MACQUARIE University

Biochemical Signalling -1 Cornerstone of Survival in Cell Biology

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Objectives

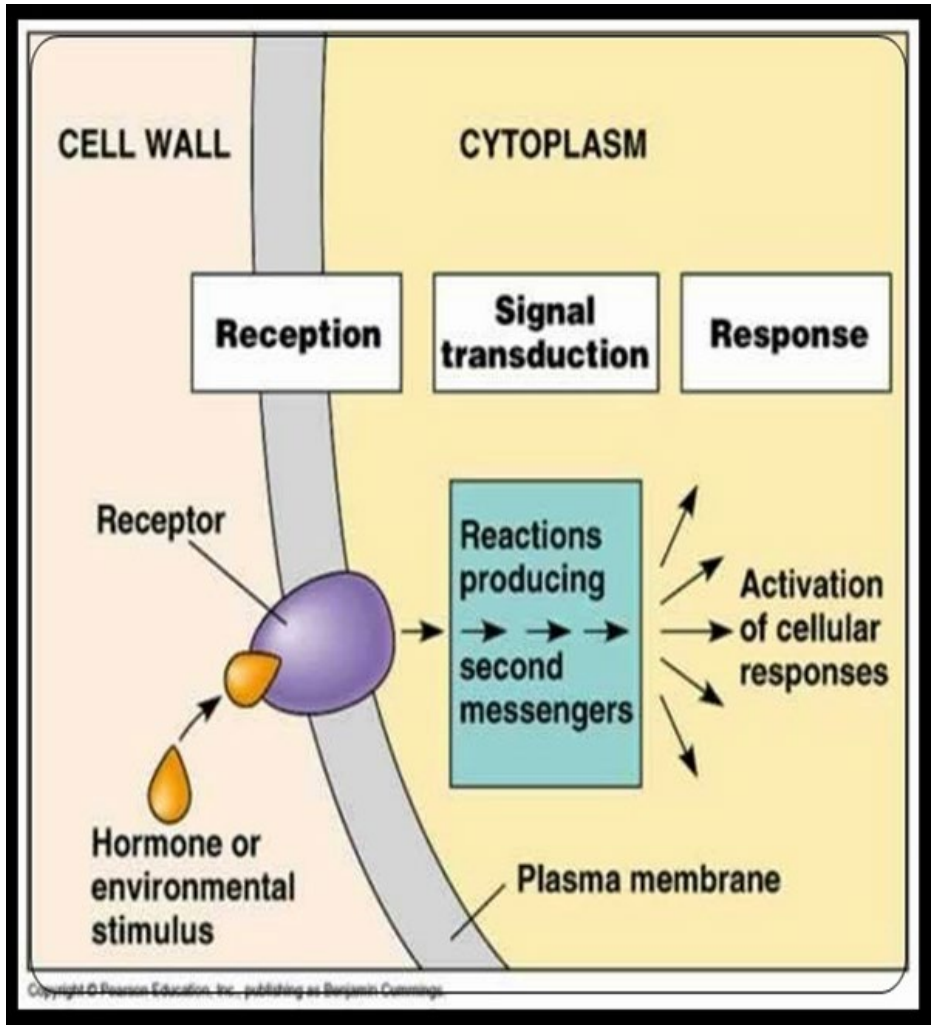
- Understand the Basics of Biochemical Signalling
  - Why we need then?
  - Steps in cascades
  - Consequences for the cell
- Hormones are signalling molecules
- Receptors that bind hormones
  - Receptor Tyrosine Kinases
- *Textbook Chapter 20*

Why do we need biochemical signaling?

- Membranes form “walls”
- How does the cell then communicate external changes e.g. food sources or dangerous situations?
  - Resources are running low inside the cell
  - Resources are available outside the cell
  - An invading organism is trying to break down the wall
  - A toxic chemical is in blood stream
- Internal communication also required
  - Time to make new cells or repair old ones.
  - Stop making new cells or genes.
  - Need to export excess materials or store them elsewhere.

Metabolic strategies

- In general, every signalling pathway consists of:
  - **a receptor protein** that specifically binds **a hormone or other ligand**,
  - a mechanism for **transmitting** the ligand-binding event **to the cell interior**, and
  - a series of **intracellular responses** that may involve the synthesis of a second messenger and/or chemical changes catalyzed by kinases and phosphatases.
- **These signalling pathways** often involve **enzyme cascades**, in which a succession of events **amplifies the signal**.



Steps in Signal Transduction Cascades

1. **Signal release** (primary message) in response to a physiological state.
2. **Reception:** signalling molecule binds to a **receptor**, usually an integral membrane protein.
3. **Transduction**: conveying the primary message to the cell interior by creating an intracellular second message.
   - The original signal may need to be ***amplified*** to have **a rapid effect**.
4. **Response**: Activation of effector molecules by the second messenger that result in a physiological response.
5. **Switching off:** Terminating the signal cascade – we do not want continuous signalling.

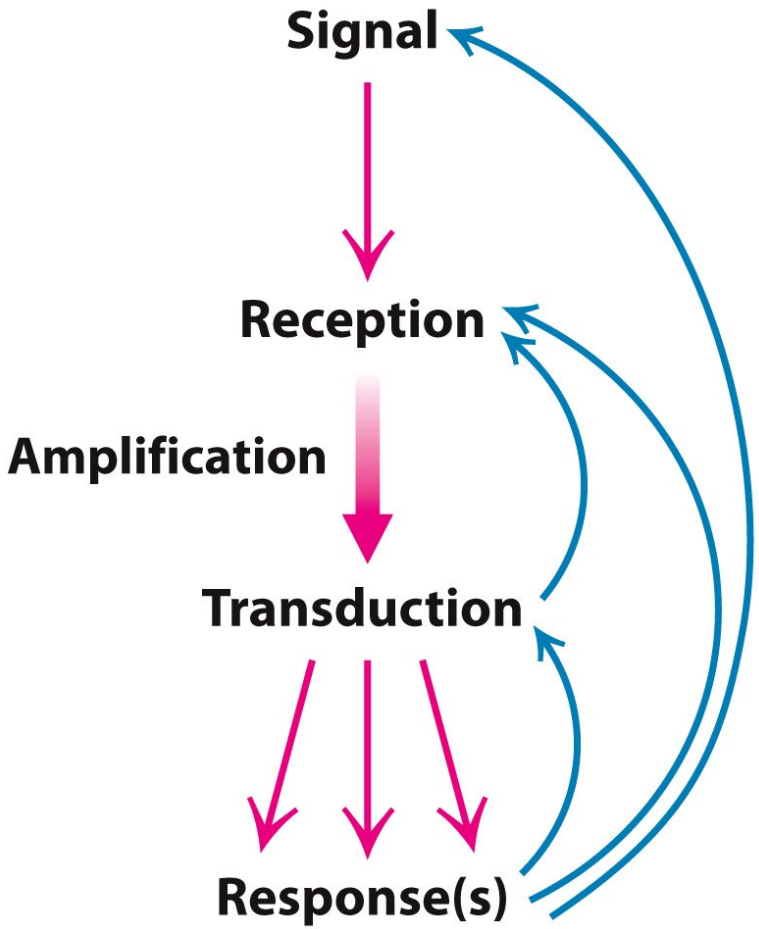
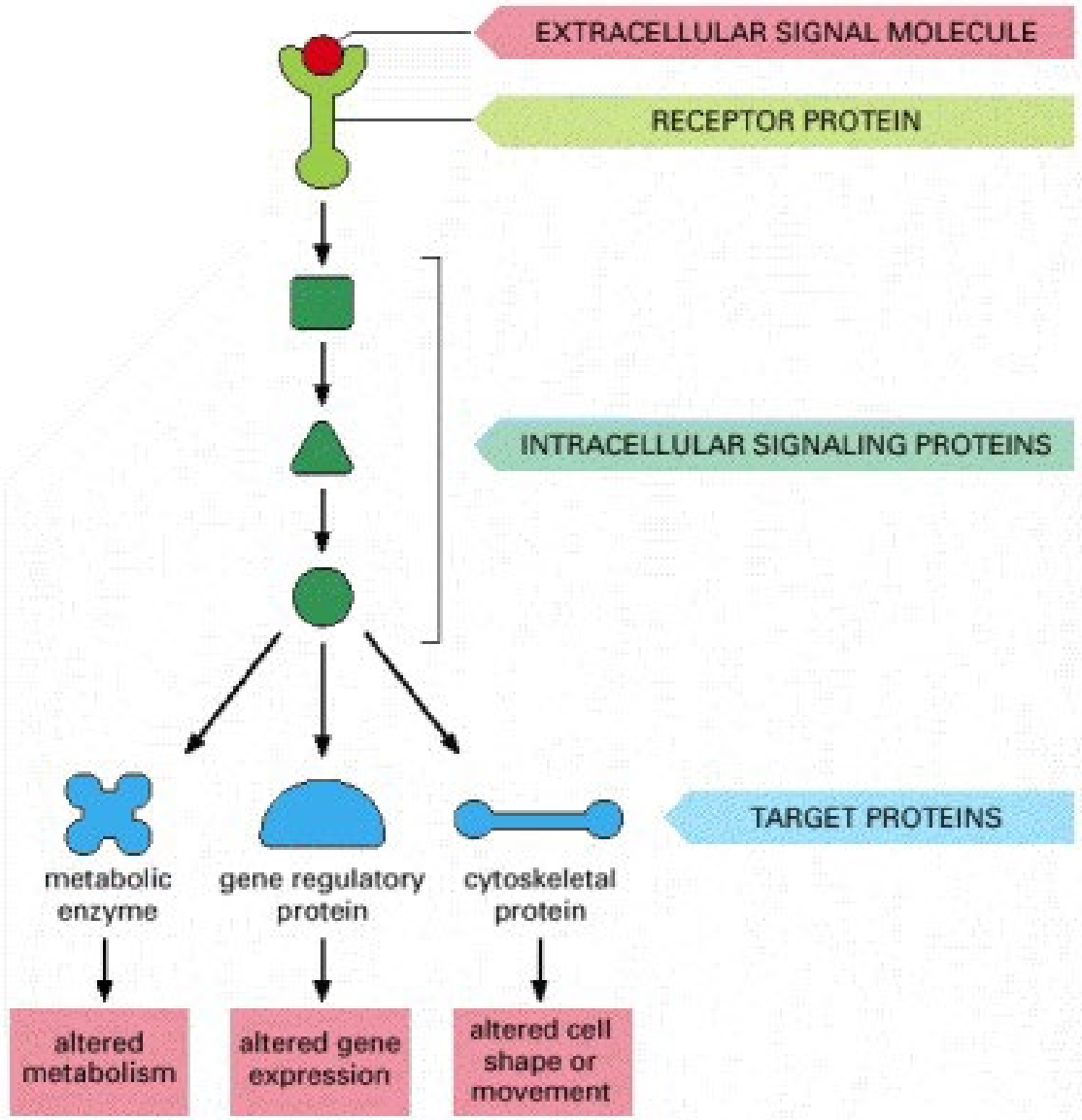


Figure 14.2
Biochemistry, Eighth Edition

Consequences of Signalling Cascades



1. **Changes in <u>metabolic activity</u>**
2. Changes to gene expression
3. Triggers for growth, differentiation or movement.

