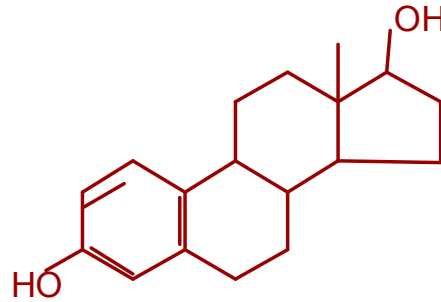
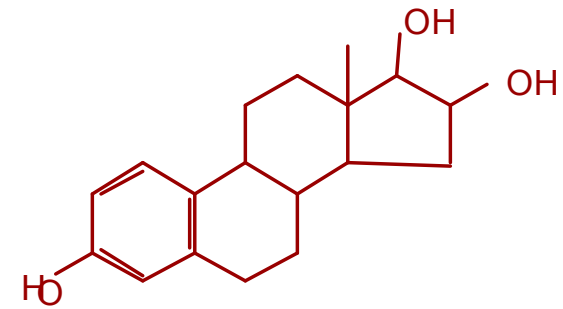

Natural and environmental estrogens

Estrogens:



17-β-estradiol



estriol

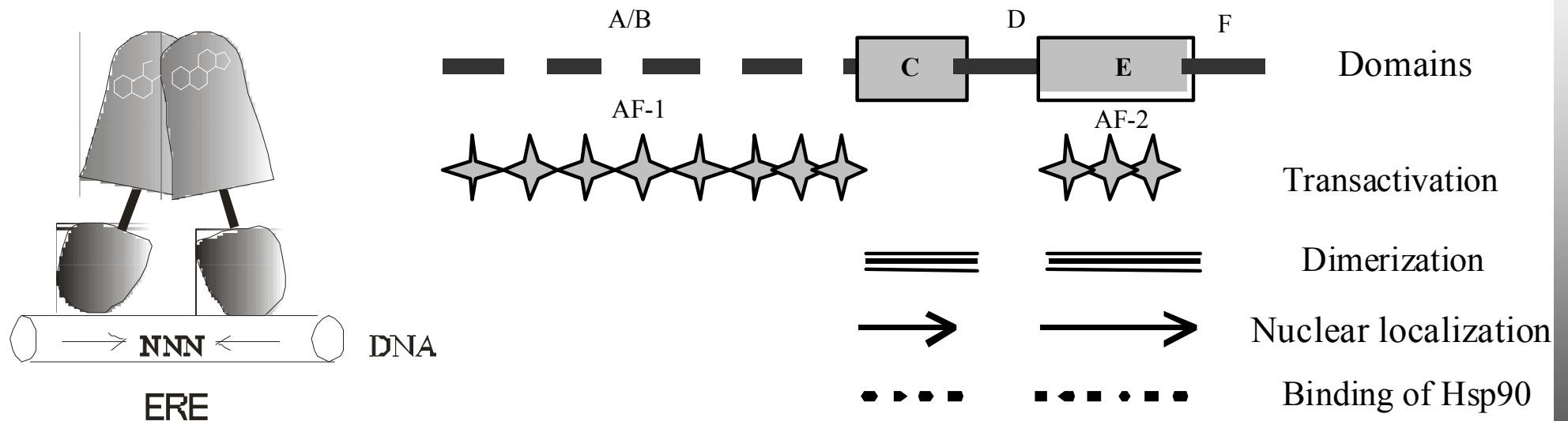
- play a key role in female hormone regulation and signalling
- are responsible for metabolic, behavioural and morphologic changes occurring during stages of reproduction
- are involved in the growth, development and homeostasis of a number of tissues
- control the bone formation, regulation of homeostasis, cardiovascular system and behaviour
- regulate production, transport and concentration of testicular liquid and anabolic activity of androgens in males

Estrogen receptor:

