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CONCEPT: INTRODUCTION TO BIOSIGNALING

- _____: the ability for all *cells* to produce, receive & respond to external signals/conditions.
  - □ Allows for the *response to stimuli* & effective cellular *communication*.

Types of Signaling Molecules:
- Antigens
- Growth Factors
- Hormones
- Light
- Mechanical Touch
- Neurotransmitters

Signal Transduction

- *Signal* _____: cell process that _____ *signals/information* into a *chemical change/response*.
  - □ Requires a *minimum* of _____ key components:
    1) a _____: a small molecule that specifically binds & forms a complex with a *biomolecule/receptor*.
    2) a _____: typically, an integral membrane protein that changes *conformation* upon ligand binding.
- NOTE: *receptor-ligand* interactions are *protein-ligand* interactions, so be sure to check out those previous videos!

$$K_d = \frac{[R]\,[L]}{[RL]}$$

PRACTICE: A sample of cells has a total receptor concentration of 10 mM and a free ligand concentration of 15 mM. If 25% of the receptors are occupied with ligand under these conditions, calculate the receptor-ligand dissociation constant (K_d).

a) 7 mM.
b) 38 mM.
c) 12 mM.
d) 45 mM.

CONCEPT: INTRODUCTION TO BIOSIGNALING

PRACTICE: Which hormone from the plot below shows the highest binding affinity for the receptor?

a) Hormone X.
b) Hormone Y.
c) Hormone Z.

5 Features of Biosignaling Transduction Systems

PRACTICE: Which of the following statements regarding signal transduction pathways in cells is FALSE?

a) A ligand, such as a hormone, binds to a specific cell surface receptor on a target cell.
b) Signal transduction cascades, often involving protein kinases, amplify a signal intracellularly.
c) A receptor changes conformation upon ligand binding, transmitting a signal across the cell membrane.
d) Signal transduction cascades directly transmit a single stimulus to one, single target, identically in all cells.
e) Phosphatases remove phosphoryl groups from polypeptides, regulating a cell's response.

CONCEPT: INTRODUCTION TO BIOSIGNALING

Types of Kinases

●Moving forward, we will see that _____ play a large role in biosignaling to create the cell response.

□ Recall: *Kinases*: enzymes that _____ their substrates (utilizing ATP).

□ Phosphorylation leads to _____ *activity* of the target (activation or inhibition).

●Two of the most common classes of kinases are _____/_____ *Kinases* & _____ *Kinases*.

□ *Ser/Thr Kinases*: phosphorylate _____ & _____ residues on their targets (make up ~25% (¼) of all kinases).

□ *Tyr Kinases*: phosphorylate _____ residues on their targets.

PRACTICE: Why is the activation of a protein kinase an important step in signal transduction?

a) Kinases bind to receptors and prevent binding of the ligand.

b) Kinases prevent signal amplification by degrading specific enzymes in the cell.

c) Kinases can activate or inactivate proteins by phosphorylation.

d) Kinases are secreted by a cell as a signaling molecule.

Types of Biosignaling Receptors

●_____ major types of integral membrane protein receptors involved in most signal transduction pathways:

1 **G** Protein-**C**oupled **R**eceptors (_____). 2 **R**eceptor **T**yrosine **K**inases (_____).

●GPCRs & RTKs transduce extracellular signals via fundamentally _____ mechanisms.

EXAMPLE: GPCRs vs. RTKs.

CONCEPT: INTRODUCTION TO BIOSIGNALING

Map of Lesson on Biosignaling Pathways

CONCEPT: G PROTEIN-COUPLED RECEPTORS

- **G** Protein-**C**oupled **R**eceptor (_____): a _____ that *associates/couples* with a _____ *protein*.
  - □ *GPCR* is an *integral membrane protein* that consists of _____ transmembrane α-helices (*7-TMS proteins*).
  - □ GPCRs have an *extra*cellular _____-terminal & an *intra*cellular _____-terminal.
- *G proteins* (*GTP-binding proteins*): intracellular *lipid-linked proteins* that hydrolyze _____ & have 3 subunits (α, β , γ).
  - □ **G**uanosine-**T**ri-**P**hosphate (_____): *similar* in function to **a**denosine-**tri**phosphate (_____).

3 Essential Components of GPCR Signal Transduction

1. _____: transmembrane receptor that changes *conformation* upon ligand-binding to activate G protein.
2. ___-*Protein*: intracellular peripheral protein that replaces GDP for GTP when activated & *slowly* hydrolyzes GTP.
   - □ Contains _____ subunits (α, β , and γ) which _____ to α and β-γ subunits *upon GTP binding*.
3. _____ *Enzyme*: membrane protein produces a *secondary messenger* upon activation by α G-protein.
   - □ _____ *messengers* affect downstream targets that lead to *cell response*.

CONCEPT: G PROTEIN-COUPLED RECEPTORS

PRACTICE: A G protein-coupled receptor is comprised of ____ transmembrane α-helix/helices:

a) 7. b) 8. c) 1. d) 10. e) 6. f) 14.

PRACTICE: G protein-coupled receptors (GPCRs) are transmembrane proteins with ligand binding sites on the extracellular side of the membrane (near its N-terminal). What is the role of the G-protein in the GPCR signaling pathway?

a) G proteins phosphorylate GPCRs.
b) G proteins bind extracellular ligands and facilitate the transport into the cell.
c) G proteins activate enzymes that transduce the signal throughout the cell.
d) G proteins activate transcription of genes.
e) G proteins facilitate dimerization of receptor monomers.

Stimulatory vs. Inhibitory G Proteins (G_s vs G_i)

- _____ types of G proteins classified according to their _____ on the *effector enzyme*.
  1) _____ *G proteins* (G_s): *stimulate/activate* the effector enzyme to create _____ secondary messenger.
  2) _____ *G proteins* (G_i): *inhibit/in*activate the effector enzyme to create _____ secondary messenger.

PRACTICE: G_i is the inhibitory G protein of various effector enzymes. If a toxin released from a bacterial infection enters cells & covalently modifies the α-subunit of G_i so that it can no longer bind GTP, which of the following is a likely result?

a) Increase in the synthesis of the secondary messenger molecule.
b) Decrease in the synthesis of the secondary messenger molecule.
c) The inhibitory G protein α-subunit will not dissociate from the β-γ subunits.
d) Only A and C are true.
e) Only B and C are true.

CONCEPT: STIMULATORY ADENYLATE CYCLASE GPCR SIGNALING

- *Adenylate Cyclase GPCR System*: a classic _____ step example/prototype of a GPCR signal transduction pathway.
  - □ Allows cells to generate a "fight or flight" response to the hormone _____ (*adrenaline*).
    - **1**) Hormone signaling ligand (*epinephrine*) binds GPCR (*β-adrenergic GPCR*) causing a _____ *shift*.
    - **2**) GPCR conformational shift _____ G protein (G_s) by promoting α subunit to *release* GDP & bind GTP
    - **3**) With bound GTP, ___ subunit of G_s _____ from β-γ subunits & *diffuses* to nearby *effector enzyme*.
    - **4**) Activated/GTP-bound α-subunit of G_s binds & _____ the effector enzyme (*adenylate cyclase*).
    - **5**) Activated effector enzyme (*adenylate cyclase*) converts ATP → _____ *messenger* (*cAMP*).
      - □ cAMP $\xrightarrow{\text{Activates}}$ ***p**rotein **k**inase **A*** (_____) ⟶ *Cell* _____ (ex. ↑ breakdown of glycogen/fat).
    - **6**) G protein α subunit slowly _____ itself (& the effector enzyme) by *hydrolyzing* its GTP → GDP.
      - □ α subunit assembles its original, *inactive* form & ligand *dissociates* from GPCR to _____ pathway.

CONCEPT: STIMULATORY ADENYLATE CYCLASE GPCR SIGNALING

How to Remember Stimulatory Adenylate Cyclase GPCR Signaling?

Story: Cell wants to go ***cAMP***ing (*secondary messenger*) & ***C****ook smores* (***C****ell response*)!

1. Pack the ***L***uggage (***L***igand binding to GPCR).
2. ***Alpha****-male* (G protein *α*-subunit) takes charge, *exchanging* **empty Gas-can** with **full Gas-can** (**G**TP exchange).
3. *Alpha*-male leaves to fill-up ***AC***ura (***A***denylate ***C***yclase) (dissociation of G protein α-subunit).
4. *Alpha*-male fills-up ***AC***ura gas tank & *starts engine* (***A***denylate ***C***yclase *activation*).

5a. ***AC***ura goes to ***cAMP*** site (***cAMP*** production).

5b. Upon arrival to ***cAMP*** site, they ***P***ar***K*** the ***A***cura (***PKA***) & can now ***C****ook smores* (***C****ell Response*)!

PRACTICE: Which of the following represents the best order of signal transduction for the β-adrenergic receptor?

a) Hormone → receptor tyrosine kinase → G-protein → protein kinase A → cAMP → cellular effects.

b) Ligand → receptor → secondary messenger → G-protein → protein kinase A → cellular effects.

c) Hormone → G-protein → receptor → cAMP → protein kinase A → cellular effects.

d) Hormone → receptor → G-protein → second messenger → protein kinase A → cellular effects.

