Frontiers in Reproductive Endocrinology
Serono Symposia International

Mechanisms of Hormone Action: Peptide Hormones

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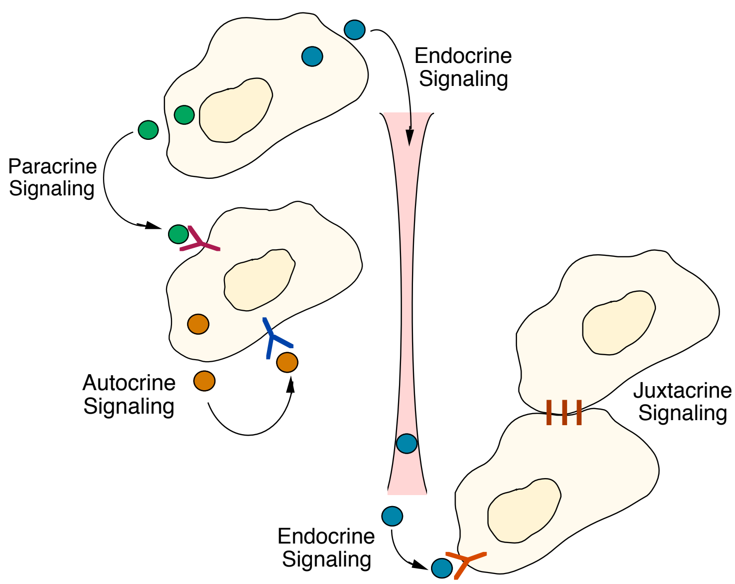
Mechanisms of Cell Communication



Emergence of Key Concepts in Hormone Action

Berthold, 1849

Castrated cockerels and restored male sex characteristics by replacing testes in the abdomen

"Endocrinology"

Starling, 1905

Stimulation of pancreatic enzyme secretion by humoral factor (secretin) from intestinal extracts

"Hormone"

Langley, 1906

Action of nicotine and curare on the 'receptive substance' of the neuromuscular junction

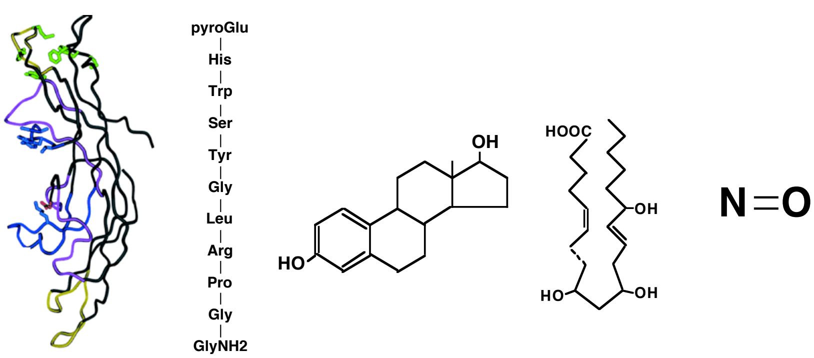
"Receptor"

Sutherland, 1962

Glycogen metabolism and hormonal activation of liver phosphorylase enzyme by cAMP

"Second Messenger"

Structural Diversity in Reproductive Hormonal Signaling Molecules



FSH	GnRH	Estradiol	PGF2α	Nitric Oxide
Protein	Peptide	Steroid	Eicosanoid	Gas
203 aa	10 aa	MW 272	MW 330	MW 30

Measuring Receptor-Ligand Interaction

$$R\ (\text{receptor}) + H\ (\text{hormone}) \underset{k_d}{\overset{k_a}{\rightleftharpoons}} RH\ (\text{complex})$$

$K_a = \frac{[RL]}{[R][L]}$ (units are moles $^{-1}$)

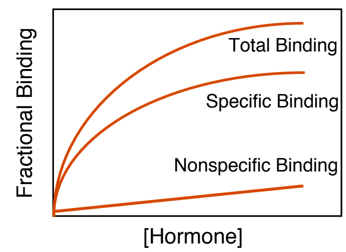
$K_d = \frac{[R][L]}{[RL]}$ (units are moles)