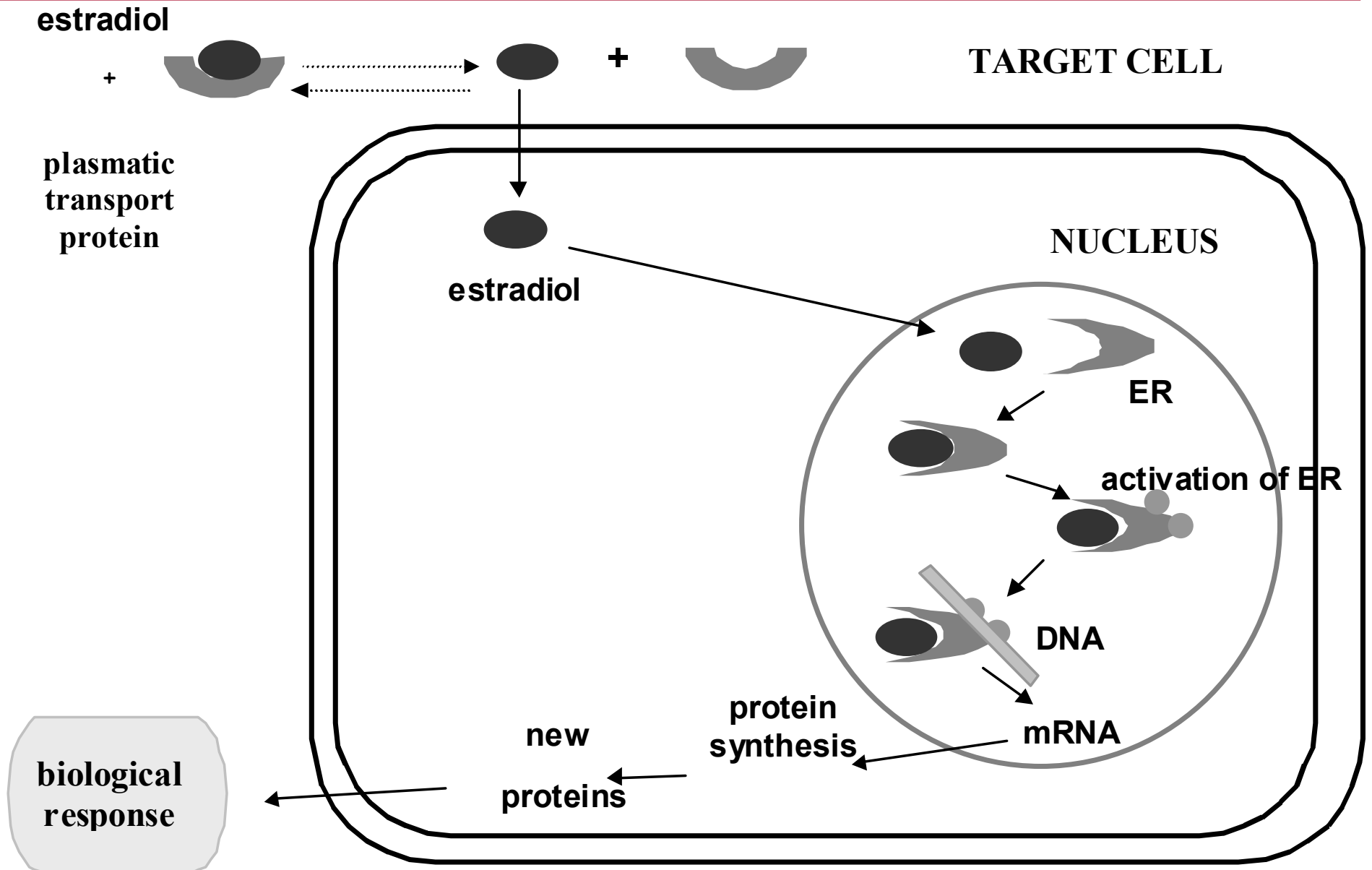
- a member of the nuclear hormone receptor superfamily
- a ligand – inducible transcription factor
- subtype: ER- α (in breast, ovary, brain, liver, bone and cardiovascular system, adrenals, testis and urogenital tract)

ER- β (in kidneys, prostate and gastrointestinal tract)