CONCEPT: STIMULATORY ADENYLATE CYCLASE GPCR SIGNALING

PRACTICE: Which of the following molecules is NOT involved in signal transduction of the β-adrenergic GPCR?

a) GTP.
b) ATP.
c) cAMP.
d) cGMP.
e) GDP.

PRACTICE: Which of the following reactions does the enzyme adenylate cyclase catalyze?

a) cATP to cAMP.
b) GTP to cGMP.
c) ATP to cADP.
d) cGMP to GTP.
e) ATP to cAMP.

PRACTICE: In response to a ligand binding a GPCR, a particular G protein (G_s) activates adenylate cyclase. If a cell has a mutation where the G protein can no longer hydrolyze GTP, which of the following would be the result?

a) Cellular levels of cAMP would go down in response to ligand binding.
b) Cellular levels of cAMP would go up independent of ligand binding.
c) The ligand would not be able to bind to the receptor.
d) Cellular levels of cAMP would not change.
e) Adenylate Cyclase would always remain inactive.

PRACTICE: When epinephrine binds to its GPCR activating the cAMP cascade, all of the following happen EXCEPT:

a) GDP dissociates from the G protein as GTP associates.
b) The alpha-subunit of the G protein activates adenylate cyclase and cAMP is produced.
c) G protein is activated and diffuses away from the cell membrane into the nucleus.
d) The alpha-subunit of the G protein hydrolyzes GTP and becomes inactive.
e) The alpha-subunit of the G protein bound to GDP reassociates with the beta & gamma-subunits.

CONCEPT: cAMP & PKA

Production & Function of cAMP

●Recall: the effector enzyme, *adenylate cyclase*, converts ATP to **C**yclic **A**denosine **M**ono-**P**hosphate (_____).

□ *cAMP*: *secondary messenger* that _____ *cAMP-dependent* ***P****rotein* ***K****inase* ***A*** (_____).

□ Therefore, cAMP functions as an *allosteric* _____ (+) of PKA.

Activation of cAMP-Dependent Protein Kinase A (PKA)

●Recall: _____: enzymes that utilize energy (ex. ATP) to *phosphorylate* a substrate.

□ In absence of cAMP, PKA is an inactive *heterotetramer* (2 **R**egulatory & 2 **C**atalytic subunits: R_2C_2).

□ _____ *cAMPs* bind regulatory PKA subunits to release _____ *catalytically active PKA subunits*.

□ Catalytically active PKA subunits _____ Ser/Thr residues on target proteins to alter their activity.

CONCEPT: cAMP & PKA

EXAMPLE: Order each of the steps in the activation of PKA (by numbering them 1-4 ; first step is already numbered for you) starting after adenylate cyclase activation.

a) Cytosolic cAMP concentration increases. ___1___
b) The free catalytic subunits phosphorylate proteins on their Ser of Thr residues. _____
c) The regulatory subunits of PKA dissociate from the catalytic subunits. _____
d) Two cAMP molecules bind to each PKA regulatory subunit. _____

PRACTICE: Which of the following statements about protein kinase A (PKA) is false?

a) PKA binds a total of four molecules of cAMP, two molecules on each of the regulatory (R) subunits.
b) PKA binds a total of four molecules of cAMP, one on each of the four subunits.
c) Once active, the catalytic (C) subunits dissociate and phosphorylate target proteins.
d) When inactive, PKA is a tetramer of two regulatory (R) and two catalytic (C) subunits.

Inactivation of cAMP & PKA

●Recall: _____ activity in the G protein α-subunit _____/*terminates* GPCR signaling pathways.

●_____ other events also allow termination of GPCR signaling:

1 cAMP's signaling affect is "*turned* _____" by ↓ [cAMP] using the enzyme *cAMP phosphodiesterase*.

□ *cAMP* _____: converts cAMP → _____ (AMP does *not* activate PKA).

2 PKA's activity is *reversed* by *Ser/Thr* _____ (which remove phosphate groups from substrates).

CONCEPT: cAMP & PKA

PRACTICE: Which of the following is/are associated with cAMP binding to cAMP-dependent protein kinase A (PKA)?

a) III and IV.
b) II, III, and IV.
c) I, II, III, and IV.
d) I and II.
e) I, II, and III.

I. cAMP binds to the regulatory subunits.

II. Tetrameric regulatory subunits and catalytic subunits dissociate.

III. Catalytic subunits phosphorylate proteins with specific Ser and Thr residues.

IV. cAMP is membrane bound via a phosphoinositol covalent attachment.

PRACTICE: What is the function of a phosphatase?

a) A phosphatase removes phosphorylated amino acids from proteins.
b) A phosphatase removes the phosphate group from phosphorylated amino acid residues in a protein.
c) A phosphatase phosphorylates serine, threonine, and tyrosine residues in a protein.
d) A phosphatase degrades secondary messengers in the cell.

PRACTICE: What could be the result of a mutation in the R subunits of cAMP-dependent protein kinase A (PKA) that inhibits formation of the R_2C_2 protein complex?

a) PKA will always remain in the inactive state.
b) cAMP would drastically decrease PKA activity.
c) PKA will always remain in the active state.
d) No effective change occurs.

PRACTICE: The image below is a schematic representation of PKA activation from epinephrine binding. Based on the provided numbers in the diagram, how many subunits of catalytically active PKA will there be?

a) 1,000 molecules.
b) 200 molecules.
c) 100 molecules.
d) 50 molecules.
e) 400 molecules.

CONCEPT: INHIBITORY ADENYLATE CYCLASE GPCR SIGNALING

- Recall: some G proteins _____ the *effector enzyme*.
  - □ *Same* pathway as before, but α subunit of G-protein (G_i) ultimately _____ activity of the effector enzyme.
- *Integration* of stimulatory & inhibitory GPCR pathways can _____ the activity of the *effector enzyme*.

EXAMPLE: Adenylate Cyclase Activity Regulated by Inhibitory GPCR Pathway.

PRACTICE: The addition of the nonhydrolyzable GTP-analog, GTPγS (shown below), is a common cell culture technique. If only affecting the inhibitory pathway, what effect would GTPγS have on cellular cAMP levels?

a) cAMP levels increase because adenylate cyclase can no longer hydrolyze GTP.
b) cAMP levels decrease because G_s remains in its inactive state and can no longer bind GDP.
c) cAMP levels decrease because G_i remains in its active state since it can no longer hydrolyze GTP.
d) cAMP levels increase because G_i remains in its inactive state since it can no longer hydrolyze GTP.

PRACTICE: If a chemical is an inhibitor of the enzyme adenylyl cyclase, which of the following steps in the GPCR signaling pathway would be directly blocked?

a) Activation of gene transcription.
b) Exchange of GTP for GDP.
c) Ligand bound receptor activation.
d) Synthesis of the secondary messenger cAMP.

CONCEPT: INHIBITORY ADENYLATE CYCLASE GPCR SIGNALING

GPCR Desensitization

- When a signal is _____ present (repeated exposure), cells need the ability to *desensitize*.
- _____: *dampening* of the cellular response EVEN WHEN the primary messenger/ligand *persists*.
  - □ **β-A**drenergic GPC**R K**inase (_____): _____ C-terminal Serines on *ligand-bound* GPCR.
  - □ **β-Arr**estin (_____): protein that binds phosphorylated GPCR, _____ its interaction with G protein.
  - □ β-Aarr can initiate temporary _____ of GPCR in vesicles (making GPCR inaccessible).

1) Epinepherine _____ β-Adrenergic GPCR.

2) β.A.R.K. _____ β-Adrenergic GPCR.

3) β-Arrestin _____ G-protein association with GPCR & triggers _____.

EXAMPLE: A cell line expressing the β-Adrenergic GPCR is incubated in epinephrine for 5 minutes. The cells are then lysed and the GPCR is purified. To determine if β-arrestin is bound to the GPCR, the purified GPCR solution is examined by Western Blot using an antibody against β-arrestin (Lanes #1 & 2). The experiment is repeated, but this time prior to and during epinephrine addition, the cells are incubated in an inhibitor that blocks β-Adrenergic GPCR phosphorylation by the GPCR kinase βARK (Lanes #3 & 4). Results are shown below. What conclusion can be made from the results/data?

a) In order for β-arrestin to bind, the receptor must be exposed/bound to its ligand.
b) In order for β-arrestin to bind, the receptor cannot be exposed/bound to its ligand.
c) In order for β-arrestin to bind, βARK must be bound to the receptor first.
d) In order for β-arrestin to bind, the inhibitor must first bind to the receptor.

