# Estrogen and Estrogen Receptors

Estrogen as a Hormone
Estrogen Receptors as Mediators of Estrogen Responses
Estrogens and Estrogen Receptors in Breast Cancer

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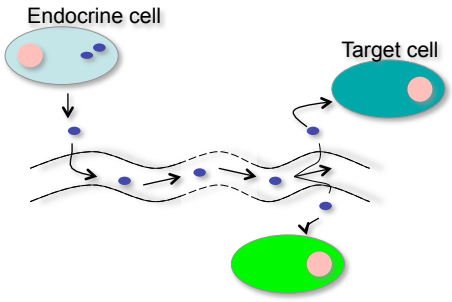
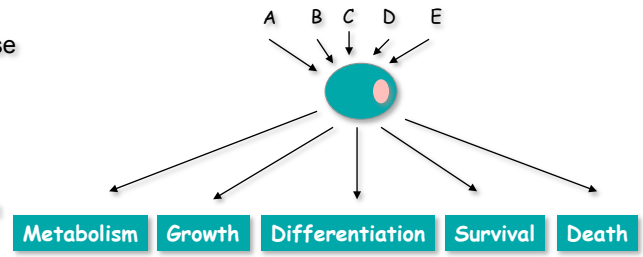
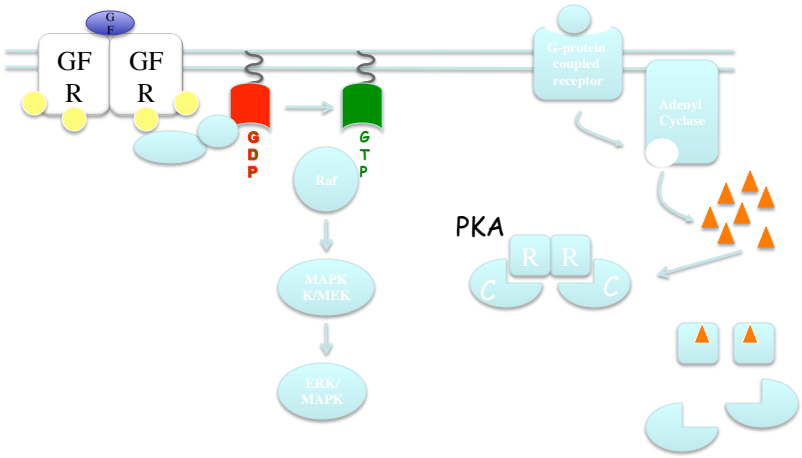
# Hormones

- What is a *hormone*?
  - Insulin
  - Growth Hormone
  - LH, FSH
  - *ESTROGEN*
  - Progesterone
- What do hormones do?
  - Regulate cellular processes
- Estrogens in Human Disease
  - Breast Cancer
  - Uterine Cancer
  - Ovarian Cancer
  - Osteoporosis
  - Cardiovascular System

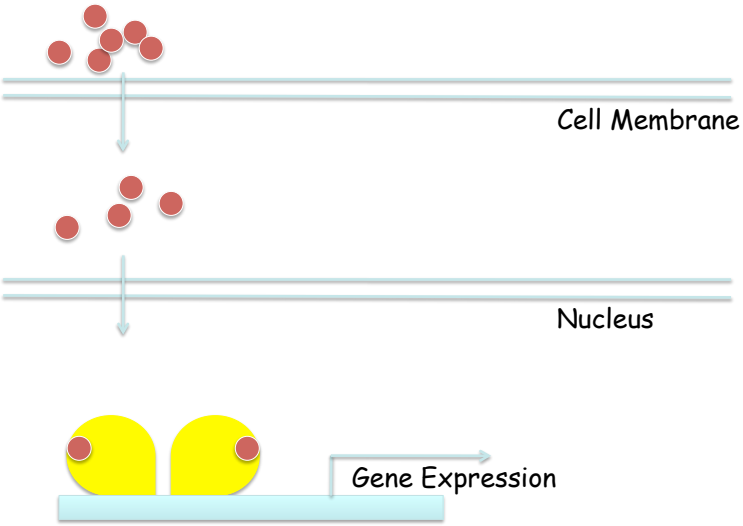


## Rapid Signaling from the Cell Surface



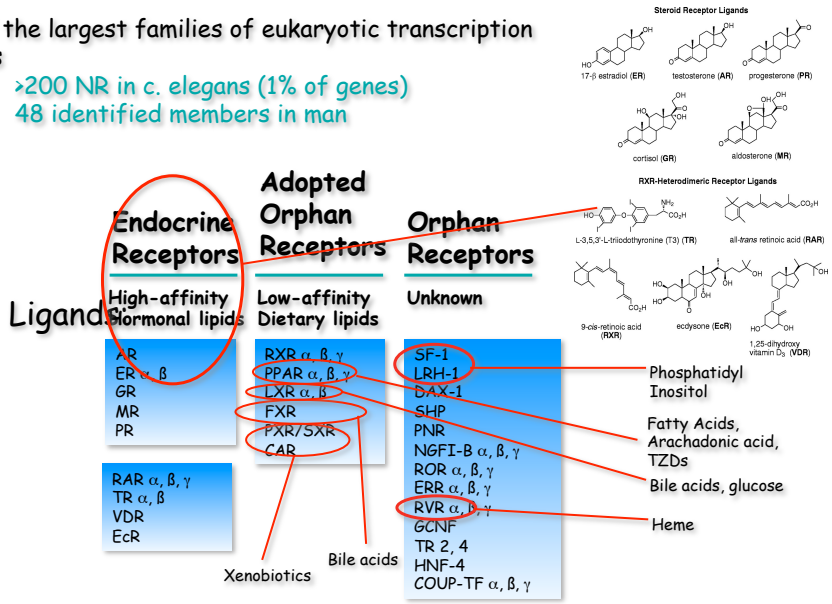

## Estrogen Signalling Occurs through Regulation of Gene Expression Estrogen Receptors



# Nuclear Receptor Superfamily

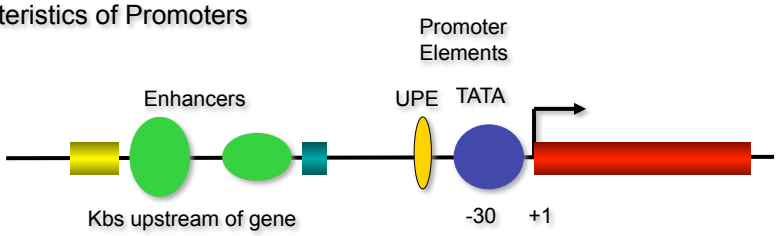
One of the largest families of eukaryotic transcription factors

>200 NR in c. elegans (1% of genes)
48 identified members in man

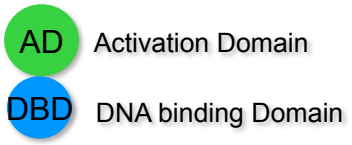



# Transcriptional Regulation of Protein Coding genes

Characteristics of Promoters

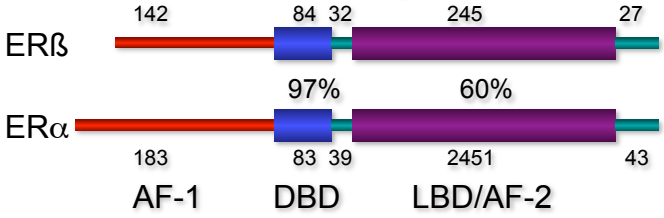


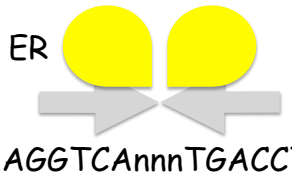

Characteristics of a Transcription Factor

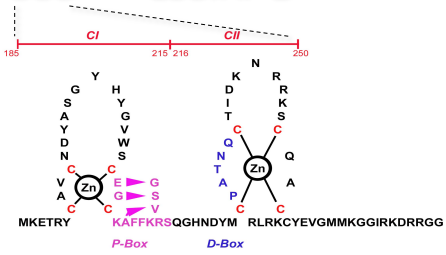



Signal Sensor
Regulates transcription

# Mechanisms of Action of Estrogen Receptors



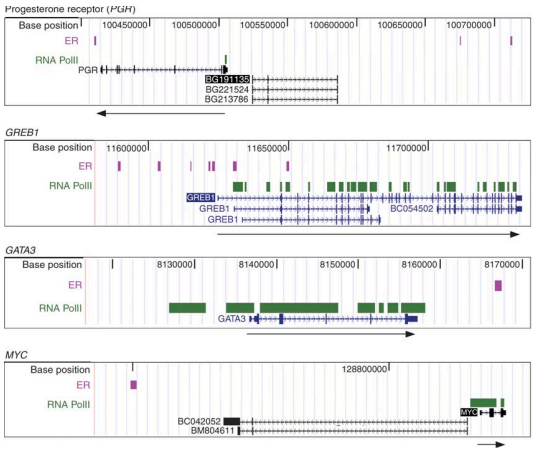





*Welboren et al 2009 EMBO J 28: 1418-1428*

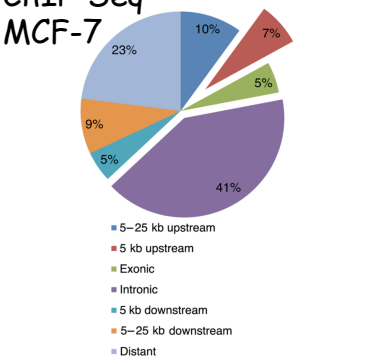
# DNA Binding by the Estrogen Receptor

## ChIP-chip (MCF-7 cells)




*Carroll et al 2006 Nature Genetics 38: 1289-1297*

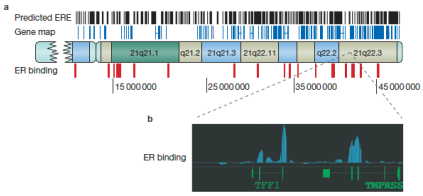

## ChIP-Seq MCF-7




*Welboren et al 2009 EMBO J 28: 1418-1428*

# DNA Binding by the Estrogen Receptor

70,000 EREs in the human genome can be identified by sequence analysis
→ 15,000 of these map within 15 kb of transcription start sites
→ 660 of these conserved in human and mouse genomes.



