Cell Biology
Lecture 9

Cell Signaling - principles

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Alberts • Johnson • Lewis • Morgan • Raff • Roberts • Walter

Molecular Biology of the Cell

Sixth Edition

Chapter 15

Cell Signaling, Part I

Pages: 813-831

Course overview – Tentative schedule

Date	Lecture		Chapters & Topics	Assignments
25.10.	1	Part 1	*Course overview,* DNA, Chromosomes, Genome, Ch. 4	
27.10.	2 -G		Replication, Repair, Recombination, Ch. 5	
1.11.	3		From DNA to protein, Ch. 6	
3.11.	4		Control of gene expression, Ch. 7	
8.11.	5	Part 2	Membrane structures, Ch. 10 Membrane transport, Ch. 11	**Assignment I (Essay) Draft I** (8.11.)
10.11.	6 -G		Intracellular compartments and protein sorting, Ch. 12	
15.11.	7		Intracellular compartments and protein sorting, Ch. 12 **Susanna Mäkinen, Solar Foods**	**Assignment II – Draft I (15.11.)**
17.11.	8		Membrane Traffic, Ch. 13 **iGEM team 2023**	*+iGEM intro*
22.11.	9	Part 3	Cell signalling, Ch. 15	**Assignment II – Peer review** (22.11.)
24.11.	10 -G		Cell signalling, Ch. 15	**Assignment I (Essay) Draft II** (24.11.)
29.11.	11		Cell cycle, Ch. 17 **Jere Weltner, Folkhälsan**	
1.12.	12		Apoptosis, Ch. 18	**Assignment II – final version** (1.12.)
7.12.	EXAM		December 7th	
8.12.	Final version essay		December 8th	**Assignment I (Essay) Final version** (8.12.) Aim at finishing before exam date. Use last days for polishing.

LEARNING OUTCOMES

- Can describe the principles of cell signaling and apply these to evaluate realistic cases of signaling

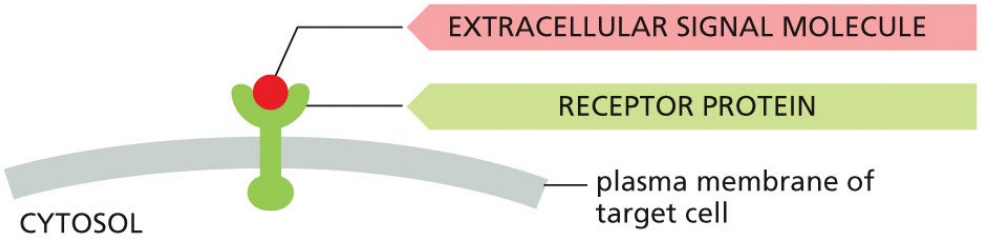
CONTENTS

- **Principles of cell signaling**
- Signaling through G-protein-coupled receptors
- Signaling through enzyme-coupled receptors

PRINCIPLES OF CELL SIGNALING

Communication between cells in multicellular organisms
→ **signaling pathways:**

- **Activated by an extracellular signal molecule,** typically binding to a **receptor** protein that is embedded in the plasma membrane of the target cell

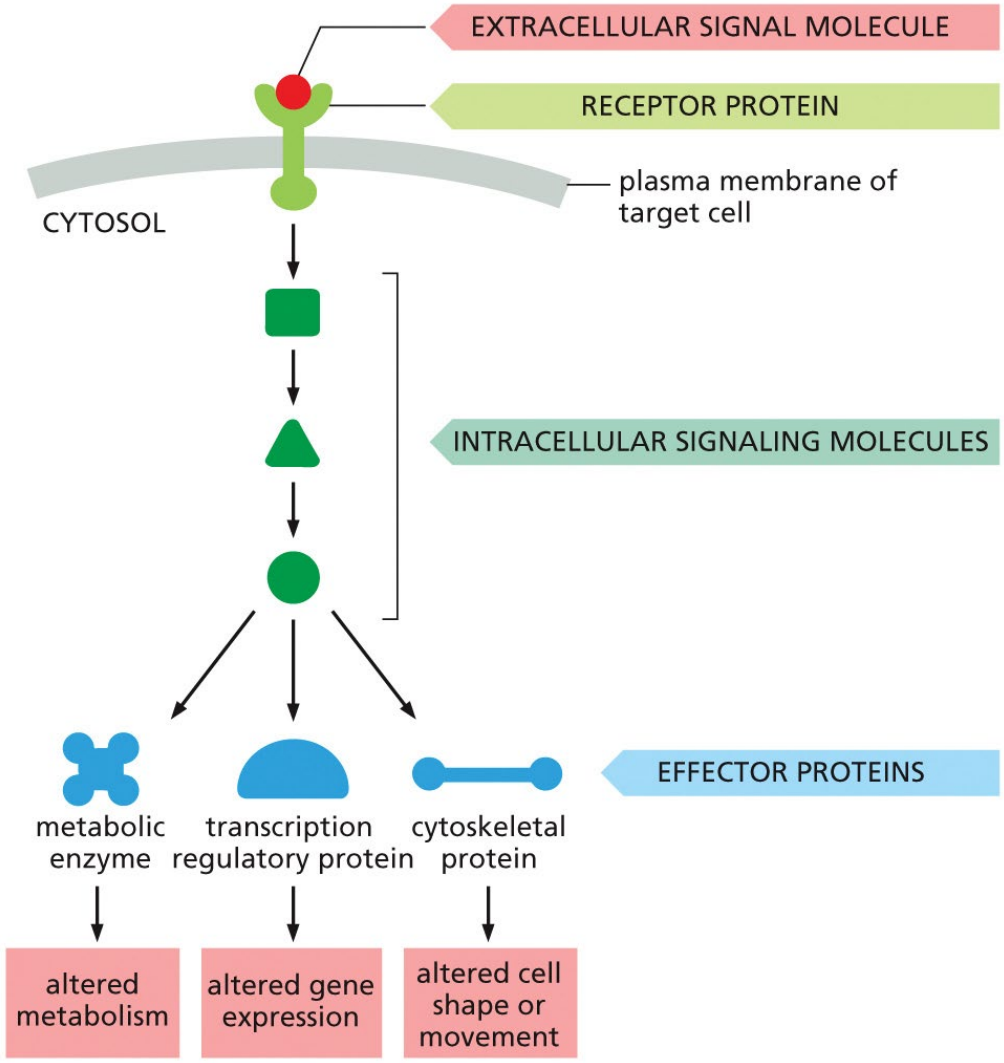


Signal molecule can be protein or peptide, but also amino acid, nucleotide, steroid etc., even dissolved gas such as carbon monoxide

PRINCIPLES OF CELL SIGNALING

Communication between cells in multicellular organisms
→ **signaling pathways:**

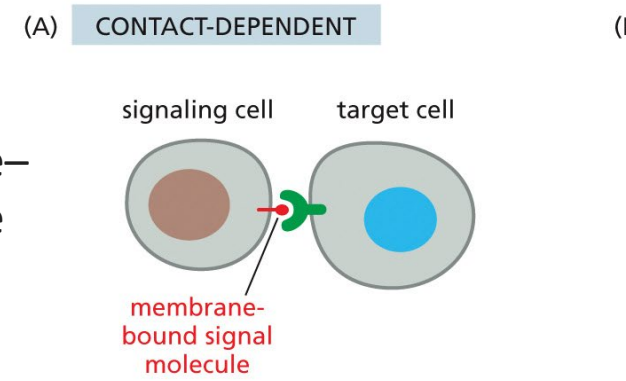
- **Activated by an extracellular signal molecule,** typically binding to a **receptor** protein that is embedded in the plasma membrane of the target cell
- The receptor activates one or more **intracellular signaling pathways**, involving a series of **signaling proteins** and small chemical messengers
- Finally, one or more of the intracellular signaling molecules alters the activity of **effector proteins** and thereby the behavior of the cell

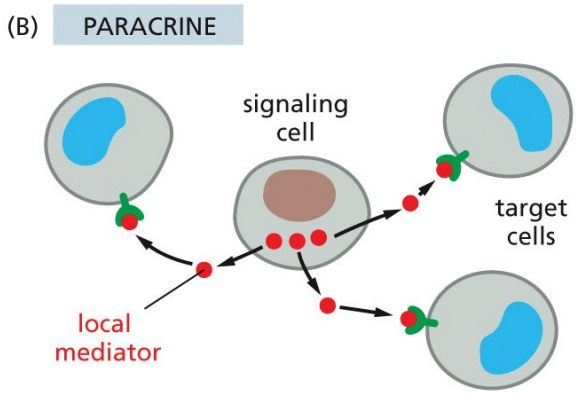


PRINCIPLES OF CELL SIGNALING

- Extracellular signals can act over short or long distances

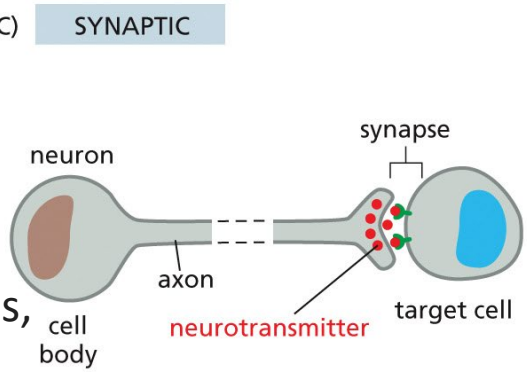
Direct membrane–membrane contact

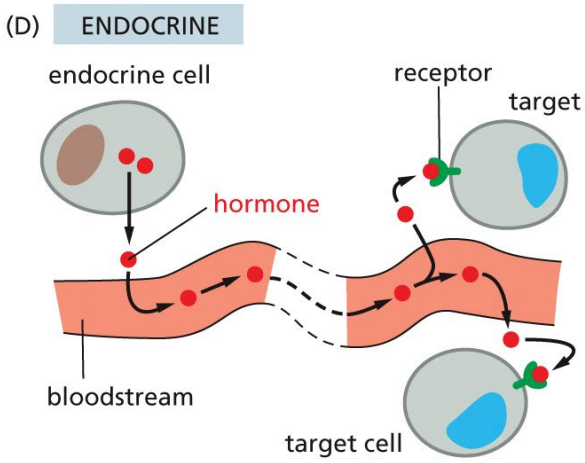




Local mediators act on neighboring cells

Signals transmitted electrically far away along axons, trigger secretion of neurotransmitters

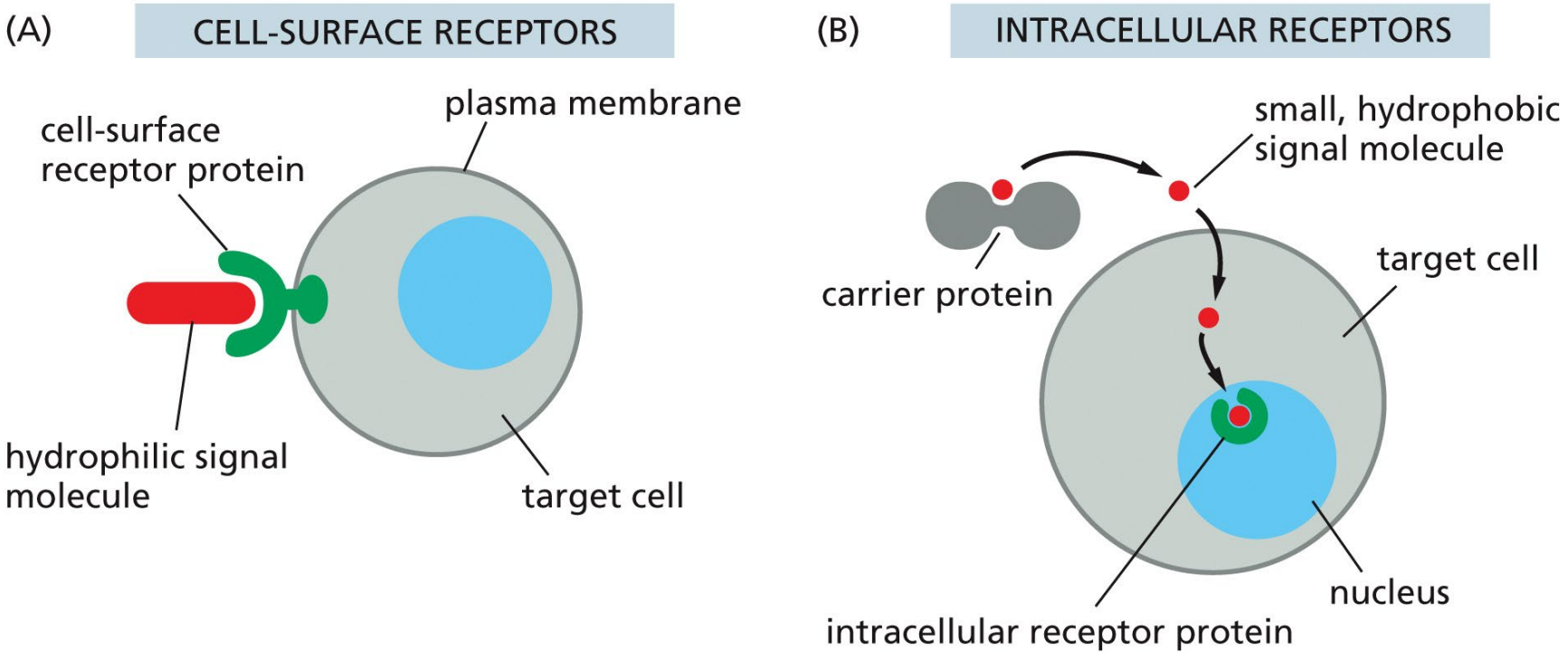




Endocrine cells secrete hormones into the bloodstream for distribution throughout the body

PRINCIPLES OF CELL SIGNALING

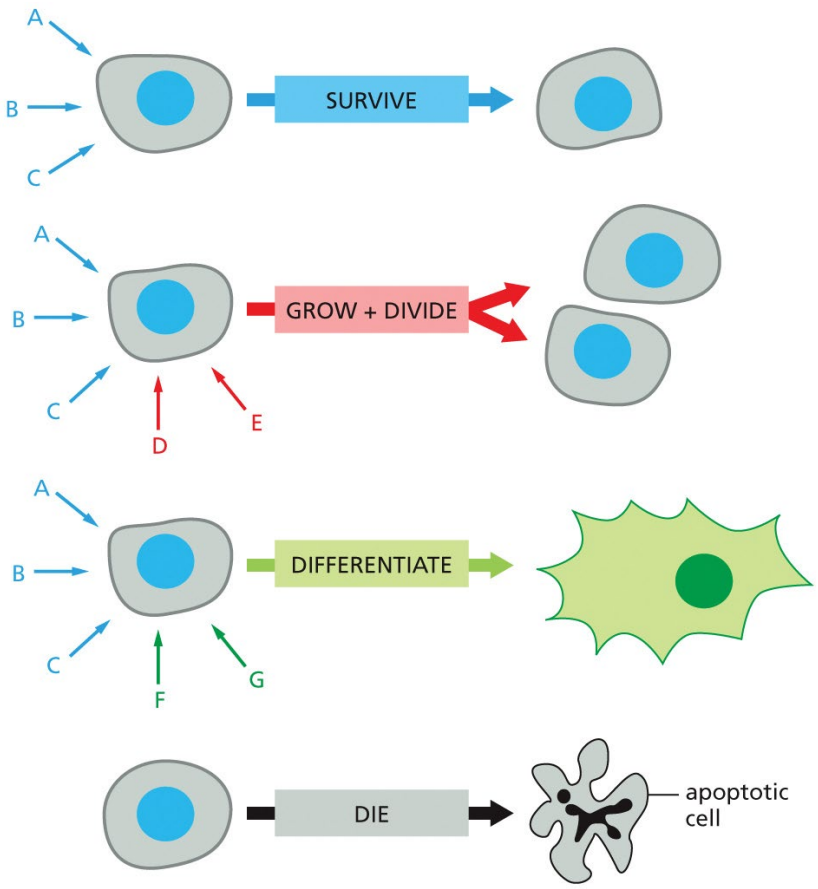
- Receptors can be on the cell surface or intracellular



- In both cases, high *specificity* and *affinity*

PRINCIPLES OF CELL SIGNALING

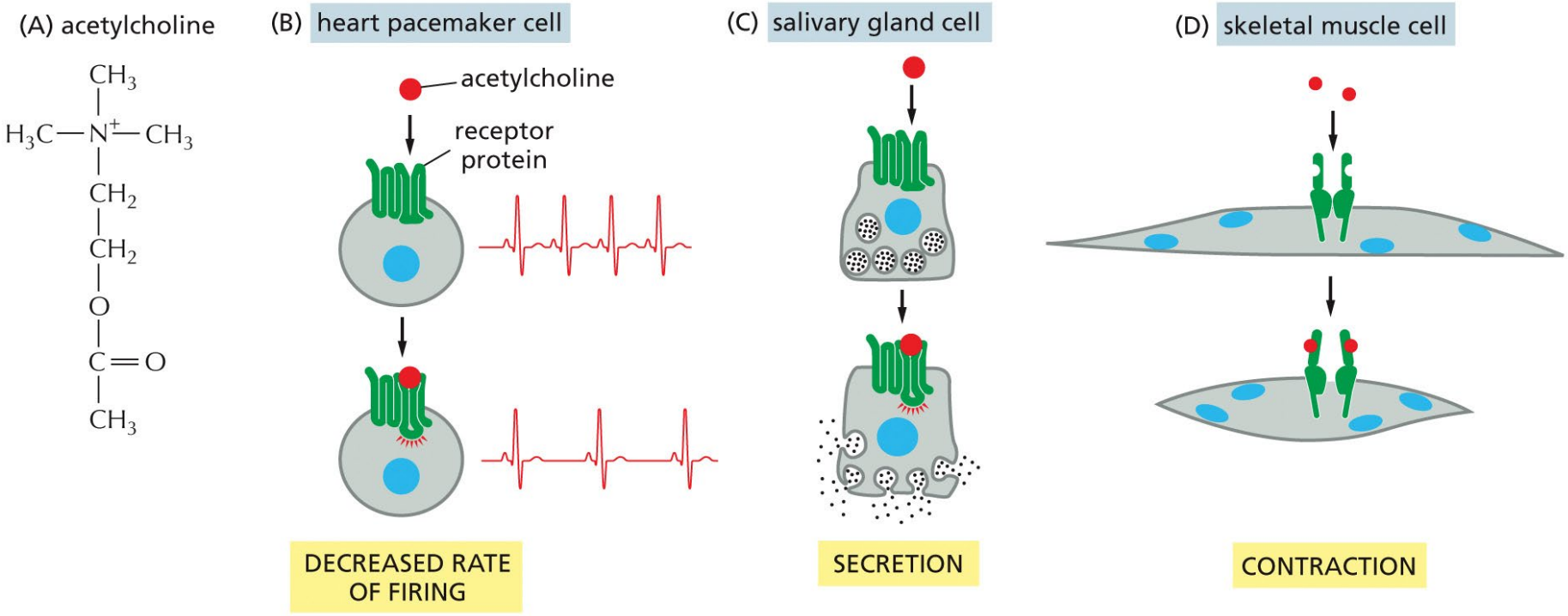
- Each cell is programmed to respond to specific combinations of extracellular signals



