XIV) Signaling.

A) The need for Signaling in multicellular organisms

B) yeast need to signal to respond to various factors

C) Extracellular signaling molecules bind to receptors

1) most bind to receptors on the cell surface

2) some pass through membranes

3) There are different types of extracellular signaling molecules

4) most signaling molecules are secreted by exocytosis

5) others diffuse through the membrane

6) others remain bound to the surface

D) Receptors

1) usually they are on the cell surface

2) sometimes they are inside the cell

E) four major types of signaling

1) paracrine signaling

2) synaptic signaling

3) endocrine signaling

4) autocrine

F) Cells connected by gap junctions can share small signaling molecules

1) allow the exchange of small signaling molecules

2) no exchange of macromolecules

3) important roles in development

G) Different types of signals can trigger different effects in a given cell

1) differentiation

2) proliferation

3) survival

4) specific cellular functions

H) different cells may respond differently to the same signal

1) response depends on cell surface receptor

2) response depends on the environment

3) intracellular machinery

4) ex - acetylcholine (a neurotransmitter)

I) Signaling by Nitric Oxide

1) NO synthase produces NO by deamination of arginine

2) Role of NO in causing smooth muscles to relax

a) sequence of events

b) medical relevance

3) other functions of NO

a) production by activated macrophages and neutrophils

b) NO is released by many nerve cells

4) mechanisms of action of NO

a) diffuses out of the cell, through the membrane

b) diffuses directly into neighboring cells

c) acts only locally

d) binds to enzymes within the target cells

e) production of cGMP

J) Signaling via intracellular receptors

1) small hydrophobic molecules bind internal receptors

2) types of ligands that bind internal receptors

a) cortisol

b) steroid sex hormones

c) vitamin D

d) thyroid hormone

e) retinoids

3) steroid hormones are made bind carrier molecules in blood

4) intracellular receptors (steroid receptors) bind DNA

a) cortisol receptor is localized in the cytosol

b) retinoid receptors are in the nucleus

c) primary and secondary responses

5) steroid hormones can have different responses in different cells

a) only some cells have the receptors

b) different cells have different transcription factors

c) different combinations of tx factors are required to activate a given gene

K) There are three major classes of cell surface receptors

1) ion channel-linked receptors (transmitter gated channels)

a) synaptic signaling between electrically excitable cells

b) neurotransmitters transiently open and close the ion channel

2) G protein linked receptors (the largest family)

