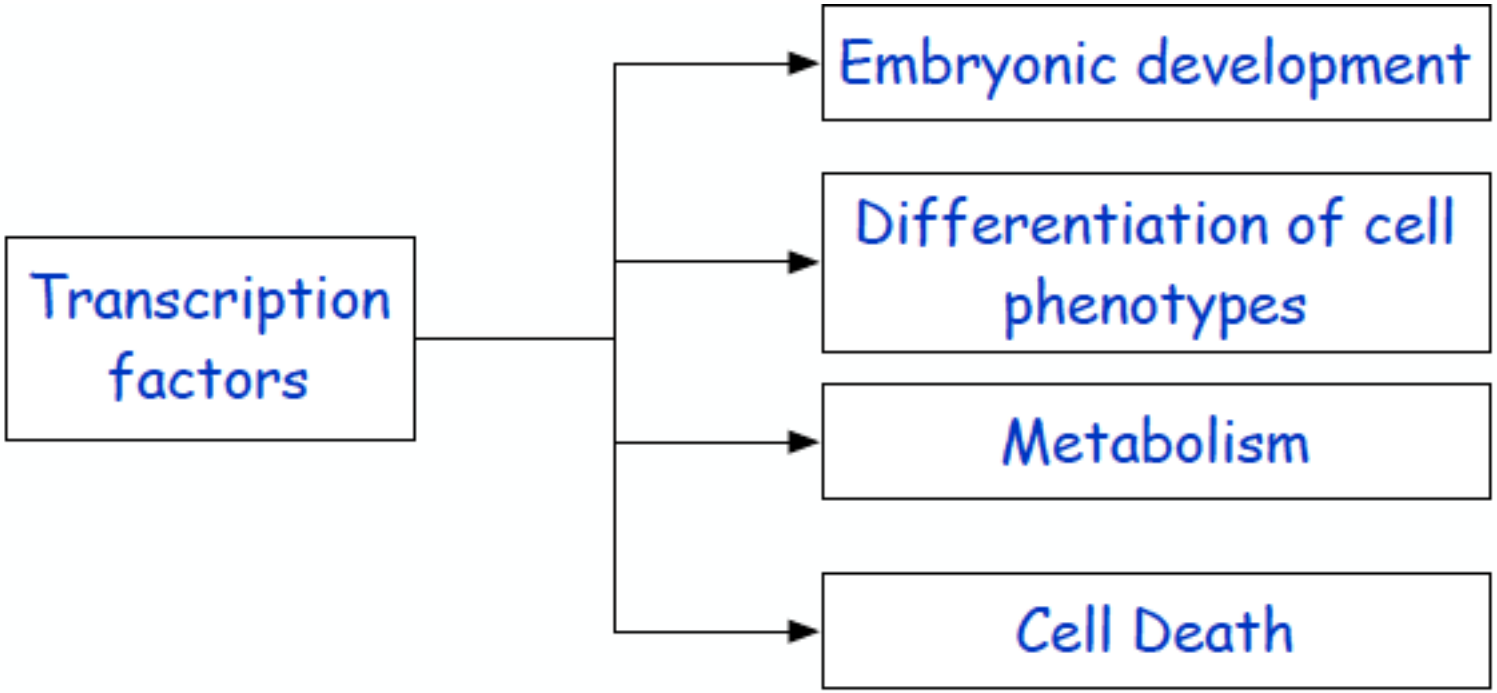
Nuclear receptor signaling

- Receptors
- Ligands
- Ligand binding
- Response - Induction gene expression
- Endocrine disruptors

Nuclear receptors

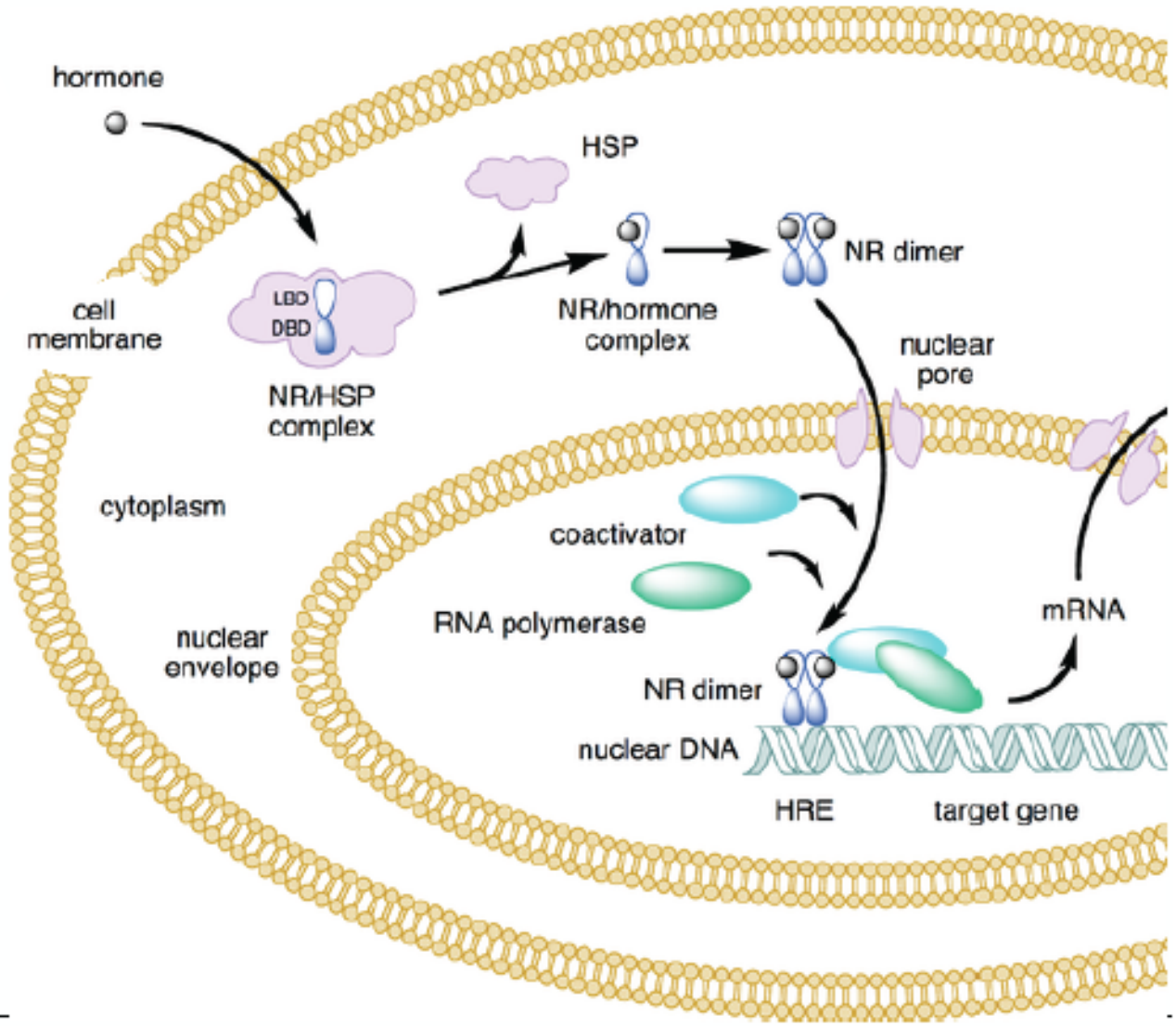
General characteristics:

