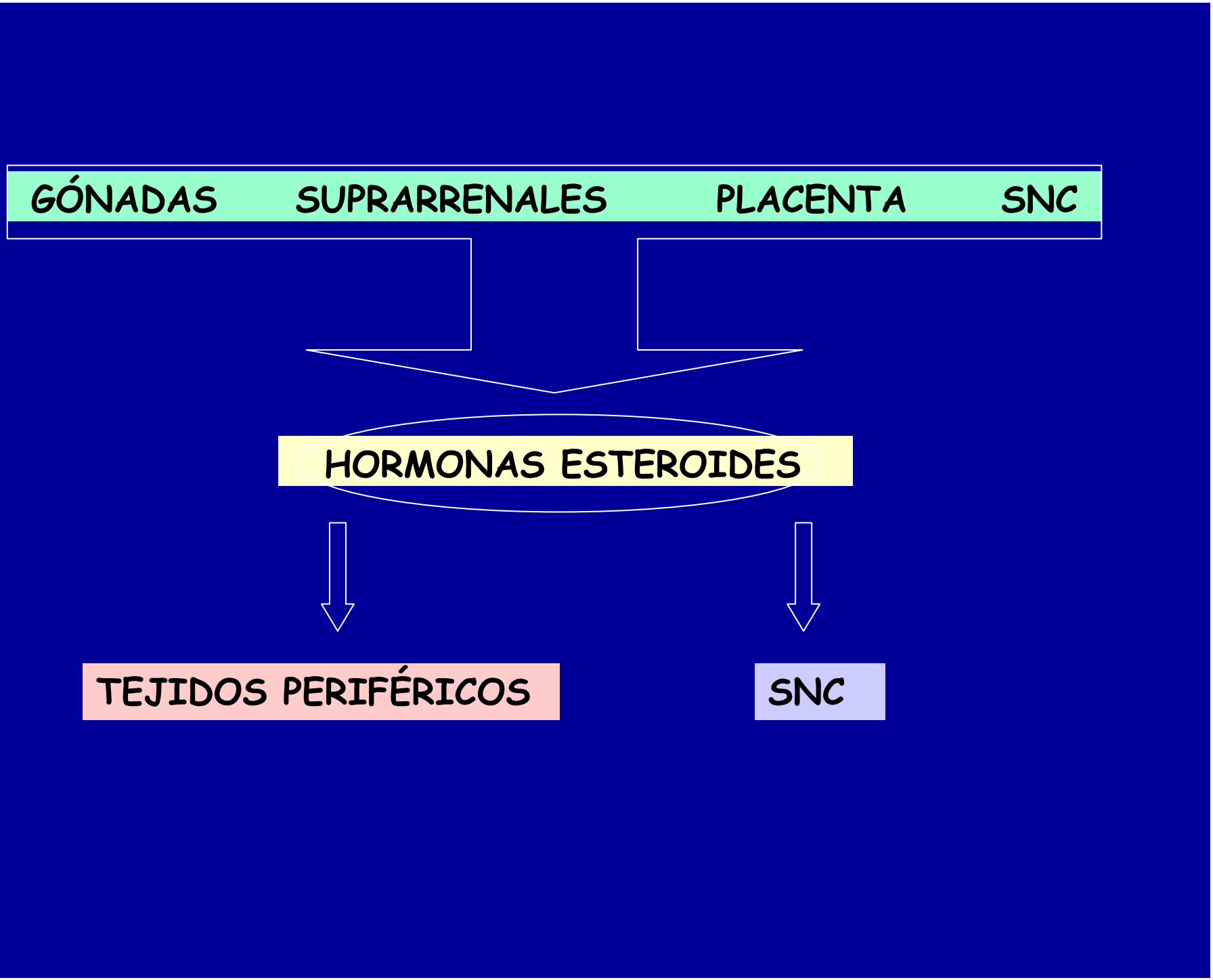
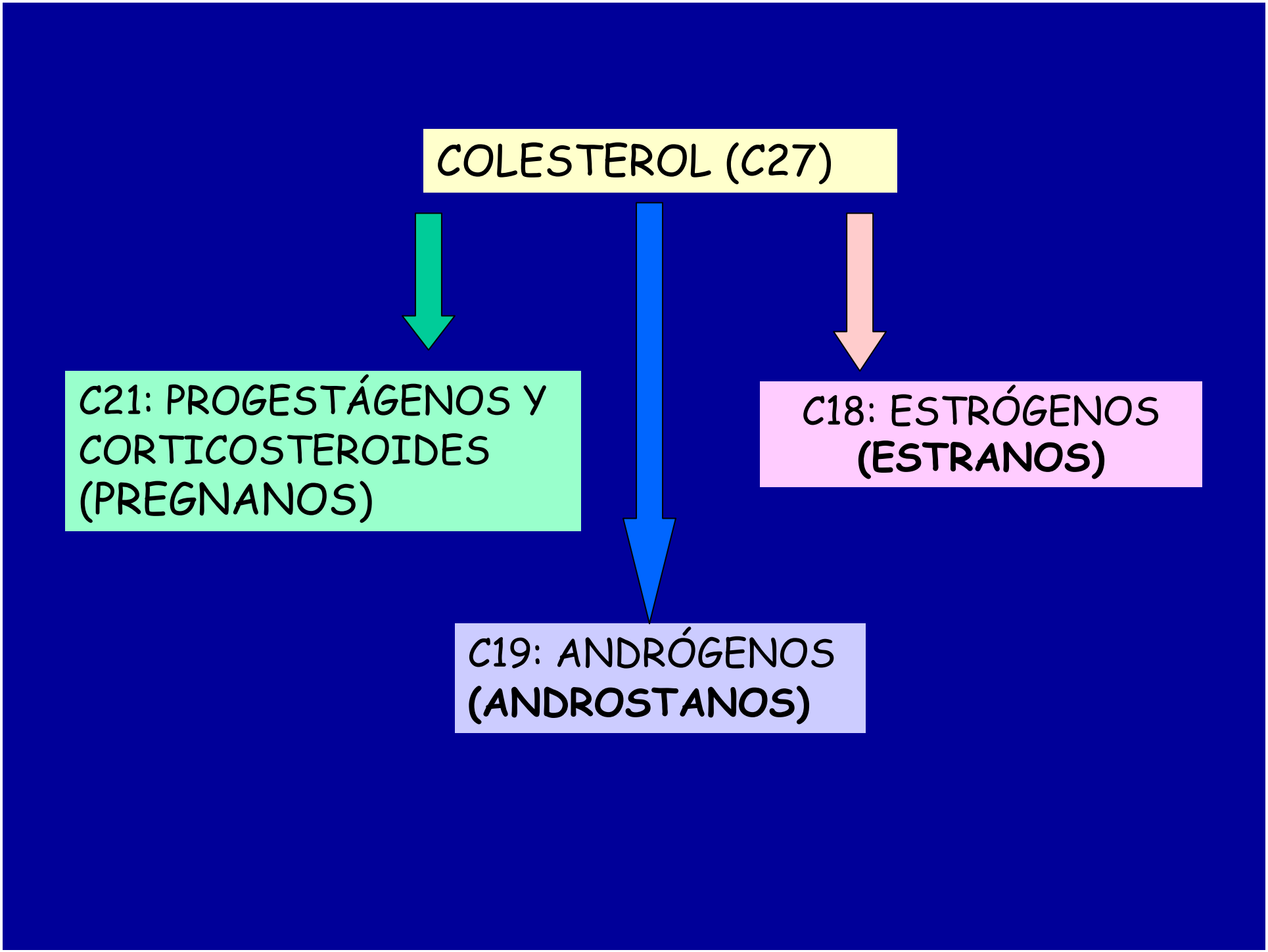
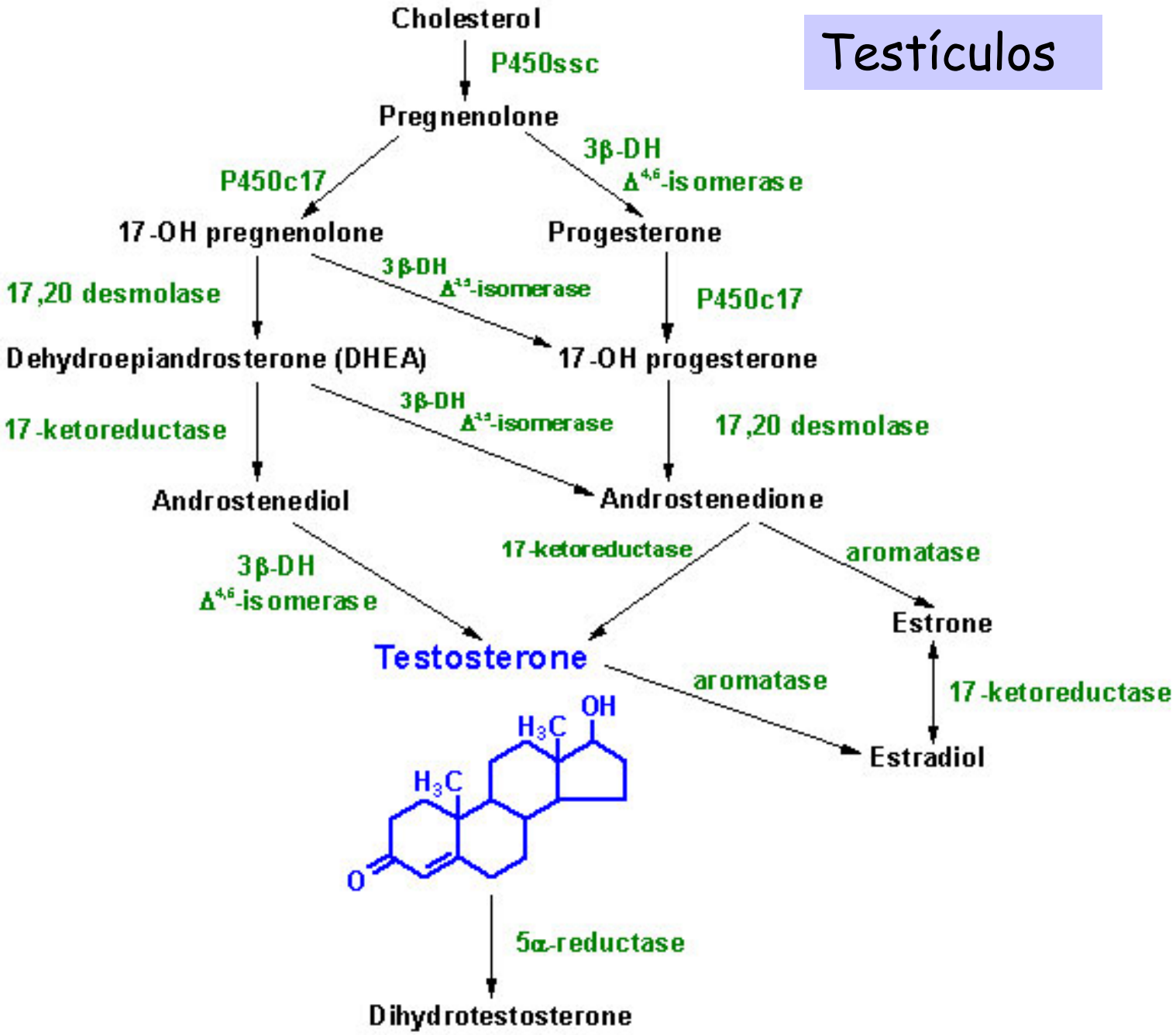
"INTERACCIÓN HORMONAS ESTEROIDEAS/RECEPTOR: MECANISMO DE ACCIÓN"

Dr. Sergio Ghersevich
Laboratorio de Estudios Reproductivos
Fac. de Cs. Bioquímicas y Farmacéuticas
Universidad Nacional de Rosario