CONCEPT: INHIBITORY ADENYLATE CYCLASE GPCR SIGNALING

PRACTICE: How does GPCR termination differ from GPCR desensitization?

a) GPCR termination promotes GPCR signaling while GPCR desensitization overexpresses the GPCR.
b) GPCR desensitization involves degradation of the GPCR while GPCR termination promotes GTPase activity of G_α.
c) GPCR termination blocks the GTPase activity of G_α while GPCR desensitization promotes ligand binding.
d) GPCR termination resets the signaling pathway in the absence of ligand, whereas GPCR desensitization dampens the cell response even in the presence of ligand.

PRACTICE: Which of the following statements about beta-arrestin and GPCRs is TRUE?

a) Beta-arrestin phosphorylates activated GPCRs.
b) Beta-arrestin can cause desensitization of a signal by inducing GPCR exocytosis.
c) Beta-arrestin is pre-bound to inactive GPCRs (no ligand bound).
d) Beta-arrestin binds to phosphorylated residues on the carboxy-terminus region of GPCRs.
e) Beta-arrestin binds to phosphorylated residues on the amino-terminus region of GPCRs.

PRACTICE: Which of the following are involved in the desensitization of the β-adrenergic receptor?

a) β-adrenergic receptor kinase.
b) β-arrestin.
c) Protein Kinase A.
d) βARK.
e) GAPs.
f) A and B only.
g) A, B and D only.
h) A, B, and E only.

CONCEPT: DRUGS & TOXINS AFFECTING GPCR SIGNALING

- _____ bacterial toxins target G-proteins & indirectly *increase* activity of the effector enzyme (*adenylate cyclase*).
  1) _____ *Toxin*: inhibits GTPase activity of G_s (*stimulating* G protein α subunit), causing *cholera*.
     - □ Permanently _____ G protein α subunit & thus *overstimulates* adenylate cyclase activity.
  2) _____ *Toxin*: inhibits GDP-GTP exchange in G_i (*inhibiting* G protein α subunit), causing *whooping cough*.
     - □ Permanently _____ G_i protein α subunit (preventing adenylate cyclase *inhibition*).

Pedal to the Metal **Broken Brakes**

EXAMPLE: Cholera toxin blocks GTP hydrolysis of the stimulating G_s protein α subunit, whereas pertussis toxin prevents the interaction of the inhibiting G_i protein α subunit with adenylate cyclase. What is the effect of these toxins on the intracellular concentration of cAMP?

a) Cholera toxin increases cAMP concentration, while pertussis toxin decreases cAMP concentration.
b) Cholera toxin decreases cAMP concentration, while pertussis toxin decreases cAMP concentration.
c) They both would lead to an increase in cAMP concentration.
d) They both would lead to a decrease in cAMP concentration.

PRACTICE: Cholera toxin increases the cellular cAMP levels by:

a) Binding to and activating GPCRs.
b) Altering the activity of stimulatory G_s proteins.
c) Inhibiting phosphodiesterase activity.
d) Binding to and inhibiting adenylyl cyclase.
e) Altering the activity of inhibitory G_i proteins.

CONCEPT: DRUGS & TOXINS AFFECTING GPCR SIGNALING

PRACTICE: Pertussis toxin is produced by *Bordetella pertussis,* the bacterium that causes whooping cough. Pertussis toxin catalyzes the addition of ADP-ribose to G_i which 'locks' it in the GDP-bound state. If the uninhibited, toxin free GPCR pathway normally results in decreased glycogen synthesis, then what would be the effect of pertussis toxin?

a) It would decrease contraction.
b) It would decrease glucose production.
c) It would further decrease glycogen production.
d) It would increase the rate of endocytosis.

Agonists vs. Antagonists

●Many clinical drugs are developed to act either as _____ or _____-agonists to various receptors.

□ *Agonist*: structural analogs that bind receptors & _____ the effect of the original/natural ligand.

□ *Antagonist*: analogs that bind receptor without triggering normal effect & thus _____ effects of agonists/ligands.

□ Antagonists function similarly to _____ enzyme inhibitors (*blocking* the "active site").

EXAMPLE: Caffeine acts as an Antagonist to the Adenosine Receptor.

PRACTICE: Caffeine is a molecule that binds to _____ receptors, inhibiting their response.

a) Tyrosine.
b) Adenosine.
c) Adenine.
d) Cytosine.
e) Asparagine.
f) Dopamine.

PRACTICE: Caffeine is a(n) _____ of adenosine that blocks its receptors from activation.

a) Inhibitor.
b) Antagonist.
c) Agonist.
d) Activator.

CONCEPT: RECAP OF ADENYLATE CYCLASE GPCR SIGNALING

EXAMPLE: Which of the following statements about the β-adrenergic receptor signaling system is correct?

a) Epinephrine binding the β-adrenergic receptor activates $G_{s\alpha}$ by replacement of GDP for GTP. Activated $G_{s\alpha}$ activates phospholipase C resulting in the release of inositol triphosphate (IP3). Ca^{2+} is released from the endoplasmic reticulum (ER) and along with diacylglycerol activates protein kinase C.

b) Epinephrine binding the β-adrenergic receptor activates $G_{s\alpha}$ by replacement of GDP for GTP. Activated $G_{s\alpha}$ activates adenylate cyclase resulting in the synthesis of cAMP. cAMP activates protein kinase A.

c) Insulin binding the β-adrenergic receptor activates $G_{s\alpha}$ by replacement of GDP for GTP. Activated $G_{s\alpha}$ activates adenylate cyclase resulting in the synthesis of cAMP. cAMP activates protein kinase A.

d) Insulin binding the β-adrenergic receptor activates $G_{s\alpha}$ by replacement of GDP for GTP. Activated $G_{s\alpha}$ activates phospholipase C resulting in the release of inositol triphosphate (IP3). Ca^{2+} is released from the endoplasmic reticulum (ER) and along with diacylglycerol activates protein kinase C.

PRACTICE: Which of the following does not bind to G-proteins?

a) GTP. b) GDP. c) GPCRs. d) cGMP.

CONCEPT: PHOSPHOINOSITIDE GPCR SIGNALING

●***P**hosphoinositide* (or *Inositol-Phospholipid*) ***S**ignaling **P**athway* (_____): another classic example of a *GPCR signaling*.

- □ Mediates a _____ of hormonal effects (ex. epinephrine, angiotensin, vasopressin).
- □ Many functions including blood platelet & injured-cell signaling to stimulate growth & injury-repair process.

●*Phosphoinositide signaling* requires these _____ components:

3 Phosphoinositide Secondary Messengers

●PSP generally involves production of _____ *secondary messengers*:

1) **I**nositol-1,4,5-*tri***p**hosphate (IP__). 2) 1-2-**dia**cyl**gl**ycerol (_____). 3) *Calcium* Ions (Ca^{2+}).

EXAMPLE: Phosphoinositide Secondary Messengers:

PRACTICE: Which of the following are secondary messengers produced in the PSP pathway?

a) cGMP & K^+. b) IP_2 & IP_3. c) IP_3 & DAG. d) DAG & cAMP.

CONCEPT: PHOSPHOINOSITIDE GPCR SIGNALING

●Many variations of **P**hosphoinositide **S**ignaling **P**athways (*PSP*), but can be condensed into _____ general steps:

α-Adrenergic Pathway

Same as Before

1. Hormone signaling ligand (*epinephrine*) binds GPCR (*α-adrenergic receptor*) causing a _____ *shift*.
2. GPCR conformational shift _____ G protein (G_q) by promoting α subunit to *release* GDP & bind GTP.
3. With bound GTP, ___ subunit of G_q _____ from β-γ subunits & *diffuses* to nearby *effector enzyme*.

PSP Pathway

New

4. The activated/GTP-bound α-subunit binds & *activates* the effector enzyme (***p**hospho**l**ipase **C***, _____).
5. Activated *PLC* hydrolyzes its substrate, ***p**hosphat**i**dylinositol-4,5-bis**p**hosphate* (_____).
   - □ Active *PLC* generates two _____ *messengers* (_____ & _____).
6. *IP_3* diffuses in cytoplasm to *endoplasmic reticulum* to bind/open _____ *transport channels*, ___ *cytosolic* [Ca^{2+}].
   - □ Ca^{2+} $\xrightarrow{\text{Activates}}$ *Calmodulin* (_____) $\xrightarrow{\text{Activates}}$ *cytosolic protein kinases* ⟶ *Cell Response*.
7. *DAG* diffuses in membrane &, along with released Ca^{2+}, *activates* a membrane-bound ***p**rotein **k**inase **C*** (_____).

Same as Before

8. G protein α subunit slowly _____ itself (& *PLC*) by *hydrolyzing* its GTP → GDP.
   - □ α subunit assembles its original, *inactive* form & ligand *dissociates* from GPCR to _____ pathway.

CONCEPT: PHOSPHOINOSITIDE GPCR SIGNALING

How to Remember Phosphoinositide GPCR Signaling?

Story: Cell wants to *save the day* (***Cell Response***) from *"**Pip** the Troll"* and his ***2*** weapons (***PIP₂***).