Nuclear receptors at the start of many processes

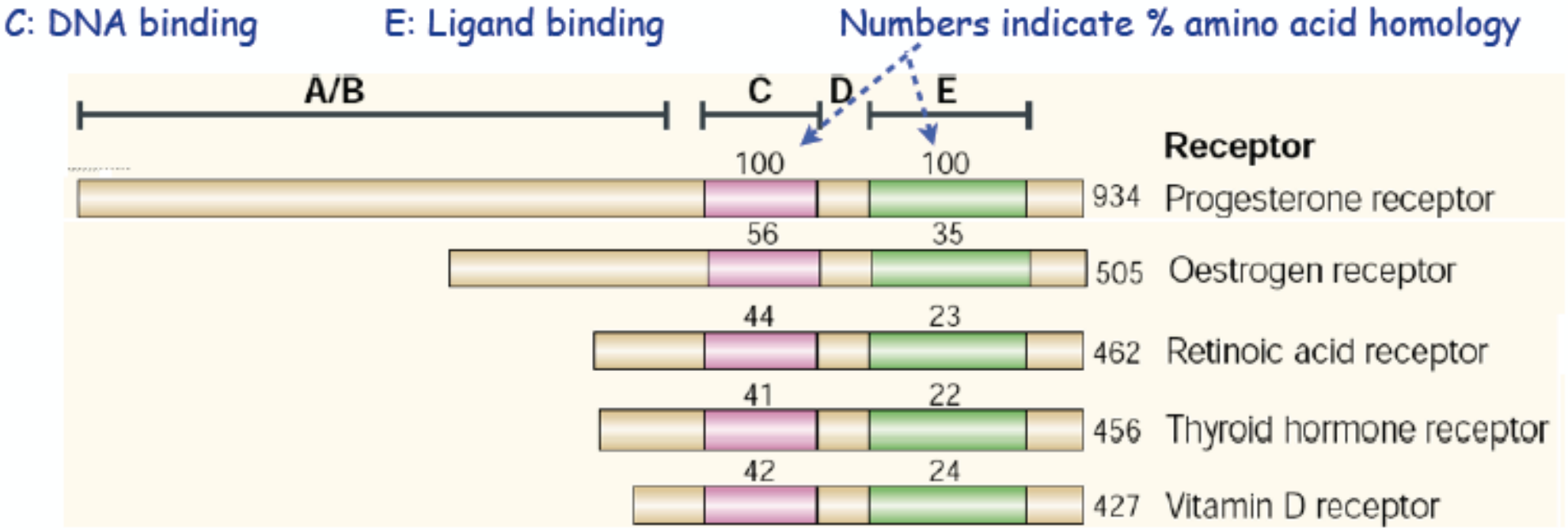


Nuclear receptor signalling route

Nuclear receptors are "ligand-regulated modulators of transcription"

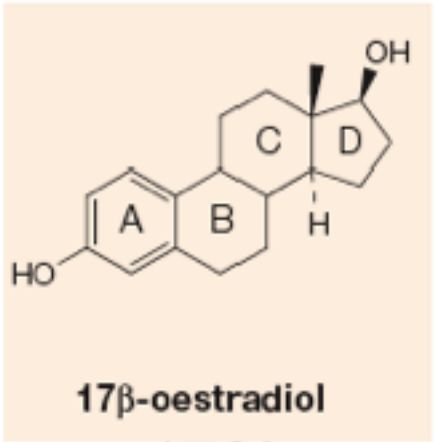


Nuclear receptors - Similar domain structure



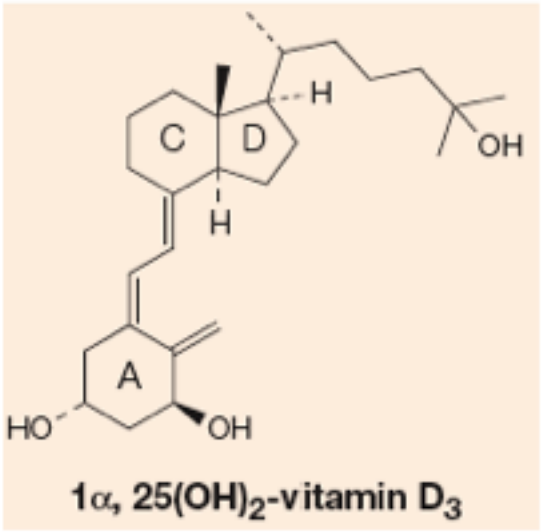
Tata Nat Rev Mol Cell Biol 2002

Ligands for nuclear receptors

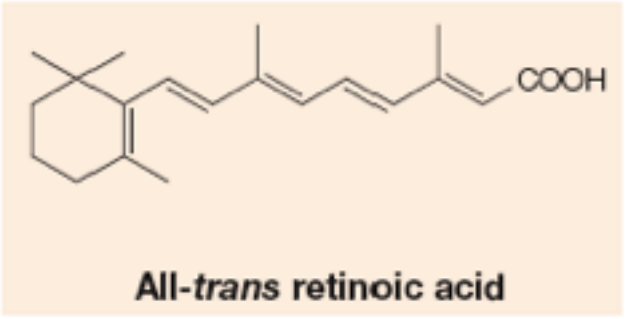


17β-oestradiol

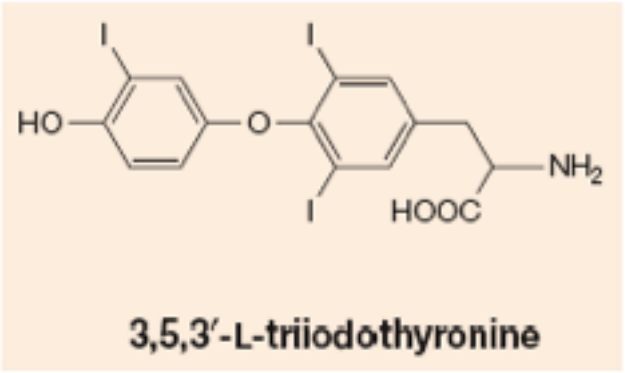
(E2)