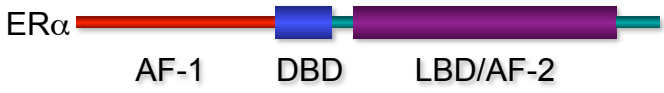

Graphical representation of binding sites of the oestrogen receptor on chromosome 21, as determined by ChIP-on-chip



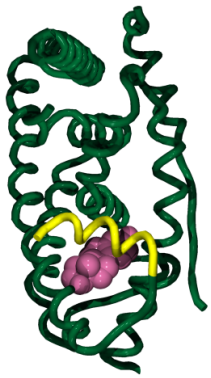
ChIP-Seq showed that the human genome (MCF-7 cells) have:
10,205 ER binding sites
68% had EREs
596 genes (59% up, 41% down)

*Bourdeau et al. 2004 Mol Endocrinol. 18: 1411-1427; Carroll et al 2006 Nature Genetics 38: 1289-1297; Welboren et al 2007 Mol Oncology 1: 138-143; Dietz & Carroll 2008 Expert Reviews in Molecular Medicine; Welboren et al 2009 EMBO J 28: 1418-1428*

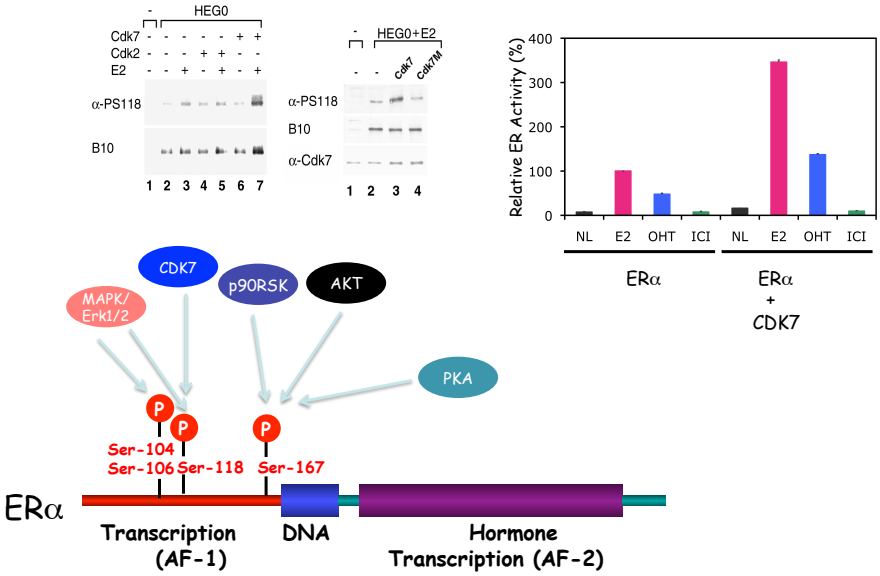
# Mechanisms of Action of Estrogen Receptors




- Estrogen binds to the Hormone Binding Domain, aka Ligand Binding Domain (LBD)
- Ligand binding results in Receptor Activation
- This involves enables the receptor binding to EREs in promoters of estrogen responsive genes and consequent stimulation of gene expression

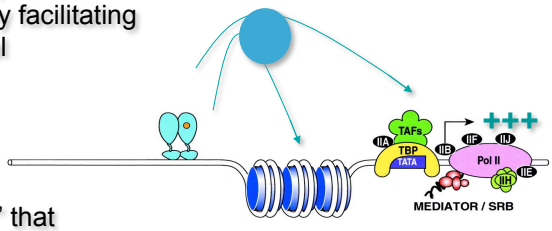


# Mechanisms of Action of Estrogen Receptors




# Transcription Regulation by Estrogen Receptors

- Liganded Estrogen Receptor stimulates transcription by facilitating recruitment of the general transcription machinery

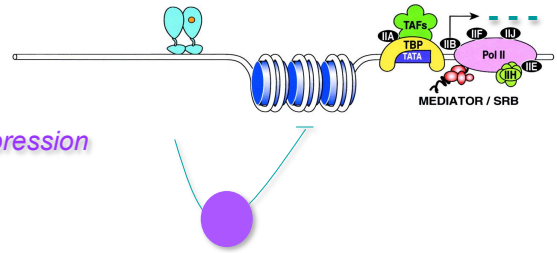



- ER recruits "*co-activators*" that remodel chromatin
  - enables strong *stimulation of transcription*

*Adapted from Glass & Rosenfeld (2000) Genes & Dev. 14: 121*

## Transcription Regulation by Estrogen Receptors

- Antagonist-occupied estrogen receptor recruits "*co-repressors*" that remodel chromatin




- to *repress gene expression*

*Adapted from Glass & Rosenfeld (2000) Genes & Dev. 14: 121*

## How does Estrogen Receptor-α Regulate Gene Expression?

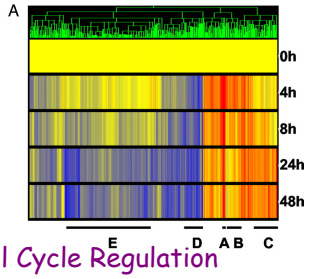
ERα is a transcription factor.

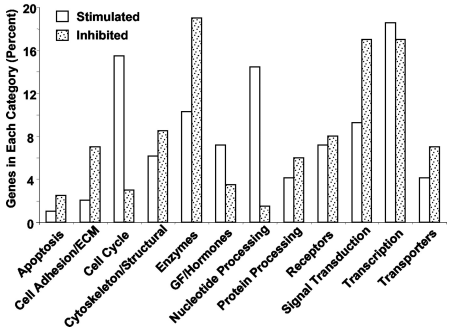
What kind of genes are likely to be estrogen-regulated?

- Transcription factors
- Cell Cycle Regulation
- DNA Replication
- Growth Factors

# Gene Profiling for Estrogen-Responsive Genes

## Gene Expression Microarray Analysis of MCF7 breast Cancer Cell Line




## Cell Cycle Regulation

TABLE 2. E2 up-regulated (top) and down-regulated (bottom) genes involved in cell cycle and apoptosis

| 4 h | 8 h | 24 h | 48 h | Gene name | GenBank no. |
|---|---|---|---|---|---|
| 5.6 | | 0.9 | 1.3 | Cyclin D1 | NM_053056 |
| 3.1 | 3.7 | 3.6 | 3.0 | *Cell division cycle 6 homolog* | NM_001254 |
| 1.0 | 1.2 | 3.0 | | *sion cycle 2* | NM_001786 |
| 0.9 | 1.3 | 2.6 | | *efficient 5* | NM_006739 |
| 0.8 | 1.3 | 2.6 | | *lymerase, α* | NM_002689 |
| 1.2 | 1.8 | 2.5 | | *efficient 2* | NM_004526 |
| 1.0 | 1.2 | 2.4 | | ase-associated tyrosine- and threonine- | NM_04268 |
| | | | | fic cdc2-inhibitory kinase (Myt1) | |
| 1.0 | 1.4 | 2.3 | | *efficient 3* | NM_002388 |
| 0.9 | 1.2 | 2.3 | | 13 | NM_001267 |
| 1.0 | 1.8 | 2.1 | | *sion factor C4* | NM_002916 |
| 1.1 | 1.5 | 2.1 | | *ssociating cell nuclear antigen* | NM_002592 |
| 0.9 | 1.7 | 2.0 | 1.6 | DNA polymerase, ε2 | NM_002692 |
| 1.1 | 0.9 | 2.0 | 3.6 | *Survivin* | NM_001168 |
| 0.7 | 1.1 | 1.7 | 2.5 | *Cell division cycle 20 homolog* | NM_001255 |
| 1.2 | 0.9 | 2.0 | 2.4 | BUBr budding uninhibited by benzimidazoles | NM_004336 |
| | | | | 1 homolog | |
| 0.9 | 0.9 | 1.4 | 2.2 | Serine/threonine kinase 6 (AIK) | NM_003600 |
| | | | | *BCL2-antagonist/killer 1* | NT_007592 |
| 0.4 | | | | BCL2-interacting killer (apoptosis-inducing) | NM_001197 |
| 0.4 | | | | *BTG family, member 2* | NM_006763 |
| 0.4 | | | | Cyclin G2 | NM_004354 |
| 0.5 | | | | *Cyclin-dependent kinase inhibitor 1A (p21, Cip1)* | NM_000389 |
| 0.8 | 0.3 | 0.3 | 0.3 | *Immediate early response 3* | NM_003815 |
| 0.6 | 0.4 | 0.4 | 0.5 | Retinoblastoma-like 2 (p130) | NM_005611 |
| 0.7 | 0.7 | 0.4 | 0.6 | *Checkpoint suppressor 1* | NM_005197 |
| 0.7 | 0.6 | 0.5 | 0.4 | BTG family, member 1 | NM_017351 |
| 1.0 | 0.6 | 0.5 | 0.7 | CASP2 and RIPK1 domain containing adaptor | NM_003805 |
| | | | | with death domain | |
| 0.6 | 0.6 | 0.5 | 0.8 | Caspase 9, apoptosis-related cysteine protease | NM_001229 |

Values are fold change at each time point relative to control and are organized by time course of regulation (early to late), with the first time of significant regulation *boxed*. Gene names in *bold* appear to be novel and not previously known to be E2 regulated. Those in *italics* have been verified by real-time PCR.

