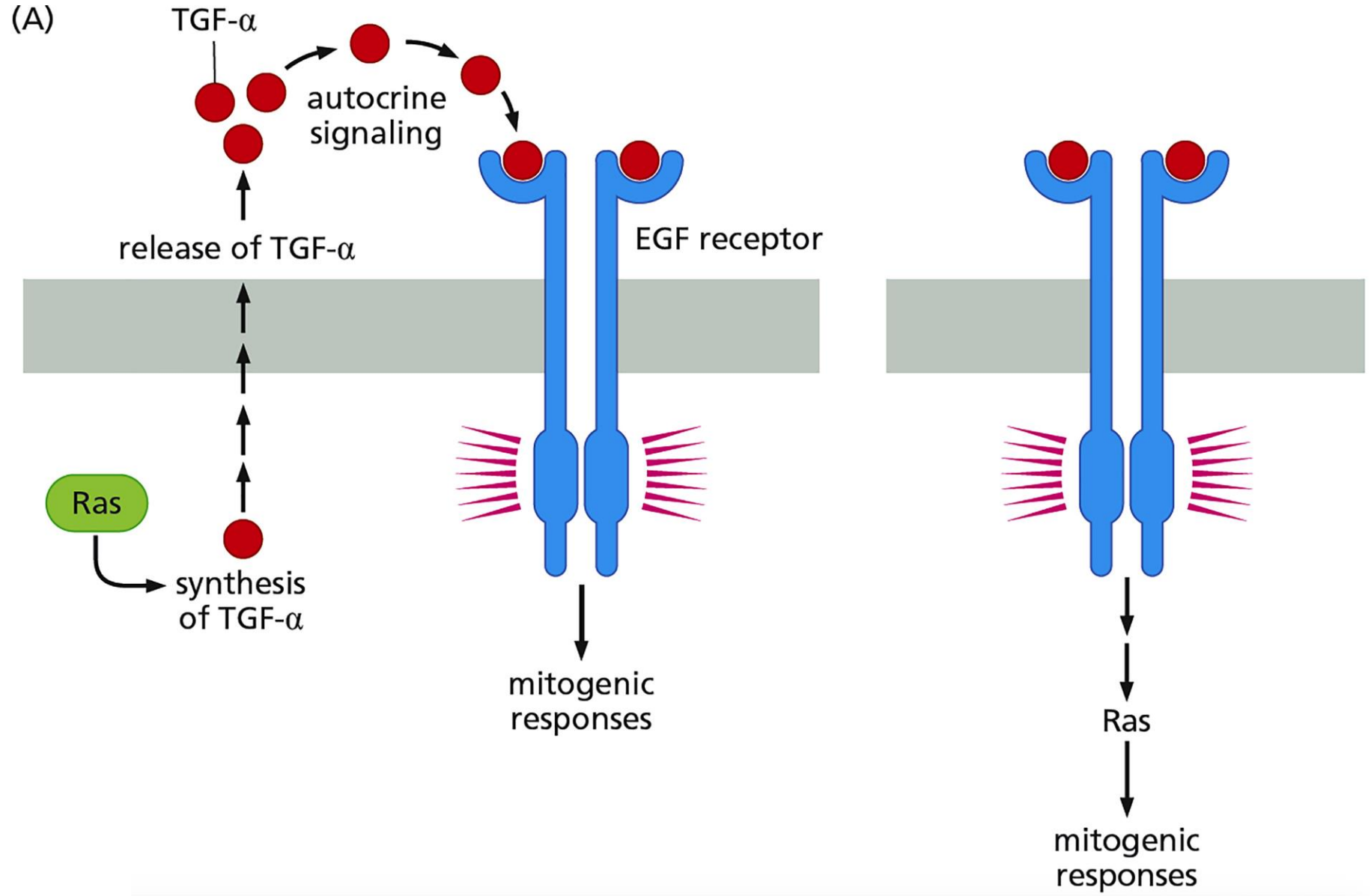


The structure of RAS protein

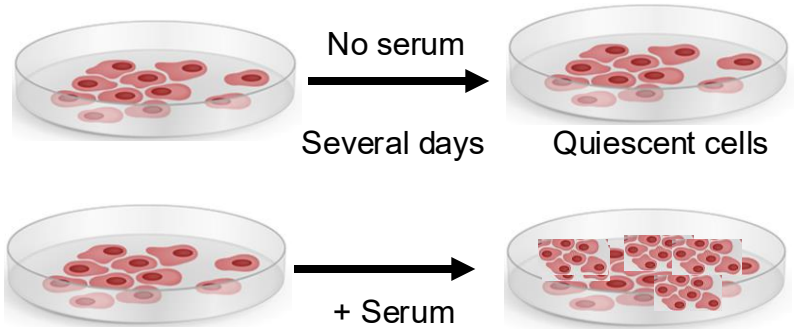
Mutant Ras loses GTPase function

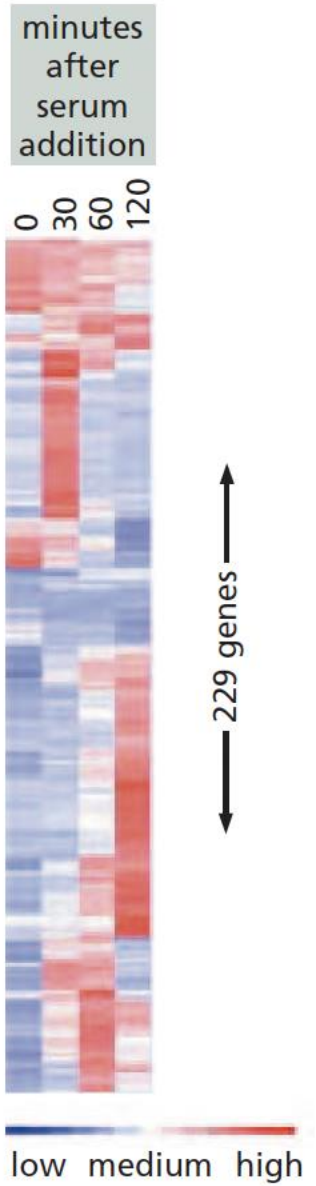


Alternative mechanisms of transformation by Ras



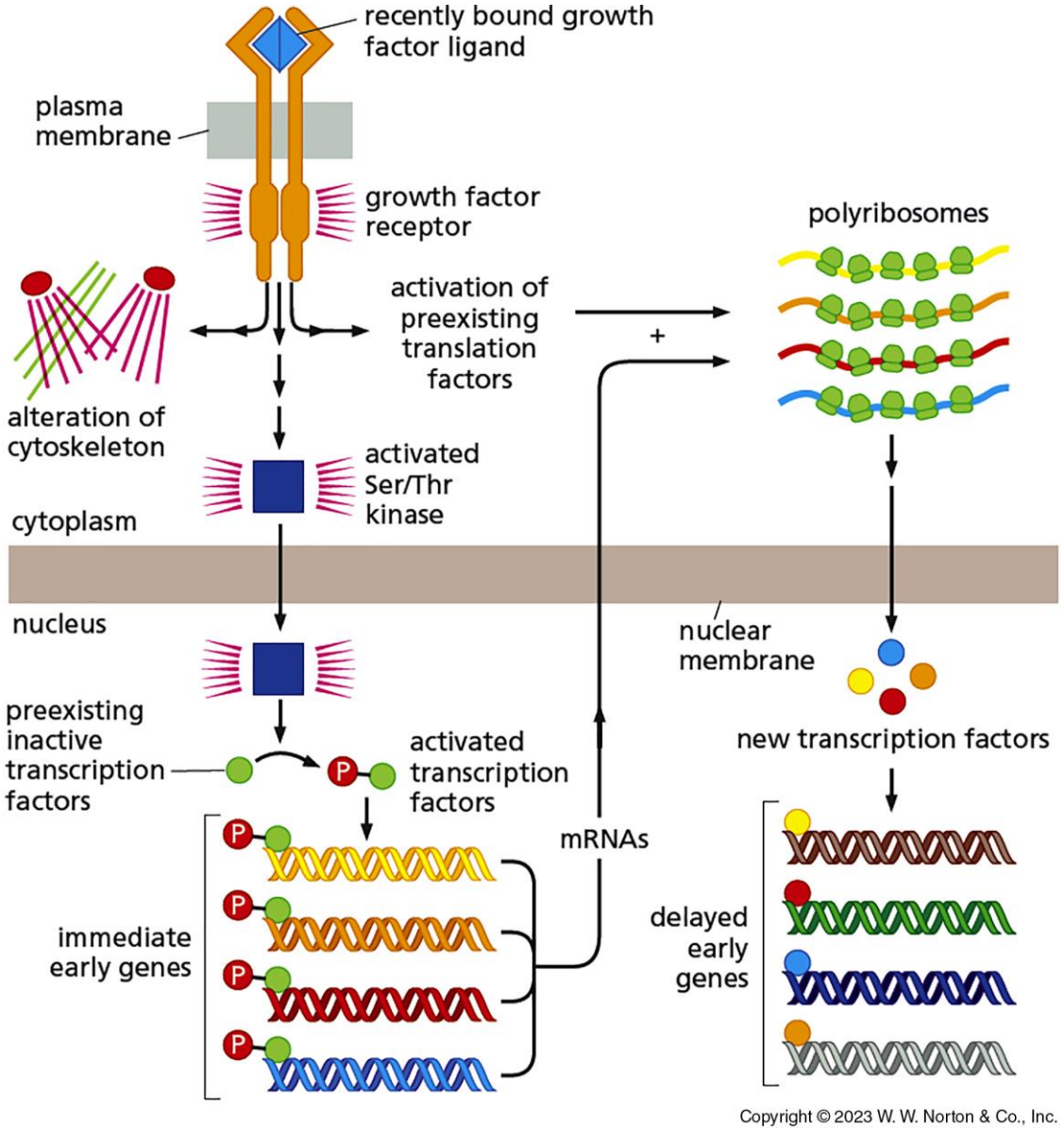
Expression of immediate and delayed early genes