1α, 25(OH)$_2$-vitamin D$_3$



All-*trans* retinoic acid



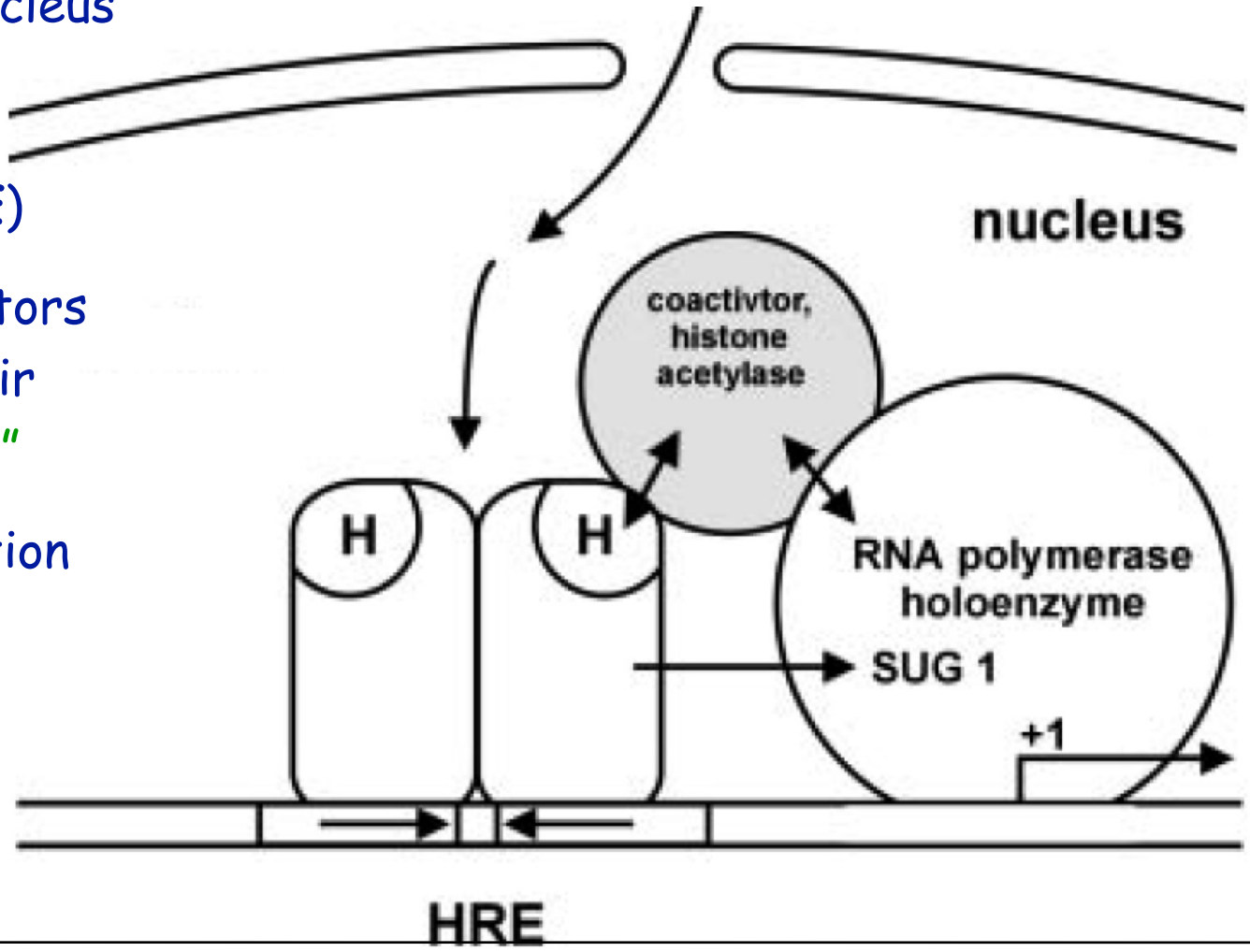
3,5,3′-L-triiodothyronine

Estrogen receptor signalling

Nuclear receptors are "ligand-gated modulators of transcription"

- Binding of ligand induces structural changes
- Receptor forms a dimer
- Dimer is imported into nucleus
- Binding to hormone response element (**HRE**)
- Recruitment of co-activators which interacts via their "Nuclear Receptor Box"
- Initiation gene transcription

=> Protein synthesis

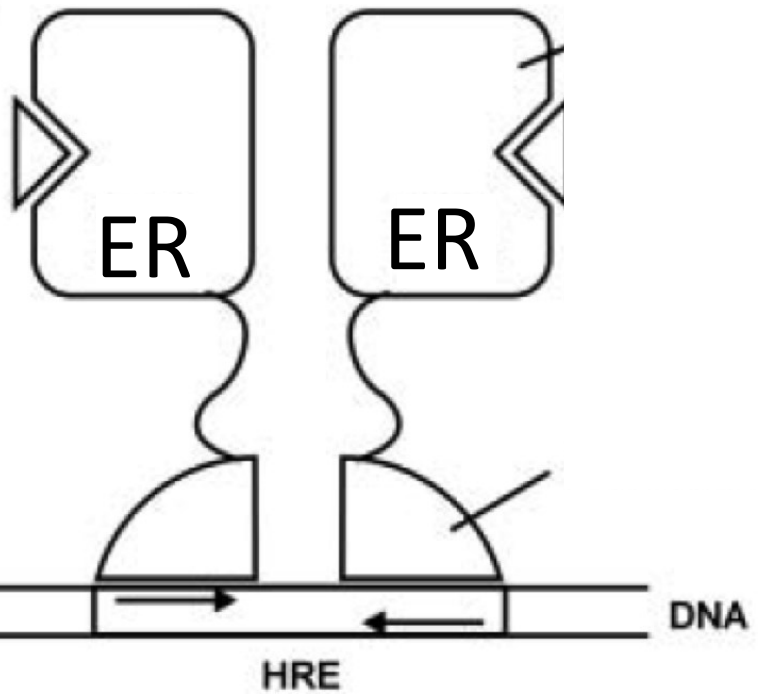


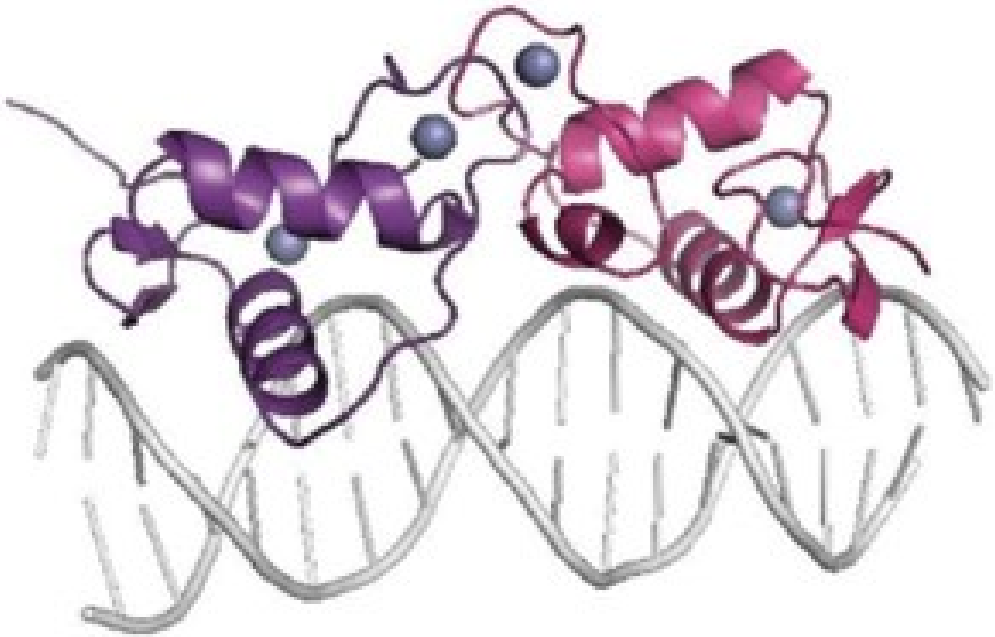
Estrogen receptor binding to DNA

- Hormone response element is a palindromic DNA sequence up-stream of promotor of estrogen-induced proteins