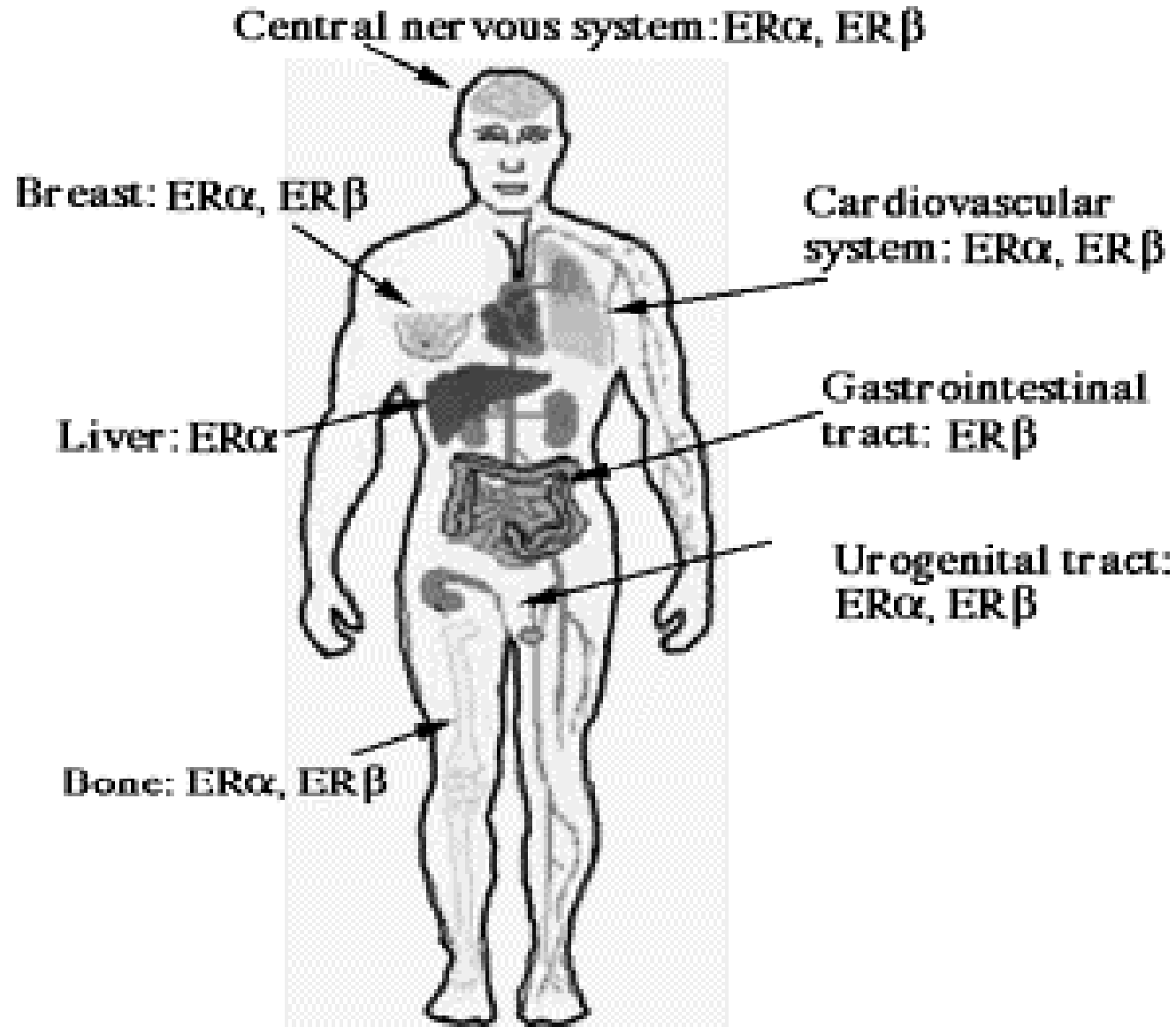
ER- γ (in fish)



Mechanism of action of the estrogen hormones



ESTROGEN RECEPTORS - ER- α & ER- β :



biosynthesis and release of estrogens



e.g. modulation of CYP11A and/or CYP19 activities

binding to plasmatic transport proteins



e.g. down-regulation of ER protein levels

binding to nuclear estrogenic receptor (ER)



activation of ER

(dissociation of associated heat shock proteins, formation of homodimers)



e.g. modulation of other nuclear receptors
(PPAR/RXR, RXR/TR)