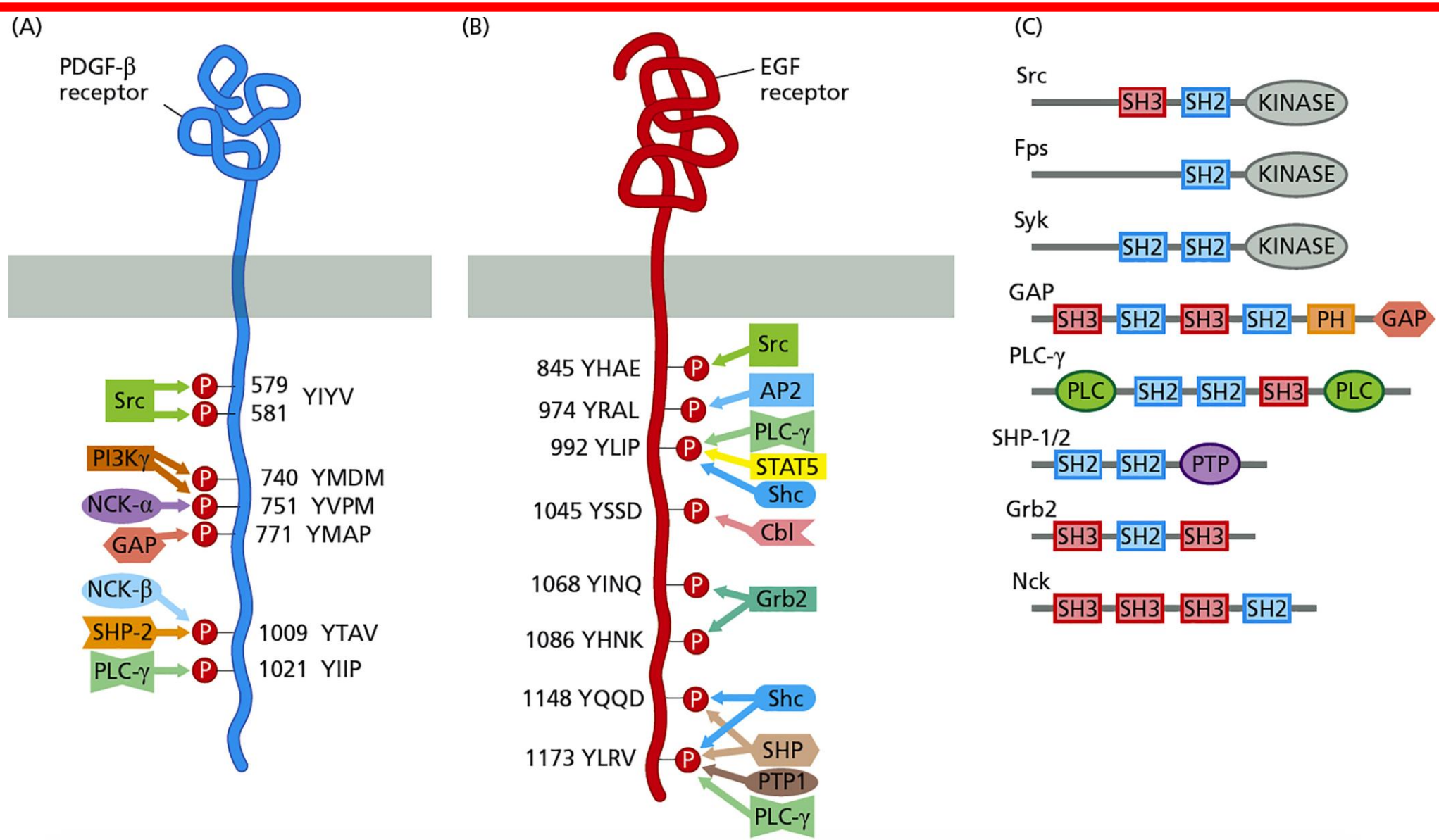




- Within 9-12 cells began to replicate their DNA
- Then another few hours later initiate cell division

Immediate early genes:
myc, fos and *jun*
Cytokines: organize cellular cytoskeleton





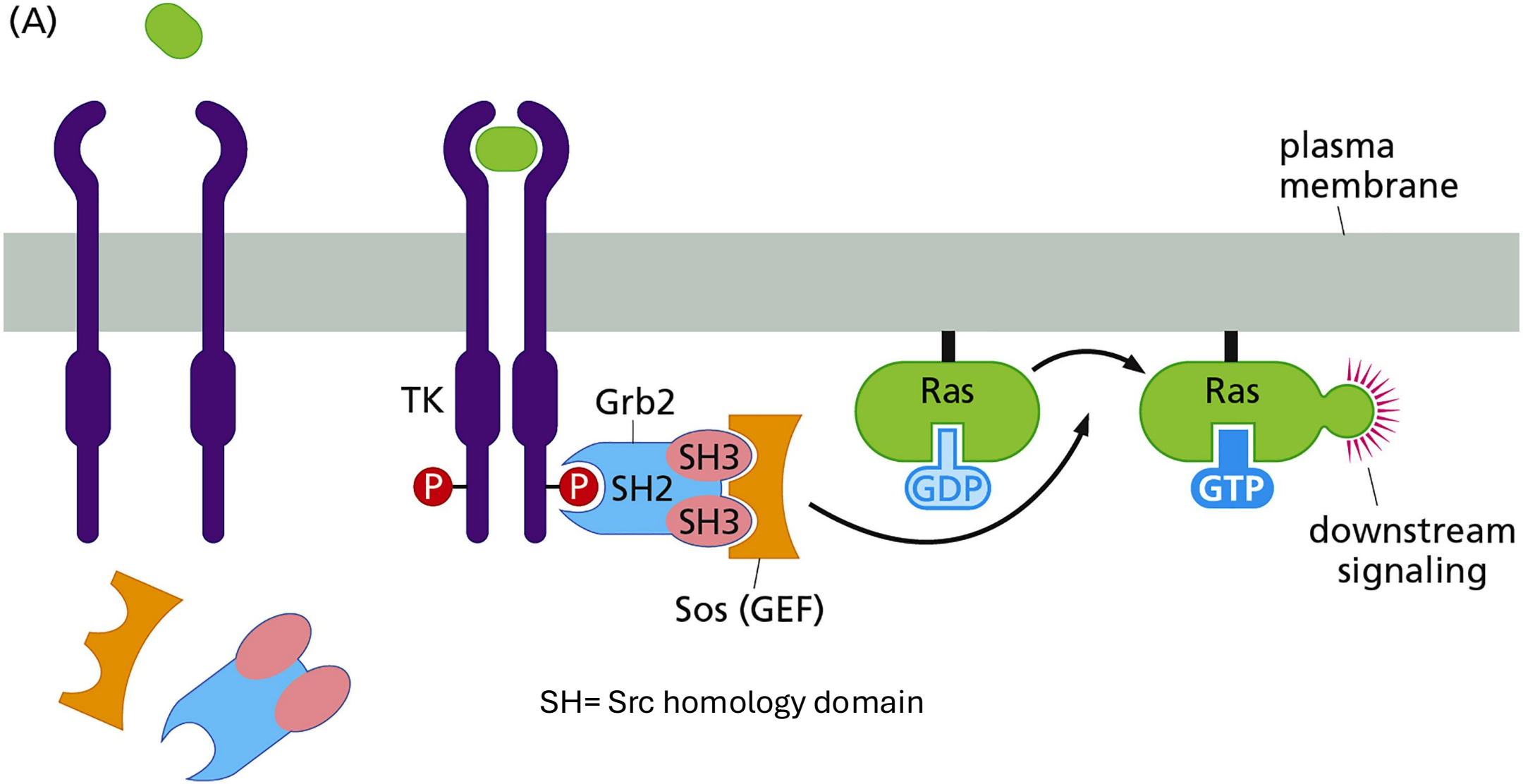
(A and C, adapted from T. Pawson, *Adv. Cancer Res.* 64:87–110, 1994. B, adapted from R. Sordella et al., *Science* 305:1163–1167, 2004.)