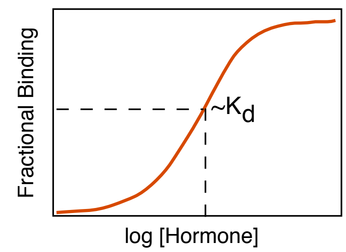
Total R, $R_T = [R] + [RL]$, so: $K_d = \frac{[R_T\text{-}RL][L]}{[RL]}$

Rearrange to: $[L] = \frac{[RL](K_d + [L])}{[R_T]}$

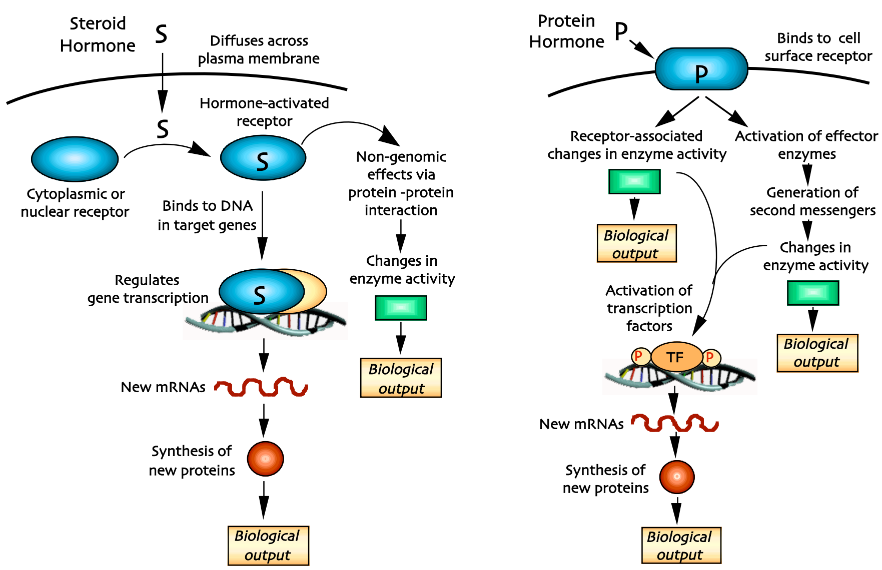
Fraction of receptor ocupied by ligand: $\frac{[RL]}{[R_T]} = \frac{[L]}{K_d + [L]} = \frac{1}{1 + \frac{K_d}{[L]}}$

→ At 50% occupancy (1/2), $K_d = [L]$





General Mechanisms of Action of Steroid and Peptide Hormones



Major Classes of Peptide Hormone Receptors

G Protein-Coupled Receptors
Largest family (>800), Highly Diverse

Receptor Tyrosine Kinases
"Growth Factor" Receptors

Receptors that Recruit Tyrosine Kinases
Cytokine Receptors/Stat Pathway

Receptor Serine/Threonine Kinases
TGF□/Smad Pathway

Developmental Pathway Receptors
Hedgehog/Wnt/DSL Ligands

"Other Pathways"
Diverse and Growing

Families of G Protein-Coupled Receptors

Family A: Rhodopsin-Like Rs
Group I: Olfactory, Adenosine, Melanocortin Rs
Group II: Adrenergic, Muscarinic, Serotonin, Dopamine Rs
Group III: Neuropeptide Rs and Vertebrate Opsins
Group IV: Bradykinin Rs and Invertebrate Opsins
Group V: Peptide, Glycoprotein Hormone and Chemokine Rs
Group VI: Melatonin and Orphan Rs

FSH,LH, Relaxin, GnRH Receptors

Family B: Secretin-Like Rs
Group I: Calcitonin and CRF Rs
Group II: PTH and PTHrP Rs
Group III: Glucagon, Secretin, VIP, GHRH Rs