binding of the activated receptor complex to specific DNA motifs - EREs

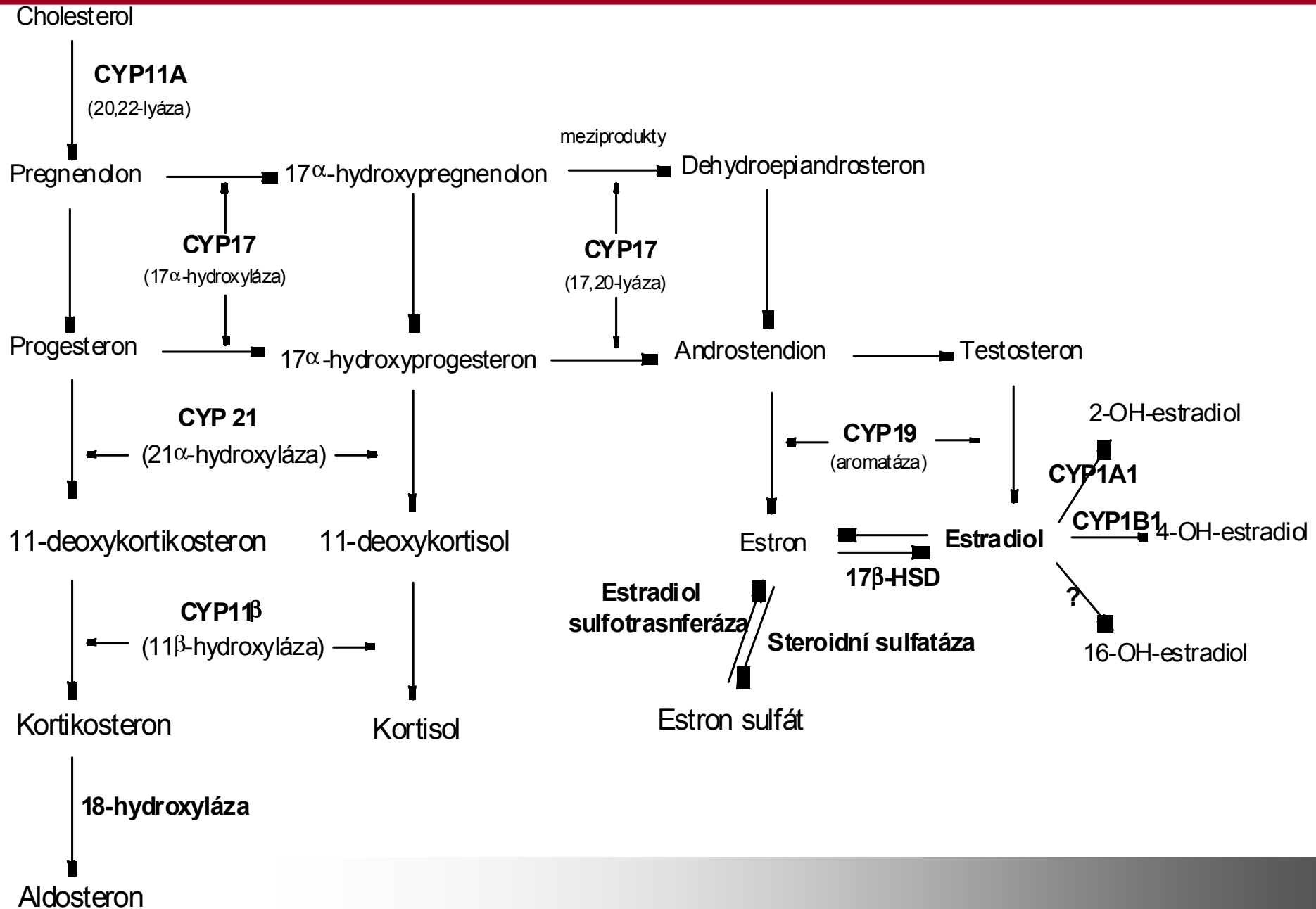


chromatin rearrangement and transcription of estrogen-inducible genes



effects at the cellular, tissue, organ, organism, and/or population level

Synthesis and metabolism of estrogens



Cross-talk between estrogen signalling pathways and other receptors

- estrogen signalling pathways and other members of nuclear receptor superfamily
- estrogen signalling pathways and AhR
- estrogen signalling pathways and receptors for EGF and insuline

Environmental estrogens (xenoestrogens, exoestrogens)

- are a diverse group of substances that do not necessarily share any structural resemblance to the prototypical estrogen (17 β -estradiol) but evoke effects resembling those of estrogen
- **estrogenic substances (estrogen agonist)**
- **estrogen-like substances**
- **ANTI-estrogenic substances**

Exoestrogens - examples (1)

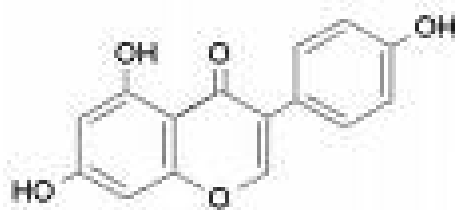
Natural products

genistein

naringenin

coumestrol

zearalenone



Environmental pollutant

DDT

kepone

PCBs/OH-PCBs

PAHs and dioxins

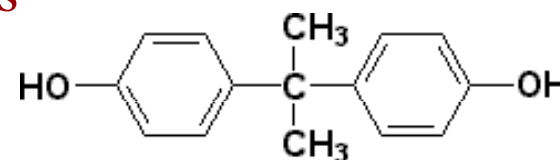
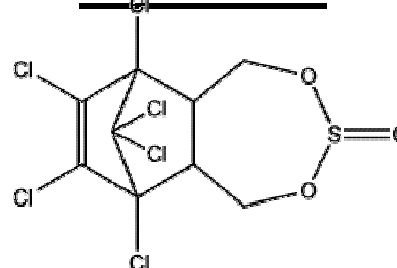
Industrial chemicals

