Estrogen (ER) receptor biology: Implications for HRT?

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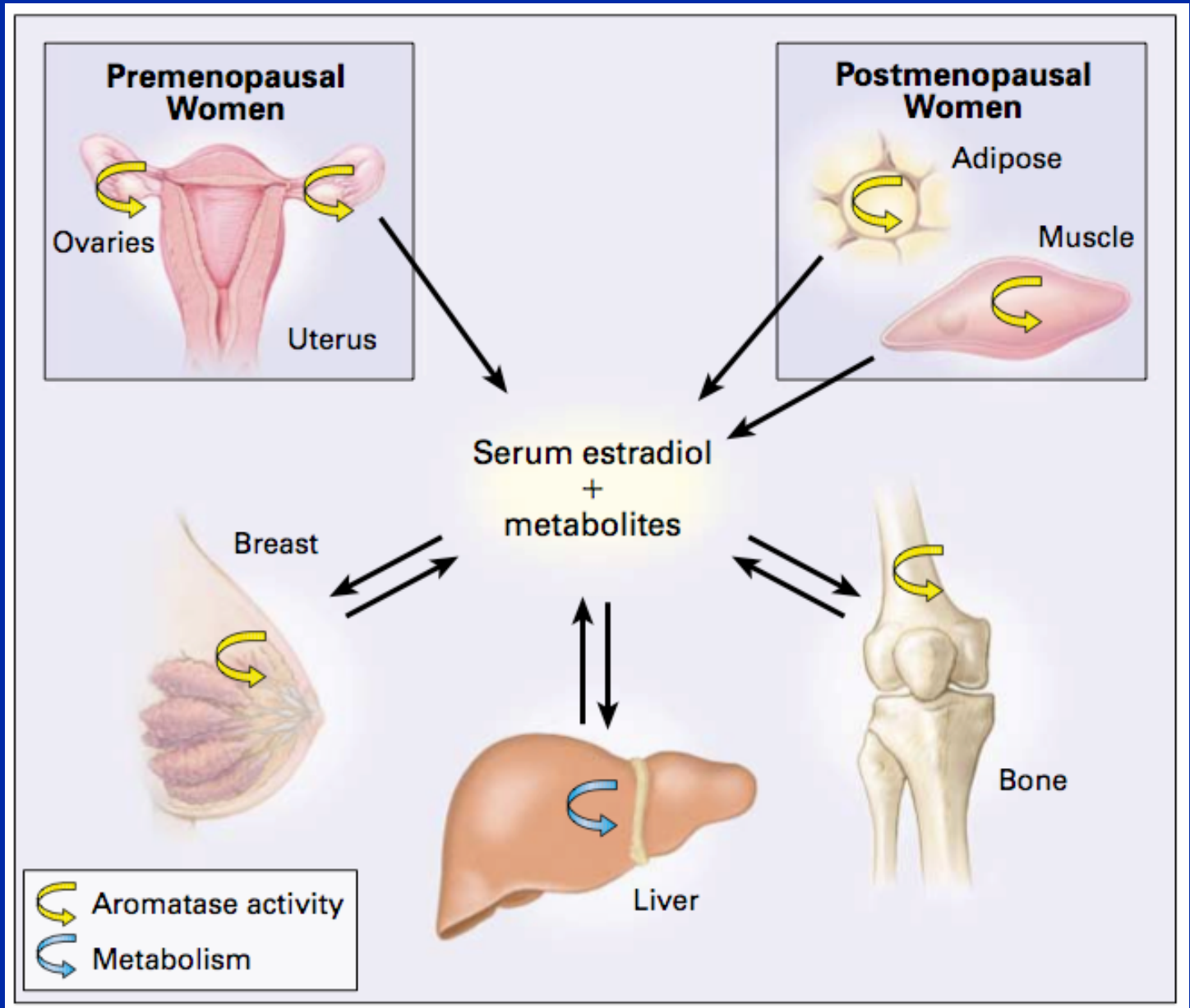


Figure 2. Effects of Whole-Body and Locally Synthesized Estrogen on Multiple End Organs. Arrows indicate sites of conversion of androgen to estrogen.

Estrogens prevent postmenopausal bone-loss

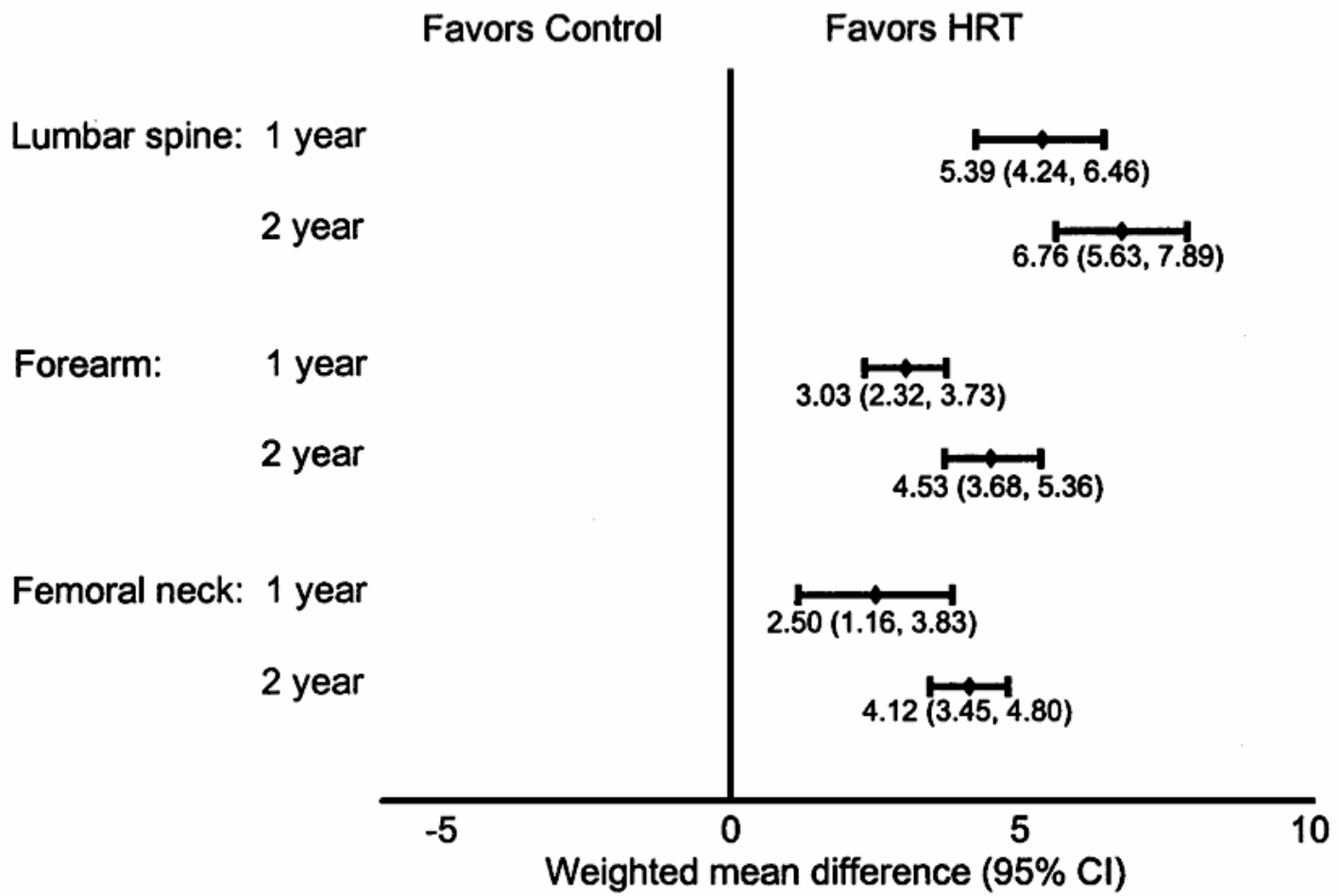
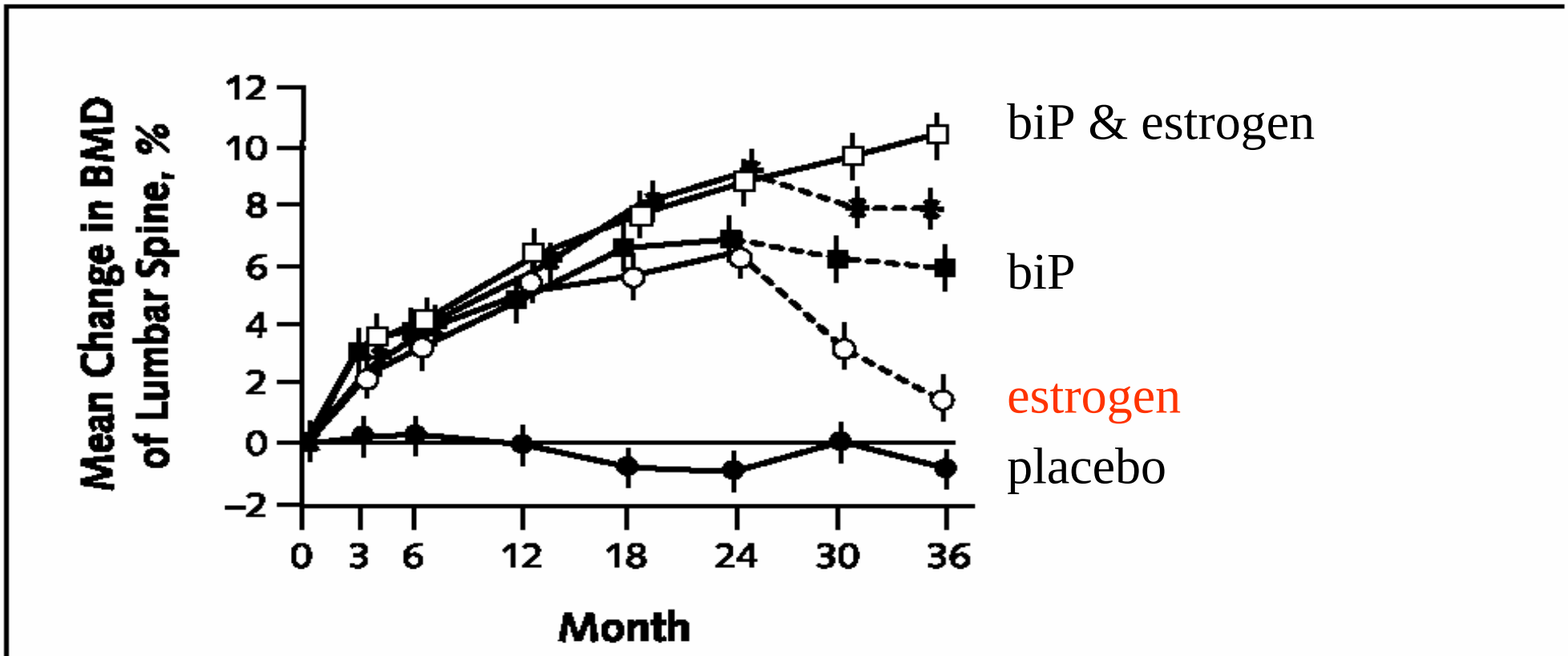


FIG. 3. Weighted mean difference in percent change in bone density after treatment with HRT.

HRT & osteoporosis

Figure 3. Mean percentage change from baseline to year 3 in bone mineral density (*BMD*).



Ann Intern Med. 2002;137:875-883.

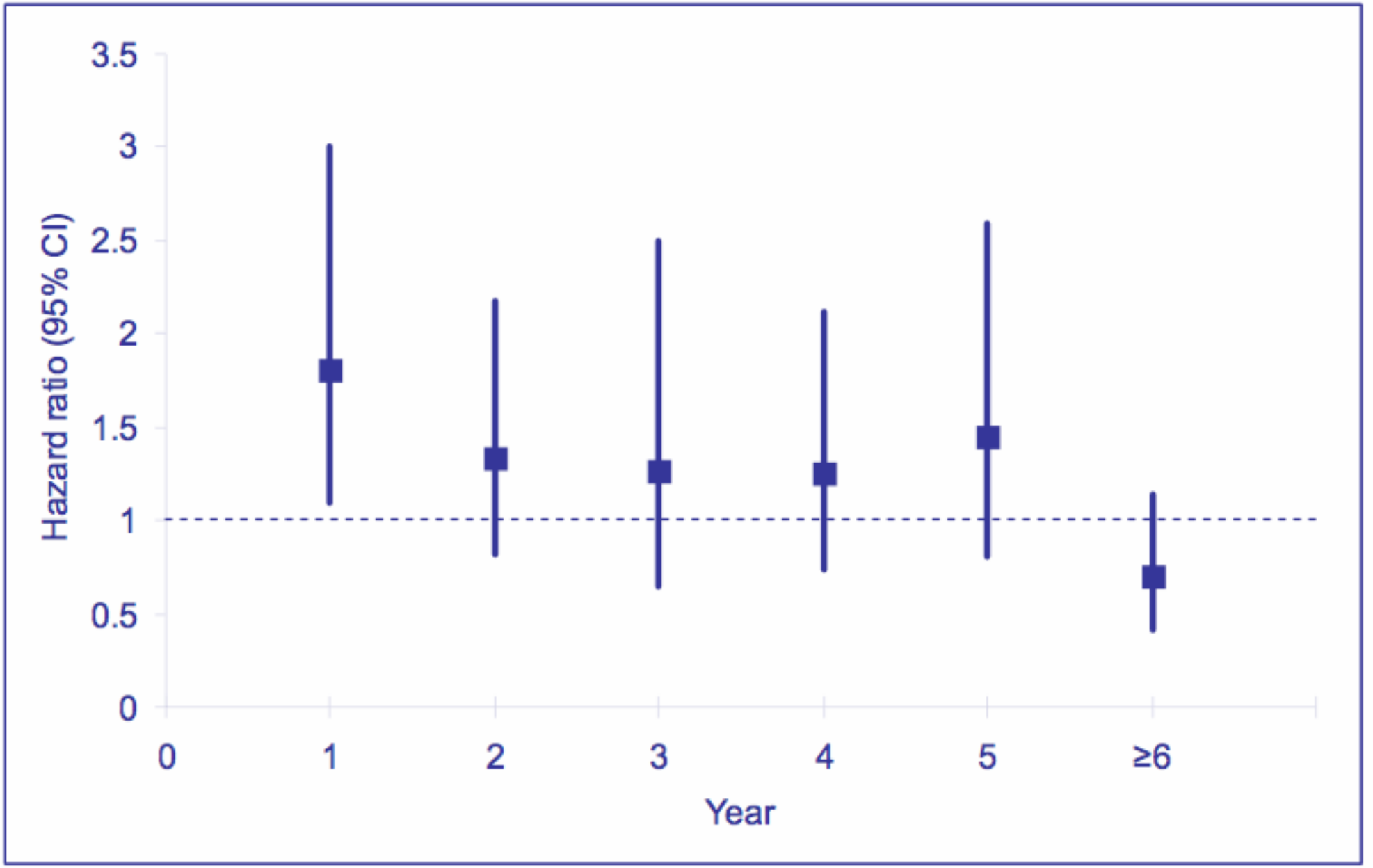


Fig. E001: Comparison of risk of CHD between estrogen plus progestin therapy vs. placebo according to duration of follow-up.

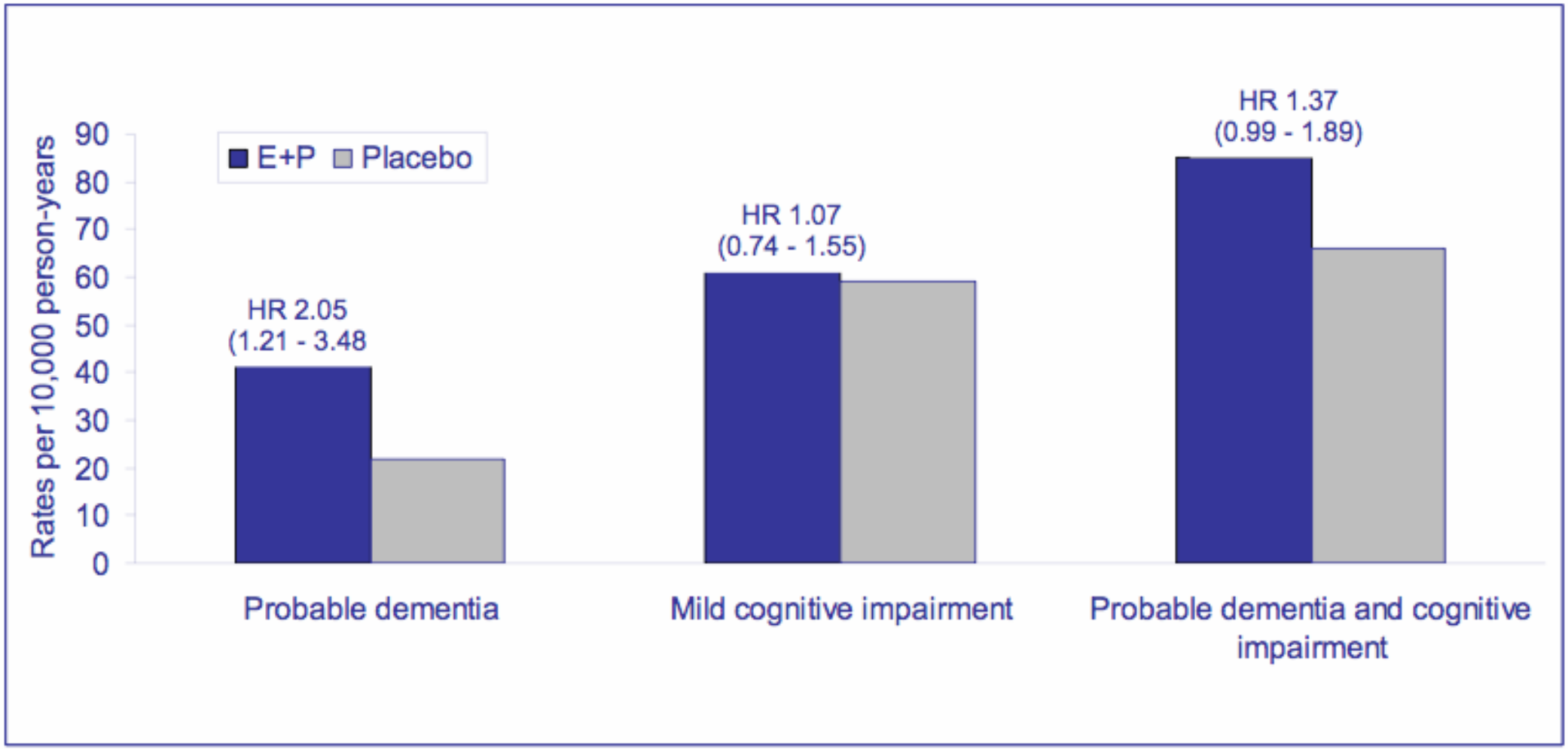


Fig. E002: Comparison of rates of probable dementia and mild cognitive dementia between estrogen plus progestin therapy vs. placebo. HR: hazard ratio, number in brackets: 95% confidence interval

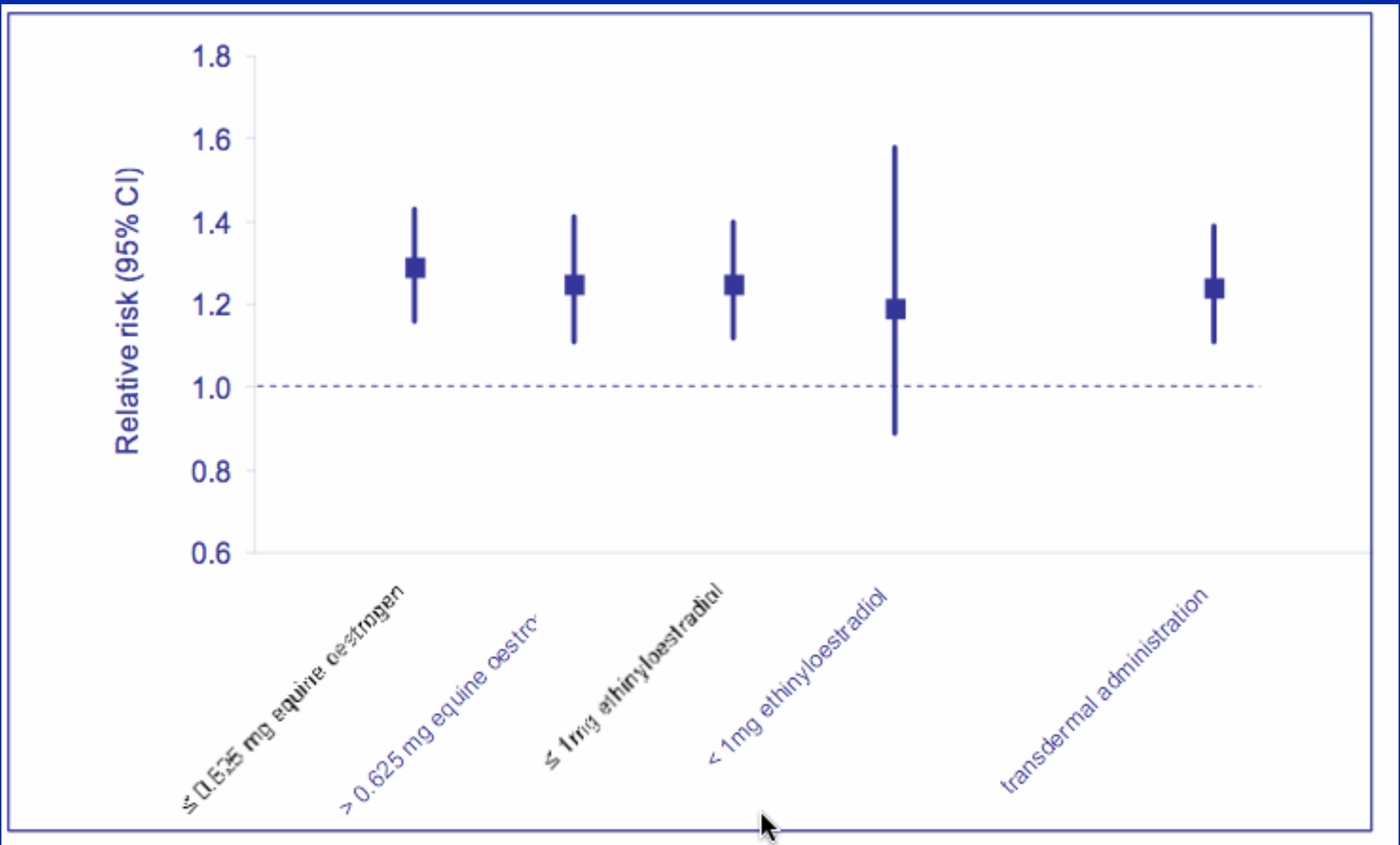


Fig. E003: Relative risk of breast cancer by therapy, dose and formulation of oestrogen only HRT relative to never users