- Combination of signals allows having less different receptors, yet multiple different responses
- To add up complexity, also inhibitory pathways

PRINCIPLES OF CELL SIGNALING

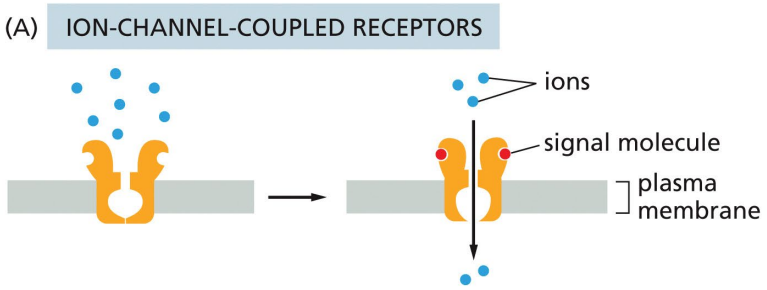
Various responses induced by the neurotransmitter acetylcholine



- Same type of acetylcholine receptor (a G-protein-coupled receptor)
- Intracellular signals produced are interpreted

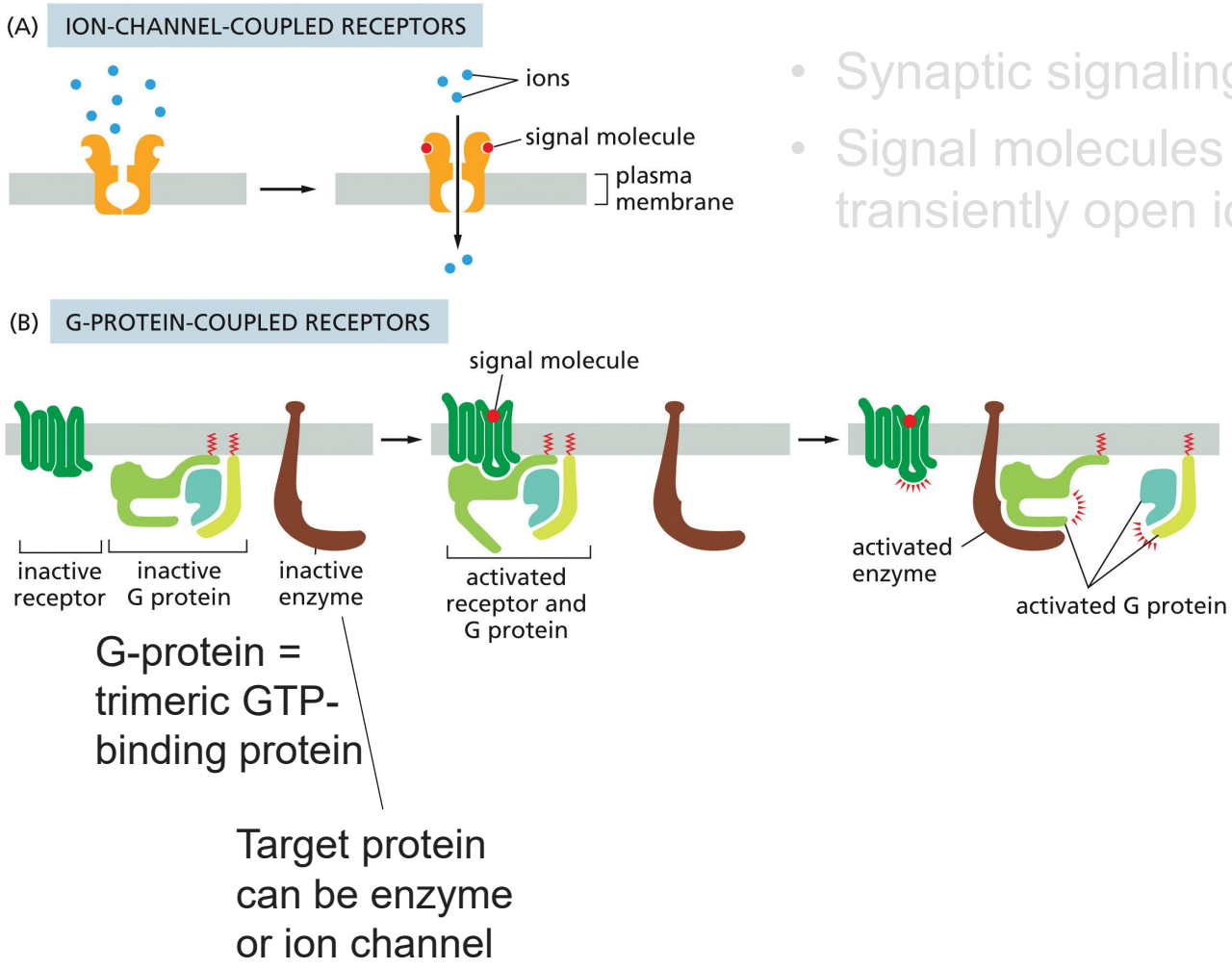
- Receptor protein is different (an ion-channel-coupled receptor)

THREE CLASSES OF CELL-SURFACE RECEPTOR PROTEINS



- Synaptic signaling between nerve cells
- Signal molecules (neurotransmitters) transiently open ion channel

THREE CLASSES OF CELL-SURFACE RECEPTOR PROTEINS



- Synaptic signaling between nerve cells
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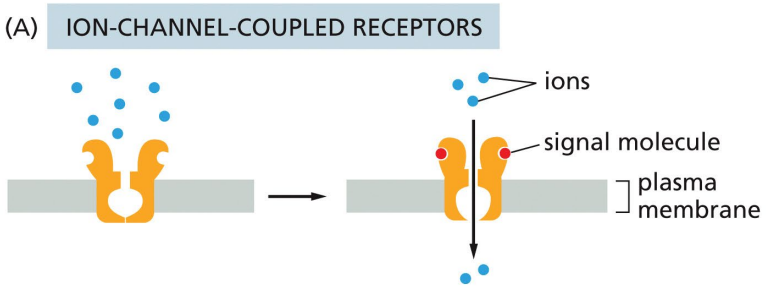
- G-protein mediates signal from receptor to target protein

THREE CLASSES OF CELL-SURFACE RECEPTOR PROTEINS



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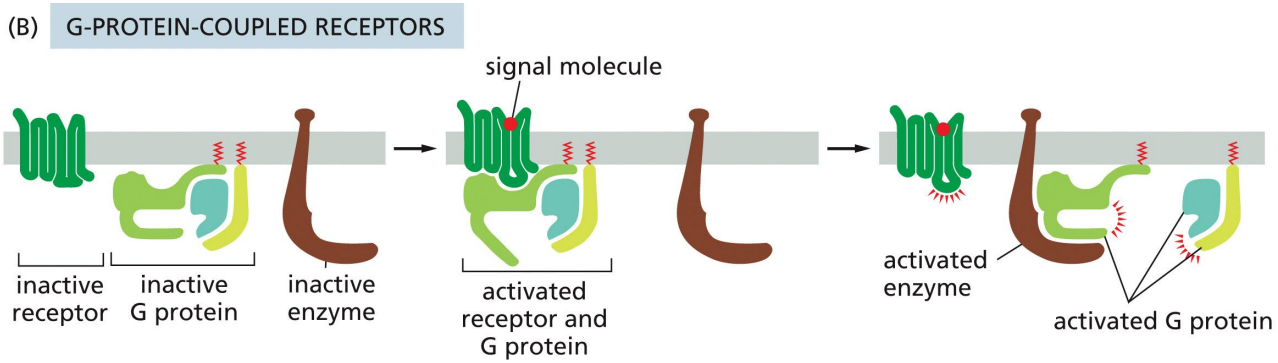

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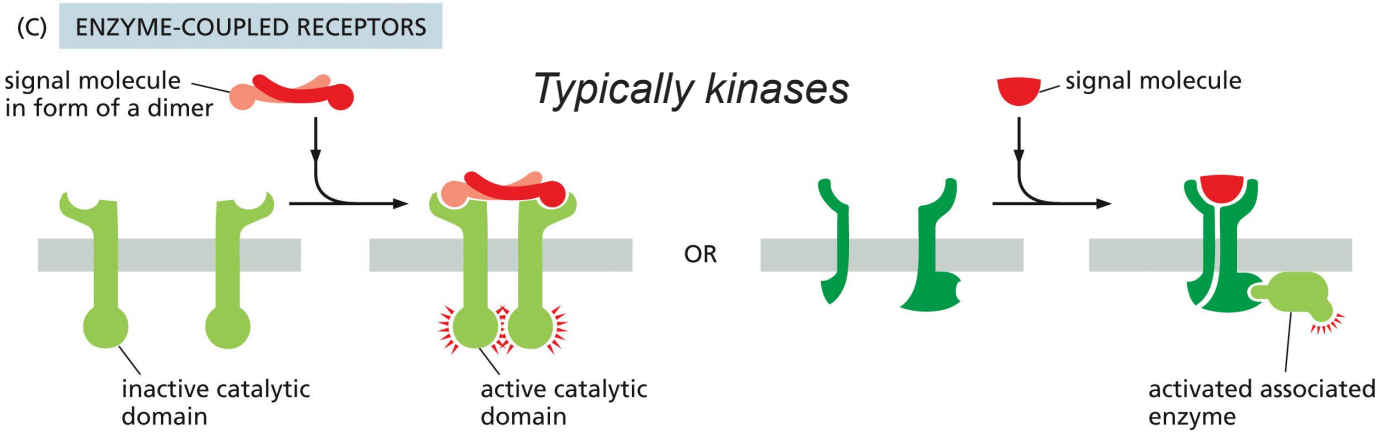
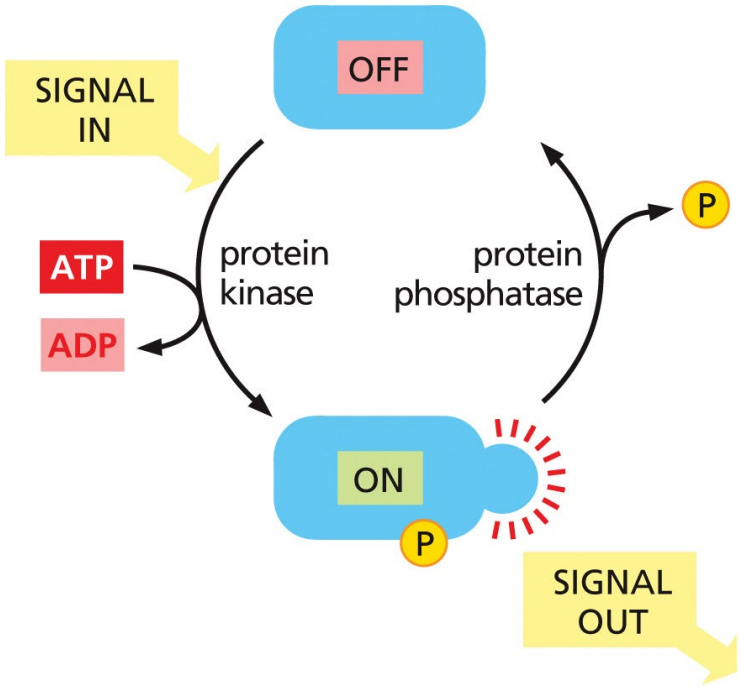


- Receptor is an enzyme that act directly or by activating an associated enzyme

PRINCIPLES OF CELL SIGNALING

- Cell-surface receptors relay signals via *intracellular signaling molecules* (“second messengers”)
- **Intracellular signaling proteins act as molecular switches**



(A) SIGNALING BY PHOSPHORYLATION

- *30-50% proteins in human phosphorylated*
- *Ser/Thr kinases (majority)*
- *Tyr kinases*
- *Kinase cascades*

PRINCIPLES OF CELL SIGNALING

- Cell-surface receptors relay signals via *intracellular signaling molecules* (“second messengers”)
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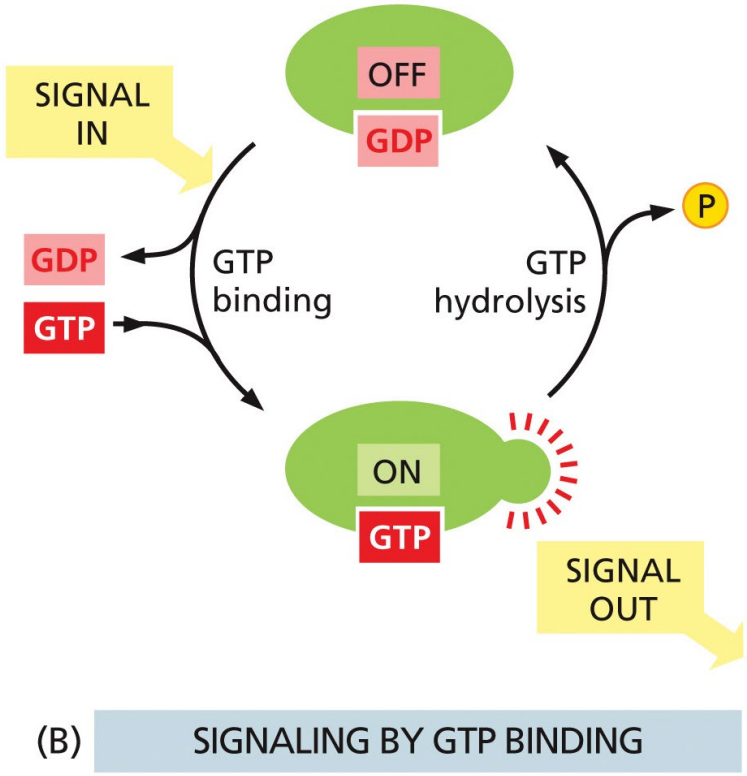
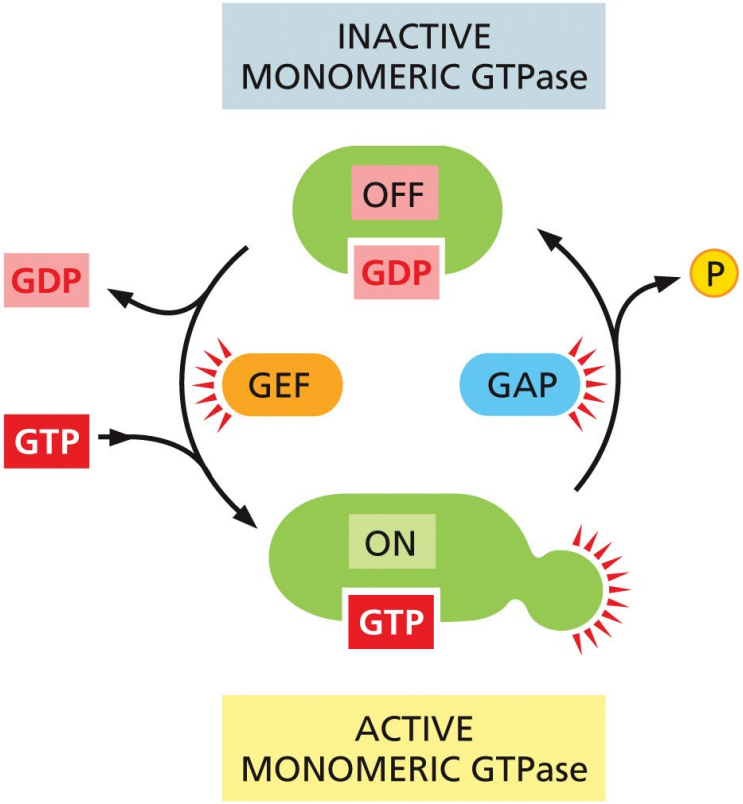


(B) SIGNALING BY GTP BINDING

- *G-proteins*
- *Monomeric GTPases*

PRINCIPLES OF CELL SIGNALING

- *G-proteins*
- ***Monomeric GTPases***



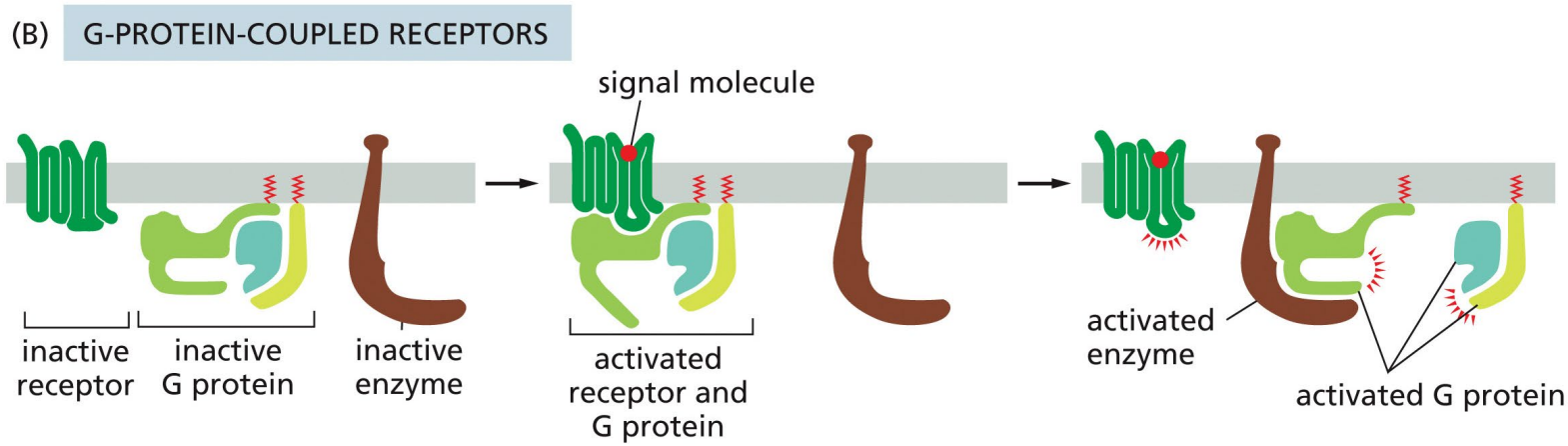
- *GAP= GTPase activating protein*
- *GEF=guanine nucleotide exchange factor*

- **GAPs inactivate** the protein by stimulating it to hydrolyze its bound GTP to GDP
  - GDP remains tightly bound to the inactivated GTPase
- **GEFs activate** the inactive protein by stimulating it to release its GDP
  - The concentration of GTP in the cytosol is 10 times greater than the concentration of GDP → the protein rapidly binds GTP and is thereby activated.

