Mechanisms of Hormone Action: Steroid Hormones

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General Mechanisms of Action of Steroid and Peptide Hormones

**Steroid Hormone**
- Diffuses across plasma membrane
- Binds to DNA in target genes
- Cytoplasmic or nuclear receptor
- Regulates gene transcription
- New mRNAs
- Synthesis of new proteins
- Biological output

**Protein Hormone**
- Binds to cell surface receptor
- Receptor-associated changes in enzyme activity
- Activation of effector enzymes
- Generation of second messengers
- Changes in enzyme activity
- Biological output

**Non-genomic effects via protein-protein interaction**
- Changes in enzyme activity
- Biological output

**New mRNAs**
- Synthesis of new proteins
- Biological output
Select Families of Nuclear Hormone Receptors

Steroid Receptors
- Estrogen Receptor (ER)
- Androgen Receptor (AR)
- Progesterone Receptor (PR)
- Glucocorticoid Receptor (GR)
- Mineralocorticoid Receptor (MR)

Mevalonate Pathway
- Liver X Receptor (LXR)
- Pregnan X Receptor (PXR)
- Farnesoid X Receptor (FXR)

Non-Steroid Hormones
- Thyroid Hormone Receptor (TR)
- Vitamin D Receptor (VDR)
- Retinoic Acid Receptor (RAR)
- 9-Cis Retinoic Acid Receptor (RXR)
- Ecdysone Receptor (EcR)

Peroxisome Proliferators
- PPARα (fibrates)
- PPARδ (thiazolidinediones)
- PPAR γ

Orphan Receptors
- ERR family
- NGFI-B family
- COUP TF family
- NGFI-B family
- RVR family
- SF-1
- LRH-1
- Dax-1
- HNF-4
- GCNF

Ex-Orphan Receptors
- Benzoate X Receptor (BXR)
- Steroid and Xenobiotic Receptor (SX)
- Constitutive Androstane Receptor (CAR)
- Also RXR, LXR, FXR, PPARs

Metabolic Pathways of Nuclear Receptor Ligands

Chawla et al Science 294:1866, 2001
Domain Structure of Nuclear Receptors and Dimerization and DNA Binding Properties

<table>
<thead>
<tr>
<th>Domain</th>
<th>AF-1</th>
<th>DBD hinge</th>
<th>LBD</th>
<th>AF-2</th>
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<tbody>
<tr>
<td>ER</td>
<td>A/B</td>
<td>C</td>
<td>D</td>
<td>E</td>
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</tbody>
</table>

Homodimeric Binding to Inverted Repeats
Steroid Receptors
ER, GR, MR, AR, PR
Some Orphan Receptors

Heterodimeric Binding to Direct Repeats
With RXR Partner
Non-Steroid Receptors
TR, RAR, VDR, PPAR

Monomeric Binding to Half Site
Orphan Receptors
Ligand Binding Unknown
SF-1, NGF-1-B, ERR

DNA Binding by Nuclear Hormone Receptors

Sanchez et al, BioEssays 24:244, 2002
DNA Binding by Nuclear Hormone Receptors

ER LBD Homodimer  RAR-RXR LBD Heterodimer  NGFI-B LBD Monomer

Hormone Enters Nucleus And Interacts with Receptor
Receptor Activation And Dimerization

HATs  Coactivators  Local Chromatin Remodeling

Activation of Target Gene Transcription
Basal Txn Factors

Cytoplasm
Inactive Receptor Complex
hsp90  hsp70

Nuclear Receptor Signaling Pathways
Nuclear Receptor Coactivators and Corepressors

**Coactivators**
- SRC-1, NCoA-1
- GRIP-1, TIF-2, SRC-3
- pCIP, ACTR, SRC-3
- Many others

**Integrators/Complexes**
- CBP, p300
- TRAPs, DRIPs
- SWI/SNF
- pCAF/CARM

**Corepressors**
- NCoR
- SMRT
- REA
- TRUP, SURF-3

** SRC-1**

<table>
<thead>
<tr>
<th>bHLH</th>
<th>PAS-A</th>
<th>PAS-B</th>
<th>RID</th>
<th>AD</th>
<th>Q-rich</th>
<th>HAT</th>
<th>RID</th>
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<td>759</td>
<td>907</td>
<td>955</td>
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<td>1440</td>
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** NCoR**