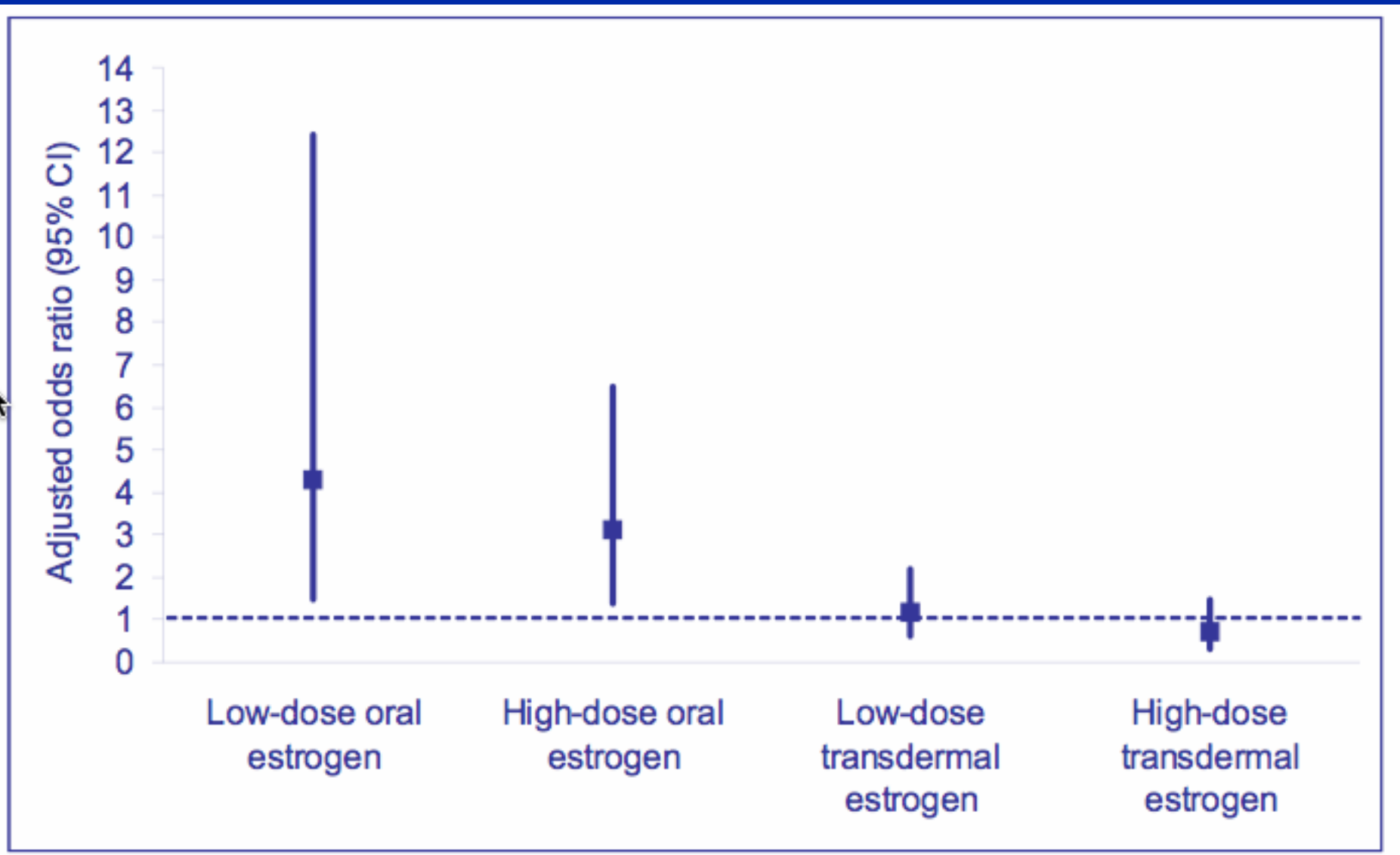


Fig. E004: Odds ratio of venous thromboembolism by ERT regimen and estrogen dose

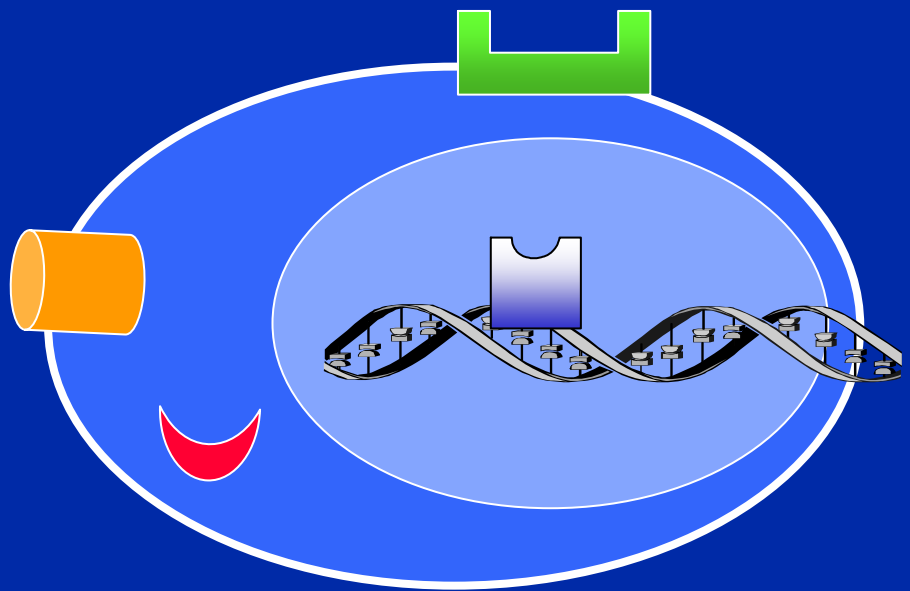
Can estrogen actions be tissue-and gene- specific?

How do estrogens act ?

Molecular targets for pharmacotherapy

- membrane receptors 50%
- enzymes 20%
- hormones, growth actors 15%
- ion channels 5%
- nuclear receptors **2%**
- other 5%

n=500



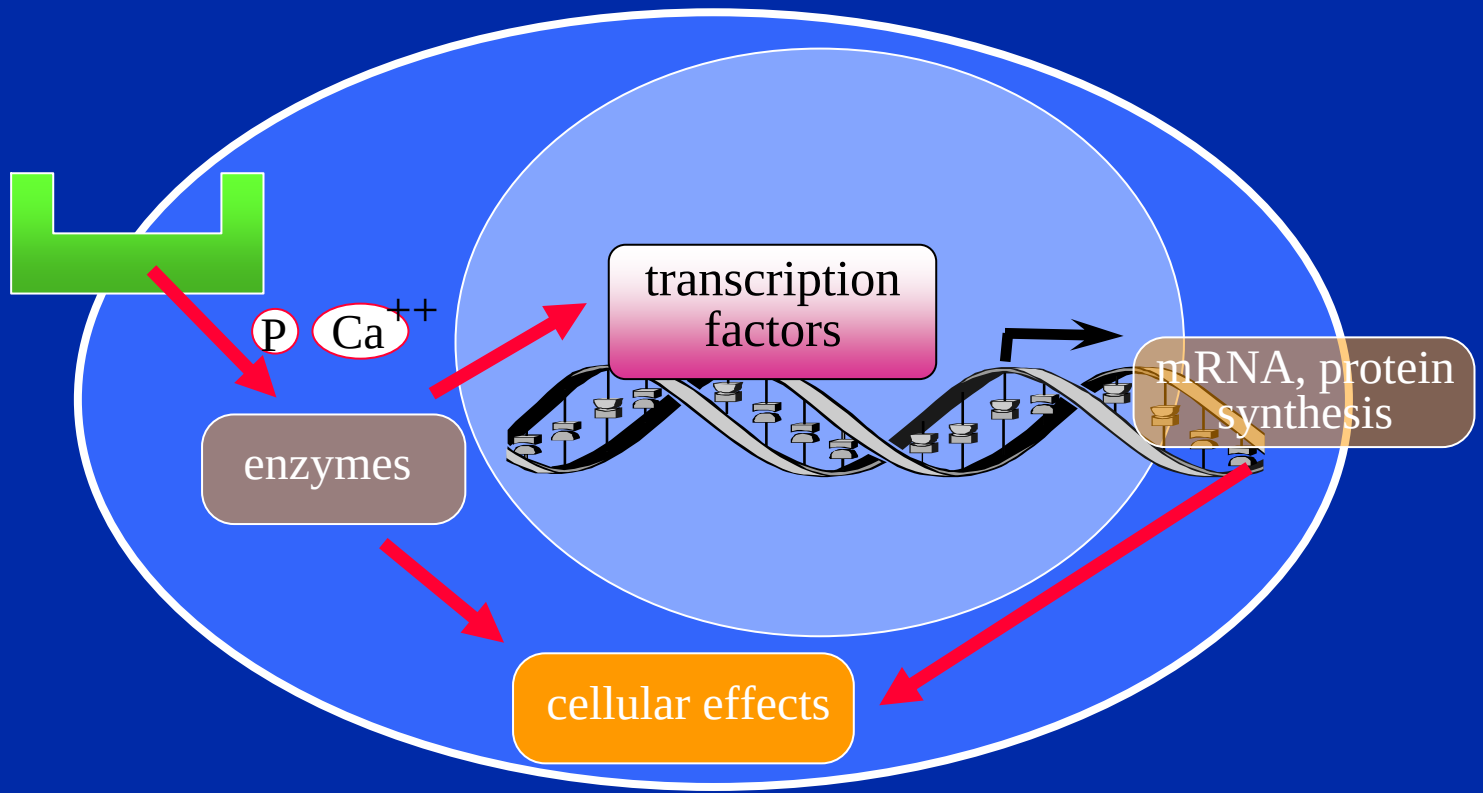
Membrane receptors as pharmaclogical targets

peptides, proteins, catech.

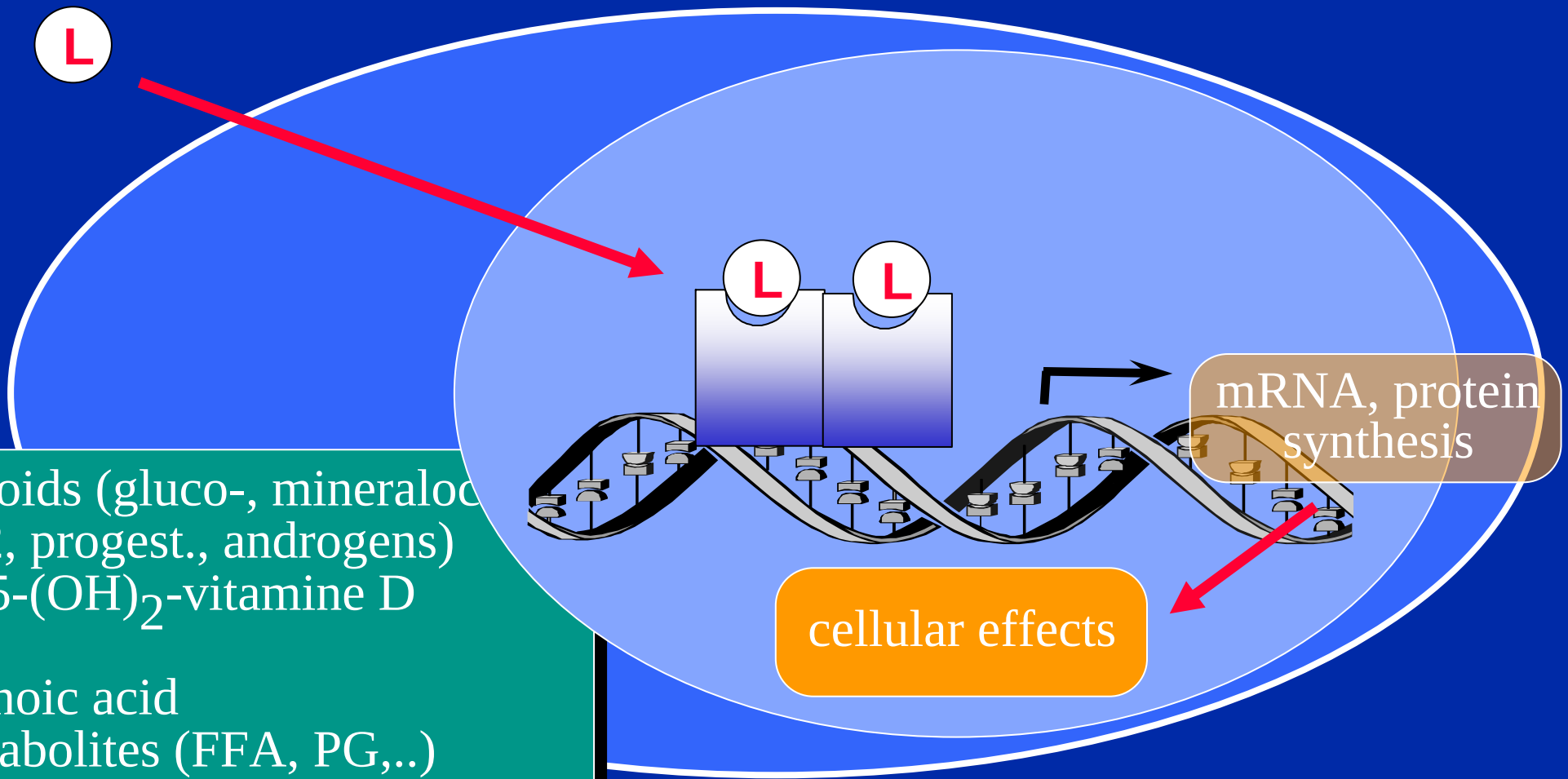
insulin, oxytocin, LH-RH, LH, FSH, PRL
adrenaline et analogues,
G-CSF, EPO, TPO

antago-nists

β-blockers
ARB
somatostatin



Nuclear receptors



steroids (gluco-, mineraloc
E2, progest., androgens)
1,25-$(OH)_2$-vitamine D
T3
retinoic acid
metabolites (FFA, PG,..)

orphan receptors

ER binding sites

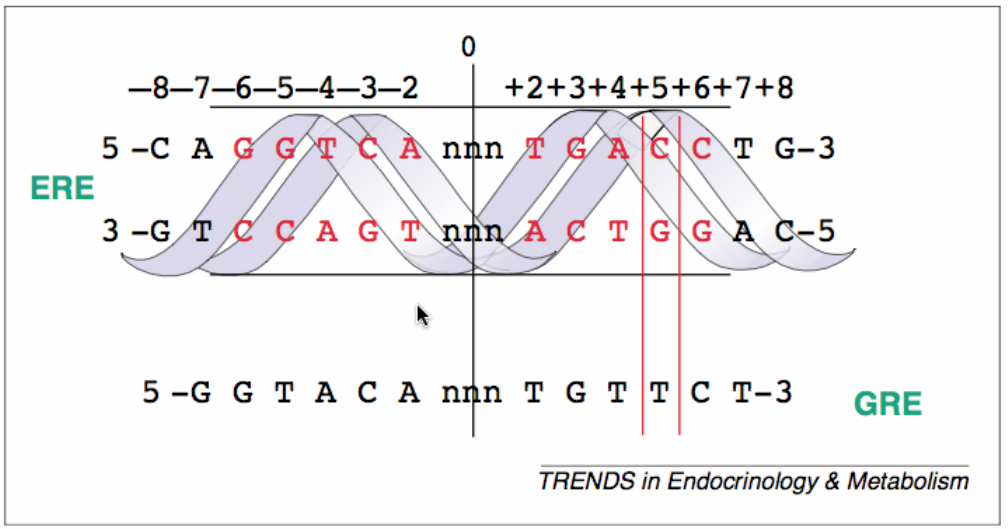


Figure 1. Sequence of the ERE and GRE. **(a)** A consensus ERE has been derived from several highly estrogen-responsive sequences from the African clawed frog *Xenopus laevis* genes encoding vitellogenin A1, A2, B1, B2 and the chicken apo-VLDL II gene. It is a 13 bp perfect palindromic inverted repeat with a 3 bp spacing of variable bases (red). **(b)** The sequence of the consensus GRE [11]. As indicated, replacement of the adenine base at position +4 by thymine results in the generation of a GRE. Positions +2, +3 and +6 are conserved in both the ERE and GRE. Abbreviations: ERE, estrogen response element; GRE, glucocorticoid response element.

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Nuclear receptors as pharmacological targets

AGONISTS

T3, prednisone, E_2,
progesterone, testosterone,
vitamine D
isotretinoine (Roaccutan®)
all-*trans* RA (ATRAC)
raloxifene, tamoxifene
glitazones
PUFA

ANTAGONISTS

flutamide, cyproterone acetate
RU486
tamoxifene, raloxifene

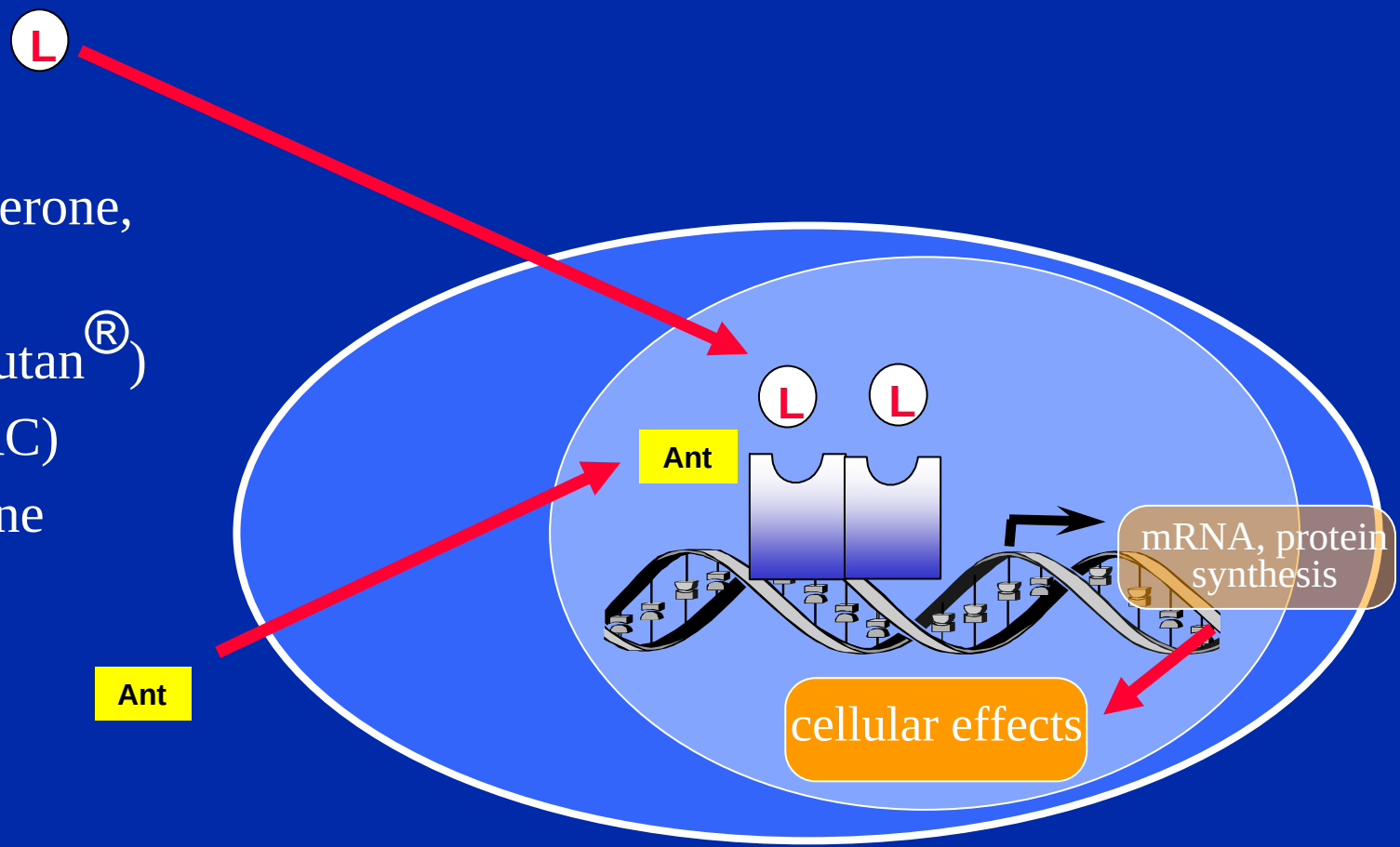


Table 1. Summary of reproductive phenotypes observed in adult male and female estrogen receptor knockout mice

	Male ERKO	*Female ERKO*
Gametogenesis	Disrupted spermatogenesis; reduced sperm counts, motility, and viability	Oogenesis blocked at secondary follicle stage; hemorrhagic, cystic, and atretic follicles
Steroid hormones	1.8-fold higher T levels	E_2 and T significantly elevated
Gonadotropins	In normal range for WT	NS
Accessory sex structures	Normal development+	Mammary agenesis; decreased uterine size and absence of responses to E_2
Behavior	Normal mounting behaviors, decreased intromissions, and ejaculations	NS

E_2, estradiol; ERKO, estrogen receptor knockout; NS, data not shown; T, testosterone; WT, wild-type.

Hereditary mutations in nuclear receptors

ERα — E_2 resistance (recessive)

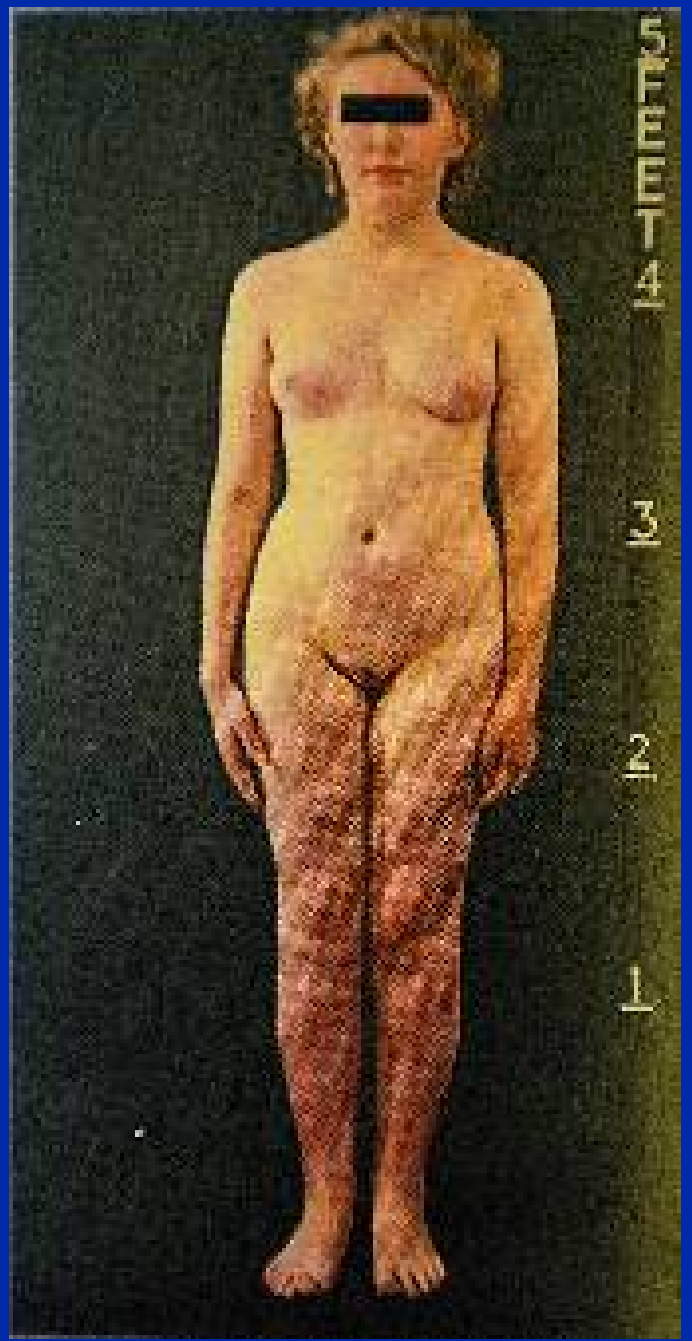
AR — $[Gln]_n$ — T resistance (X chr.)
(Reifenstein, testicular feminisation, Kennedy syndrome)

GR — GC resistance (recessive)

TR — TH resistance (**dominant**)

VDR — VDDR II (recessive)