Signalling Consequences

Each cell is programmed to respond to specific combinations of extracellular signals

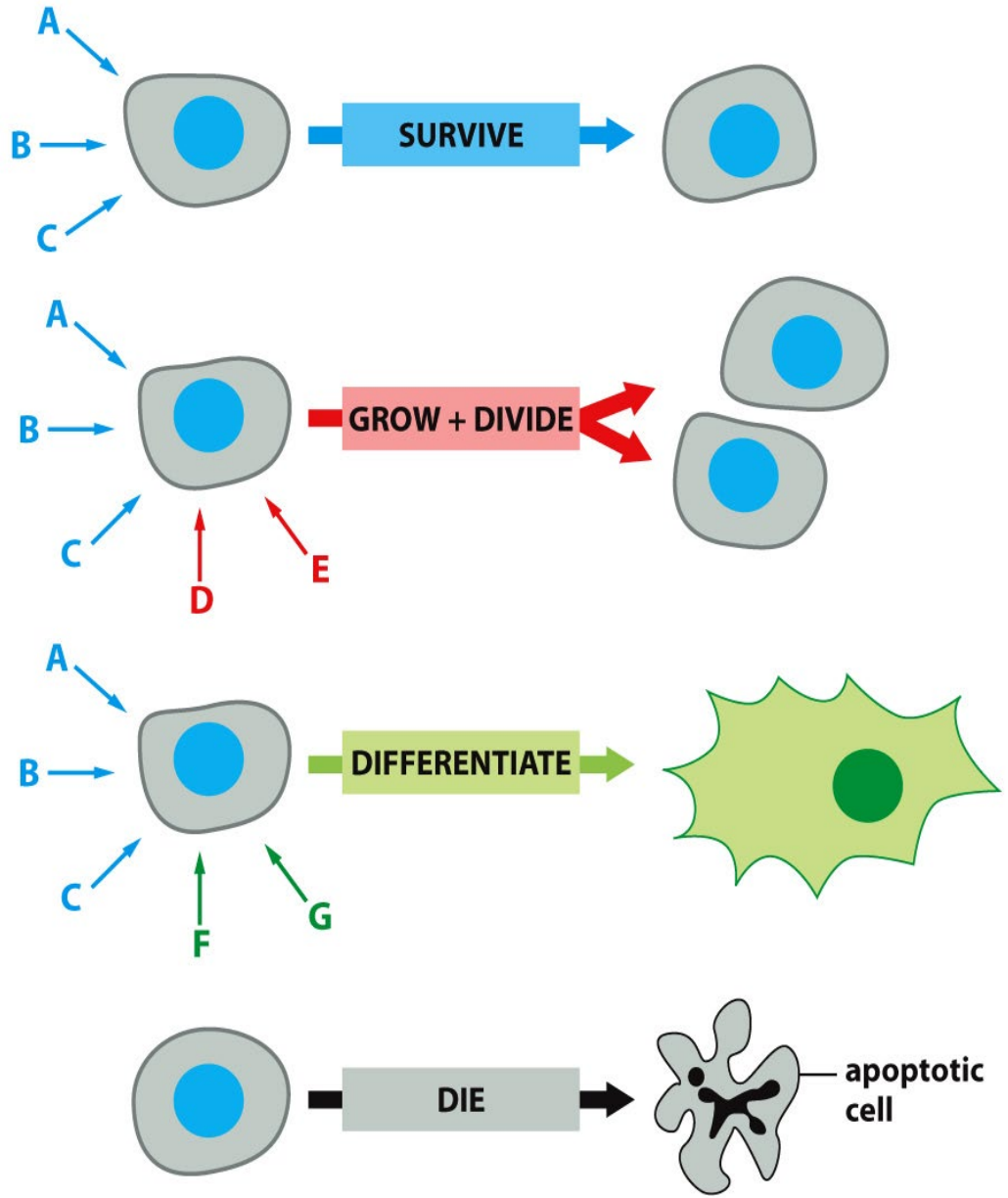


Figure 15-4 Molecular Biology of the Cell 6e (© Garland Science 2015)

Different responses from the same signal

Each cell is programmed to respond to specific combinations of extracellular signals: aceylcholine is the signal here

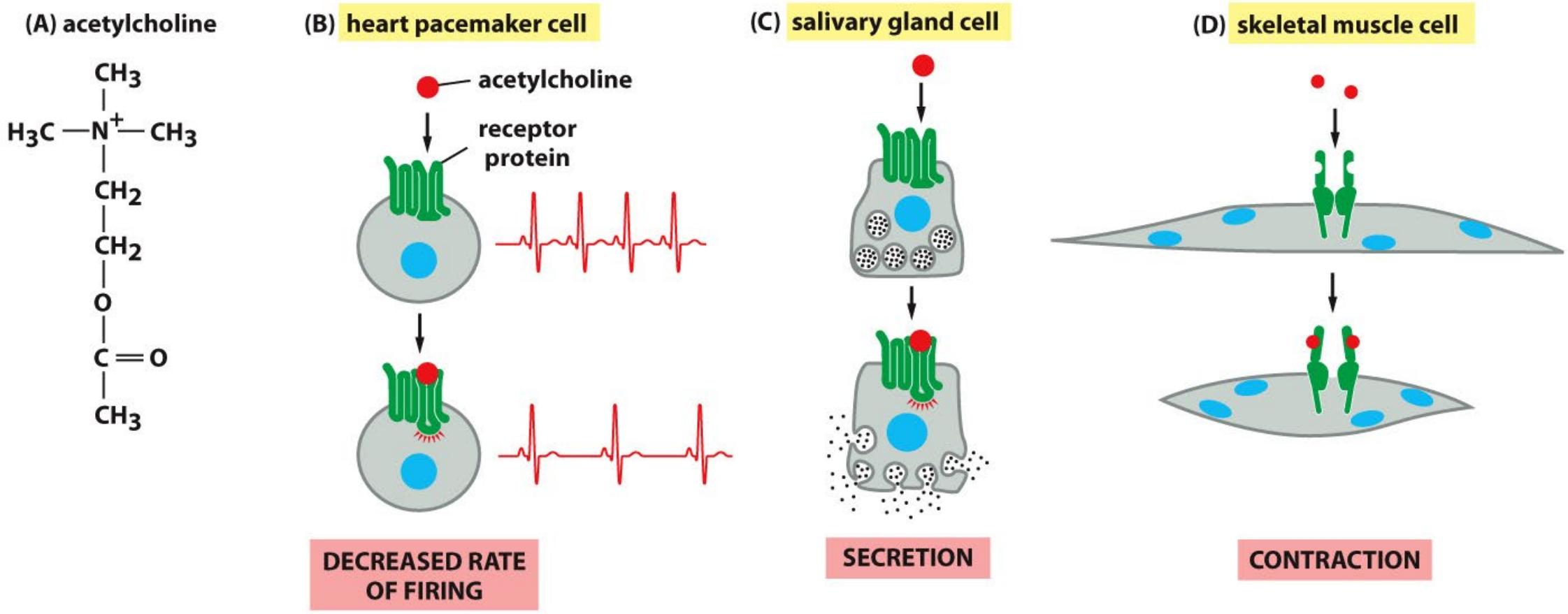


Figure 15-5 Molecular Biology of the Cell 6e (© Garland Science 2015)

Signalling Basics Summary

1. The signal molecule binds to a receptor protein (which is usually embedded in the plasma membrane)
2. This binding activates an intracellular signalling pathway involving many signalling proteins.
3. These intracellular signalling proteins interact with a target protein.
4. The target protein reacts to change the behaviour of the cell.

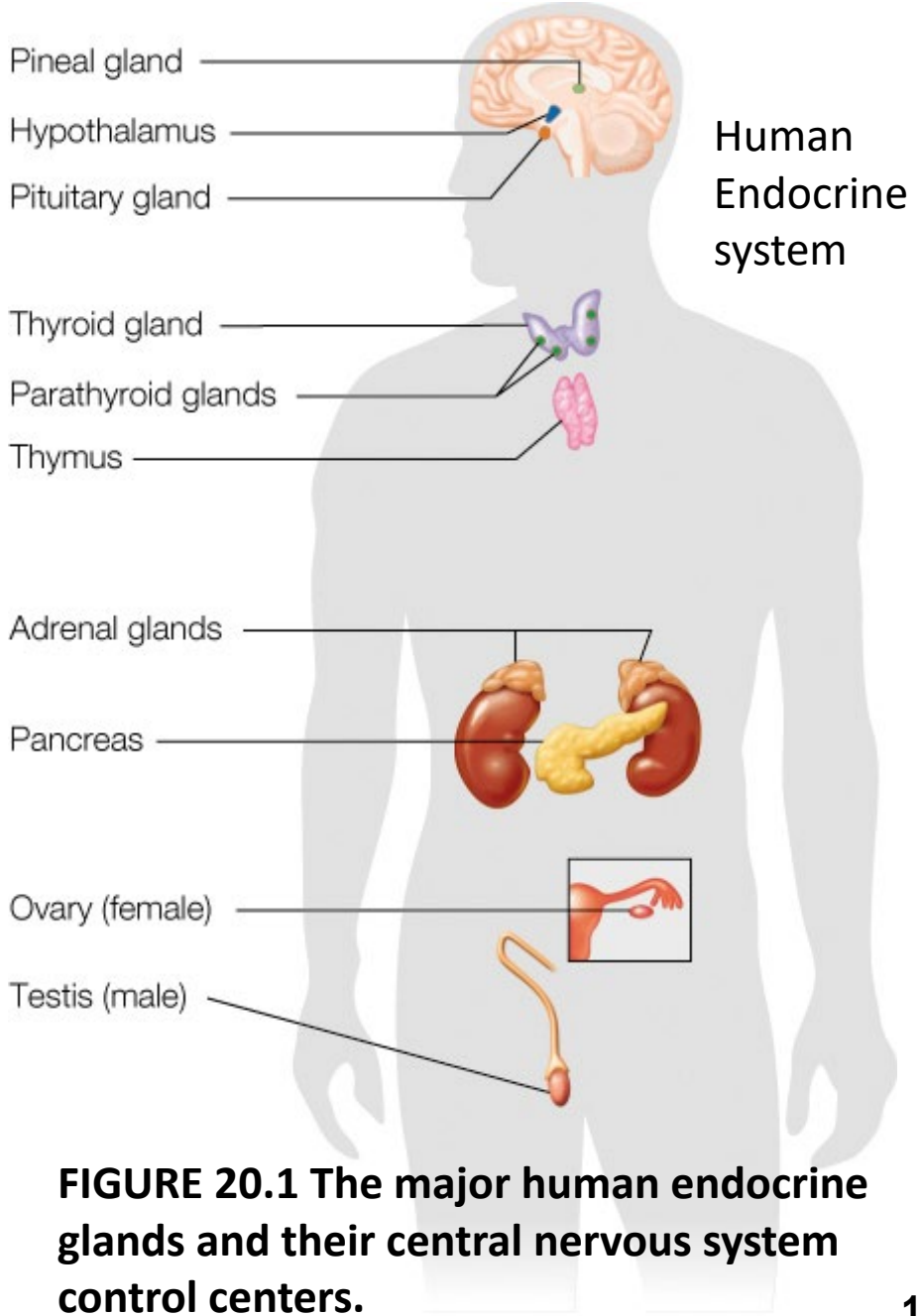
Endogenous signalling molecules: Hormones

- In vertebrates, hormones are small molecules secreted by endocrine glands, which travel through the bloodstream and bind to specific receptors on or in target cells
- Chemically, hormones include
  - peptides or polypeptides (insulin, glucagon)
  - steroids (glucocorticoids and sex hormones)
  - amino acid derivatives (catecholamines such as epinephrine and thyroxine, derived from tyrosine)

Endocrine Hormones: our own signals

Several hormones are involved in the coordination of carbohydrate metabolism:

1. epinephrine, secreted from the adrenal gland, promotes metabolic energy generation in muscle
2. glucagon, secreted by the pancreas, acts upon liver to promote gluconeogenesis
3. insulin, secreted by the pancreas, promotes uptake of glucose into cells



Human Endocrine system

FIGURE 20.1 The major human endocrine glands and their central nervous system control centers.