PRINCIPLES OF CELL SIGNALING

- ***G-proteins***
- *Monomeric GTPases*

- *GAP= GTPase activating protein*
- *GEF=guanine nucleotide exchange factor*



- *Activated G-proteins act as GEFs*

PRINCIPLES OF CELL SIGNALING

- **Intracellular signals must be specific and robust in a noisy cytoplasm.** Ways to ensure specificity:

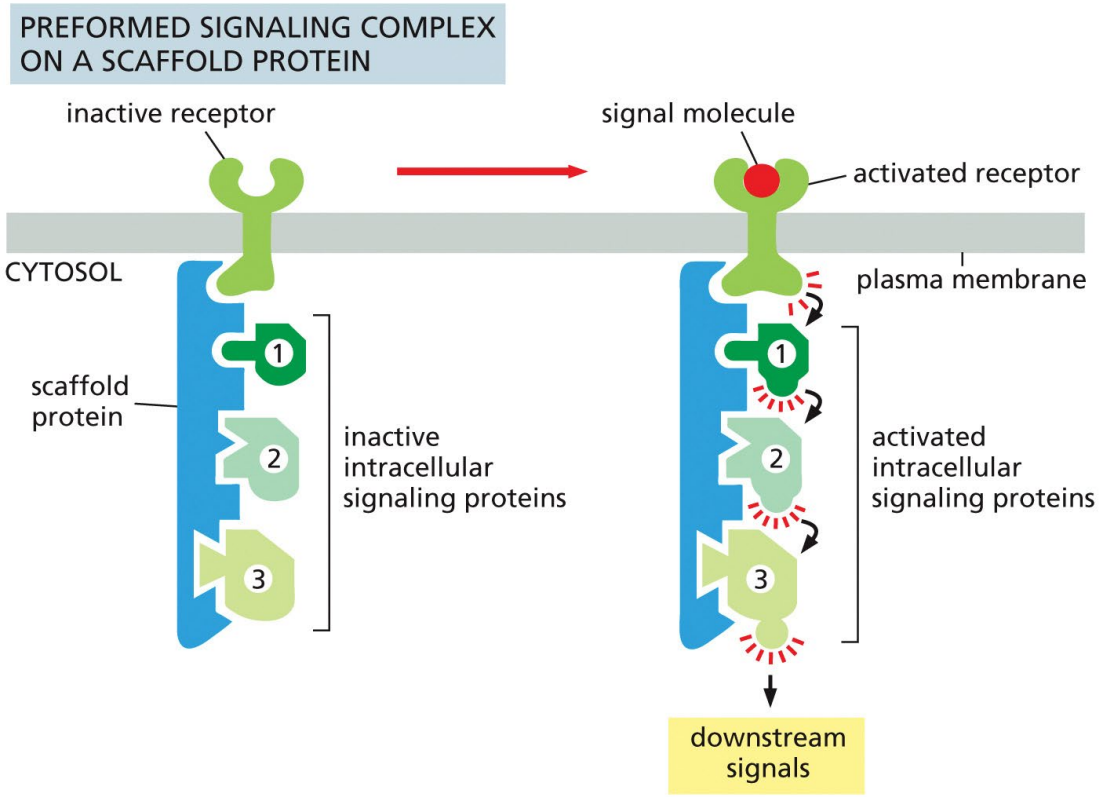
1. Spesificity of **catalytic site** towards specific residue
2. Selection of **consensus sequence** near the active site
3. **Docking sites** that provide enforced proximity and allosteric regulation of a correct kinase–substrate pair
4. **Localization**, increases local concentrations. May be achieved by **condensation**
5. **Scaffolds**, form dynamic ternary complexes with kinases and substrates
6. **Competition** between correct and wrong residues
7. **Multisite phosphorylation** and **kinetic proofreading**, minimize the harm by wrong phosphorylations

Modified from: https://www.nature.com/articles/nrm2203

PRINCIPLES OF CELL SIGNALING

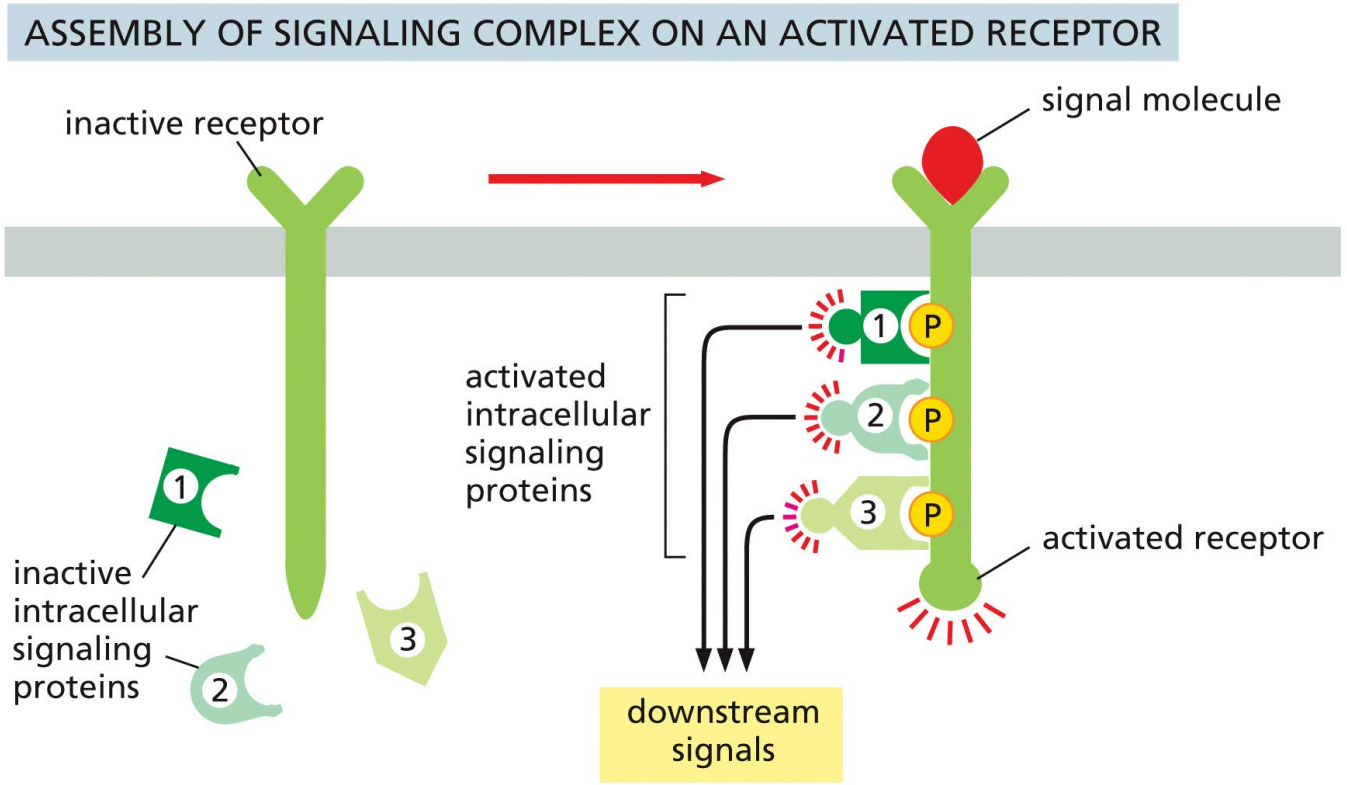
- Intracellular signaling complexes form at activated cell-surface receptors:
  1. Preformed signaling complexes on a scaffold protein
  2. Assembly of signaling complex on an activated receptor
  3. Assembly of signaling complex on phosphoinositide binding site
- Helps to enhance specificity

1. Preformed signaling complexes on a scaffold protein



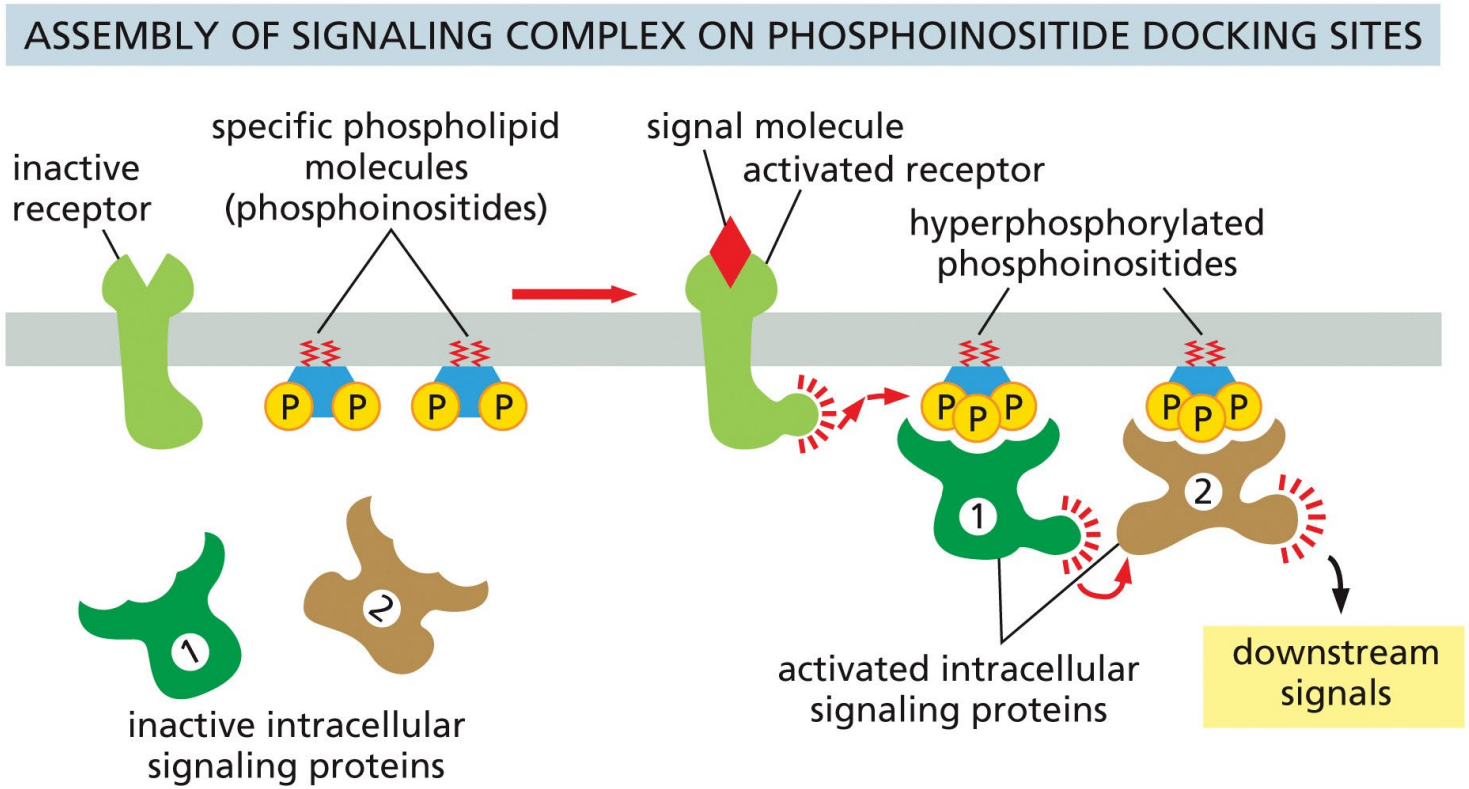
A receptor and some of the intracellular signaling proteins it activates in sequence are **preassembled into a signaling complex** on the inactive receptor by a large *scaffold protein*

2. Assembly of signaling complex on an activated receptor



- A signaling complex assembles *transiently* on a receptor only after the **binding of an extracellular signal molecule has activated the receptor**
- Typically, **activated receptor phosphorylates itself** *at multiple sites*, which then act as docking sites for intracellular signaling proteins

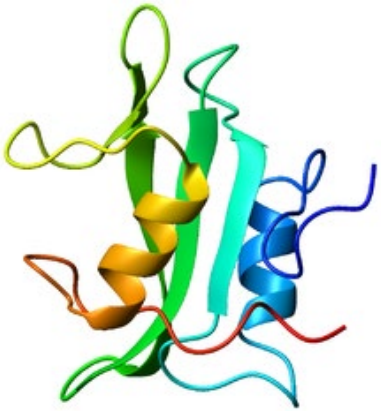
3. Assembly of signaling complex on phosphoinositide binding site



- *Activation of a receptor* leads to the increased **phosphorylation of specific phospholipids** (phosphoinositides) in the adjacent plasma membrane
- **PIPs serve as docking sites** for specific intracellular signaling proteins, which can now interact with each other

PRINCIPLES OF CELL SIGNALING

- Modular interaction domains mediate interactions between intracellular signaling proteins

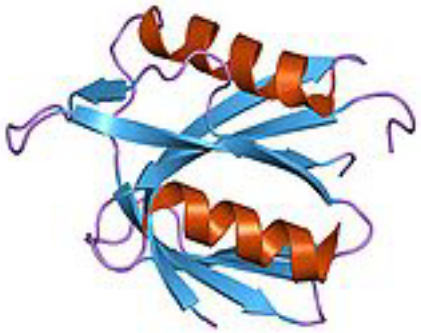


SH2
Src homology 2

Phosphorylated tyrosine

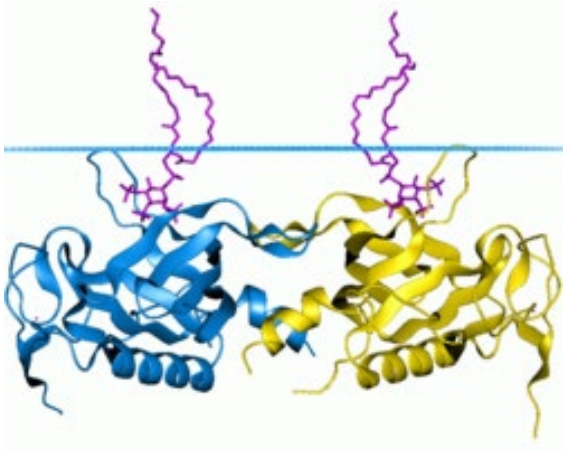
SH3
Src homology 3

Pro-rich regions



PTB
Phosphotyrosine binding

Phosphorylated tyrosine

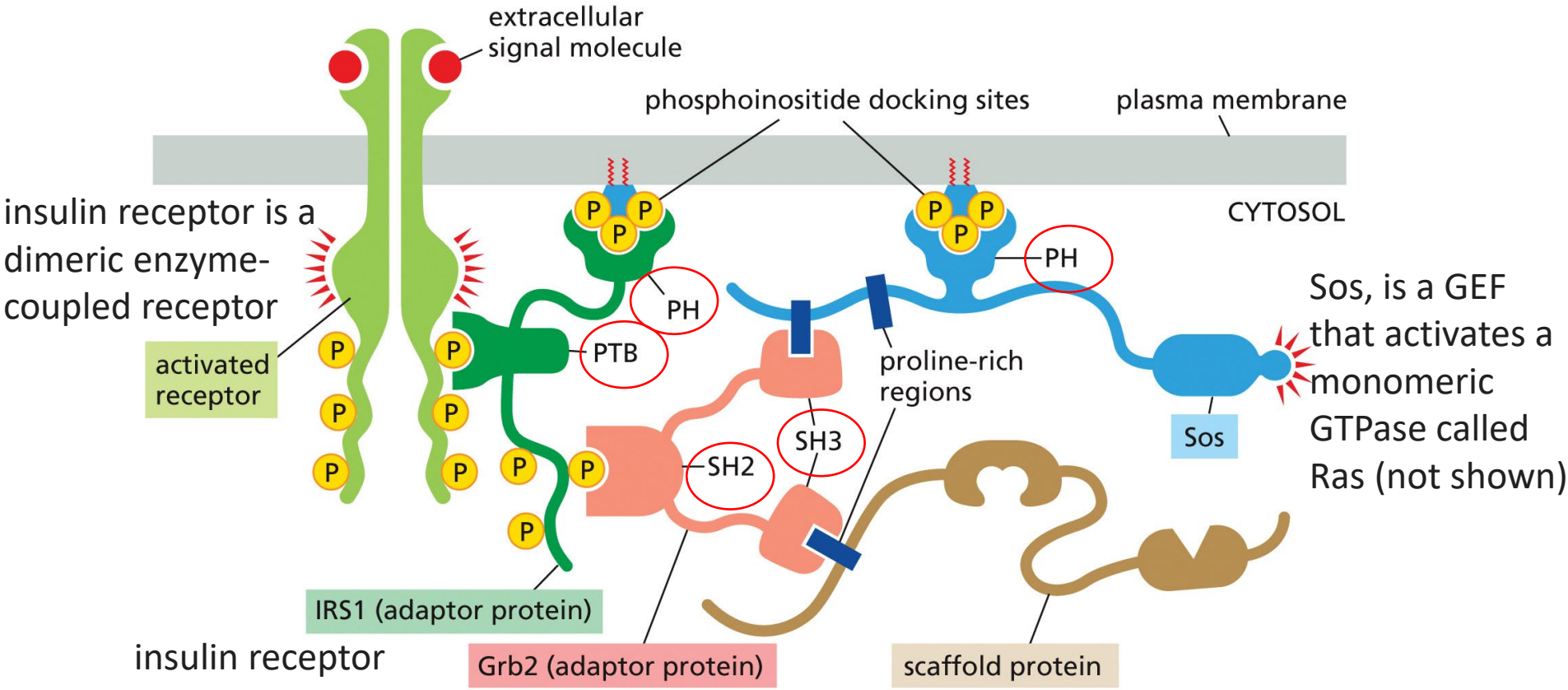


PH
Pleckstrin homology

Phosphoinositides

PRINCIPLES OF CELL SIGNALING

- Modular interaction domains guide the formation of a specific signaling complex