Cyclin D1

DNA Pol α
Cyclin A2
PCNA
Survivin

Bcl2 Antagonists
P21, Cip1

Frasor et al 2003

## Growth Factors/Cytokines

TABLE 3. E2 up-regulated (top) and down-regulated (bottom) genes that encode growth factors, cytokines, and hormones

| 4 h | 8 h | 24 h | 48 h | Gene name | GenBank no. |
|---|---|---|---|---|---|
| 5.1 | | | | *IGF-binding protein 4* | NM_001552 |
| 4.9 | | | | *Amphiregulin* | NM_001657 |
| 3.9 | | | | Chemokine ligand 12 (stromal cell-derived factor 1) | NM_000609 |
| 2.8 | | | | VEGF | NM_003376 |
| 2.2 | | | | Stanniocalcin 2 | NM_003714 |
| 1.3 | | | | *WNT1-inducible signaling pathway protein 2* | NM_003881 |
| 0.9 | | | | IGF-binding protein 5 | NM_000599 |
| 0.5 | | 1.0 | 0.7 | IL-4 | NM_000589 |
| 0.8 | | 0.4 | 0.5 | Pre-B-cell colony-enhancing factor | NM_005746 |
| 0.6 | | 0.5 | 0.4 | TGFβ3 | NM_003239 |
| 0.7 | | 0.5 | 0.5 | Platelet-derived growth factor β polypeptide | NM_002608 |
| 0.6 | | 0.6 | 0.3 | BMP4 | NM_001202 |
| 0.9 | | 0.6 | 0.2 | Stanniocalcin 1 | NM_003155 |
| 0.8 | | 0.6 | 0.2 | Inhibin, βB (activin AB$_\beta$ polypeptide) | NM_002193 |

Values are fold change at each time point relative to control and are organized by time course of regulation (early to late), with the first time of significant regulation *boxed*. Gene names in *bold* appear to be novel and not previously known to be E2 regulated. Those in *italics* have been verified by real-time PCR.

IGFBP4
Amphiregulin
Chemokine ligand 12
VEGF

TGFβ3
PDGFβ
BMP4
Inhibin

# Gene Profiling for Estrogen-Responsive Genes

## Signal Transduction

TABLE 4. E2 up-regulated (top) and down-regulated (bottom) genes that encode receptors and signal transduction proteins

| 4 h | 8 h | 24 h | 48 h | Gene name | GenBank no. |
|---|---|---|---|---|---|
| 4.7 | 3.7 | 3.4 | 1.6 | Ret/Ptc | NM_000323 |
| 4.4 | 1.2 | 1.3 | 0.6 | Retinoic acid receptor α | NM_000964 |
| 4.0 | 2.8 | 3.7 | 3.7 | RAB31 | NM_006868 |
| 3.6 | 1.3 | 0.9 | 0.7 | RAS guanyl-releasing protein 1 | NM_005739 |
| 3.0 | 2.8 | 3.3 | 4.3 | *Calcitonin receptor* | NM_001742 |
| 2.8 | 2.2 | 2.3 | 1.8 | Ret/Ptc1 | |
| 2.6 | 1.4 | 1.6 | 2.1 | Adenylate cyclase 9 | NM_001116 |
| 2.6 | 1.9 | 1.3 | 1.5 | Growth arrest and DNA damage-inducible β | NM_015675 |
| 2.5 | 2.9 | 1.6 | 1.5 | Glycine receptor β | NM_000824 |
| 2.3 | 1.4 | 1.6 | 1.4 | Tetraspan 5 | NM_005723 |
| 2.1 | 1.8 | 2.7 | 2.1 | *Prostaglandin E receptor 3* | NM_000957 |
| 2.0 | 1.1 | 0.9 | 0.9 | Sterile-related serine/threonine kinase | NM_014720 |
| 2.0 | 1.4 | 1.3 | 1.5 | Osteoclast-stimulating factor 1 | NM_012383 |
| 1.9 | 0.9 | 1.8 | 1.7 | Janus kinase 1 | NM_002227 |
| 1.5 | 1.9 | 2.7 | 2.6 | Protein phosphatase 3, regulatory subunit Aβ | NM_002716 |
| 1.5 | 1.8 | 4.0 | 1.9 | Chemokine-binding protein 2 | NM_001296 |
| 0.3 | 0.3 | 0.3 | 0.2 | *B-cell linker* | NM_013314 |
| 0.4 | 0.6 | 0.6 | 0.4 | *Chemokine (C-X-C motif) receptor 4* | NM_003467 |
| 0.4 | 0.4 | 0.3 | 0.2 | *Erythropoietin receptor* | NM_000121 |
| 0.4 | 0.1 | 0.0 | 0.0 | *IL-1 receptor, type I* | NM_000877 |
| 0.4 | 0.4 | 0.3 | 0.3 | Protein tyrosine phosphatase, nonreceptor type 12 | NM_002836 |
| 0.4 | 0.3 | 0.2 | 0.1 | *Dual specificity phosphatase 4* | NM_001394 |
| 0.4 | 0.6 | 0.6 | 0.8 | Serine/threonine kinase 3 (STE20 homolog) | NM_006281 |
| 0.5 | 0.7 | 0.6 | 0.6 | CDC42 effector protein (Rho GTPase binding) 4 | NM_012121 |
| 0.5 | 0.5 | 0.6 | 0.5 | Growth factor receptor-bound protein 10 | NM_005311 |
| 0.5 | 0.6 | 0.5 | 0.5 | Phosphoinositide-3-kinase, regulatory subunit, polypeptide 3 | NM_005029 |
| 0.5 | 0.5 | 0.5 | 0.5 | Cas-Br-M (murine) ecotropic retroviral transforming sequence b | NM_004351 |
| 0.5 | 0.4 | 0.5 | 0.5 | MAPK kinase 6 | NM_002758 |
| 0.8 | 0.4 | 0.4 | 0.5 | *Notch homolog 3 (Drosophila)* | NM_000435 |
| 0.6 | 0.4 | 0.3 | 0.1 | Amnesia Ab | NM_001139 |
| 0.6 | 0.4 | 0.3 | 0.3 | Cornuckle virus and adenovirus receptor | NM_001338 |
| 0.7 | 0.4 | 0.3 | 0.4 | Dual specificity phosphatase 1 | NM_004417 |
| 0.6 | 0.4 | 0.3 | 0.2 | Serine/threonine kinase 6 | NM_006282 |
| 0.6 | 0.4 | 0.3 | 0.2 | *RAB3B, GTP-binding protein 1* | NM_002866 |
| 0.6 | 0.5 | 0.5 | 0.4 | Phosphatidylinositol (4,5) bisphosphate 5-phosphatase, A | NM_004325 |
| 0.8 | 0.6 | 0.5 | 0.6 | IQ motif containing GTPase-activating protein 1 | NM_003870 |
| 0.7 | 0.5 | 0.5 | 0.2 | Retinoic acid-induced 3 | NM_003979 |
| 0.8 | 0.5 | 0.5 | 0.5 | *v-rel-b2* | NM_004445 |
| 0.9 | 0.8 | 0.3 | 0.7 | T cell receptor γ locus | NG_001336 |
| 0.9 | 0.6 | 0.3 | 0.2 | Parvalbumin | NM_002854 |
| 0.6 | 0.9 | 0.3 | 0.4 | Interferon-γ receptor 2 (interferon-γ transducer 1) | NM_005534 |
| 0.6 | 0.6 | 0.4 | 0.4 | RABβA, member RAS oncogene family | NM_004251 |
| 0.6 | 0.6 | 0.4 | 0.5 | Dinculin domain receptor family, member 1 | NM_001854 |
| 1.0 | 0.7 | 0.4 | 0.6 | PTEN-induced putative kinase 1 | NM_032409 |
| 0.6 | 0.6 | 0.4 | 0.5 | Protein phosphatase 1, regulatory (inhibitor) subunit 3C | NM_005398 |
| 0.7 | 0.6 | 0.4 | 0.5 | Son of sevenless homolog 2 (*Drosophila*) | NT_025892 |
| 0.7 | 0.6 | 0.4 | 0.5 | RABβA, member RAS oncogene family | NM_002869 |
| 0.8 | 0.6 | 0.4 | 0.5 | Low density lipoprotein receptor | NM_000527 |
| 0.8 | 0.6 | 0.4 | 0.5 | Syndecan 4 (amphiglycan, ryndecan) | NM_002999 |
| 0.7 | 0.9 | 0.4 | 0.8 | TGFβ receptor II | NM_003242 |
| 1.0 | 0.7 | 0.4 | 0.8 | Inositol hexaphosphate kinase 1 | NM_153279 |
| 0.9 | 0.6 | 0.4 | 0.9 | Dual specificity phosphatase 5 | NM_004090 |
| 0.7 | 0.6 | 0.5 | 0.6 | Retinoid X receptor α | NM_002957 |
| 0.9 | 0.7 | 0.4 | 0.5 | Protein tyrosine phosphatase, receptor type, K | NM_002844 |
| 0.9 | 0.6 | 0.5 | 0.5 | Leptin receptor gene-related protein | NM_017526 |
| 0.7 | 0.8 | 0.5 | 0.8 | Reticulocalbin 2, EF-hand calcium-binding domain | NM_002902 |
| 0.6 | 0.7 | 0.5 | 0.6 | Phosphoinositide-3-kinase, class 2, β polypeptide | NM_002646 |
| 0.7 | 0.6 | 0.5 | 0.6 | *v-rel-b2*, homolog 2 (SRES) | NM_001982 |
| 0.6 | 0.7 | 0.5 | 0.4 | *ras* homolog gene family, member E | NM_005168 |
| 0.8 | 0.8 | 0.6 | 1.0 | Dual specificity tyrosine (Y) phosphorylation-regulated kinase 2 | NM_003583 |
| 0.9 | 0.8 | 0.5 | 0.7 | Serine/threonine kinase 10 | NM_005891 |
| 0.8 | 0.6 | 0.5 | 0.5 | Phosphoinositol 3-phosphate-binding protein-3 | NM_014935 |
| 0.9 | 0.6 | 0.7 | 0.5 | GTP cyclohydrolase 1 feedback regulatory protein | NM_005283 |