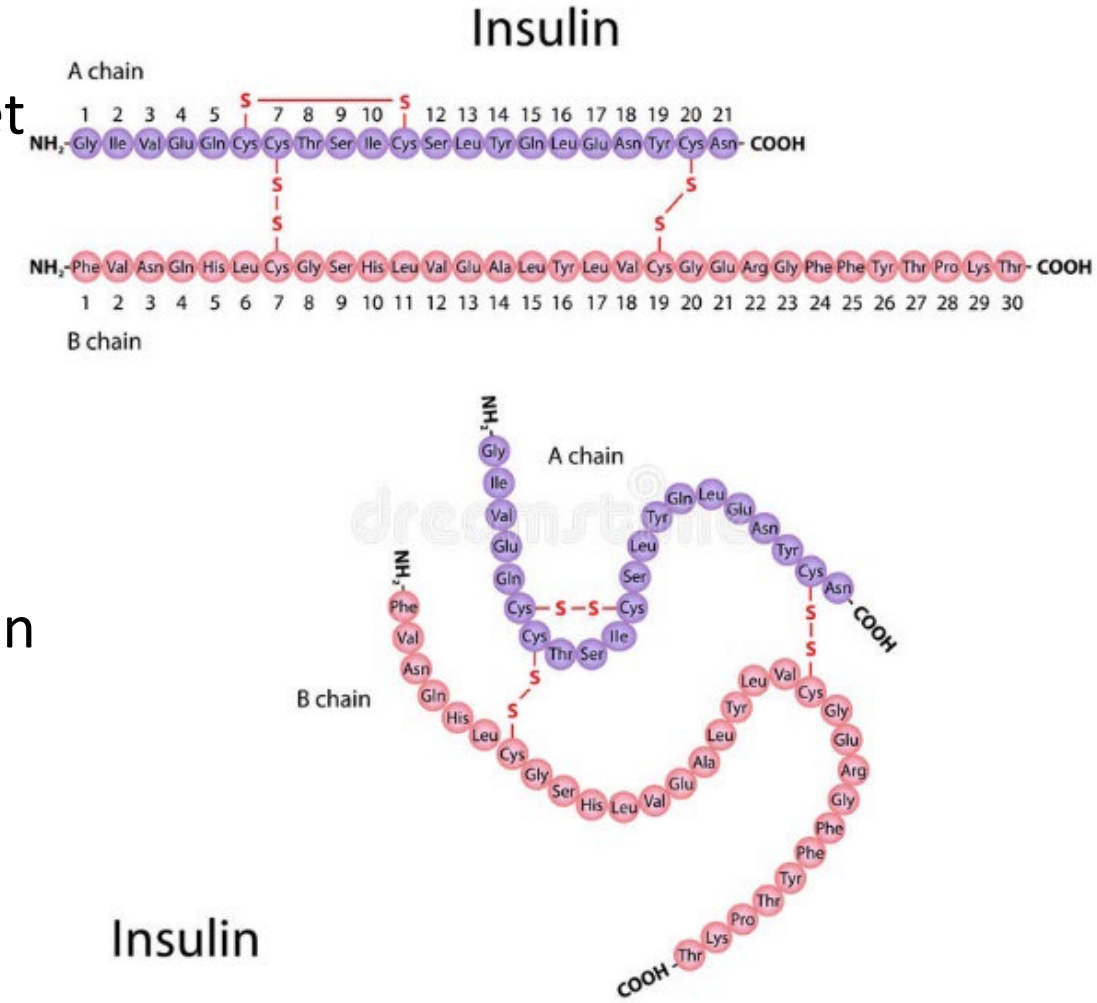
Pancreatic Islet Hormones Control Fuel Metabolism

3 polypeptide hormones released by the Islet cells

1. α cells: **glucagon** (29 residues)
2. β cells: **insulin** (51 residues)
3. δ cells: **somatostatin** (14 residues)

Glucagon and **insulin** have opposite effects on sugar metabolism (next slide).

Somatostatin inhibits the release of both glucagon and insulin, as well as other hormones such as growth hormones.



Control of Fuel Metabolism in Mammals

- Maintenance of blood glucose levels
  - ➢critical to brain function
- Major hormones:
  - insulin
  - glucagon
  - Epinephrine (under stress)

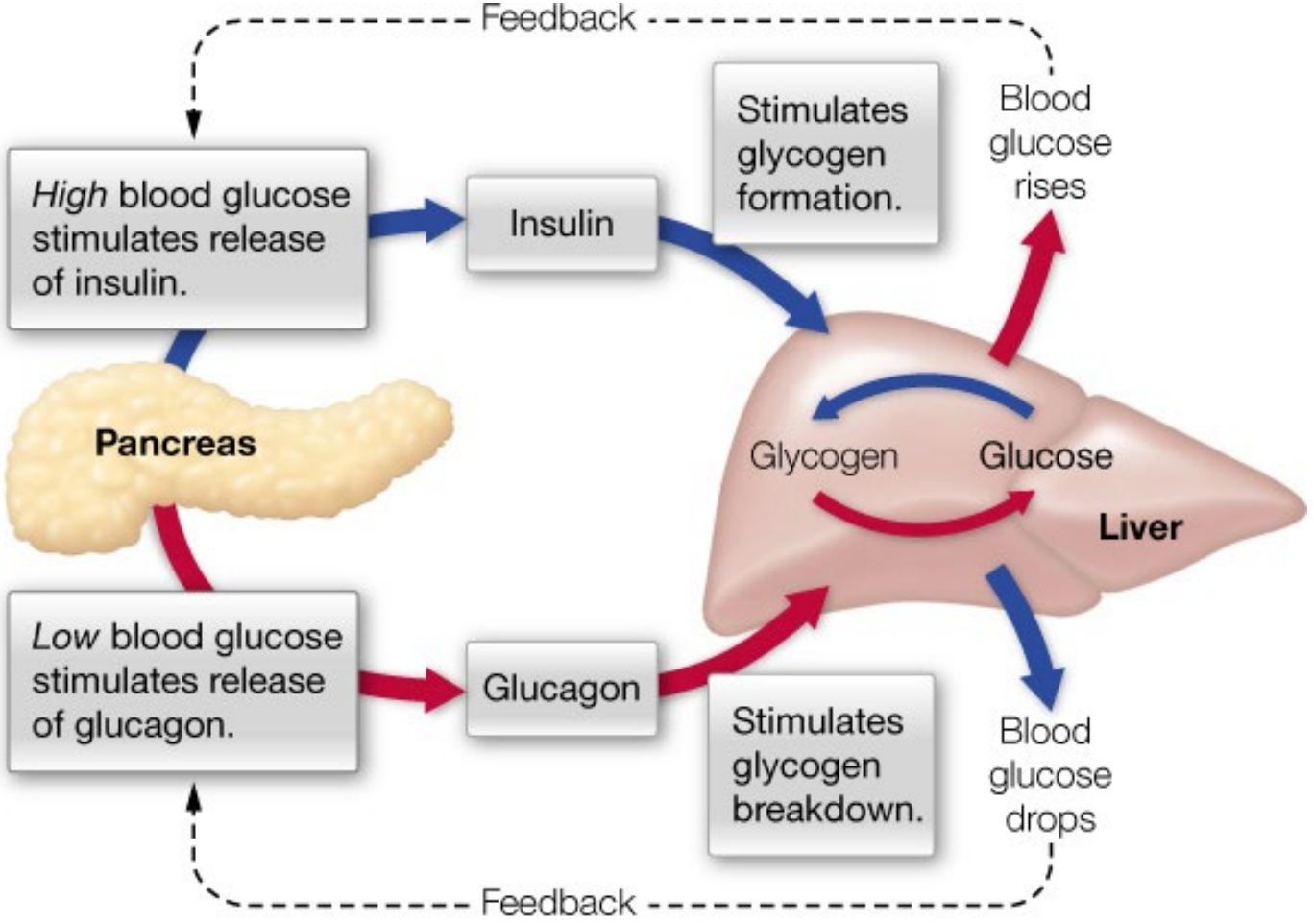
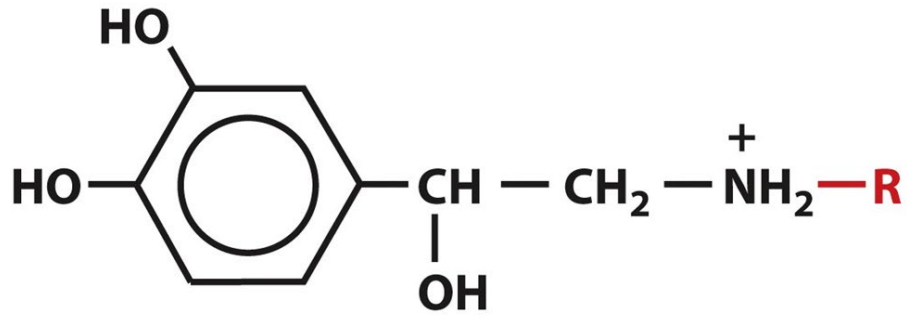


FIGURE 17.2 Aspects of the control of blood glucose levels by pancreatic secretion of insulin and glucagon.