PRINCIPLES OF CELL SIGNALING

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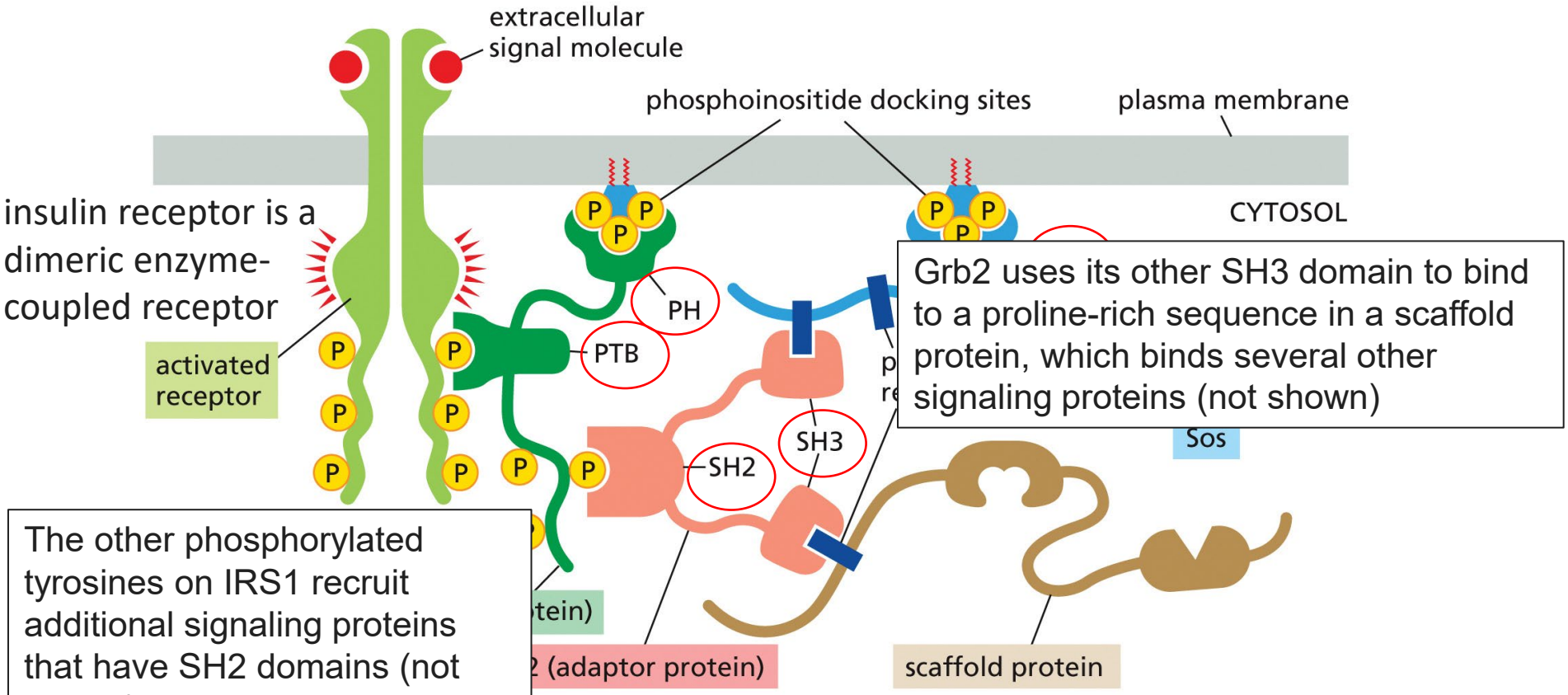
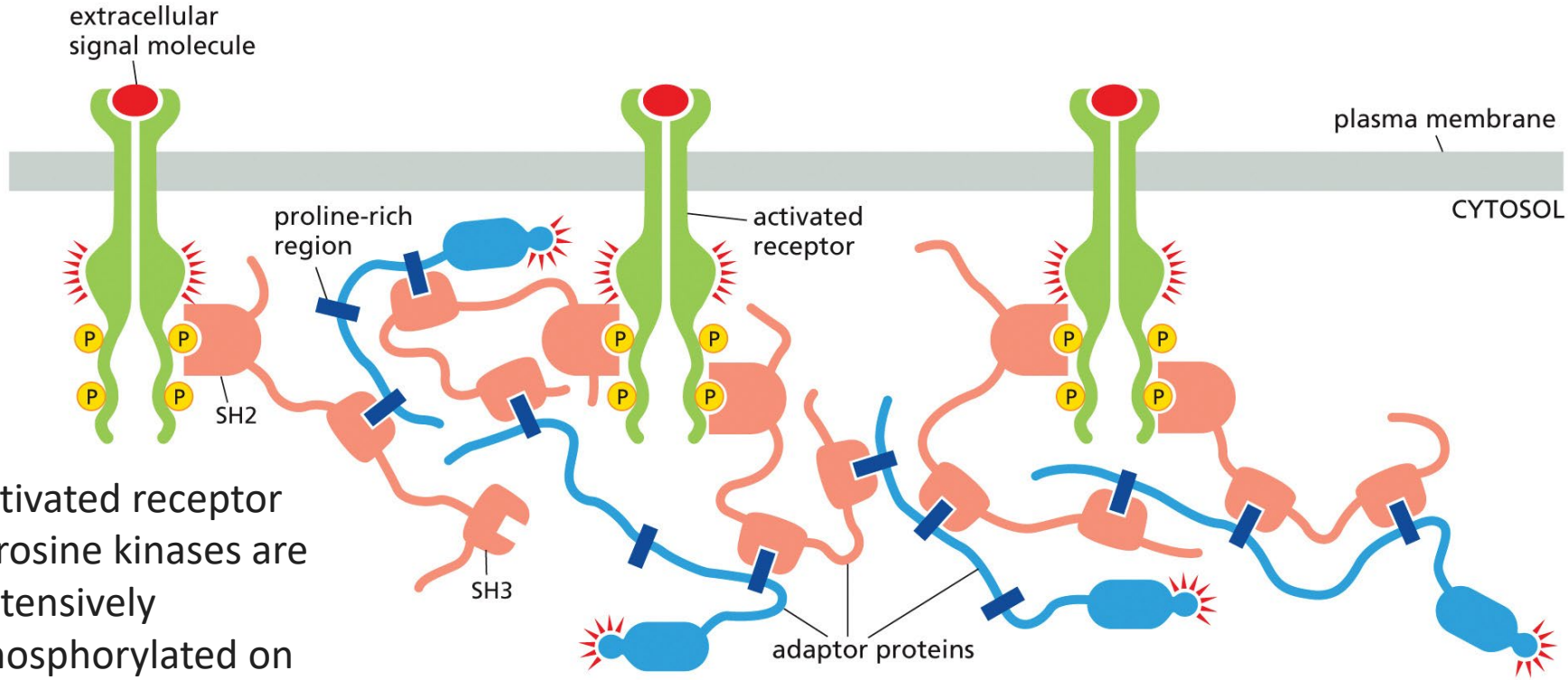


Formation of large receptor clusters by multivalent interactions among signaling proteins

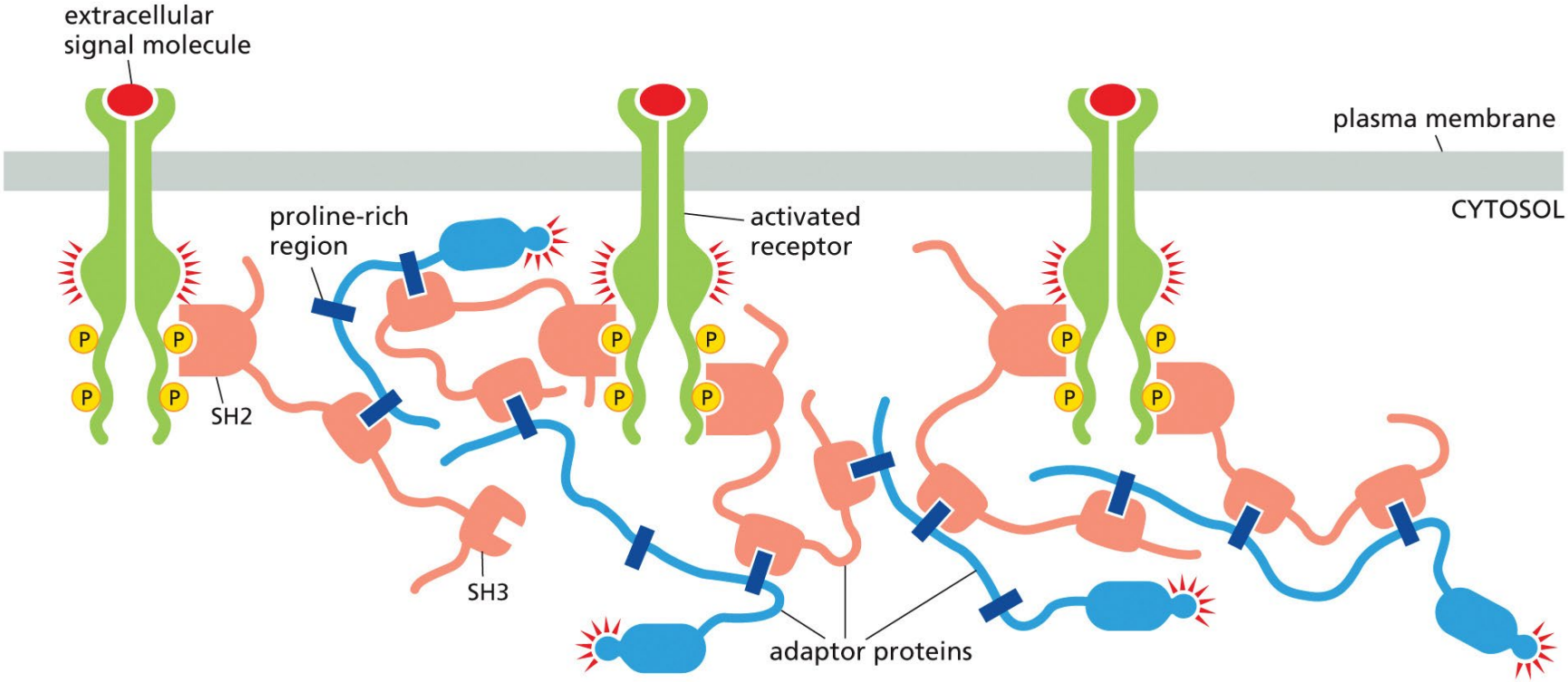


activated receptor tyrosine kinases are extensively phosphorylated on disordered regions in the receptor tails

two adaptor proteins:

- One contains one SH2 domain, which binds phosphorylated tyrosines on the receptors, and two SH3 domains.
- The other contains three proline-rich regions that can bind to SH3 domains, plus a protein kinase domain.

Formation of large receptor clusters by multivalent interactions among signaling proteins



Numerous multivalent binding interactions can occur among the three components in this system, generating a cross-linked protein matrix or condensate in which the protein kinases of the receptor and adaptor protein are concentrated, potentially providing a more effective signal output.

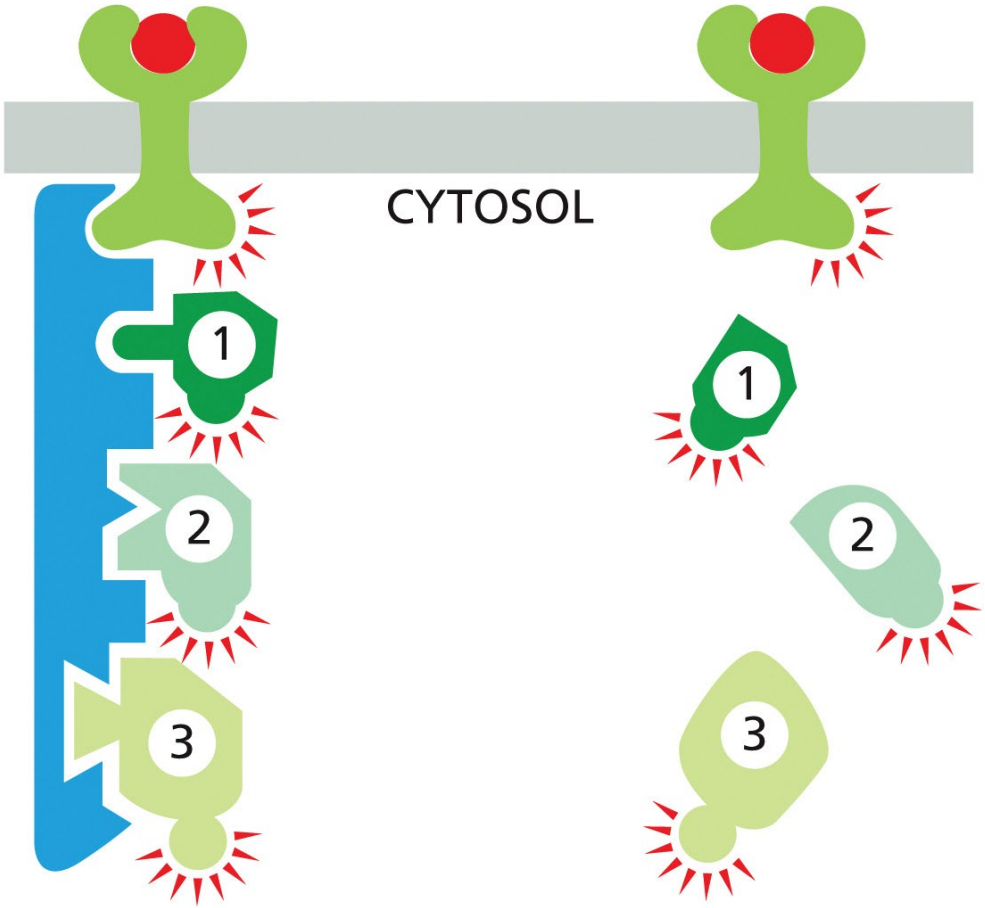
The cross-linking of the matrix can be enhanced further by including adaptor proteins with domains that interact with modified phospholipids in the membrane.

Question

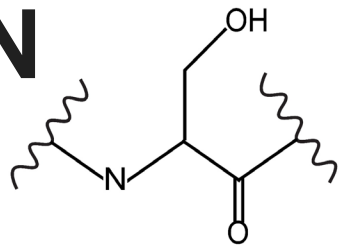
A kinase cascade organized by a scaffold protein or composed of freely diffusing components

Compare:

- Amplification
- Speed
- Crosstalk between pathways

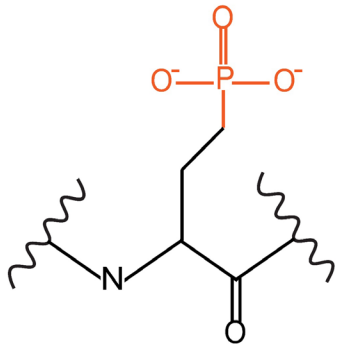


PROTEIN PHOSPHORYLATION AND KINASES



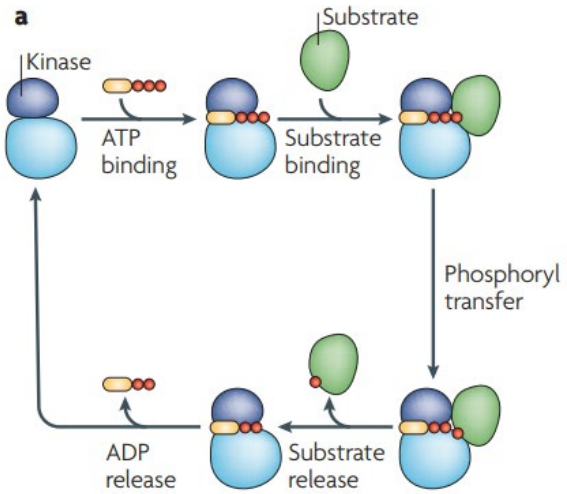
Peptidyl serine

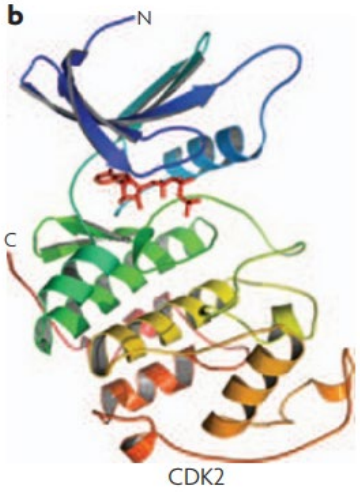
- Protein phosphorylation is the most common post-translational modification
- Affects almost every basic cellular process



Peptidyl phosphoserine

- *Ser/Thr kinases (majority)*
  - cAMP/cGMP, diacylglycerol, and Ca^{2+}/calmodulin
- *Tyr kinases*
  - *Typically, receptor kinases or receptor associated kinases*