Values are fold change at each time point relative to control and are organized by time course of regulation (early to late), with the first time of significant regulation *boxed*. Gene names in *bold* appear to be novel and not previously known to be E2 regulated. Those in *italics* have been verified by real-time PCR.

## Transcriptional Regulators

TABLE 5. E2 up-regulated (top) and down-regulated (bottom) genes that encode transcription factors and transcriptional coregulators

| 4 h | 8 h | 24 h | 48 h | Gene name | GenBank no. |
|---|---|---|---|---|---|
| 15.5 | | | | ; C4 | NM_153633 |
| 6.6 | | | | ng factor, runt domain, α-subunit 2; translocated to, 3 | NM_005187 |
| 4.1 | | | | *response 3* | NT_008300 |
| 3.8 | | | | | NM_006252 |
| 3.6 | | | | *ptor interacting protein 1* | NM_003489 |
| 3.5 | | | | ster 1 | NT_009984 |
| 3.1 | | | | ; C6 | NM_004503 |
| 2.8 | | | | ; C8 | NM_018653 |
| 2.3 | 2.0 | 1.5 | 1.1 | c-myc | NM_002467 |
| 2.3 | 1.4 | 1.3 | 1.8 | TGFβ-induced factor 2 | NM_021809 |
| 2.3 | 1.7 | 1.8 | 1.9 | ELL-related RNA polymerase II elongation factor | NM_012081 |
| 2.2 | 2.3 | 2.0 | 1.5 | TATA element modulatory factor 1 | NM_007114 |
| 2.1 | 2.1 | 1.9 | 1.9 | *c-myb* | NM_005375 |
| 2.1 | 2.1 | 1.8 | 2.1 | Retinoblastoma-binding protein 7 | NM_002893 |
| 2.0 | 1.0 | 0.6 | 0.4 | | NT_008186 |
| 1.7 | 1.2 | 2.8 | 3.1 | BRCA1 | NM_003466 |
| 1.0 | 1.6 | 2.6 | 3.0 | er 2, early onset | NM_000059 |
| 0.9 | 1.7 | 1.9 | 2.7 | er 1, early onset | NM_007294 |
| 0.5 | 0.4 | 0.4 | 0.4 | MAD, mothers against decapentaplegic homolog 6 | NM_005585 |
| 0.4 | 0.5 | 0.4 | 0.3 | Core promoter element-binding protein | NM_012300 |
| 0.4 | 0.5 | 0.3 | 0.3 | Inhibitor of DNA binding 2 | NM_002166 |
| 0.4 | 0.7 | 0.5 | 0.4 | *Nuclear receptor coactivator 2* | NM_006540 |
| 0.4 | 0.4 | 0.5 | 0.2 | Nuclear receptor subfamily 2, group C, member 1 | NM_002297 |
| 0.5 | 0.7 | 0.7 | 0.8 | *Transcriptional intermediary factor 1* | NM_003852 |
| 0.6 | 0.4 | 0.4 | 0.3 | Aryl-hydrocarbon receptor nuclear translocator 2 | NM_014862 |
| 0.8 | 0.4 | 0.6 | 0.7 | *junB* protooncogene | NM_002229 |
| 0.8 | 0.4 | 0.1 | 0.4 | MAX dimerization protein 4 | NM_006454 |
| 0.6 | 0.5 | 0.4 | 0.4 | Interferon regulatory factor 6 | NM_006147 |
| 0.6 | 0.5 | 0.3 | 0.4 | *Special AT-rich sequence-binding protein 1* | NM_002971 |
| 0.7 | 0.5 | 0.7 | 0.5 | δ sleep-inducing peptide, immunoreactor | NM_004989 |
| 0.7 | 0.5 | 0.5 | 0.5 | *HMG box-containing protein 1* | NM_012257 |
| 0.7 | 0.5 | 0.5 | 0.5 | LPS-induced TNF-α factor | NM_004903 |
| 0.8 | 0.5 | 0.4 | 0.5 | Basic helix-loop-helix domain containing, class B, 2 (DEC1) | NM_003670 |
| 0.8 | 0.5 | 0.4 | 0.5 | SRY (sex determining region Y)-box 9 | NM_000346 |
| 1.0 | 0.6 | 0.3 | 0.1 | Aml1-Evi-1 | S69002 |
| 0.6 | 0.7 | 0.3 | 0.3 | Nuclear factor related to κB-binding protein | NM_006185 |
| 0.6 | 0.6 | 0.4 | 0.4 | Inhibitor of DNA binding | NM_002165 |
| 0.6 | 0.6 | 0.4 | 0.5 | *Proline rich 2* | NM_006813 |
| 0.7 | 0.6 | 0.4 | 0.5 | *MAD, mothers against decapentaplegic homolog 3* | NM_005902 |
| 0.7 | 0.6 | 0.4 | 0.4 | Zinc finger protein 217 | NM_006526 |
| 0.6 | 0.6 | 0.5 | 0.6 | *Nuclear receptor coactivator 3* | NM_006534 |
| 0.6 | 0.6 | 0.5 | 0.6 | TGFβ-stimulated protein TSC-22 | NM_006022 |
| 0.7 | 0.6 | 0.5 | 0.4 | Basic transcription element-binding protein 1 (KLF9) | NM_001206 |
| 0.7 | 0.6 | 0.5 | 0.4 | Pre-B-cell leukemia transcription factor 1 | NM_002585 |
| 0.8 | 0.6 | 0.5 | 0.9 | Regulatory factor X, 5 | NM_000449 |
| 0.8 | 0.6 | 0.5 | 0.5 | RING1 and YY1-binding protein | NM_012234 |
| 0.7 | 0.7 | 0.5 | 0.5 | Hepatocyte nuclear factor 3, α | NM_004496 |
| 0.8 | 0.7 | 0.5 | 0.7 | Ets1 repressor factor | NM_005404 |
| 0.9 | 0.7 | 0.5 | 0.4 | Forkhead box O4A | NM_001455 |
| 0.9 | 0.8 | 0.5 | 0.6 | Thyroid transcription factor 1 | NM_003317 |
| 1.1 | 0.8 | 0.5 | 0.6 | Zinc finger protein 36, C3H type-like 2 | NM_006887 |
| 0.8 | 0.9 | 0.6 | 0.5 | Iroquois homeobox protein 5 | NM_005853 |
| 0.8 | 0.8 | 1.0 | 0.5 | Hairy homolog (*Drosophila*) | NM_005524 |

Values are fold change at each time point relative to control and are organized by time course of regulation (early to late), with the first time of significant regulation *boxed*. Gene names in *bold* appear to be novel and not previously known to be E2 regulated. Those in *italics* have been verified by real-time PCR.

Homeo box C4, C6, C5
c-fos
c-myc
myb

BRCA1
BRCA2

## What kind of Genes are Estrogen-Regulated?

ERα is a transcription factor.

What kind of genes are likely to be estrogen-regulated?