Name of domain	Ligand	Examples of proteins carrying this domain
SH2	phosphotyrosine	Src (tyrosine kinase), Grb2 (adaptor protein), Shc (scaffolding protein), SHP-2 (phosphatase), Cbl (ubiquitylation)
PTB	phosphotyrosine	Shc (adaptor protein), IRS-1 (adaptor for insulin RTK signaling), X11 (neuronal protein)
SH3	proline-rich	Src (tyrosine kinase), Crk (adaptor protein), Grb2 (adaptor protein)
14-3-3	phosphoserine	Cdc25 (CDK phosphatase), Bad (apoptosis regulator), Raf (Ser/Thr kinase), PKC (protein kinase C Ser/Thr kinase)
Bromo	acetylated lysine	P/CAF (transcription co-factor), chromatin proteins
PH[b]	phosphorylated inositides	PLC-δ (phospholipase C-δ), Akt/PKB (Ser/Thr kinase), BTK

[a]At least 32 distinct types of binding domains have been identified (see Supplementary Sidebar 6.3). This table presents six of these that are often associated with transduction of mitogenic signals.

[b]The phosphoinositide-binding groups include, in addition to the PH domain, the Fab1, YOTB, Vac1, EEA1 (FYVE), PX, ENTH, and FERM domains.