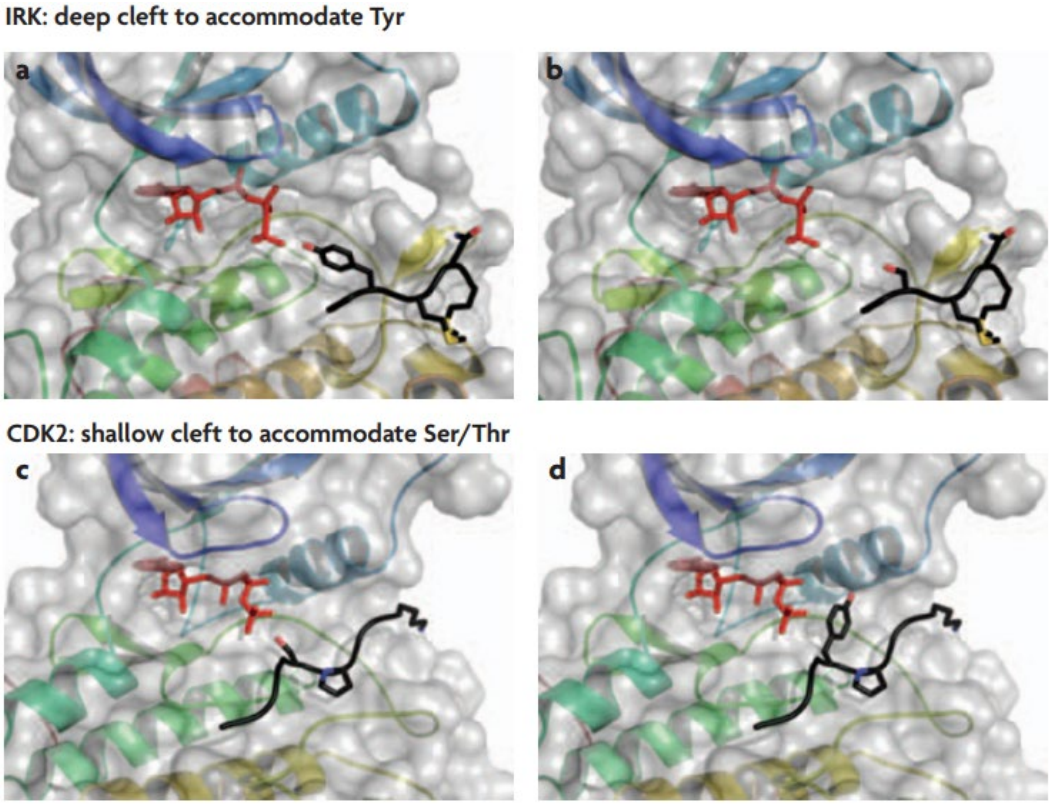


https://www.nature.com/articles/nrm2203

SPECIFICITY OF PHOSPHORYLATION

Protein kinases phosphorylate typically 1-~hundreds of residues out of ~700,000 potential target residues → a range of mechanisms to ensure specificity

1. Depth of the kinase catalytic cleft → tyrosine or serine/threonine residues



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SPECIFICITY OF PHOSPHORYLATION

2. Local interactions near the phosphorylation site → consensus sequences

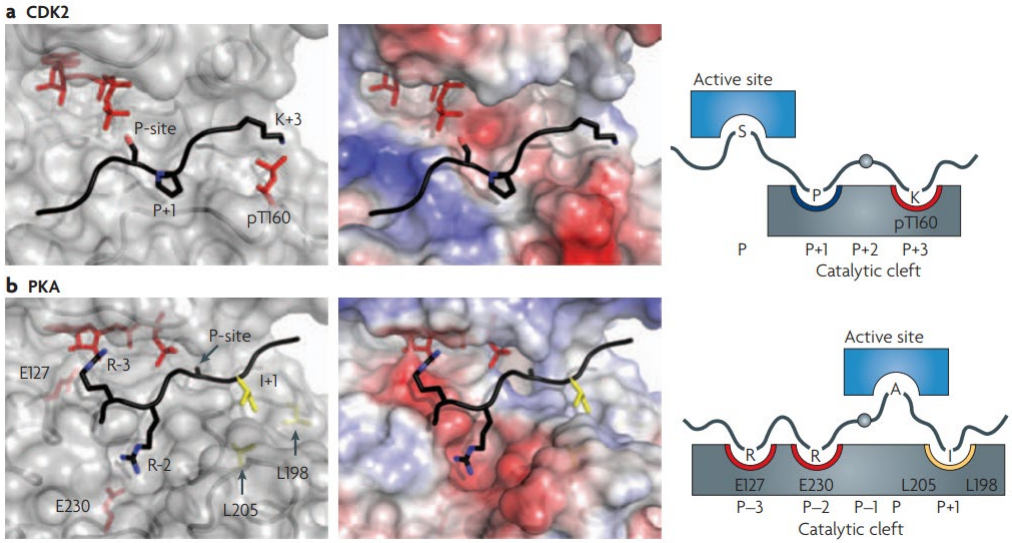


Table 1 | **Consensus phosphorylation sites of some protein kinases**

Kinase	Full name	Consensus phosphorylation site	Refs
PKA	Protein kinase A or cAMP-dependent protein kinase	R-R-X-S/T-Φ	5,39
CDK	Cyclin-dependent kinase	S/T-P-X-K/R	5,39
ERK2	Extracellular-regulated kinase-2	P-X-S/T-P	5,136
CK1*	Casein kinase-1	pS-X-X-S/T	5,137
CK2‡	Casein kinase-2	S/T-D/E-X-E/D	5,138
GSK3	Glycogen synthase kinase-3	S-X-X-X-pS	5,139
CaMK2	Calmodulin-dependent protein kinase-2	R-X-X-S/T	5,136
ABL	Abelson murine leukaemia virus tyrosine kinase	I/V/L-Y-X-X-P/F	5,140
EGFR	Epidermal growth factor receptor	E-E-E-Y-F	5,141
Src	Rous sarcoma virus tyrosine kinase	E-E-I-Y-E/G-X-F	5,141
IRK	Insulin receptor tyrosine kinase	Y-M-M-M	5,141
PKB/AKT	Protein kinase B	R-X-R-X-X-S/T	142
PKD	Protein kinase D	L/I-X-R-X-X-S/T	40
PIM1–3	Proviral integration site kinases 1–3	R-X-R-X-X-S/T	40,143

*CK1 is a well-conserved Ser/Thr-specific protein kinase, the regulation and function of which are incompletely understood. ‡CK2 is also a well-conserved Ser/Thr kinase that is unrelated to CK1 and is implicated in the regulation of diverse biological phenomena. pS, phosphorylated Ser; X, any residue; Φ, hydrophobic residue.

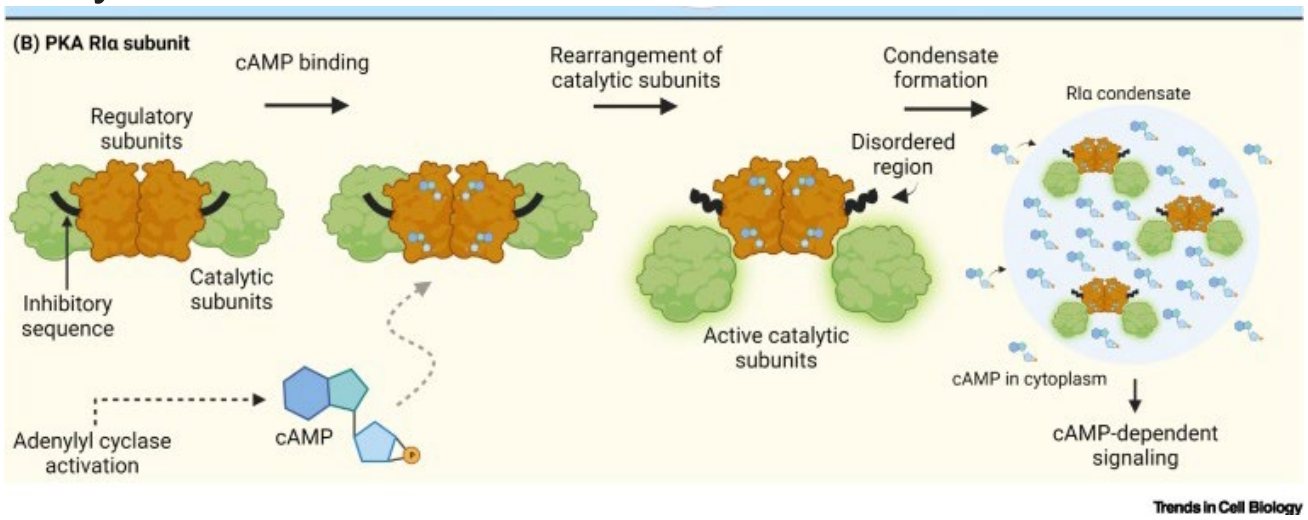
https://www.nature.com/articles/nrm2203

SPECIFICITY OF PHOSPHORYLATION

3. Docking sites are separated from the catalytic site of the kinase and the phosphorylation site of the substrate, can provide enforced proximity and allosteric regulation of a correct kinase–substrate pair

4. Localization restricts kinases to a subset of substrates and increases local kinase concentrations.

5. Scaffolds, which form dynamic ternary complexes with kinases and substrates and might contribute to kinase specificity in several ways.

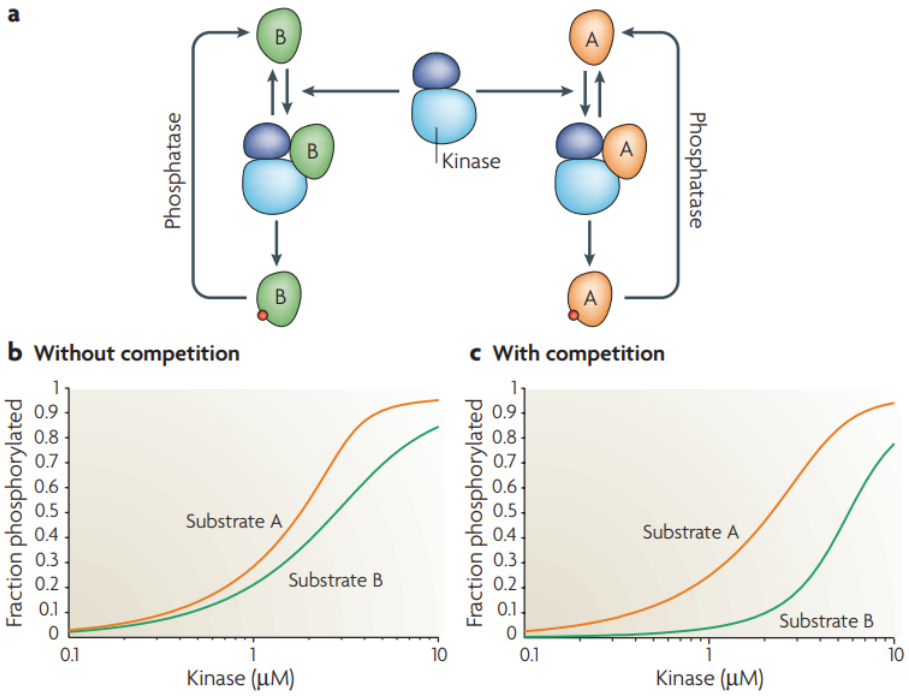


https://www.nature.com/articles/nrm2203

https://www.cell.com/trends/cell-biology/fulltext/S0962-8924(22)00260-4

SPECIFICITY OF PHOSPHORYLATION

6. Competition ensures that the correct residues are targeted for phosphorylation. This mechanism can suppress the phosphorylation of off-target substrates and can add thresholds and temporal ordering to phosphorylation responses.



7. Multisite phosphorylation and kinetic proofreading, minimize the consequences of aberrant phosphorylations

https://www.nature.com/articles/nrm2203

PRINCIPLES OF CELL SIGNALING

- The relationship between signal and response varies in different signaling pathways
  - Timing (speed of response)
  - Sensitivity
  - Dynamic range
  - Persistence
  - Signal processing

PRINCIPLES OF CELL SIGNALING

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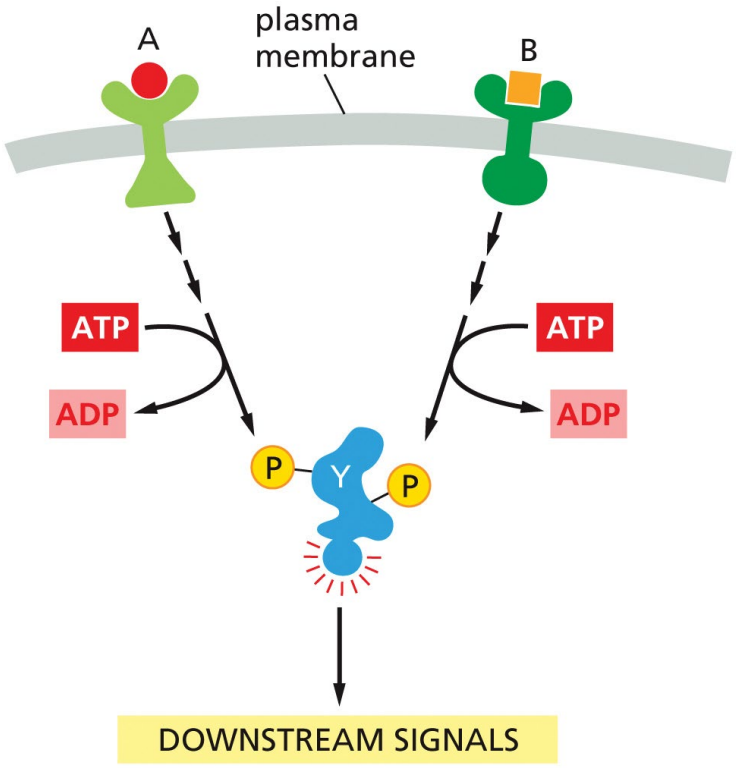
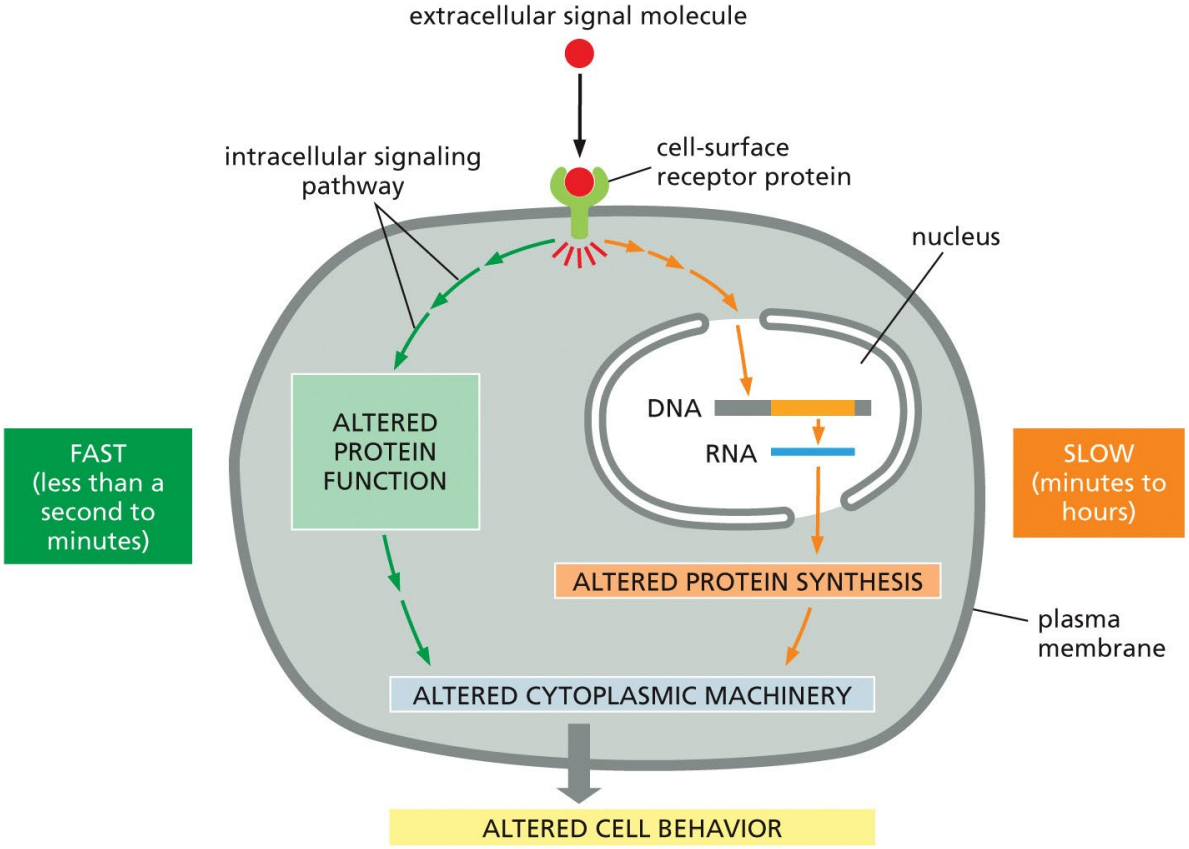
  - Sensitivity
  - Dynamic range
  - Persistence
  - Signal processing
  - Coordination of multiple responses for one signal
  - **Integration (of multiple signals)**
    - **Including AND gates**