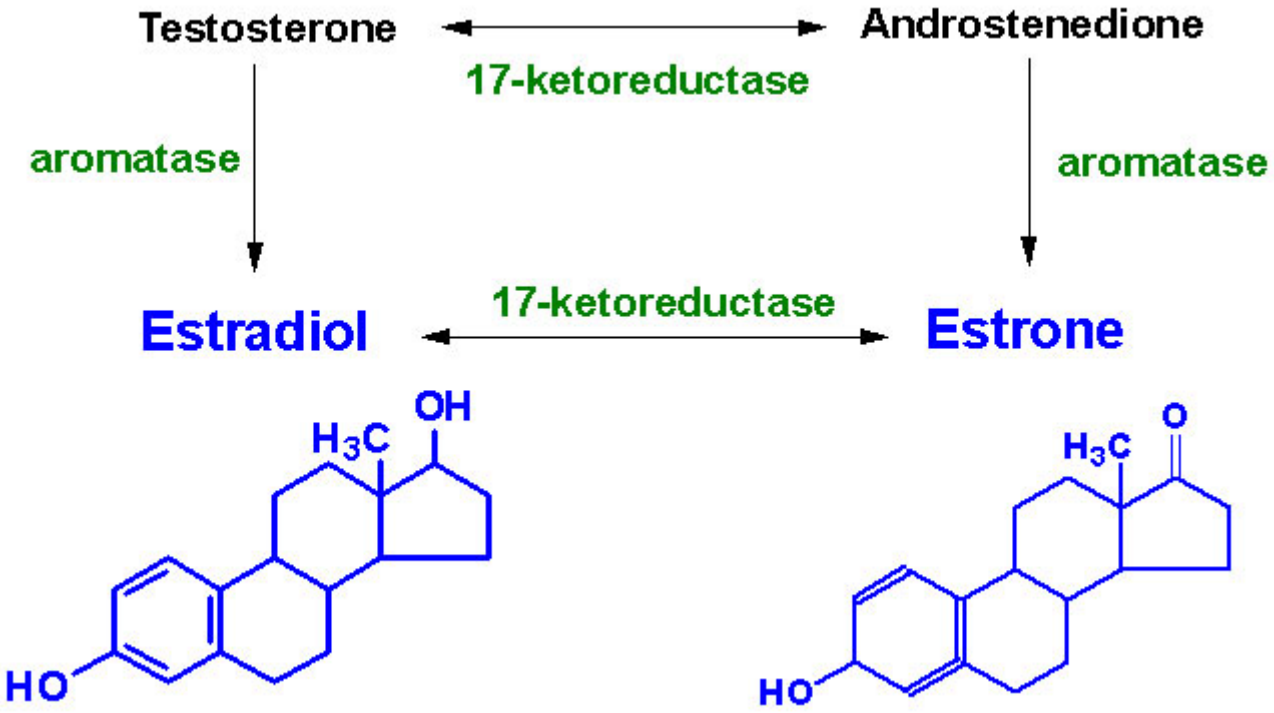

GÓNADAS
SUPRARRENALES
PLACENTA
SNC
HORMONAS ESTEROIDES
TEJIDOS PERIFÉRICOS
SNC


COLESTEROL (C27)
C21: PROGESTÁGENOS Y CORTICOSTEROIDES (PREGNANOS)
C18: ESTRÓGENOS (ESTRANOS)
C19: ANDRÓGENOS (ANDROSTANOS)

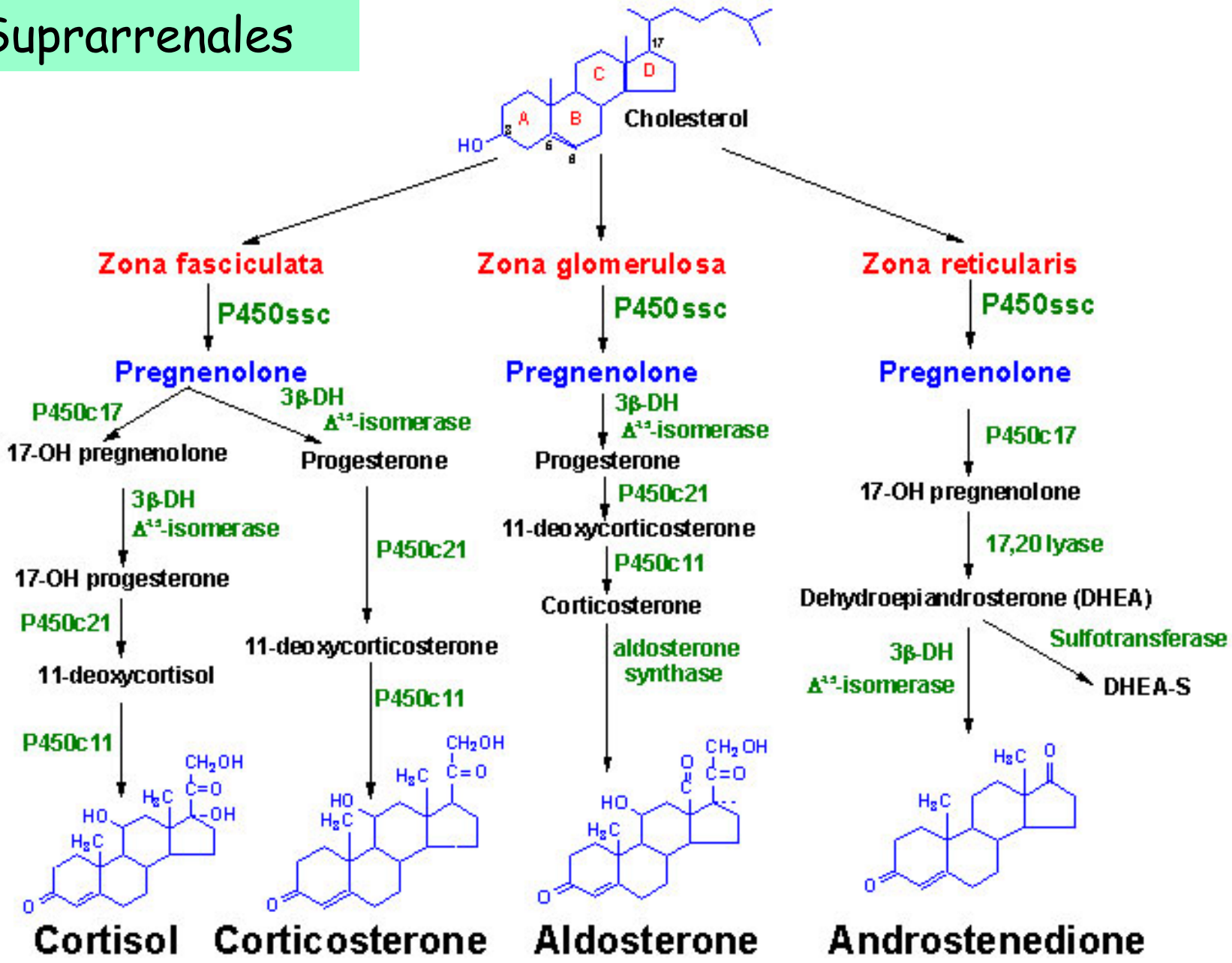
Testículos


Cholesterol
P450ssc
Pregnenolone
3β-DH
Δ4,6-isomerase
P450c17
17-OH pregnenolone
Progesterone
3β-DH
Δ5,4-isomerase
17,20 desmolase
P450c17
Dehydroepiandrosterone (DHEA)
17-OH progesterone
3β-DH
Δ5,4-isomerase
17-ketoreductase
17,20 desmolase
Androstenediol
Androstenedione
17-ketoreductase
aromatase
3β-DH
Δ4,6-isomerase
Estrone
Testosterone
aromatase
17-ketoreductase
Estradiol
OH
H3C
H3C
O
5α-reductase
Dihydrotestosterone

Ovarios

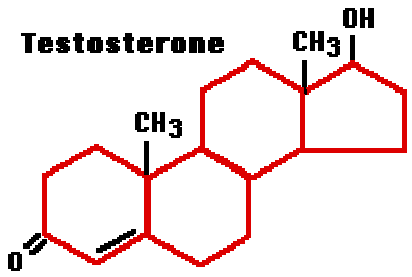

Testosterone
Androstenedione
17-ketoreductase
aromatase
aromatase
Estradiol
17-ketoreductase
Estrone
OH
H3C
HO
O
H3C
HO