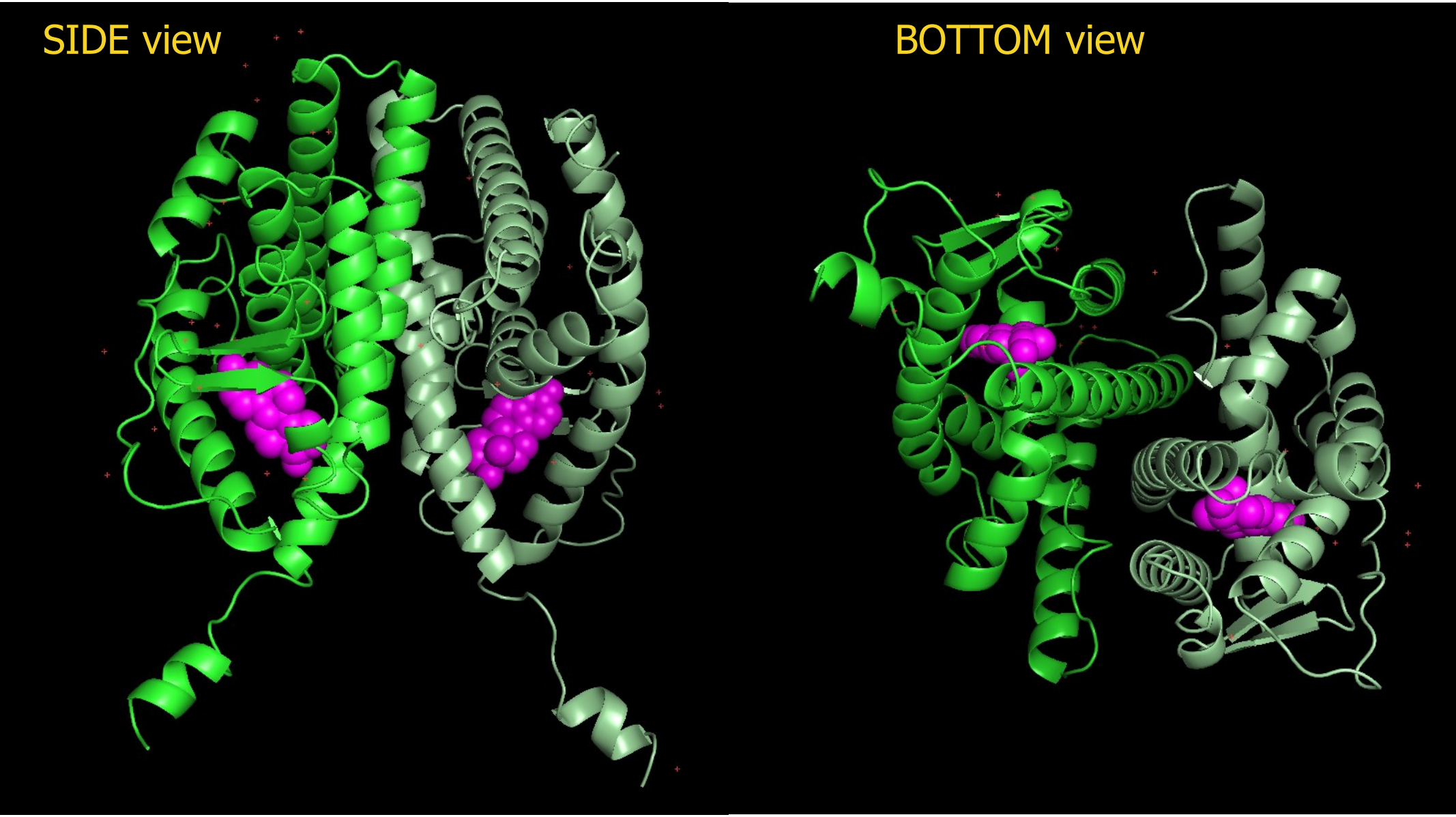
```
5'-AGGTCAxxxTGACCT
   :::::::::::::::
   TCCAGTxxxACTGGA-5'
```

- DNA binding domain of receptor has 2 Zn-fingers to place the HRE recognition helix_1 in the major groove of DNA'



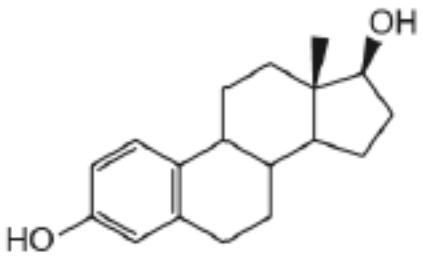


Binding of oestrogen receptor dimer

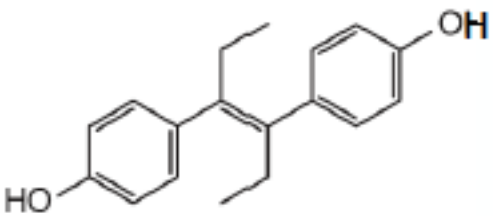


Natural and xenobiotic oestrogen receptor ligands

Full agonists



Oestradiol (ER)



Diethylstilbestrol (ER)