Bisphenol A

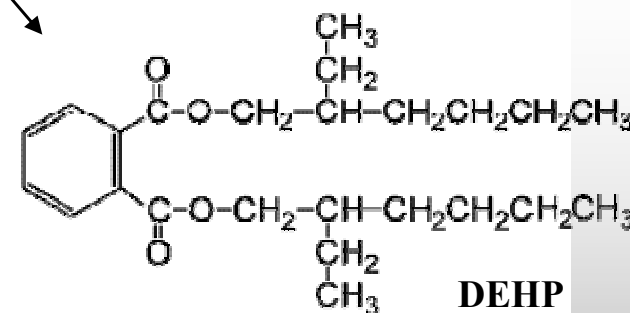
Nonionic surfactants

Pthalate esters

endosulfan



bisphenol A



DEHP

Pharmaceuticals

Ethinyl estradiol

Diethylstilbestrol

gestodene

norgestrel

Exoestrogens - Relative Potencies to bind to ER α (REPs)

Chemical group	Substance	REP
Endogenous hormones	Estradiol	1
	Estriol	$6,3 \cdot 10^{-3}$
	Testosteron	$9,6 \cdot 10^{-6}$
Phytoestrogens	Cuomestrol	$6,8 \cdot 10^{-3}$
	Genistein	$4,9 \cdot 10^{-4}$
Pesticides	o,p'-DDT	$1,1 \cdot 10^{-6}$
PCBs	2,4,6-trichlorobiphenyl-4'-ol	$1 \cdot 10^{-2}$
	2,5-dichlorobiphenyl-4'-ol	$6,2 \cdot 10^{-3}$
	3,3',5,5'tetrachlorobiphenyl-4,4'-diol	$1,6 \cdot 10^{-4}$
alkylphenoles	4-tert-oktylphenol	$3,6 \cdot 10^{-6}$
phthalates	butylbenzylphthalate	$4 \cdot 10^{-6}$

REP (RElative Potencies) of selected compounds related to 17- β -estradiol derived from reporter yeast assay

Toxicity assessment - in vivo and in vitro methods

Assay (ref.)	Exposure type	Detects ER-dependent agents?	Detects non-ER-dependent agents?	Distinguishes agonist versus antagonist?	Pharmacokinetic and metabolism included?
Receptor-based assays					
Receptor binding assay (27)	Cell lysate	Yes	No	No	No
Receptor activation assay (32-34)	Cells in vitro	Yes	No	Yes ^a	No
In vitro estrogen-regulated response assays					
MCF-7 cell proliferation assay (41)	Cells in vitro	Yes	Limited	Yes ^a	No
Induction assays (46,48)	Cells in vitro	Yes	Limited	Yes ^a	No
DNA synthesis assays (47)	Cells in vitro	Yes	Limited	Yes ^a	No
In vivo estrogen-regulated response assays					
Uterotrophic response assay (49)	Whole animal	Yes	Limited	Yes ^a	Yes
Vaginal cornification assay (50)	Whole animal	Yes	Limited	Yes ^a	Yes
Vaginal opening (11)	Whole animal	Yes	Limited	Yes ^a	Yes
Uterine fluid imbibition (11)	Whole animal	Yes	Limited	Yes ^a	Yes
Uterine epithelial hypertrophy (51)	Whole animal	Yes	Limited	Yes ^a	Yes
Inhibition of steroid synthesis assays					
In vitro ovarian steroid assay (55)	Minced tissue	No	Yes	Yes	No
Ex vivo ovarian steroid assay (56)	Whole animal	No	Yes	Yes	Yes

^aDetection of antagonists requires use of additional groups with test material + estradiol.

In vitro assay

- competitive ligand binding assay
- cell proliferation assay
- endogenous protein expression (or enzyme activity) assay
- reporter gene assay

In vivo assay

- **uterotropic assay**
- **vaginal cornification assay**
- **standard test procedures for reproductive and developmental toxicity (e.g. FETAX)**
- **production estrogen-inducible proteins (e.g. vitellogenin and zona radiata protein)**