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<tr>
<th>Repression Domains</th>
<th>Su(H)D</th>
<th>Su(H)D</th>
<th>RID</th>
<th>RID</th>
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</thead>
<tbody>
<tr>
<td>SID1</td>
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<td>92</td>
<td>751</td>
<td>1035</td>
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<tr>
<td>SID2</td>
<td>1</td>
<td>1944</td>
<td>2453</td>
<td></td>
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</table>

Coregulators and Chromatin Remodeling

Inactive
- Corepressors
- HDACs

Active
- Coactivators
- HATS/MTs
- Tn5 Complexes

Remodeling
- Chromatin Modifying ATPases

Inactive Corepressors HDACs

Active Corepressors HDACs

Remodeling

Hormone
Some Nuclear Receptor Ligand-Binding Domains


Structures of Agonist and Antagonist Bound Estrogen Receptor Ligand Binding Domain

Shiau et al Cell 95:927-937, 1998
Complexities in Steroid Hormone Action: Estrogen

<table>
<thead>
<tr>
<th>Receptor Genes</th>
<th>Receptor Variants</th>
<th>Interactors</th>
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</thead>
<tbody>
<tr>
<td>ER-α and ER-β</td>
<td>Diverse promoters</td>
<td>ERR Orphan Receptors</td>
</tr>
<tr>
<td></td>
<td>Alternative RNA splicing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Post-translational modification</td>
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</tr>
</tbody>
</table>

Multiple Mechanisms
- Genomic actions
- Complex response elements
- Non-genomic actions

Coregulators
- Tissue specific coregulators
- Combinatorial coregulator code
- Agonist versus antagonist actions

Tissue-Specific Responses

SERMS

Generation of Diversity in Nuclear Receptors: Multiple Estrogen Receptor Genes

<table>
<thead>
<tr>
<th></th>
<th>AF-1</th>
<th>DBD</th>
<th>LBD</th>
<th>AF-2</th>
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<tbody>
<tr>
<td>ER-α</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Homology:</td>
<td>23%</td>
<td>86%</td>
<td>24%</td>
<td>58%</td>
</tr>
<tr>
<td>ER-β</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homology:</td>
<td>86%</td>
<td>24%</td>
<td>58%</td>
<td>12%</td>
</tr>
</tbody>
</table>

ER-α predominates: Kidney, Adrenal, Pituitary, Testis, Epididymis
Equivalent expression: Mammary gland, Uterus, Bone, Heart, Gut, Brain
ER-β predominates: Prostate, Ovary, Lungs, Bladder
Generation of Diversity in Nuclear Receptors: Multiple Estrogen Receptor Gene Promoters

Human ER-[] Promoters

Potential Implications:
- Tissue- or cell-specific ER expression
- Developmental-specific ER expression
- Alternative splicing of ER transcripts


Generation of Diversity in Nuclear Receptors: Multiple Estrogen Receptor Splice Variants

Shupnik, J Neuroendocrinol 14:85, 2002
Generation of Diversity in Nuclear Receptors: Phosphorylation of the Estrogen Receptor

In response to estrogen binding:

AF-1  DBD  AF-2

CDK2  Ser  Ser  MAPK?
104/106 118

In response to second messenger pathways:

AF-1  DBD  AF-2

MAPK  Ser  Ser  RSK  Ser  PKA
118  167  236

Lannigan, Steroids 68:1, 2003

Generation of Diversity in Nuclear Receptors: Interaction with Other Nuclear Receptor Pathways

ER[]  ER[]  ERR[]  ERR[]

ER[]  RER[]

AF-1  DBD  LDB/AF-2

68%  37%

ERE  AGGTGAnnnTGACCT  ERRE  TnAAGGTC

Estradol  DES/OHT  Natural Ligands?