Suprarrenales



ESTEREOISÓMEROS: DIFIEREN EN LA DISPOSICIÓN ESPACIAL DE SU ESTRUCTURA

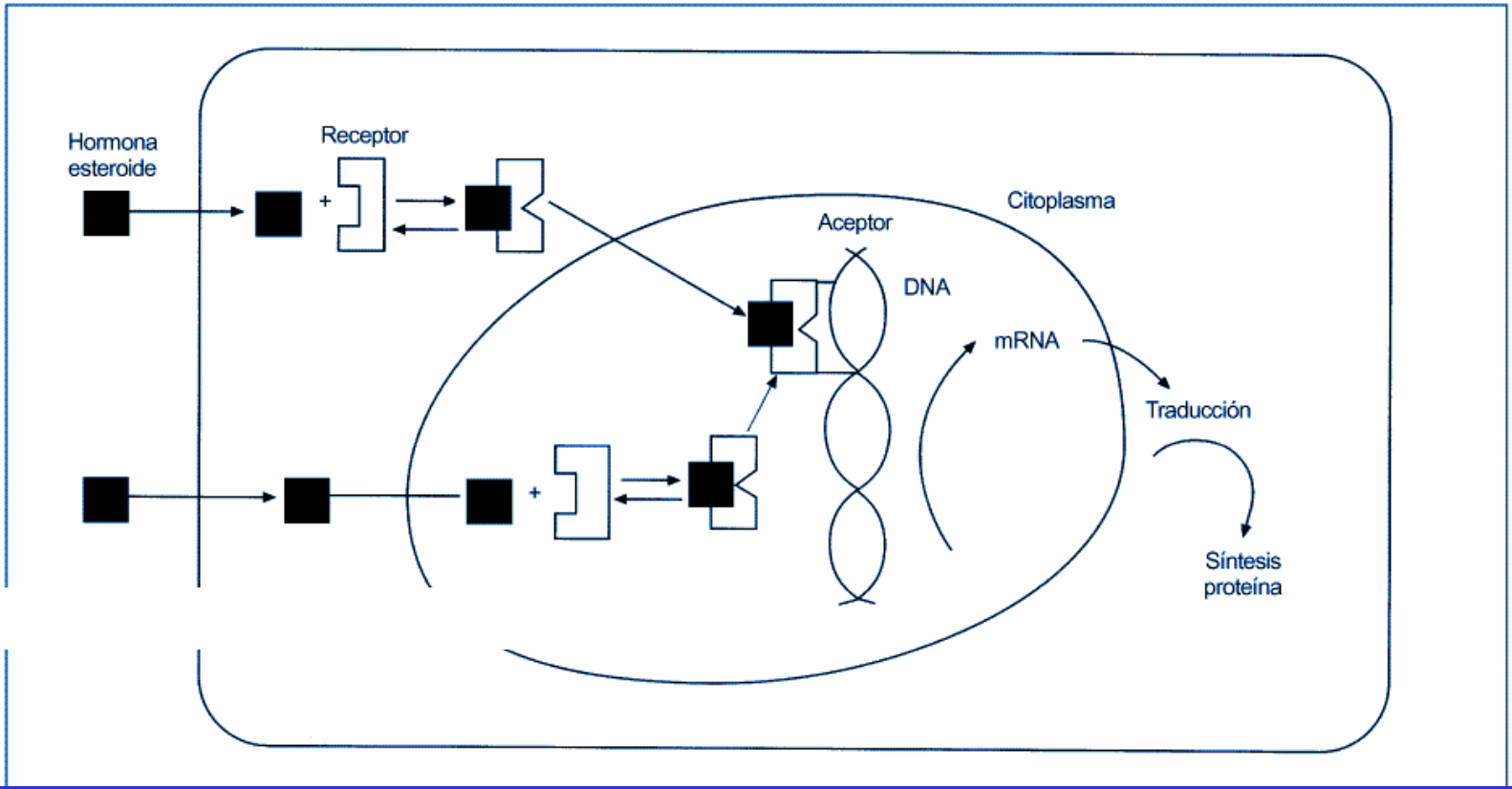
DISTINTA ACTIVIDAD BIOLÓGICA



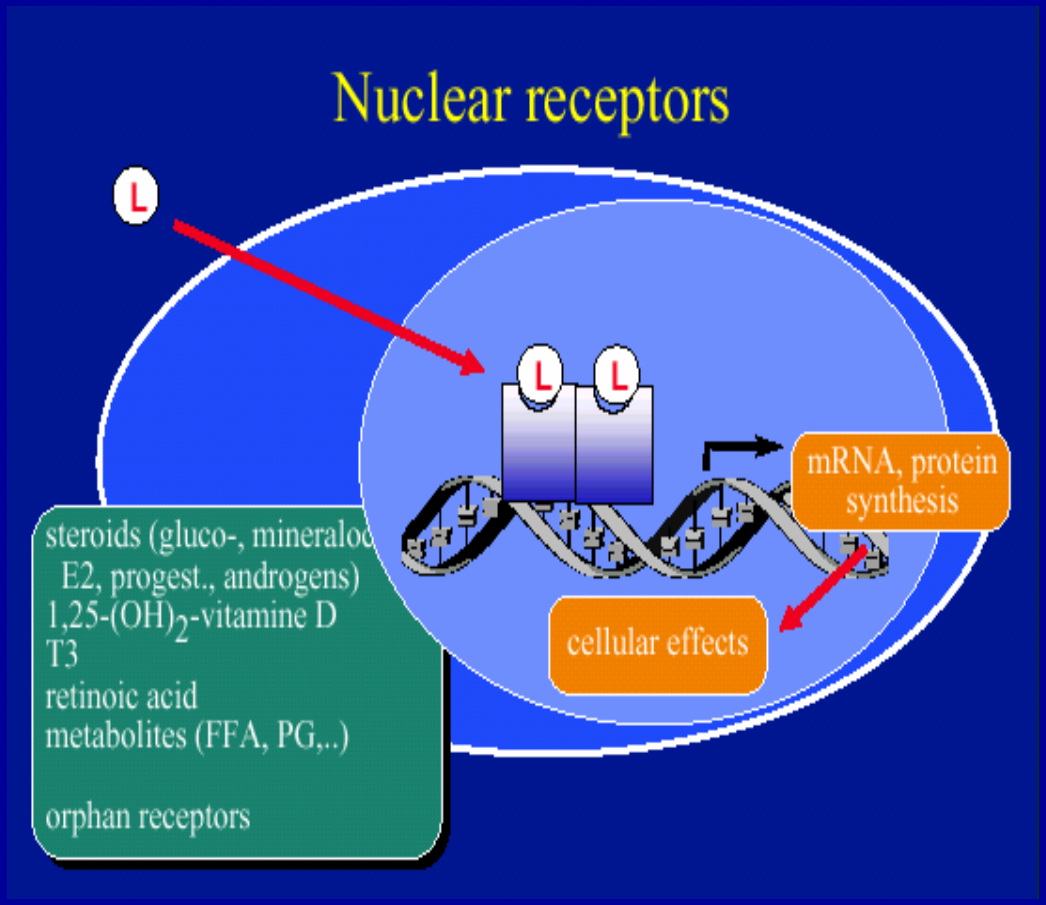
Testosterona ⟶ 17ß-hydroxy-androst-4-ene-3-ona

MECANISMOS DE ACCIÓN SOBRE TEJIDOS BLANCO:

-Directo a través de unión a receptor.
-Conversión previa a metabolito activo.
-Otros mecanismos.



Receptores esteroideos

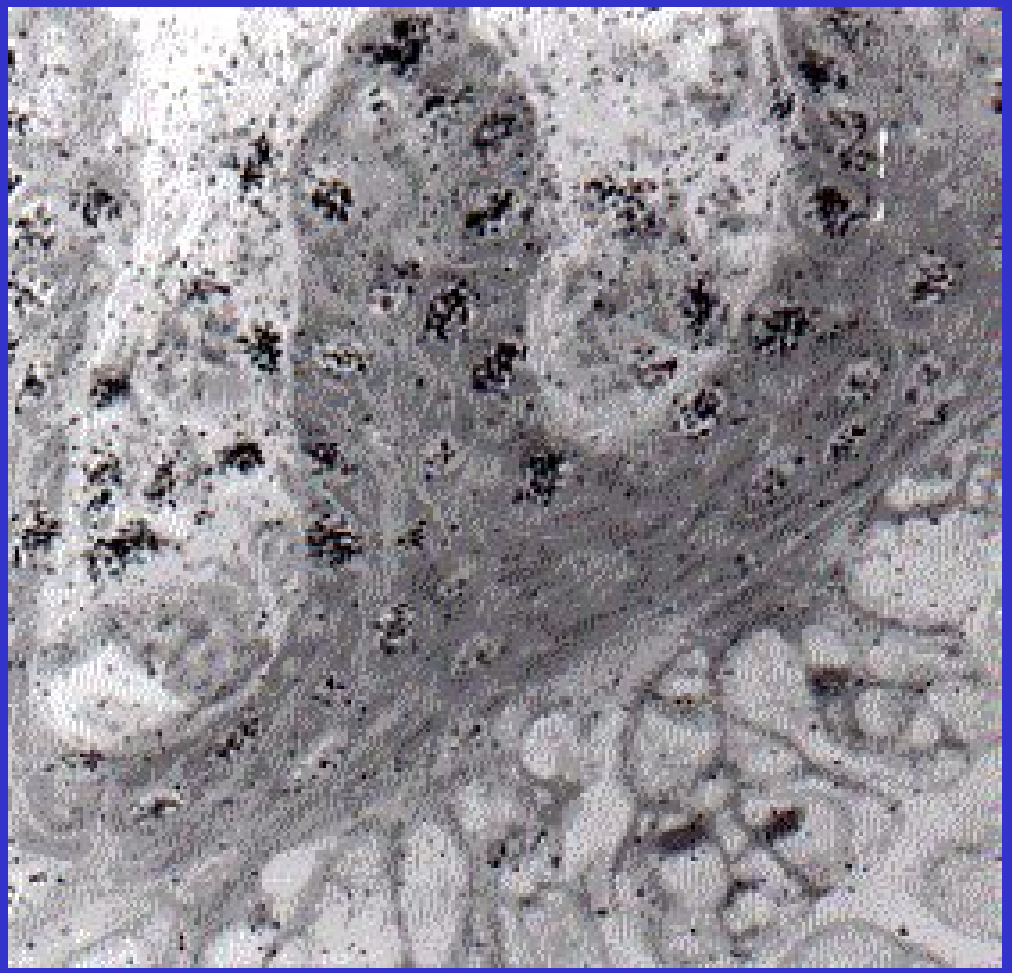


RECEPTORES DE HORMONAS ESTEROIDES:

Pertenecen a la superfamilia de proteinas receptoras de esteroides y hormonas tiroideas.

Se localizan a nivel citoplásmico o nuclear.

Células endometriales con el núcleo marcado con progesterona radioactiva

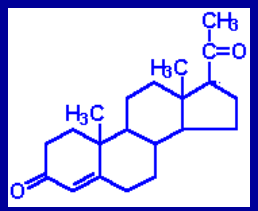


Subfamilias de receptores de esteroides según su función:

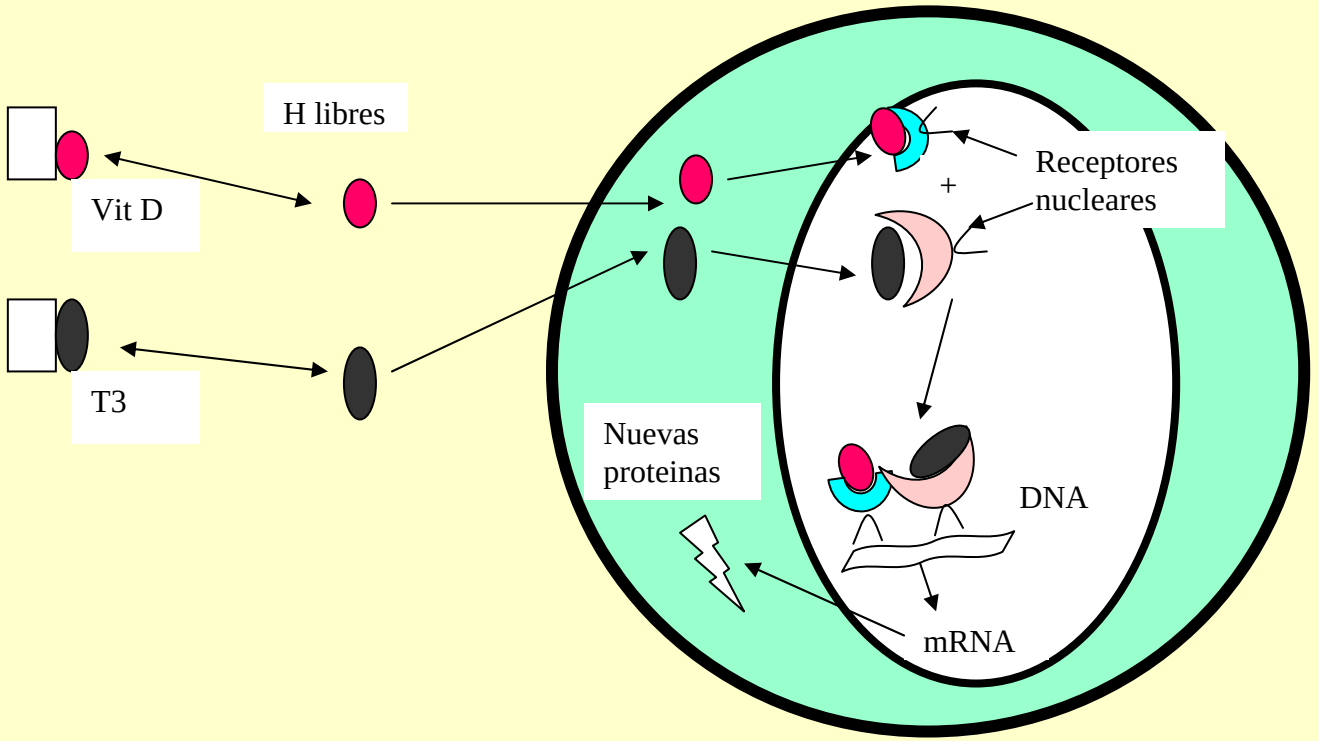
Clase I: receptores relacionados a hormonas tiroideas (T3R, RAR, VDR y peroxisome proliferator-activated receptors o PPARs). (HETERODÍMEROS)

Clase II: RXR, HNF-4 y algunos receptores huérfanos.