THE SPEED OF A RESPONSE

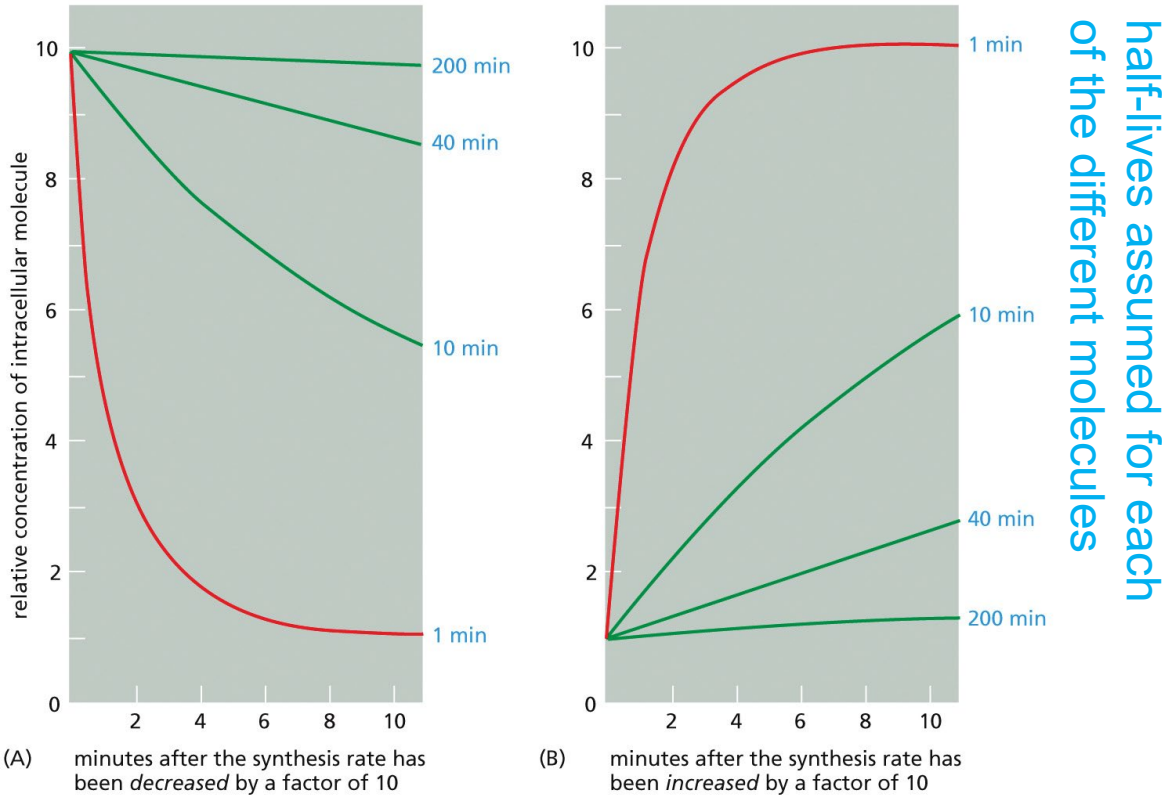
- The **speed** of a response depends on the **nature** of the signaling molecules



THE SPEED OF A RESPONSE AND TURNOVER

- The **speed** of a response depends on the **turnover** of signaling molecules

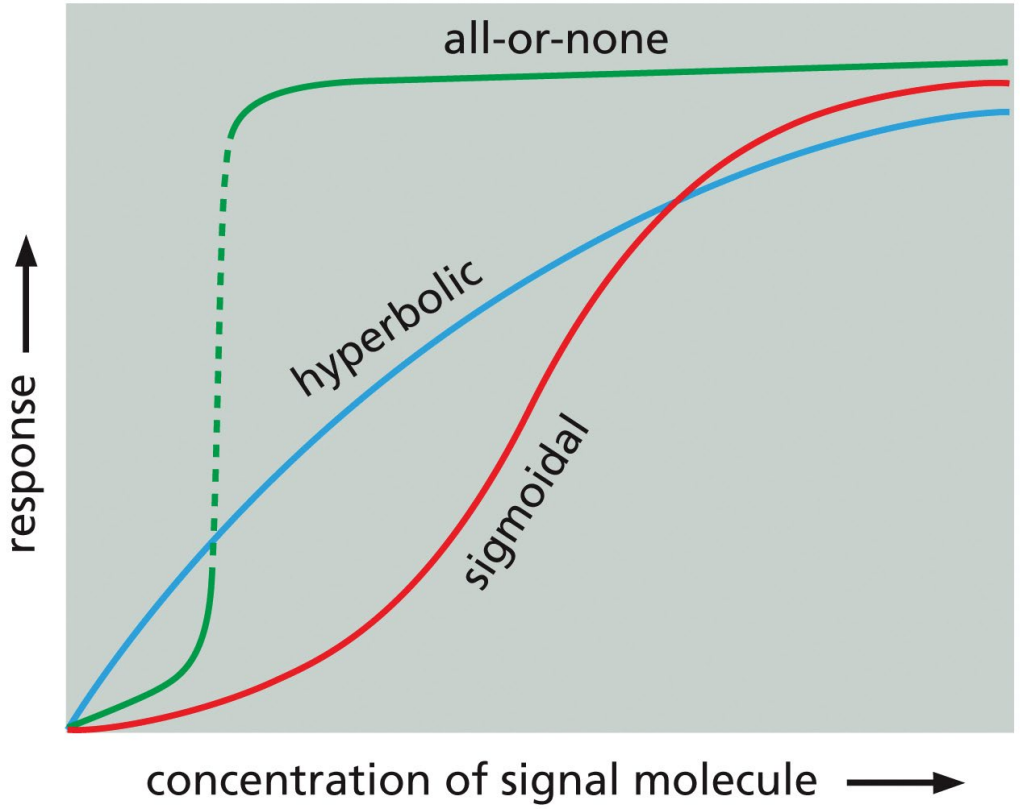
- The synthesis rates are (**A**) decreased or (**B**) increased by a factor of 10
- The concentrations of molecules that are degraded rapidly *(red lines)* change quickly, the concentrations of those that are degraded slowly *(green lines)* change proportionally more slowly



PRINCIPLES OF CELL SIGNALING

- Cells can respond abruptly to a gradually increasing signal

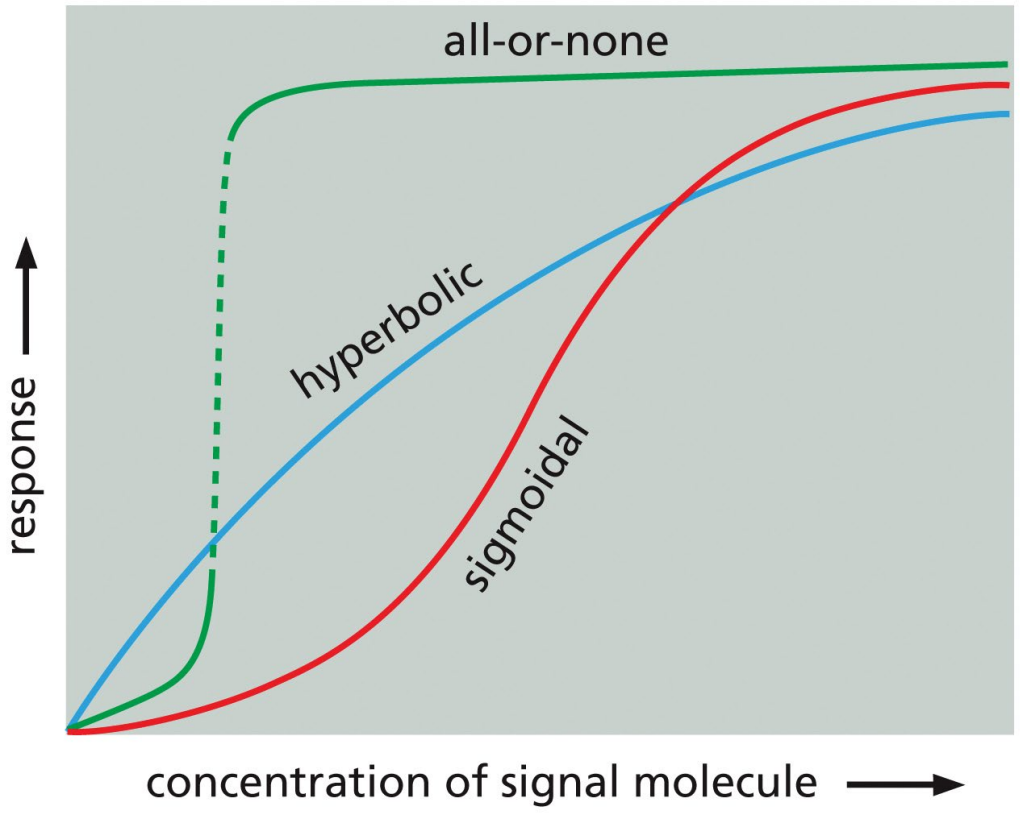
- Response increases gradually as the concentration of extracellular signal molecule increases
- Eventually reaching a plateau (signaling pathway saturated)
- =*Hyperbolic* response curve
- E.g. hormones



PRINCIPLES OF CELL SIGNALING

- Cells can respond abruptly to a gradually increasing signal

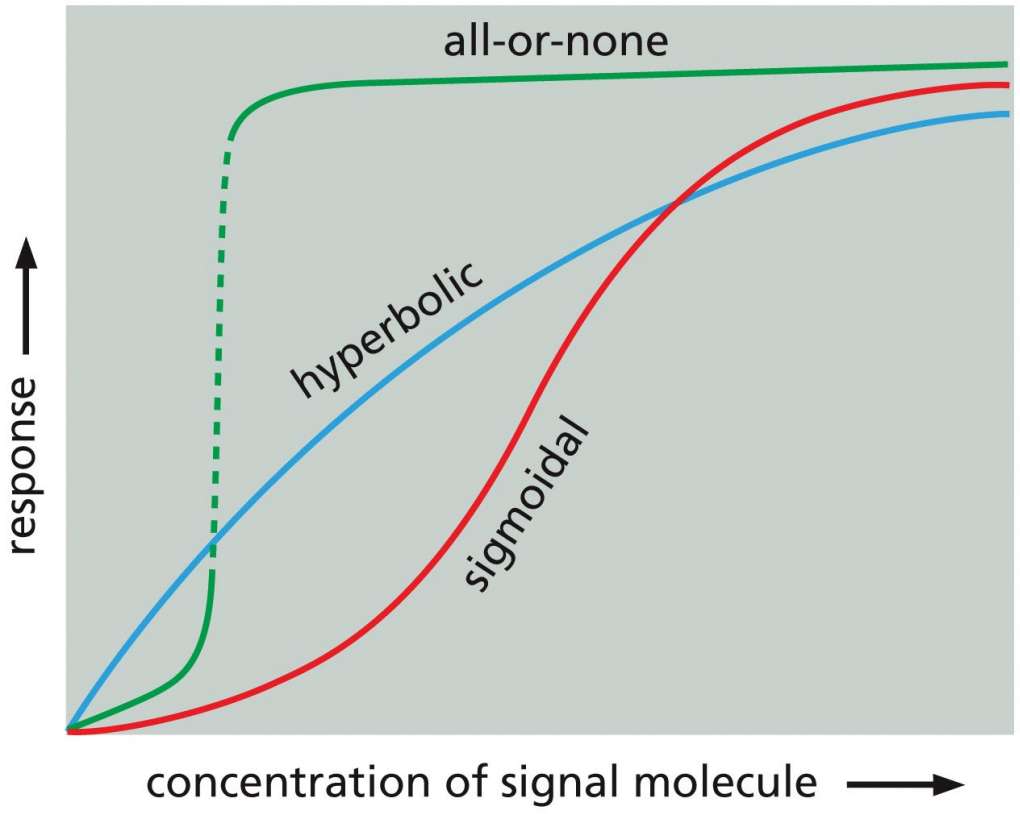
- Response is switchlike
- The cell switches completely between a low and high response
- Typical to control cell states



PRINCIPLES OF CELL SIGNALING

- Cells can respond abruptly to a gradually increasing signal

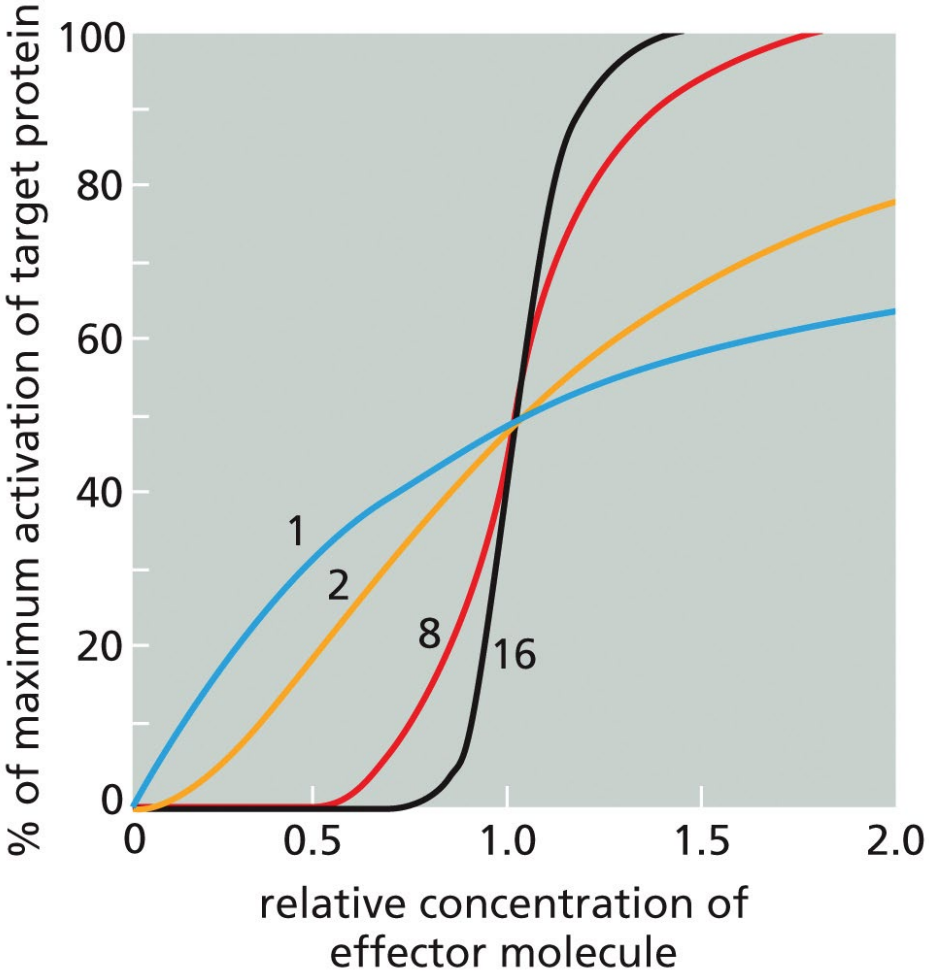
- The signaling system reduces the response at low signal concentrations
- Produces a steeper response at some intermediate signal concentration
- = *Sigmoidal* response curve
- Examples, allostreric regulation (next slide) or simultanous activation and inhibition of opposite reactions



Activation curves for an allosteric protein as a function of effector molecule concentration

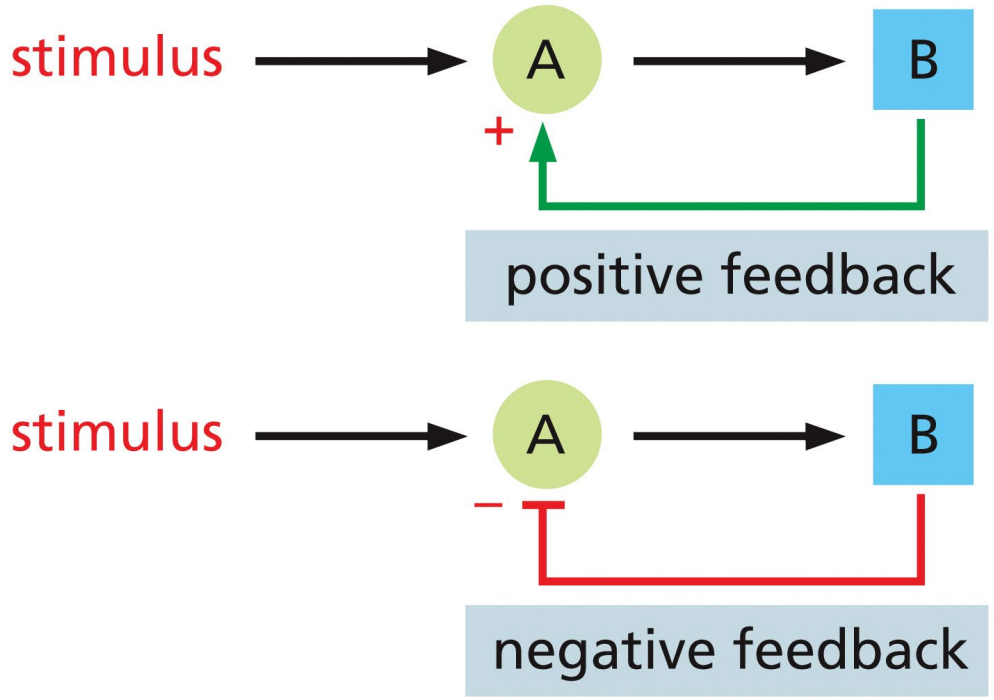
- The curves show how the sharpness of the activation response increases with an increase in the number of effector molecules that must be bound simultaneously to activate the target protein

- An example of **sigmoidal response**
- E.g. phosphorylation of multiple sites or binding of multiple signalling molecules required



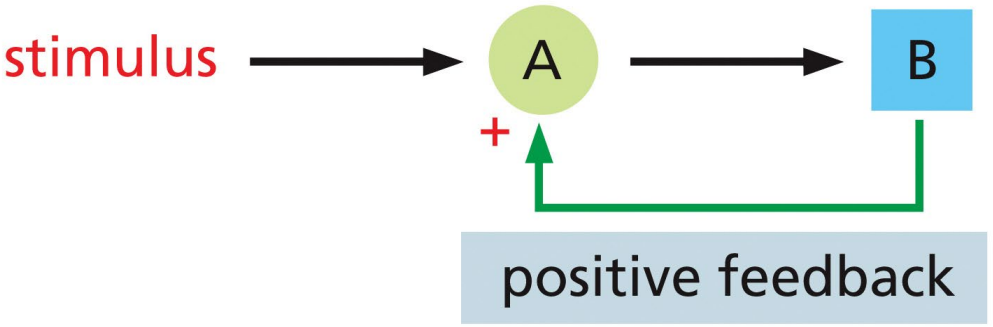
POSITIVE AND NEGATIVE FEEDBACK

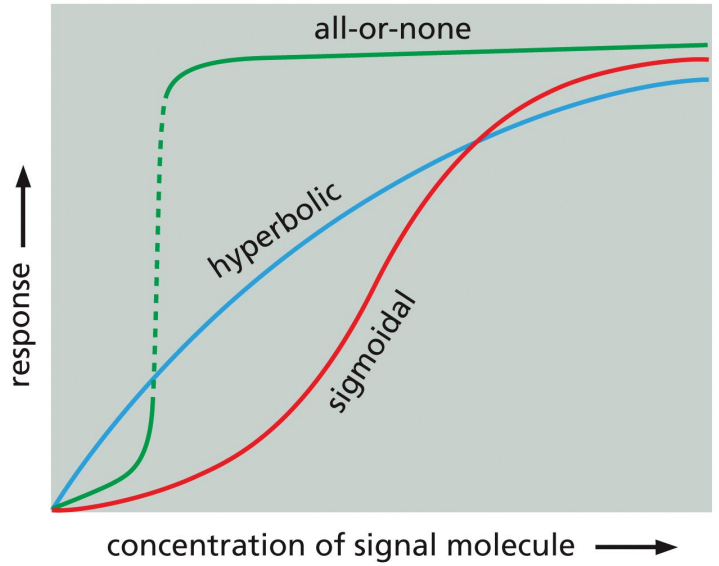
- Positive feedback can generate an all-or-none response
- Negative feedback is a common feature of intracellular signaling systems