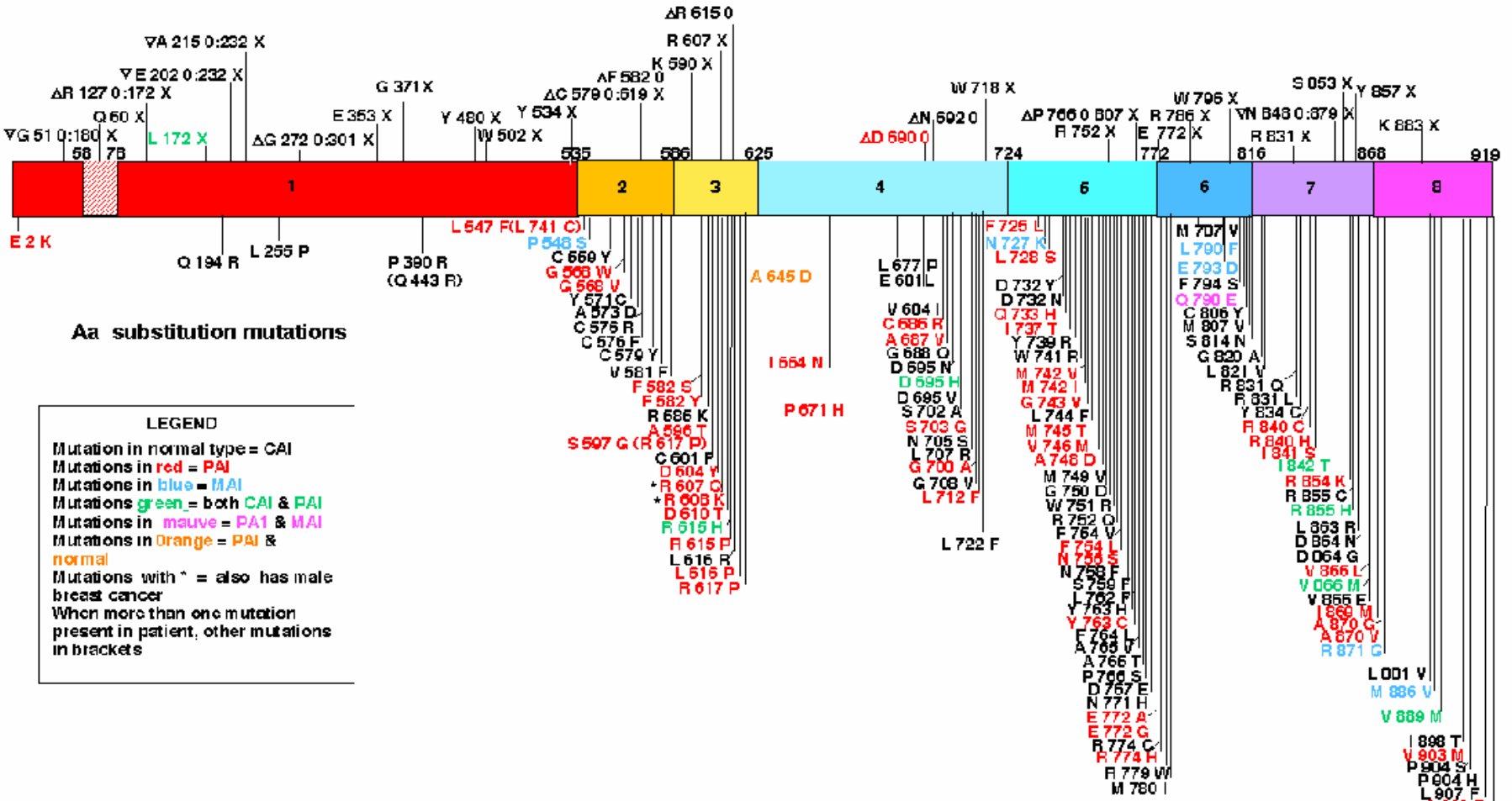
Testicular feminization



XY

ANDROGEN RECEPTOR GENE MUTATIONS IN AIS 26-11-98

Premature termination, deletion,or insertion mutations

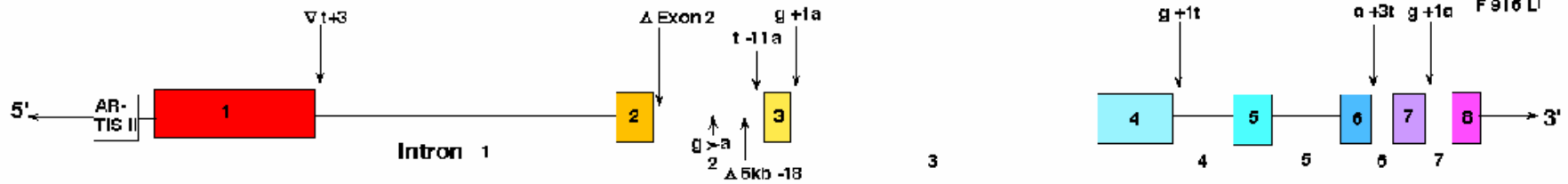


Aa substitution mutations

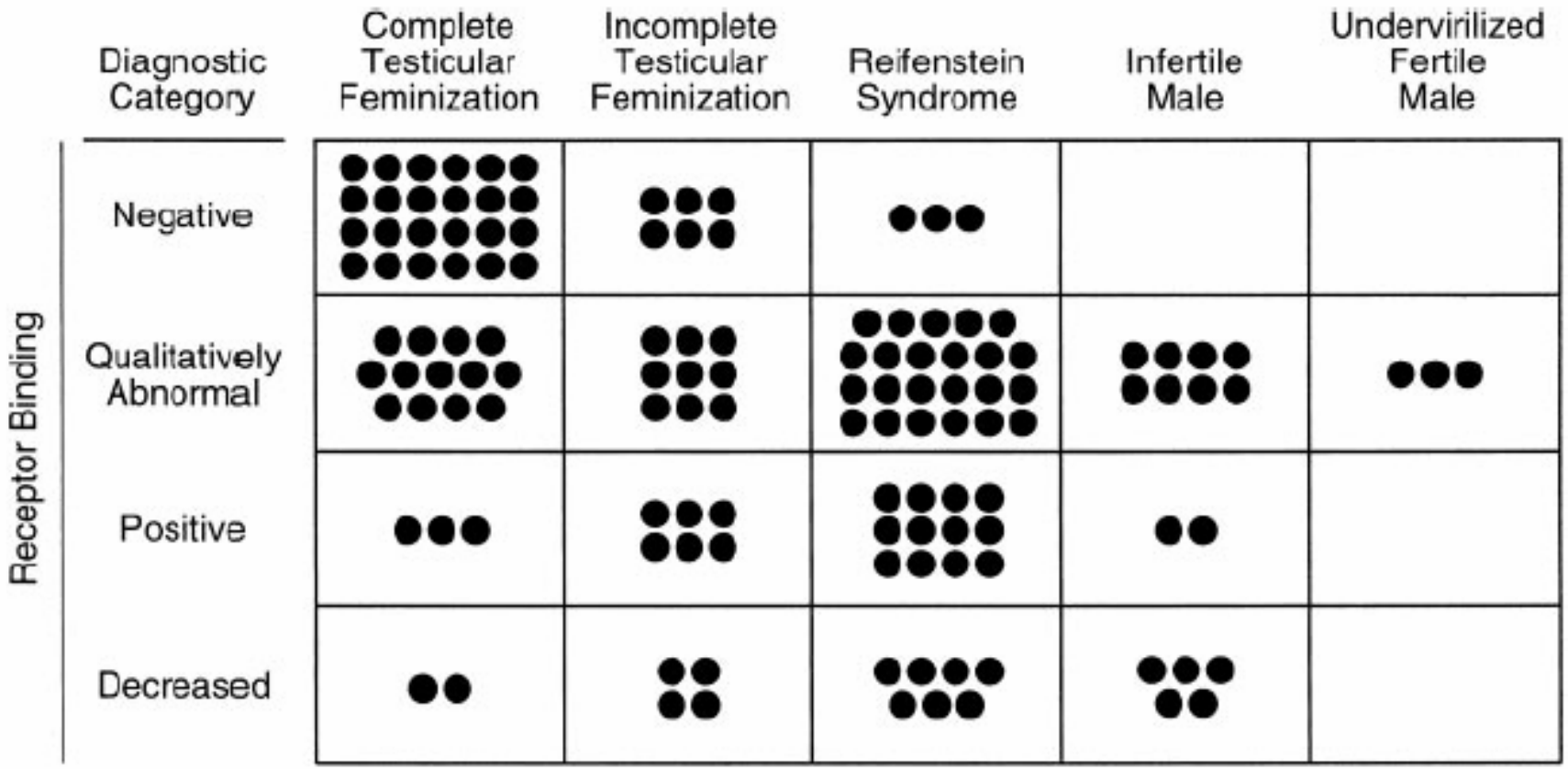
LEGEND

Mutation in normal type = CAI
Mutations in red = PAI
Mutations in blue = MAI
Mutations green = both CAI & PAI
Mutations in mauve = PA1 & MAI
Mutations in Orange = PAI & normal
Mutations with * = also has male breast cancer
When more than one mutation present in patient, other mutations in brackets

Location of splicing and untranslated region mutations



Location of intron mutations

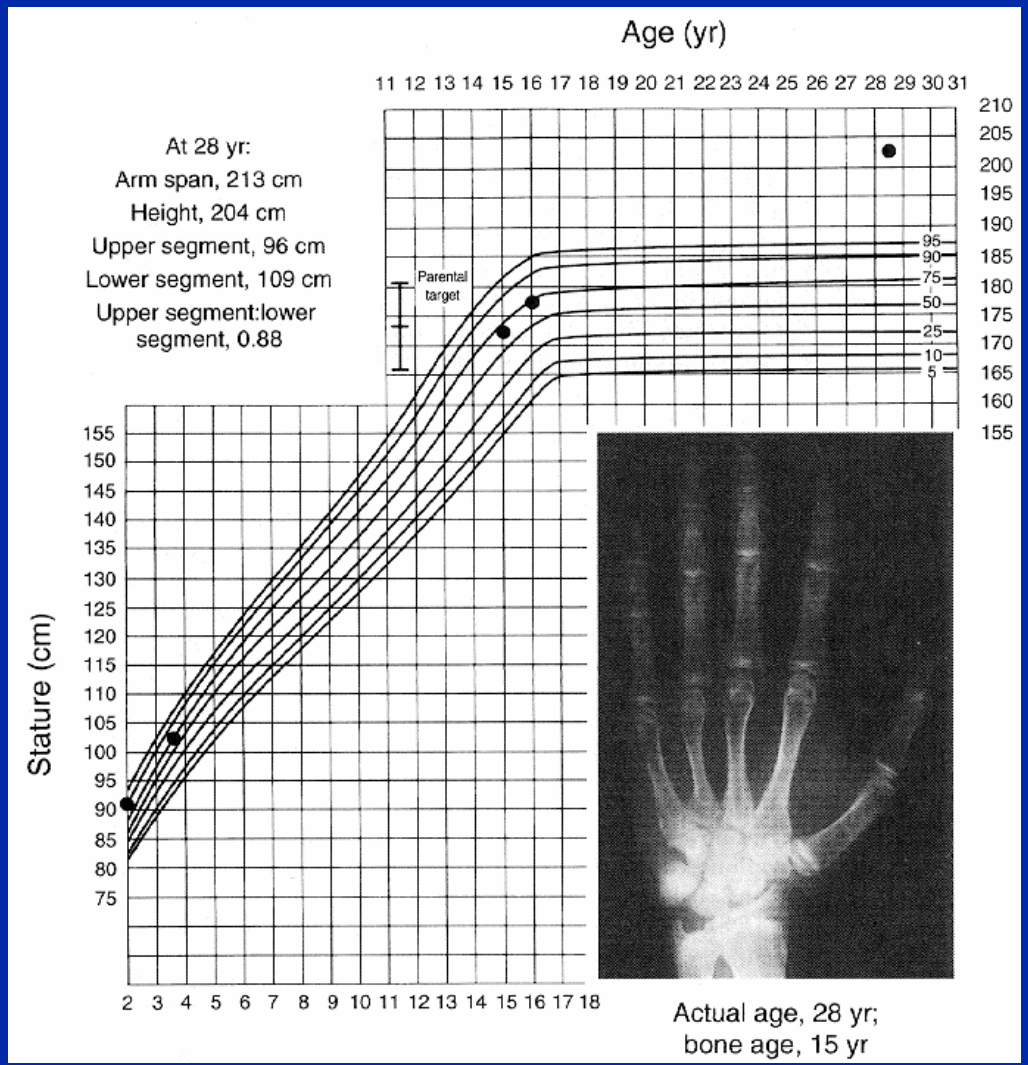


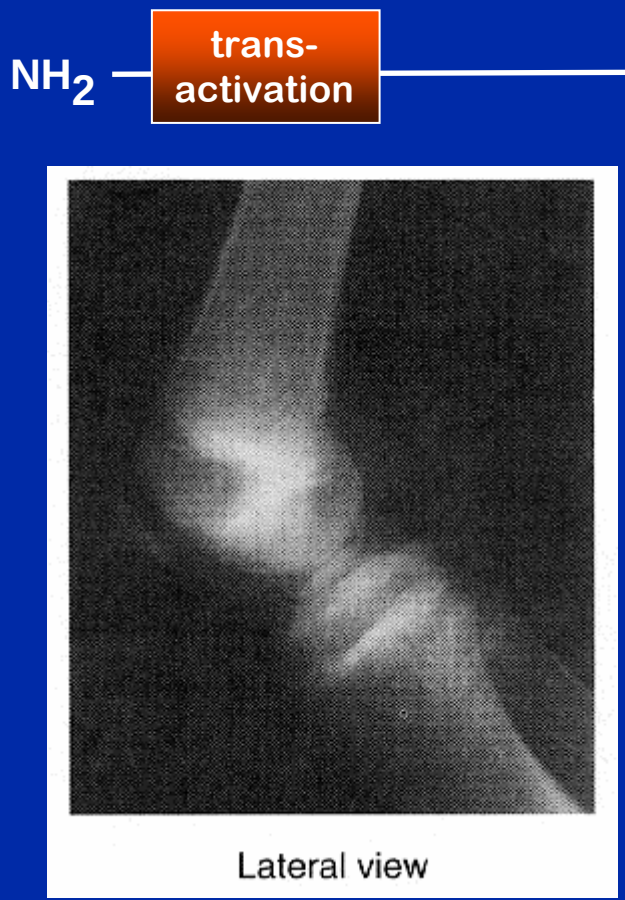
Rec Prog Horm Res 57: 181

Spino-bulbar Muscular Atrophy

- Expansion of a Gln-repeat in the N-terminal trans-activation domain
- Clinical presentation:
  - delayed onset (30-50 y) of progressive atrophy of spinal and bulbar muscles (cramps, tremor, weakness of the tongue, facial muscles and prox. limb girdle muscles)
  - mild, late-onset androgen resistance
- Pathogenesis:
  1. dysfunctional AR
  2. accumulation of a toxic protein ? (in males only)

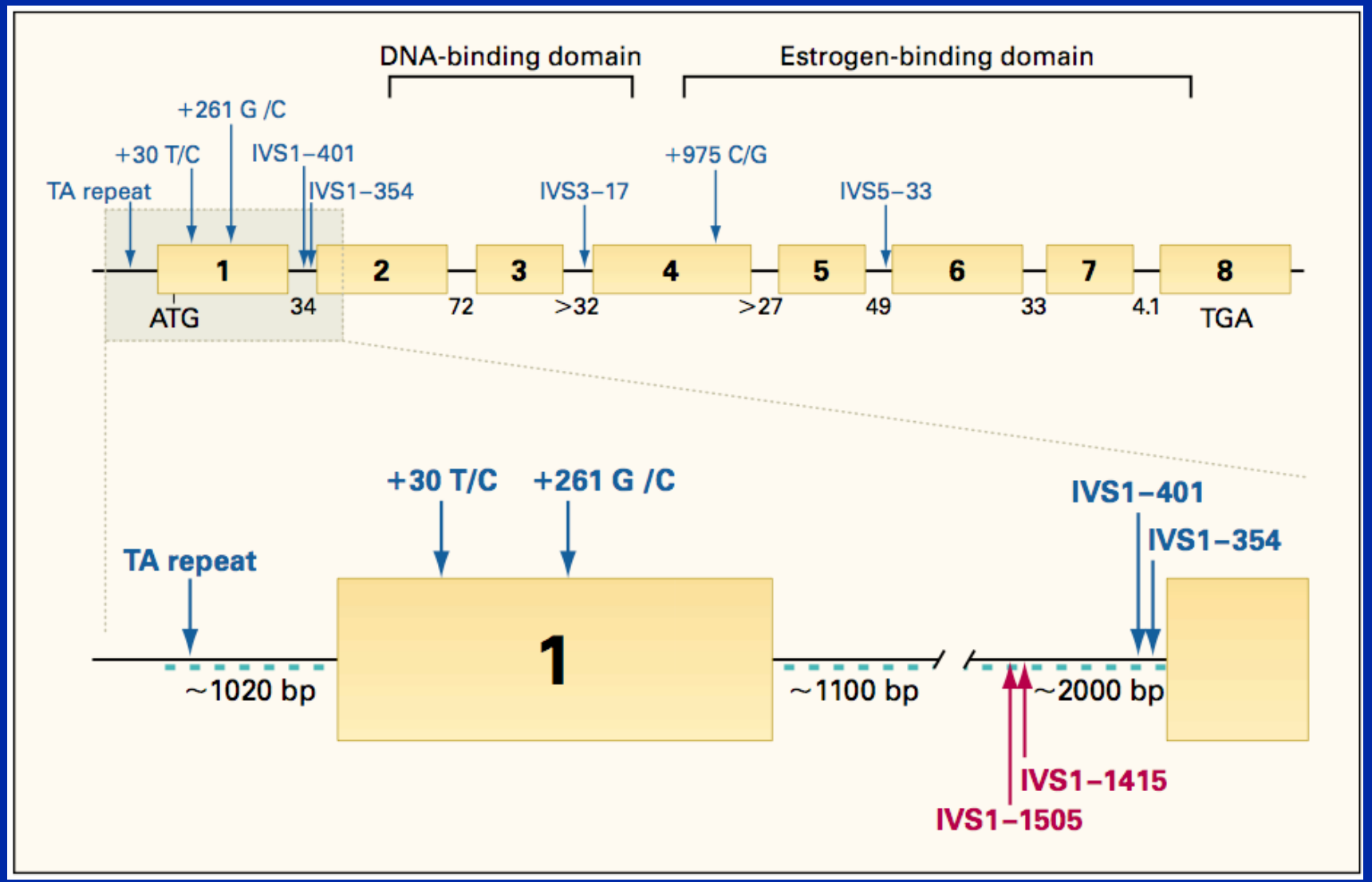
Estrogen resistance





NEJM, 331: 1056f (1994)

ER polymorphisms in humans



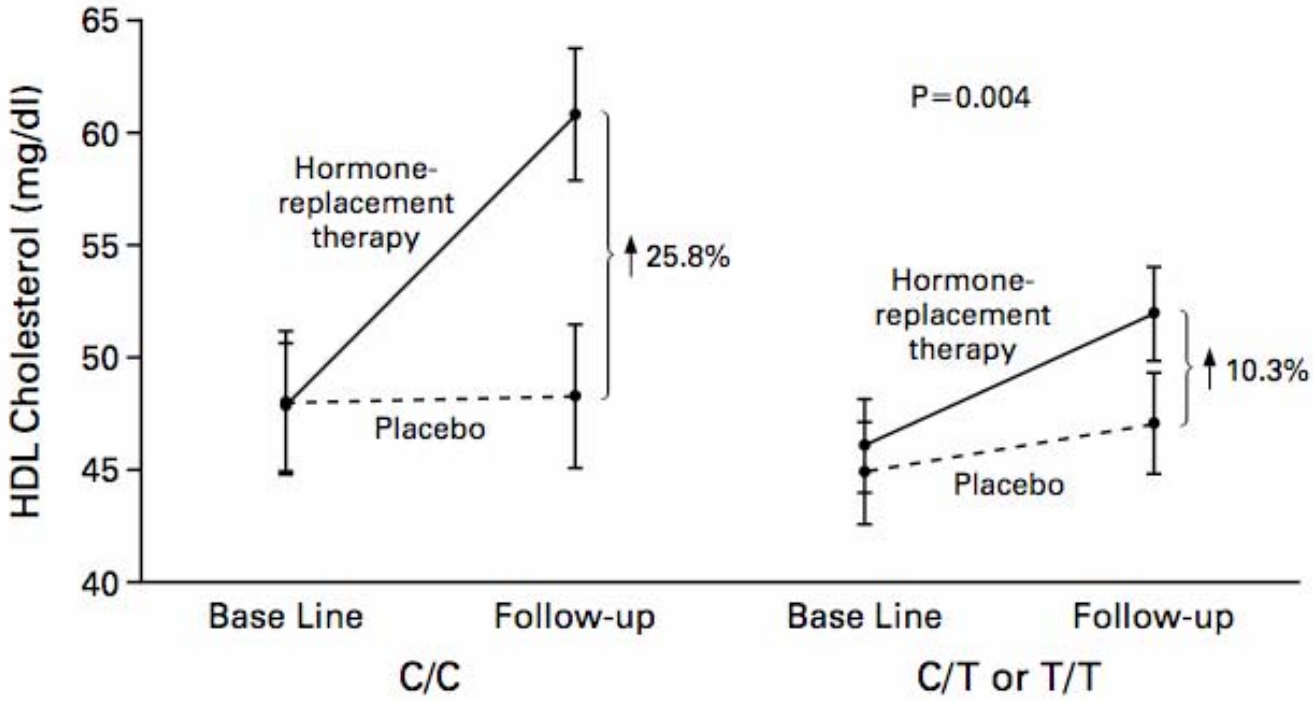


Figure 2. Mean (±SE) High-Density Lipoprotein (HDL) Cholesterol Levels at Base Line and Follow-up among Women in the Estrogen Replacement and Atherosclerosis Trial According to Study Group and Human Estrogen Receptor α IVS1−401 Genotype, with Adjustment for Age, Race or Ethnic Group, Body-Mass Index, Diabetes Status, Smoking Status, Frequency of Exercise, and Alcohol Intake.

The P value is for the treatment-by-genotype interaction. To convert values for cholesterol to millimoles per liter, multiply by 0.02586.