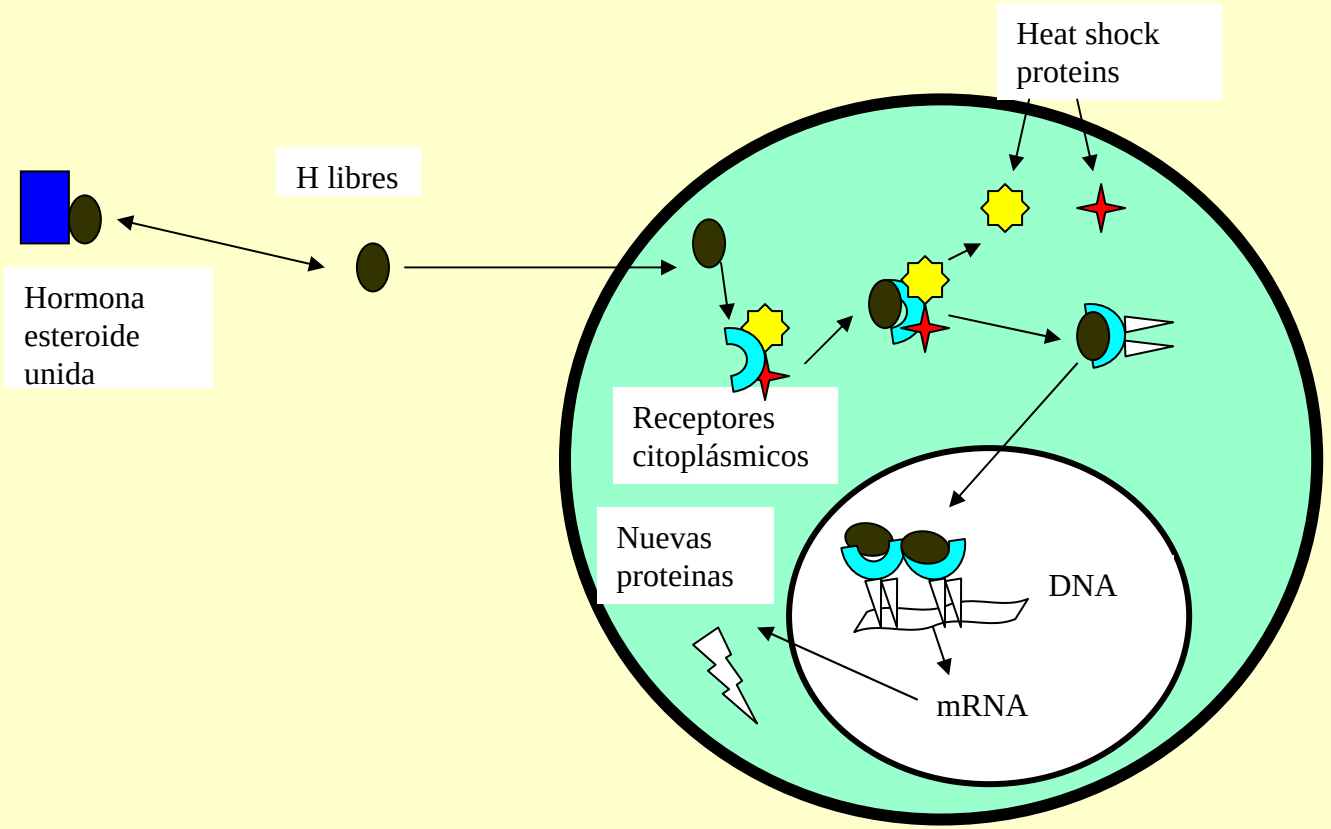
Clase III: receptores esteroideos clásicos. Incluyen GR, AR, MR, PR y ER. (HOMODÍMEROS)

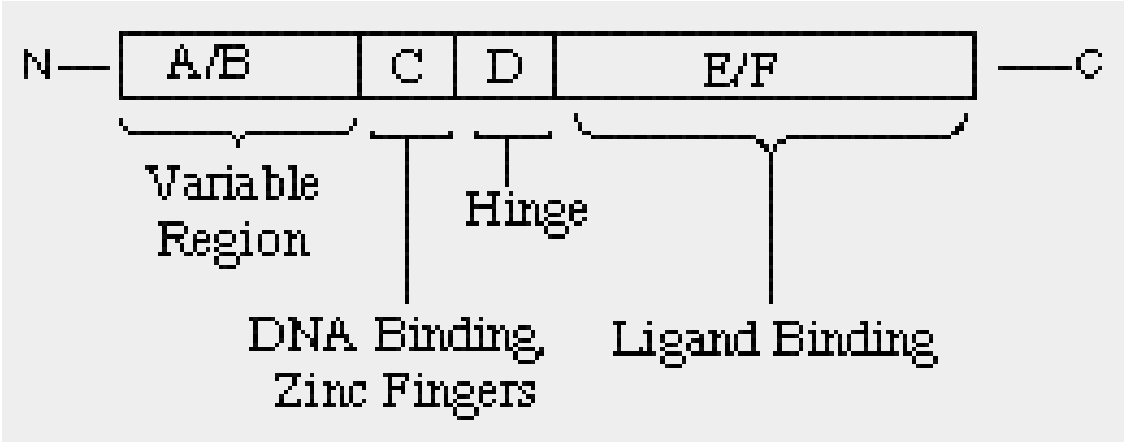


Mecanismo de activación receptores esteroides Clase I



Mecanismo de activación receptores esteroides Clase III





Dominios funcionales de los receptores esteroideos:

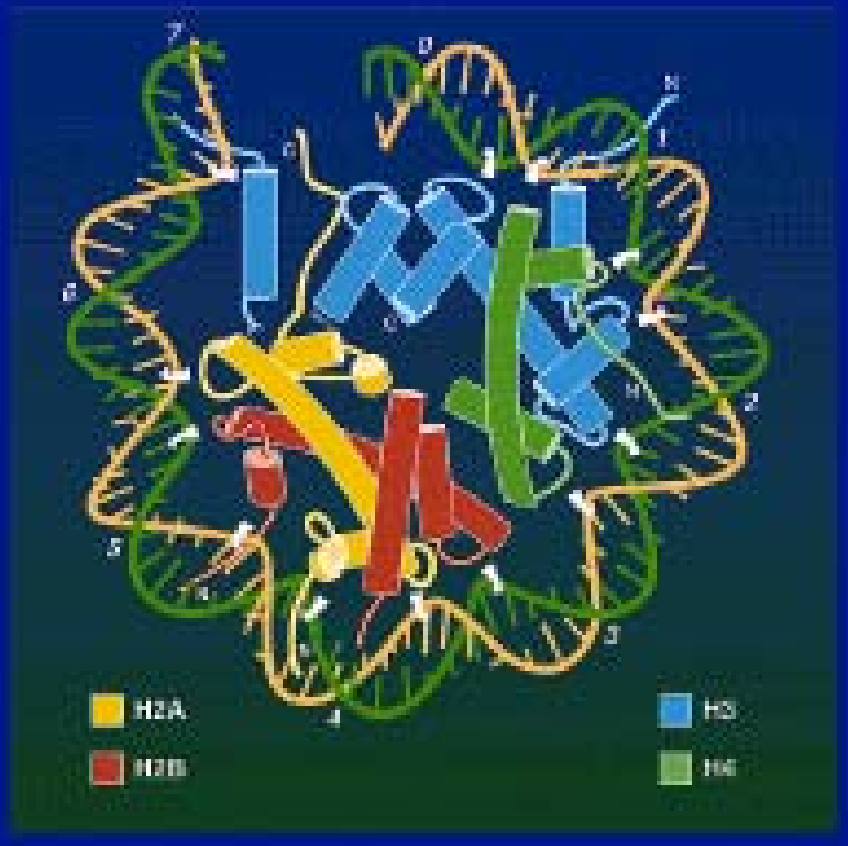
Dominio A/B: región N-terminal. Contiene el dominio de transactivación AF-1 independiente del ligando.

Dominio C: región zinc-finger de unión al DNA (reconocimiento de secuencias específicas: HRE).

Dominio D: región eje o bisagra.

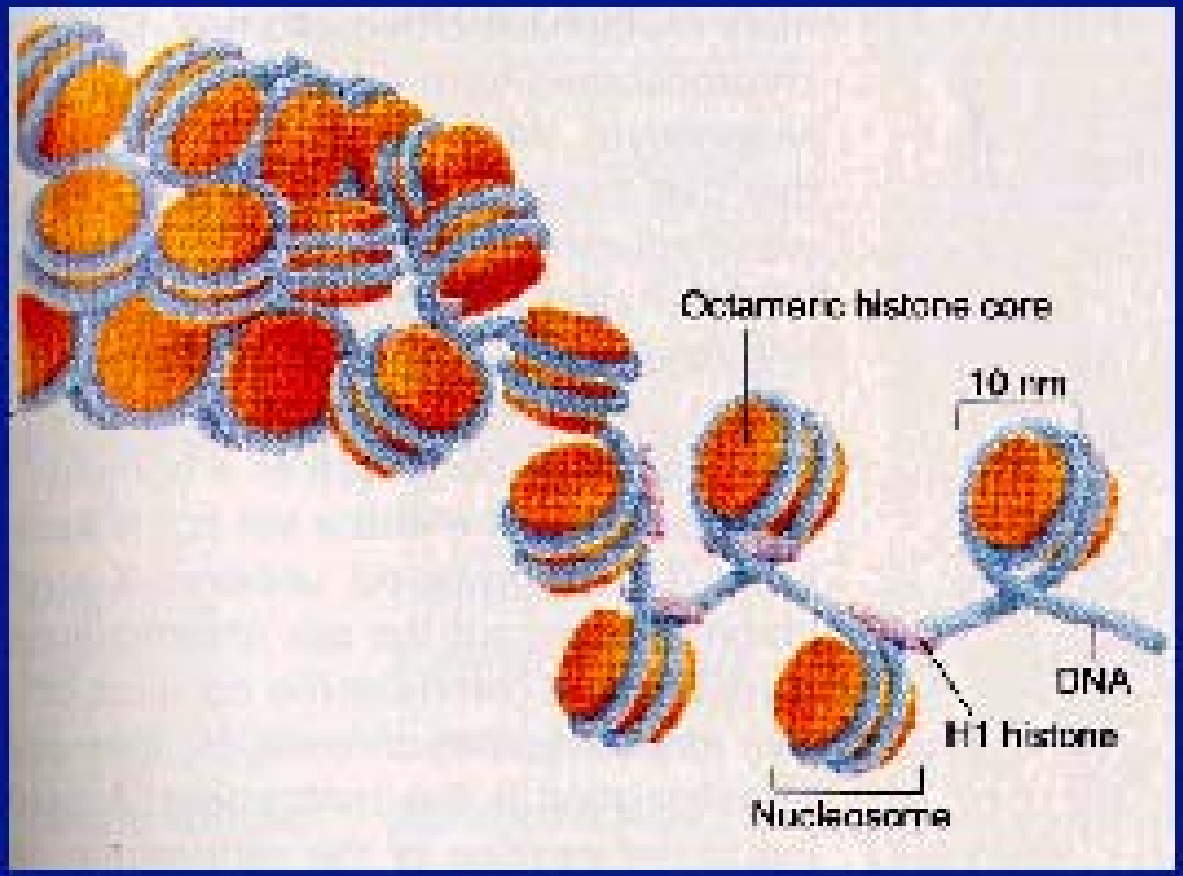
Dominio E/F: región C-terminal que contiene el dominio de unión al ligando y la superficie de dimerización. Contiene el dominio de transactivación AF-2 dependiente del ligando.