POSITIVE FEEDBACK

- **Positive feedback can generate an all-or-none response**
- May be bistable – exists in on-state or off-state that *persists after original signal level drops*
- *E.g. cell memory in the differentiation of cells - permanent change without a change in DNA*





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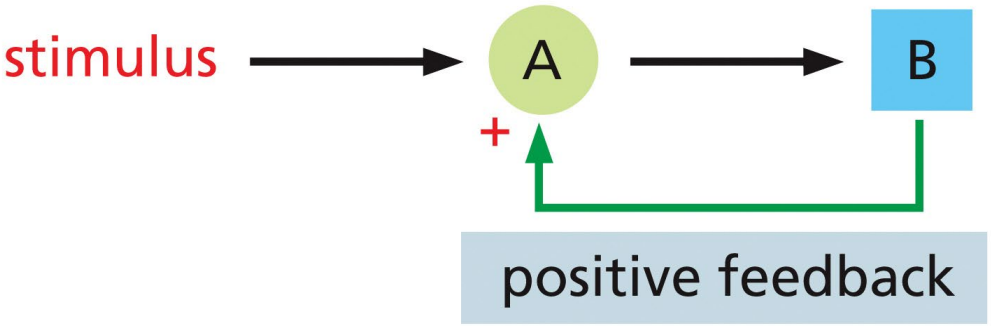
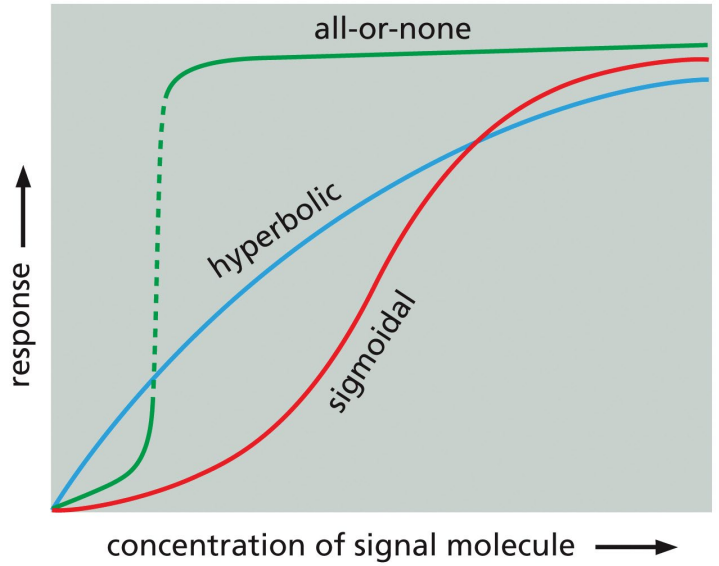
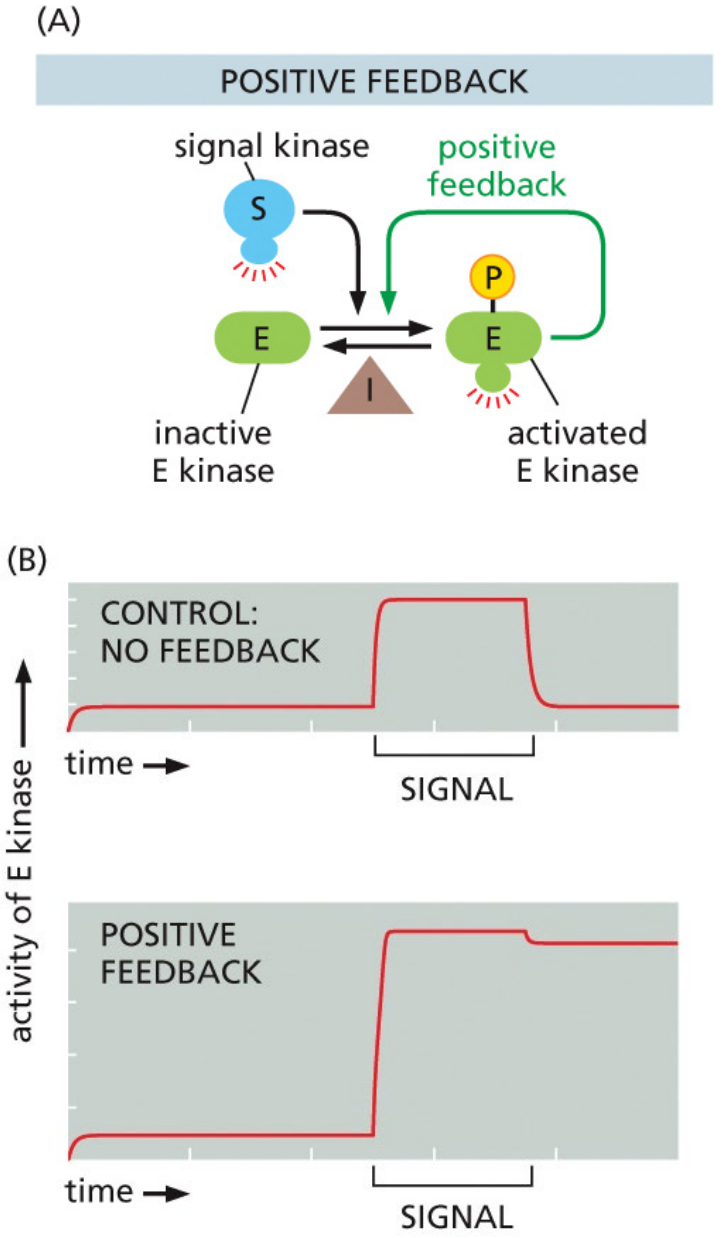




- The **input signal** is an **activated protein kinase** (S) that *phosphorylates* and thereby *activates* another protein kinase (E)
- (A **protein phosphatase** (I) *dephosphorylates* and *inactivates* the activated E kinase)

- **Without feedback**, the *activity of the E kinase* is *proportional to the level of stimulation* by the S kinase

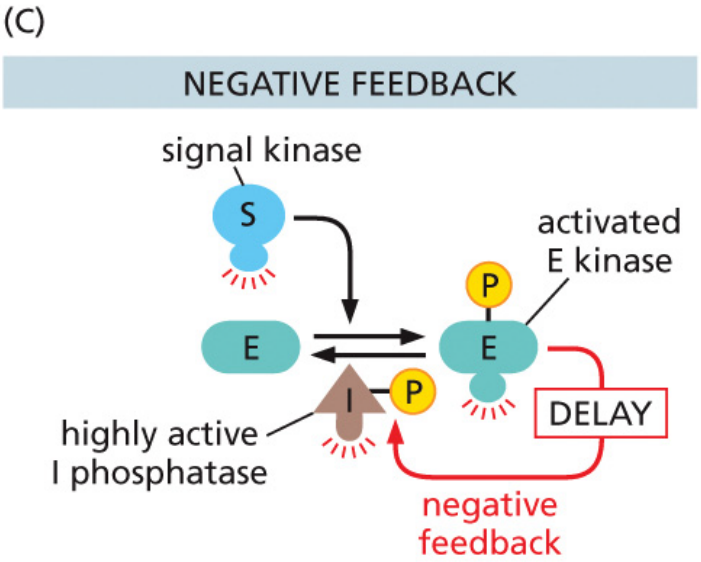
- **With the positive feedback loop**, the *transient stimulation* by S kinase *switches the system from an "off" state to an "on" state*, which then *persists* after the stimulus has been removed



time during which the input signal (activated S kinase) is present

NEGATIVE FEEDBACK

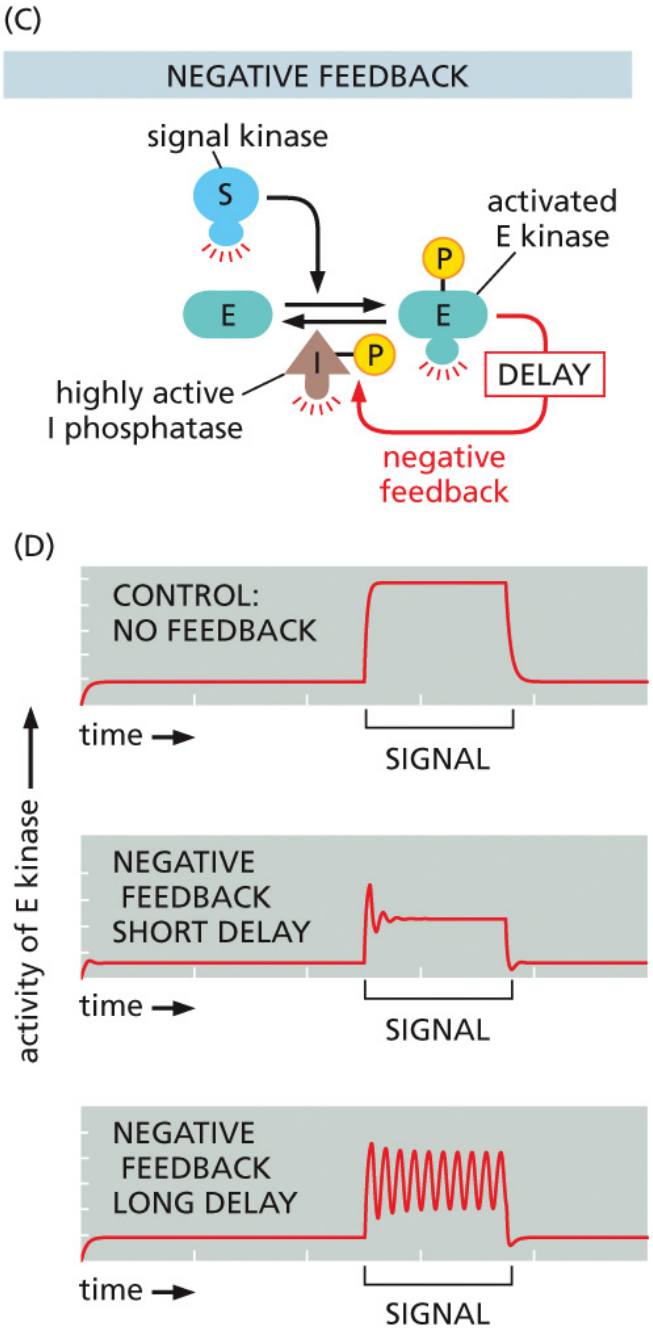
- The **input signal** is an **activated protein kinase** (S) that *phosphorylates* and thereby *activates* another protein kinase (E)
- A **protein phosphatase** (I) *dephosphorylates* and *inactivates* the activated E kinase



NEGATIVE FEEDBACK

- **Without feedback**, the *activity of the E kinase* is *proportional to the level of stimulation* by the S kinase

- **Short delay**: the system shows a response when the signal is first increased, but the feedback quickly dampens the response—which then declines to some intermediate level at which the input signal and feedback are balanced

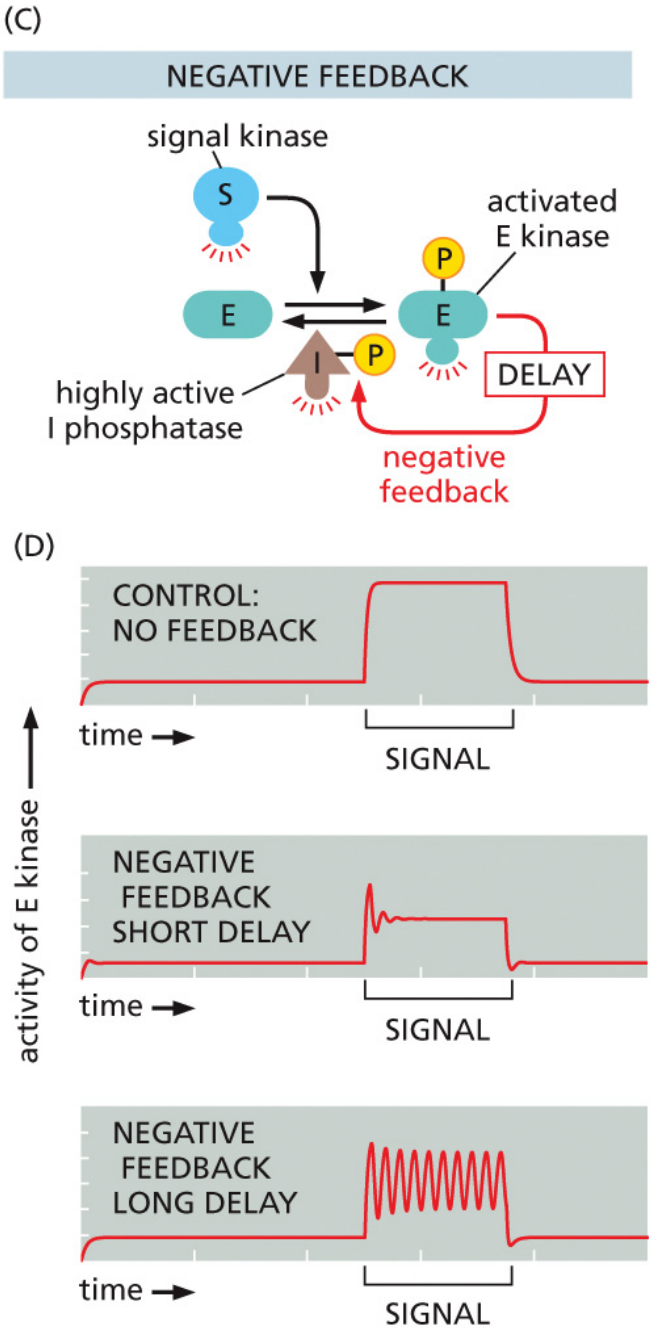


NEGATIVE FEEDBACK

- **Without feedback**, the *activity of the E kinase* is *proportional to the level of stimulation* by the S kinase

- **Long delay**: the response rises unopposed at first, allowing kinase activity to reach maximum levels before it feeds back to shut itself off. Then the sudden drop in activity removes the negative feedback, unleashing another pulse of kinase activity

- If conditions are right, the result is **sustained oscillations** for as long as the stimulus is present



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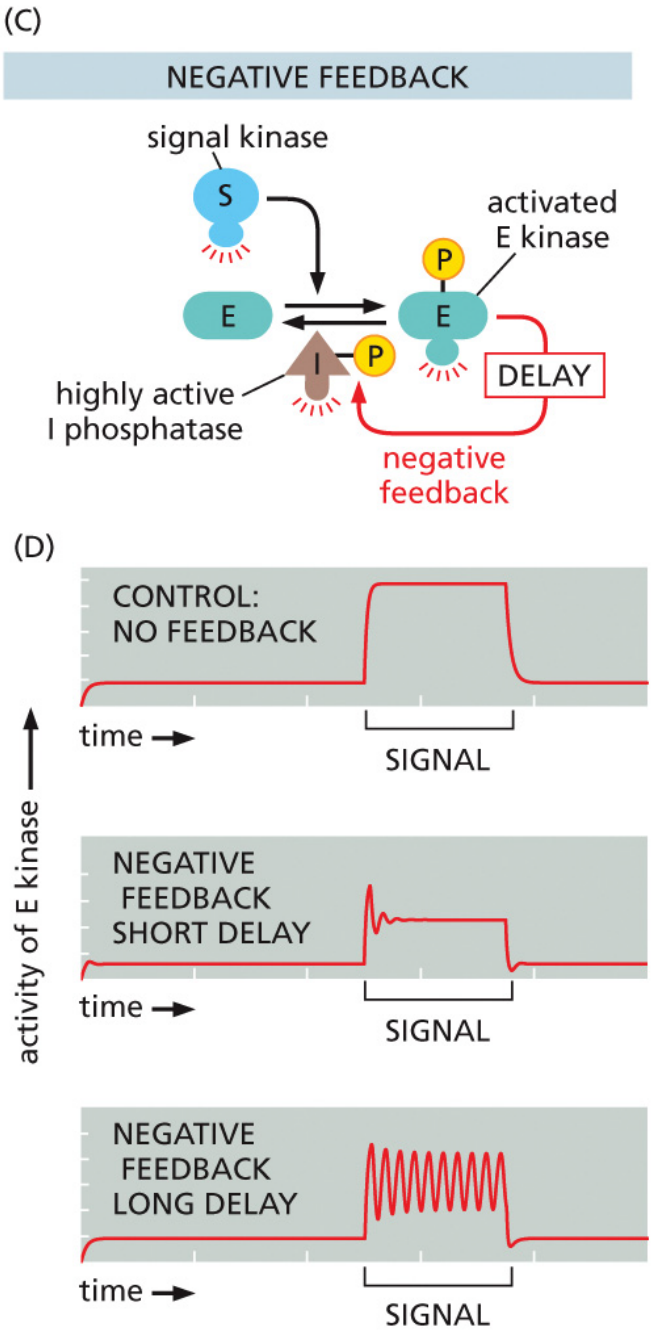
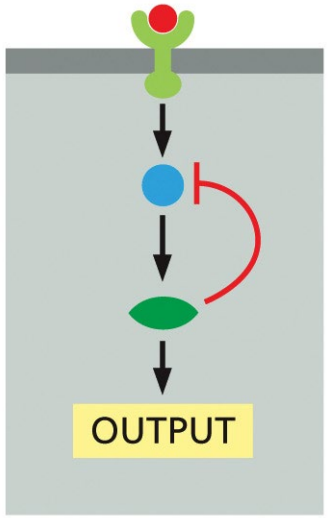
- **Short delay – adaptation, response to change**