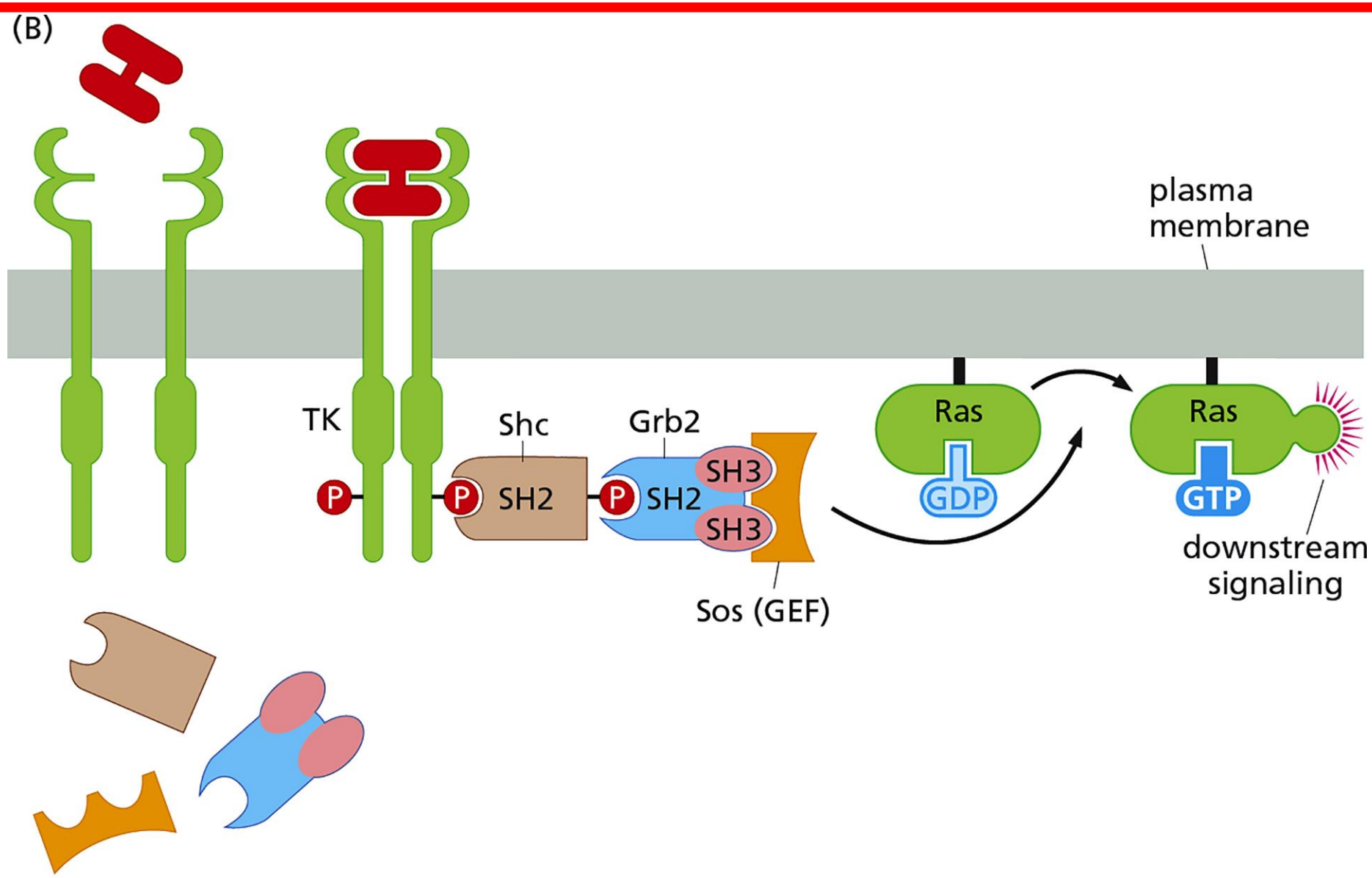
Intermolecular links by Grb2 and Shc



SH= Src homology domain

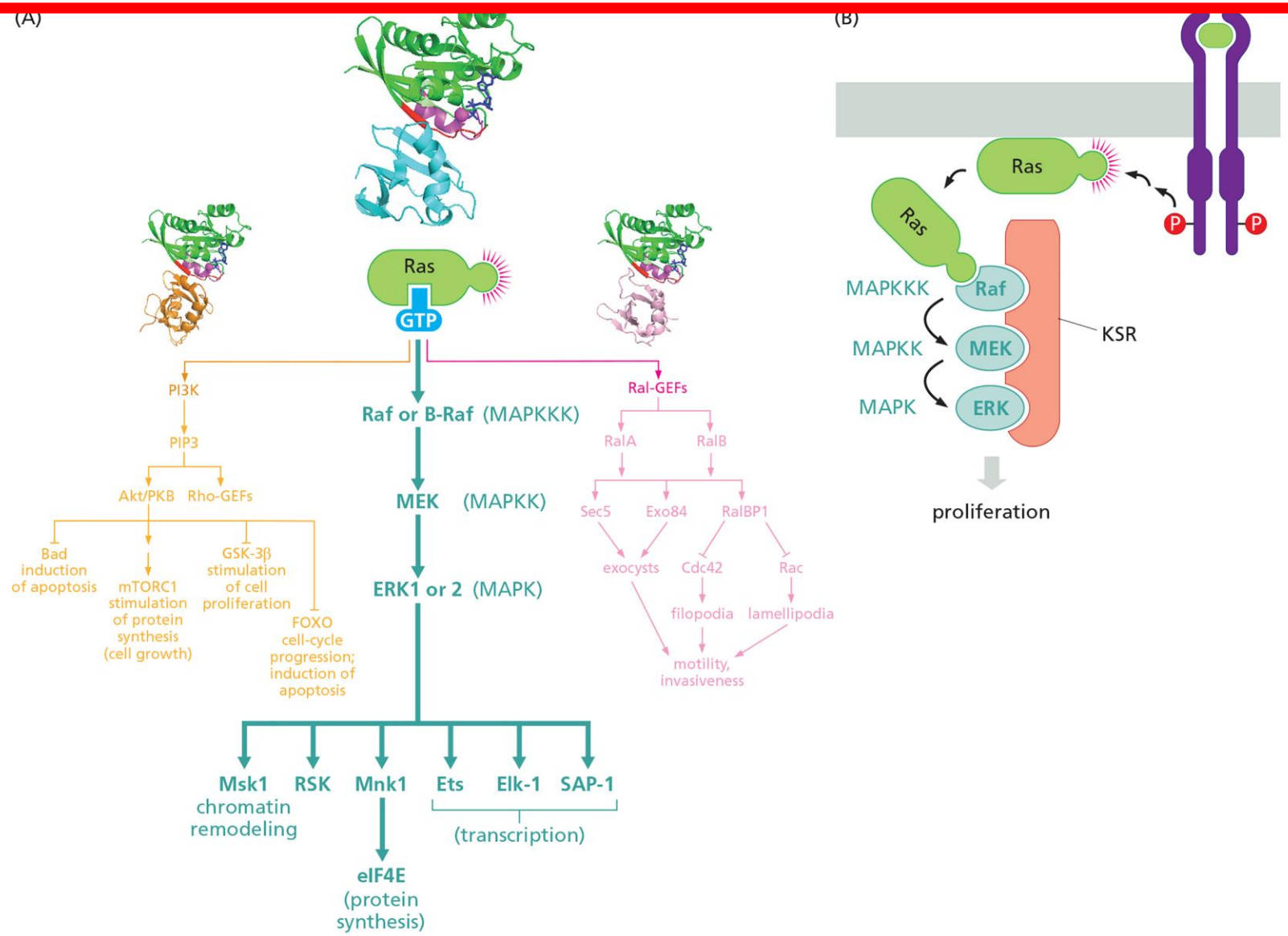
(Adapted from G.M. Cooper, Oncogenes, 2nd ed. Sudbury, MA: Jones and Bartlett, 1995. www.jblearning.com. Reprinted with permission from Jones and Bartlett.)

Alternative mode of association between Grb2 and Shc



(Adapted from G.M. Cooper, Oncogenes, 2nd ed. Sudbury, MA: Jones and Bartlett, 1995. www.jblearning.com. Reprinted with permission from Jones and Bartlett.)

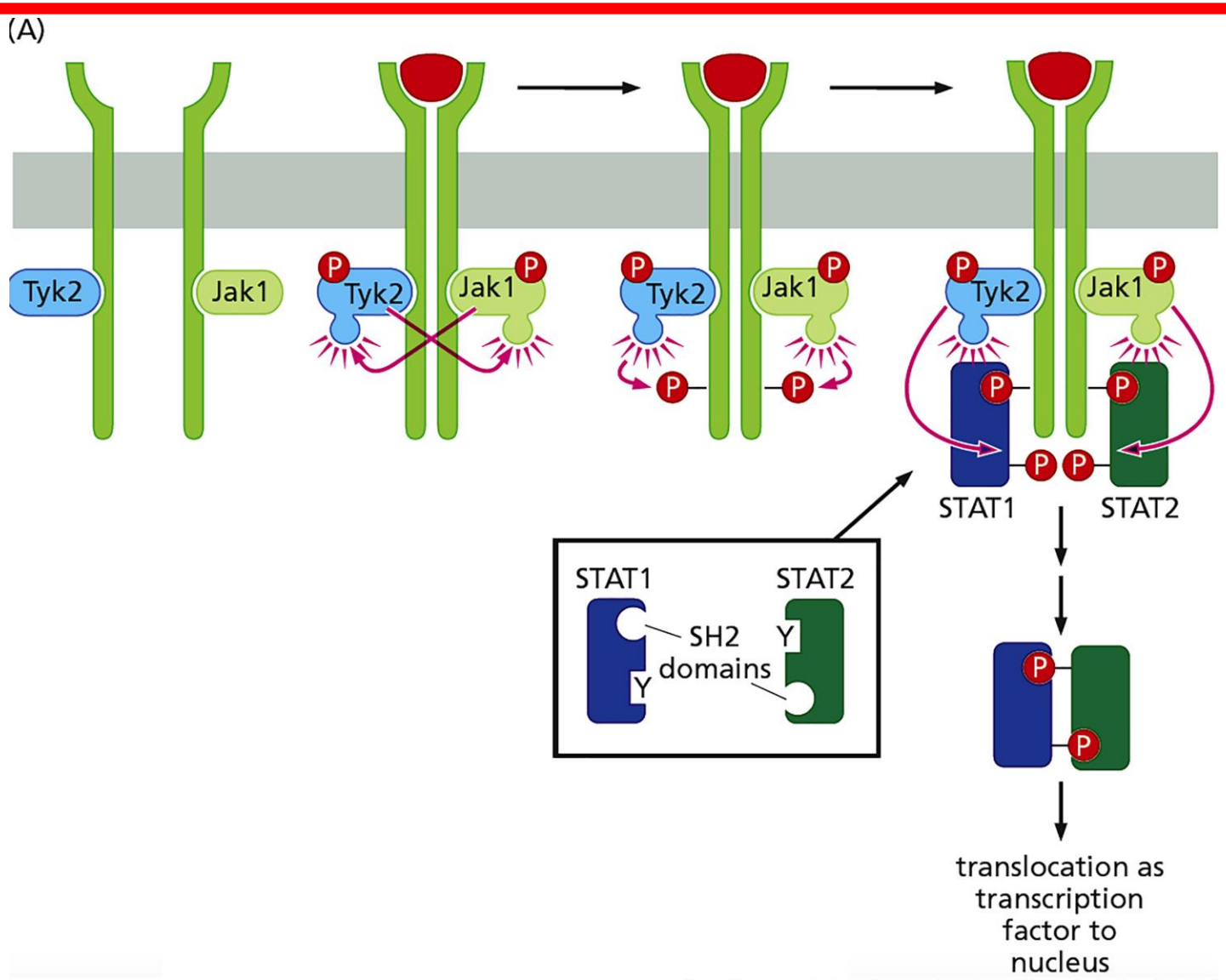
Ras-Raf-MAP kinase pathway



(A, adapted from M.E. Pacold et al., *Cell* 103:931–943, 2000; N. Nassar et al., *Nature* 375:554–560, 1995; and L. Huang et al., *Nat. Struct. Biol.* 5:422–426, 1998.
B, adapted from M.C. Good, J.G. Zalatan and W.A. Lim, *Science* 332, 680–686, 2011.)