Cromatina nuclear

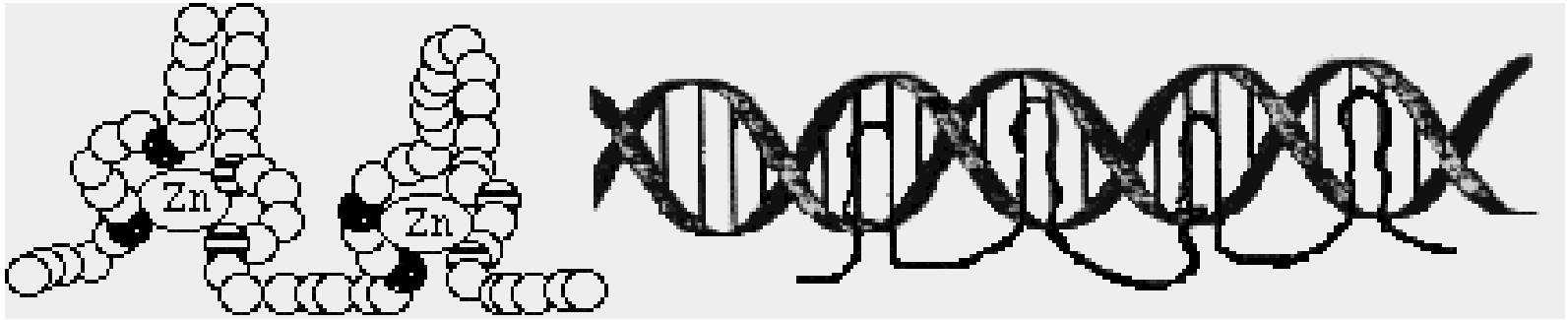


Nature
389: 251f
1997

APERTURA DE LA CROMATINA



Interacción Zinc-finger protein-DNA



- Cisteína
- Histidina o cisteína

Sitios de unión del ER

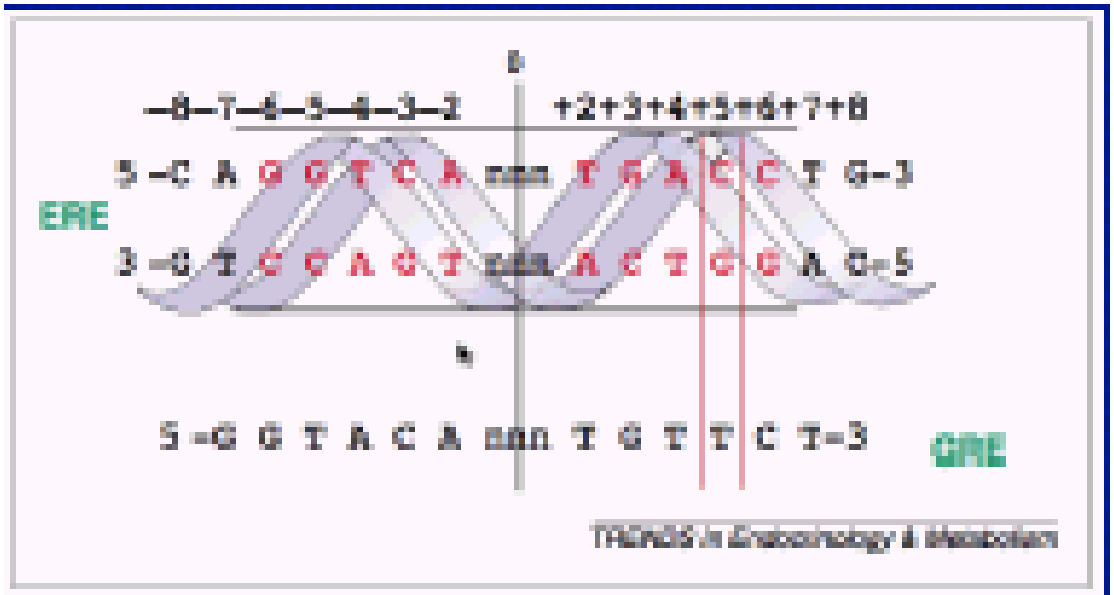


Figure 1. Sequence of the ERE and GRE. (a) A consensus ERE has been derived from several highly estrogen-responsive sequences from the African clawed frog *Xenopus laevis* genes encoding vitellogenin A1, A2, B1, B2 and the chicken apo-VLDL II gene. It is a 13 bp perfect palindromic inverted repeat with 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 +6 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.