Epinephrine and Norepinephrine Prepare the Body for Action



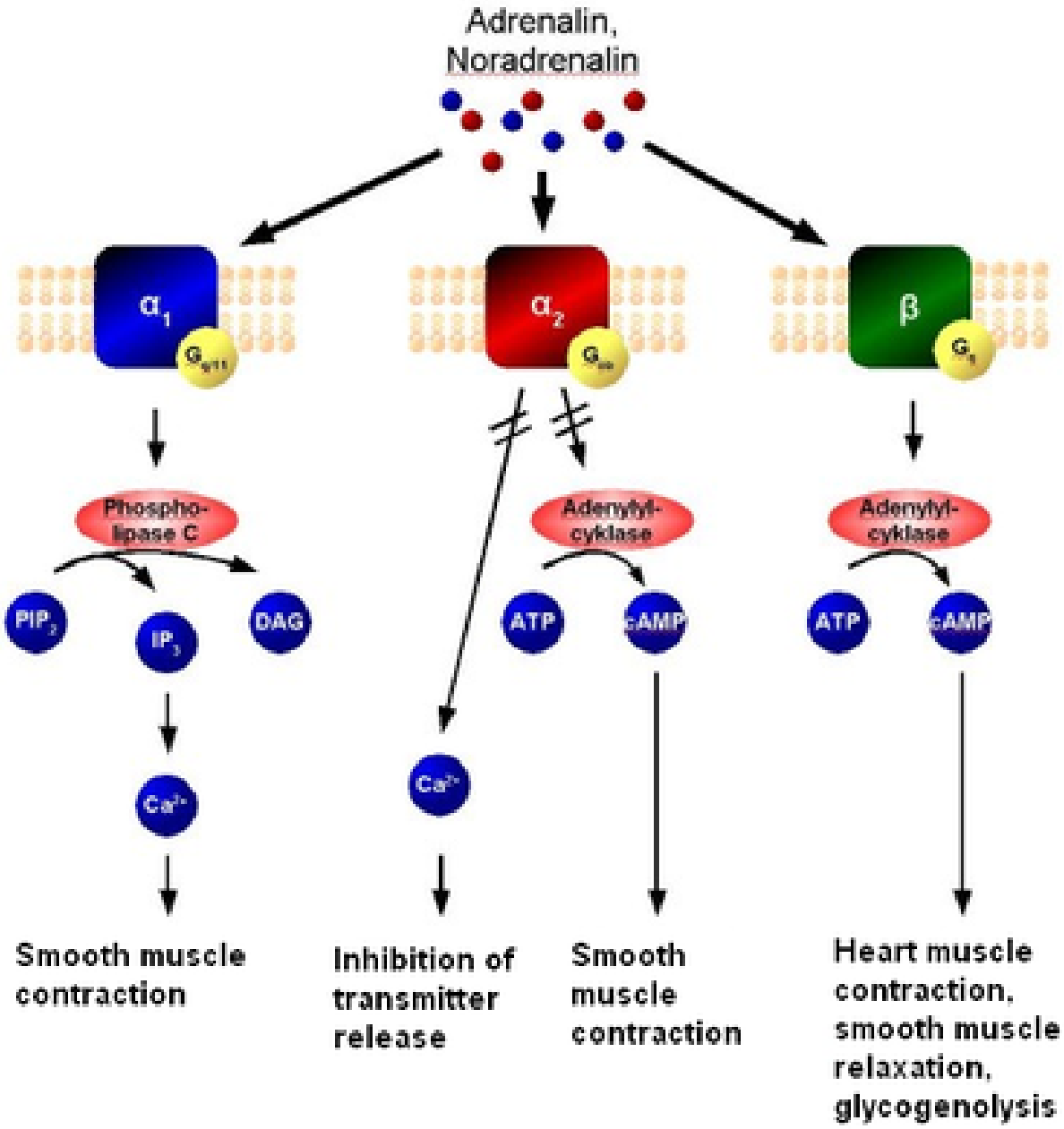
R = H **Norepinephrine (noradrenalin)**

R = CH_3 **Epinephrine (adrenalin)**

- The **medulla** (core) of the **adrenal glands** makes two ***catecholamine hormones****:*
  1. **norepinephrine (noradrenalin)** and
  2. its methyl derivative **epinephrine (adrenalin)**
- These bind to membrane-bound α- and β-adrenergic receptors on the different tissues, with different biological responses.
- The main function of these hormones is to overcome normal regulation for “flight-or-fight” responses.

Epinephrine and Norepinephrine: Biological Function

- β-receptors increase sugar breakdown in liver, lipid breakdown in adipose tissue, and smooth muscle relaxation
  - ❖bronchodilation: treatment for asthma).
- α-receptors activate smooth muscle contraction in peripheral organs
  - ❖decrease high blood pressure and protect against heart disease
- Essentially, releasing energy resources to prepare the body for action!



Hormone transport and functions

- Transported from endocrine cells to receptors on target cells, by blood.

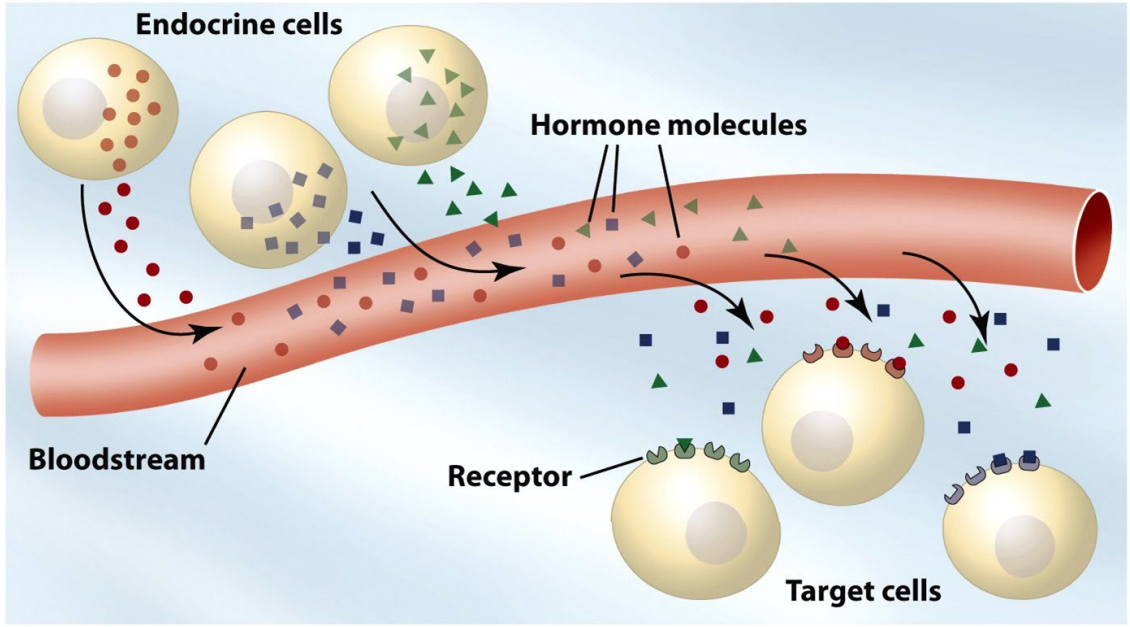
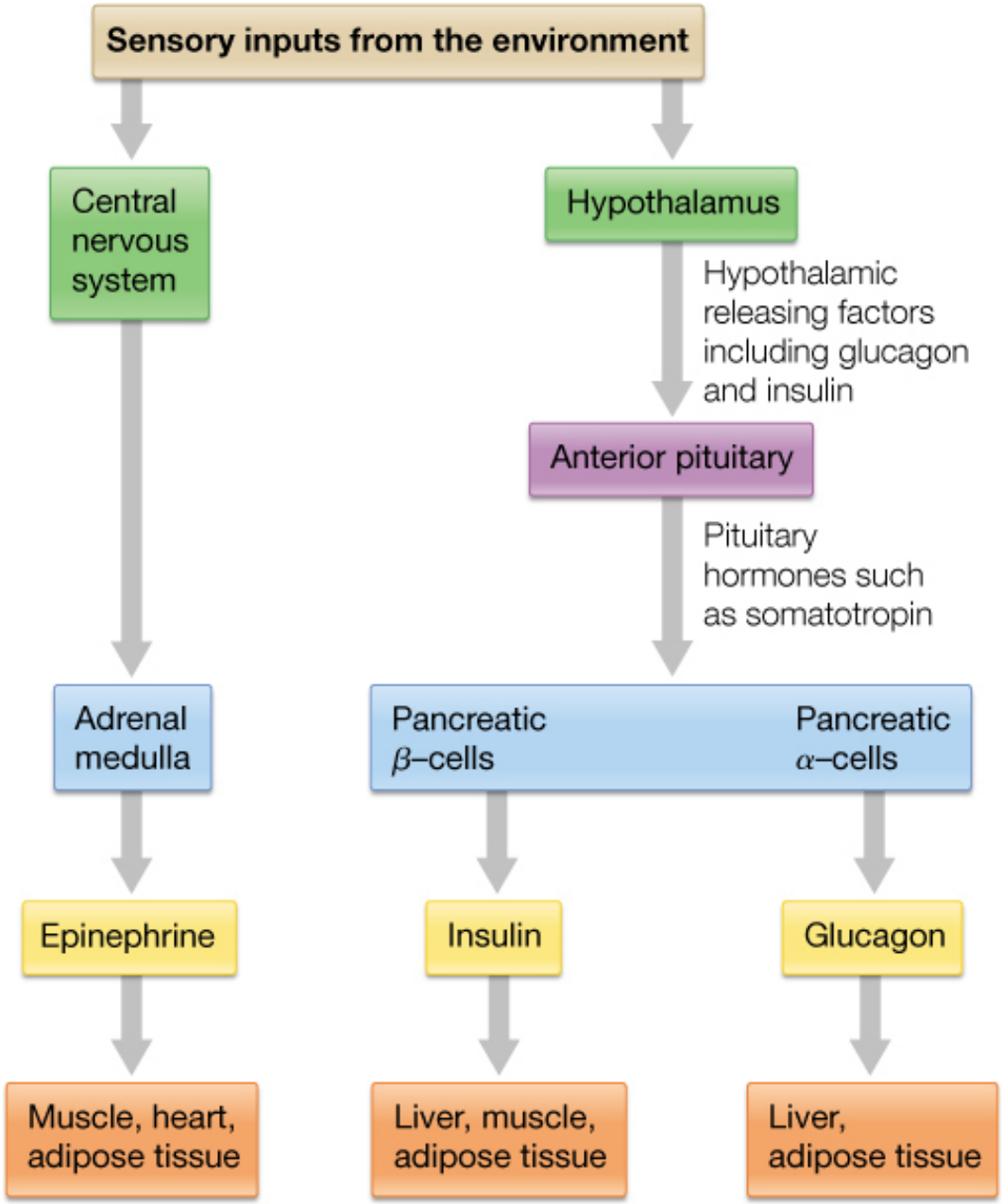


Figure 13-2

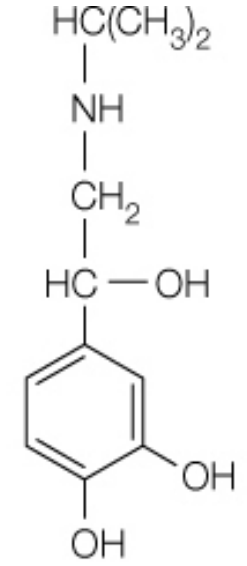
- Biological function of hormones:
  1. Maintain homeostasis: steady state of nutrients. E.g. insulin and glucagon control blood sugar levels, under normal conditions.
  2. Provide rapid response to external factors. E.g. epinephrine and norepinephrine for the “fight or flight” response.
  3. *Regulate cyclic and developmental programs. E.g. sex hormones monitor maturation, sexual differentiation, menstruation and pregnancy.*