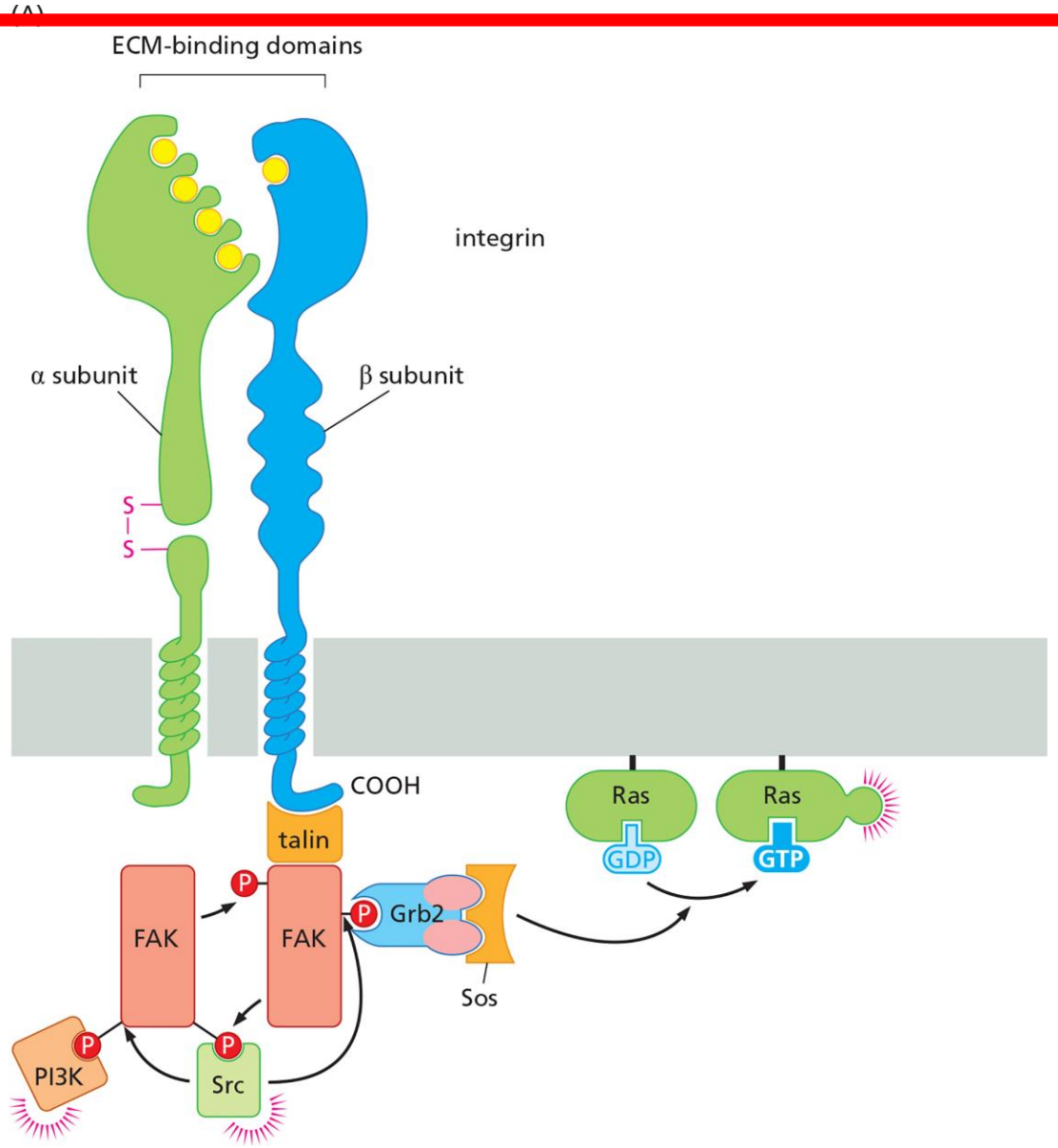
JAK-STAT pathway

Cytokine receptors



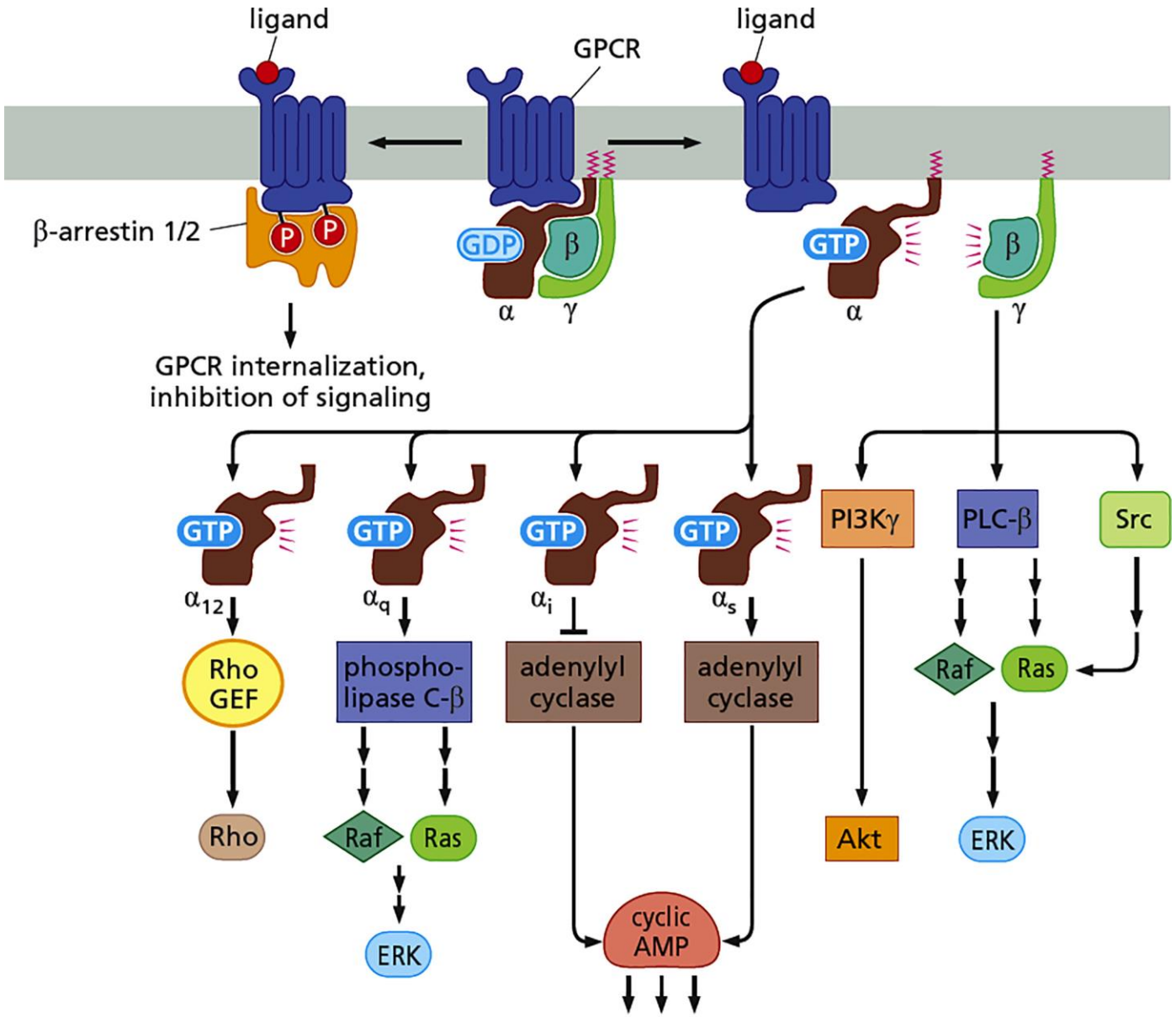
(From J. Bromberg et al., *Cell* 98: 295–303, 1999. With permission from Elsevier.)

Integrin signaling



(Adapted from C. Miranti and J. Brugge, *Nat. Cell Biol.* 4:E83–E90, 2002. With permission from Nature.)

G-protein-coupled receptors signaling



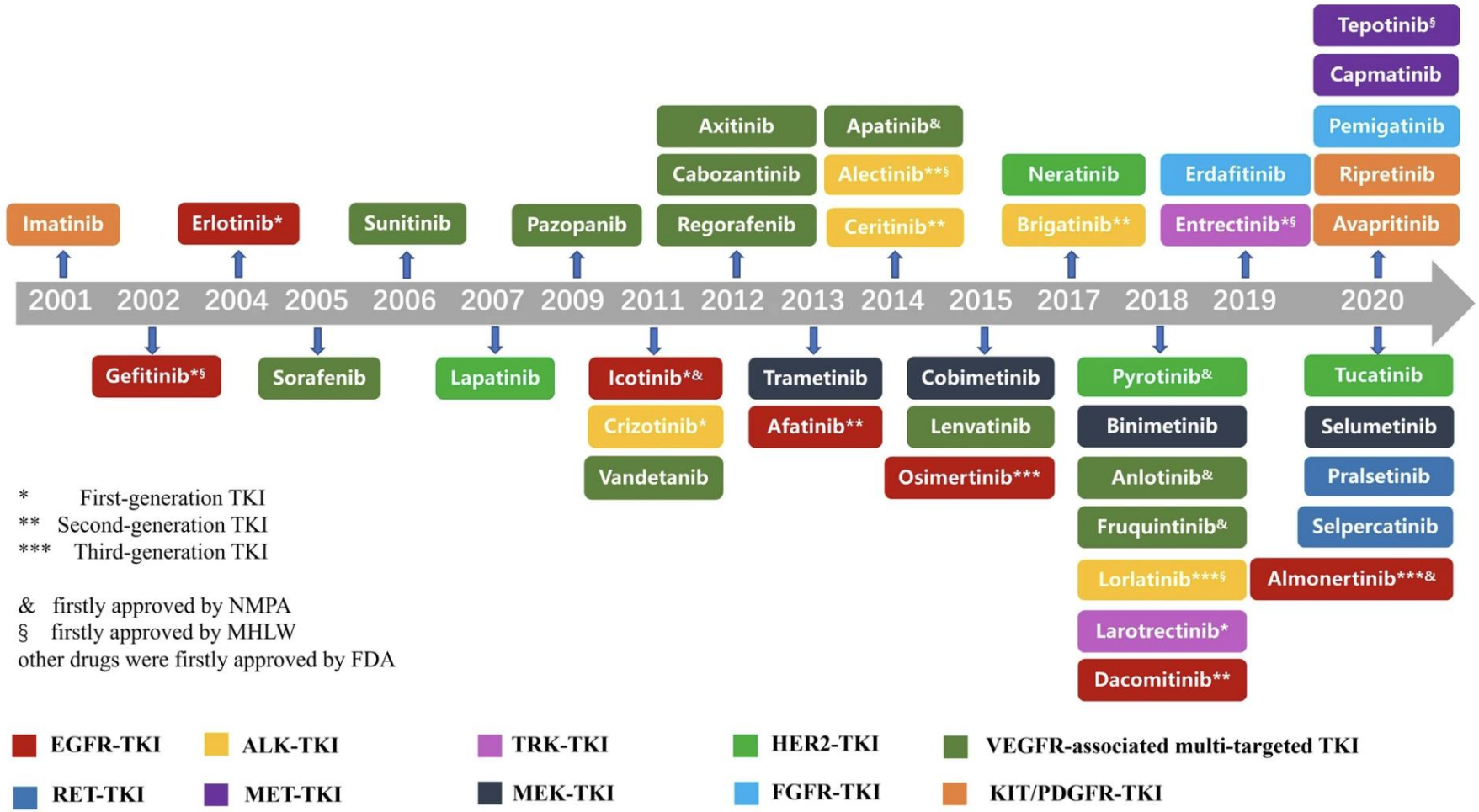
G-protein-coupled receptors and G proteins involved in human cancer pathogenesis