- Transcription factors
- Cell Cycle Regulation
- DNA Replication
- Growth Factors

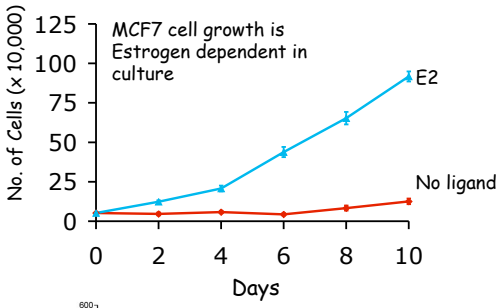
The expression of some genes is activated by estrogen
Other genes are repressed by estrogen

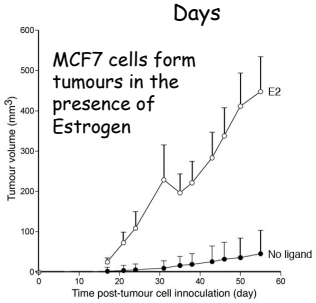
The expression of some genes is rapidly altered by estrogen
The regulation of other genes is slower

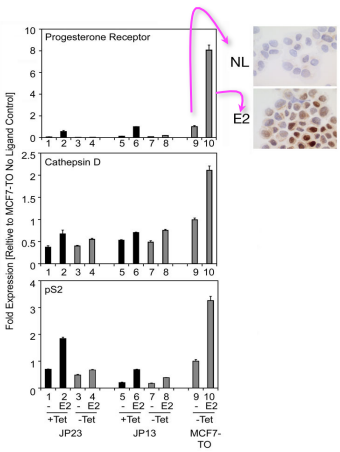
- 'Weaker' promoters?
- Indirect regulation? These genes may be regulated by other estrogen-responsive genes

Thus, estrogen responses are mediated by estrogen receptors acting to provide coordinate regulation of a large number of genes - involving stimulation and inhibition of gene expression

## How does Estrogen Receptor-α Regulate Gene Expression?



## Summary

- Estrogen Action is Mediated by Estrogen Receptors
- Ligand binding is mediated by the Ligand binding Domain (LBD) aka Hormone Binding Domain
- Transcription Regulation by NR is mediated by two transcription activation functions – AF1, AF-2
- In many cases AF-1 activity is regulated by phosphorylation – e.g. ER
- AF-2 activity requires ligand binding.
- Ligand binding promotes a conformational change that "activates" the LBD
- ER regulates gene expression by binding to Estrogen Response Elements in promoters of regulated genes – often EREs are located far from transcription start sites
- Gene Expression by Estrogen Receptors requires the co-ordinated recruitment of many transcriptional co-regulator protein complexes that modify and remodel chromatin, to allow transcriptional effects

## Estrogen Action in Normal Physiology and in Human Disease

## Effects of Estrogen Receptor Gene Knockout in Reproduction

| Tissue | α | ß | αß |
|---|---|---|---|
| Mammary | Immature-ductal rudiment | Normal structure | Immature-ductal rudiment |
| Fertility | Males infertile<br>Females infertile | Males fertile | Both sexes infertile<br>Females subfertile<br>infrequent pregnancies<br>small litter sizes |
| Pituitary | LH production elevated<br>Prolactin low | Normal | LH production elevated |
| Ovary | Elevated estrogen, T;<br>Follicles don't mature;<br>hemorrhagic cystic follicles<br>begin developing at puberty<br>due to LH elevation | Reduced no. of corpora lutea, inefficient ovulation<br>Normal gonadotrophin and steroid levels | Progressive degeneration of germ cells, loss of granulosa cells;<br>Elevated LH, estrogen, Testosterone |
| Uterus | Immature. Insensitive to estrogen, no epithelial proliferation<br><br>No implantation | Normal responses to estrogen<br><br>Pregnancies occur and are carried to term | Like αERKO<br><br>Like αERKO |
| Testes | Progressive fluid retention and dilation of semineferous tubules, eventaul loss of sperm | Normal | Like αERKO |

## Effects of Estrogen Receptor Gene Knockout in Reproduction

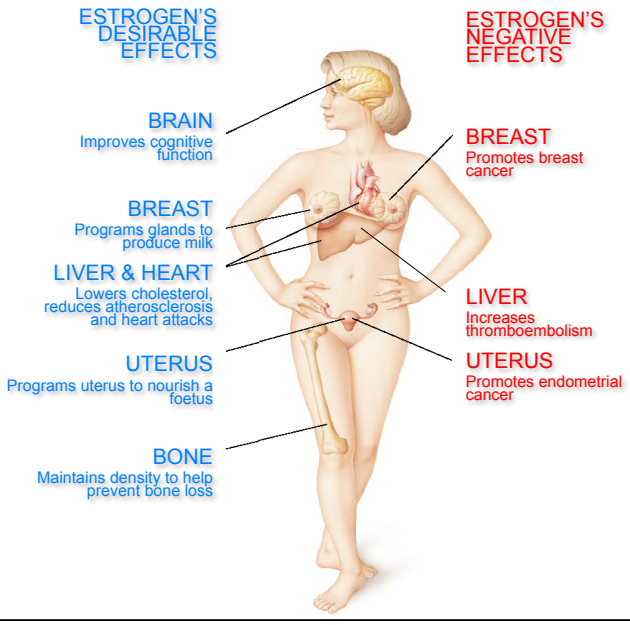
Aromatase KO (ArKO) Mice:

Immature mammary glands,
Immature uteri,
haemorrhagic cysts,
males infertile due to loss of spermatids
Deficiencies is sexual behaviour in males (mounting) - also seen in αßERKO

# Estrogen Target Tissues

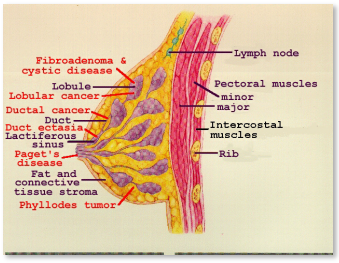
*Osteoporosis*:
Estrogen is important to maintain bone in premenopausal women. After menopause, hormone replacement therapy is often recommended to prevent the development of osteoporosis.

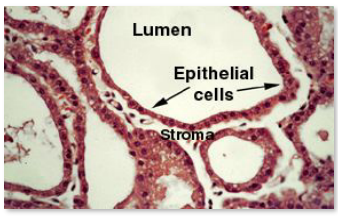
*Atherosclerosis*:
Estrogen lowers low-density lipoprotein (LDL) cholesterol levels and raises high-density lipoprotein (HDL) cholesterol levels. Following menopause, women are at the same risk for coronary heart disease as men.

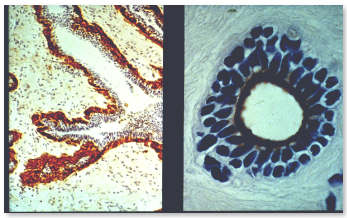



# Breast Cancer Development

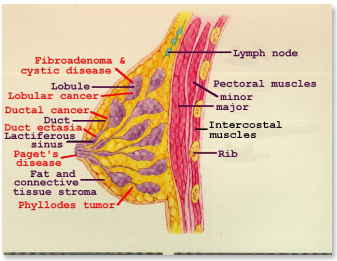
Breast cancer: Carcinoma [tumour of epithelial cells]

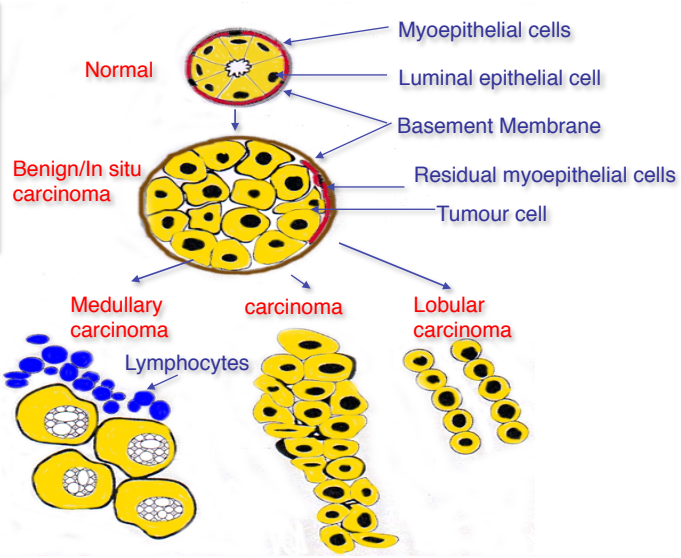


# Schematic diagram of the progression of normal to malignant breast




# Breast Cancer - the Statistics

| Cancer Site | Cases | Percentage of All Cases |
|---|---|---|
| Lung (C33-34) | 1,608,055 | 12.7 |
| Breast (C50) | 1,384,155 | 10.9 |
| Colorectum* (C18-21) | 1,235,108 | 9.8 |
| Stomach (C16) | 988,602 | 7.8 |
| Prostate (C61) | 899,102 | 7.1 |
| Liver (C22) | 749,744 | 5.9 |
| Cervix Uteri (C53) | 530,232 | 4.2 |
| Oesophagus (C15) | 481,645 | 3.8 |
| Bladder (C67) | 382,660 | 3 |
| Non-Hodgkin Lymphoma (C82-85 and C96) | 356,431 | 2.8 |
| Leukaemia (C91-95) | 350,434 | 2.8 |
| Corpus Uteri (C54) | 288,387 | 2.3 |
| Pancreas (C25) | 278,684 | 2.2 |
| Kidney (C64-66) | 273,518 | 2.2 |
| Lip and Oral Cavity (C00-08) | 263,020 | 2.1 |
| Brain and Central Nervous System (C70-72) | 237,913 | 1.9 |
| Ovary (C56) | 224,747 | 1.8 |
| Thyroid (C73) | 213,179 | 1.7 |
| Malignant Melanoma (C43) | 199,627 | 1.6 |
| Larynx (C32) | 150,677 | 1.2 |
| Other Sites | 1,566,634 | 12.4 |

| Cancer Site | Deaths | Percentage of All Cancer Deaths |
|---|---|---|
| Lung (C33-34) | 1,376,579 | 18.2 |
| Stomach (C16) | 737,419 | 9.7 |
| Liver (C22) | 695,726 | 9.2 |
| Colorectum* (C18-21) | 609,051 | 8.1 |
| Breast (C50) | 458,503 | 6.1 |
| Oesophagus (C15) | 406,533 | 5.4 |
| Cervix Uteri (C53) | 275,008 | 3.6 |
| Pancreas (C25) | 266,669 | 3.5 |
| Prostate (C61) | 258,133 | 3.4 |
| Leukaemia (C91-95) | 257,161 | 3.4 |
| Non-Hodgkin Lymphoma | 191,599 | 2.5 |
| Brain and Central Nervous System | 174,880 | 2.3 |
| Bladder (C67) | 150,282 | 2 |
| Ovary (C56) | 140,163 | 1.9 |
| Lip and Oral Cavity (C00-08) | 127,654 | 1.7 |
| Kidney (C64-66) | 116,368 | 1.5 |
| Gallbladder (C23-24) | 109,587 | 1.4 |
| Other Pharynx (C09-10 and C12-14) | 95,550 | 1.3 |
| Larynx (C32) | 81,892 | 1.1 |
| Corpus Uteri (C54) | 73,854 | 1 |
| Other Sites | 962,191 | 12.7 |