CoA  CoR

ERE  ERRE  Other

Cell-Specific Responses

Giguere, Trends Endocrinol Metab 13:220, 2002
Generation of Diversity in Nuclear Receptors: Multiple Mechanisms of Action

**Genomic Actions**
- Classical
  - E2 → ERE
- Non-Classical
  - E2 → SP1/AP1 → NF-κB
- Ligand-Independent
  - E2 → ERE

**Non-Genomic Actions**
- Membrane-Bound ER
- Novel ER?
- Interaction with Other Membrane Proteins

Falkenstein et al, Physiol Rev 52:523, 2000

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Generation of Diversity in Nuclear Receptors: Combinatorial use of Coregulatory Proteins

**CoActivators**
- p160/SRC family
- CBP/p300
- TRAP/DRIP
- “SRA”

**CoRepressors**
- NCoR/SMRT
- Sin3/HDACs
- “REA”

Differential Regulation of Receptors
- Tissue-Specific Expression
  - “Combinatorial Code”
- Factor-Specific
  - Modification
    - (Phosphorylation, Methylation)

Receptor and Tissue-Selective Effects
Selective Estrogen Receptor Modulators (SERMs)

Examples of SERMs:
- Tamoxifin (breast cancer)
  - Antagonist in breast, but agonist in bone/endometrium
- Roloxifene (osteoporosis)
  - Agonist in bone, antagonist in breast/endometrium

Determinants of SERM Action:
Each ligand (SERM) will induce a unique conformation of the estrogen receptor that impacts its interaction with coregulatory proteins.
Each tissue or cell type will contain a unique complement of coregulatory proteins and a distinct pattern of activation state of these proteins.

Cell-Selective Actions of SERMs

Antiestrogenic
- Surface Silent
  - ER, CoR, CoA
  - Gene Silent

Estrogenic
- Surface Signaling
  - EGFR, HER2/neu, tks
  - Gene Activation
  - AF-2, AF-1, CoR, CoA, ER

*Jordan Cancer Cell 11:215, 2002*
**Additional Pathways of Intracellular Hormone Action**

### Extrinsic Signals
- **Arylhydrocarbon Receptor**
  - Intracellular dioxin receptor
  - Ligand activated transcription factor
  - Binds to xenobiotic response element
- **Nitric Oxide Receptor**
  - Cytoplasmic form of guanylyl cyclase
  - h / heterodimer with heme cofactor
  - Increases cGMP and PKG activity

### Intrinsic Signals
- **Sterol Sensing**
  - Proteolysis of membrane-bound SREBP
  - bHLH domain regulates transcription
- **Oxygen Sensing**
  - Prolyl and Asn hydroxylases regulated by O_{2}
  - Hydroxylation regulates HIF-{\alpha}
  - Heterodimer with ARNT regulates transcription

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**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 oligosperma 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
Emerging and Future Issues in Hormone Action

• Cross-talk between different signaling pathways
  • Integration of multiple signals in target cell
• Generation of diverse responses from common stimuli
  • Combinatorial codes for signaling diversity
• Spatial regulation of signaling complexes
  • Temporal dynamics of cell signaling
• Discovering new signaling pathways
• Discovering ligands for orphan receptors
• Structural solutions to membrane receptors
  • Mechanistic structural studies on signaling molecules
• Genetic approaches to hormone action
  • Hormone action and human disease
  • Rationale drug design

Additional Readings on Steroid Hormone Action

• Tsai and O'Malley (1994) Molecular mechanisms of action of steroid/thyroid hormone
• Rosenfeld and Glass (2000) Coregulator codes of transcriptional regulation by nuclear receptors. J
• McKenna and O'Malley (2002) Combinatorial control of gene expression by nuclear receptors and
• Katzenellenbogen et al (2000) Estrogen receptors: selective ligands, partners and distinctive
  Bioessays 24:744.
  Cancer Cell 1:235.
  68:559.
• Chawla et al (2001) Nuclear receptors and lipid physiology: opening the X-files. Science
  294:1867.
  Endocrinol 16:1135.
• Yudt and Cidlowski (2002) The glucocorticoid receptor: coding a diversity of proteins and responses
  57:339.