G protein or receptor	Type of tumor
Activating mutations affecting G proteins	
$G\alpha_s$	thyroid adenomas and carcinomas, pituitary adenomas
$G\alpha_{i2}$	ovarian tumors, adrenal cortical tumors
$G\alpha_q$	melanocyte-derived tumors, uveal melanomas
$G\alpha_{11}$	uveal melanomas
Activating mutations affecting G-protein–coupled receptors	
Thyroid-stimulating hormone receptor	thyroid adenomas and carcinomas
Follicle-stimulating hormone receptor	ovarian tumors
Luteinizing hormone receptor	Leydig cell hyperplasias
Cholecystokinin-2 receptor	colorectal carcinomas
Ca^{2+}-sensing receptor	various neoplasms
GPR98	melanomas
GRM3	melanomas
Autocrine and paracrine activation	
Neuromedin B receptor	small cell lung cancer (SCLC)
Neurotensin receptor	prostate carcinomas and SCLC
Gastrin receptor	gastric carcinomas and SCLC
Cholecystokinin receptor	pancreatic hyperplasias and carcinomas, gastrointestinal carcinomas, and SCLC
Virus-encoded G-protein–coupled receptors	
Kaposi's sarcoma herpesvirus (HHV-8)	Kaposi's sarcoma
Herpesvirus saimiri	primate leukemias and lymphomas
Jaagsiekte sheep retrovirus	sheep pulmonary carcinomas

Adapted in part from M.J. Marinissen and J.S. Gutkind, *Trends Pharmacol. Sci.* 22:368–376, 2001. With permission from Elsevier.

Inhibiting tyrosine kinase: New targeted therapies in cancer

Tyrosine kinase inhibitors: targeted therapies in cancer



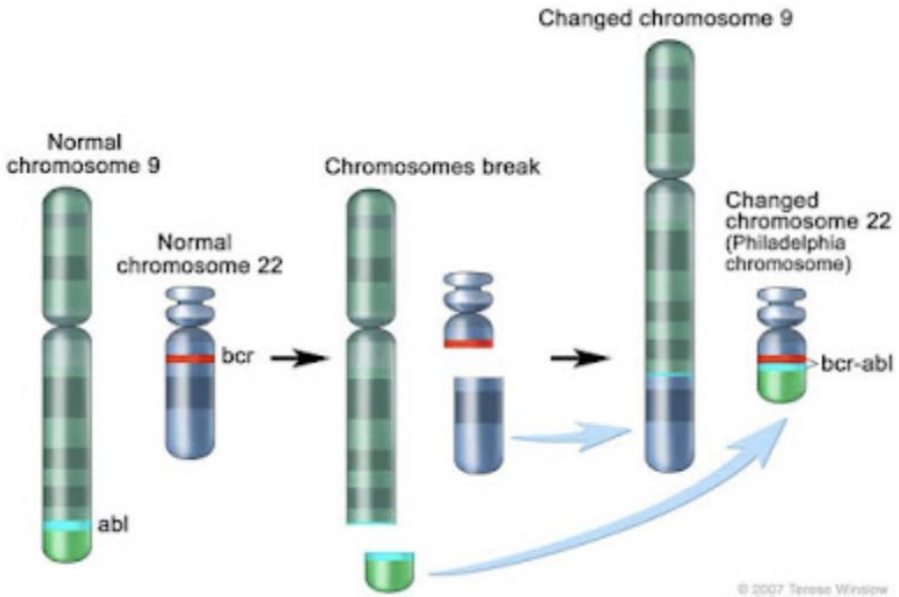
A schematic summary of the approved TKIs in 2001–2020. *NMPA* National Medical Products Administration, *MHLW* Ministry of Health, Labor and Welfare, *FDA* Food and Drug Administration

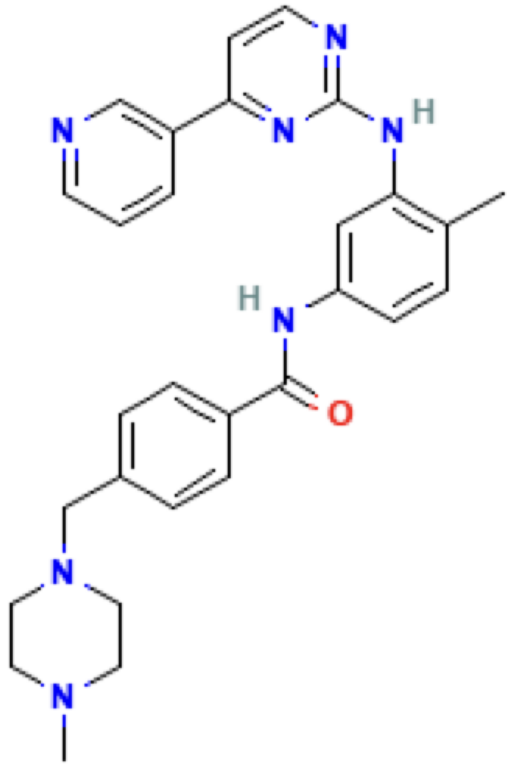
J Hematol Oncol (2020) 13:143

Imatinib: The first tyrosine kinase inhibitor approved for cancer therapy

Imatinib (Gleevec): Potent inhibitor for BCR-ABL

Initially approved for treatment of chronic myeloid Leukemia (CML)