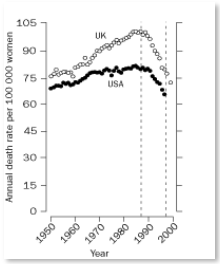
| Estimated risk at birth up to and including: | UK (2008) |
|---|---|
| age 29 | 1 in 2,000 |
| age 39 | 1 in 215 |
| age 49 | 1 in 50 |
| age 59 | 1 in 22 |
| age 69 | 1 in 13 |
| **Lifetime risk** | **1 in 8** |

- Breast cancer is the leading female cancer, accounting for almost 1 in 5 cancer deaths among women.
- 1 in 9 women in the UK and the USA will develop the disease in their lifetime.
- Breast cancer is the commonest single cause of death among women aged 35-54 years.
- In 2008: 48, 034 women developed breast cancer
- In 2008: 12, 047 women died of the disease

CANCER RESEARCH UK

## Breast Cancer - the Statistics

- Breast cancer incidence is rising.
  - 1979: 75 cases per 100, 000
  - 2000: 114 cases per 100, 000
  - A 46% increase in the incidence rate from 1979-2000




Peto *et al.* (2000) Lancet 355: 1822.

- However, mortality rates are falling.
  - 1989: 42 women per 100, 000 died
  - 2002: 30 women per 100, 000 died
  - A 28% fall in deaths (1989-2002).

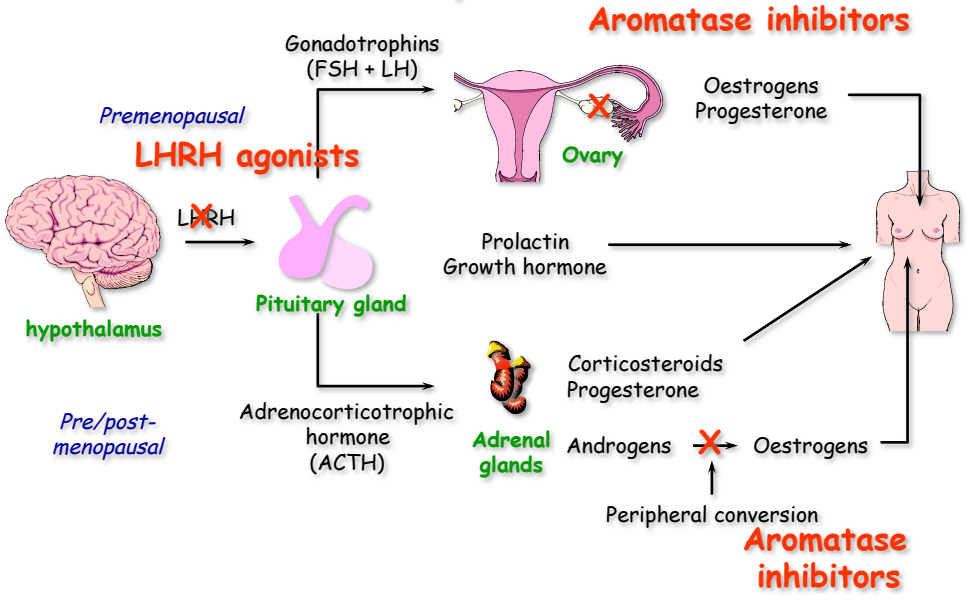
## Breast Cancer is an Hormone-Regulated Cancer

- What is the evidence that Breast Cancer is hormone-regulated?
  - Importance of Ovarian Function
    - 1870s: A. Schinzinger noted that atrophy of the breast follows cessation of ovarian function and proposed ovariectomy as a treatment for breast cancer (1870s)
    - George Beatson demonstrated that ovariectomy in pre-menopausal women resulted in disease regression and improved prognosis (1886)
  - Risk Factors - exposure to estrogens
    - Early age of onset of menarche
    - Late age to menopause
    - Age at first full-term pregnancy
    - Some forms of the contraceptive pill
    - (High estrogen-containing hormone replacement therapy)
    - (Obesity)
  - Endocrine Therapies
    - Anti-estrogens [Tamoxifen]
    - Aromatase Inhibitors [Anastrozole, Letrozole]
    - LHRH Agonists

## Breast Cancer Growth is an Hormone-Regulated Cancer

- Estrogen stimulates Breast Cancer Growth.
- Is Estrogen involved in breast cancer initiation?
  - Women with overexpression of Estrogen Receptor-α have a higher risk of developing breast cancer
  - Risk of breast cancer development in the contralateral breast is reduced by 50% in breast cancer patients receiving Tamoxifen
  - Tamoxifen prevents breast cancer development in patients at high risk
    - Tamoxifen reduces the incidence of contralateral breast cancer by 50%.
    - This has led to clinical trials investigating the utility of Tamoxifen and Raloxifene for breast cancer prevention.
    - High-risk populations have been tested.
    - Tamoxifen *vs* placebo trials showed:
      - 38% reduction in breast cancer incidence.
      - Estrogen receptor-positive breast cancers were decreased by 48%.
      - No effect for receptor-negative breast cancers.
      - Age had no effect on breast cancer reduction.

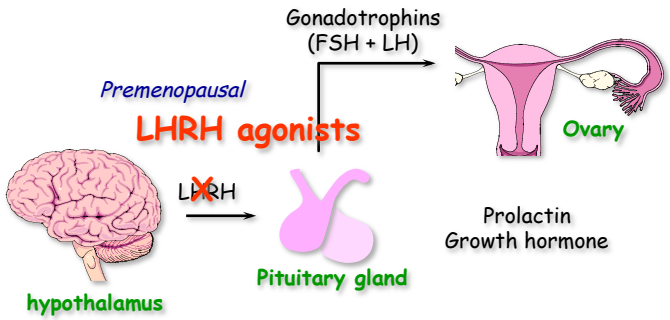
## Endocrine Therapies in Breast Cancer



# Breast Cancer Therapies Directed at Reducing Estrogen Biosynthesis

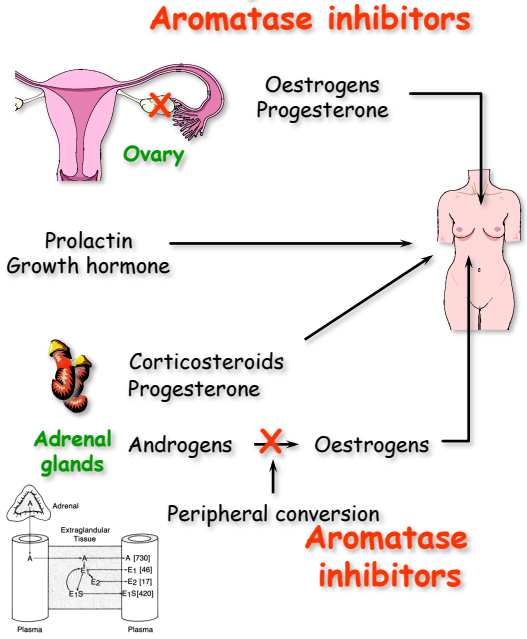
# LHRH Agonists in Breast Cancer Treatment

- LHRH Agonists such as Goserelin act by downregulating the LHRH Receptor



# Estrogen Synthesis is Mediated by Aromatase

- The Aromatase enzyme consists of a complex containing a cytochrome P450 heme containing protein as well as the flavoprotein NADPH cytochrome P450 reductase.
- Aromatase catalyzes three separate steroid hydroxylations involved in the conversion of androstenedione to estrone and testosterone to estradiol.
- Aromatase can metabolise androstenedione, which is produced by the adrenal glands. This leads to the production of Estrone Sulphate, which is circulated in the plasma.
- In pre-menopausal women the major site of estrogen synthesis is the ovary.
- In post-menopausal women androstenedione conversion occurs at peripheral sites such as fat, liver, muscle.




# Aromatase Inhibitors in Breast Cancer Treatment

A convenient classification divides the aromatase inhibitors into the mechanism-based, or suicide inhibitors (type 1) and those that are competitive inhibitors (type II).

- *Suicide inhibitors* initially compete with the natural substrate (i.e., androstenedione and testosterone) for binding to the active site of the enzyme. The enzyme, then, specifically acts on the inhibitor to yield reactive alkylating species, which form covalent bonds at or near the active site of the enzyme. Through this mechanism, the enzyme is irreversibly inactivated.
  - An example of this type of drug is “Exemestane”. Single-dose administration reveals a major reduction of plasma estrogens with this compound. Side effects associated with exemestane treatment are mild and include hot flushes, nausea, and fatigue.
- *Competitive inhibitors* bind reversibly to the active site of the enzyme and prevent product formation only as long as the inhibitor occupies the catalytic site
  - An example of this type of drug is “Anastrozole” (“arimidex”: ICI-D1033). Studies demonstrate suppression of plasma estrogen to levels approaching the limits of assay sensitivity. Anastrozole was the first aromatase inhibitor to be approved in the United States for the management of advanced breast carcinoma in postmenopausal women.