NEJM 346: 967f (2002)

Somatic mutations in nuclear receptors

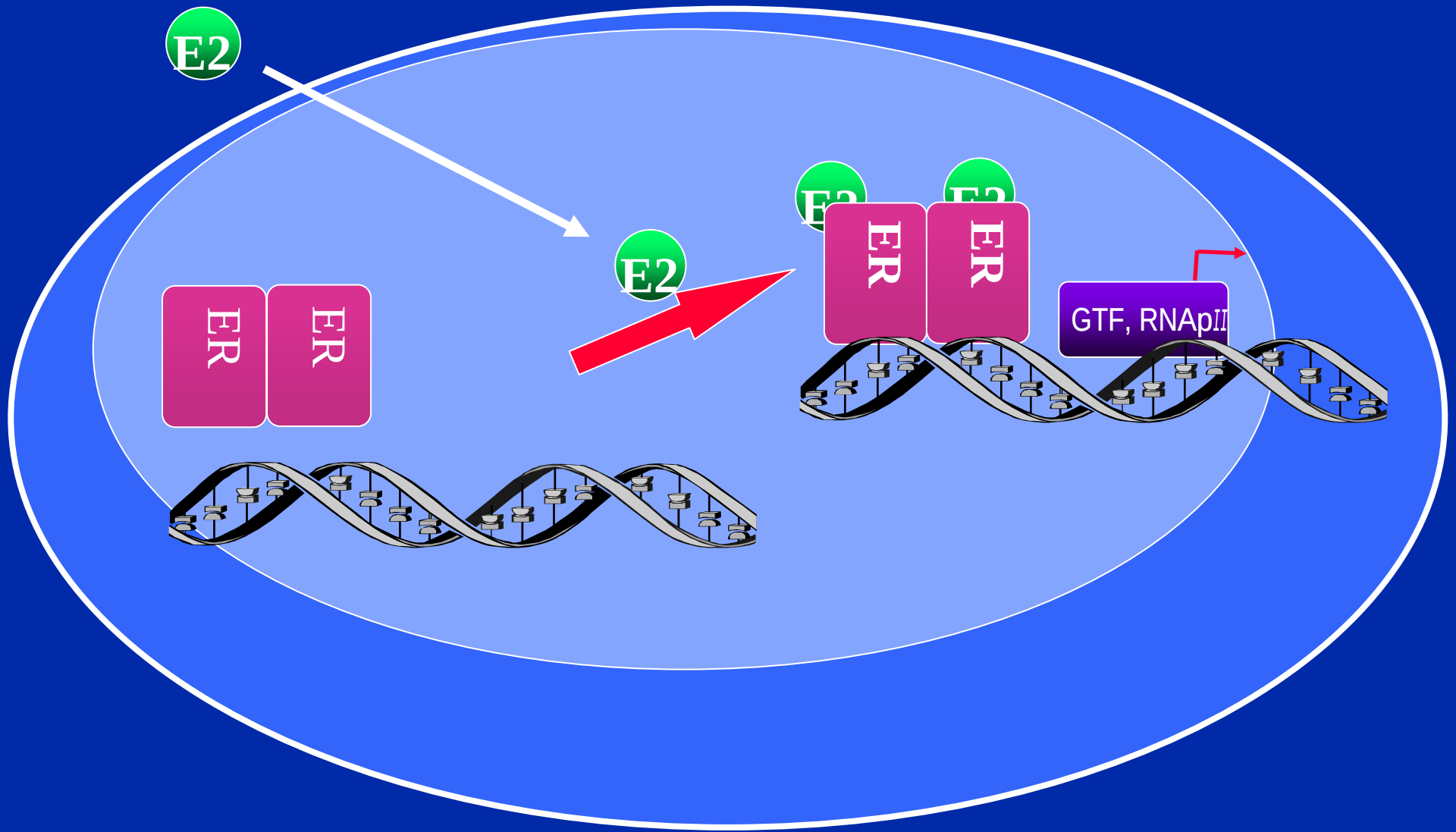
ER breast cancer: dominant negative & positive mutations
paradoxical activation by tamoxifene

GR glucocorticoid resistance ? (lymphomas, myelomas)

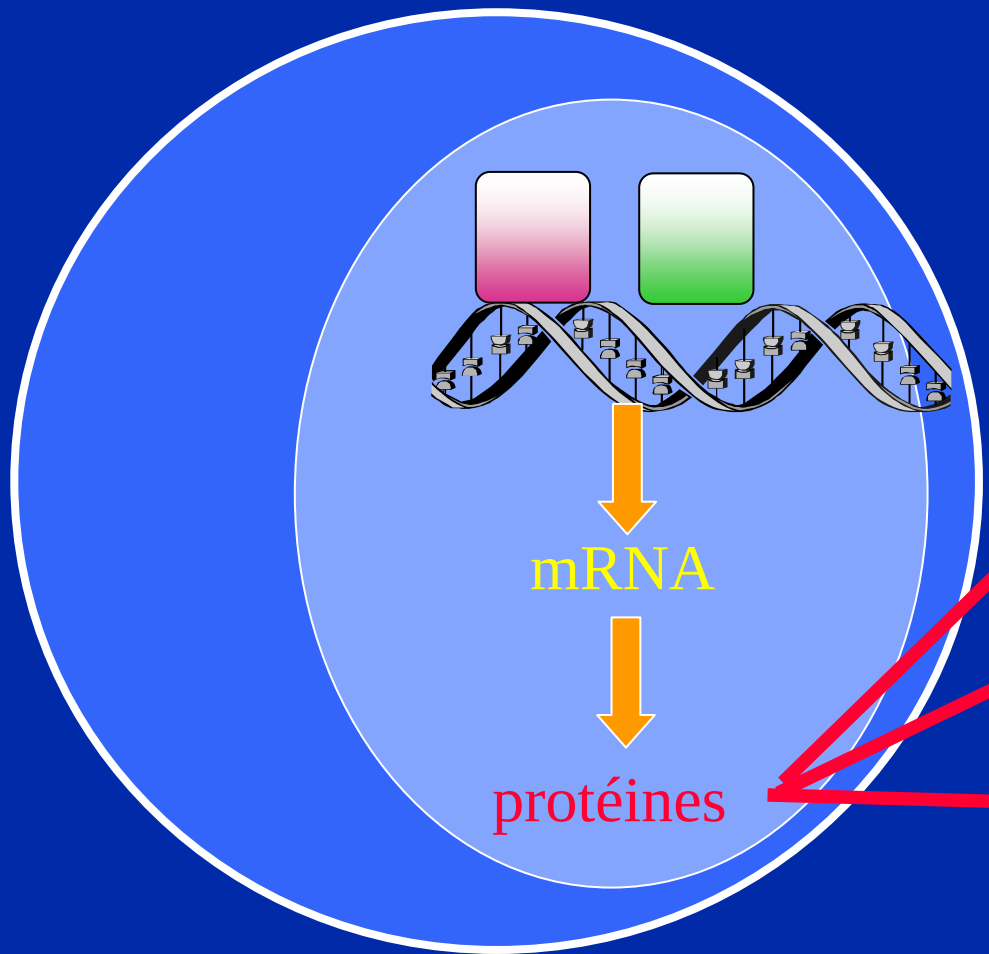
AR prostate cancer

RAR PML

Activation of ER

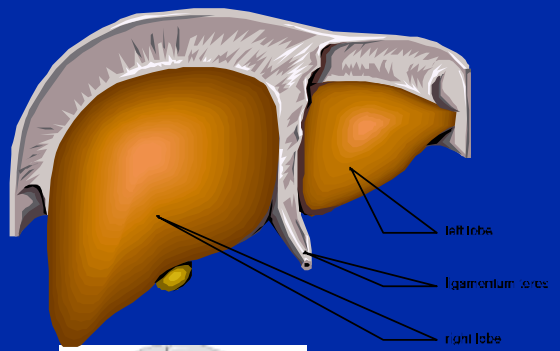


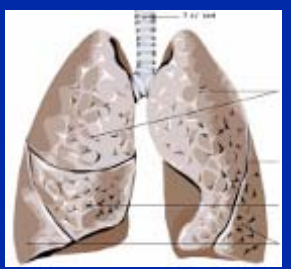
Transcriptional regulation



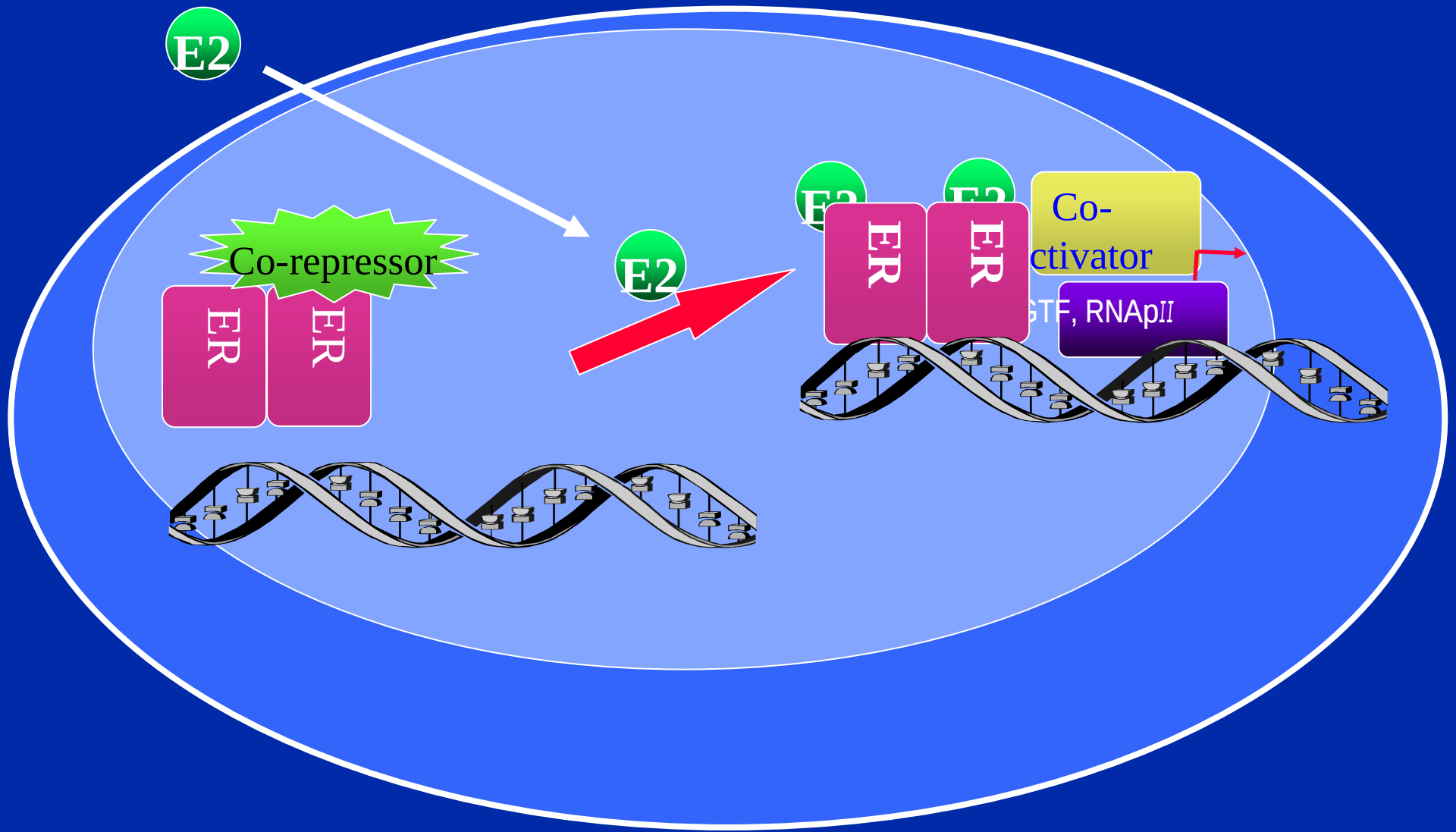
differentiation

differentiation

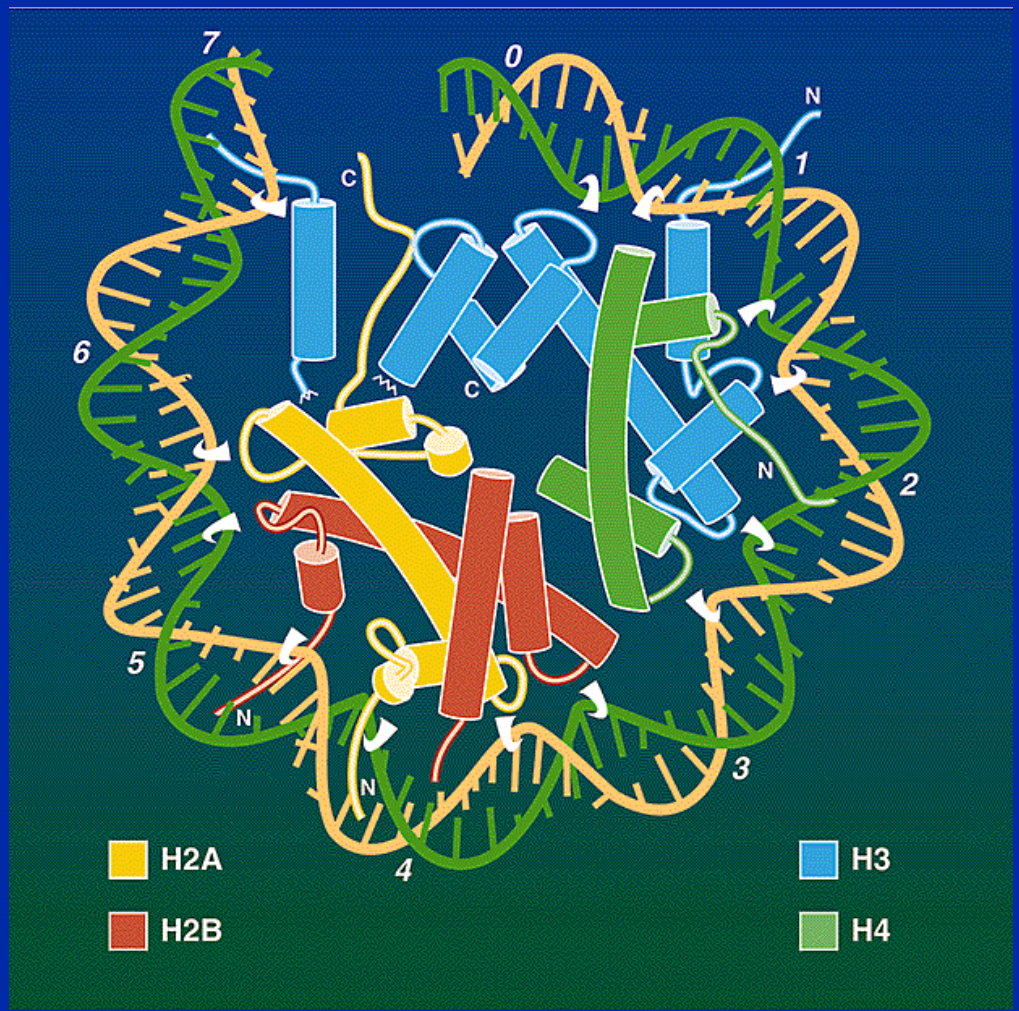




Activation of nuclear receptors

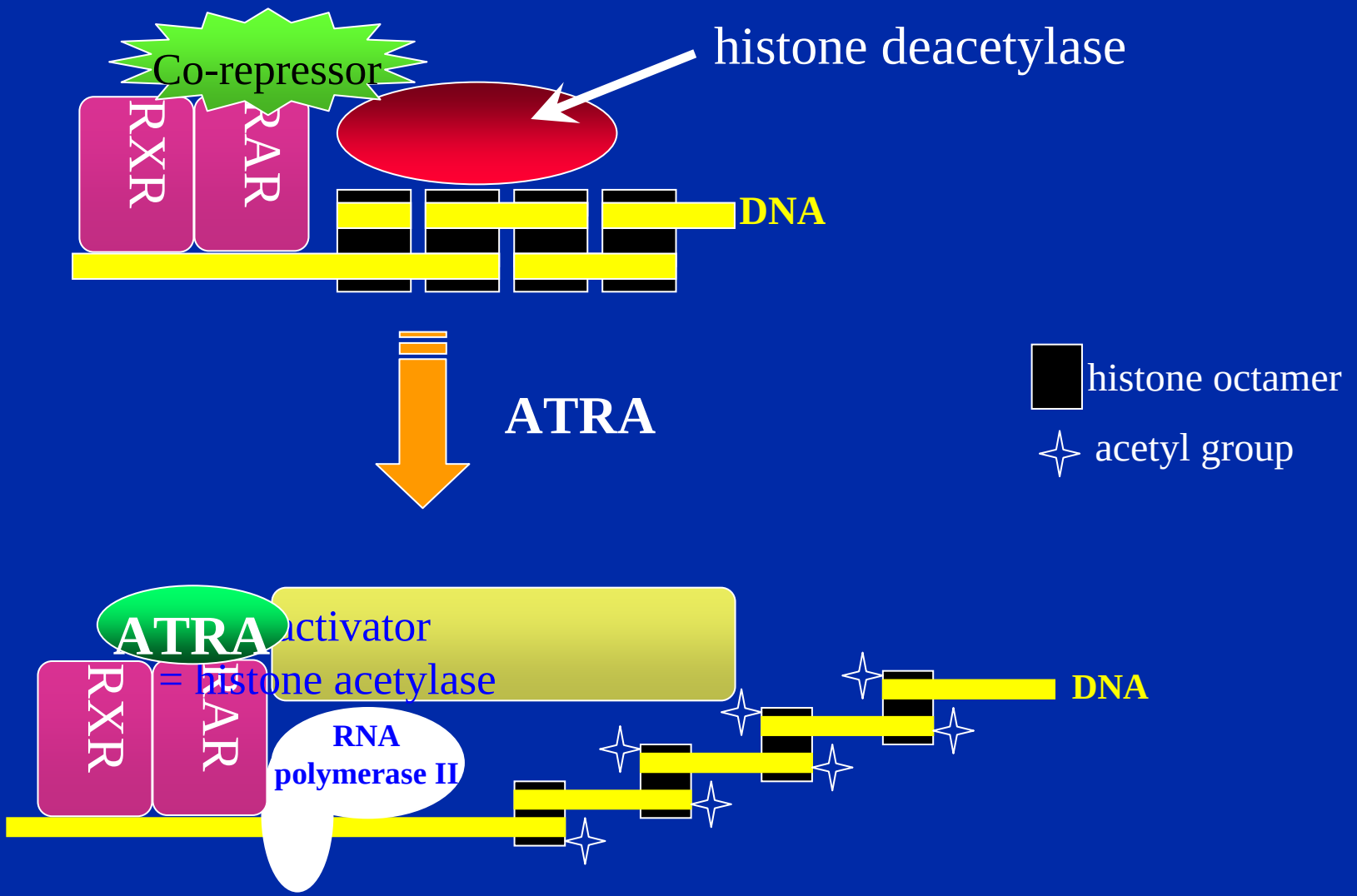


Chromatin

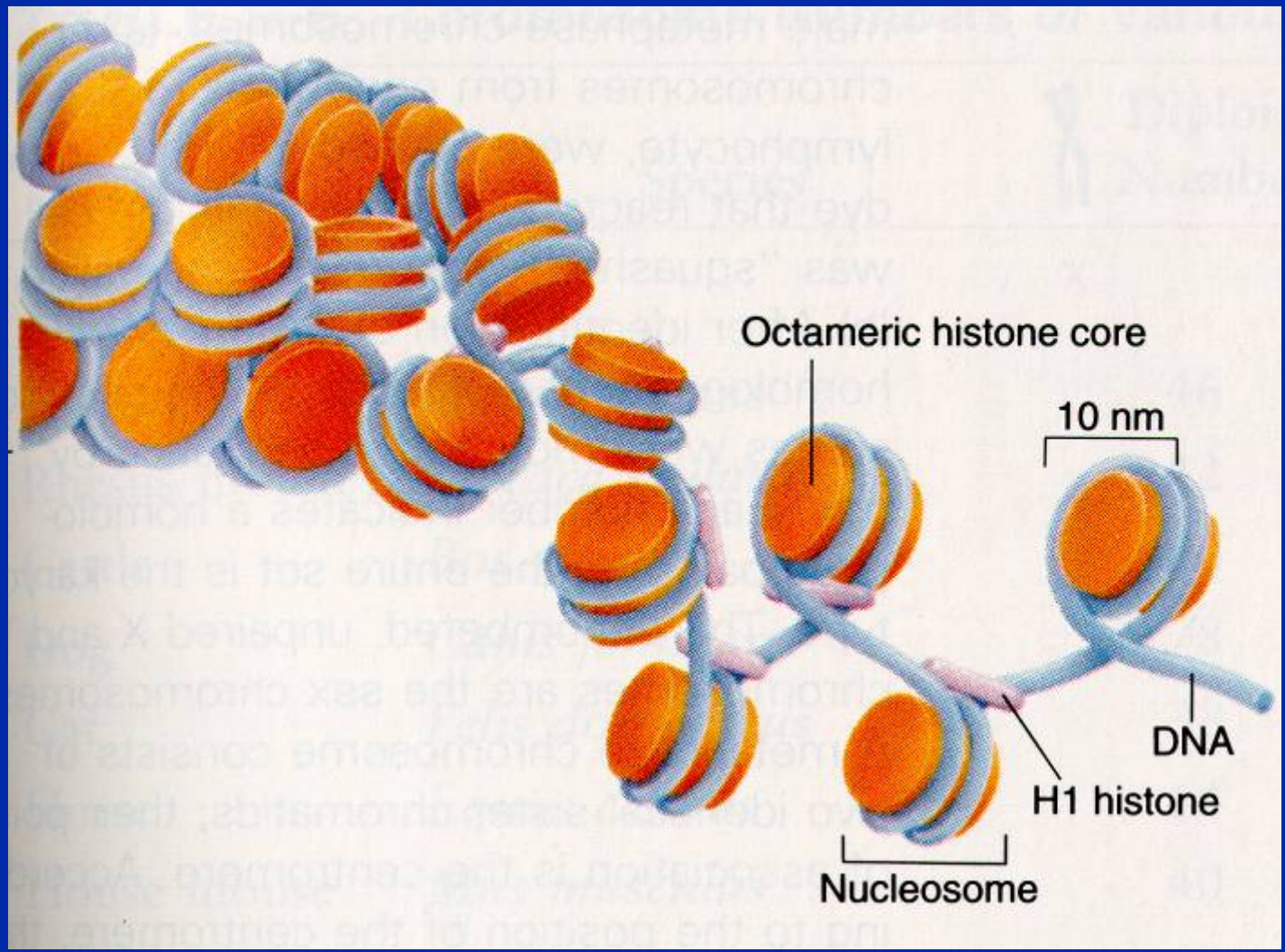


Nature
389: 251f
1997

Chromatin structure



Chromatin opening



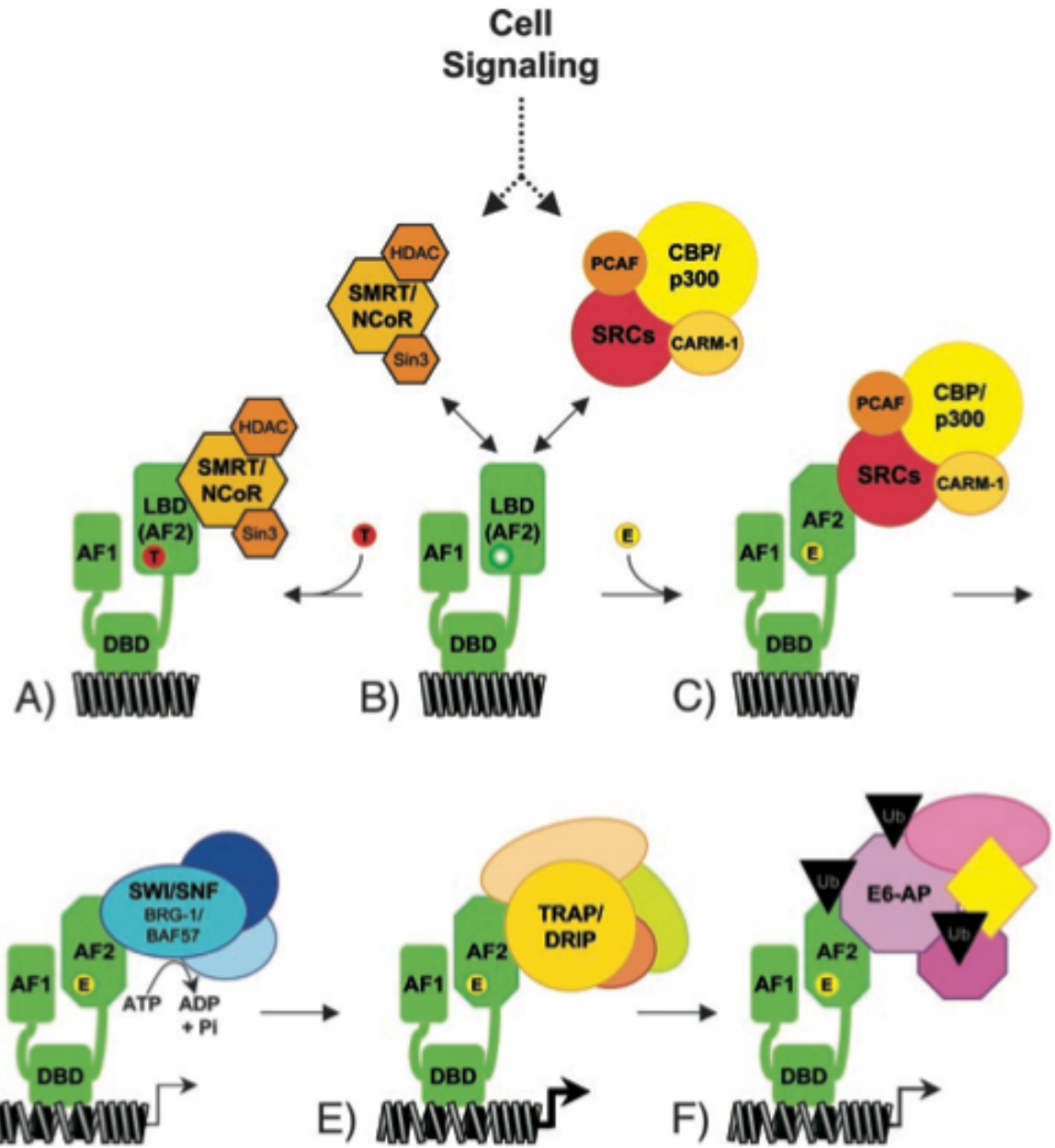


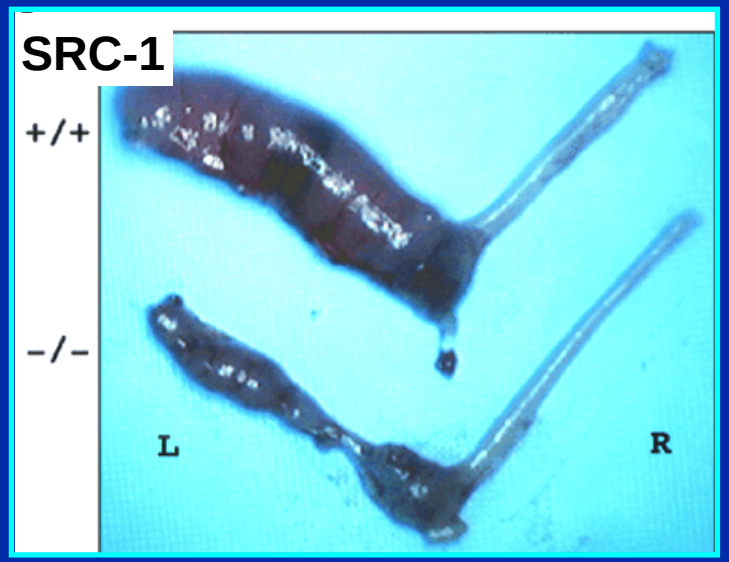
FIG. 1. Model of nuclear receptor-dependent gene expression. This represents a hypothetical schematic of the exchange of coregulators involved in activation of a gene by a steroid hormone receptor, such as ERα. Coactivators and corepressors exist in complexes in the cell and do not appear