ADAPTATION AND DESENSIZATION

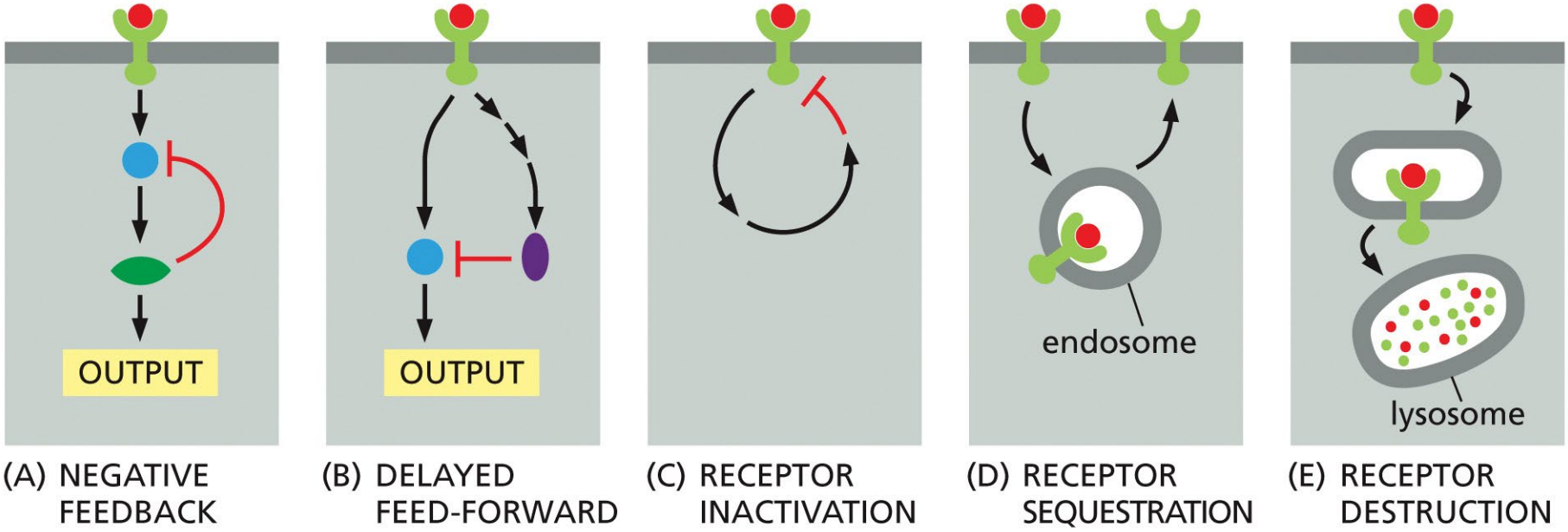
- Cells can **adjust their sensitivity to a signal** – adaptation and desensization
- **Negative feedback with a short delay** dampens the initial response to receptor activation



(A) NEGATIVE FEEDBACK

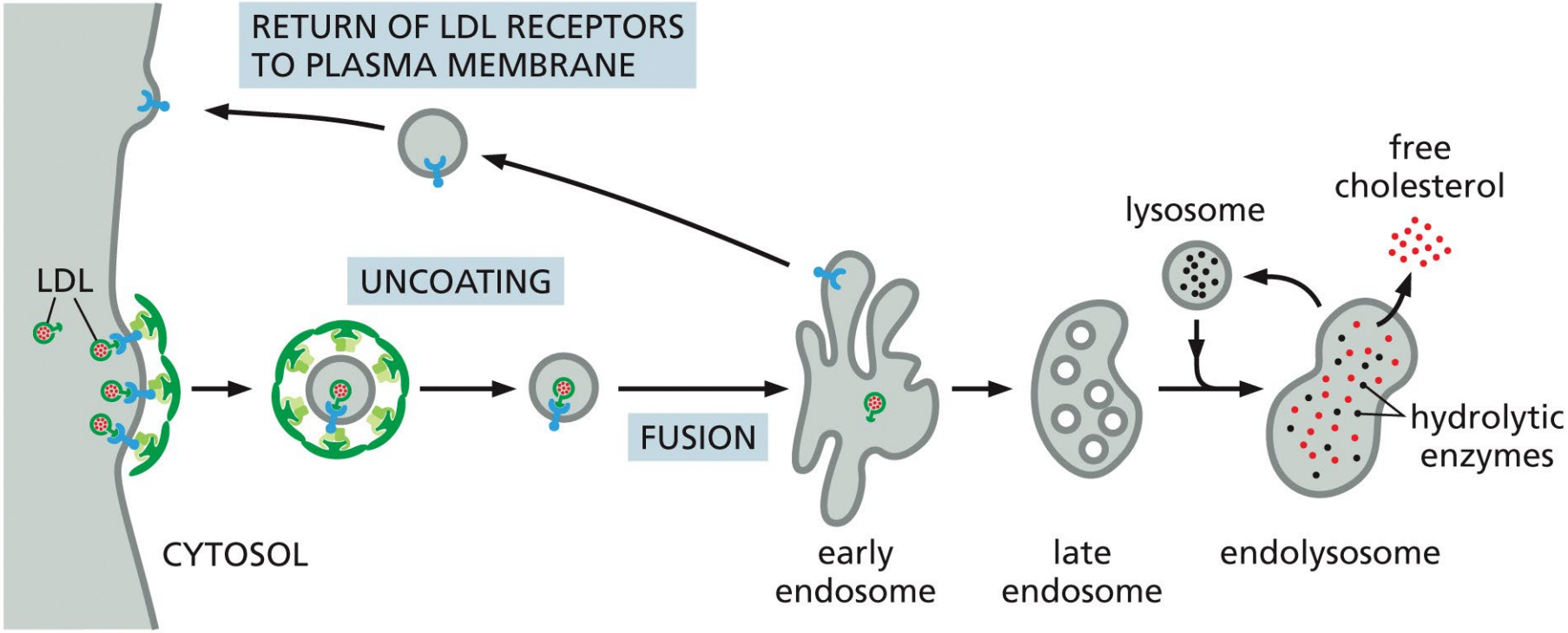
ADAPTATION AND DESENSIZATION

- Cells can adjust their sensitivity to a signal – adaptation and desensization
- In some cases, the activated receptor rapidly activates a stimulatory pathway while also initiating **a slower inhibitory pathway**—resulting in a transient output response. This is called a delayed feed-forward loop.
- Various mechanisms can **inactivate a cell-surface receptor**



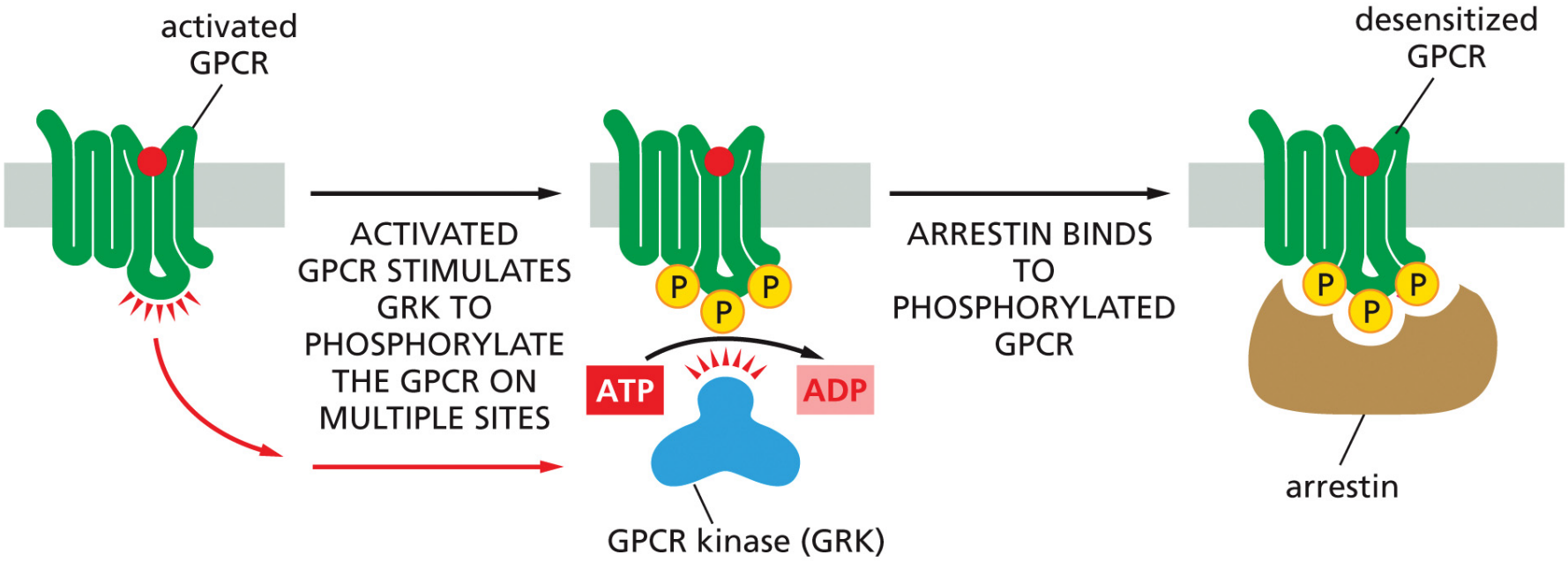
TRANSPORT INTO THE CELL FROM THE PLASMA MEMBRANE: ENDOCYTOSIS

- Specific proteins are retrieved from early endosomes and returned to the plasma



SIGNALING THROUGH G-PROTEIN-COUPLED RECEPTORS

- GPCR Desensitization depends on receptor phosphorylation



A GRK phosphorylates only activated receptors because it is the activated GPCR that turns on the GRK. The binding of an arrestin to the phosphorylated receptor prevents the receptor from binding to its G protein and also directs its endocytosis

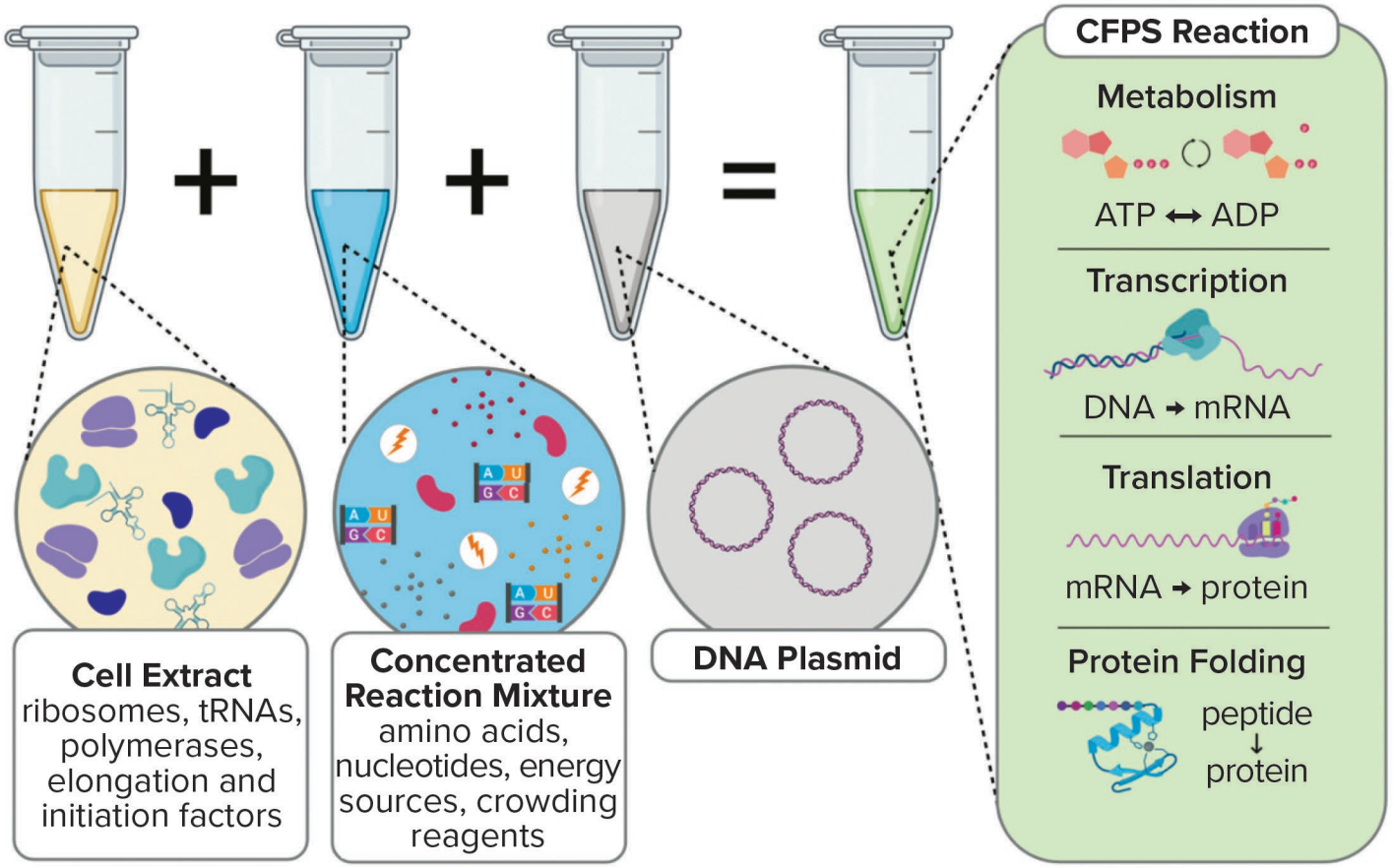
Question

Tell 3 examples of how a signal can be sharpened?

SUMMARY

- **Modular interaction domains** mediate interactions between intracellular signaling proteins
- The relationship between signal and response varies in different signaling pathways
- The **speed of a response** depends on the **turnover** of signaling molecules
- Cells can respond abruptly to a gradually increasing signal
- **Positive feedback** can generate an all-or-none response
- **Negative feedback** is a common feature of intracellular signaling systems
- Cells can adjust their **sensitivity** to a signal

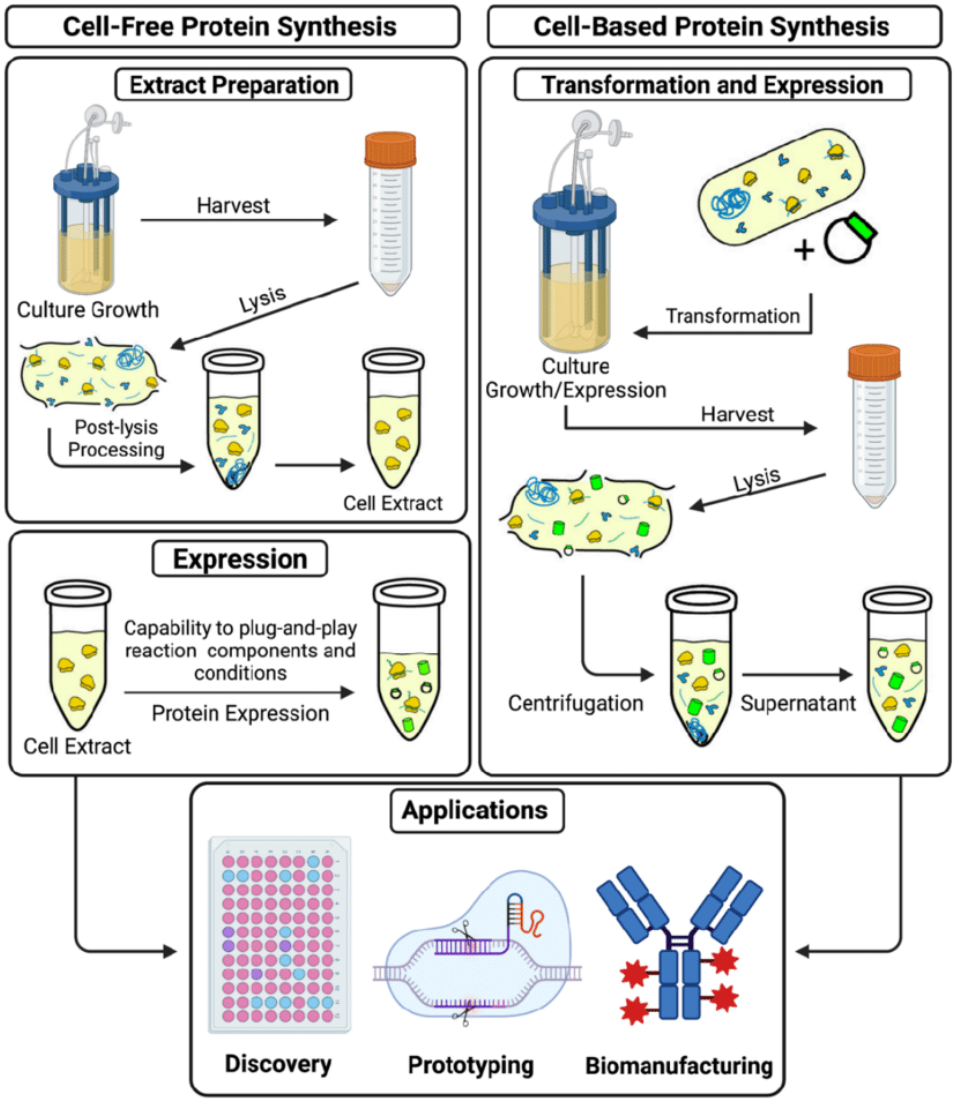
CELL-FREE PROTEIN SYNTHESIS



Brookwell, A.; Oza, J.P.; Caschera, F. Biotechnology Applications of Cell-Free Expression Systems. *Life* **2021**, *11*, 1367. https://doi.org/10.3390/life11121367

CELL-FREE VS *IN-VIVO* PROTEIN EXPRESSION

ADVANTAGES AND DISADVANTAGES OF EACH?



https://bioprocessintl.com/upstream-processing/expression-platforms/toward-a-roadmap-for-cell-free-synthesis-in-bioprocessin