## Aromatase Inhibitors in Breast Cancer Treatment

Response and time to progression in randomized trials comparing aromatase inhibitors and tamoxifen as neoadjuvant therapy or in advanced breast cancer

| Reference | Agent administered | Number of subjects | Response (%) | Clinical benefit (%) | Time to progression (months) |
|---|---|---|---|---|---|
| [9] | Letrozole | 453 | 30 | 49 | 9.4 |
| | Tamoxifen | 454 | 20 | 38 | 6.0 |
| [13] | Letrozole | 154 | 55 | – | – |
| | Tamoxifen | 170 | 36 | – | – |
| [11] | Anastrozole | 171 | 21 | 59 | 11.1 |
| | Tamoxifen | 182 | 17 | 46 | 5.6 |
| [19] | Anastrozole | 340 | 33 | 56 | 8.2 |
| | Tamoxifen | 328 | 33 | 56 | 8.3 |
| [11] | Anastrozole | 121 | 43 | 83 | – |
| | Tamoxifen | 117 | 31 | 56 | – |
| [10] | Exemestane | 61 | 41 | 57 | – |
| | Tamoxifen | 59 | 17 | 42 | – |

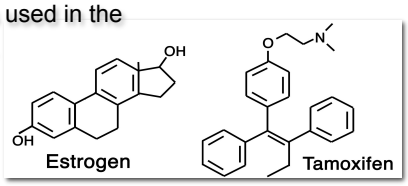
From Howell & Dowsett (2004) Breast Cancer Res. 6: 269-274

## Breast Cancer Therapies Directed at Inhibiting the Estrogen Binding by the Estrogen Receptor

## Anti-estrogens Antagonise Estrogen Action

- Anti-estrogens are synthetic molecules that compete with estrogen for binding to the estrogen receptor. Anti-estrogen binding to the estrogen receptor inhibits its activity, thereby blocking the growth stimulatory effects of estrogen on breast cancer cells.

- Lacassagne (1936) proposed the use of estrogen "antagonists", as synthetic molecules that compete with estrogen for binding to the estrogen receptor AND inhibit the activity of the receptor.
- Synthetic estrogen agonists were developed in the 1930s-1960 for use in fertility control.
- Some of these turned out to be estrogen antagonists, or antiestrogens.
- The first of these anti-estrogens, tamoxifen, is a transgeometric isomer of substituted triphenylethylene (Walpole 1966).
- Tamoxifen was approved by FDA in 1977 and is used in the treatment of pre- and post-menopausal women.




## Tamoxifen in the Treatment of Breast Cancer

- Adjuvant tamoxifen treatment for as little as 1 year reduces recurrence and death.
- Adjuvant tamoxifen for 5 years provides maximal benefit
- For patients with primary breast cancer, five years adjuvant treatment with tamoxifen provides
  - Reduced recurrence in 30 - 50% of patients
  - Reduced mortality in 25 - 30% of patients
- In metastatic breast cancer:
  - ~30% of women who present with metastatic breast cancer respond
  - In a further 20% disease stabilisation, for ~6 months.
  - However, mean response period: ~12 months
- Survival benefits with tamoxifen are similar for pre- and post-menopausal patients.
- Tamoxifen also reduces the incidence of contra-lateral breast cancer.

- Tamoxifen is the first-line adjuvant treatment for the treatment of ERα-positive breast cancer

# Estrogen Receptor is the Target for Anti-Estrogens

- Response to Tamoxifen is limited to patients with Estrogen Receptor-α positive breast cancer.
- An estrogen binding protein was identified in the early 1960s and subsequently the estrogen receptor-α (ERα) gene was cloned in 1985.
- Biochemical and immunohistochemical studies have established that 70-80% of primary breast cancers express ERα.
- Presence of ERα correlates with a better prognosis & likelihood of response to endocrine therapies.
- Current clinical practice:
  - Surgery and immunohistochemical determination of ERα status.
  - If the cancer cells are ERα-positive, the patients are offered tamoxifen for 5 years.

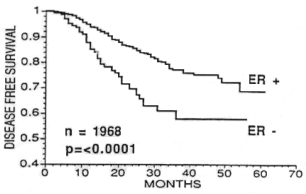
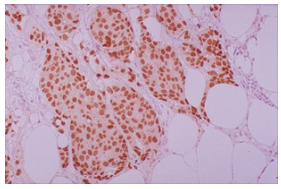


Fig. 1. Disease-Free Survival as a Function of ER Status.



# Tamoxifen in the Treatment of Breast Cancer

- Adjuvant tamoxifen treatment for as little as 1 year reduces recurrence and death.
- Maximal benefit is observed with 5 years adjuvant tamoxifen treatment.
- Five years with tamoxifen provides 50% reduction in recurrence and 26% reduction in mortality.
- Survival benefits with tamoxifen are similar for pre- and post-menopausal patients.
- Tamoxifen also reduces the incidence of contra-lateral breast cancer.

These effects of tamoxifen are observed in patients with Estrogen Receptor-α positive tumours

Tamoxifen is now the first-line adjuvant treatment in pre- and post-menopausal women with Estrogen Receptor-α positive disease

## Estrogen Target Tissues & Tamoxifen

Estrogen is important in the growth, development, differentiation and homoeostasis in many organs.

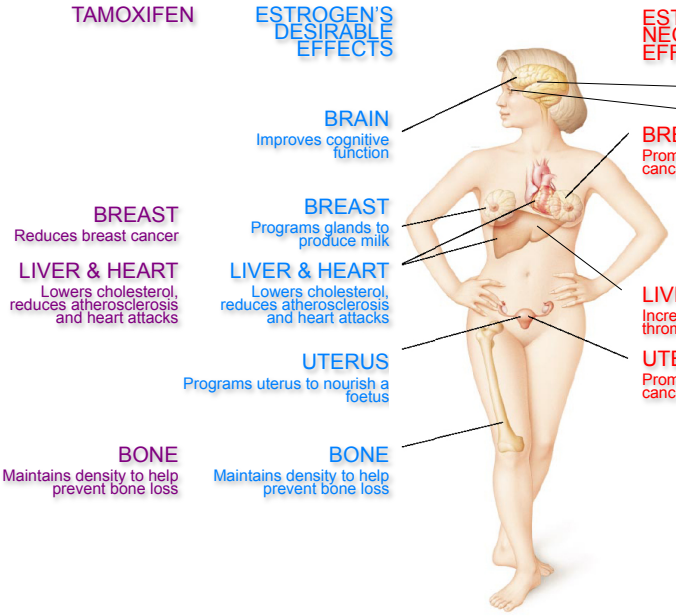
## Benefits & Problems Associated with Tamoxifen

- Tamoxifen is ANTIESTROGENIC in the breast, BUT it is an ESTROGEN in other tissues.
- *Osteoporosis*: Estrogen is important to maintain bone in premenopausal women. After menopause, hormone replacement therapy is often recommended to prevent the development of osteoporosis. Clearly, the long-term administration of an anti-estrogen has the potential to precipitate premature osteoporosis.
  Tamoxifen has estrogenic effects in bone
- *Atherosclerosis*: Eestrogen lowers low-density lipoprotein (LDL) cholesterol levels and raises high-density lipoprotein (HDL) cholesterol levels. Following menopause, women are at the same risk for coronary heart disease as men. It can be argued that the long-term administration of an anti-estrogen could produce a population at risk for premature coronary heart disease.
  Tamoxifen has estrogenic effects in the cardiovascular system
- However, undesirably,
- (Anecdotal reports) associating the administration of tamoxifen for advanced breast cancer with subsequent thromboembolic episodes.
- Tamoxifen is known to produce endometrial thickening, hyperplasia, and fibroids following several years of therapy

Tamoxifen is a partial estrogen agonist/antagonist.
Hence tamoxifen is defined as a Selective Estrogen Receptor Modulator (SERM).

How is tamoxifen estrogenic in some tissues and anti-estrogenic in other tissues?