Hierarchical Nature of Hormone Control

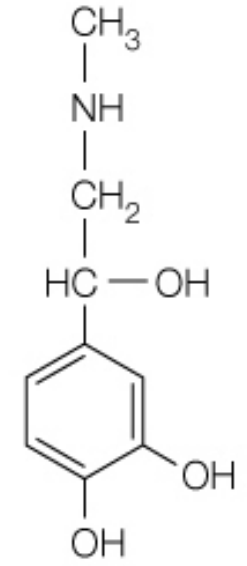
- The pituitary is the first target for most hormones and is under hypothalmic control
  - ➢Pituitary hormones then act on secondary targets
  - ➢Neural stimulation of the adrenal medulla releases epinephrine



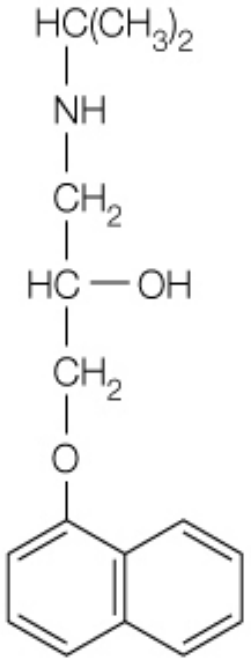
Receptors historically defined by Interactions with Drugs



Isoproterenol



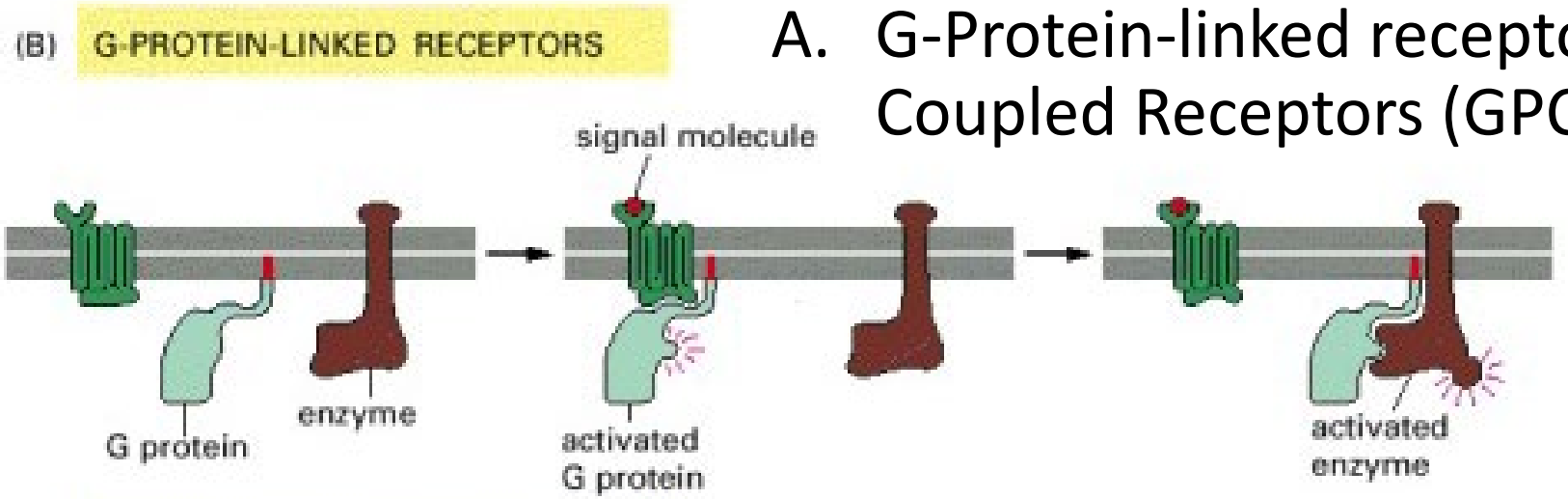
Epinephrine



Propranolol

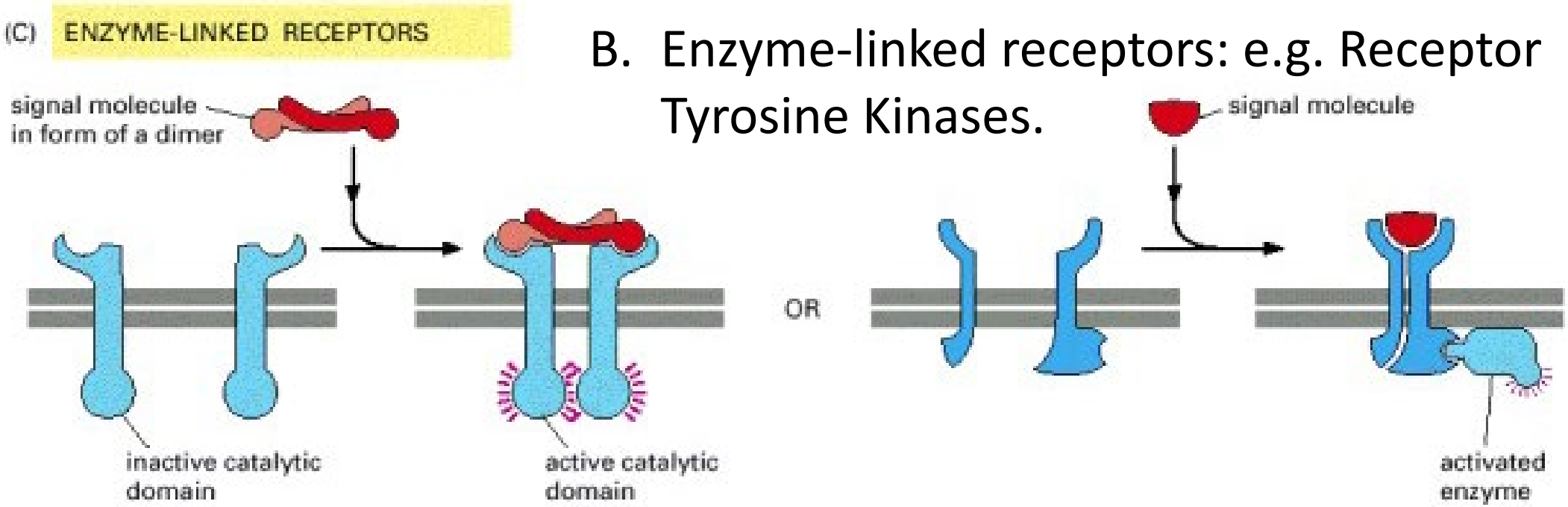
- Before the structure of hormone receptors were known, tissues and cells had been categorized pharmacologically in terms of their response to epinephrine analogs
- The epinephrine (adrenaline) analogs such as isoproterenol and propranolol acted either as:
  - agonists – agents that act similarly to epinephrine
  - antagonists – agents that block the action of epinephrine
- Propranolol antagonizes β_2-adrenergic receptors (controls blood pressure)
- Isoproterenol agonizes β_2-adrenergic receptors (used to treat asthma)

Main classes of hormone receptors



A. G-Protein-linked receptors or G-Protein Coupled Receptors (GPCRs) – next lecture

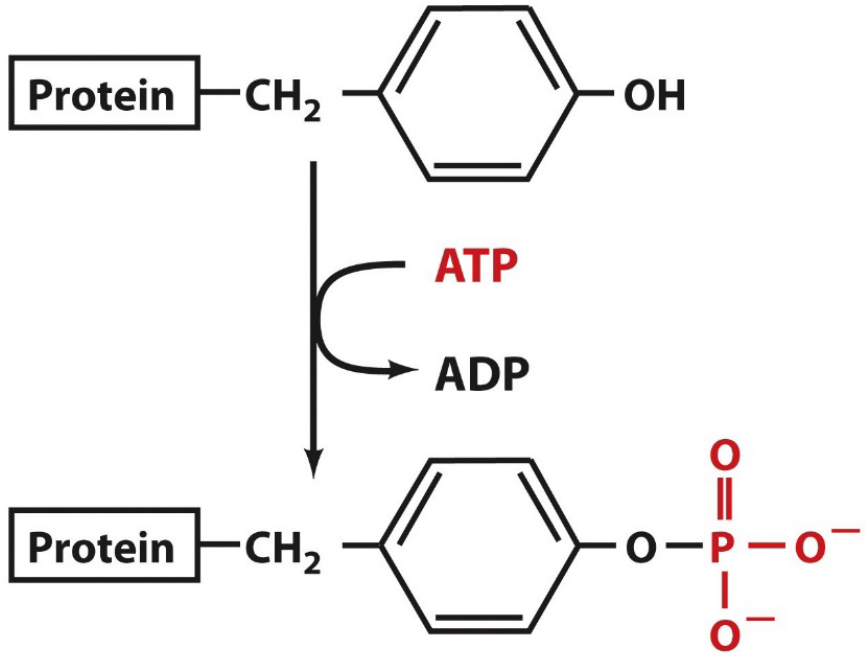
*A **G protein** is a **guanine nucleotide-binding proteins***



B. Enzyme-linked receptors: e.g. Receptor Tyrosine Kinases.

Function of Protein Tyrosine Kinases

- **Kinases** in general **add a phosphate group** to a protein **from ATP**, usually leading to ***activation***.
- **OH groups** of **Ser, Thr and Tyr** can be phosphorylated.
- **Tyrosine kinases** specifically target ***tyrosine residues*** for phosphorylation.
- **Phosphate** groups can be ***removed*** by ***phosphatases***, leading to ***deactivation***.