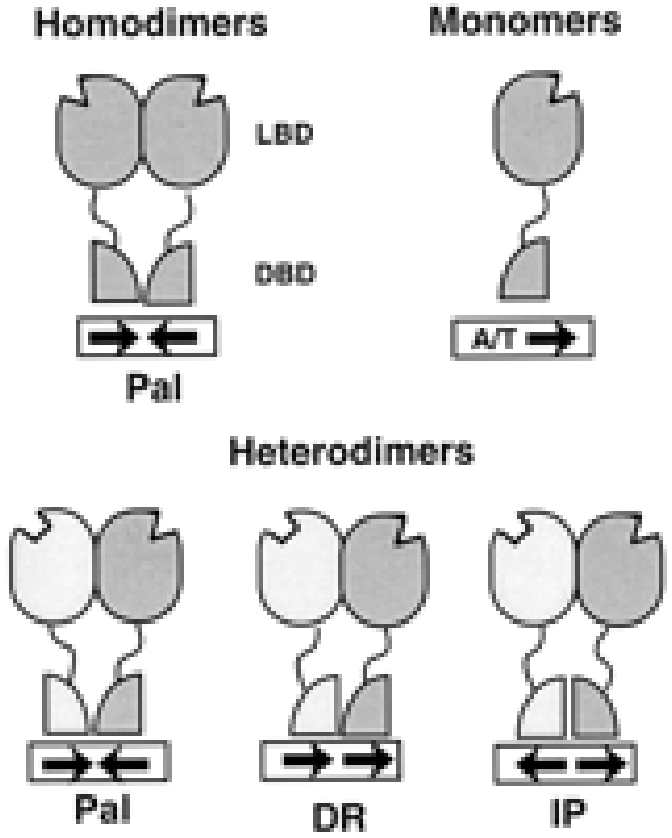
Unión de los receptores a los HRE

Pal: palíndromos

DR: repeticiones directas

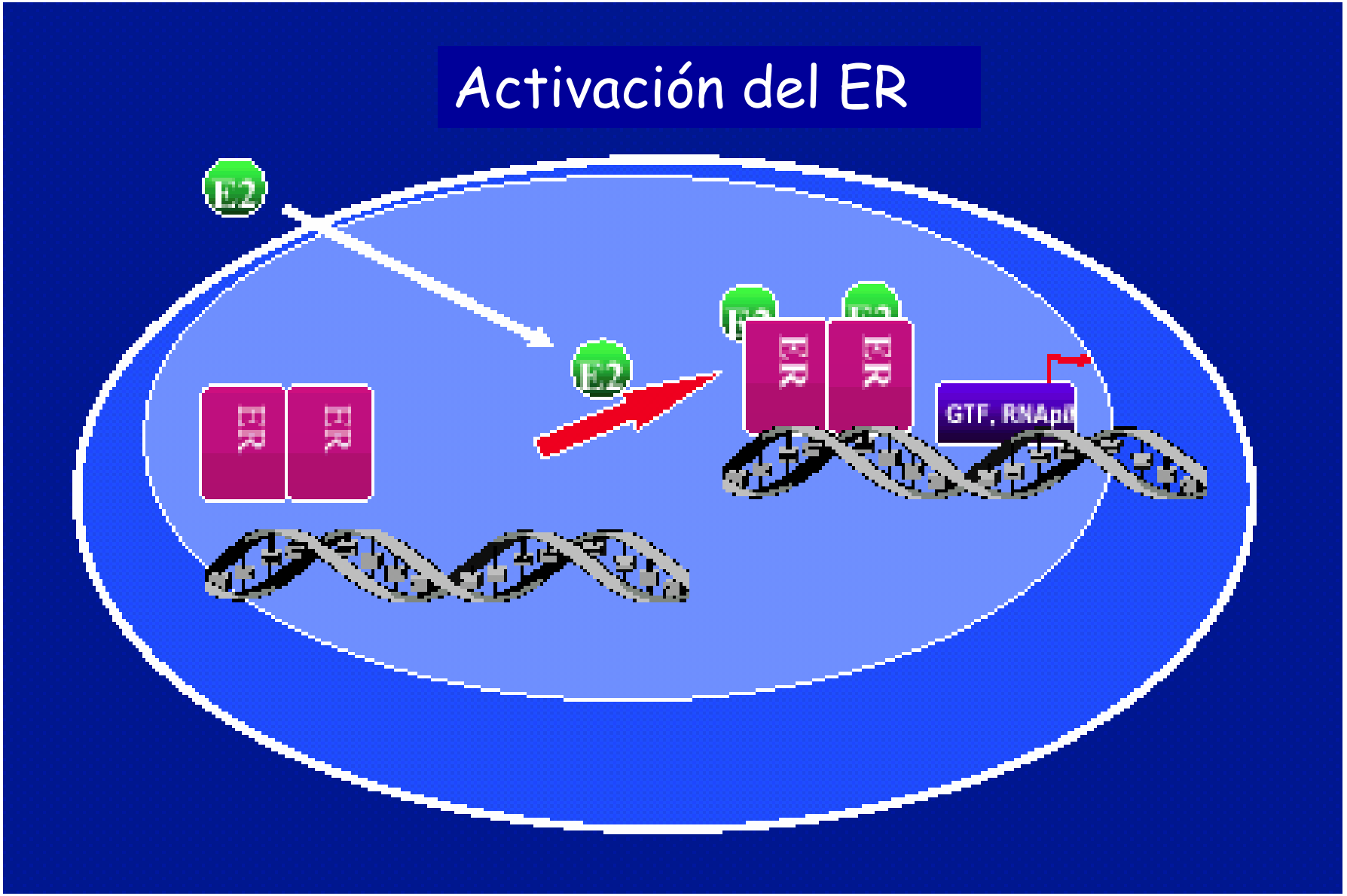
IP: palíndromos invertidos

A/T: secuencias 5' ricas en A/T

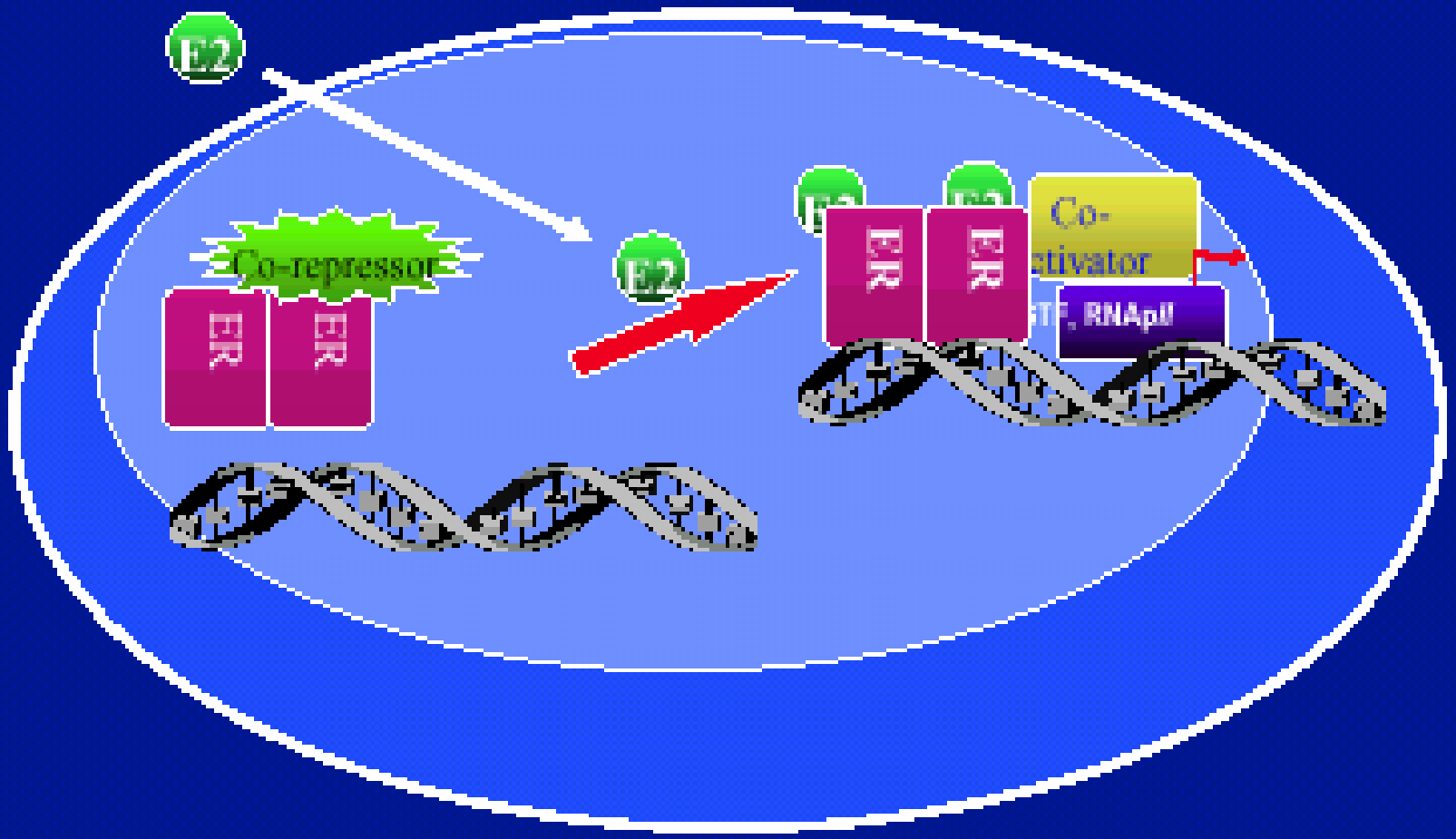


Aranda and Pascual. Physiol Rev, Vol. 81, 2001

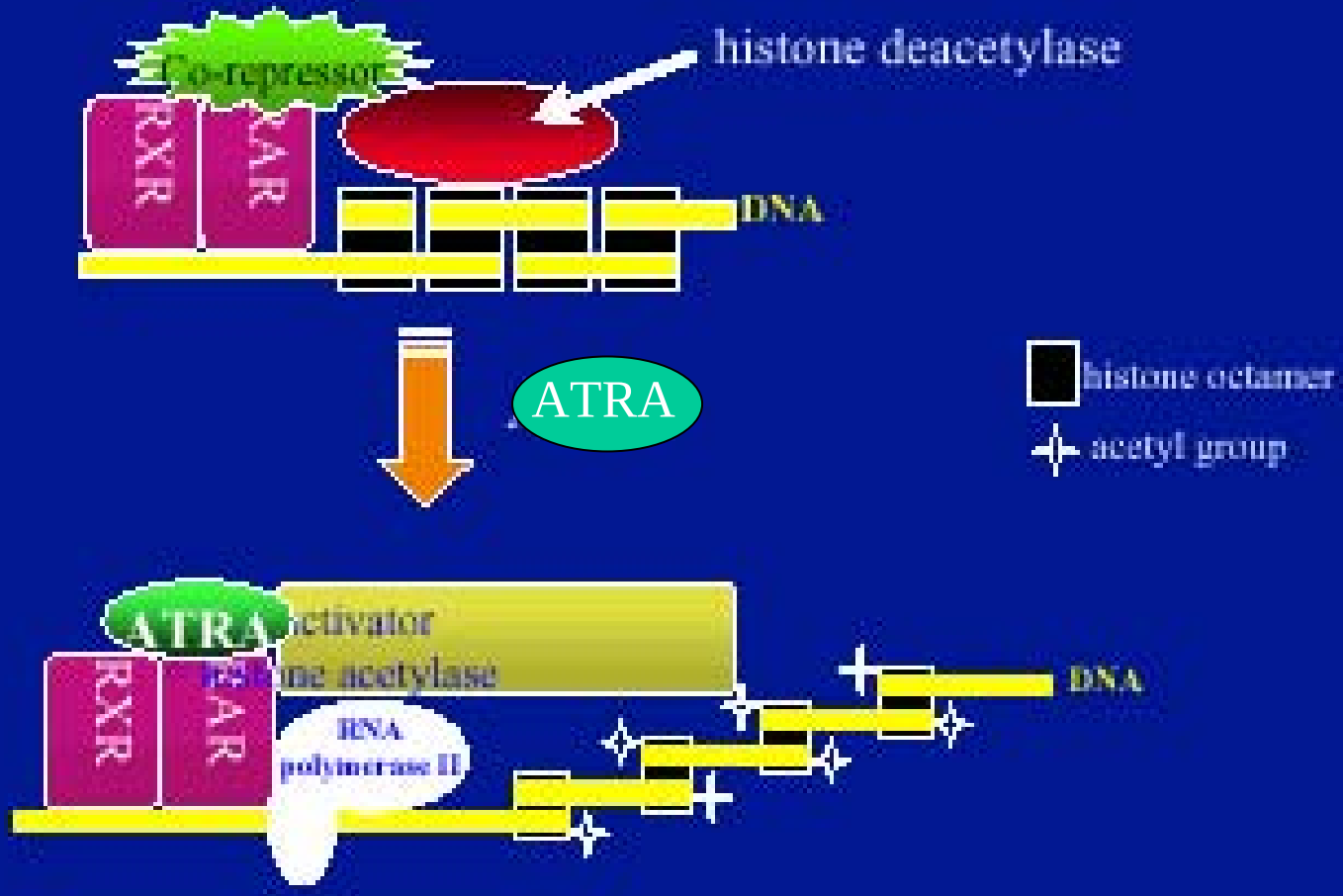
Activación del ER



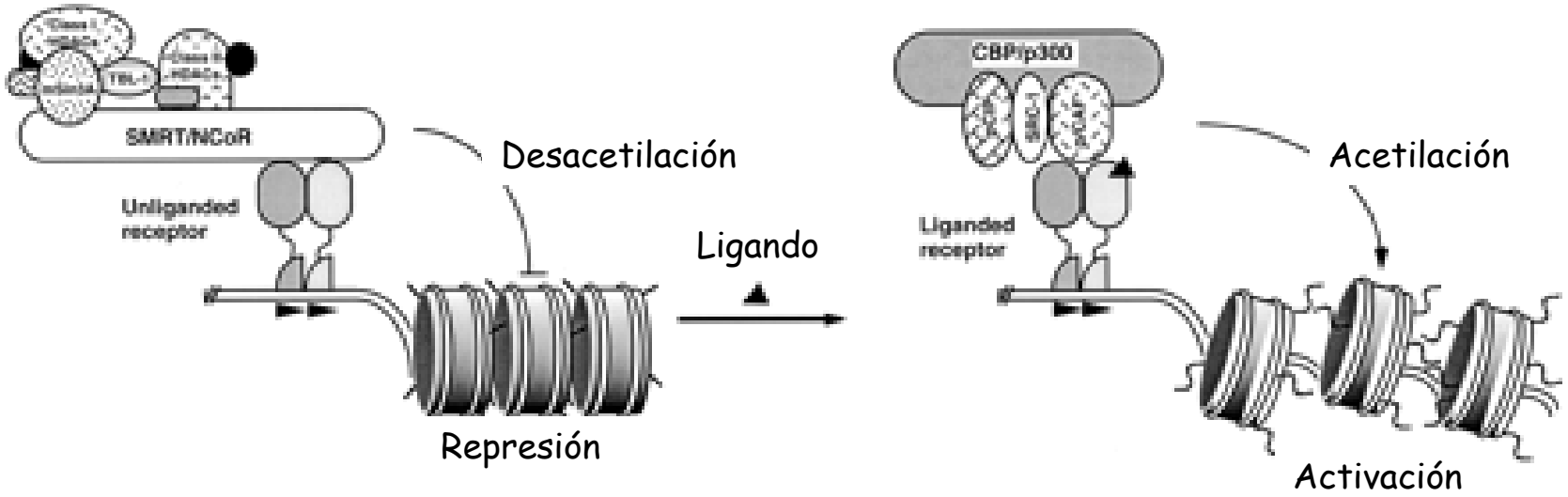
Activación de receptores nucleares



Interacción receptor-cromatina

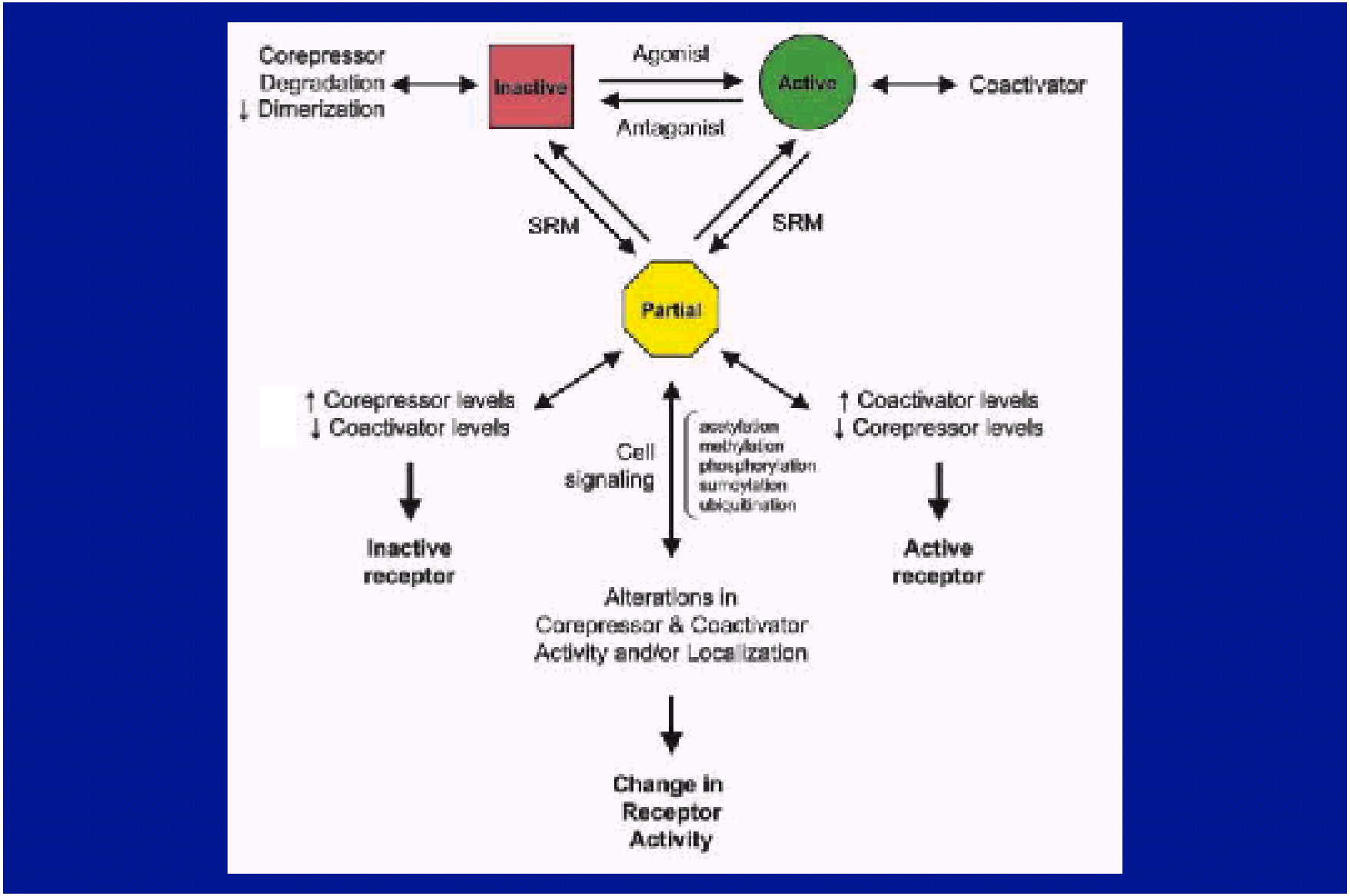


Coactivación y correpresión: acetilación de histonas

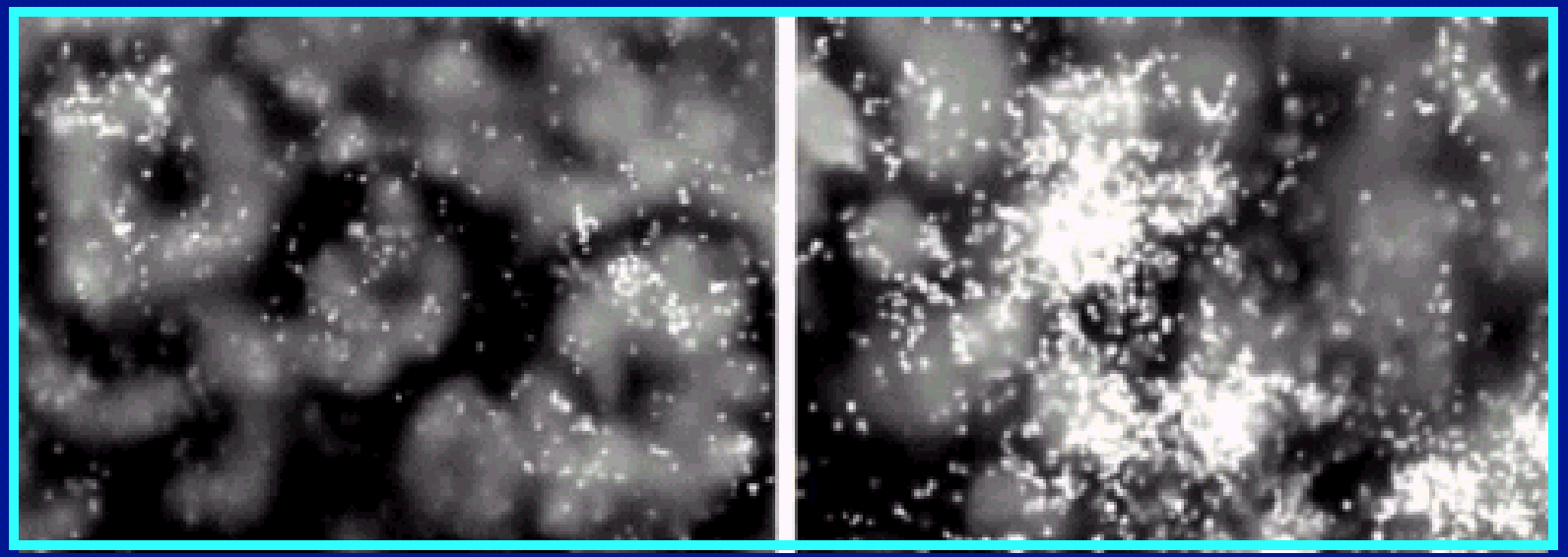


Aranda and Pascual. Physiol Rev, Vol. 81, 2001

Activación de receptores nucleares



Sobre-expresión del coactivador AIB-1 en cáncer de mamas



Células mamarias normales

Células de cáncer de mama

Science 277: 965f (1997)

Mecanismo de acción "no transcripcional" de los esteroides

- Acciones rápidas no compatibles con síntesis previa de ARN o proteínas.
- Acción en presencia de inhibidores de síntesis de ARN o proteínas.
- Acción de esteroides acoplados a proteínas de membrana.
- Acciones sobre células con cromatina super compacta.
- Acciones en células con receptores mutados inactivos.

Sitios de unión en membranas para testosterona:

- Osteoblastos
- Macrófagos
- Linfocitos T
- Células próstata
- Células vasculares

Sitios de unión en membranas para progesterona:

- Espermatozoides
- Osteoblastos
- Células granulosas
- Ovocitos

Sitios de unión en membranas para estrógenos:

- Células endometriales.
- Osteoblastos
- Neuronas
- Células musculares vasculares.
- Adipocitos.

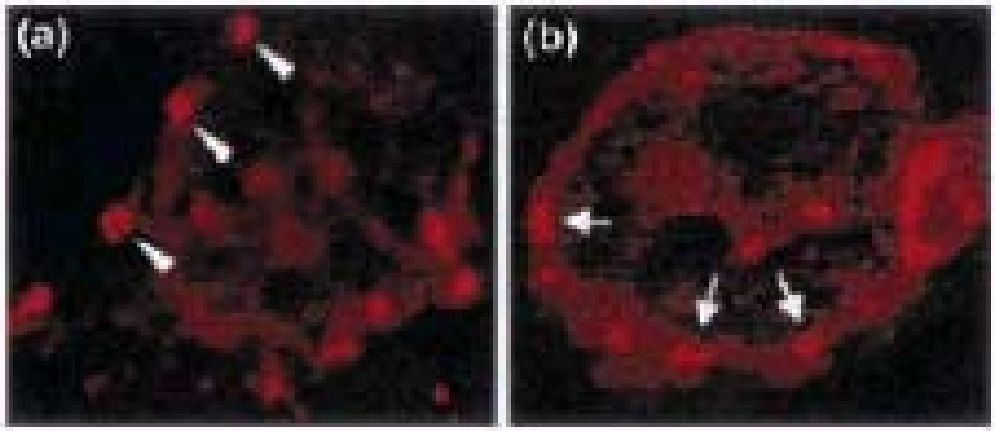