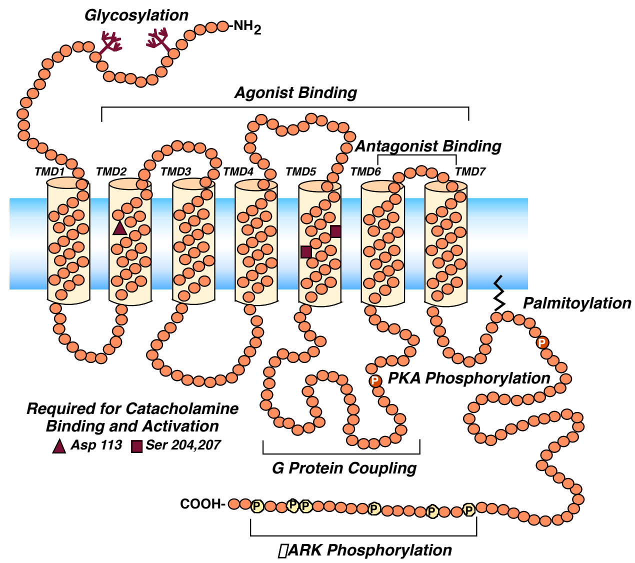
Family C: Glutamate-Like Rs
Group I: Metabotropic Glutamate Rs
Group II: Calcium Sensing Rs
Group III: Vertebrate Pheromone Rs

Family D: Fungal Pheromone Rs
Group I: Alpha Factor Pheromone Rs
Group II: A Factor Pheromone Rs

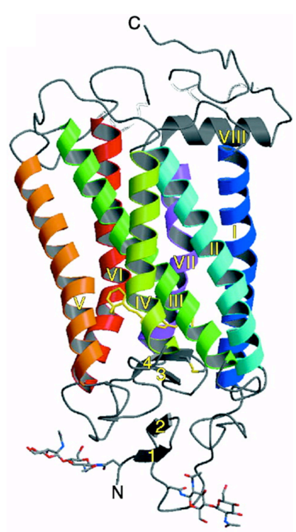
Family E: cAMP Rs
Group I: Dictyostelium cAR Rs

Frizzled/Smoothened Rs
Group I: Frizzled Rs
Group II: Smoothened Rs

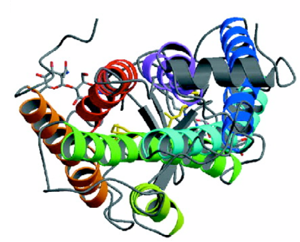
Features of a Prototypical G Protein-Coupled Receptor (GPCR), the β-Adrenergic Receptor



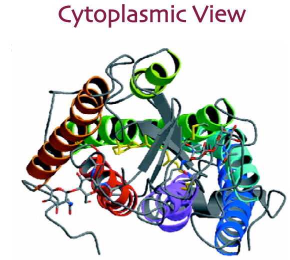
Structure of a G Protein-Coupled Receptor, Rhodopsin



Membrane-Parallel View



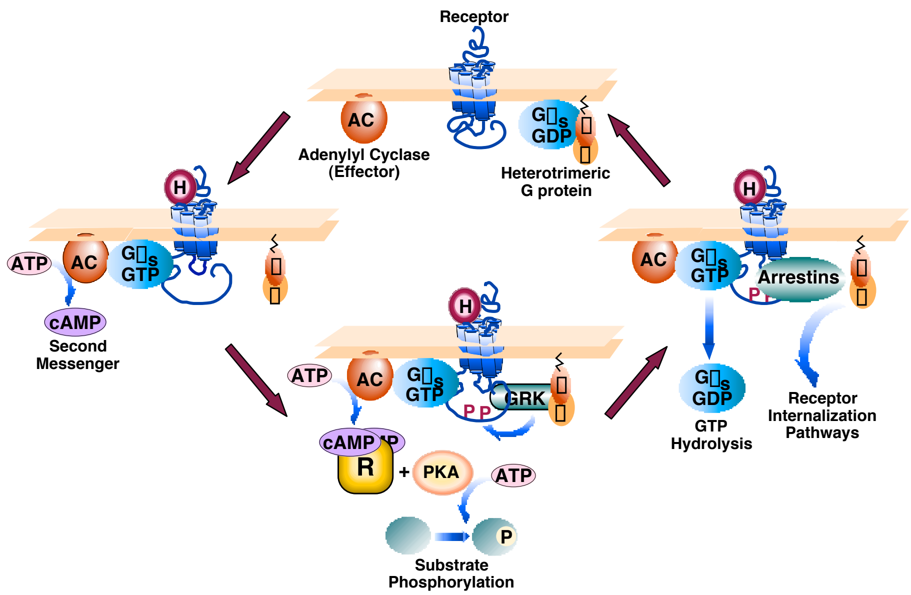
Cytoplasmic View



Extracellular View

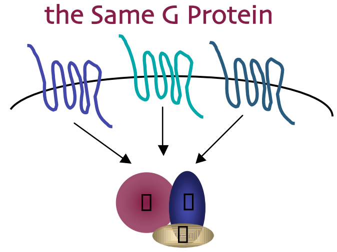
Palczewski et al, Science 289: 739, 2000