# Estrogen Target Tissues & Tamoxifen



TAMOXIFEN

BREAST
Reduces breast cancer

LIVER & HEART
Lowers cholesterol, reduces atherosclerosis and heart attacks

BONE
Maintains density to help prevent bone loss

ESTROGEN'S DESIRABLE EFFECTS

BRAIN
Improves cognitive function

BREAST
Programs glands to produce milk

LIVER & HEART
Lowers cholesterol, reduces atherosclerosis and heart attacks

UTERUS
Programs uterus to nourish a foetus

BONE
Maintains density to help prevent bone loss

ESTROGEN'S NEGATIVE EFFECTS

BREAST
Promotes breast cancer

LIVER
Increases thromboembolism

UTERUS
Promotes endometrial cancer

TAMOXIFEN

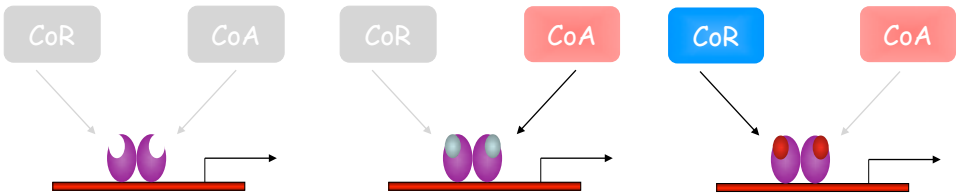
HYPOTHALAMUS
Increases vasomotor symptoms

EYE
Increases cataracts

LIVER
Increases thromboembolism

UTERUS
Promotes endometrial cancer, fibroids, polyps & vaginal discharge

# Tamoxifen is a SERM




Unliganded ER

No EXPRESSION

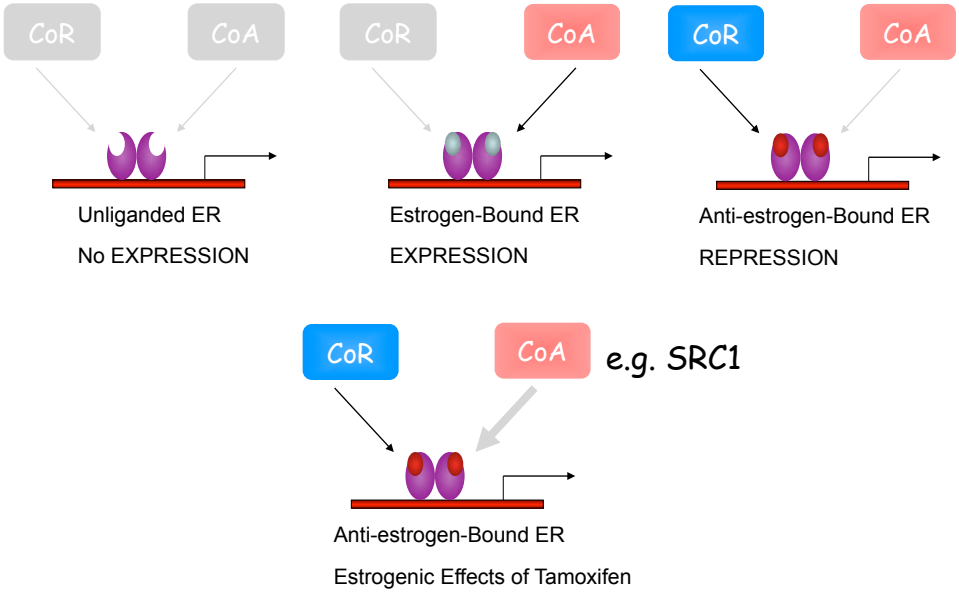
Estrogen-Bound ER

EXPRESSION

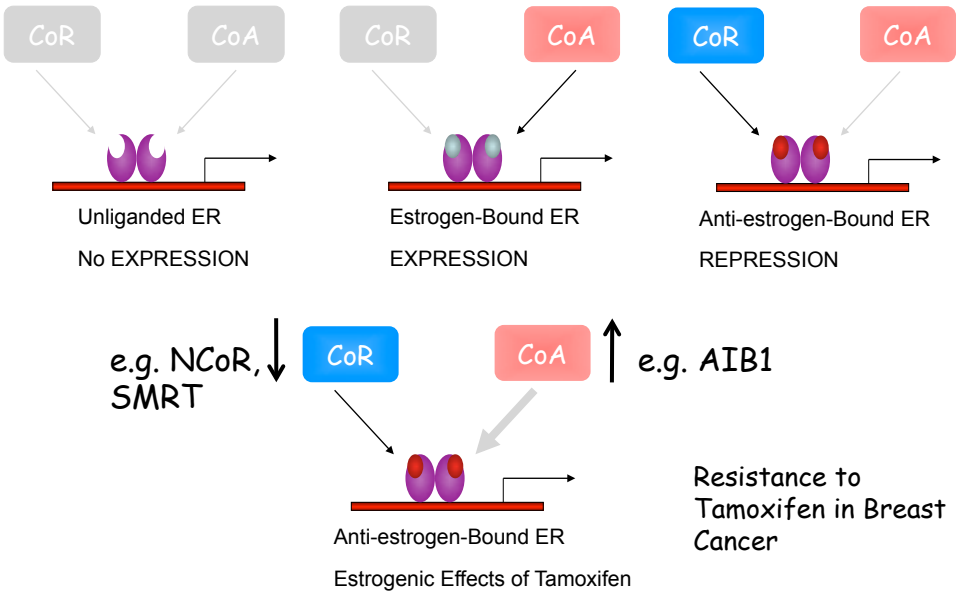
Anti-estrogen-Bound ER

REPRESSION

## Tamoxifen is a SERM




## Tamoxifen is a SERM



# Anti-Estrogens in Breast Cancer Prevention

Tamoxifen reduces the development of contra-lateral breast cancer by 50%, suggesting that it may prevent breast cancer.

ERα overexpression in the benign breast correlates with increased risk of breast cancer development.

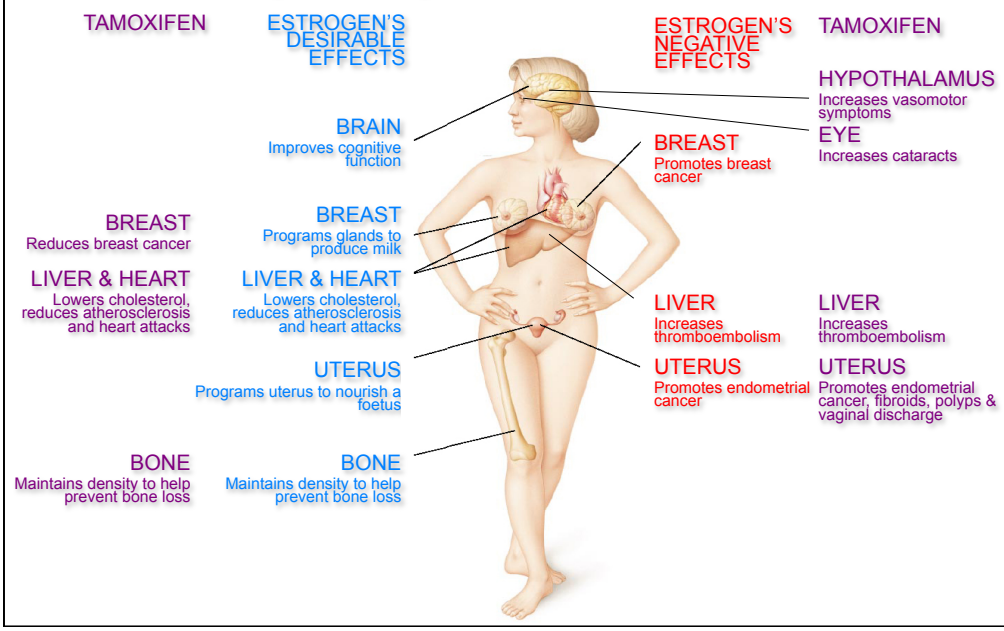
Suggest that tamoxifen could be used in breast cancer prevention

Clinical trials on women at high risk:

- NSABP P1: women with high-risk benign pathology such as atypical ductal hyperplasia,
- INT trial: women on hormone-replacement therapy
- IBIS trial: women with family history

Overall reduction in early incidence of ~40%

# Estrogen Target Tissues & Tamoxifen



# Summary

- Estrogens are important for the development and maintenance of many organs.
- As such, they are important in human disease, including breast cancer.
- Breast cancer initiation and progression is often estrogen-dependent.
- Estrogen withdrawal/anti-estrogens are central to the treatment of breast cancer.

- Estrogen actions are mediated by the estrogen receptors.
- Estrogen receptors act by regulating gene expression by recruitment to gene promoters (direct, indirect)
- Estrogen receptor target genes include genes involved in many cellular processes, including cell cycle progression, DNA replication, apoptosis.
- Anti-estrogens act by inhibiting coactivator recruitment and stimulating corepressor recruitment.
- Estrogen and anti-estrogen actions can be cell/tissue-specific.

# Reading List


**Estrogen and Estrogen Receptors in Development:**

Couse and Korach [1999] Endocrine Reviews 20: 358-417
Hewitt et al [2004] Annu Rev Physiol. 67: 85-308

**Steroid Hormones in Cancer**

Jordan and Morrow [1999] Endocrine Reviews. 20: 253-278
Ali and Coombes [2002] Nature Reviews Cancer 2: 101-112
Johnston and Dowsett [2003] Nature Reviews Cancer 3: 821-831
Ali et al [2011] Annu Rev Medicine 62:217-32
Huang and Tindall [2002] 12: 193-207

**Steroid Hormones Mechanisms**

Evans [1988] Science 240: 889-895
Beato et al. [1995] Cell 83: 851-857

**Other nuclear receptors**

Giguere [1999] Endocrine Reviews 20: 689-725
Mangelsdorf and Evans [1995] Cell 83: 841-850
Chawla et al. [2002] Science 294: 1866-1870

**Coactivators/Corepressors**

Glass and Rosenfeld [2000] 14: 121-141
Jepson et al [2000] Cell 102: 753-763
McKenna & O'Malley [2002] Cell 108: 465-474
Lonard et al. [2007] Endocrine Reviews 28: 575-587

**Gene regulation control by Estrogen Receptors**

Carroll et al. [2006] Nature Genetics 38: 1289-97
Green & Carroll [2007] Nature Reviews Cancer 7: 713-722
Metivier et al. ([2006] EMBO Reports 7: 161-167