a) receptor is linked to a trimeric G protein

b) ligand binding leads to activation of the G protein

i) can lead to activation of an enzyme, or

ii) can lead to opening of an ion channel

3) enzyme linked receptors

a) the receptors can have enzyme activity themselves, or

b) receptors can be linked to an enzyme

c) often the enzyme activity is a protein kinase

L) activation of intracellular signaling enzymes and second messengers

1) GTP binding proteins

a) monomeric

b) trimeric

2) second messengers

a) cAMP

b) calcium

3) protein kinases

a) serine/threonine kinases - most abundant

b) tyrosine kinases

c) dual specificity kinases

4) transcription factors

M) Signaling via G protein linked receptors

1) G protein linked receptors are very abundant

2) they mediate responses to many types of signals

3) they span the membrane 7 times

4) These receptors are linked to trimeric G proteins

5) trimeric G proteins and monomeric G proteins

a) trimeric G proteins have three subunits

b) all of them bind GTP and GDP

5) many G proteins linked receptors increase concentrations of second messengers

6) second messengers in turn affect specific cellular proteins

7) There are different families of trimeric G proteins

8) receptors coupled to Gs lead to an increase in cAMP

a) adrenergic receptor

b) adrenaline or noradrenalin bind adrenergic receptors

c) other hormones also bind Gs coupled receptors

d) This leads to activation of the trimeric Gs protein

e) activated α-GTP activates adenylate cyclase

f) activated adenylate cyclase produces cAMP

g) cAMP levels rise in the cell

h) cAMP triggers various cellular responses

9) <u>cAMP dependent PKA mediates effects of cAMP</u>

a) structure and regulation of PKA

i) 2 regulatory subunits and 2 catalytic subunits

ii) the two regulatory subunits bind cAMP

iii) catalytic subunits dissociate

iv) catalytic subunits phosphorylate substrates

v) they phosphorylate a variety of cellular substrates

b) PKA and glycogen metabolism in skeletal muscle cells

i) adrenal gland secretes adrenaline into the blood

ii) adrenaline binds β adrenergic receptors

iii) results in an increase in cAMP

iv) activation of PKA

v) PKA phosphorylates at least 3 targets

vi) this maximizes the amount of glucose available

vii) glucose can enter glycolysis to yield ATP

c) transcription of some genes can be increased by cAMP and PKA

i) CRE site is found in promoters of some genes

ii) PKA phosphorylates the CREB transcription factor

iii) CREB turns on transcription of genes

10) once activated, the Gs system is rapidly downregulated

a) GTPase activity of Gα is rapidly stimulated when it is bound to adenylate cyclase

b) GTP is hydrolyzed to GDP

c) Gα and adenylate cyclase are inactivated

d) Gα reassociates with β γ

ε) ready for another round

f) rapid turn on and off ensures rapid responses

11) deficiency in Gs leads to clinical disorders

a) decreased response to some hormones

b) metabolic abnormalities

c) abnormal bone development

d) usually mental retardation

12) cholera results from overactivation of Gs

a) cholera toxin alters Gs so it can not hydrolyze GTP

b) adenylate cyclase stays activated

c) increased flow of Na+ and H20 into gut

13) cAMP mediates responses to many different types of signals

14) Gi inhibits adenylate cyclase

a) α2 adrenergic receptors are Gi linked

b) these receptors can also bind adrenaline

c) Gi has the same β γ but Gαi differs from Gαs

d) upon rcptr activation both βγ and αi contribute to adenylate cyclase inhibition

i) βγ binds any free Gs in the cell

ii) βγ also directly binds the cyclase

iii) Gαi also has an inhibitory effect on the cyclase

e) Gi also has another role, in opening K+ channels

f) pertussis toxin (a bacterial toxin) inactivates Gi

15) Some G proteins regulate ion channels

a) Regulation of K+ channels in heart muscle

i) acetylcholine activates Gi in muscle

ii) (α subunit inhibits adenylyl cyclase)

iii) α also opens K+ channels in the muscle cells

iv) this makes the membranes harder to depolarize

v) reduced rate and strength of contraction

b) channel phosphorylation

- via activation of PKA, PCK, CaM Kinases, etc.

c) regulate specific cyclic nucleotides

i) olfaction

ii) vision

16) Some G protein linked receptors regulate intracellular Ca

a) cytosolic calcium concentration is normally kept low

b) Extracellular and ER calcium levels are high

c) opening of channels causes Ca+ to rush into the cell

d) this leads to activation of calcium dependent signals

e) The earliest studies of Ca+ signaling were in skeletal muscle

f) Ca+ is involved in a wide array of responses

g) Two major calcium pathways have been defined

i) in electrically excitable cells

ii) in all other cells

h) some G protein linked receptors linked to Gq activate Ca signaling

i) PIP2 plays an important role

ii) A G protein coupled receptor activates Gq

iii) Gq activates PLC-Beta

iv) PLC - beta cleaves PIP2 to IP3 and DAG

v) IP3 diffuses to the ER and binds IP3 gated Ca channels

vi) DAG activates PKC

i) Some pharmacological agents can mimic Ca signaling

i) Calcium ionophores A23187 or ionomycin mimic IP3

ii) phorbol esters mimic DAG

iii) many responses require ionophore and phorbol

j) calmodulin is a Ca receptor

i) Calmodulin has 4 binding sites for calcium

ii) calmodulin undergoes a conformational change

iii) calmodulin itself has no catalytic activity

iv) calmodulin can bind other proteins

v) CAM Kinases mediates most Ca responses

17) <u>Interactions between cAMP and Ca pathways</u>

a) Cyclic AMP phosphodiesterase and adenylyl cyclase

b) PKA can bind and affect Ca channels

c) some CAM Kinases are phosphorylated by PKA

d) CAM Kinase and PKA have some shared targets

e) phosphorylase kinase is regulated by cAMP and calcium

N) Signaling via enzyme linked receptors

1) <u>receptor guanalyl cyclases</u>

a) a small family of receptors

b) in kidney cells and smooth muscle cells of blood vessels

c) single pass membrane spanning proteins

d) contains a guanylyl cyclase catalytic domain

e) ligands are atrial natruiretic peptides (ANP)