Pathways of G Protein-Coupled Receptor Action

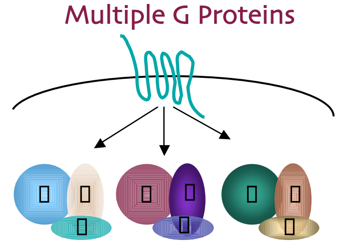


Diversity and Specificity in G Protein Signaling

Multiple Receptors Activate the Same G Protein



One Receptor Activates Multiple G Proteins



Diverse Actions of G Protein Heterotrimers

□ Signaling Diversity (G_s, G_i, G_q, G_o)

□ and □□ Effector Activation

Combinatorial Assembly

20 □, 6 □, 12 □=1440 Heterotrimers

G Protein Receptor Specificity

Carbachol → M4R → Go1□3□4 → Go

Somatostatin → SSR → Go2□1□3 → Go

Go → Ca^{++} Channel

Kleuss et al, Nature 353:43, 1991

Current Issues in G Protein Coupled Receptor Research

Receptor Trafficking
Internalization
GRK Kinases
RAMP Proteins

Receptor Signaling
Desensitization
RGS Proteins
Non G protein pathways

Receptor Diversity
Dimerization
Alternative Splicing
Modifications

Receptor Structure
Molecular Modeling
G Protein Interaction
Structure Determination

Therapeutics
Ligand Mimics
Altered Regulation
Orphan Receptors
New Pathways

Receptors with Intrinsic Kinase Activity or Associated with Kinase Activity

General Features
Type I Integral Membrane Proteins
Dimerization Critical to Activation
Receptor Phosphorylated on Activation
Signaling Proteins Bind to Phosphorylated Receptor
Proteins that Suppress Signaling

Receptor that are Tyrosine Kinases
Growth Factor Receptors
Insulin Receptor

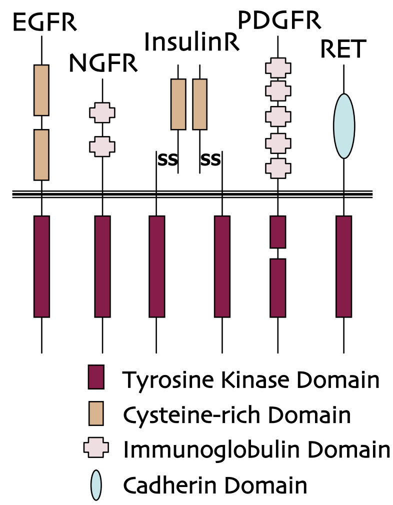
Receptors that Recruit Tyrosine Kinases
Cytokine Superfamily Receptors
Prolactin Receptor

Receptors that are Serine/Threonine Kinases
TGF□ Superfamily Receptors
MIS Receptor

Receptors that are Protein Tyrosine Kinases

Examples of RTK Families

EGF Receptor Subfamily
(EGFR, HER2/Neu)
NGF Receptor Subfamily
(TrkA, TrkB, TrkC)
FGF Receptor Subfamily
(FGFR1-4, Cek2)
HGF Receptor Subfamily
(HGFR=MET)
PDGF Receptor Subfamily
(PDGFR ▯/▯, VEGFR)
Insulin Receptor Subfamily
(InsulinR, IGF-1R)
EPH Receptor Subfamily
(Ephrin AR, Ephrin BR)
ROR Receptor Subfamily
(RET-GDNF)