"DES": estrogen supplement
=> major health problems

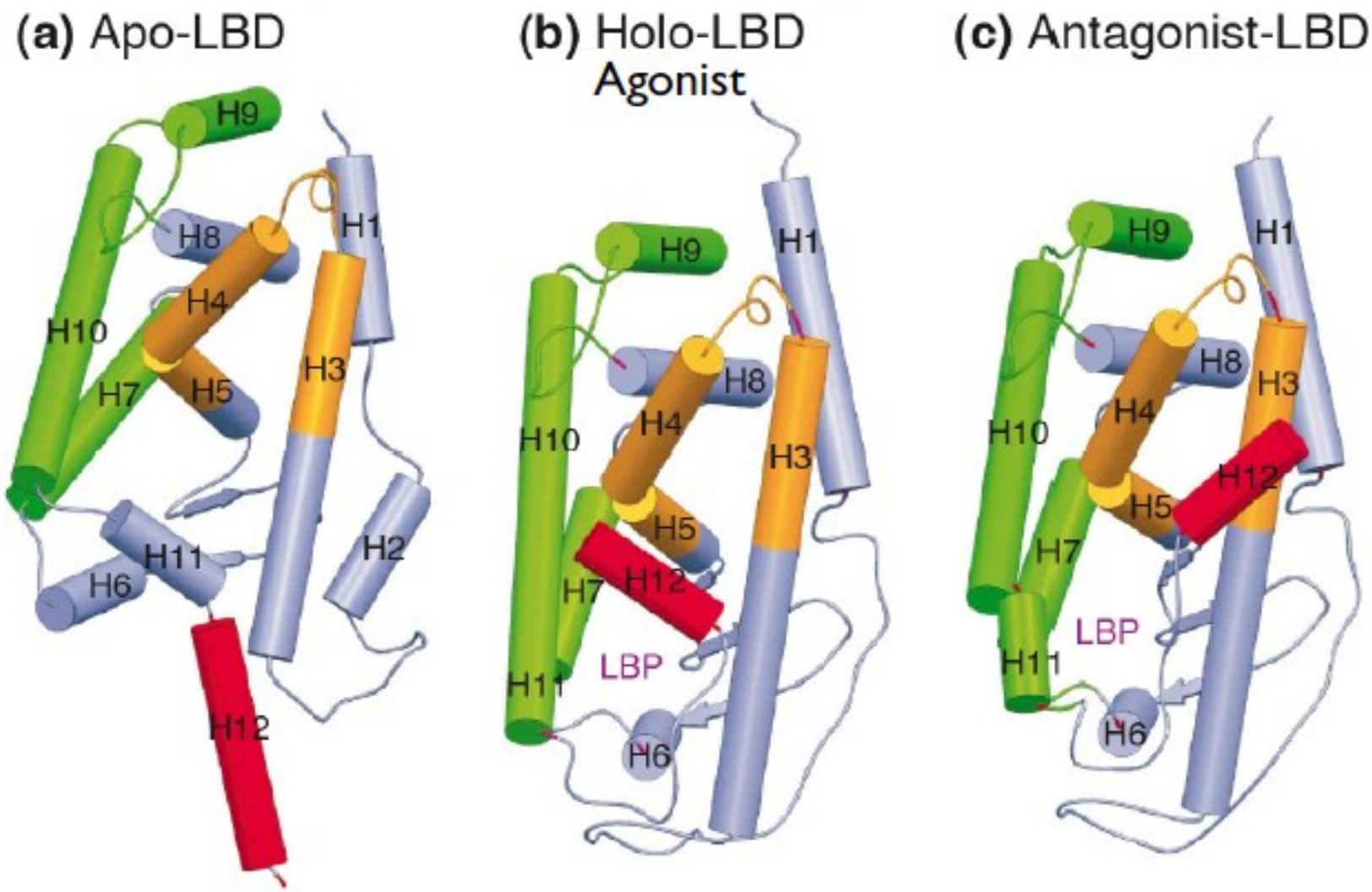
Nuclear receptor pharmacology

"The response of a given nuclear receptor to a particular ligand in a given tissue is dictated by the set of proteins with which this nuclear receptor interacts following ligand-induced allosteric alterations of activity" .

Gronemeyer, Nat Rev Drug Disc 2004

Ligand-binding domain with and without ligand

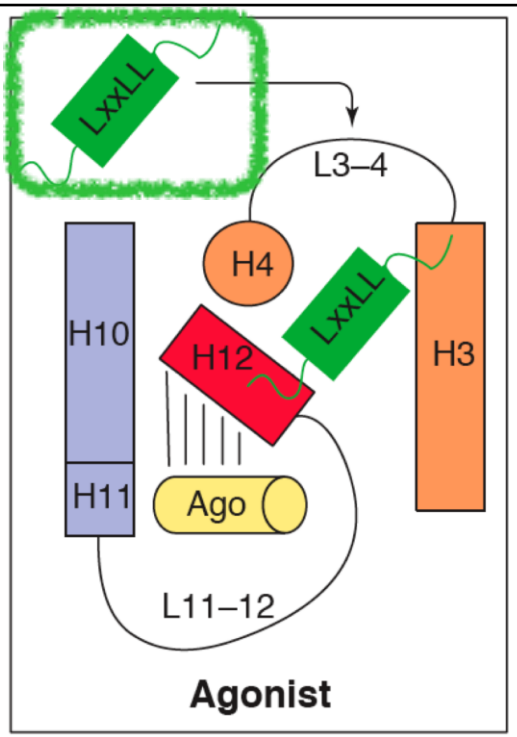
- Helix 12 & 11 have a very different position depending on the presence and type of ligand



LBP: ligand binding pocket

Bourget TiPS 2000

Position of helix 12 is pivotal



Ligand binding domain with:

Agonist: H12 strongly interacts with ligand

=> **co-activator** interacts via its Nuclear Receptor box with H3-H4 -H12 domain

Bourget TiPS 2000

Effect of ligands on the estrogen receptor

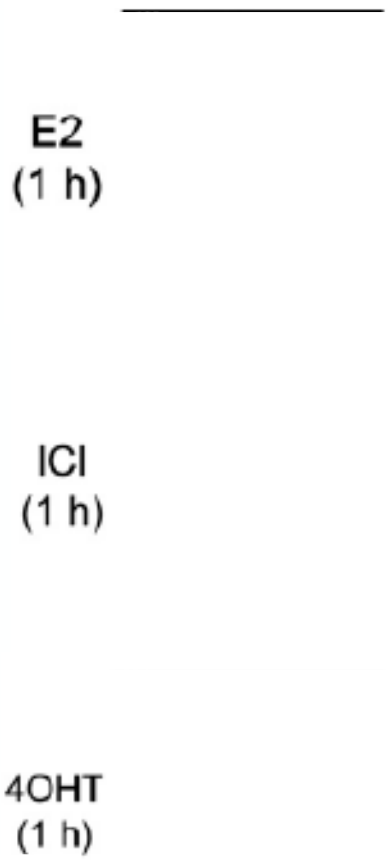
Q: What happens to receptor upon ligand exposure ?

Exp: fluorescently label receptor and look !

- t= 0 h: Label all ER-SNAP with a red fluorophore
- t = 0-1 h: Incubate 1h with buffer containing ligands

Results: Receptor remains ?

- Blank
- Agonist estrogen (E2):
- Antagonist ICI
- Partial agonist 4-OH-tamoxifen (4OHT)



Estrogen receptor system : Some numbers

- Interaction of ER with co-activator SRC2 in live cells :

Method: determination of of interaction of fluorescent protein fusions of ER and SRC2 by fluorescence cross correlation spectroscopy (FcCS).

- Observed ER concentration : ~20 nM

- Dimerization ER K_d ~ 2 - 8 nM

Savatier, Biochemistry 2010

Estrogen receptor : Numbers of genes regulated

Study	Cells	Tissue[a]	ERα expression	E2[b]	Probes on microarray[c]	Thresholds[d]	Regulated genes[e]	Up-regulated	Down-regulated
Frasor *et al.* (2003)	MCF-7	Mammary	Endogenous	4-48 h	~12,000	CS > 12	438	30%	70%
Rae *et al.* (2005)	MCF-7	Mammary	Endogenous	24 h	~12,000	FC > 1.2, P < 0.1	674	N/A	N/A
Carroll *et al.* (2006)	MCF-7	Mammary	Endogenous	3-12 h	~47,000	RMA, Welch t test	1,526	51%	49%
Kwon *et al.* (2007)	MCF-7	Mammary	Endogenous	3-12 h	~47,000	N/A	879	64%	36%
Kininis *et al.* (2007)	MCF-7	Mammary	Endogenous	3 h	~14,500	FC > 2, P < 0.05	217	56%	44%
Lin *et al.* (2007)	MCF-7	Mammary	Endogenous	12-48 h	~47,000	RMA, SAM	1,250	44%	56%
Coser *et al.* (2003)	MCF-7/BUS	Mammary	Endogenous	48 h	~12,000	FC > 2, P < 0.01	730	58%	42%
Lin *et al.* (2004)	T-47D	Mammary	Endogenous	1-24 h	~19,000	FC > 1.2, P < 0.05	386	59%	41%
Rae *et al.* (2005)	T-47D	Mammary	Endogenous	24 h	~12,000	FC > 1.2, P < 0.1	140	N/A	N/A
Rae *et al.* (2005)	BT-474	Mammary	Endogenous	24 h	~12,000	FC > 1.2, P < 0.1	33	N/A	N/A
Levenson *et al.* (2002)	MDA-MB-231	Mammary	Exogenous	24 h	~600	FC > 2	11	N/A	N/A
Stender *et al.* (2007)	MDA-MB-231	Mammary	Exogenous	4-48 h	~12,000	CS > 12	340	N/A	N/A
Monroe *et al.* (2003)	U2OS	Bone	Exogenous	24 h	~6,800	FC > 2	80	85%	15%
Kian Tee *et al.* (2004)	U2OS	Bone	Exogenous	18 h	~12,600	FC > 1.7, P < 0.05	103	65%	35%

=> very unclear....