1. ***L***ightining strike provides a warning of *danger* (***L***igand binding).
2. ***Alpha**-male* (G protein *α*-subunit) *exchanges* **empty siren battery** with **full siren battery** (GTP exchange).
3. *Alpha*-male leaves to alarm the ***P***o***L***i***C***e (***P***hospho***L***ipase ***C***) (dissociation of G protein α-subunit).
4. *Alpha*-male *tells* ***P***o***L***i***C***e to target ***PIP***'s ***2*** weapons (***PIP₂***) (***P***hospho***L***ipase ***C*** *activation*).
5. ***P***o***L***i***C***e *shoots* ***PIP***'s ***2*** weapons, separating them into ***DAG***ger & ***3 I***ce ***P***icks (***DAG*** & ***IP₃*** production).
6. ***3 I***ce ***P***icks drop to *release* ***Ca***ptain ***M***erica to help *save the day* (***Ca²⁺*** *release*, ***CaM*** *activation* & Cell Response).
7. ***DAG***ger flips toward a ***P***ac***K***ed ***C***rowd (***PKC***), who catch it & help *save the day* (***PKC*** *activation* & *Cell Response*).

CONCEPT: PHOSPHOINOSITIDE GPCR SIGNALING

EXAMPLE: Place the following steps of the PSP signaling pathway in order from 1-7:

a) Conformational shift of the G-protein and G_α exchanges GDP for GTP _____.

b) Activation of Phospholipase C _____.

c) DAG and Ca^{2+} ions activate Protein Kinase C _____.

d) Ligand binds to the GPCR _____.

e) G_α dissociates from $G_{\beta\gamma}$ _____.

f) IP_3 diffuses to the ER membrane to release Ca^{2+} into the cytoplasm _____.

g) Phospholipase C hydrolyzes PIP_2 into IP_3 and DAG _____.

PRACTICE: When epinephrine binds to a(n) _____ receptor, _____ is activated, causing an increase in the cellular concentration of _____.

a) Beta ; $G_{\alpha i}$; Inositol triphosphate.

b) Alpha ; $G_{\alpha q}$; Inositol triphosphate.

c) Beta ; $G_{\alpha i}$; Diacylglycerol.

d) Alpha ; $G_{\alpha s}$; cAMP.

e) Beta ; $G_{\alpha q}$; Diacylglycerol & Inositol triphosphate.

PRACTICE: Which of the following secondary messengers, when released by phospholipase C will directly activate PKC?

a) Inositol triphosphate.

b) Phosphatidylinositol bisphosphate.

c) Diacylglycerol.

d) cGMP.

PRACTICE: _____, when formed by phospholipase C, triggers the release of calcium from the endoplasmic reticulum?

a) Inositol triphosphate.

b) Phosphatidylinositol bisphosphate.

c) Diacylglycerol.

d) cGMP.

CONCEPT: PHOSPHOINOSITIDE GPCR SIGNALING

PRACTICE: In cells, epinephrine binding to the α-adrenergic receptor activates $G_{\alpha q}$. All of the following happen EXCEPT:

a) Protein Kinase C activity will be decreased.
b) Inositol triphosphate will cause the release of Ca^{2+} from the endoplasmic reticulum.
c) Increased binding of Ca^{2+} to the calmodulin will occur.
d) Increased Ca^{2+}-Calmodulin complex will bind to and activate calmodulin-binding proteins.
e) Protein Kinase C will be activated by increased levels of diacylglycerol.

PRACTICE: All G proteins are activated by GDP/GTP exchange (binding to GTP), but lose their activity when they hydrolyze GTP to GDP. However, different G proteins can have different roles. Which of the following is INCORRECT?

a) When active, $G_{\alpha s}$ activates adenylate cyclase.
b) When active, $G_{\alpha s}$ results in increased production of cAMP.
c) When active, $G_{\alpha i}$ results in the inhibition of adenylate cyclase.
d) When active, $G_{\alpha q}$ results in the activation of phospholipase C.
e) When active, $G_{\alpha s}$ results in increased production of inositol triphosphate.

PRACTICE: The reaction catalyzed by Phospholipase C can use all of the following EXCEPT:

a) Phosphatidylinositol bisphosphate as a substrate.
b) Diacylglycerol as a product.
c) Diacylglycerol phosphate as a product.
d) Inositol triphosphate as a product.
e) Water as a substrate.

CONCEPT: PSP SECONADRY MESSENGERS & PKC

IP_3 + DAG

●Recall: Upon activation, *PLC* catalyzes hydrolysis of _____.

□ PLC hydrolysis of PIP_2 yields _____ & _____.

□ *IP_3* diffuses through cytosol to ER surface where it binds _____ channels, triggering an ↑ in cytoplasmic [*Ca^{2+}*].

□ *DAG* remains associated with membrane &, along with released *Ca^{2+}*, *activates* _____.

Ca^{2+} & Calmodulin

●Calcium (_____) is a common intracellular signal that can trigger many biochemical processes:

□ Ca^{2+} triggered processes include *muscle* _____ & *vesicle exocytosis* in neurons.

□ HOWEVER, in order to affect its targets, Ca^{2+} generally forms a complex with _____.

●***Calm**odulin* (_____): a *Ca^{2+}-modulated* cytosolic protein that activates a variety of targets (ex. kinases).

□ CaM is activated when all _____ Ca^{2+}-binding sites are occupied.

□ Active *CaM-Ca^{2+}-complex* binds to other target proteins to _____ them.

CONCEPT: PSP SECONADRY MESSENGERS & PKC

Protein Kinase C (PKC)

●***P**rotein **K**inase **C*** (_____): *phosphorylates* Ser/Thr residues on target proteins to alter their activity (similar to PKA).

□ PKC targets include enzymes, cytoskeletal proteins, & nuclear proteins regulating gene expression.

PRACTICE: All of the following are secondary messengers in cell signaling EXCEPT:

a) IP_3.
b) DAG.
c) Protein Kinase A.
d) Calcium Ions.
e) cAMP.

PRACTICE: In the phospholipase C signal transduction pathway, which two second messengers are produced?

a) Phospholipase C and protein kinase A.
b) PIP_2 and IP_3.
c) Phospholipase C and G.
d) IP_3 and DAG.
e) cAMP and ATP.

PRACTICE: Which of the following enzymes does diacylglycerol recruit to the membrane and activate when bound?

a) Phospholipase C.
b) Protein Kinase C.
c) Adenylate Cyclase.
d) Protein Kinase A.
e) Phospholipase A.
f) Calmodulin.

PRACTICE: Which of the following statements about calmodulin is TRUE?

a) Calmodulin causes the release of Ca^{2+} ions from the endoplasmic reticulum.
b) When associated with Ca^{2+}, calmodulin is deactivated and unable to affect the activity of its targets.
c) In the presence of Ca^{2+}, calmodulin binds to and activates CaM-dependent protein kinases.
d) Calmodulin binds to and activates Protein Kinase C (PKC) when active.

CONCEPT: PSP SECONADRY MESSENGERS & PKC

Inactivation/Termination of Phosphoinositide GPCR Signaling

●Recall: GTPase activity in the G protein α-subunit *inactivates/terminates* GPCR signaling pathways.

●HOWEVER, _____ other events also help *inactivate/terminate* the *Phosphoinositde GPCR signaling pathway*:

1. *IP_3*'s signaling affect is "*turned* _____" by ↓ [*IP_3*] using the enzyme *inositol polyphosphate 5-phosphatase*.
   - □ *Inositol polyphosphate 5-*_____: converts *IP_3* → _____ (*IP_2* does not release Ca^{2+}).
2. PKC's activity is *reversed* by *Ser/Thr* _____ (which remove phosphate groups from substrates).
3. Cytoplasmic [Ca^{2+}] *decreased* by the ***S**arcoplasmic/**E**ndoplasmic **R**eticulum **C**a^{2+} **A**TPase* (_____) *pump*.

PRACTICE: Protein Kinase C (PKC) is activated when bound by:

a) Ca^{2+} which leads to cytosolic protein phosphorylation.

b) Diacylglycerol which leads to cytosolic protein phosphorylation.

c) Ca^{2+} and Diacylglycerol which leads to extracellular protein phosphorylation.

d) Ca^{2+} and Diacylglycerol which leads to cytosolic protein phosphorylation.

e) Diacylglycerol which leads to extracellular protein phosphorylation.

PRACTICE: What is the effector enzyme in the phosphoinositide signal transduction system?

a) Diacylglycerol. b) Adenylate cyclase. c) Phospholipase C. d) Protein Kinase C.

CONCEPT: PSP SECONADRY MESSENGERS & PKC

PRACTICE: All of the following are functions served by phosphatidylinositol bisphosphate EXCEPT:

a) Phosphatidyl inositol bisphosphate can be hydrolyzed to produce diacylglycerol, a second messenger.
b) Phosphatidyl inositol bisphosphate can be hydrolyzed to produce inositol trisphosphate, a second messenger.
c) Phosphatidyl inositol bisphosphate can serve as the substrate to phospholipase C.
d) Phosphatidyl inositol bisphosphate will serve as a docking site for proteins containing a SH2 domain.