God does not play dice,
he prefers Lego

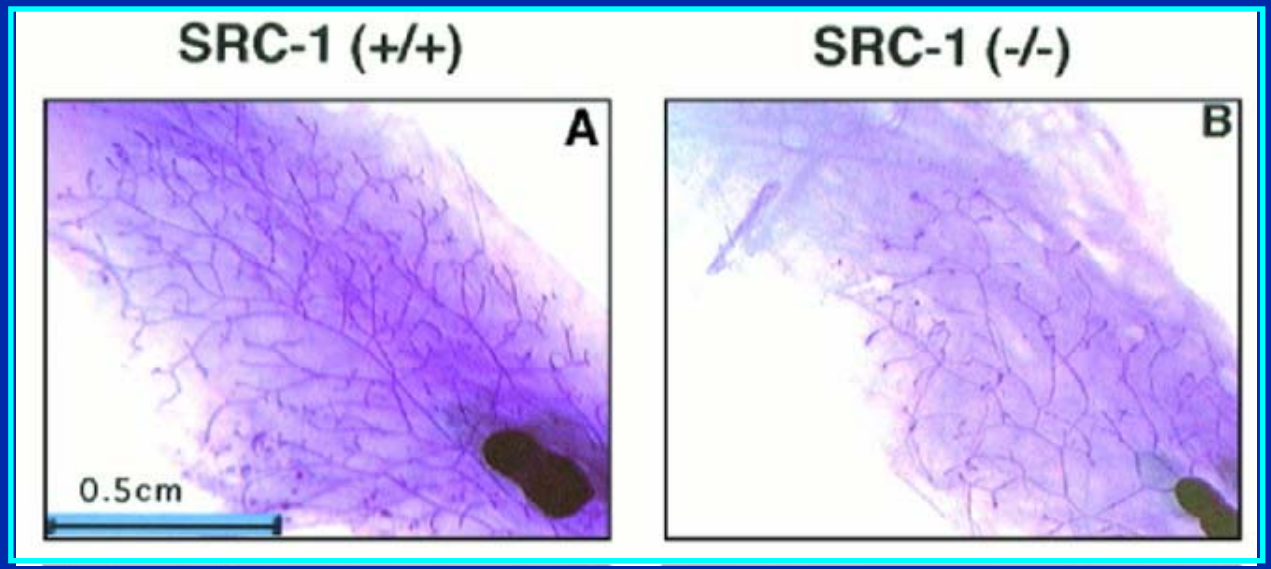
Pierre Chardin, 1997

Knock-out of the co-activator SRC-1

uterus (stimulated)

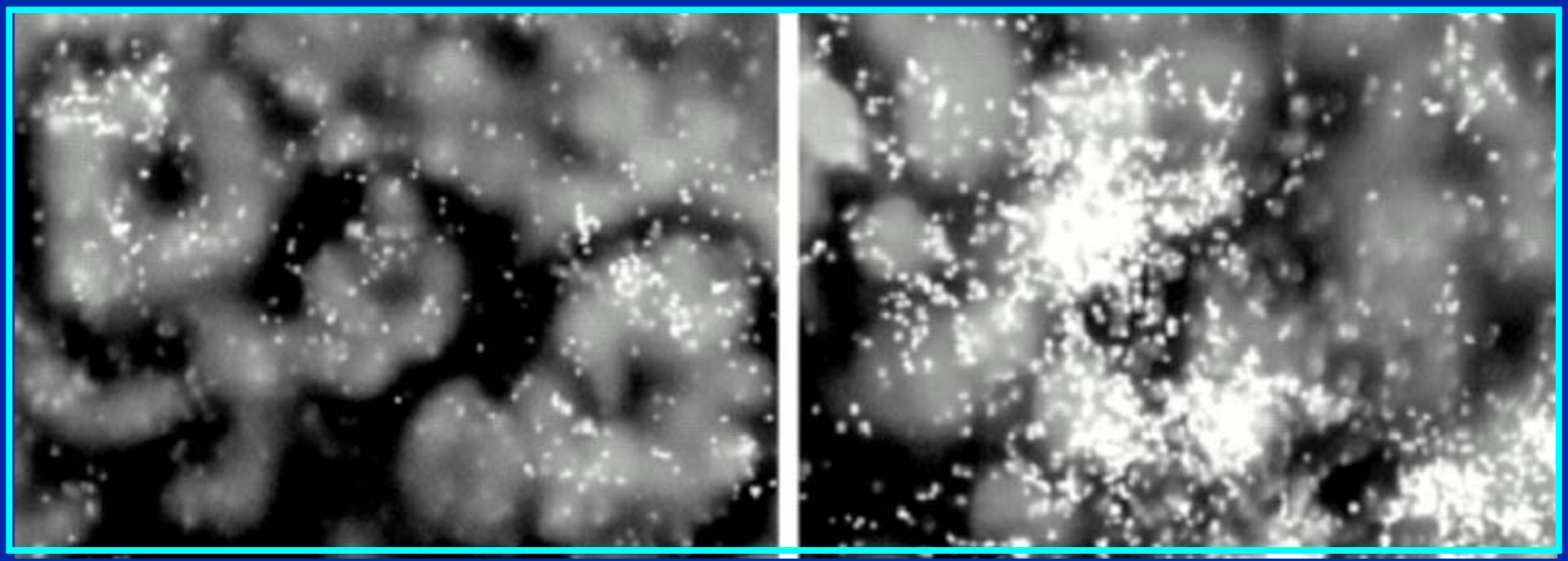


breast tissue (8 weeks)



Science 279:1922f (1998)

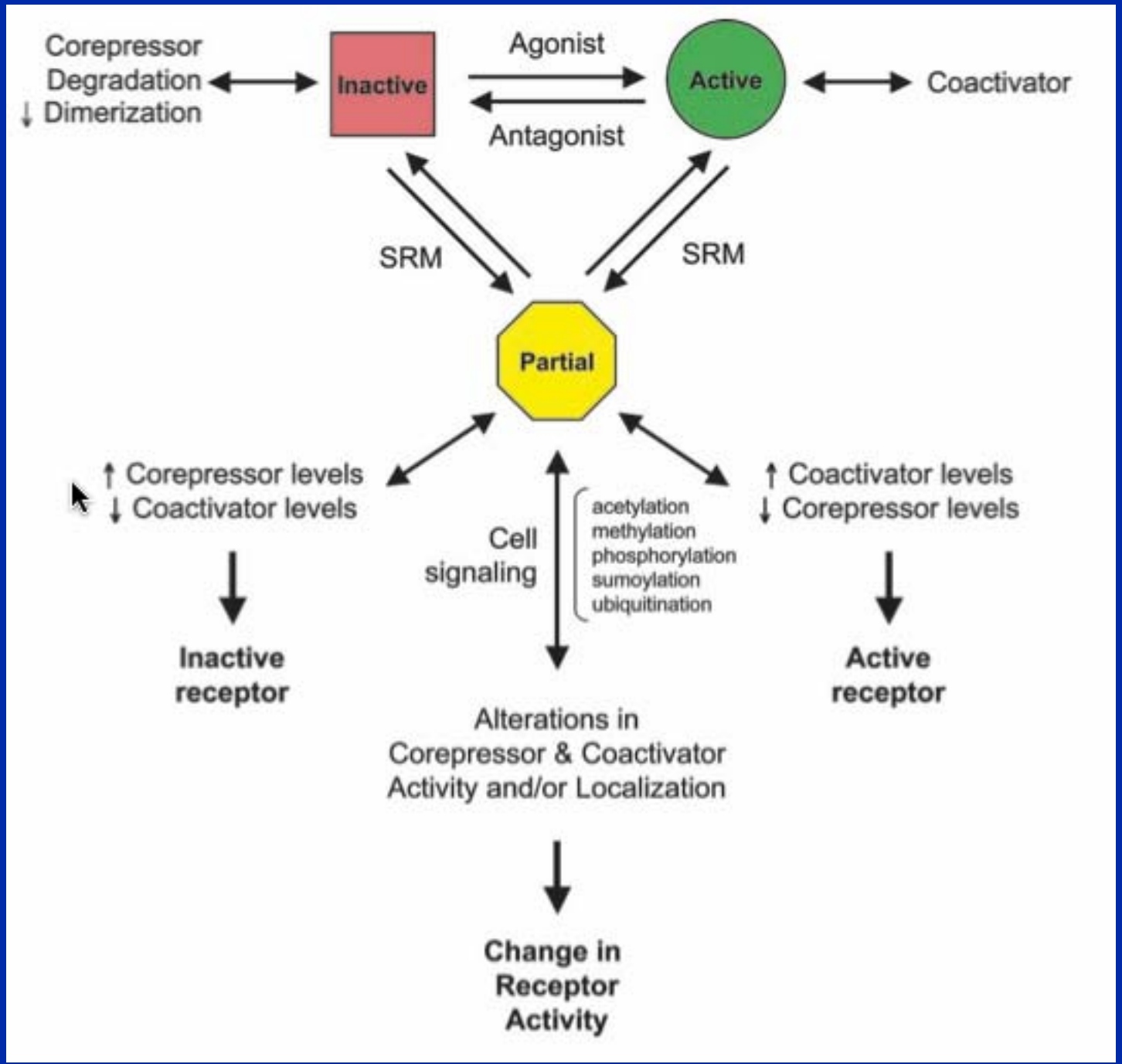
Overexpression of the co-activator AIB-1 in breast cancer



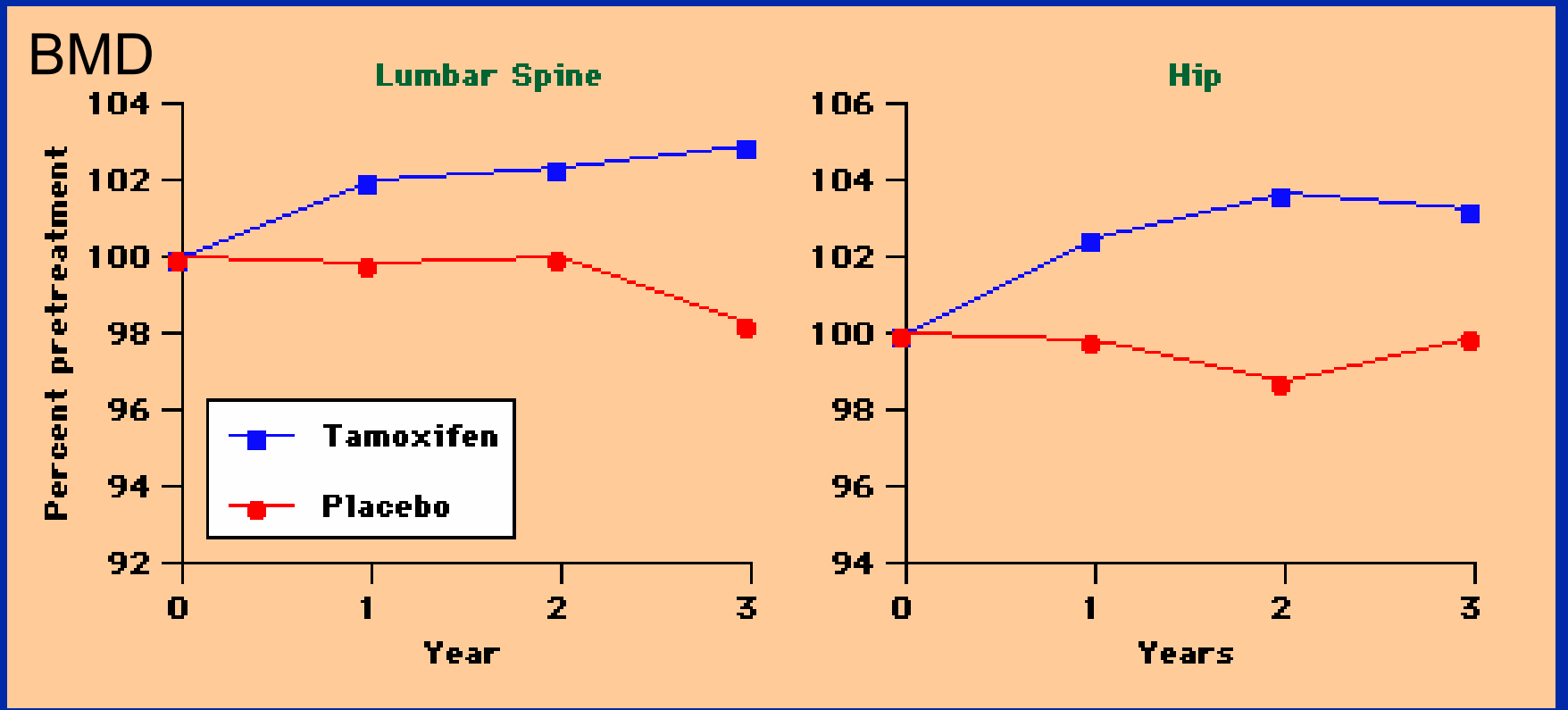
normal mammary cells

breast cancer cells

Science 277: 965f (1997)


Corepressor
Degradation
↓ Dimerization
Inactive
Agonist
Antagonist
Active
Coactivator
SRM
SRM
Partial
↑ Corepressor levels
↓ Coactivator levels
Inactive receptor
Cell signaling
acetylation
methylation
phosphorylation
sumoylation
ubiquitination
↑ Coactivator levels
↓ Corepressor levels
Active receptor
Alterations in Corepressor & Coactivator Activity and/or Localization
Change in Receptor Activity

Tamoxifene



But: endometrial hyperplasia, hot flashes

J.Clin.Oncol. 14:76f (1996)

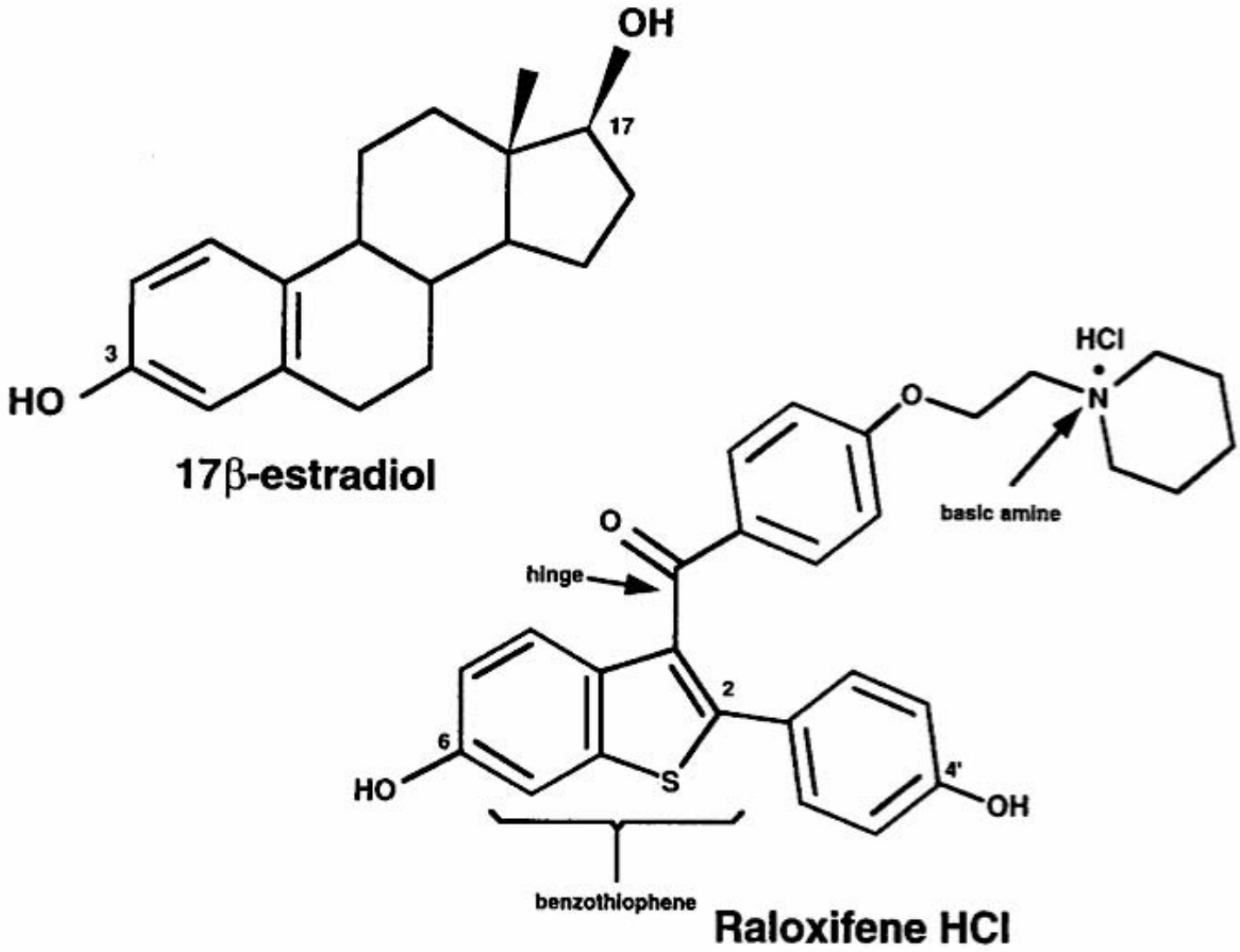
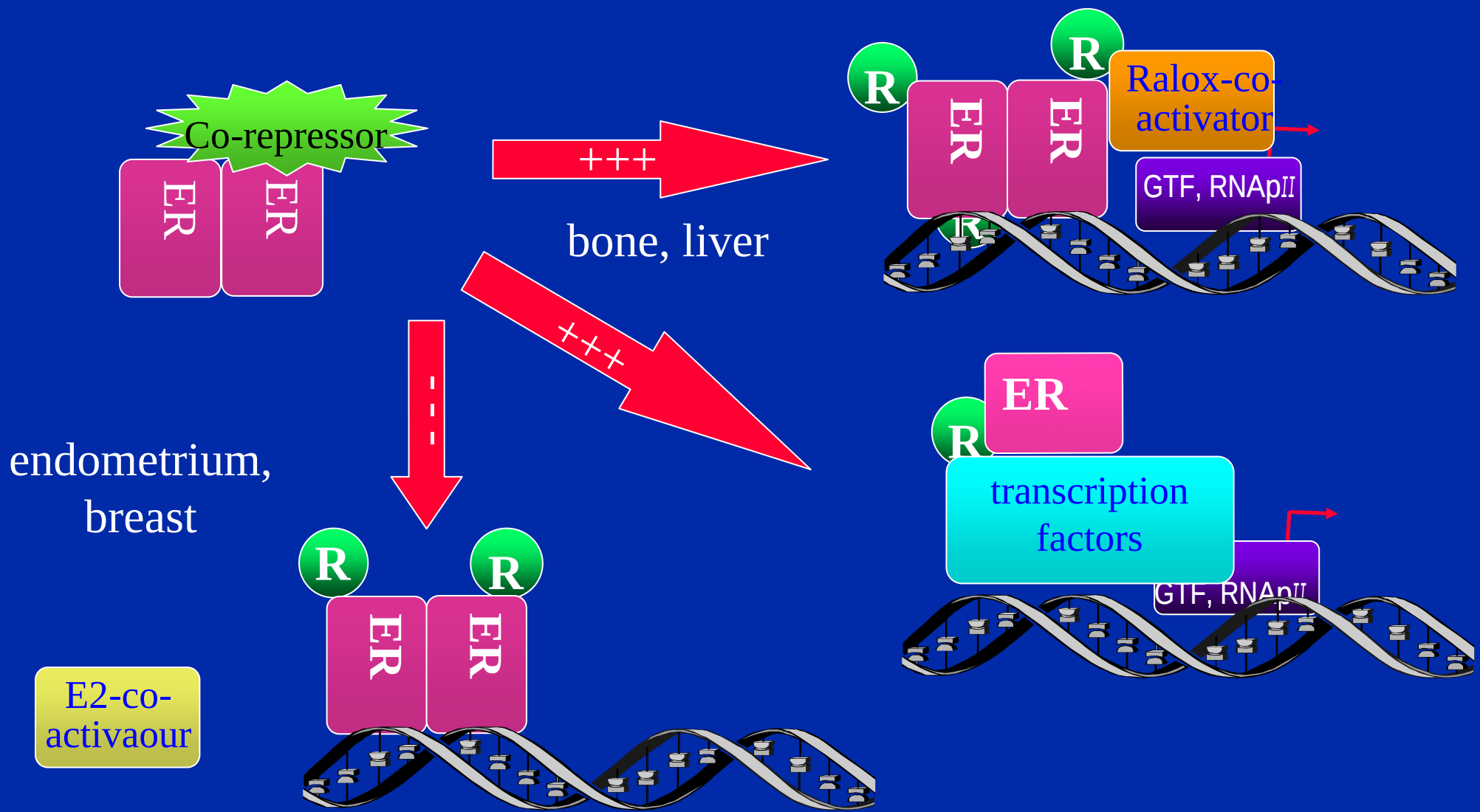


Figure. Chemical structures of raloxifene hydrochloride and 17β-estradiol.