Kininis 2008 NRS

Estrogen receptor : Numbers of binding sites on DNA

Cells	Method[a]	Microarray[b]	DNA assayed[c]	ERα antibody[d]	-E2[e]	+E2[e]	Data analysis[f]	Binding sites[g]
MCF-7	ChIP-chip	Custom, PCR-based	~9K CpG islands	HC-20, C-term.	No	24 h	$FE_1 > 2$	70
MCF-7	ChIP-chip	Affy, 21mer tiling	Chr. 21 and 22	HC-20, C-term.	No	45 min	$FE_2 > 1.5$, HMM	57
MCF-7	ChIP-chip	Custom, PCR-based	~19K promoters	HC-20, C-term.	No	45 min	$P < 0.005$	153
MCF-7	ChIP-chip	Custom, PCR-based	~12K CpG islands	D-12, N-term.	Yes	3-24 h	$FE_2 > 2$, SAM	92
MCF-7	ChIP-chip	Affy, 21mer tiling	Whole genome	HC-20, C-term.	No	45 min	MP < 10-5, MAT	3,665
MCF-7	ChIP-chip	Custom, PCR-based	~900 promoters	Custom, N-term.	Yes	45 min	FC > 1.3, TP < 0.05	47*
MCF-7	ChIP-clon.	N/A	N/A	HC-20, C-term.	No	N/A	N/A	12
MCF-7	ChIP-DSL	Custom, 40mer tiling	~16.2K promoters	HC-20, C-term. H-184, N-term.	Yes	1 h	P < 0.0001, SAM	578
MCF-7	ChIP-PET	N/A	Whole genome	HC-20, C-term.	No	45 min	PET cluster > 3	1,234

=> Between 12 and 3'665 sites per nucleus very unclear....

Kininis 2008 NRS

Estrogen receptor : Numbers of genes regulated

Number of genes of which transcription is regulated

- number of mRNA probes used : 600 - 47'000

  => >> 11 - 1'500 genes regulated

  => 30 - 85 % are up-regulated

Number of ER binding sites on DNA

=> 12 - 3'665 sites per nucleus

How many receptors ?

Kininis 2008 NRS

Nuclear receptors

- Initiation of signalling by ligand binding
- Receptor dimer
- Nuclear import
- DNA binding
- Initiation of gene transcription

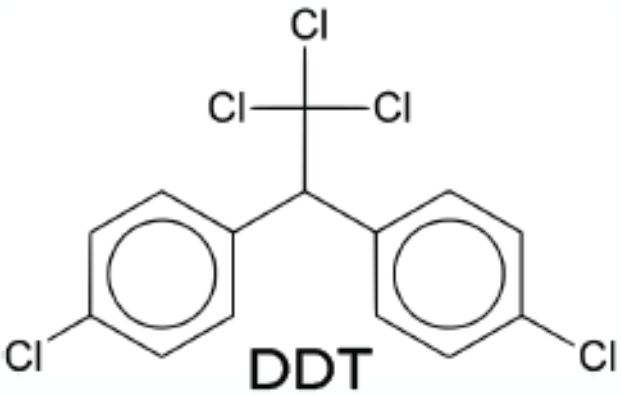
Endocrine disruptors

- Definition: Xenobiotic compounds that have effect on the endocrine system, and thus affect life

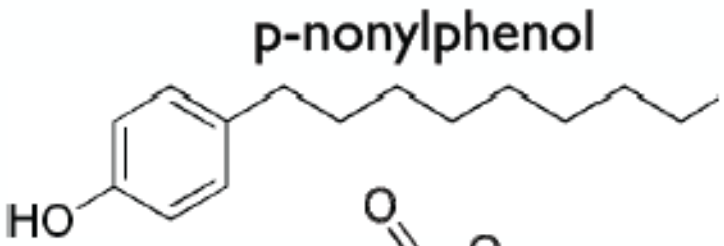
- Often the compounds originate from waste or pollution and accumulate in the environment

Examples:

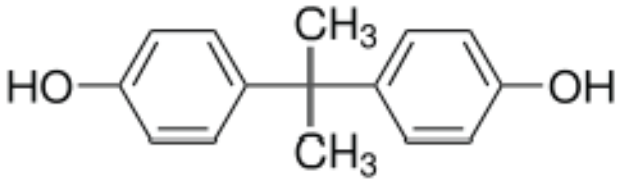
- hormones from medicine or agriculture e.g. estrogens
- plasticizers e.g. phtalates or polycholinated biphenyls (PCBs)
- surfactants e.g. nonylphenol
- pesticides e.g. dichlorodiphenyltrichloroethane (DDT)
- food e.g. coumesterol from soy



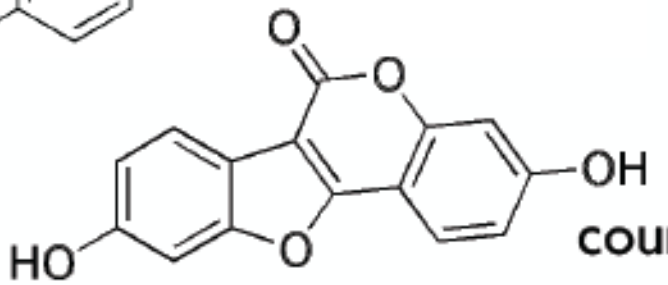
DDT



p-nonylphenol



bisphenol-A



coumesterol

Endocrine disruptors

Estrogen disruptors => feminization of male individuals

Examples:

- **Fish** in Columbia river (2000):

"Of the females sampled on the spawning grounds 84% are chromosomal males"

- **Aligators** in Florida (1990-ies): "> 90% of male are infertile"

- **Human**

- in Costa Rica: "Excessive" use of estrogen to stimulated growth of chicken severely affected development of sexual traits of boys

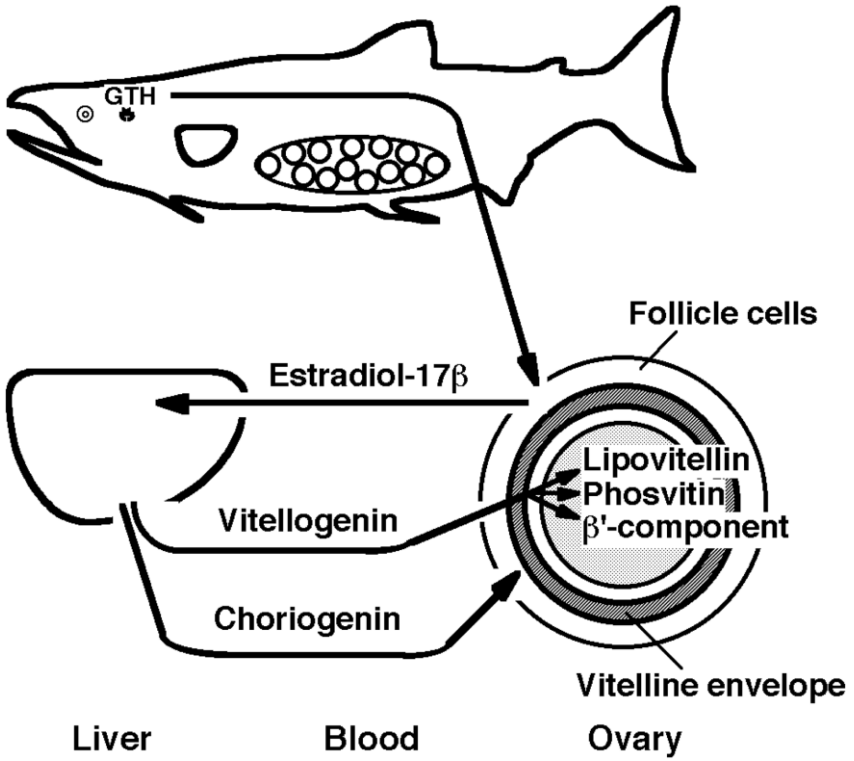
- in Europe: Sperm fertility decreased by ~50%