Receptor Tyrosine Kinase Signaling Pathway

RTK Signaling

Ligand-induced dimerization

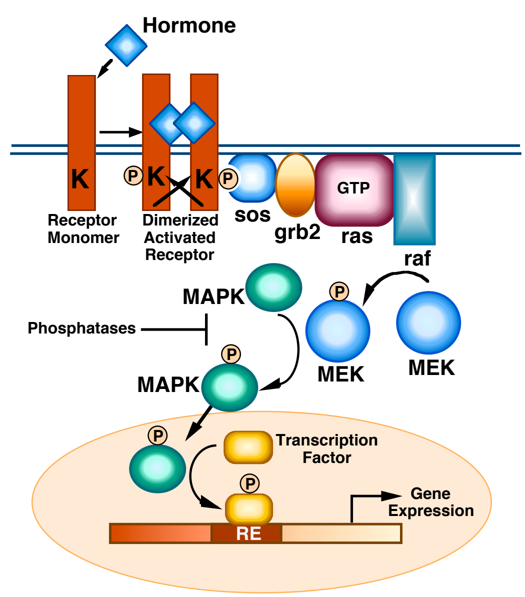
Receptor phosphorylation

Effector proteins bind receptor
- direct enzymatic function
- adaptor proteins

Initiation of kinase cascades

Changes in gene expression

Phosphatases and negative regulation



Receptors that Associate with Cytoplasmic Kinases

Families of Cytokine Receptors

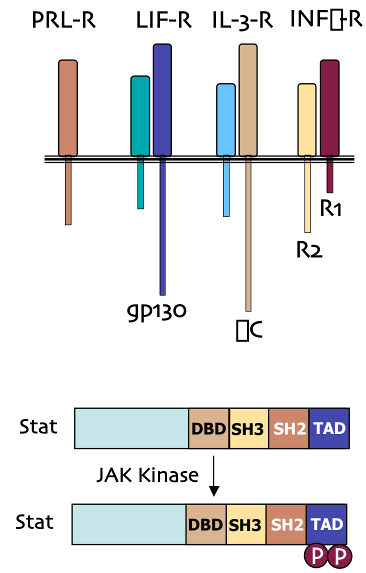
Type I: Single chain
(GH, PRL, EPO, TPO)
Type II: Shared gp130 signaling subunit
(IL-6, LIF, IL-11, CNTF)
Type III: Shared gp140□, ligand-specific □
(IL-3, IL-5, GMCF)
Type IV: Shared □c, ligand-specific □
(HGFR=MET)
Type V: Two or more distinct subunits
(IFN□/□, IFN□)

Janus Family Kinases

Jak1 (IL-2, IL-6, GCSF, INF□/□, INF□)
Jak2 (GH, PRL, EPO, IL-5)
Jak3 (IL-2. IL-4, IL-7)
Tyk2 (IL-6, LIF, INF□/□)

Stat Proteins

Stats 2 and 6
Stats 1, 3, 4, 5A, 5B



Cytokine Receptor Signaling Pathway

Cytokine Signaling

Ligand-induced dimerization

Receptor association with
JAK kinase

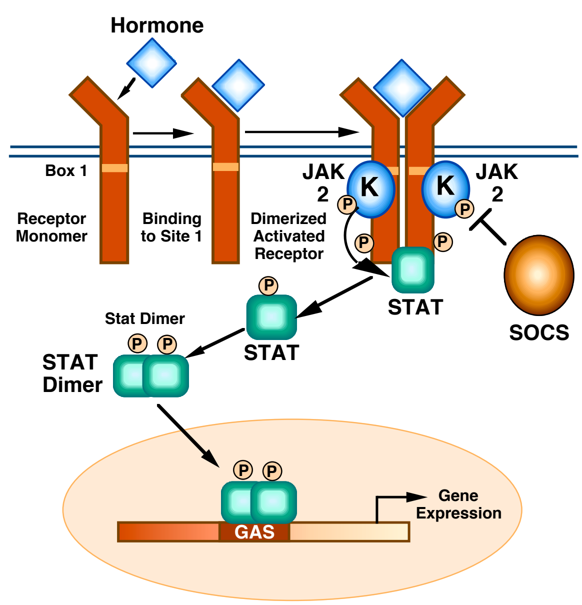
Receptor phosphorylation

Effector proteins bind receptor
- direct enzymatic function
- STAT proteins

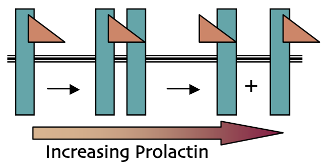
STATs phosphorylated
STATs dimerize
STATs migrate to the nucleus