SERMs

	AGONIST	ANTAGONIST
E2	all tissues	
tamoxifene	endometrium, bone, lipids	breast
raloxifène	bone, lipids	breast, endometrium
ICI 164,384		all tissues

How do SERMs work



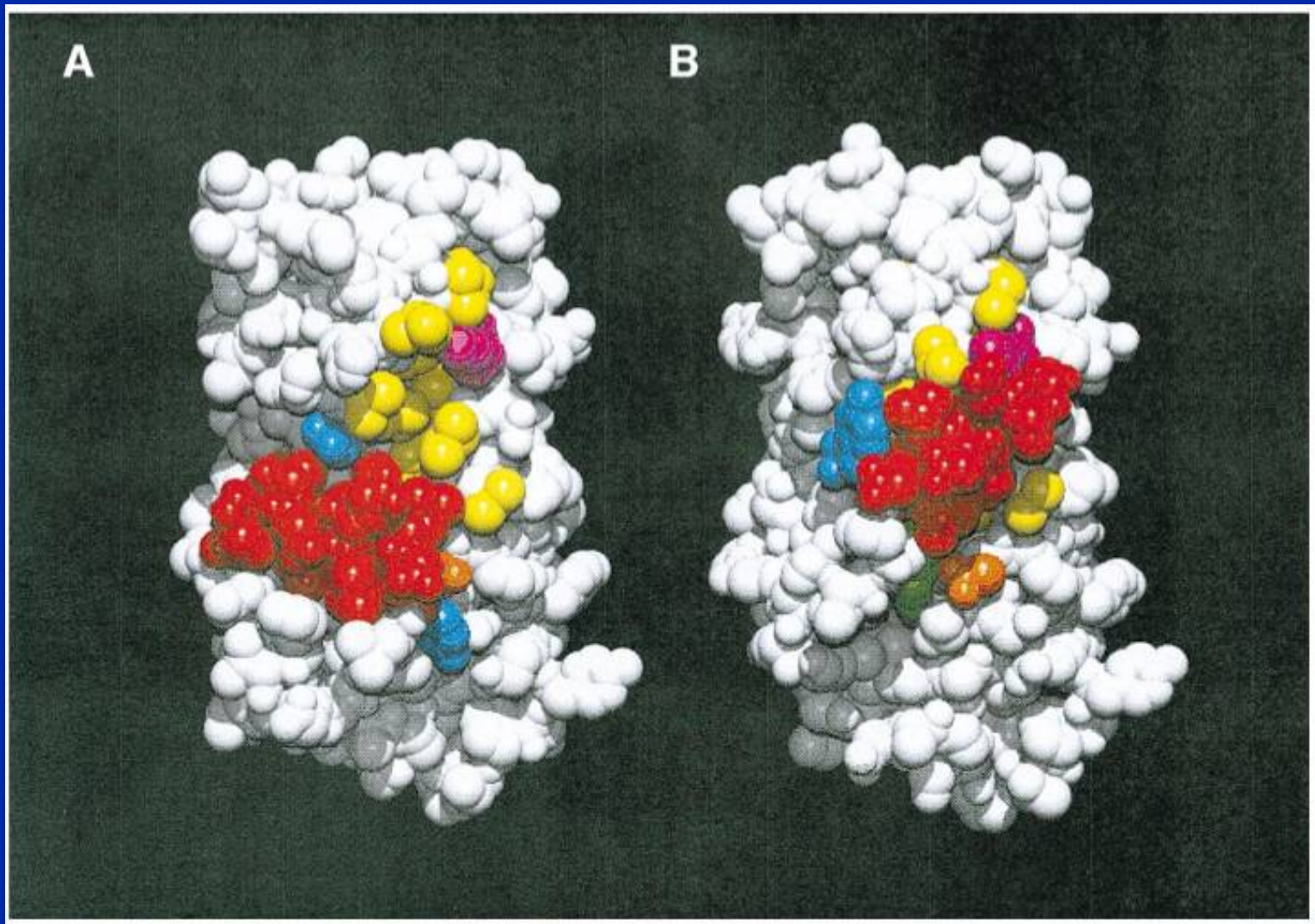
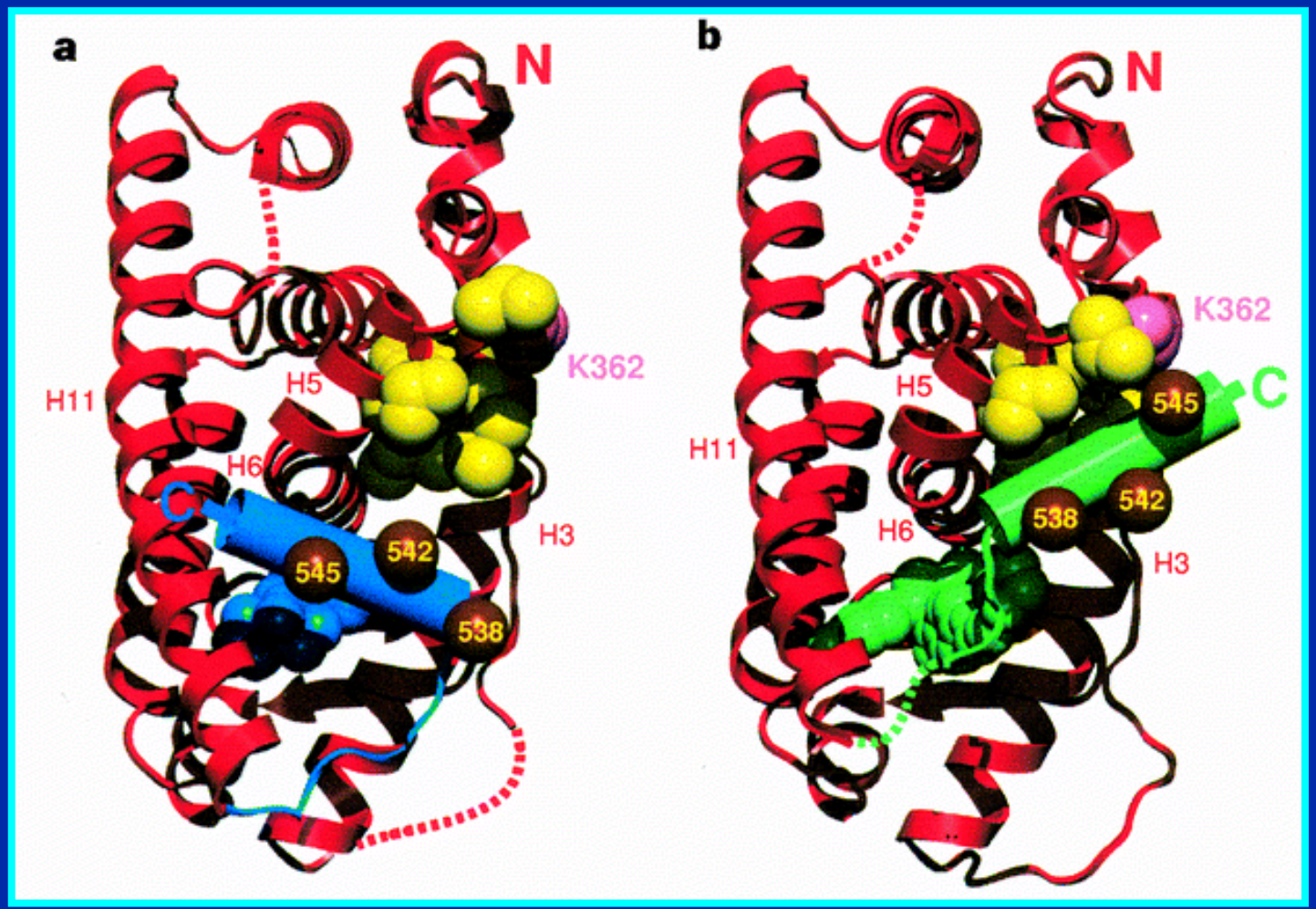


Figure 1. Structure of the human estrogen receptor-α ligand-binding domain when complexed with its natural ligand 17β-estradiol (E_2) versus the synthetic estrogen antagonist raloxifene. A space-filling model of the three-dimensional structure of the human estrogen receptor-α ligand-binding domain (ER-LBD) (Brzozowski *et al.* 1997) is depicted, complexed with (**A**) E_2 and (**B**) the antiestrogen raloxifene. Ligands bound to the receptor are completely buried in the receptor protein and, therefore, cannot be seen in the two structures shown. Helix 12 (H12), which con-

Raloxifene

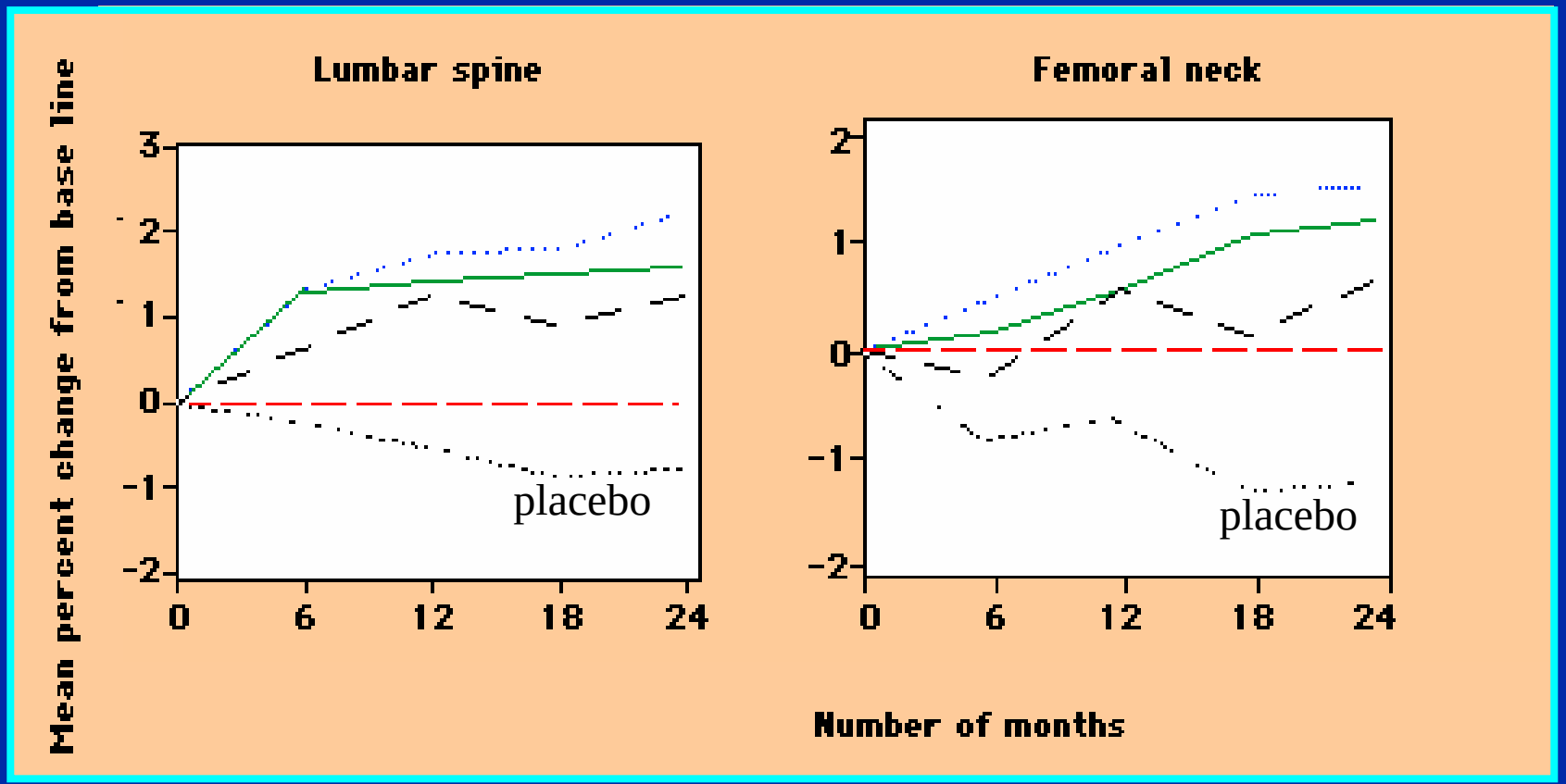


E2

raloxifene

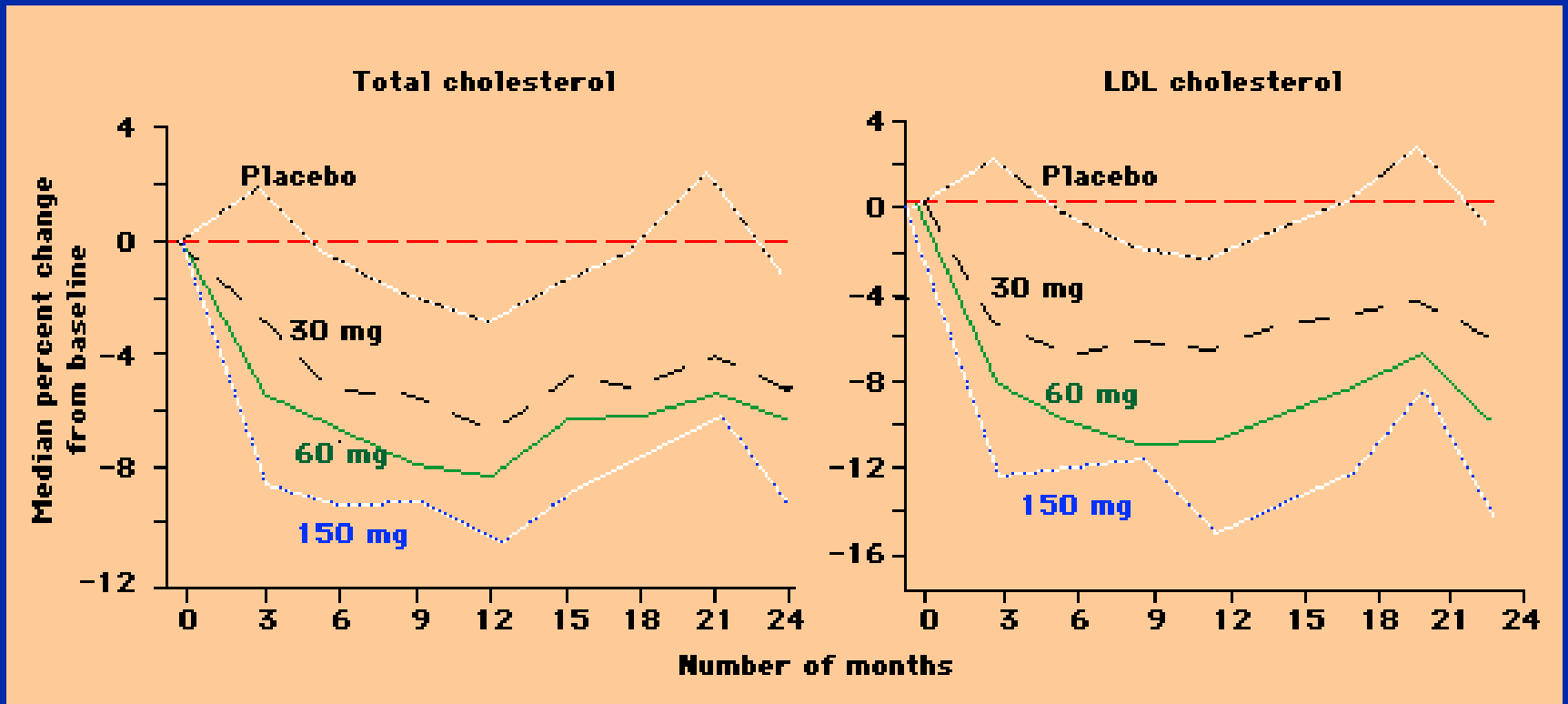
Nature 389: 753f (1997)

Effects of raloxifene on bone



Bone mineral density increases with raloxifene in postmenopausal women Administration of raloxifene at varying doses (30 mg, black dashed line; 60 mg, green line; 150 mg, blue dotted line) resulted in an increase in bone mineral density compared to placebo (black dotted line) in all sites tested over two years. Redrawn from Delmas, PD, Bjarnason, NH, Mitlak, BH, et al, N Engl J Med 1997;337:1641.

Effects of raloxifene on lipids



Serum total cholesterol and HDL cholesterol concentrations decrease with raloxifene therapy in postmenopausal women Raloxifene administered in three different doses (30 mg, black dashed line; 60 mg, green line; 150 mg, blue dotted line) resulted in significant decreases in serum total and HDL cholesterol compared to placebo (black dotted line) over the two year follow-up. Redrawn from Delmas, PD, Bjarnason, NH, Mitlak, BH, et al, N Engl J Med 1997;337:1641.

Raloxifene and Cardiovascular Events in Osteoporotic Postmenopausal Women

Four-Year Results From the MORE (Multiple Outcomes of Raloxifene Evaluation) Randomized Trial

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Context Raloxifene, a selective estrogen receptor modulator, improves cardiovascular risk factors, but its effect on cardiovascular events is unknown.

Objective To determine the effect of raloxifene on cardiovascular events in osteoporotic postmenopausal women.

Design Secondary analysis of data from the Multiple Outcomes of Raloxifene Evaluation trial, a randomized, double-blind, placebo-controlled trial conducted between November 1994 and September 1999.

Setting Outpatient and community settings at 180 sites in 25 countries.

Participants A total of 7705 osteoporotic postmenopausal women (mean age, 67 years).

Intervention Patients were randomly assigned to receive raloxifene, 60 mg/d (n=2557), or 120 mg/d (n=2572), or placebo (n=2576) for 4 years.

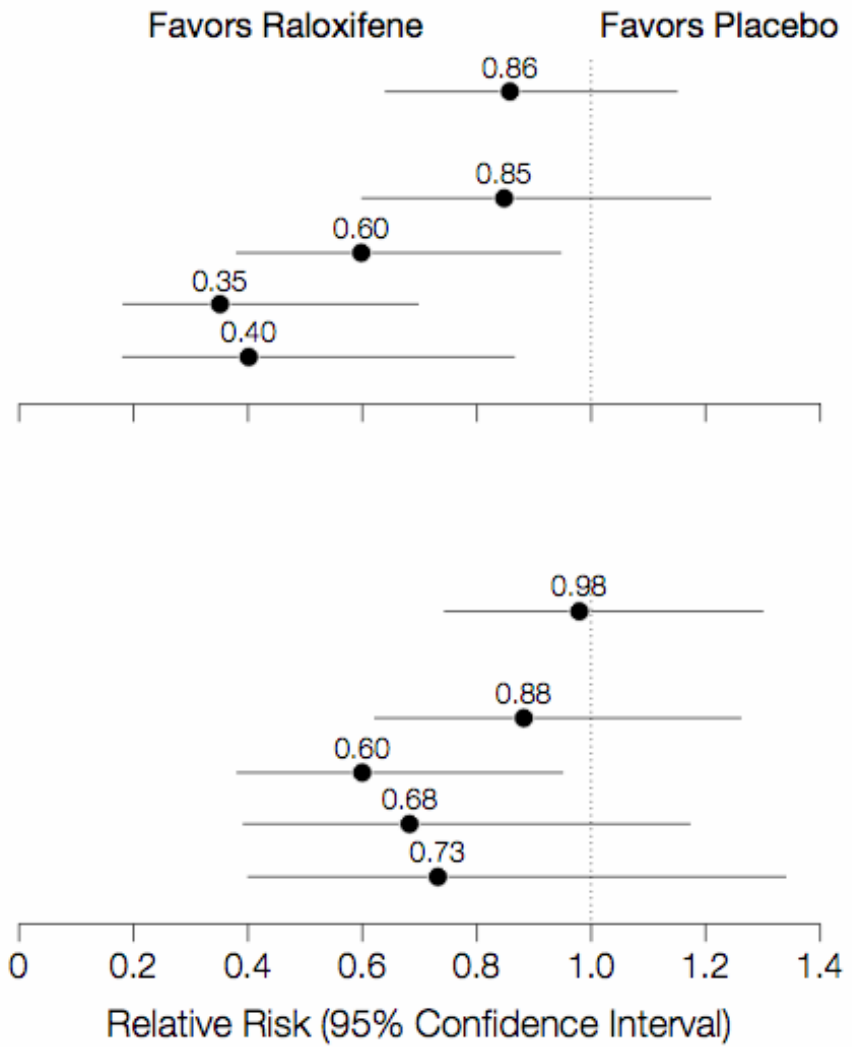
Figure 4. Relative Risk of Any Cardiovascular Events Compared by Raloxifene and Placebo

A Raloxifene 60 mg/d

	No. of Women	No. of Events
Overall	5133	178
Cardiovascular Risk Score		
≥3	1726	118
≥4	676	69
≥5	252	32
≥6	186	26

B Raloxifene 120 mg/d

	No. of Women	No. of Events
Overall	5148	190
Cardiovascular Risk Score		
≥3	1665	116
≥4	676	69
≥5	272	43
≥6	209	35



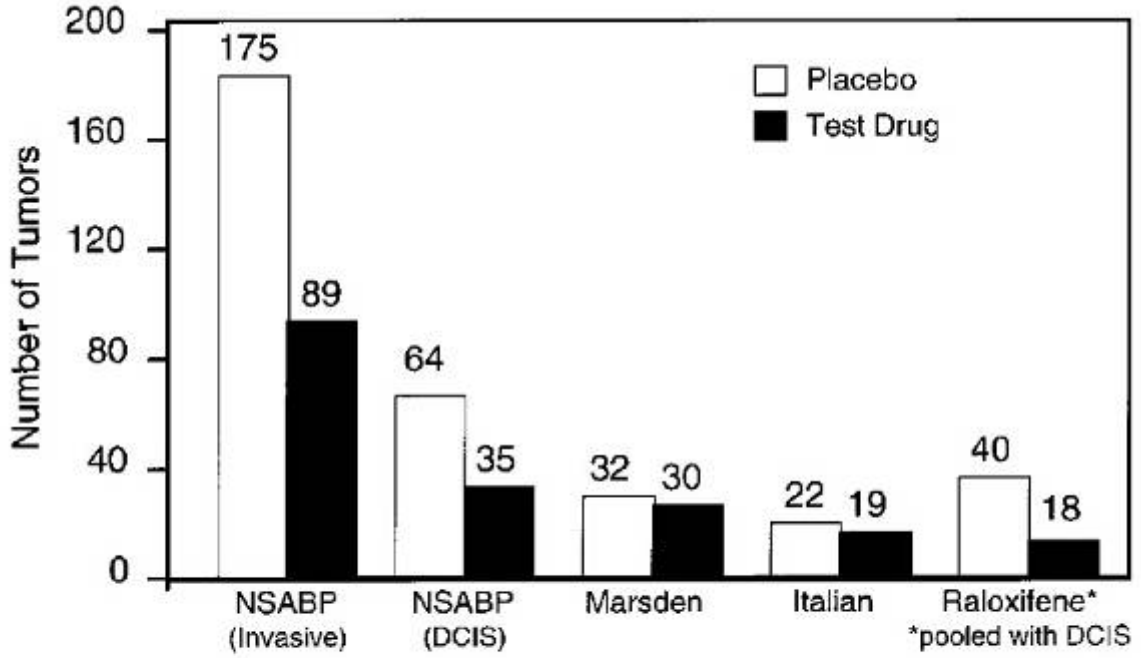


FIG. 13. A comparison of the able-to-be-evaluated events observed in the studies to reduce the incidence of breast cancer. The NSABP P-1 trial is the only prospective clinical trial designed to test the worth of an antiestrogen to prevent breast cancer in 13,388 high-risk women. The figure illustrates the effect of tamoxifen on both invasive and noninvasive (ductal carcinoma *in situ* DCIS) breast cancer. By contrast, the Royal Marsden Study is a pilot project (209) originally designed to be a toxicity evaluation (211) in 2,471 high-risk women, and the Italian study reports (210) at least one year's data from an original population of 5,408 young women of normal risk. Finally, the raloxifene data that can only be estimated from published abstracts (240, 241), constitute a secondary end point from 10,553 postmenopausal women in osteoporosis trials. The reported cases are both invasive and noninvasive breast cancers.