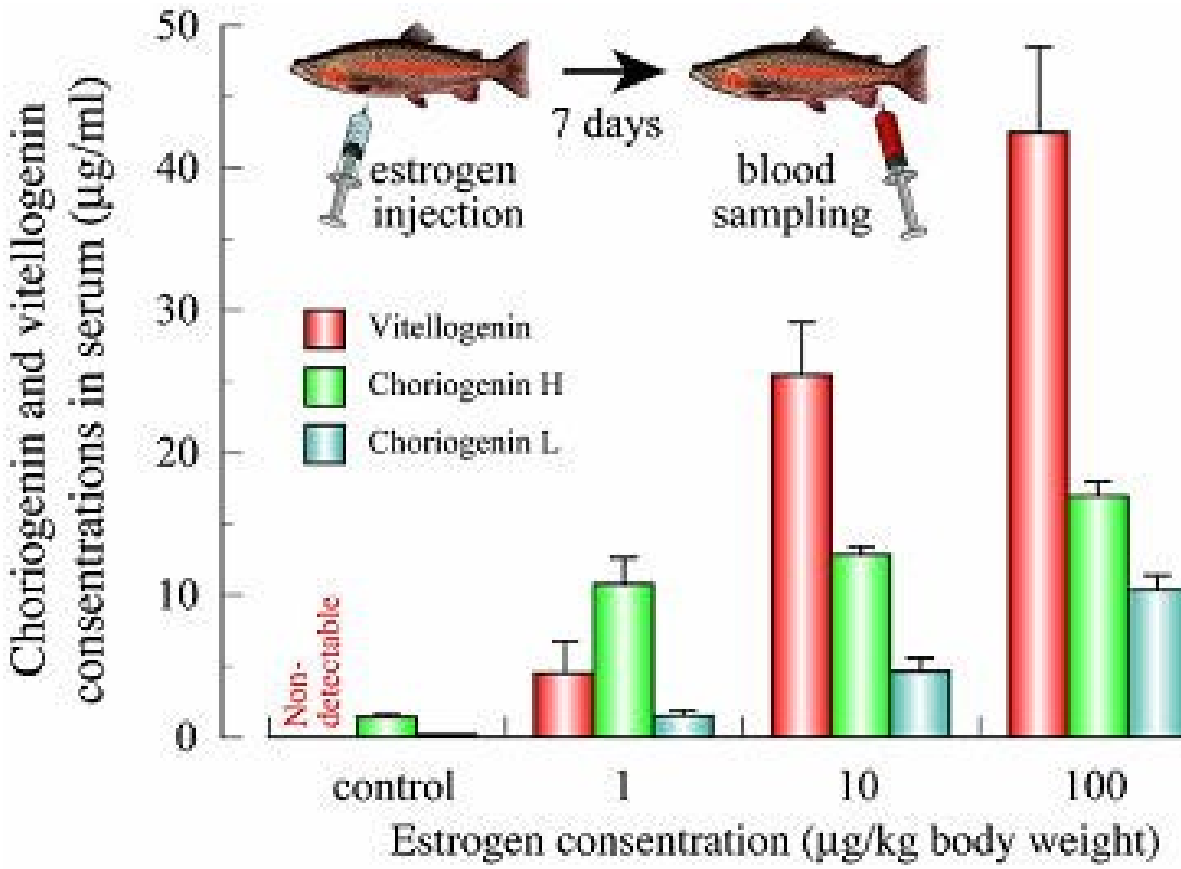
How to detect endocrine disruptors ? Use fish !

Vitellogenin is made in liver of female and secreted in blood to be used for the production of egg yolk

Its gene is under control of the estrogen hormone response element

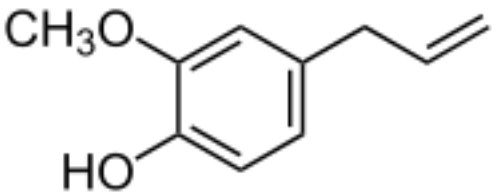
- Male fish will produce vitellogenin upon exposure to estrogenic compounds