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General Structure of RTKs

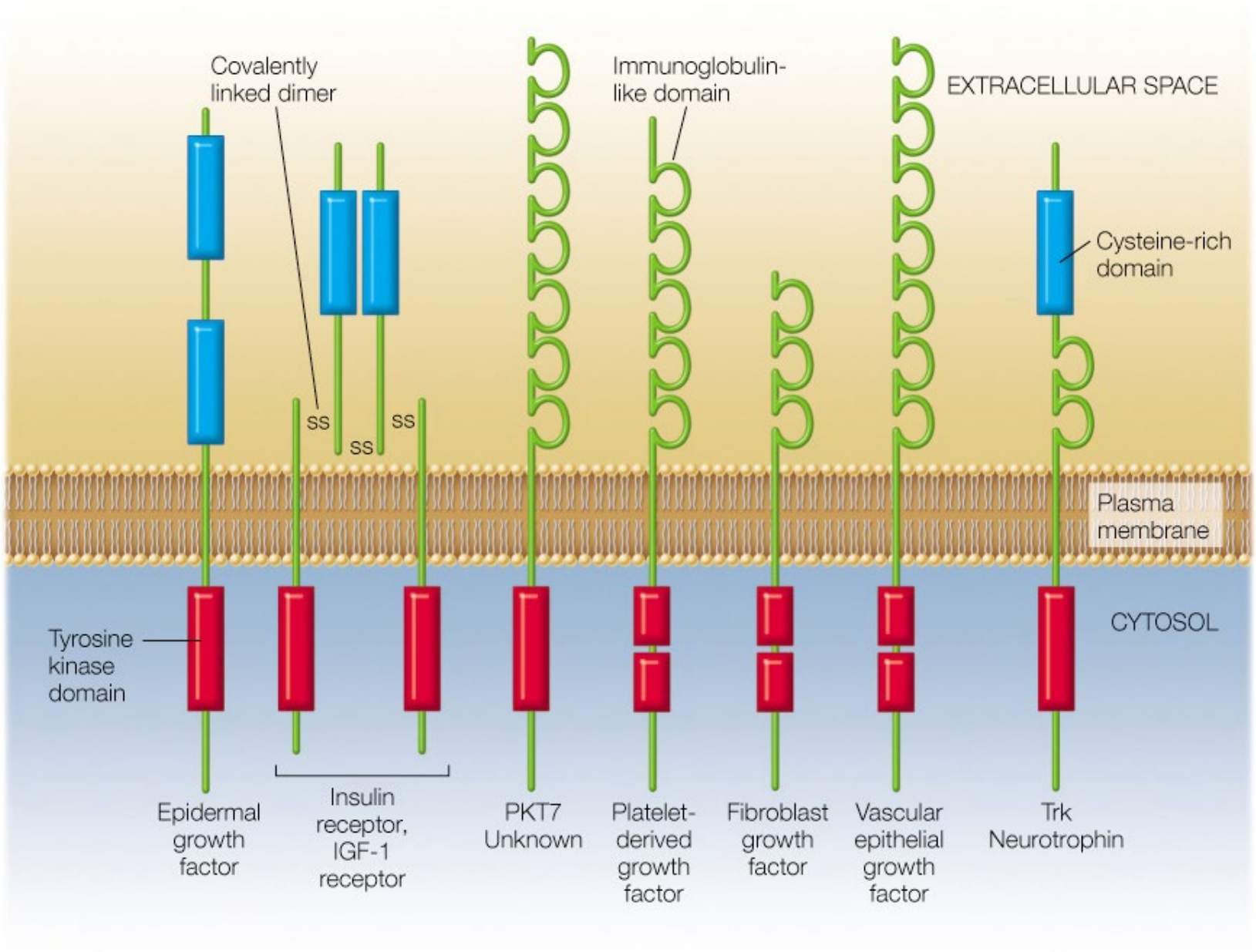


FIGURE 20.9 Representative receptor tyrosine kinase families.

Activation of the Protein Kinase Domain of the Epidermal Growth Factor (EGF) Receptor

Once the agonist is bound, the monomeric RTK dimerizes and the kinase domain is autoactivated by phosphorylation.

The activated RTK recruits intracellular signaling or adaptor proteins.

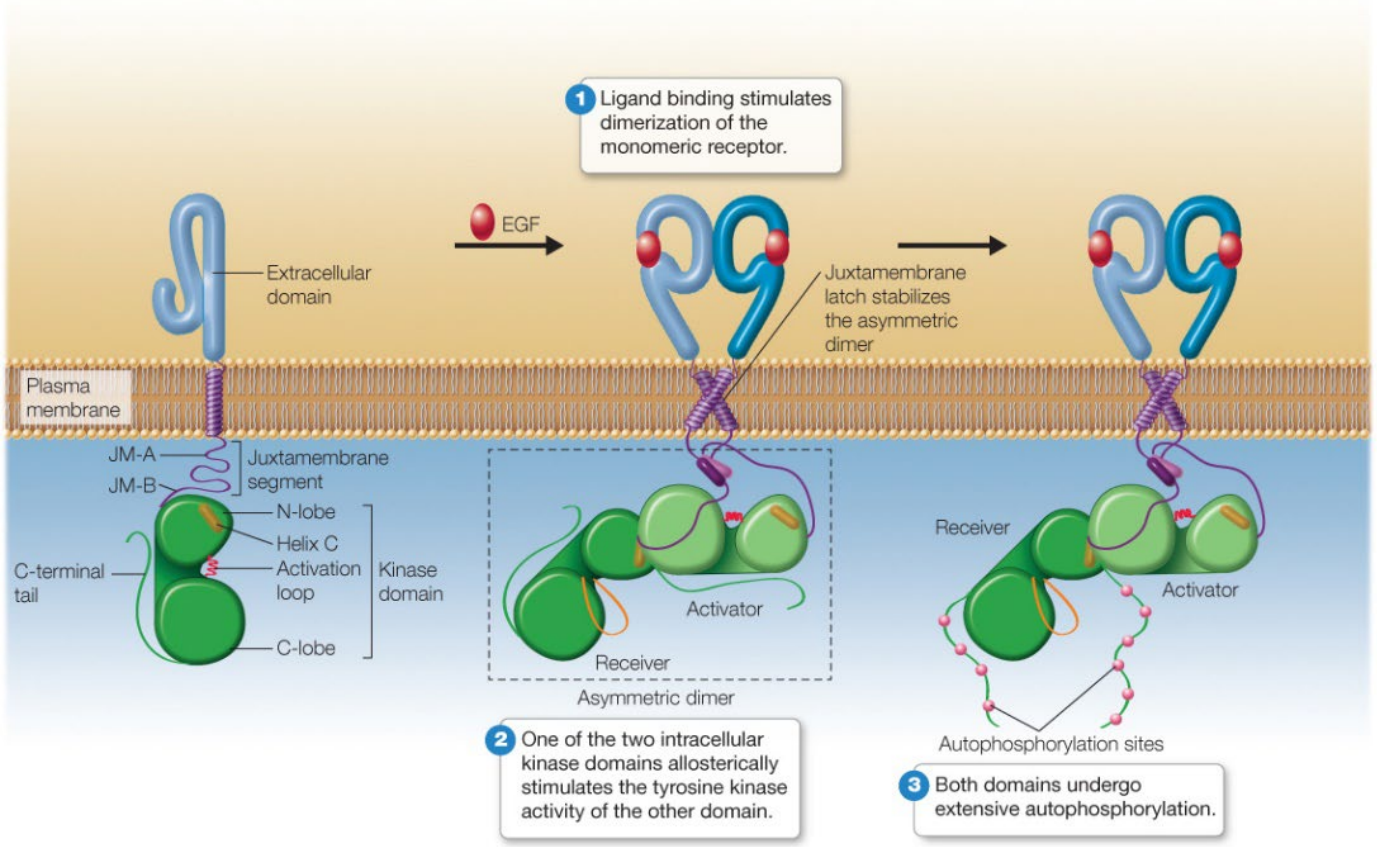
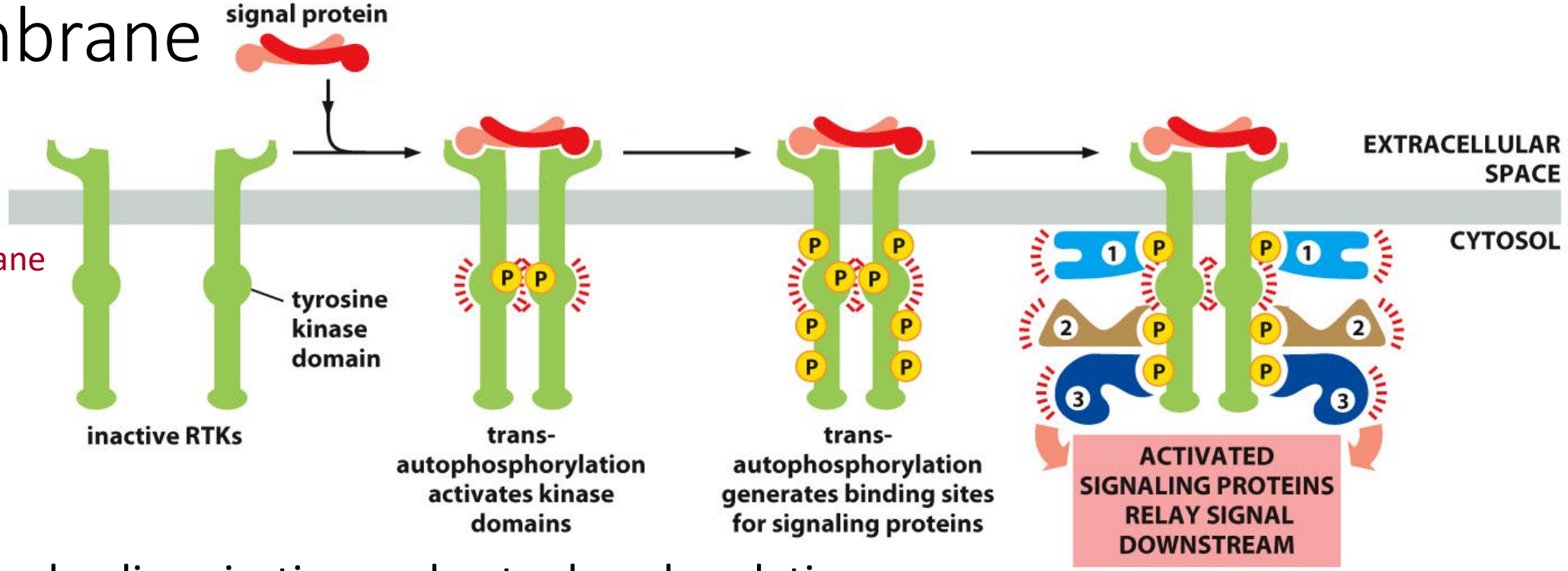


FIGURE 20.10 Activation of the protein tyrosine kinase of the EGF receptor.