PRACTICE: Diacylglycerol is a(n) _____ that promotes _____.

a) Insulin agonist ; tyrosine phosphorylation.
b) Phosphodiesterase inhibitor ; cAMP accumulation.
c) Secondary messenger ; protein kinase C activation.
d) Steroid-like hormone ; protein dimerization.
e) Secondary messenger ; phospholipase C activation.
f) Lipid hormone ; receptor dimerization.

CONCEPT: RECAP OF PHOSPHOINOSITIDE GPCR SIGNALING

PRACTICE: Appropriately match each of the following terms with their description on the right.

a) Primary messenger _____.
b) Secondary messenger _____.
c) G-protein coupled receptor _____.
d) Protein Kinase A _____.
e) $G_{\alpha s}$ _____.
f) $G_{\alpha q}$ _____.
g) GTPase activity _____.
h) Phospholipase C _____.
i) Inositol triphosphate _____.
j) Protein Kinase C _____.

1. Generates two secondary messengers.
2. Stimulated by cAMP.
3. Activates phospholipase C.
4. Message received by the cell.
5. Composed of 7 transmembrane alpha-helices.
6. Results in the re-association of G_{α} and $G_{\beta\gamma}$.
7. Intracellular chemical message that relays a signal from ligand receptor.
8. Activates adenylate cyclase.
9. Activated by diacylglycerol and Ca^{2+}.
10. Activates a Ca^{2+} channel.

CONCEPT: RECEPTOR TYROSINE KINASES

●***R**eceptor **T**yrosine **K**inase* (_____): a _____ with an intracellular *Tyrosine* (*Tyr*) _____ domain.

□ *Tyrosine Kinases*: enzymes that _____ *Tyr* residues on specific target proteins.

3 Domains of RTK Monomers

Dimerization & Autophosphorylation of RTKs

●Several RTK classes show they can & usually exist as *monomers*, but always form _____ when *activated*.

1. *Ligand Binding*: _____ molecule binds to RTK.
2. *Dimerization*: a *conformational change* causes _____ RTKs to _____ (if not already dimerized).
   - □ *Ligand-binding* &/or *RTK dimerization* _____ *activates* both *Tyr kinase* domains.
3. *Auto-phosphorylation*: *partial activation* allows Tyr kinase domains to _____*-phosphorylate* each other.
   - □ Leads to _____ *activation* of Tyr kinase domains.
4. RTK Phosphorylates Targets: autophosphorylated RTK can phosphorylate other _____ proteins.
   - □ *Cascade* of phosphorylation results in *changes to metabolism & gene expression* → Cell _____.

EXAMPLE: RTK Dimerization & Autophosphorylation.

CONCEPT: RECEPTOR TYROSINE KINASES

PRACTICE: Which of the following statements is NOT true of receptor tyrosine kinases?

a) Monomeric receptors are often induced to dimerize upon ligand binding.

b) The activated receptors attract and activate G proteins to continue the GPCR signaling pathway.

c) The cytoplasmic side of RTKs contains a kinase enzyme domain that is partially activated upon ligand binding.

d) Activated RTKs autophosphorylate themselves and can attract proteins with SH2 domains.

SH2 Domains Bind Phosphorylated Tyr

●Proteins with a ***S**rc **H**omology **2*** (_____) domain directly _____ *phosphorylated* _____ residues.

□ NOTE: SH2 domains do _____ bind phosphorylated Ser/Thr residues (only phosphorylated Tyr).

□ SH2 can either bind directly to (1) *autophosphorylated RTK* or (2) *phosphorylated target proteins*.

●Proteins with SH2 domains can have a wide _____ of functions, allowing RTKs to elicit a *variety* of cell responses.

SH2 binds RTK _____

SH2 binds Target Protein—Ⓟ

PRACTICE: Proteins that contain a src homology 2 domain (SH2 domain):

a) Have a phosphoserine residue that causes them to bind to other proteins.

b) Have a phosphotyrosine residue that causes them to bind to other proteins.

c) Bind to any proteins that have a phosphothreonine group.

d) Bind to proteins that have a phosphotyrosine residue and a specific conformation.

e) Binds to proteins that have 2 sulfhydryl groups.

CONCEPT: INSULIN

●_____: a 51-amino-acid peptide hormone involved in classic RTK pathways.

□ Signals a "*well-*_____" or "*fuel* _____" state.

□ Initially secreted as a _____ (*proinsulin*) by *pancreatic β-cells* in response to a *meal*.

PRACTICE: Proinsulin:

a) Is synthesized in three parts and then assembled for storage.

b) Is more active than insulin.

c) Is synthesized as a single chain, folded and then cleaved into insulin.

d) Stimulates insulin secretion.

Insulin's 3 Primary Biological Effects

●After eating a meal, insulin can signal many effects, but generates _____ primary biological effects:

1. _____ *blood-glucose* concentration.
2. _____ cell growth by *regulating* gene expression.
3. *Increases* the synthesis of specific _____.

PRACTICE: Which of the following is NOT a biological effect of insulin after eating a large meal?

a) Increases the concentration of blood glucose.

b) Regulates gene expression to activate cell growth.

c) Stimulates the production of certain lipid molecules.

d) Decreases the concentration of blood glucose.

CONCEPT: INSULIN

1) Insulin Decreases Blood [Glucose]

●After a high-glucose meal, insulin's primary job is to help _____ [glucose] in blood in the following ways:

1. Insulin ____ *glucose transporter* (_____) expression, which imports blood glucose into cells.
2. Insulin indirectly affects cytosolic enzyme activity (via signaling) to convert *free glucose* to _____.
   - □ This ↓ free [glucose] inside cell facilitates diffusion of blood glucose into cells (_____ [glucose] in blood).

PRACTICE: What is the primary function of the peptide insulin?

a) To stimulate transport of glucose out of the cell and into the bloodstream.
b) To stimulate transport of proinsulin out of the pancreas and into the bloodstream.
c) To stimulate transport of glucose out of the bloodstream and into the cell.
d) The transport of epinephrine from the bloodstream to the cell receptor.

2) Insulin Stimulates Cell Growth

●When [insulin] is high, insulin can also act as a _____ *factor*.

- □ *Growth factor*: a biological substance that _____ cellular growth, healing, &/or differentiation.
- □ Insulin as a growth hormone uses a _____ signaling pathway than when it lowers blood [glucose].

EXAMPLE: Insulin Acts as a Growth Factor to Stimulate Cell Growth.

CONCEPT: INSULIN

PRACTICE: The hormone insulin can function as a(n) _____.

a) Secondary messenger.
b) Adapter protein.
c) Effector enzyme.
d) Growth factor.

3) Insulin Increases Lipid Synthesis

●Insulin has a large effect on _____ synthesis, which we'll discuss more in a later chapter.

PRACTICE: What is the effect of insulin on lipid fatty acid synthesis?

a) It activates regulatory proteins that inhibit synthesis of fatty acids.
b) It increases the synthesis of fatty acids resulting in higher amounts of fat storage.
c) In causes fatty acids degradation by liposomes.
d) It decreases fat storage by converting fatty acids to energy.
e) None of the above.

PRACTICE: Which of the following is NOT an effect of insulin?

a) It increases the rate of fatty-acid synthesis and storage.
b) It decreases the concentration of blood glucose.
c) It stimulates cell growth and division.
d) In stimulates the release of proinsulin from the pancreas.

CONCEPT: INSULIN RECEPTOR

●Insulin signaling beings with _____ steps:

1 *Ligand Binding*: Insulin does _____ enter cells, but instead binds an *insulin* _____.

- □ **Ins**ulin **R**eceptor (_____): a specific type of RTK activated by *insulin* binding.
  - □ INSR has _____ protein subunits (2 α & 2 β) linked via _____ *bonds*.
  - □ Unusual because even in the *unliganded* state, INSR already exists as two αβ _____.

2 *INSR Auto-phosphorylation*: tyrosine kinase domains in the INSR _____ subunits cross-phosphorylate each other.

EXAMPLE: Insulin Receptor.

EXAMPLE: For the insulin receptor to transduce the signal inside the cell:

a) The G-alpha subunit needs to bind GTP to activate adenylate cyclase.
b) The G-alpha subunit needs to bind GTP to activate phosphodiesterase.
c) It needs to activate its tyrosine kinase domains via autophosphorylation, after binding the insulin ligand.
d) It needs to recruit a tyrosine kinase to auto-phosphorylate the cytoplasmic domain after dimerization.
e) It needs to recruit a tyrosine phosphatase to phosphorylate the cytoplasmic domain after dimerization.

PRACTICE: The insulin receptor is an example of a _____:

a) G-protein coupled receptor.
b) Receptor tyrosine kinase.
c) Receptor tyrosine phosphatase.
d) Membrane channel protein.
e) Receptor threonine kinase.

CONCEPT: INSULIN RECEPTOR

INSR Substrates (IRS)

●*Insulin* ***R****eceptor* ***S****ubstrates* (_____): peptides that are the main targets of fully active autophosphorylated INSR.