Table 3. Tissue-Selective Estrogenic Effects of Raloxifene

Tissue	Agonistic Effects	Antagonistic Effects	Uncertain
Skeleton	Yes		
Lipids	Yes		
Hemostasis	Yes		
Breast		Yes	
Uterus		Yes	
Vasomotor		Yes	
Ovary			Yes
Pituitary gland and brain			Yes

Another type of SERMs: Endocrine disrupters

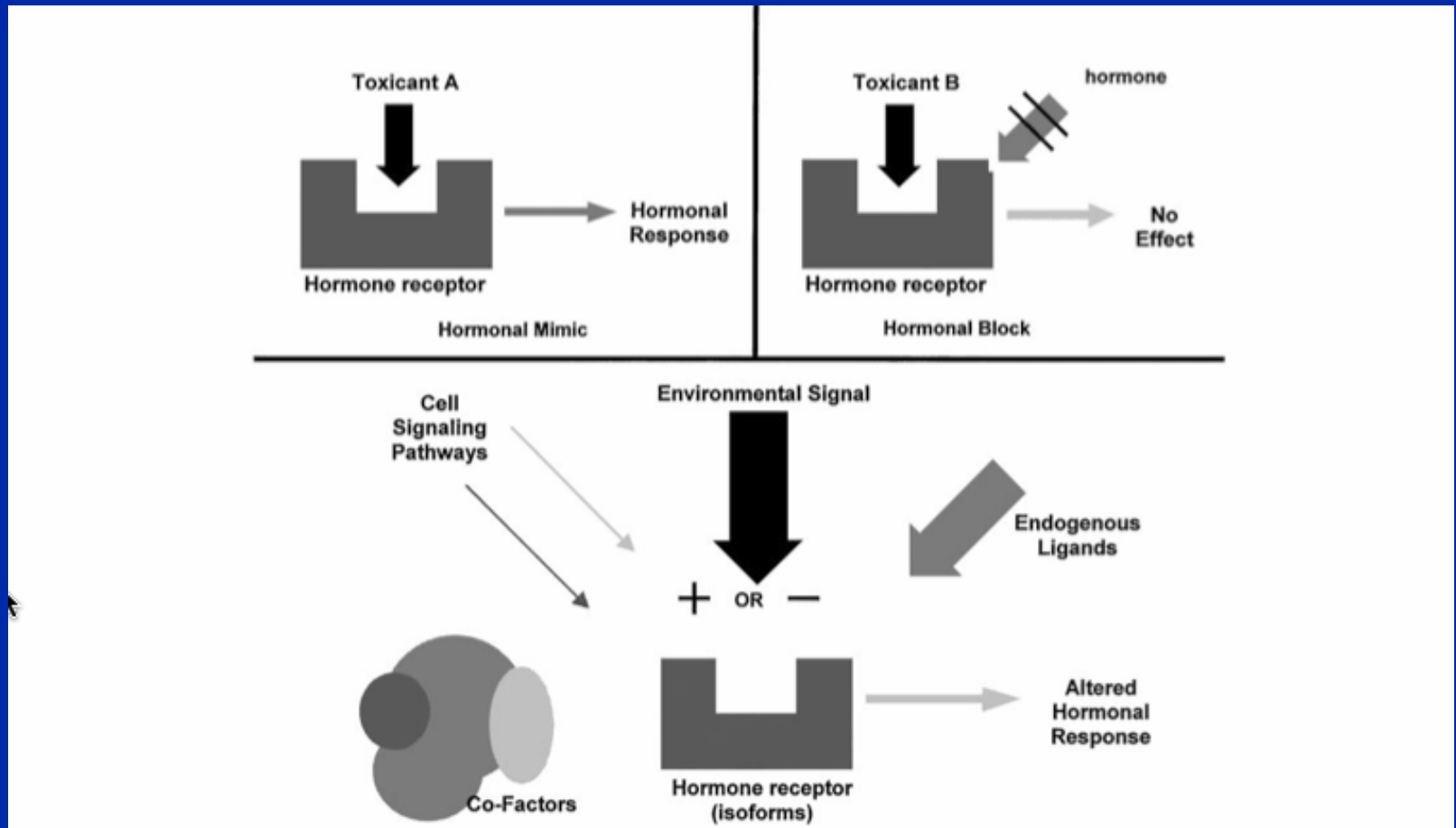


FIG. 6. Functional or receptor-based toxicology. The practice of using steroid hormone receptors to determine the hormonal or antihormonal activity of environmental chemicals is more than a decade old (49). The relatively simple concept presented then (*upper panels*), must now accommodate the advances in knowledge over the last 10 yr. These include the convergence of activating ligands and cellular signals on the ER (50), the multiple isoforms of the ER (51), contributions of coactivators and corepressors, and the gradation in response (50).

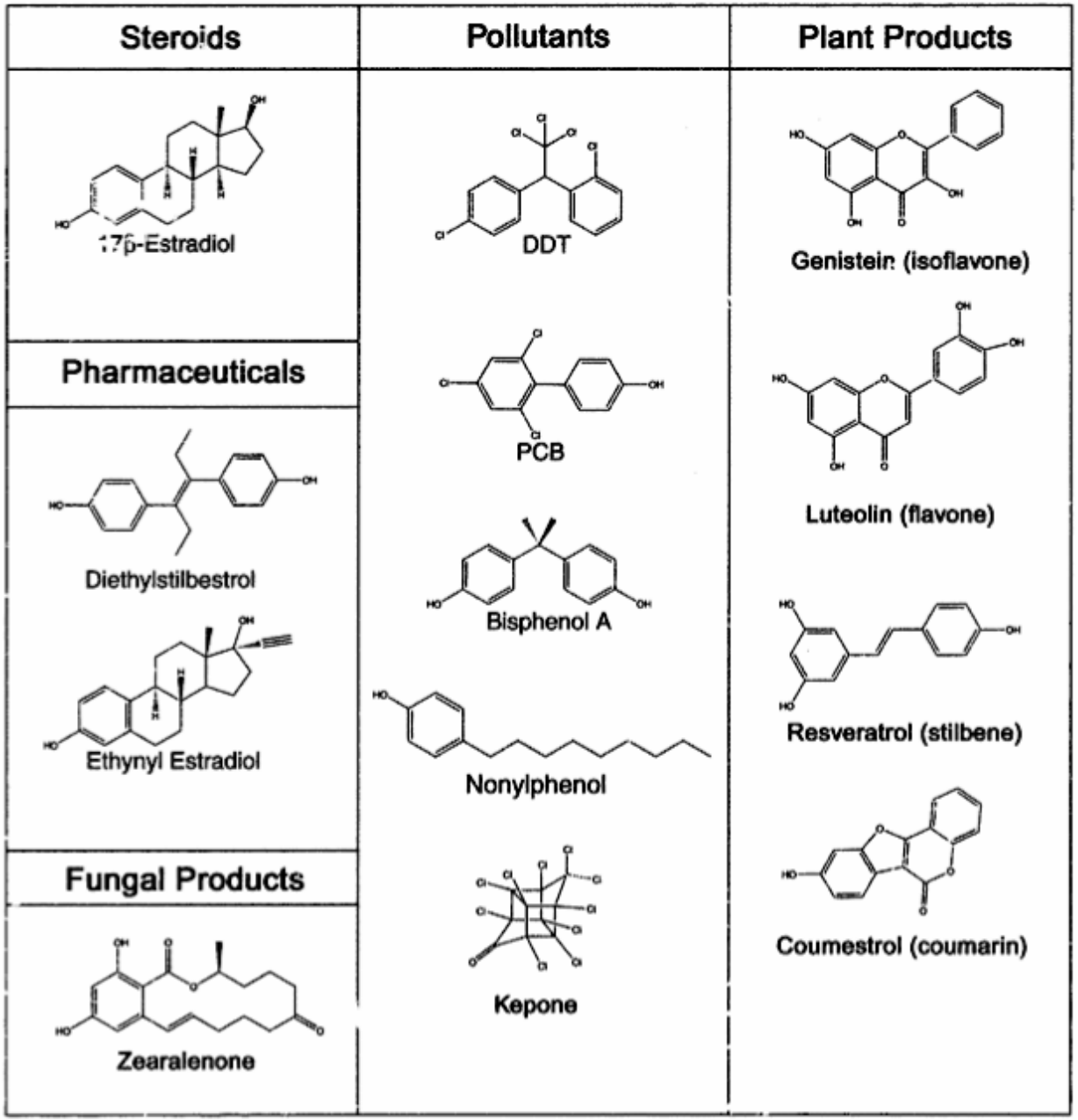


FIG. 5. Chemicals found in the environment reported to be estrogenic. This list is not comprehensive, but illustrates representative structures of estrogenic compounds from various sources. Information on these compounds is contained in the text.

TABLE 1. Environmental hormonal activities

Hormonal activity	Environmental	
	Hormone	Antihormone
Estrogen	Yes, many[a]	Yes, few[a]
Progestin	?	?
Androgen	Yes, few[b]	Yes, many[c]
Gluccocorticoid	?[d]	?
Mineralocorticoid	?	?
Retinoid	Yes, one	?
Thyroid	?[e]	?

[a] See representative structures in Fig. 5.

[b] Androstenedione, the product of bacterial metabolism of stigmasterol; see Fig. 3.

[c] See representative structures in Fig. 2.

[d] Arsenic is reported to block the GR_I activation at the receptor binding level (23).

[e] PCB congeners elicit a thyroid hormone-like response, but no binding data for the thyroid hormone receptor is available (21). One study that evaluated binding of chlorinated hydrocarbons to the thyroid hormone receptor and thyroid binding proteins did not demonstrate specific receptor binding, while binding to transthyretin was of the same affinity as T_4 (22).

Office of Prevention, Pesticides,
and Toxic Substances

Endocrine Disrupters Screening and Testing Advisory Committee (EDSTAC)

1995

Cloning of a second ER...

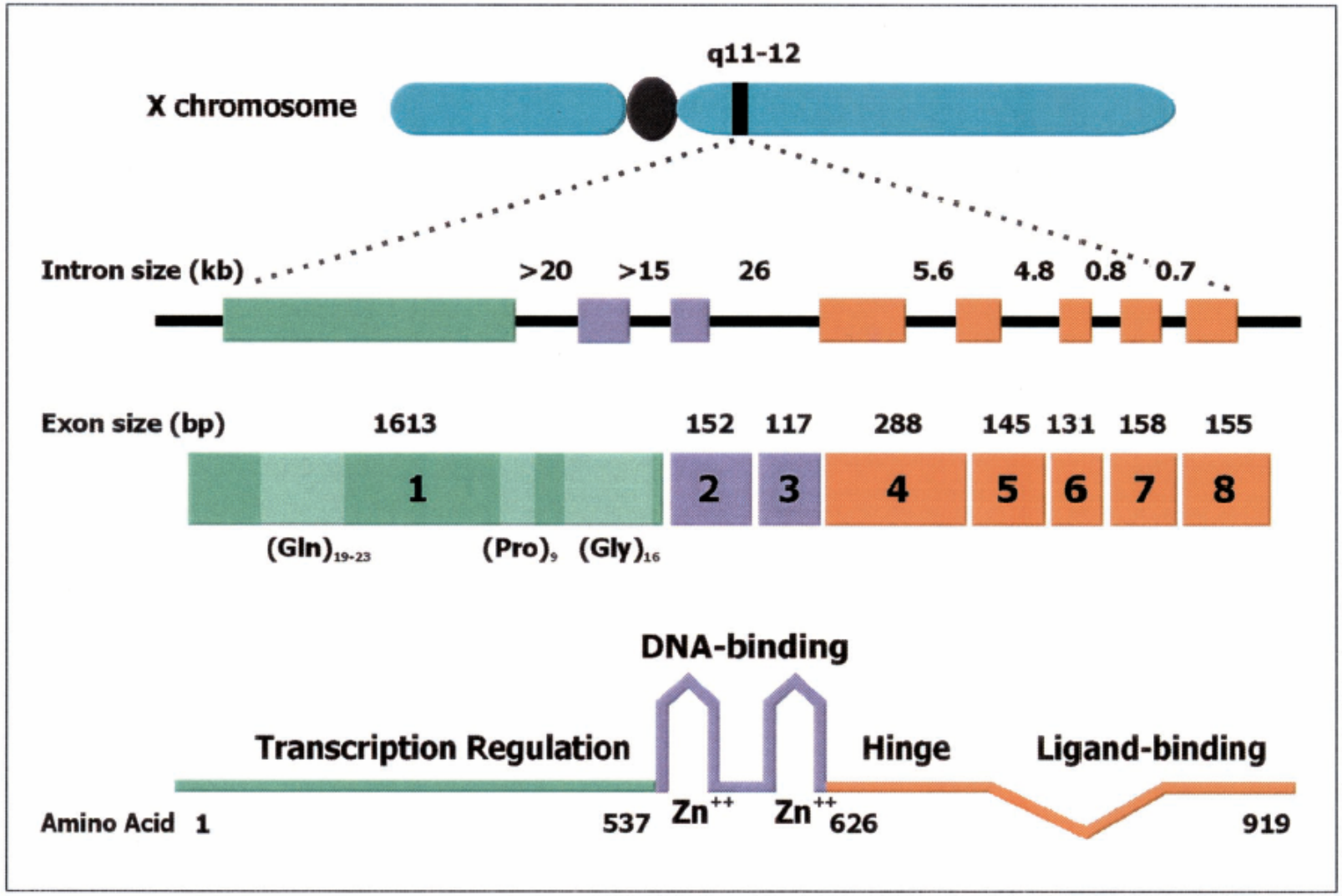


Fig 1. Genomic organization of the *AR* gene is shown. The genome spans more than 80 kb that includes the exonic organization shown in the second panel. Location of three codon repeat regions in the first exon that codes for the N-terminal domain is shown in the third panel. The diagram of the protein structure demonstrates how the exon organization translates into discrete functional regions of the receptor. Adapted from Quigley et al.[7]

Role of the different ER subtypes ?

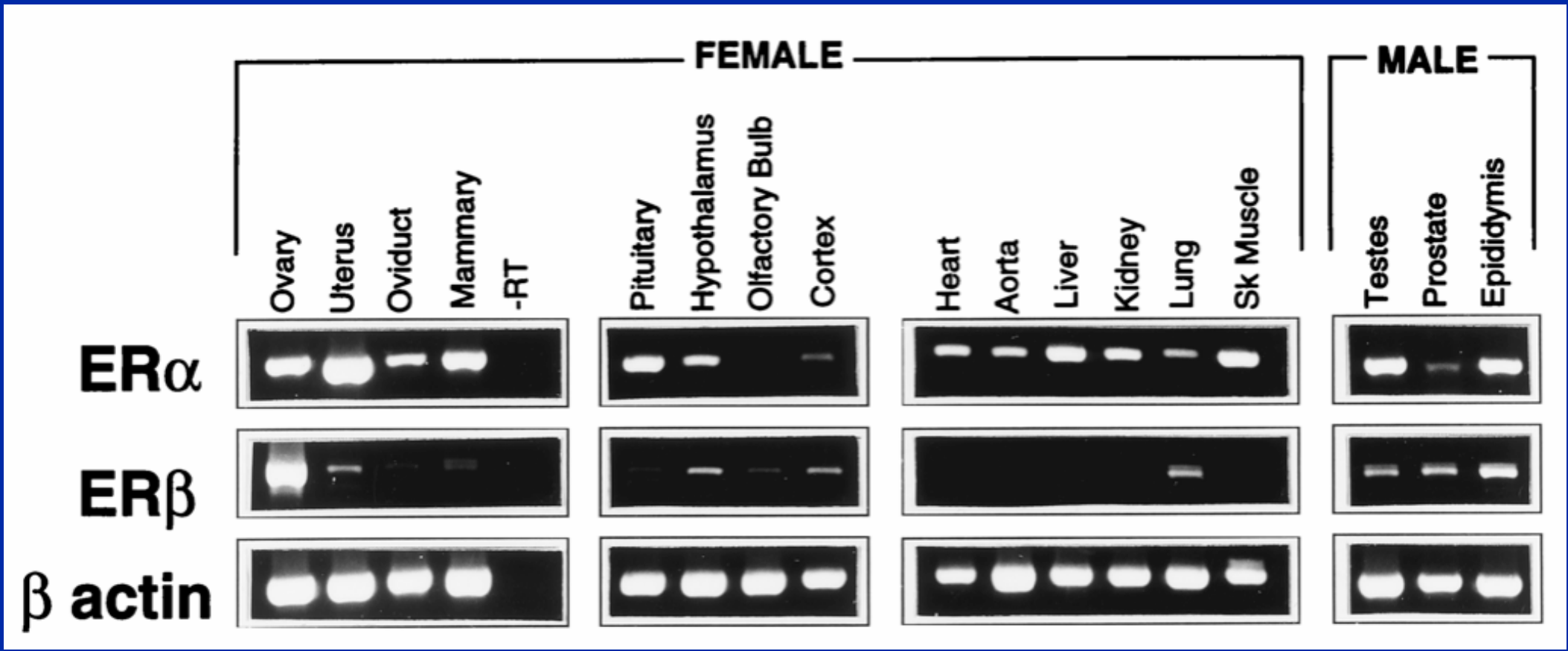


Table 1. Tissue distribution and relative abundance of ERα and ERβ mRNA in various tissues of the rat

	ERα	*ERβ*
Bone	+	+
Bladder	–/+	+
Uterus	+	+
Ovary	–/+	+
Prostate	+	+
Testis	+	+
Epididymis	+	+
Gastrointestinal tract	–	+
Kidney	+	+
Liver	+	–
Breast	+	+
Heart	+	+
Vessel wall	+	+
Immune system	?	+
Lung	–	+
Pituitary	+	+
Hippocampus	–	+
Hypothalamus	+	+

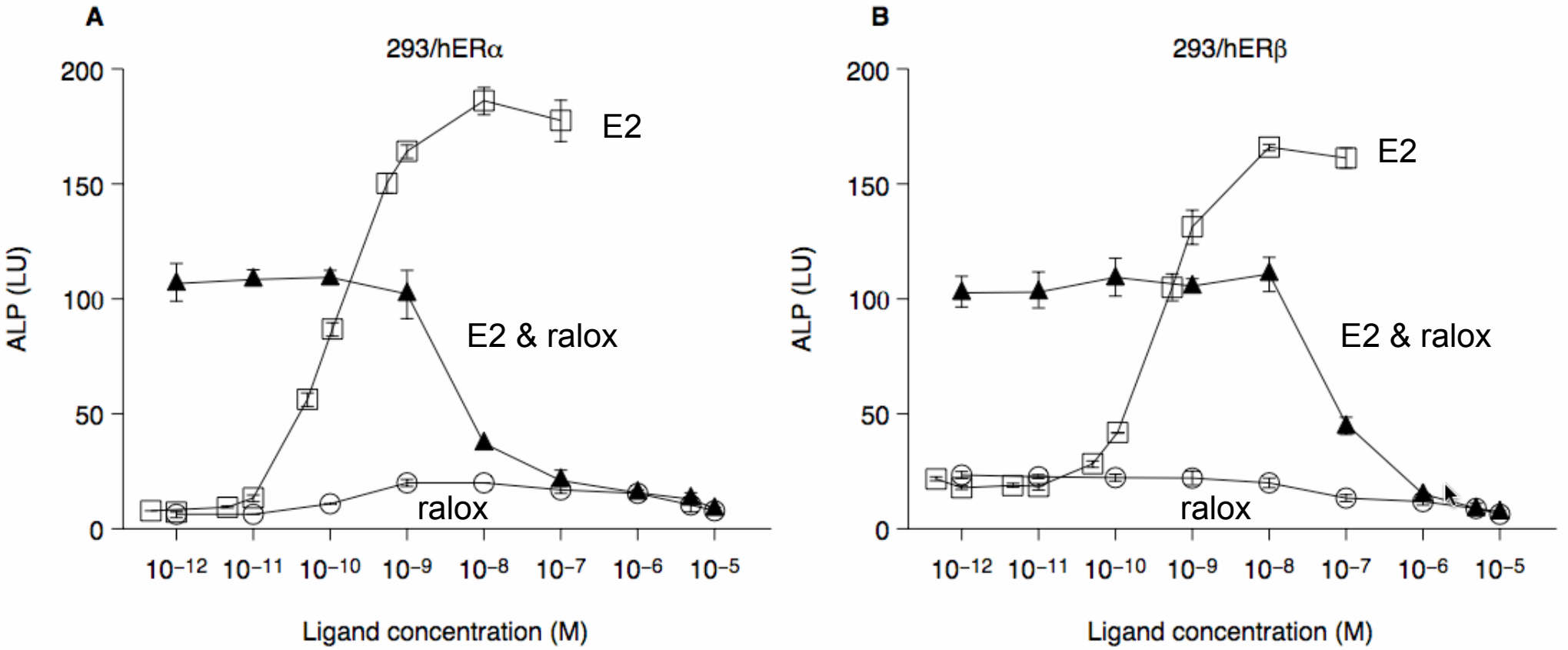


Figure 2. Effect of the natural ligand 17β-estradiol (E_2) and the synthetic antagonist raloxifene on regulation of target gene expression in genetically engineered cells expressing (**A**) the human estrogen receptor α (ERα) and (**B**) human ERβ. The human kidney epithelial cell line, 293, has been transformed to express the human ERα or ERβ constitutively (Barkhem *et al.* 1998). In addition, the two receptor-expressing cells harbor an estrogen-responsive reporter gene transcription unit, stably integrated into the cellular genome (Barkhem *et al.* 1998). The expression of the

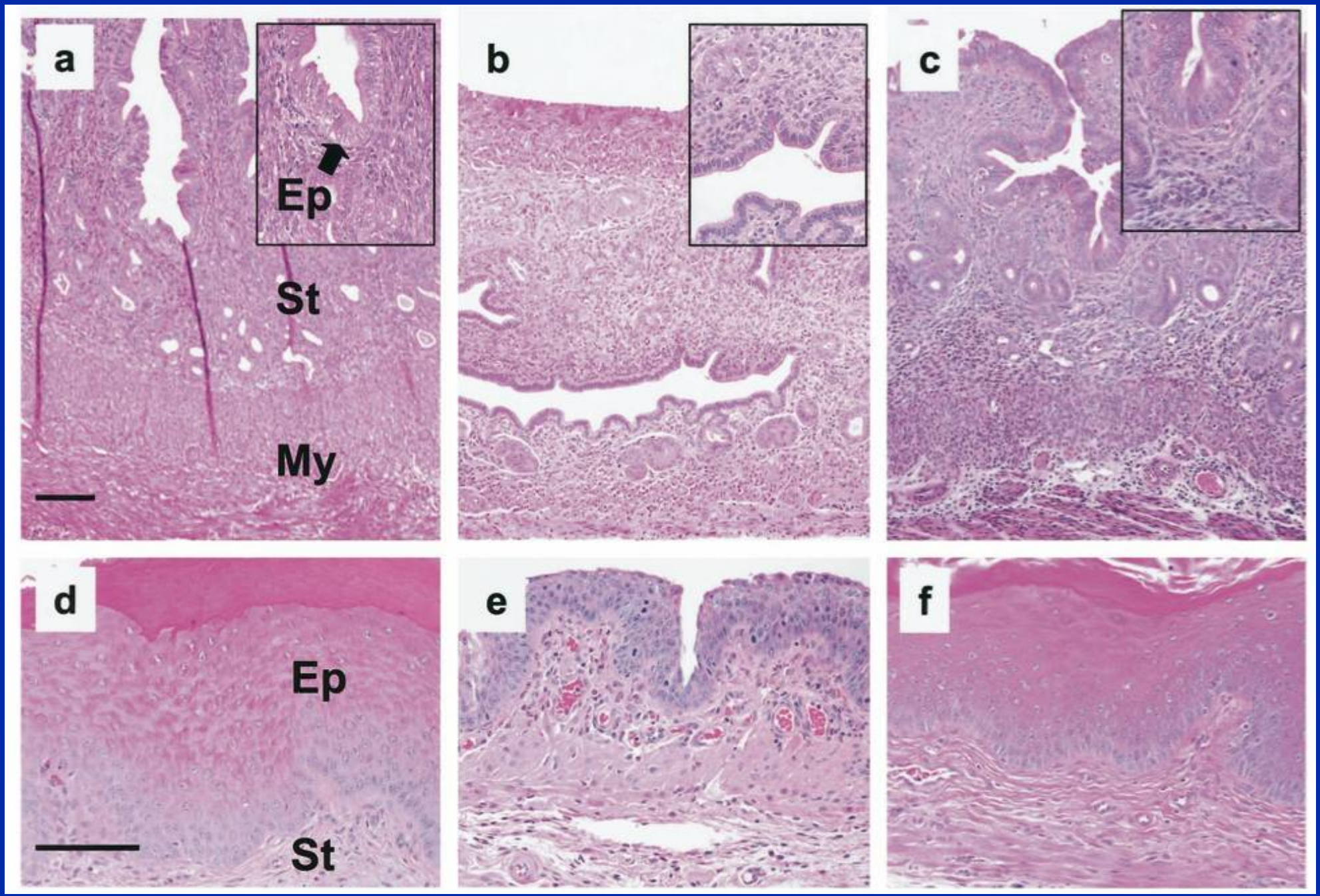

a
Ep
St
My
b
c
d
Ep
St
e
f

TABLE 2. Serum hormone levels in adult wild-type and αERKO mice

Hormone	Female		Male	
	Wild-type (SEM)	αERKO (SEM)	Wild-type (SEM)	αERKO (SEM)
Gonadal steroids				
Estradiol (pg/ml)[b]	29.5 ± 2.5	84.3 ± 12.5[a]	11.8 ± 3.4	12.9 ± 3.4
Progesterone (ng/ml)[b]	2.3 ± 0.6	4.0 ± 1.1	0.5 ± 0.3	0.3 ± 0.1
Testosterone (ng/ml)	0.4 ± 0.4	3.2 ± 0.6	9.3 ± 4.0	16.0 ± 2.3
Anterior pituitary				
LH (ng/ml)	0.3 ± 0.04	1.7 ± 0.3[a]	2.4 ± 1.2	3.7 ± 0.7
FSH (ng/ml)	4.9 ± 0.6	5.4 ± 0.7	26.0 ± 1.4	30.0 ± 1.1
PRL (ng/ml)	18.8 ± 10.7	3.5 ± 1.3	nd	nd

nd, Not determined.