Receptor Tyrosine Kinases (RTKs) Convey Signals across the Membrane

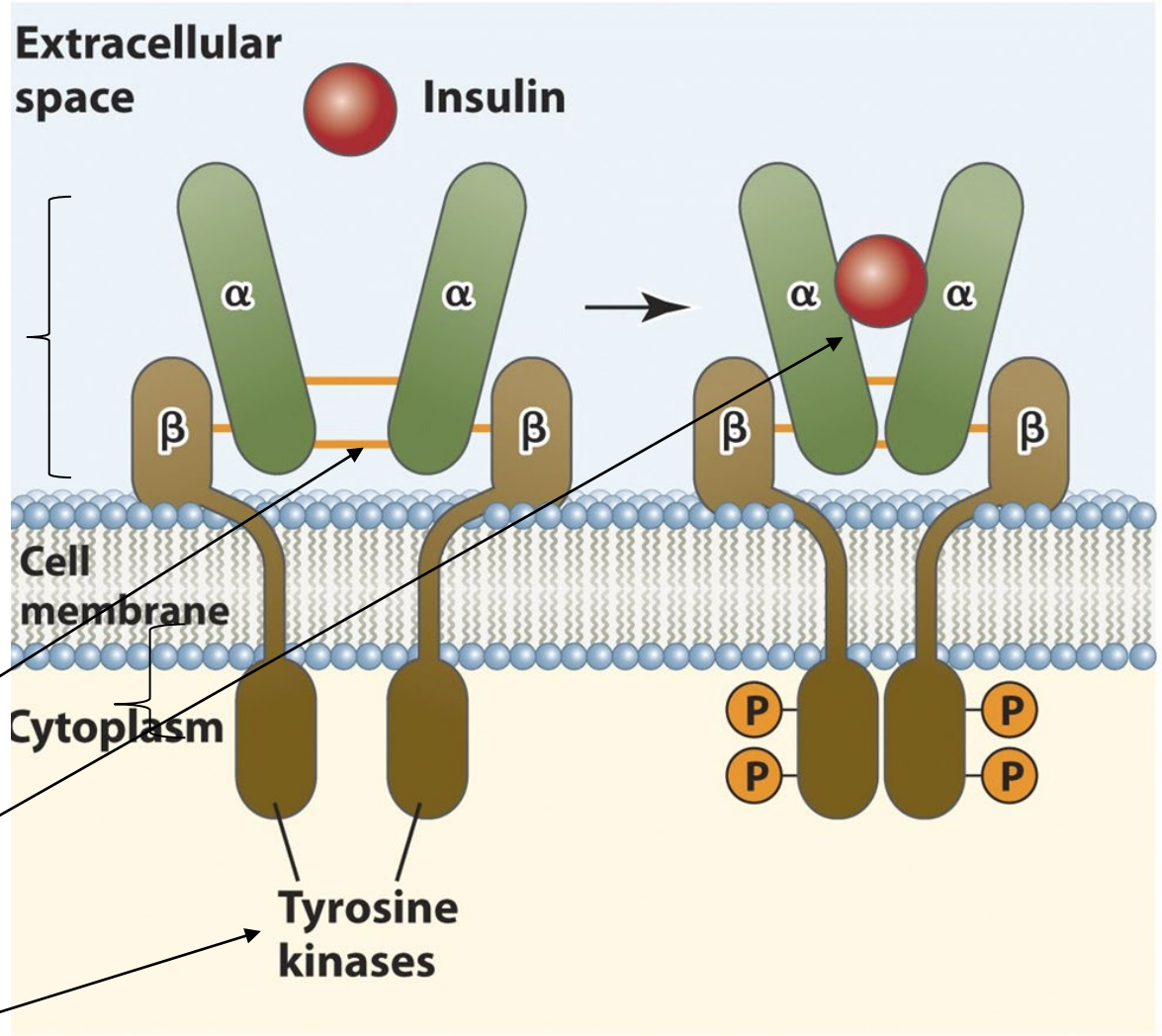
Single transmembrane domain



- Activation by dimerization and autophosphorylation.
- Adaptor proteins containing SH2 and SH3 domains link RTKs with other signalling proteins, such as G proteins, and additional kinases, to amplify the signal.
- After the signal is conveyed, deactivation is by protein phosphatases which remove phosphate groups from proteins.

Insulin Receptor is an RTK

- All RTKs possess a single membrane spanning domain, an extracellular ligand-binding domain and a cytoplasmic domain with tyrosine kinase activity
- The insulin receptor is an RTK but is unique in that, it exists as a covalently-linked dimer even in the absence of ligand
  - Subunits α2β2 linked by disulfide bonds (yellow bars).
  - Extracellular subunits form the insulin-binding site.
  - Cytoplasmic portions are tyrosine kinases that are activated by insulin binding.



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Signal Pathways Involving the Insulin Receptor

- Leads to a huge amplification of the original signal
- Called a signalling cascade

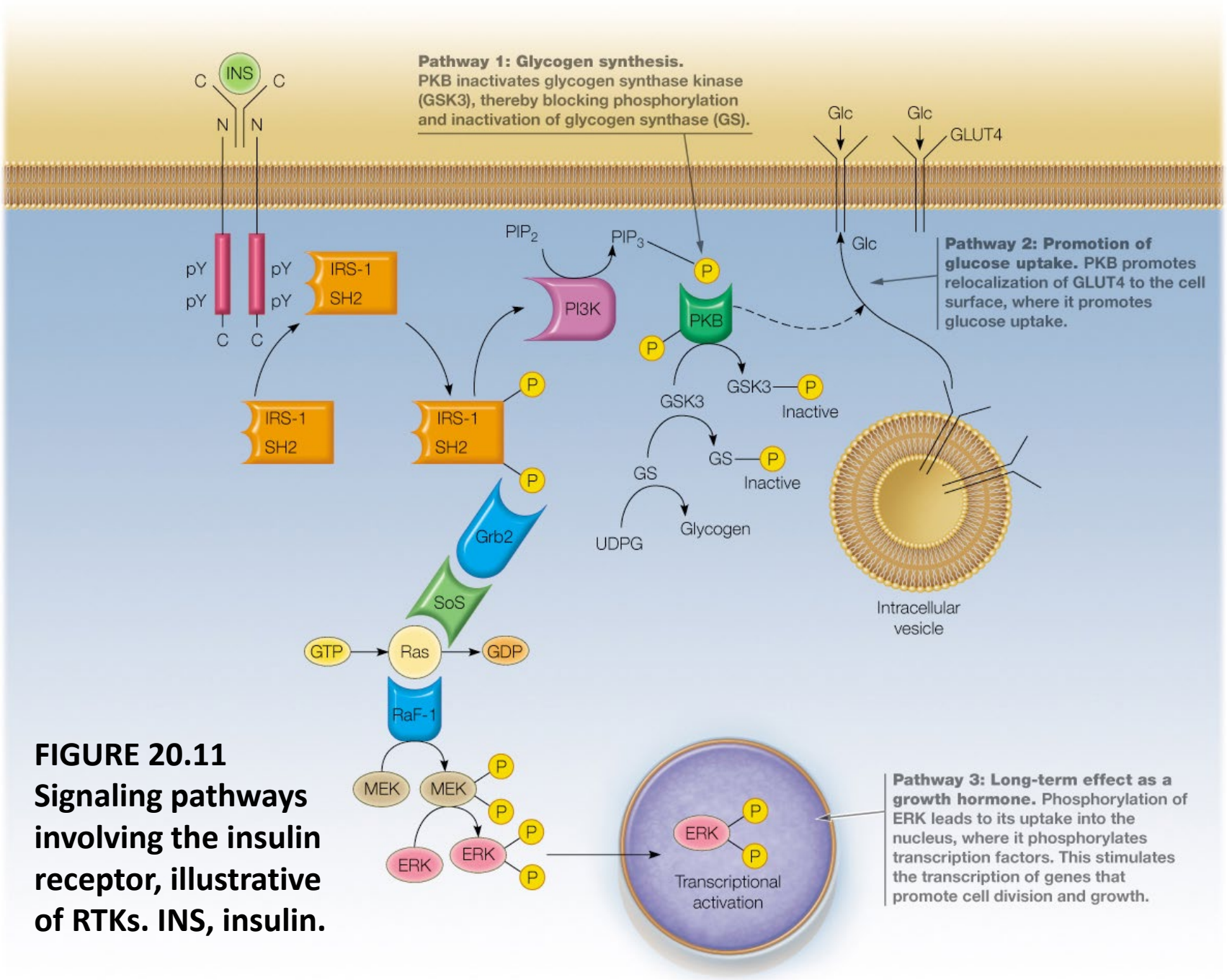


FIGURE 20.11 Signaling pathways involving the insulin receptor, illustrative of RTKs. INS, insulin.

Kinase Cascades Relay Signals to the Nucleus

- Growth factor binds to its RTK receptor → activation
- G protein, Ras, gets activated.
- This starts the kinase cascade, leading to gene expression in the nucleus.
  - ➢Domains such as SH2 and SH3 are important to pass on the signal.
- Important for cancers: chemo drugs are inhibitors of the kinase cascade.
- Deactivation by GTPase-activating proteins (GAPs).

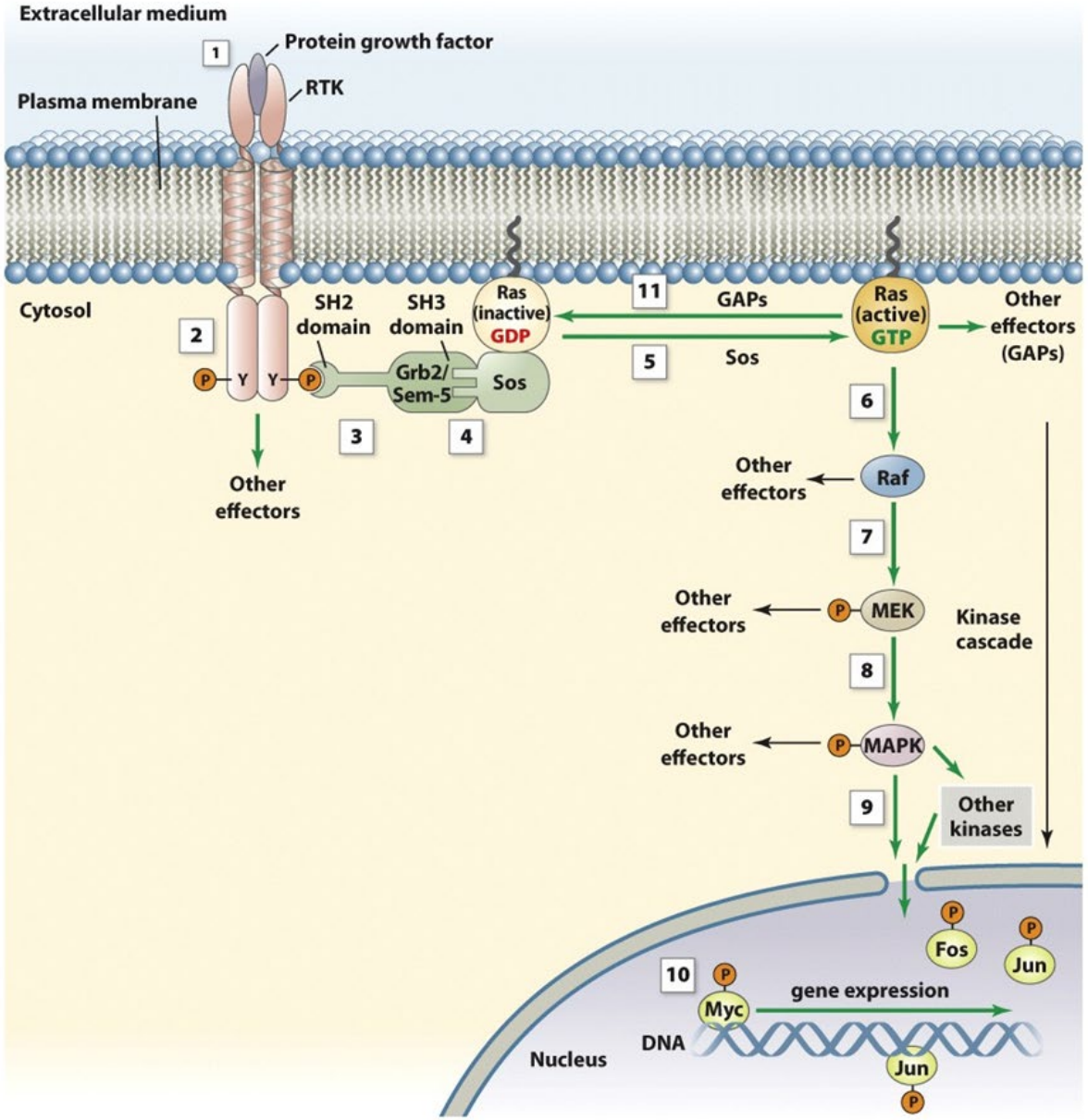
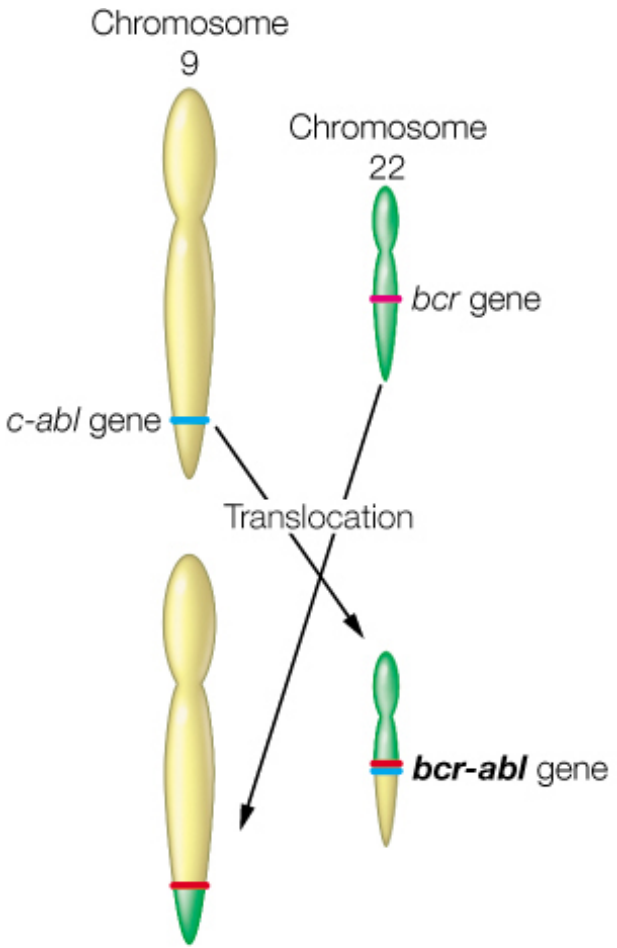