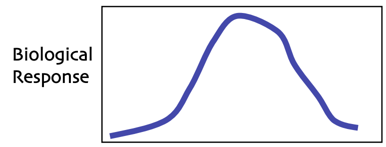
Changes in gene expression

SOCS proteins and negative regulation

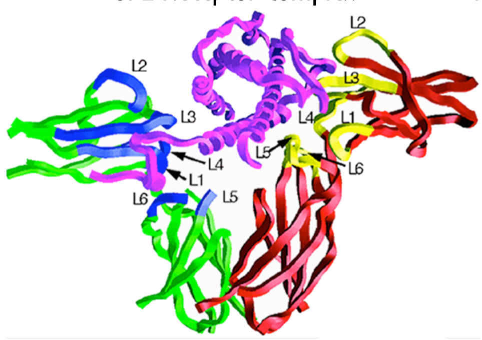


Prolactin-Induced Receptor Dimerization

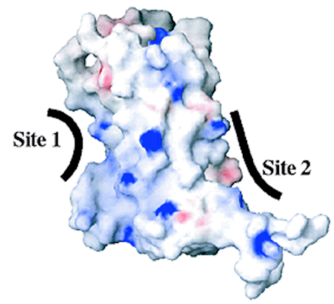




oPL-Receptor Complex



Receptor Interaction Sites on oPL

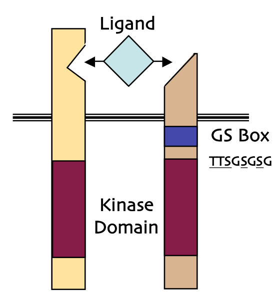


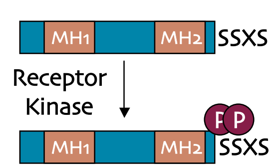
Elkins et al, Nature Structural Biology 7:808, 2000

Receptors with Serine/Threonine Kinase Activity

Ligand	Type II Receptor	Type I Receptor
TGF-β	TβR-II	TβR-I
Activin	ActR-IIB	ActR-IB
MIS	AMHR	ActR-I
BMP2	BMPR-II	BMPR-IA
BMP7	ActR-II	BMPR-1B
GDF5	ActR-IIB	ActR-I
Dpp	Punt	Tkv, Sax

Receptor Smads	Co-Smads	Inhibitory Smads
1,2,3,5,8 Mad Sma2,3	Smad 4 Medea Sma-4	Smads 6,7 Dad





Serine/Threonine Receptor Signaling Pathway

TGF Family Signaling

Ligand binds to type II receptor

Type I and II receptor associate

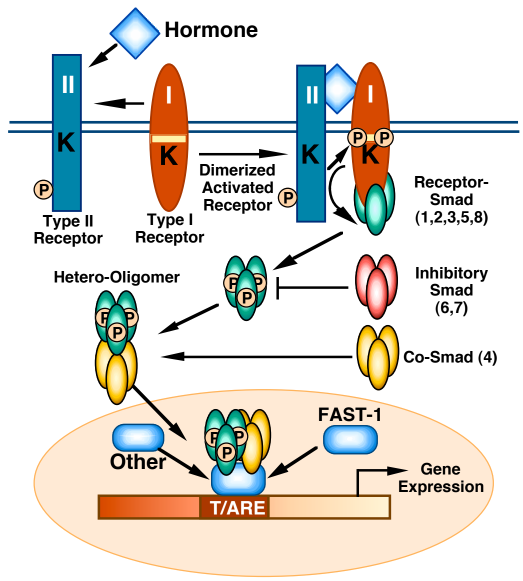
Type I receptor phosphorylation

Effector proteins bind receptor
- direct enzymatic function
- Smad proteins

Smads phosphorylated
Smads heterodimerize
Smads migrate to the nucleus
Smads bind other TFs

Changes in gene expression

Inhibitory Smads and negative regulation



Some Key Developmental Signaling Pathways

Ligand	Receptor	To Nucleus	Partner	Comments
Delta/Serrate/ Lag2	Notch	Notch (Cytoplasmic Domain)	CSL	Receptor proteolysis CSL Repressor to Activator
Wnt Family	Frizzled	-catenin	LEF/TCF	-catenin stabilized TCF Repressor to Activator
Hedgehog (Shh,Dhh,Ihh)	Patched	Ci/Gli (Full-Length)	CBP	Patched/Smoothened Complex Ci-75 Repressor in Basal State

Notch: Oogenesis in Drosophila (expressed in mammalian ovary)
Notch2: Placental trophoblast development