[a] ǂtest, wild-type *vs.* ERKO, $P < 0.001$.

[b] These values in the female are different than those reported in Ref. 123, which were carried out on pooled sera. The values above are the means from assays on individual samples and therefore are more likely to reflect the true levels in the two genotypes.

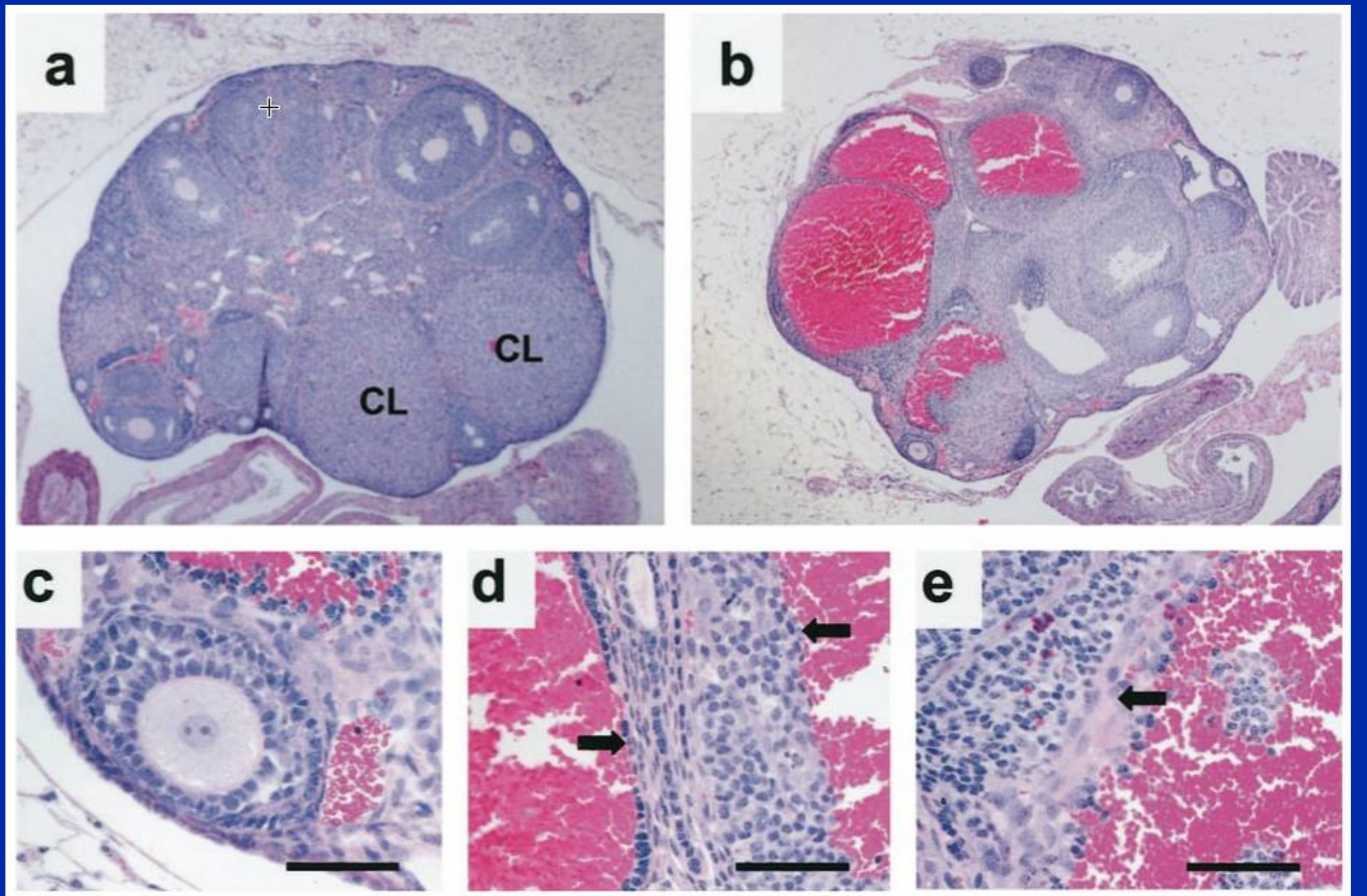

a
CL
CL
b
c
d
e

TABLE 3. Fertility and superovulation data in the βERKO female mice

Genotype	Continuous mating results			Superovulation results		
	n	Litters per female (SEM)	Pups per litter (SEM)	n	Oocytes per female (SEM)	Range
Wild-type	6	2.8 ± 0.4	8.8 ± 2.5	10	33.7 ± 4.8	9–57
Heterozygous	nd	nd	nd	11	52.5 ± 5.7[a]	20–77
βERKO	11	1.7 ± 1.0[a]	3.1 ± 1.8[b]	11	6.0 ± 1.5[a]	0–13

nd, Not determined.
[a] $P < 0.05$, Student's two tailed t-test *vs.* wild-type
[b] $P < 0.001$, Student's two tailed t-test *vs.* wild-type

Table 1. Estrogen receptors (ERs) as novel targets for disease

Target tissue	Estrogen receptor present	Disease	Suggested pharmaceutical	Refs
Uterus	ERα	Uterine cancer	ERα antagonist	[9]
Prostate stroma	ERα	Benign prostatic hyperplasia	ERα antagonist	[43]
Ovary theca cells	ERα	Polycystic ovary syndrome	ERα agonist	[9]
Bone	ERα	Osteoporosis	ERα agonist	[9]
Breast epithelium	ERα, ERβ, ERβcx[a]	Breast cancer	ERα antagonist and/or ERβ	[62]
Breast stroma	ERβ		agonist	[58]
Brain	ERα, ERβ	Stroke	ERα agonist	[11,33]
		Hypertension	ERα agonist	
		Obesity	ERβ agonist	
		Dementia	ERβ agonist	
Sympathetic ganglia	ERβ	Hypertension	ERβ agonist	[19]
		Bladder control	ERβ agonist	
Colon	ERβ	Colon cancer	ERβ agonist	[14]
Prostate epithelium	ERβ, ERβcx[a]	Prostate cancer	ERβ agonist	[45]
Ovarian granulosa cells	ERβ	Infertility, polycystic ovarian syndrome	ERβ agonist	[10]
Dorsal raphe	ERβ	Depression	ERβ agonist	[11]
Bone marrow	ERβ	Leukaemia	ERβ agonist	[20]

[a]A splice variant of ERβ.

Table 2. Differential actions of ERα and ERβ on different promoters and with different ligands[a]

Ligand	ER	Interaction site			
		ERE	AP-1[b]	Sp1	NF-κB promoter[c]
E2	ERα	↑	↑	↑ RARα1 promoter [24]; ↓ IGF-1 promoter [25]	↓
	ERβ	↑	NC	No change in RARα1 promoter; ↑ IGF-1 promoter	NC
Tamoxifen	ERα	↓	↓	↓ RARα1 promoter	↓
	ERβ	↓	↑	↑ RARα1 promoter	NC

[a]Abbreviations: AP-1, activating protein 1; E2, 17β-estradiol; ER, estrogen receptor; ERE, estrogen response element; IGF-1, insulin-like growth factor 1; NC, no change; NF-κB, nuclear factor κB; RAR, retinoic acid receptor; Sp1, GC-box binding protein; ↑, increased activity; ↓, decreased activity.

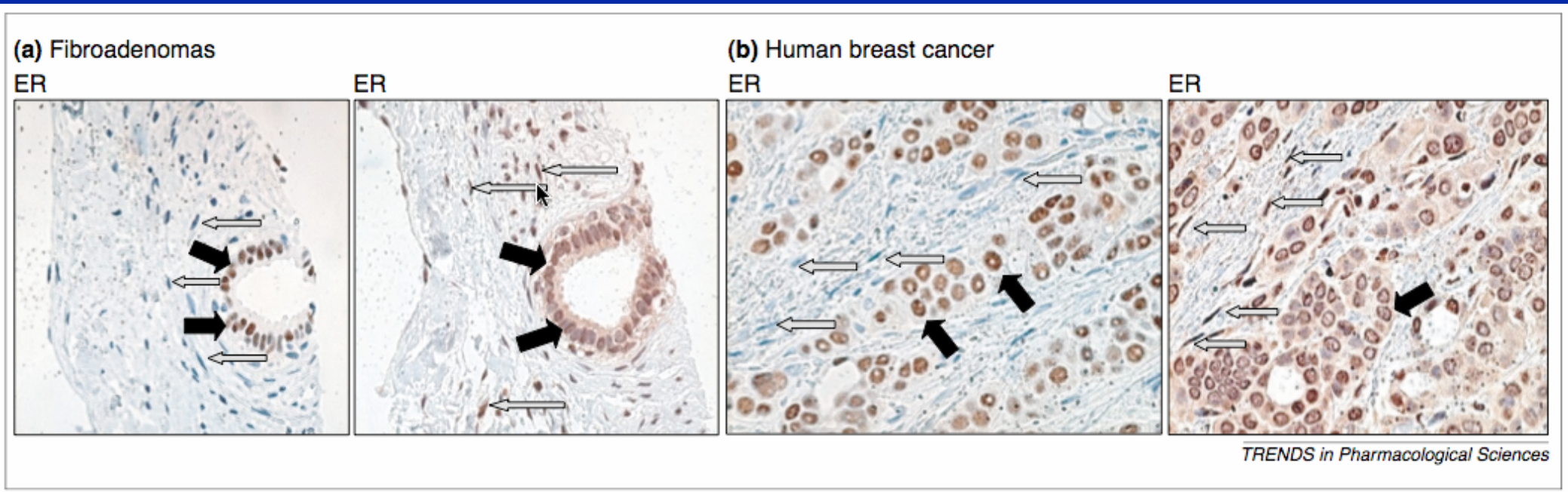
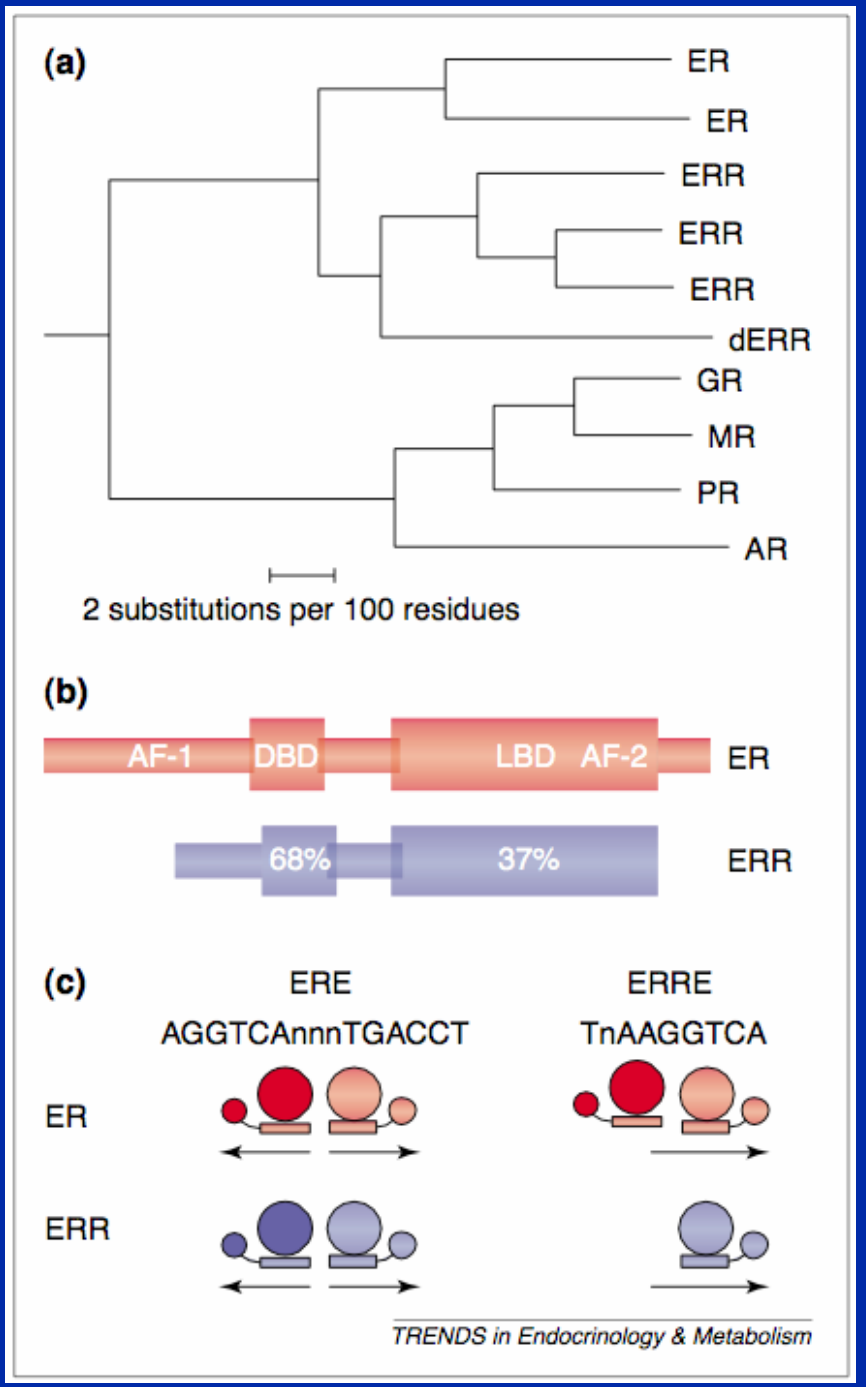
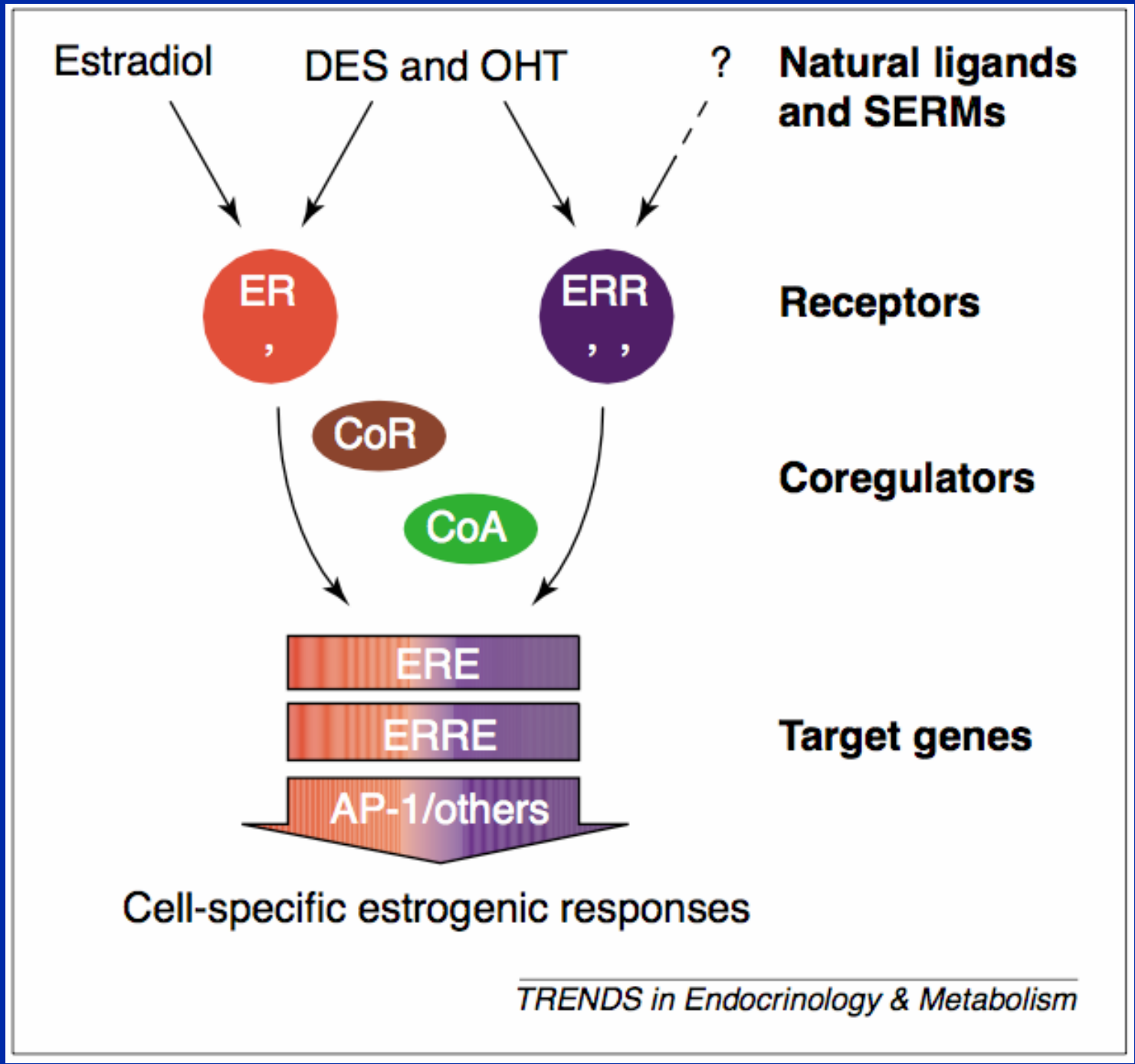


Fig. 1. **(a)** Estrogen receptor (ER) expression in fibroadenomas. ERα expression (brown) is exclusively epithelial (black arrows) with no stromal expression (grey arrows), whereas ERβ is expressed in both epithelial and stromal cells. **(b)** ER expression in human breast cancer. Note the intense staining for ERα in epithelial cells (black arrows) but no staining of the stroma (grey arrows). Intense ERβ (brown) staining is present in both epithelial and stromal cells. In both tissues, ERα expression was detected using a monolonal antibody (NovoCastra), whereas ERβ expression was detected using a polyclonal antibody (Upstate). All slides were lightly counterstained with hemotoxylin (blue). Reproduced, with permission, from the Society for Endocrinology [62].

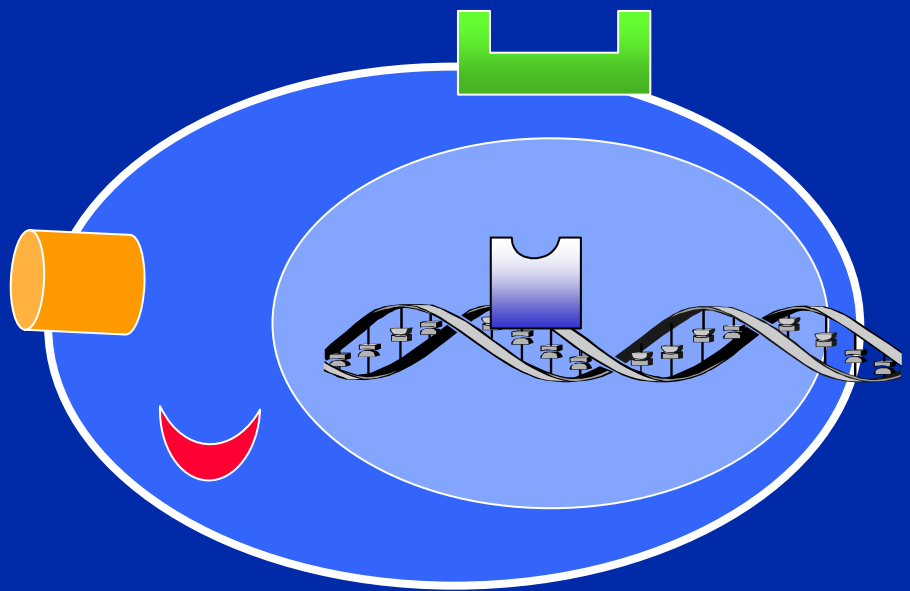

(a)
ER
ER
ERR
ERR
ERR
dERR
GR
MR
PR
AR
2 substitutions per 100 residues
(b)
AF-1
DBD
LBD
AF-2
ER
68%
37%
ERR
(c)
ERE
AGGTCAnnnTGACCT
ERRE
TnAAGGTCA
ER
ERR
TRENDS in Endocrinology & Metabolism


Estradiol
DES and OHT
?
Natural ligands and SERMs
ER ,
ERR , ,
Receptors
CoR
CoA
Coregulators
ERE
ERRE
AP-1/others
Target genes
Cell-specific estrogenic responses
TRENDS in Endocrinology & Metabolism

Molecular targets for pharmacotherapy

- membrane receptors 50%
- enzymes 20%
- hormones, growth actors 15%
- ion channels 5%
- nuclear receptors **2%**
- other 5%

n=500



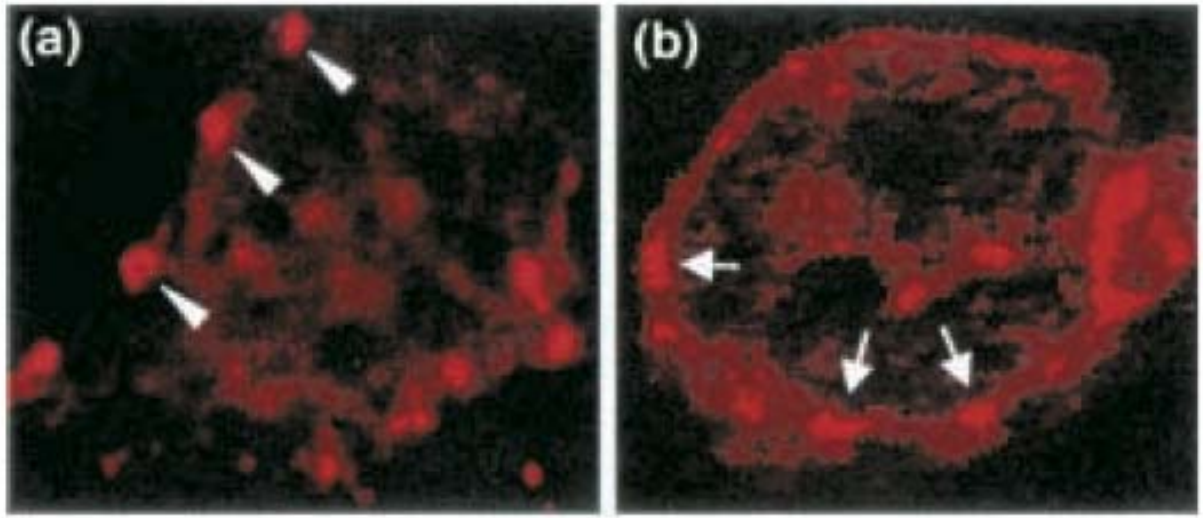


Fig. 1 Confocal analysis of ligand-labelling and immunocytochemical staining of cultured mouse midbrain neurones. **(a)** Living cells were exposed to 17-β-oestradiol coupled to hemisuccinate-BSA-FITC at a steroid concentration of approximately 1 nM for 5 min followed by a brief washing step and subsequent fixation. Note the presence of labelled clusters at the surface of the cell soma (arrowheads). Pre-incubation with unlabelled oestrogen completely prevented this staining. **(b)** Immunocytochemistry with an polyclonal antiserum specific for the nuclear oestrogen receptor-α. The arrows point at clusters of ER-α associated with the neuronal surface (magnification × 550).

Table 1. Potential Mechanisms by Which Selective Estrogen Receptor Modulators Produce Tissue-Selective Effects*

Differences in binding affinities to the estrogen receptor (22)
Differences in mechanisms of binding to the estrogen receptor (23, 28)
Differential changes in estrogen receptor structure after ligand binding (29)
Differential activation of the activation domains of the estrogen receptor (30)
Differences in the kinetics of estrogen receptor interaction with specific DNA elements (31)
Interaction of the estrogen receptor with different DNA response elements (31, 32)
Interaction with different coactivators and co-repressors in gene transcription (32)
Interference of estrogen receptor–associated proteins with the estrogen receptor (33)
Estrogen receptor–independent nongenomic effects (34)
Interaction of ligands with different estrogen receptor subtypes (estrogen receptor-α and estrogen receptor-β) (38, 42)
Different patterns of tissue expression of estrogen receptor subtypes (estrogen receptor-α and estrogen receptor-β) (38–41)

'This is not a pipe'

R. Magritte, La trahison des images 1929