□ *IRS-1*: a *secondary messenger* that transmits the insulin receptor signal as an _____ *protein*.

□ *Adapter proteins*: _____ enzymatic activity & serve mainly as a _____ to bring other proteins together.

●Depending on the cell type & conditions, IRS-1 can induce _____ other signaling pathways.

□ Insulin's *RTK signaling pathway* results in *IRS-1 activation*, but then _____ at this point.

PRACTICE: insulin binds to a receptor that:

a) I, III and V.
b) II and IV.
c) I, II and V.
d) II, IV and V.
e) IV and V.

I. Is coupled to a G protein.

II. Possesses tyrosine kinase activity.

III. Possesses serine/threonine phosphatase activity.

IV. Interacts with the adapter protein IRS-1.

PRACTICE: Which of the following is TRUE concerning the interaction between the insulin receptor and IRS-1:

a) The IRS protein phosphorylates the insulin receptor.
b) The β-subunits of the receptor bind to insulin, where each subunit has a separate insulin binding site.
c) Each β-subunit acts as a serine kinase and auto-phosphorylates the other subunit.
d) IRS is phosphorylated by the tyrosine kinase domains in the β-subunits of the insulin receptor.
e) IRS contains phosphorylated threonine residues that directly bind to insulin.

CONCEPT: INSULIN SIGNALING ON GLUCOSE METABOLISM

●Recall: Insulin affects *glucose metabolism* by: 1) ↑ _____ *expression* & 2) ↑ _____ *synthesis*.

□ Recall: both help to _____ [glucose] in the blood.

□ Insulin signals ↓ blood [glucose] via the following pathway: PI3K → PIP_3 → PDK1 → PKB.

Insulin Signaling Decreases Blood [Glucose]

●_____ steps in insulin's RTK signaling cascade to decrease blood glucose levels:

1. After a high-glucose meal, *insulin* is released & binds its receptor, which *phosphorylates* & *activates* _____.
2. Active IRS-1 binds SH2 domain of ***P**hospho**i**nositide **3** **K**inase* (_____) to *activate* it.
3. ***P**hosphatidyl**i**nositol 4,5-bis**p**hosphate* (PIP___) $\xrightarrow{PI3K}$ ***P**hosphatidyl**i**nositol 3,4,5-tris**p**hosphate* (PIP___).
4. PIP_3 *binds* **P**rotein **K**inase **B** (_____ or Akt).
5. **P**IP_3-**D**ependent **K**inase **1** (_____) *phosphorylates* & *activates* PKB.
6. Active PKB phosphorylates targets controlling _____ *expression* & _____ *synthesis*.
   - □ Both events help _____ blood [glucose].

EXAMPLE: If you delete the PKB gene from insulin-responsive cells, what would you likely observe in insulin's presence?

a) PI3K is not activated.

b) PDK1 is not activated.

c) GLUT4 is not expressed & is retained in intracellular vesicles.

d) Phosphatidylinositol is cleaved by phospholipase C.

CONCEPT: INSULIN SIGNALING ON GLUCOSE METABOLISM

Insulin Signaling Activates Glycogen Synthesis

- ***G**lycogen **S**ynthase* (GS): enzyme that synthesizes _____ (converting glucose into glycogen).
  - □ Usually ***G**lycogen **S**ynthase **K**inase **3*** (_____) *phosphorylates* & _____ GS.
  - □ HOWEVER, active PKB *phosphorylates* & _____ GSK3 (which allows *activation* of GS).

EXAMPLE: Insulin Activates Glycogen Synthesis.

7 PKB phosphorylates & inhibits _____.

8 Inactive GSK3 can no longer inhibit GS.

9 Active GS synthesizes _____.

PRACTICE: Place the following insulin signaling transduction events in order of occurrence (event 9 is provided).

a) Full activation of PKB/Akt _____.
b) Activated PI3K converts PIP_2 to PIP_3 _____.
c) GLUT4 expressed in the cell membrane ___9___.
d) Ligand/insulin binding to the insulin receptor _____.
e) GSK3 is phosphorylated & inactivated _____.
f) Activated PDK1 phosphorylates & activates of PKB/Akt _____.
g) IRS-1 is phosphorylated & activated by the insulin receptor _____.
h) Autophosphorylation of the insulin receptor _____.
i) PI3K is activated upon its SH2 domain binding phosphorylated IRS-1 _____.
j) Glycogen synthase converts glucose to glycogen _____.
k) PIP_3 molecules laterally diffuse to bind PKB/Akt & PDK1 _____.

CONCEPT: INSULIN SIGNALING ON GLUCOSE METABOLISM

How to Remember Insulin RTK Signaling on Glucose Metabolism?

1. **IRS** gives a tax refund (**IRS-1**).
2. Cell buys a ***PI***e & puts a fake $***3K*** label on it (***PI3K***) to prank PIP (*activation* of ***PI3K***).
3. ***PIP*** first offers $***2***k for a *bite*, but then agrees to pay $***3***k (***PIP2*** *conversion* to ***PIP3***).
4. Bank tellers are shocked when they hear PIP ***P***aid thousands ($***K***) for a ***B***ite (***PKB***).
5. ***P***IP ***D***eposits ***1K*** (***PDK1***) at the bank but bank tellers (***PKB***) handle the transaction (***PDK1*** *activates* ***PKB***).
6. Bank tellers *increase* PIP's savin***GS*** (***G***lycogen ***S***ynthesis) & *express* order ***GLUT***en ***F***ree (***GLUT4***) donut.

CONCEPT: INSULIN SIGNALING ON GLUCOSE METABOLISM

EXAMPLE: Place the following insulin signal transduction events in order of occurrence (1-8).

a) Activation of PI3K _____.
b) Activation of the insulin receptor _____.
c) Activation of PDK1 _____.
d) GLUT4 transporter expressed in the membrane _____.
e) Conversion of PIP_2 to PIP_3 _____.
f) Binding of insulin to the receptor _____.
g) Full activation of PKB _____.
h) Phosphorylation of IRS-1 _____.

PRACTICE: All of the following are true of the reaction catalyzed by PI3K EXCEPT:

a) Phosphatidylinositol bisphosphate is a substrate of PI3K.
b) Inositol triphosphate is a substrate of PI3K.
c) ATP is a substrate of PI3K.
d) Phosphatidylinositol triphosphate is a product of PI3K.
e) ADP is a product of PI3K.

PRACTICE: Which of the following results would you predict to occur if a mutation changes a Tyr residue of the Insulin Receptor to Ala?

a) Inability to take up glucose from the bloodstream.
b) Constant uptake of glucose from the bloodstream.
c) Upregulated glycogen synthesis and GLUT4 transport to the membrane.
d) B and C.

PRACTICE: IRS-1 is an essential adaptor protein in the insulin signaling pathway. If IRS-1 was under expressed in muscle cells, what effect would you expect to see on glycogen synthesis?

a) Protein kinase B would remain inactive, resulting in increased glycogen synthesis.
b) Protein kinase B would be overstimulated, resulting in increased glycogen synthesis.
c) Protein kinase B would remain inactive, resulting in decreased glycogen synthesis.
d) Protein kinase B would be overstimulated, resulting in decreased glycogen synthesis.

CONCEPT: INSULIN SIGNALING AS A GROWTH FACTOR

●Recall: In addition to regulating glucose metabolism, insulin can also act as a _____ *factor*.

□ Insulin growth factor RTK signaling triggers the following pathway: Grb2→ Sos → Ras → MAPK.

Insulin Growth Factor Signaling via Ras Pathway

●Recall: Insulin results in active IRS-1, which acts as a _____ leading to many pathways (ex. *Ras Pathway*).

●_____: a lipid-linked monomeric G-protein existing in *two* forms: GDP-bound (*inactive*) & _____-bound (_____).

●A _____-step cascade of protein interactions *activates* Ras during insulin signaling.

1. Active IRS-1 serves as binding site for the SH2 domain of the *adaptor protein* _____.
2. Grb2's SH3 domain serves as binding site for the protein _____, forming an *IRS1-Grb2-Sos complex*.
3. Bound Sos is a ***G**uanine **E**xchange **F**actor* (_____): catalyzes replacement of GDP with GTP on Ras.

□ *Active Ras* regulates cell growth via activation of a _____ *signaling cascade*.

□ Similar pathways can also be initiated by a _____ of other *growth factors* (ex. EGF & PDGF).

EXAMPLE: G proteins such as Ras are activated when:

a) Bound to GDP.
b) Bound to GTP.
c) Phosphorylated at a serine or threonine residue.
d) Phosphorylated at a tyrosine residue.

PRACTICE: In the insulin signaling pathway to activate the protein Ras, activated IRS-1 binds to _____.

a) Adenylate cyclase. b) SH2 domain of Grb2. c) PLC. d) GTP-bound Ras. e) Insulin.