EGFR mutations in lung cancer

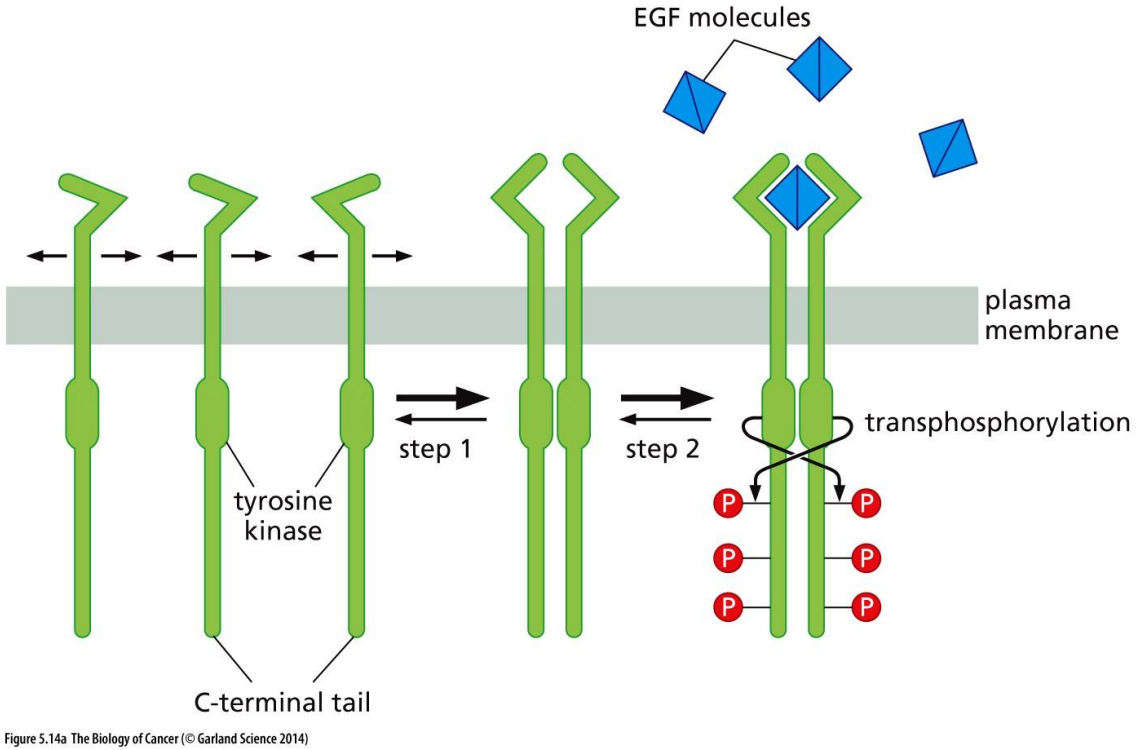
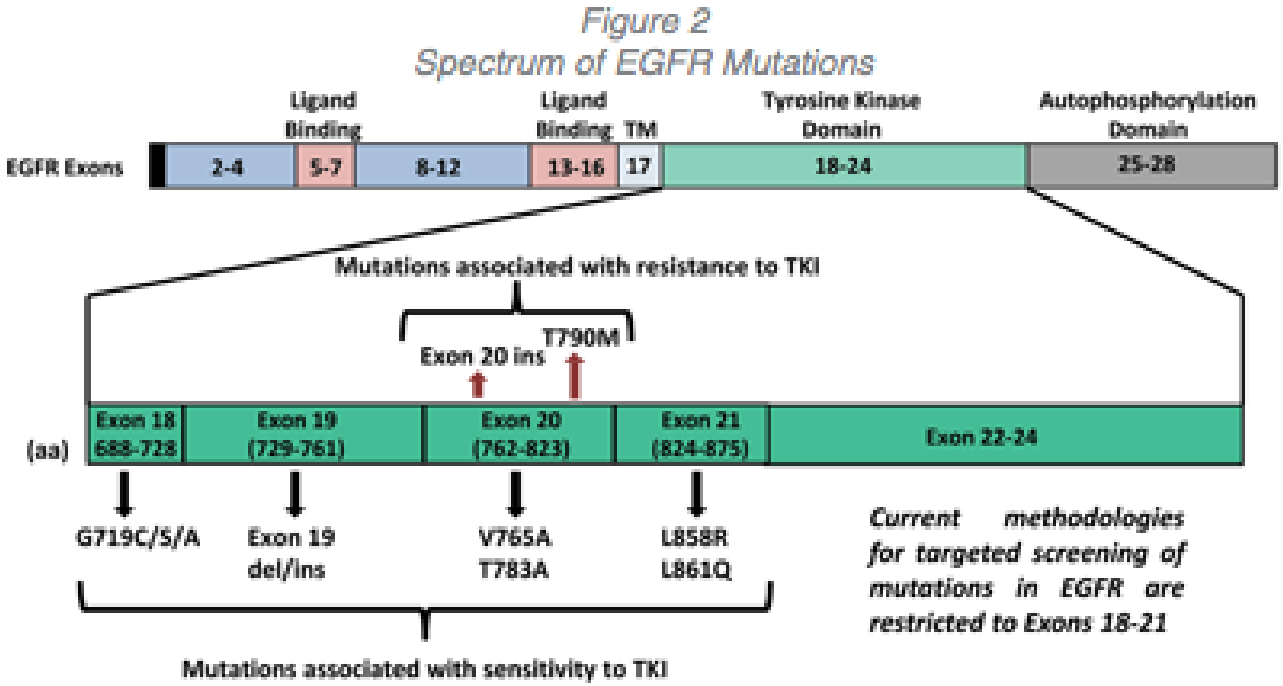


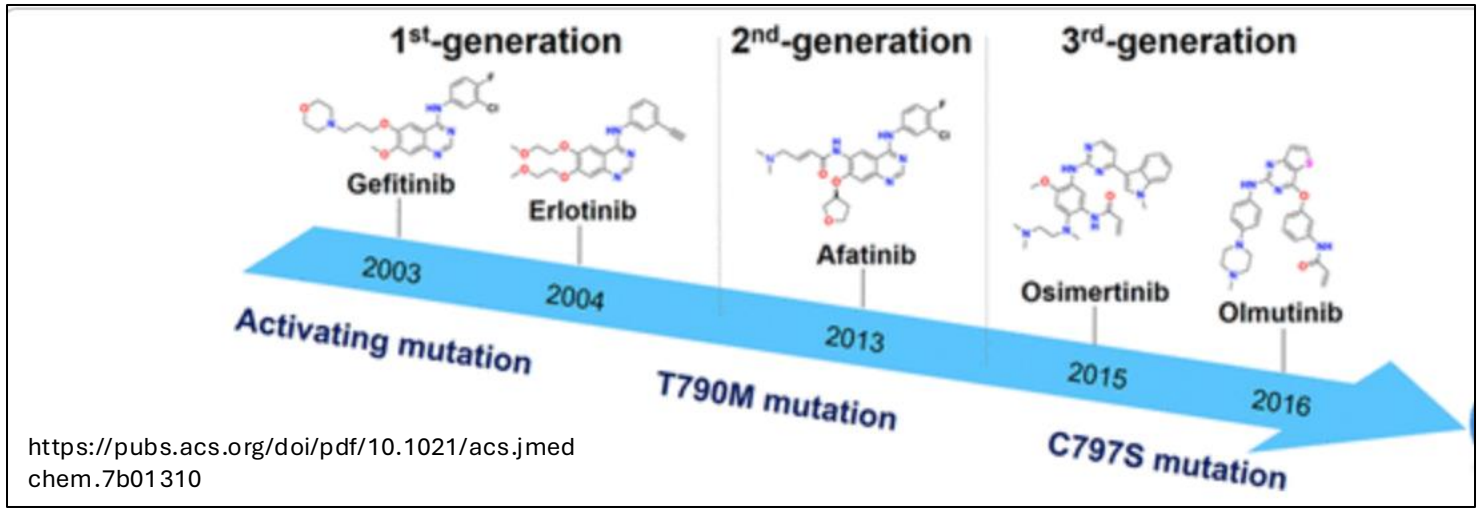
Figure 5.14a The Biology of Cancer (© Garland Science 2014)



- Exon 19 deletion and L858R activation mutation constitute 90% of all EGFR activating mutation in lung cancer
- Ligand-independent activation

https://www.aacc.org/cln/articles/2013/october/egfr-mutations

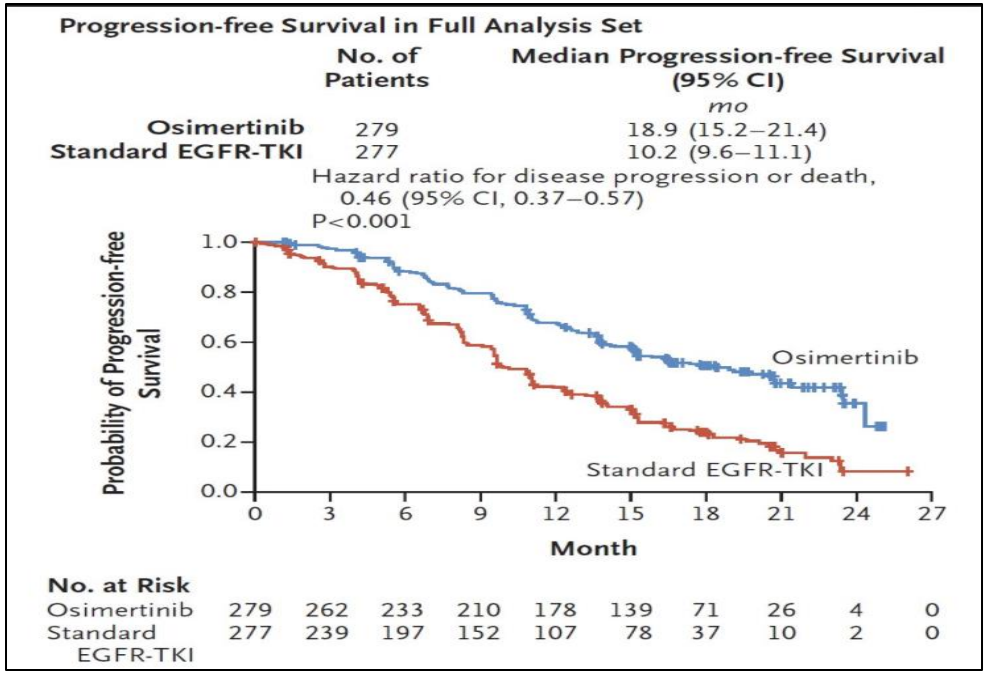
EGFR mutant lung cancer



https://pubs.acs.org/doi/pdf/10.1021/acs.jmedchem.7b01310

Third generation TKI AZD9291(Osimertinib):