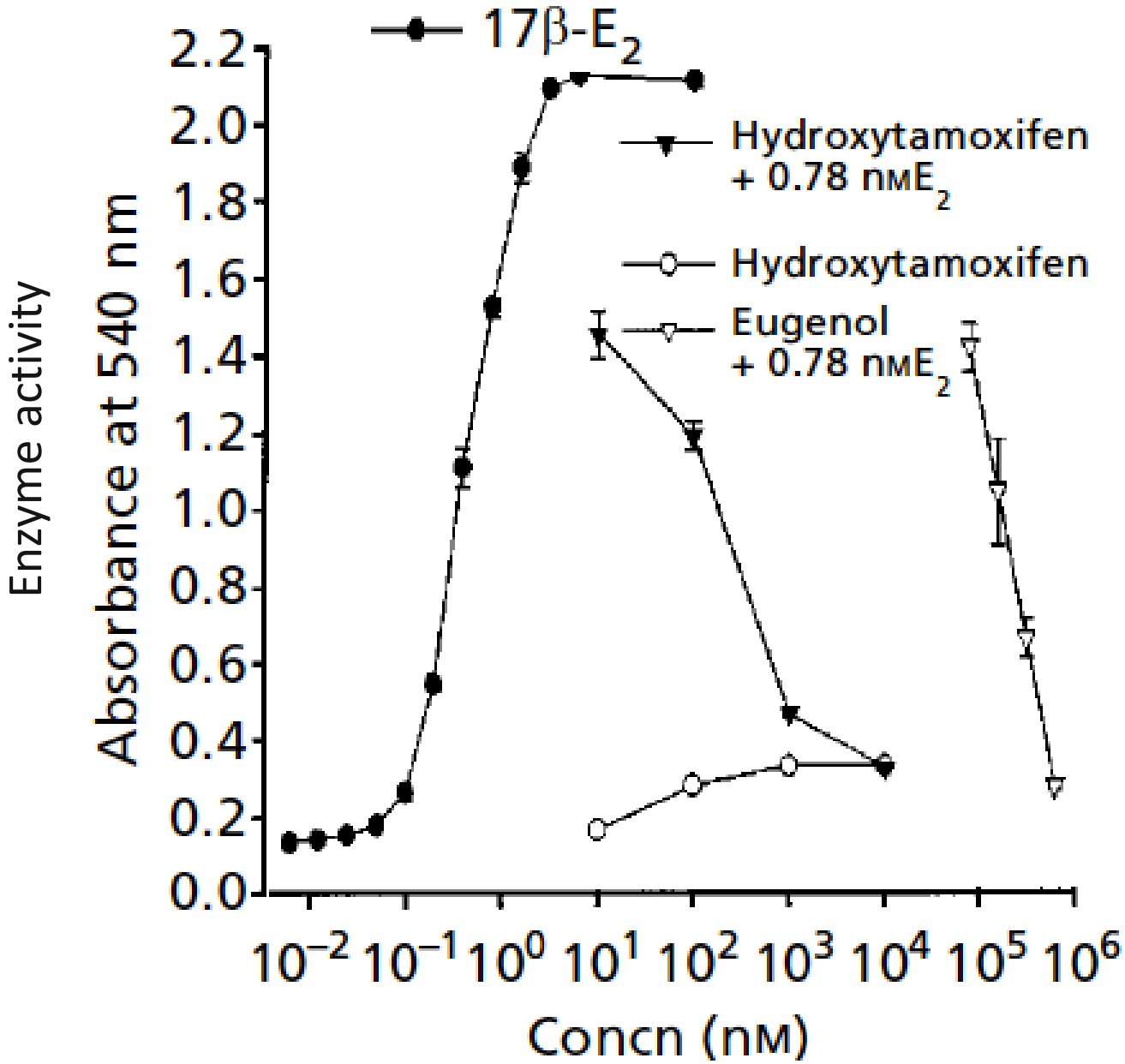


Effects of common compounds on ER

Eugenol, major component of the essential oil of cloves



Effects of common compounds on ER



=> Eugenol inhibits ER-activation by estradiol

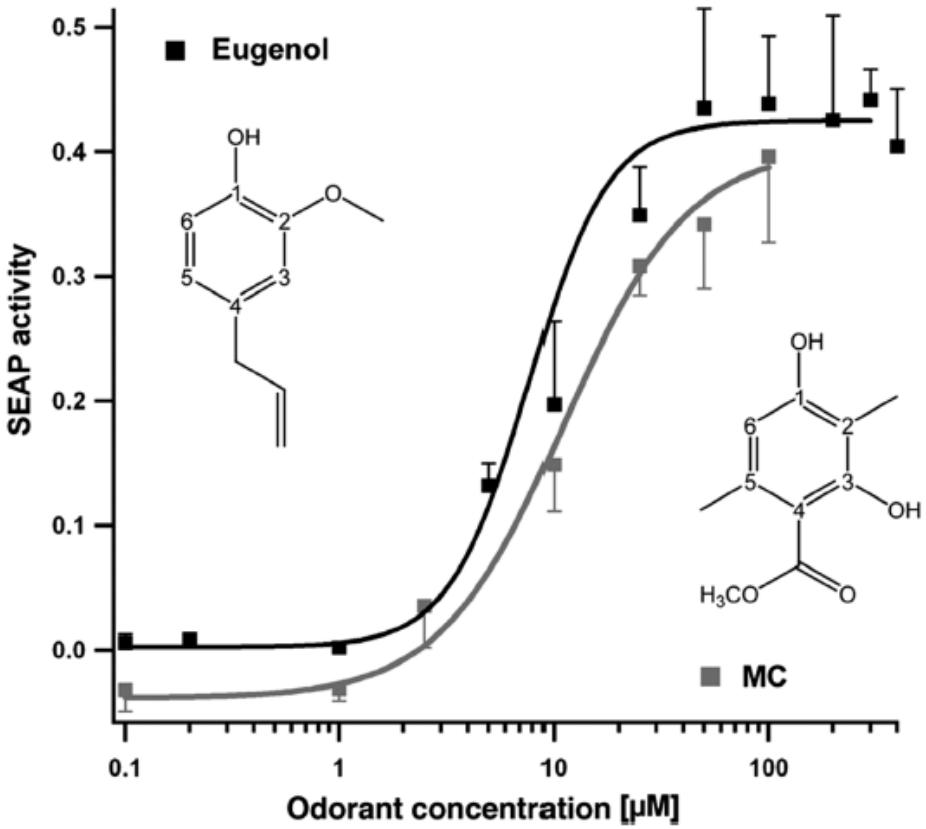
IC_{50} ~100 µM

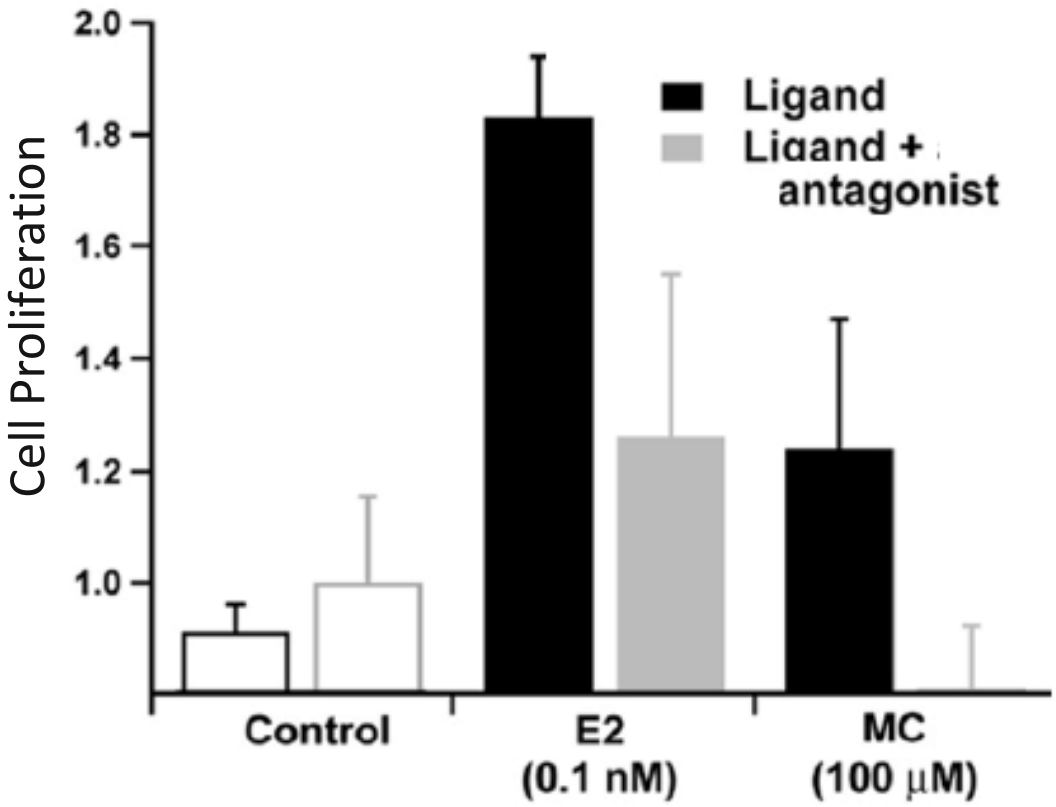
Howes 2002 J Phama Phamac

Effects of common compounds on ER

Perfume components:

- act on odorant receptors
  here eugenol GPCR
- some act on ER too !!





Reporter gene assay

MC : Mousse cristal, a perfume component

Pick 2009 J B C

Endocrine disruptors

- A large diversity of components affect signalling through nuclear receptors
- Many components used at "innocent" concentrations can have effects due to accumulation

Nuclear receptors

- Initiation of signaling by ligand binding
- Receptor dimer
- Nuclear import
- DNA binding
- Initiation of gene transcription

Endocrine disruptors

- A large diversity of components affect signaling through nuclear receptors
- Many components used at "innocent" concentrations might have effects due to accumulation

Nuclear receptors - Further reading

Weikum "The nuclear receptor superfamily : A structural perspective"
Protein Science 2018

Fox "Chemical communication threatened by Endocrine-Disrupting Chemicals"
Environmental Health Aspects 2004

Birnbaum "When environmental chemicals act like uncontrolled medicine"
TEM 2013