f) binding leads to production of cGMP

g) cGMP binds cGMP dependent protein kinase

h) cGMP dependent protein kinase phosphs substrate

i) in kidney cells this stimulates excretion of Na+ and H20

j) relaxation of smooth muscle cells on walls of blood vessels

k) leads to lower blood pressure

2) <u>Receptor tyrosine kinase (RTK) family of receptors</u>

a) a large family of receptors

b) activation of RTKs

i) single pass membrane spanning

ii) There are many types of receptor TKs

iii) ligand binding usually causes dimerization

iv) cytoplasmic domains then phosphorylate each other

v) P-Tyr binds downstream SH2 domains

c) Ras superfamily of small GTPases help relay signals

i) blocking Ras can inhibit proliferation

ii) constitutively active Ras can stimulate proliferation

iii) constitutively active Ras can promote cancer

iv) GAPs inactivate Ras

v) GNRPs (GEFs) activate Ras

vi) Ras signaling is highly conserved in evolution

d) drosophila eye development and RTK signaling

i) drosophila eye is made up of around 800 ommatidia

ii) ommatidia cells develop from an epithelial sheet

iii) sev (son of sevenless) mutant has R7 missing

iv) Boss (bride of sevenless) has the same phenotype

v) Ras

vi) sos

vii) DRK (downstream of receptor Kinases)

viii) Ras activates downstream signaling pathways

e) MAP Kinase pathways

i) Highly conserved pathways

ii) signal from the cell surface to the nucleus

iii) activated by a wide range of stimuli

iv) mediate many cellular changes

v) can be activated by both RTK and GPRs

vi) types of signaling enzymes involved

vii) MAP Kinase pathways in yeast

viii) Three major mammalian MAP Kinase pathways

f) Ca^{2+} signaling can be activated by RTKs via PLC gamma

i) PLC gamma can bind RTKs via its SH2 domain

ii) PLC gamma cleaves PIP2 to IP3 and DAG

iii) pathway to Ca^{2+} release

g) RTKs can activate PI3-kinase

i) one form can be activated by G proteins

ii) one form can be activated by RTKs

iii) phosphorylation of PIP2 gives rise to PIP3

iv) PIP3 is also a second messenger

3) <u>Tyrosine Kinase (TK) associated receptors</u>

a) These receptors have no catalytic activity on their own

b) several types of receptors are tyrosine kinase linked

c) TK associated receptors often interact with src family TKs

d) structure of src

e) Molecular interactions of src

g) Src family members can also bind to RTKs via SH2

h) Src family members activate many of the same types of pathways as RTKs

e) Janus family of non-receptor TKs also bind some receptors

i) growth hormone receptors

ii) prolactin receptors

iii) some cytokine receptors

iv) include JAK1, JAK2, Tyk2

v) more poorly characterized

4) Occasionally receptors can be protein tyrosine phosphatases

a) ex CD45 is found on the surfaces of wbcs

b) CD45 plays a role in the activation of B and T lymphocytes by foreign antigen

c) dephosphorylate specific proteins in response to ligand stimulation

d) ex, CD45 may dephosphorylate Lck

5) serine/threonine protein kinases such as the TGFB superfamily

a) functions can vary

i) suppress proliferation

ii) stimulate ECM synthesis

iii) stimulate bone formation

iv) chemotaxis

b) these receptors are serine/threonine kinases

O) Ligand induced cascades lead to amplification of the signal

P) Many signaling enzymes are proto-oncogenesQ) Signal Transduction and the Cytoskeleton

1) The activities of most cells are also directly affected by cell adhesion and the organization of the cytoskeleton

2) integrins can function as cell surface receptors

3) integrins bind FAK

4) FAK becomes phosphd on tyr

5) src probably binds to an autophosphorylation site on FAK

6) src phosphorylates additional sites on FAK

7) SH2 domain containing proteins bind FAK

8) activation of downstream signaling pathways

R) Adaptation

1) When exposed to a stimulus for a prolonged period, their response decreases

2) Receptor downregulation

3) receptor phosphorylation

4) change in concentration of activity intracellular signaling enzymes