CONCEPT: INSULIN SIGNALING AS A GROWTH FACTOR

MAPK

- ***M**itogen-**A**ctivated-**P**rotein-**K**inases* (_____): enzymes that phosphorylate their substrates, leading to cell growth.
  - □ _____: type of growth factor that induces *mitosis* & *cell division*.
- MAPK enzymes can be regulated & phosphorylated by other _____.

EXAMPLE: MAPK Kinases.

Ras Pathway Activates the MAPK Signaling Pathway

- Recall: Ras pathway results in an active, GTP-bound Ras that initiates a _____-step MAPK signaling cascade.
  - 4 Active Ras-GTP directly *activates* _____ (a MAPKKK).
  - 5 Active Raf-1 phosphorylates two Ser residues, *activating* _____ (a MAPKK).
  - 6 Active MEK phosphorylates a Ser & Tyr, *activating* _____ (a MAPK).
  - 7 Active ERK enters _____ & regulates activity of *transcription* _____ related to cell growth.
    - □ *Transcription Factors*: proteins that induce _____ (DNA → RNA) of specific target genes.
    - □ ERK transcription factor targets include Elk-1, SRF, Jun, Fos, Myc.

CONCEPT: INSULIN SIGNALING AS A GROWTH FACTOR

EXAMPLE: All of the following are true in the Ras and MAPK pathways EXCEPT:

a) Grb2 changes conformation and binds to the next protein (Sos) in the signal transduction pathway.

b) Sos acts as a GAP in the activation of the protein Ras.

c) Ras exchanges GDP for GTP and activates Raf-1, the first kinase in the MAPK pathway.

d) The MAPK pathway regulates transcription factors that regulate specific genes.

Termination of Insulin Signaling as a Growth Factor

●Ras possesses *GTPase activity* which serves to _____ the signal & reset the pathway to the inactive state.

●Ras's *GTPase* activity is very _____; HOWEVER, it can be *sped up* with help from other proteins.

□ ***G**TPase-**A**ctivating **P**rotein* (_____): increases rate of Ras's GTP hydrolysis (thus *inactivating* Ras).

●*Phosphatases* remove phosphate groups, reversing kinase activity & helping to _____ the pathway.

PRACTICE: The monomeric G-protein Ras is activated by _____, while _____ helps turn Ras off.

a) Grb2 ; MAPK.

b) Sos ; GAP.

c) IRS-1 ; GAP.

d) MAPK ; Raf-1.

e) GAP ; Sos.

f) GEF ; Sos.

CONCEPT: INSULIN SIGNALING AS A GROWTH FACTOR

How to Remember Insulin RTK Signaling as a Growth Factor?

1. ***IRS*** gives a tax refund (***IRS-1***).
2. Cell buys ***2*** orders on ***Gr***u***b***-hub (***Grb2***).
3. When food arrives, cell adds hot ***S***auce (***Sos***).
4. Hot Sos *activates* a ***Ras***h (***Ras***).
5. Cell calls Dr. ***Raf***ael (***Raf-1***)….
6. To ***M***a***KE*** (***MEK***)….
7. ***ERK***ing-pain (***ERK***) go-away.

PRACTICE: Place the insulin RTK signaling events in order from 1-7:

a) Ligand binding results in autophosphorylation of the receptor tyrosine kinase _____.

b) MAPKK (Mek) phosphorylates MAPK (Erk) _____.

c) Sos binds then activates Ras _____.

d) Receptor tyrosine kinase phosphorylate IRS-1, which binds the SH2 domain of Grb2 _____.

e) Ras activates MAPKKK (Raf-1) _____.

f) MAPK (Erk) activates transcription factors to regulate mRNA synthesis _____.

g) MAPKKK (Raf-1) phosphorylates MAPKK (Mek) _____.

CONCEPT: INSULIN SIGNALING AS A GROWTH FACTOR

PRACTICE: Ras is estimated to be responsible for 30% of human cancers. Most mutations in Ras prevent the protein from binding GTPase-activation proteins (GAPs). What is the effect of this mutation on the signal transduction?

a) The GAP protein will activate too many Ras proteins.
b) The Ras protein cannot be turned "off" by GTP hydrolysis, so the signaling pathways remains activated.
c) The Ras protein cannot be turned "on" so the signaling pathway cannot be activated.
d) The Ras protein is turned "on" by the action of a protein kinase rather than GTP exchange.

PRACTICE: Insulin is one of the hormones/growth factors that will activate the Ras and MAP kinase pathway. Concerning this system, all of the following are true EXCEPT

a) Grb2 binds to the phosphorylated IRS-1.
b) Grb2 binds to the phosphorylated IRS using its SH2 domain.
c) Grb2 uses its SH3 domain to bind the GEF, Sos.
d) Grb2 binds directly to the serine residues of the insulin receptor's alpha-subunits.
e) The beta-subunits of the insulin receptor are phosphorylated on tyrosyl residues.

PRACTICE: All of the following are true in the Ras and MAPK pathway EXCEPT:

a) The occupied growth factor receptor autophosphorylates serine residues.
b) Grb2 uses its Src homology 2 domain to bind to the phosphorylated IRS-1.
c) Grb2 binds Sos in the Ras signal transduction pathway.
d) In the MAPK pathway, Raf-1 phosphorylates MEK.
e) In the MAPK pathway, ERK regulates transcription factors that regulate mRNA synthesis.

PRACTICE: Guanine Exchange Factors (GEFs) bind Ras-GDP and promote dissociation of bound GDP to be replaced by GTP. GTPase Activating Proteins (GAPs) bind Ras-GTP and stimulates GTPase activity of Ras. How is the downstream signal affected by the presence of GEFs and GAPs?

a) Signaling pathway increases in the presence of GAPs and decreases in the presence of GEFs.
b) Signaling pathway increases in the presence of GEFs and decreases in the presence of GAPs.
c) GAPs and GEFs both decrease the signaling pathway.
d) GAPs and GEFs both increase the signaling pathway.
e) GAPs and GEFs have no downstream effect on the Ras signaling pathway.

CONCEPT: RECAP OF INSULIN SIGNALING

PRACTICE: Match the following terms with their correct descriptions:

a) Insulin _____.
b) Receptor tyrosine kinase _____.
c) Grb2 _____.
d) Sos _____.
e) Ras _____.
f) IRS _____.
g) Phosphoinositide 3 Kinase _____.
h) PIP_3-Dependent Kinase 1 _____.
i) Akt/PKB _____.

1. Ligand.
2. Activates Ras.
3. Adaptor protein in insulin signaling.
4. Fully activates Akt/PKB.
5. Binds to IRS-1 and forms IP_3.
6. Insulin Receptor.
7. Promotes expression of GLUT4 to membrane.
8. Monomeric G-protein.
9. Binds to IRS-1 and Sos.

PRACTICE: After a thorough investigation, one would likely find that many hormones like epinephrine, insulin, & glucagon:

a) Can only regulate key cytosolic enzymes in metabolic pathways.
b) Can only regulate the activity of nuclear transcription factors.
c) Can regulate key cytosolic enzymes in metabolic pathways and regulate the activity of nuclear transcription factors.

CONCEPT: RECAP OF INSULIN SIGNALING AS A GROWTH FACTOR

PRACTICE: How is Ras activity turned off?

a) It is turned off by phosphorylation.
b) It is turned off by hydrolysis of its bound GTP to GDP.
c) It is turned off by hydrolysis of its bound GDP to GTP.
d) It is turned off by hydrolysis of its bound GTP to GMP.

PRACTICE: In a tumor cell line, Raf-1 is mutated such that it is constitutively activated by Ras even in the absence of insulin signaling. How can you inhibit the growth of this tumor cell line?

a) Blocking insulin receptor autophosphorylation.
b) Inhibiting the kinase activity of ERK.
c) Preventing the production of cAMP.
d) Blocking the recruitment of PI3K to the plasma membrane by IRS-1.

CONCEPT: JAK-STAT SIGNALING

●*JAK-STAT Signaling Pathway*: a variation of RTKs that _____ covalently-bound *Tyrosine Kinase domains*.

- □ HOWEVER, they can still _____-*covalently* recruit a soluble, cytosolic *Tyrosine Kinase* (JAK2).
- □ ***Ja**nus **K**inase **2*** (_____): a *tyrosine kinase* that phosphorylates & activates *STAT*.
- □ ***S**ignal **T**ransducers & **A**ctivators of **T**ranscription* (_____): a specific transcription factor.

Erythropoietin Signals via JAK-STAT Pathway

●**Erythro**po**ietin** (_____): a 165-amino-acid protein *cytokine* released by *kidneys* to induce *hemopoiesis* in bone marrow.

- □ _____: small, soluble proteins regulating development/differentiation/proliferation of cells (mainly blood).

Hemopoiesis: formation of _____ (*red blood cells*).

- □ EPO (& other cytokines) commonly signal via a JAK-STAT pathway.

PRACTICE: Erythropoietin is a cytokine produced in the _____ and released into the _____ to promote cell growth.

a) Liver ; Kidneys.
b) Kidneys ; Bone marrow.
c) Kidneys ; Liver.
d) Liver ; Bone marrow.