- **Irreversible: C797 (Exon 20)**
- **Mutant-EGFR specific: Exon 19 Del, T790M**
- **Brain-penetrant**

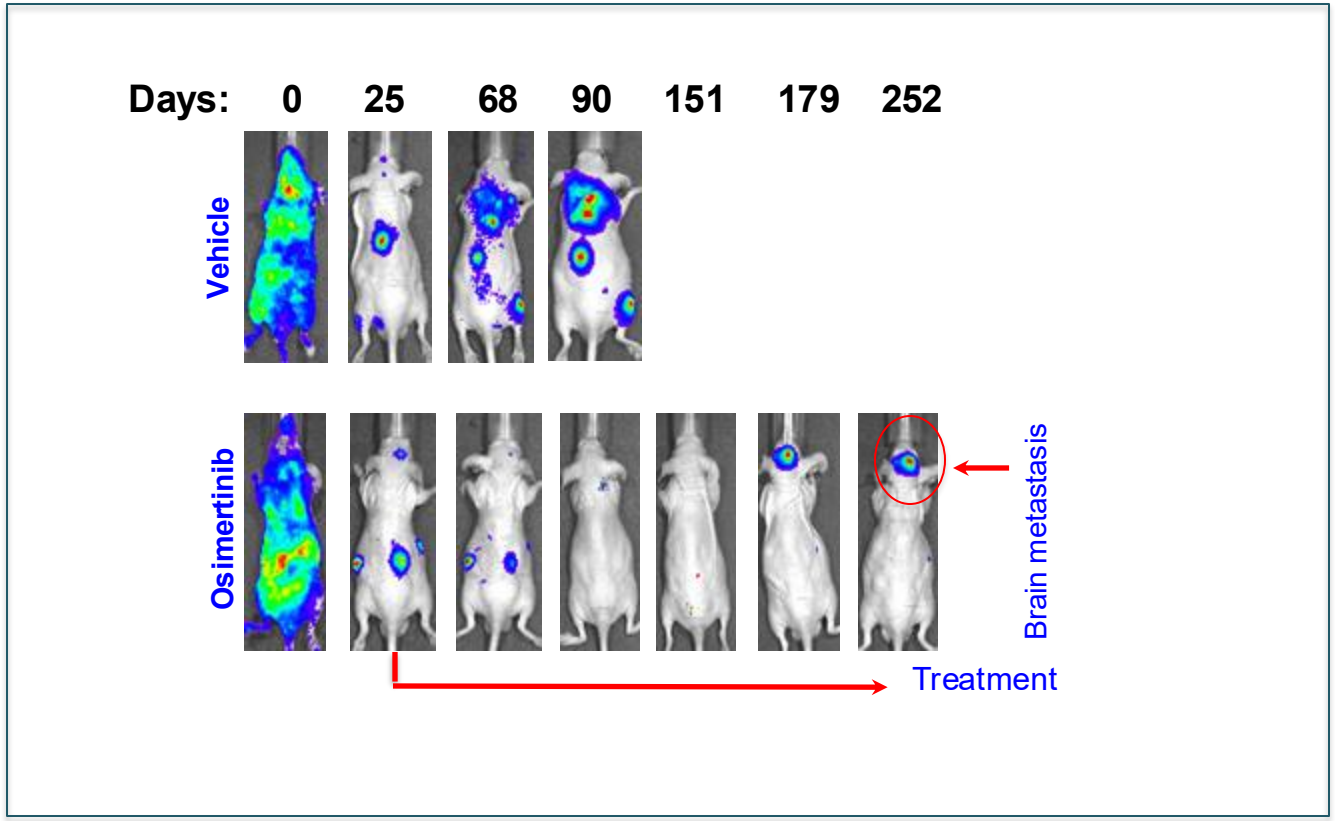


FLAURA trial, NEJM, 2018

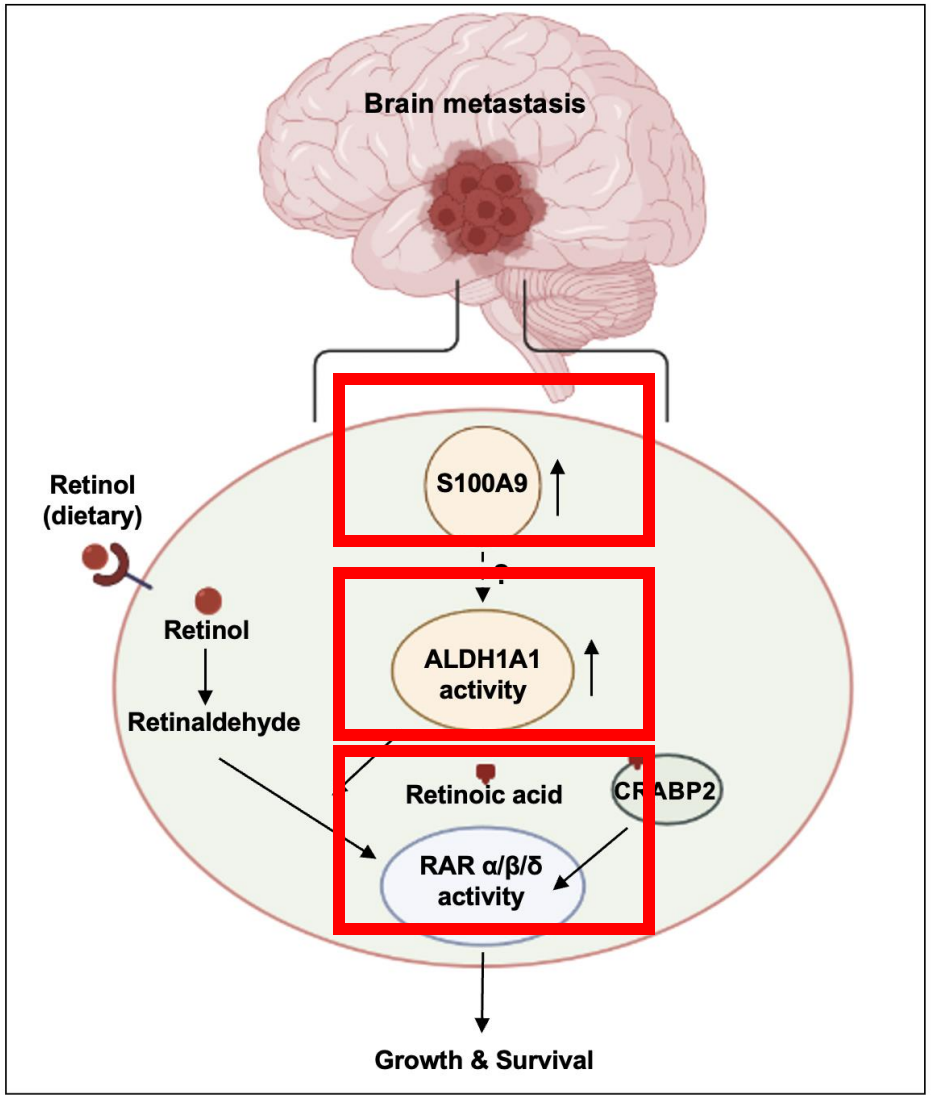
Resistance to targeted therapies

A major challenge in the treatment of cancer patients

Striking response to EGFR-inhibitor (osimertinib) followed by lethal brain relapse From EGFR-mutant lung cancer cells



S100A9-ALDH1A1-retinoic acid pathway promotes both brain metastasis and osimertinib therapy resistance



Biswas et al., *Cancer Discovery*, 2022