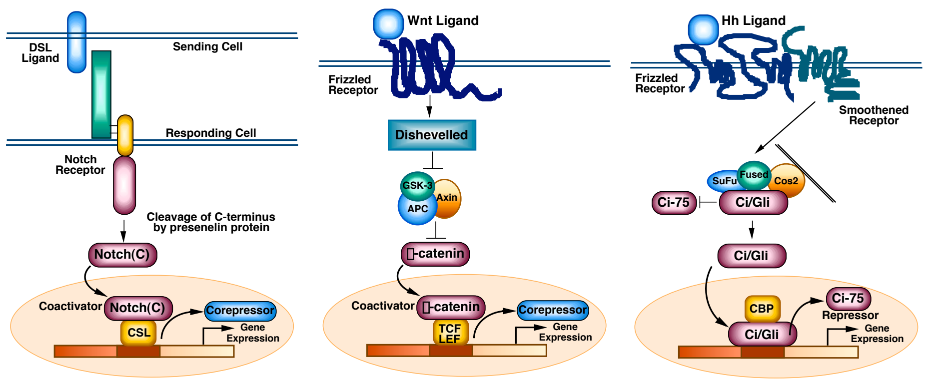
Wnt4: Roles in gametogenesis, Mullerian duct development
Wnt5a: Development of the external genitalia
Wnt7a: Development of the oviduct and uterus

Dhh: Maturation of testis, Seroli-Leydig cell interations
Shh: Pituitary cell type determination

Commonalities in Developmental Signaling Pathways

Famlies of related ligands
Involvement of proteolysis
Signaling to the nucleus
Switching from repression to activation

Notch Signaling | Wnt Signaling | Hh Signaling



Additional Pathways of Peptide Hormone Action

Plasmaprotein Receptors
LDL-R, Transferrin-R, M6P/IGF-II-R
Endocytosed, deliver cargo to lysosome
Recycled to cell surface, highly regulated

Guanylyl Cyclase Receptors
Inactive kinase/active guanylyl cyclase domains
Sperm receptors for egg peptides in sea urchin
Mammalian natriuretic peptides and enterotoxins

Ligand-Gated Ion Channels
nACh-R, GABA-R, Glutamate-R, ATP-R
Multisubunit, 4 TMD proteins
Major targets for therapeutics

Tyrosine Phosphatase Receptors
Transmembrane tyrosine phosphatases
Ligands unknown, cell-cell interactions
Activate signaling via src gamily kinases

Tumor Necrosis Factor Receptors
Lead to caspase activation and apoptosis
TNFR1/Fas signal via death domain
TNFRII/CD40 signal via TRAF proteins

Receptors Activating NF-κB
Interleukin-I and TNF receptors
Leads to activation of IκB kinase
Major pathway of inflammation

Mutations of Hormones, Receptors and Signaling Proteins in Reproductive Disease

Hormones

FSH	Delayed puberty, primary amenorrhea in females; male hypogonadism
LH	Luteal insufficiency, infertility in female; delayed puberty, azoospermia in male
MIS	Persistence of Mullerian duct derivatives in males

Receptors

GnRH-R	Partial to complete hypogonadotropic hypogonadism, males and females
FSH-R	Primary or secondary amenorrhea in females, variable/mild oligospermia in males
LH-R (Loss)	Amenorrhea or oligomenorrhea in females, range of defects to complete feminization in males
LH-R (Gain)	Male-limited precocious puberty, no phenotype in females
Estrogen R	Normal puberty, tall stature and unfused epiphyses in male
Androgen R	Many mutations, broad range of phenotypes to complete feminization in males
MIS R-II	Persistence of Mullerian duct derivatives in males
RET	Multiple endocrine neoplasia type 2

Signaling Proteins

Gs protein □	McCune-Albright Syndrome (gain), male precocious puberty (loss/gain)
Gi protein □	Ovarian and adrenal tumors?
Smads	Mutations in many cancers, including Smad4 mutation in seminoma testicular germ cell tumor

Transcription Factors

Dax-1	Hypogonadotropic hypogonadism/adrenal failure in male
SF-1	XY sex reversal/adrenal failure
Prop-1	Variable hypogonadotropic hypogonadism in males and females

Additional Readings on Peptide Hormone Action

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