Fig. 1 Confocal analysis of ligand-labelling and immunocytochemical staining of cultured mouse midbrain neurones. **(a)** Living cells were exposed to 17-β-oestradiol coupled to hemisuccinate-BSA-FITC at a steroid concentration of approximately 1 nM for 5 min followed by a brief washing step and subsequent 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).

Mecanismo de acción no genómico de las hormonas esteroideas

- Regulación de receptores acoplados a proteína G.
- Regulación de canales iónicos.
- Regulación de quinasas:

-MAPK, ERK ½, p38, etc

-Tirosinas quinasas.

Mecanismo de acción no genómico de las hormonas esteroideas

Activación de MAPK por E2

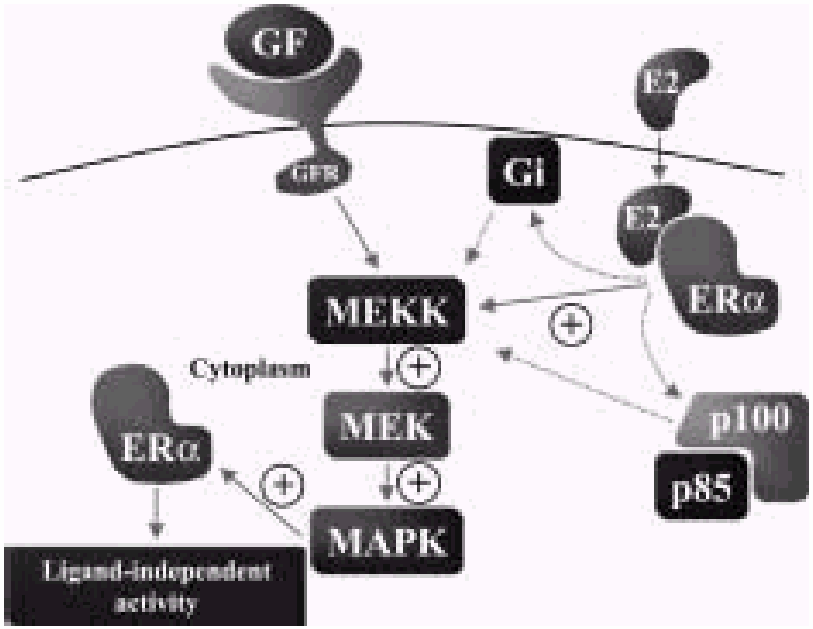


Figure 2 ERs and MAPKs. Binding of growth factors (GF) to the specific membrane receptors induces activation of the MAPK cascade (MEKK → MEK → MAPK). Active MAPK phosphorylates and activates ER independently of the presence of E_2. On the other side, E_2-bound ER triggers the activation of the three-step ERK 1/2 MAPK module. ER activation of MAPK is finely modulated by Gi protein (Gi)-dependent and PI3K (p85/p100)-dependent intracellular pathways.

Eur J Endocrinol (2003), 148

Mecanismo de acción no genómico de las hormonas esteroideas

Activación de la NOS por E2

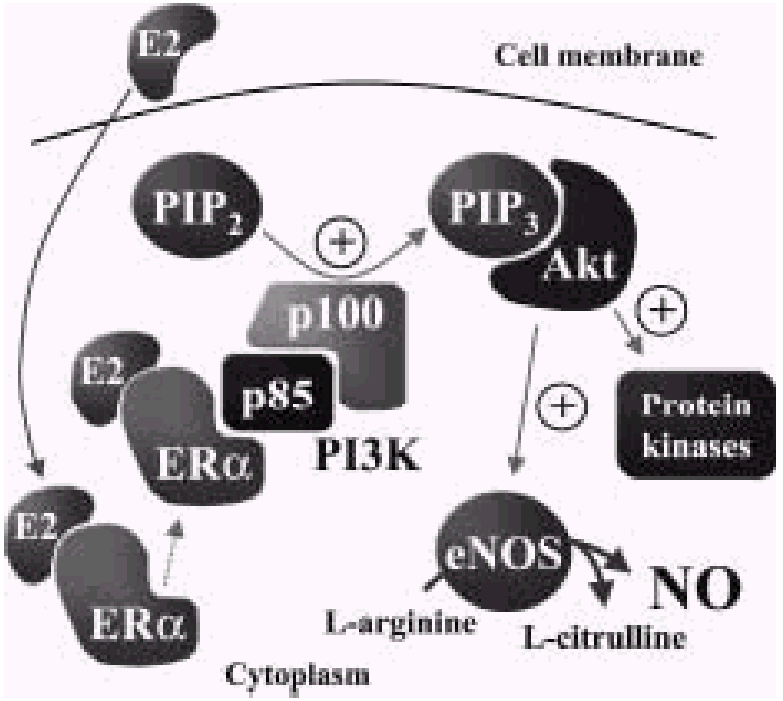


Figure 3 ER and PI3K. Ligand (E_2)-bound ER interacts with the regulatory subunit (p85) of PI3K (p85/p110). The catalytic subunit (p110) is thus activated, leading to phosphorylation of phosphoinositides (PIP_2, PIP_3) on the D-3 position of the inositol ring. PIP_3 is recognized by specific docking sites on protein kinases, which trigger the activation of protein kinase Akt. Akt regulates, via phosphorylation, several intracellular enzymes (protein kinases), including eNOS, leading to increased NO synthesis.