CONCEPT: JAK-STAT SIGNALING

JAK-STAT Pathway

●JAK-STAT pathway occurs in _____ steps:

1. EPO ligand binds *Cytokine Receptor*, causing it to _____.
2. *Dimerized Cytokine Receptor* recruits & activates _____.
3. *Activated JAK2* _____ Cytokine Receptor (a process which *resembles* autophosphorylation).
4. SH2 domain of STAT5 _____ *phosphorylated Cytokine Receptor* (bringing STAT5 closer to JAK).
5. JAK2 _____ STAT5,
6. Phosphorylated STAT5 _____ with another phosphorylated STAT5.
7. STAT5 dimerization exposes a signal, transporting it to the _____ to function as a *transcription factor*.

PRACTICE: When the JAK-STAT signaling cascade is activated, which of the following occurs?

a) Adenylate cyclase produces cAMP.
b) Ras GTPase activity is activated.
c) Activated STAT5 is translocated to the nucleus.
d) Grb2 binds Sos.

PRACTICE: How could a cell inhibit the JAK-STAT signal transduction pathway?

a) Dephosphorylating cytokine receptor tails using phosphatases.
b) Dephosphorylating STAT dimers using phosphatases.
c) Using proteins that bind STAT dimers to inhibit their DNA binding.
d) All of the above are ways to inhibit the JAK-STAT signaling pathway.

CONCEPT: JAK-STAT SIGNALING

How to Remember JAK-STAT Signaling?

1. ____**ucky** (**L**igand binds, and receptor dimerizes)
2. ____**ack's** (**J**AK activation)
3. ____**ig** (**P**hosphorylation of JAK)
4. ____**potted** (**S**TAT5 recruitment)
5. ____**olka** (**P**hosphorylation of STAT5)
6. ____**otted** (**D**imerization of STAT5)
7. ____**ruffles** (**T**ranscription Regulation)

EXAMPLE: List the following steps of the JAK-STAT pathway in order of their occurrence (from 1-7):

a) SH2 domain of STAT5 binds to the phosphorylated receptor _____.
b) Recruitment and activation of JAK2 by the receptor _____.
c) Regulation of transcription by dimerized STAT5 _____.
d) JAK2 phosphorylation of the receptor _____.
e) EPO binds stimulating receptor dimerization _____.
f) Dimerization of two phosphorylated STAT5 proteins _____.
g) JAK2 phosphorylation and activation of STAT5 _____.

PRACTICE: Which of the following statements is not true of the JAK-STAT signal transduction pathway?

a) JAK2 is both a protein kinase and a target of phosphorylation.
b) The ligand initiates a kinase cascade by phosphorylation of the JAK2 protein.
c) Receptor dimerization is required for recruitment & phosphorylation by JAK2.
d) Phosphorylated STAT5 protein enters the nucleus and functions as a transcription factor.
e) The receptor must be phosphorylated in order to bind cytoplasmic STAT5 protein.

CONCEPT: LIPID HORMONE SIGNALING

●Recall: _____: *signaling* molecules released by a cell/gland that can *travel* & affect *distant* cells in other areas.

□ *Paracrine Hormones*: travel _____ distances & only act on *nearby* cells in the vicinity of its synthesis.

□ *Endocrine Hormones*: released into bloodstream & travel _____ distances to their target cell.

PRACTICE: What is the major difference between paracrine and endocrine signaling?

a) Paracrine signaling regulates nearby cells, and endocrine signaling regulates target cells far away.
b) Paracrine signaling regulates target cells far away, and endocrine signaling regulates nearby cells.
c) Paracrine signaling regulates target cells with action potentials, endocrine signaling regulates with hormone signals.
d) Endocrine signaling regulates target cells with action potentials, paracrine signaling regulates with hormone signals.
e) None of the above are correct.

PRACTICE: Upon secretion, a chemical messenger (hormone) binds its receptors on other cells that were nearby. The hormone was secreted in such low concentration that it did not have any effect on distant cells, even though those distant cells had the appropriate receptors. We would best classify this hormone as a(n):

a) Endocrine hormone.
b) Paracrine hormone.
c) Autocrine hormone.
d) Both an autocrine hormone and an endocrine hormone.

CONCEPT: LIPID HORMONE SIGNALING

Lipid Hormones Diffuse Through Plasma Membranes

●Most hormones are *proteins* (ex. insulin), *amino-acid derivatives* (ex. epinephrine) or *steroids* (ex. estrogen).

□ Recall: _____ *Hormones*: hydrophobic molecules derived from cholesterol.

●*Lipid hormones* commonly bind _____ receptors inside cells (either in the cytoplasm or nucleus).

□ _____*-lipid hormones* bind exclusively to _____ portions of receptors in plasma membrane.

PRACTICE: The main difference between hormones that have intracellular receptors and those that have cell membrane receptors is that the former tend to be _____:

a) Larger.
b) Charged.
c) Amino acid derivatives.
d) Proteins.
e) Hydrophobic.

CONCEPT: SUMMARY OF BIOSIGNALING

PRACTICE: Which of the following is NOT associated with GPCR signaling?

a) Ras monomeric GTPase gets activated.

b) Replacement of GDP for GTP causes dissociation of the heterotrimeric G-protein α-subunit from $\beta\gamma$ subunits.

c) Adenylate cyclase is activated, causing an increase in the concentration of cyclic AMP (cAMP).

d) Secondary messengers are released leading to signal amplification.

e) GPCRs can become phosphorylated and desensitized via binding of an inhibitory protein.

f) All are associated with GPCR signaling.

PRACTICE: Which of the following statements is FALSE?

a) Activation of the α-adrenergic receptor leads to increased levels of Calcium in the cytoplasm.

b) Cholera toxin causes the inhibition of G_s GTPase activity, leading to an overstimulated signal.

c) Upon receptor autophosphorylation, JAK2 then binds to the receptor via its SH2 homology domains.

d) 4 cAMP molecules are required to activate protein kinase A.

e) Phosphorylated and dimerized STAT5 acts as a transcription factor in the nucleus.

CONCEPT: SIGNALING DEFECTS & CANCER

- _____ in signaling pathways can cause them to fail, leading to *disease*.
- _____, a disease characterized by *uncontrollable/inappropriate* cell growth, is associated with signaling defects.

Types of Genes Regulating Cell Growth

- In a healthy, normal cell, _____ types of genes regulate cell growth:
  1) _____-*oncogene*: provide signals that promote appropriate cell division (green light for cell division).
     - □ Ex. gene encoding _____ *G-protein*.
  2) *Tumor*-_____ *Gene*: provide signals to inhibit cell division (red light for cell division).
     - □ Ex. genes encoding _____ reversing kinase activity.

Oncogenes & Mutated-Tumor-Suppressor-Genes Promote Cancer

- Though proto-oncogenes are essential, they are susceptible to *mutations* that generate _____.
  - □ *Oncogene*: _____ gene that promotes unrestrained cell growth (*cancer*).
- The proto-oncogene encoding _____ is one of the most commonly *mutated* in human cancer tumors.
  - (1) Most common *mutation* in cancer tumors is loss of Ras's intrinsic _____ activity (keeping Ras active).
  - (2) *Mutations* in *tumor-suppressor genes* (ex. phosphatases) can also lead to _____ development.

CONCEPT: SIGNALING DEFECTS & CANCER

PRACTICE: Is there a difference between oncogenes and tumor suppressor genes?

a) Yes, oncogenes are genes that can cause cancer when they become mutated to become proto-oncogenes, whereas tumor suppressor genes play no role in cancer.
b) Yes, oncogenes prevent cancer from forming unless they are mutated to become proto-oncogenes, whereas tumor suppressor genes stimulate the formation of cancer even in the absence of mutation.
c) No, oncogenes and tumor suppressor genes both stimulate the development of cancer, even in the absence of their becoming mutated.
d) Yes, oncogenes are mutated versions of genes that promote abnormal cell division (such as mutated Ras), whereas tumor suppressor genes are genes that normally inhibit cell division.
e) No, since both types of genes contribute to the development of cancer, there is no difference between them.

PRACTICE: The protein product of the Ras oncogene is a mutated Ras protein. All of the following would be true EXCEPT**:**

a) The Ras protein is a G-protein and functions as an internal clock.
b) G-proteins have evolved to stay active for a certain length of time.
c) Ras protein is active in cell growth and division.
d) Ras can mutate so that it is less active as a GTPase.
e) A less active GTPase would mean less stimulation of the MAP kinase pathway.

PRACTICE: How does a proto-oncogene differ from an oncogene?

a) Proto-oncogenes code for proteins that regulate expression of structural genes; oncogenes code for nucleic acids involved in cell division.
b) Proto-oncogenes control normal cell division; oncogenes contribute to the development of cancer.
c) When oncogenes become damaged, they become proto-oncogenes.
d) Oncogenes activate tumor suppressor genes; proto-oncogenes repress tumor suppressor genes.
e) Proto-oncogenes cause cells to self-destruct when damaged; oncogenes cause damaged cells to repair themselves.