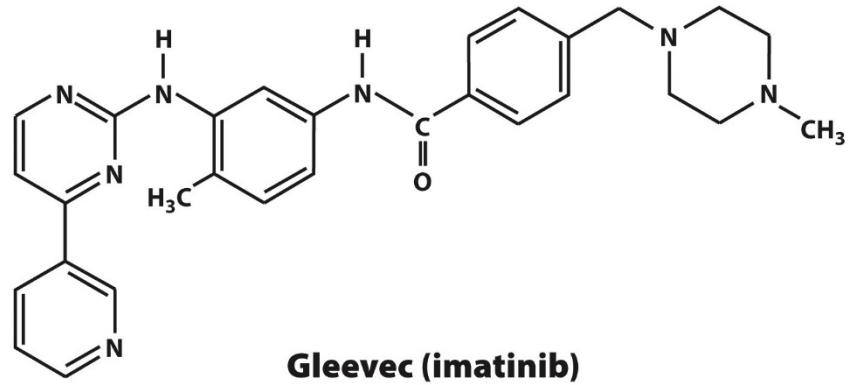
Figure 13-7

PTKs are Anticancer targets



Formation of a novel protein kinase gene (*bcr-abl*) by chromosomal translocation

- The hallmark of chronic myelogenous leukemia (CML) is a chromosomal translocation (Philadelphia chromosome)
- Here two kinase genes fuse to form Bcr-Abl protein, in which Abl is **always** switched on.
- ***Gleevec*** traps Abl in an inactive conformation by occupying its ATP-binding site.
- Gleevec causes remission in >90% of CML Patients.

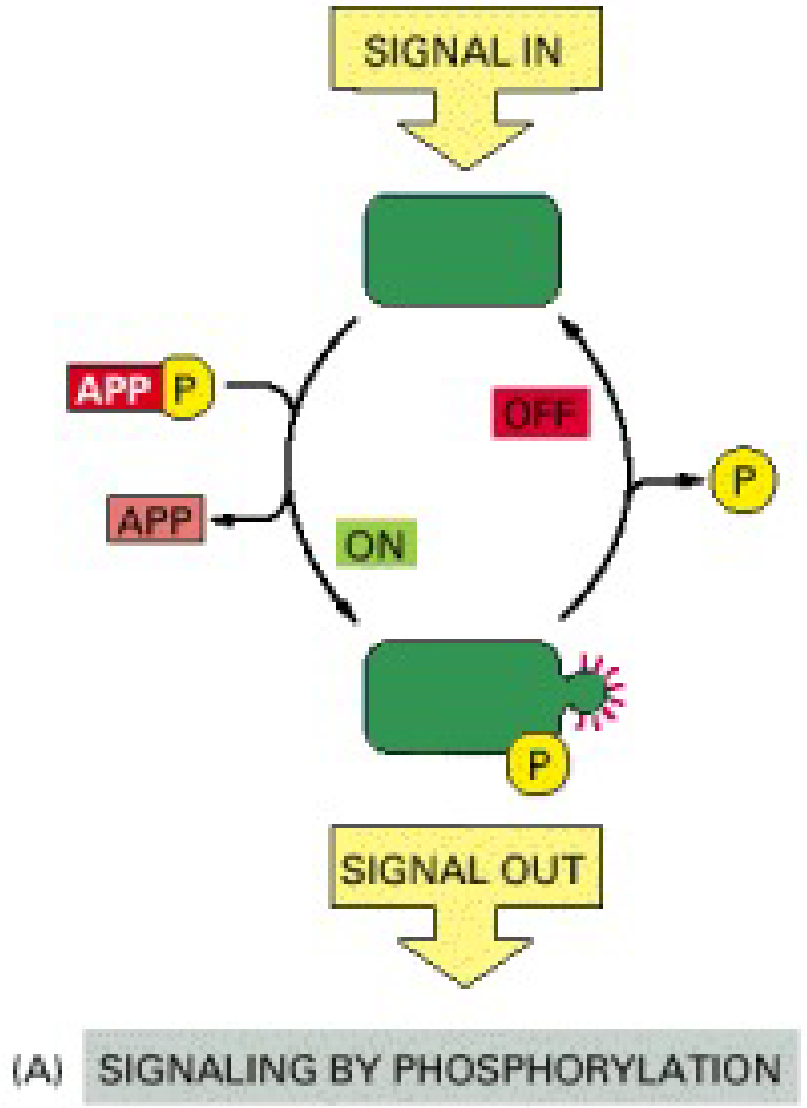


Gleevec (imatinib)

Other soluble kinases are also anticancer targets: we are working on ATP-mimics functioning as PKC inhibitors with the Liu group.

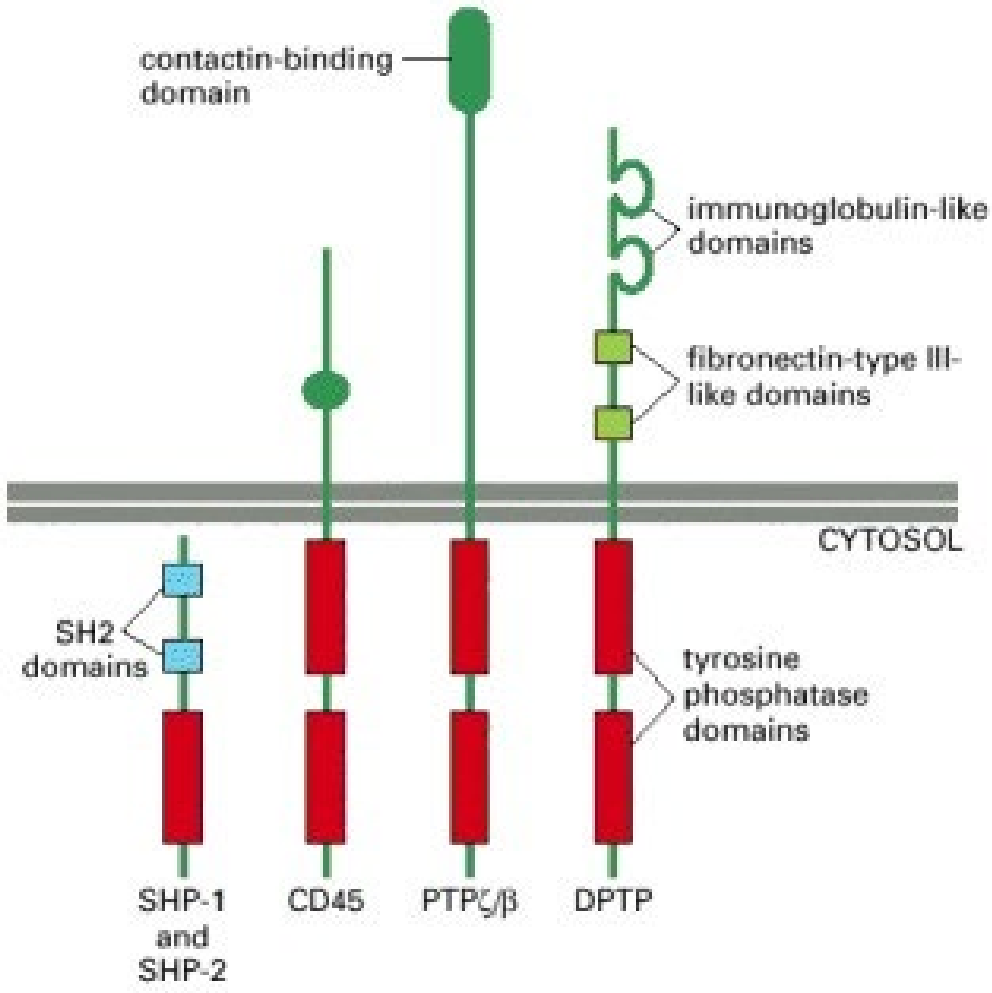
Kinases and Phosphatases

- A signalling protein is activated by the addition of a phosphate group and inactivated by the removal of the phosphate.
- **ON:** The phosphate is added covalently to the signaling protein by a **protein kinase**.
- **OFF:** After signalling, the phosphate is removed by a **protein phosphatase**.



Protein Phosphatases are Also Signalling Proteins

- Kinases that are activated by phosphorylation need to be deactivated.
- The intracellular signals turned “on” by kinases are turned “off” by phosphatases.
- Phosphate groups attached to Ser, Thr or Tyr side chains are hydrolysed.
- We have 2 types of phosphates: soluble and membrane-embedded.



Receptor Tyrosine Kinase Summary

- The first step is ligand binding to two individual receptor subunits, pulling then together, forming dimers (exception insulin receptor, which is a constitutive dimer).
- RTK subunits then phosphorylate each other, to become active as protein tyrosine kinases (insulin receptor is activated by insulin binding).
- Adaptor proteins containing SH2 and SH3 domains can link an RTK with G proteins and additional kinases that operate as a cascade.
- Protein phosphatases participate in signaling pathways by removing phosphoryl groups from receptors and target proteins.