Mecanismo de acción no genómico de las hormonas esteroideas

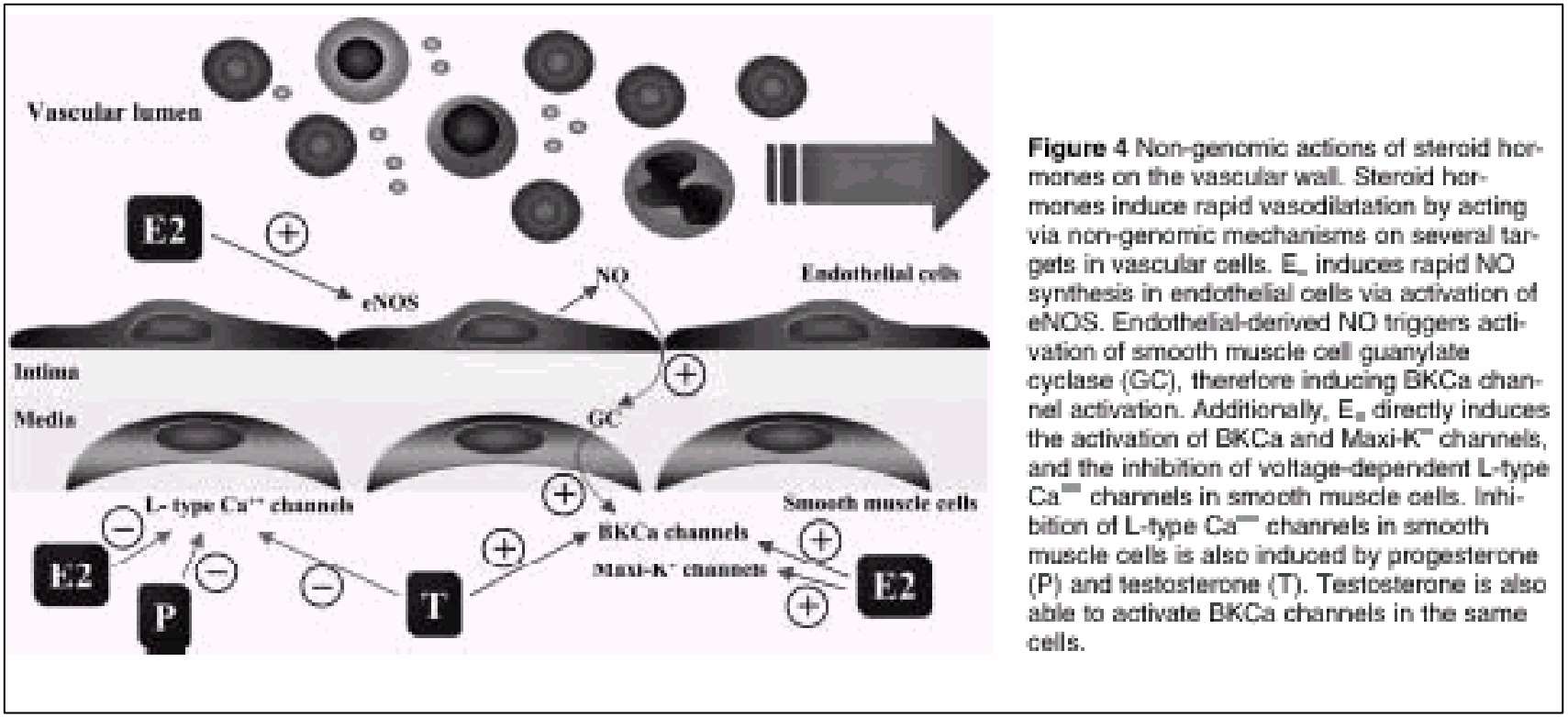


Figure 4 Non-genomic actions of steroid hormones on the vascular wall. Steroid hormones induce rapid vasodilatation by acting via non-genomic mechanisms on several targets in vascular cells. E_2 induces rapid NO synthesis in endothelial cells via activation of eNOS. Endothelial-derived NO triggers activation of smooth muscle cell guanylate cyclase (GC), therefore inducing BKCa channel activation. Additionally, E_2 directly induces the activation of BKCa and Maxi-K^+ channels, and the inhibition of voltage-dependent L-type Ca^{++} channels in smooth muscle cells. Inhibition of L-type Ca^{++} channels in smooth muscle cells is also induced by progesterone (P) and testosterone (T). Testosterone is also able to activate BKCa channels in the same cells.

Acción sobre los vasos sanguíneos

Mecanismo de acción no genómico de las hormonas esteroideas

Acción sobre las neuronas

Acción anestésica y ansiolítica por modulación receptor GABA Tipo A

Neuroprotección

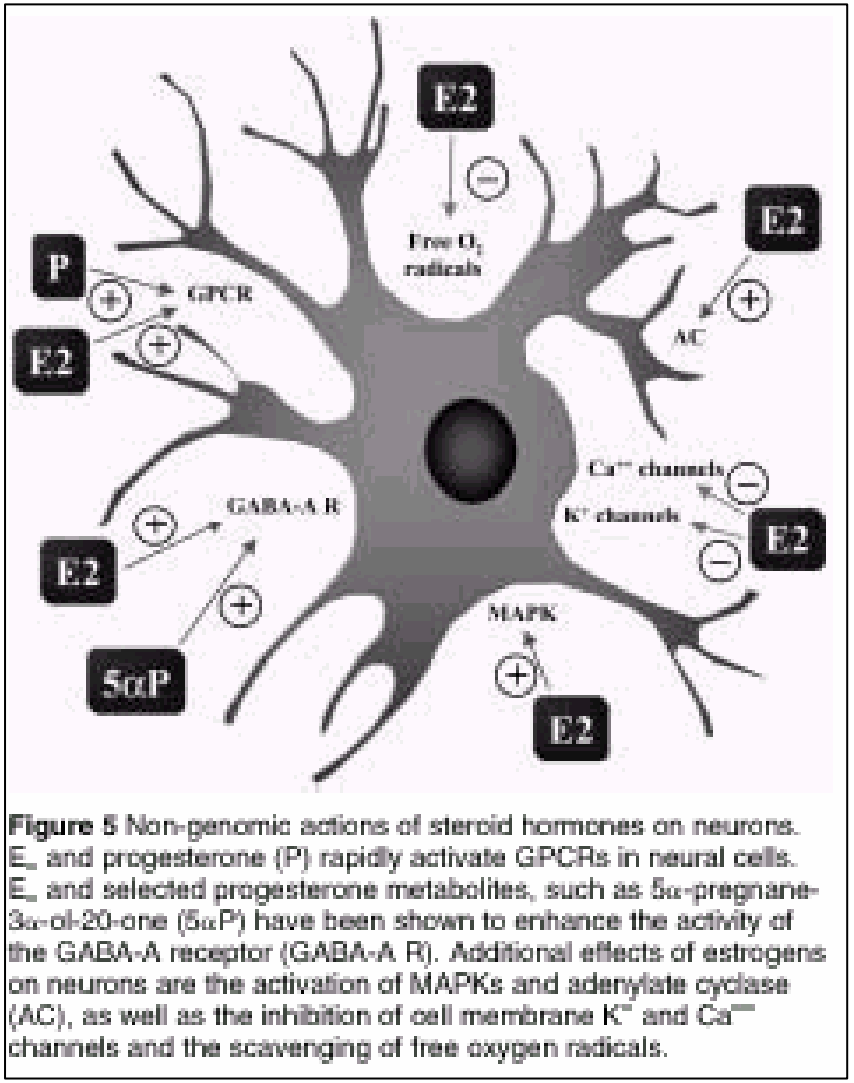
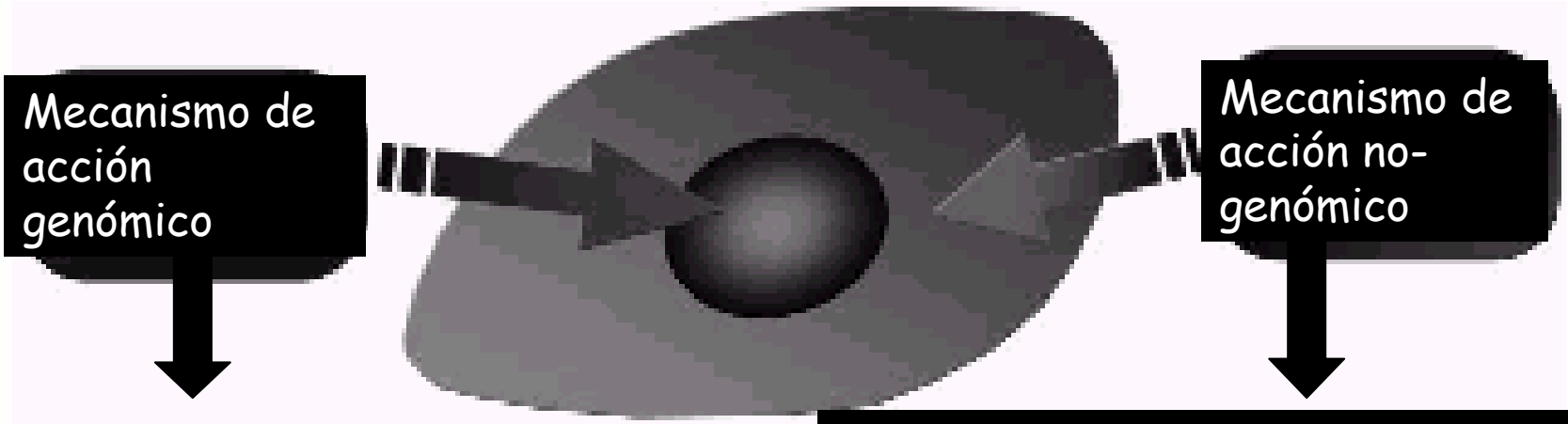


Figure 5 Non-genomic actions of steroid hormones on neurons. E_2 and progesterone (P) rapidly activate GPCRs in neural cells. E_2 and selected progesterone metabolites, such as 5α-pregnane-3α-ol-20-one (5αP) have been shown to enhance the activity of the GABA-A receptor (GABA-A R). Additional effects of estrogens on neurons are the activation of MAPKs and adenylate cyclase (AC), as well as the inhibition of cell membrane K^+ and Ca^{++} channels and the scavenging of free oxygen radicals.

Mecanismo de acción de las hormonas esteroideas

Célula blanco



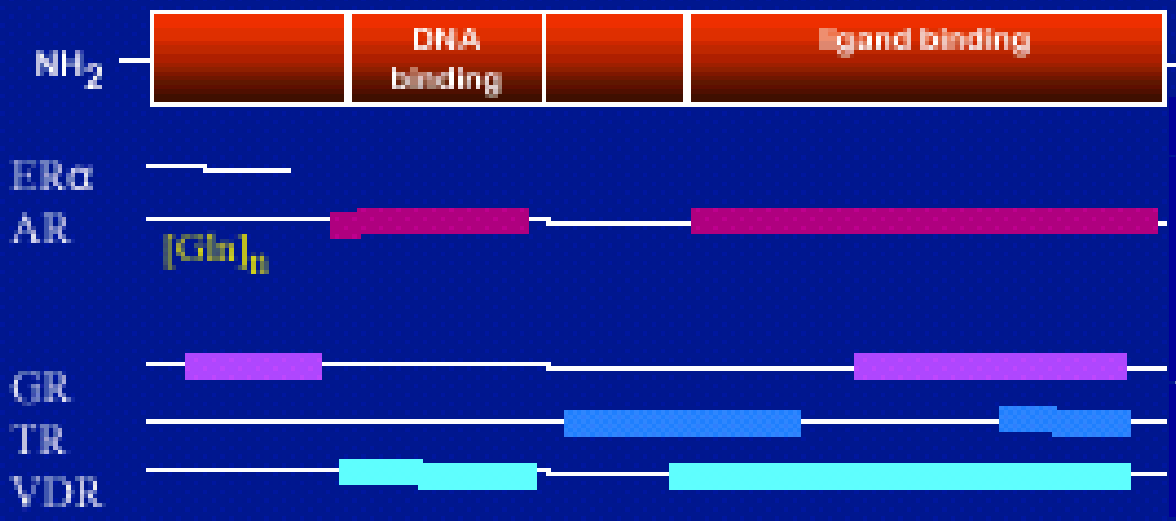
Mecanismo de acción genómico

- Expresión de genes y síntesis de nuevas proteínas.
- Mecanismo de comienzo relativamente lento.
- Programa celular de medio y largo plazo.
- Organización de redes celulares para funciones complejas.

Mecanismo de acción no-genómico

- Activación/represión de proteínas celulares pre-existentes.
- Mecanismo de comienzo rápido.
- Adaptación rápida a los cambio en los niveles hormonales.
- Modificaciones dinámicas del programa celular de largo plazo.

Mutaciones hereditarias en receptores nucleares



Resistencia a E2 (recesiva)

Resistencia a T (crom X)
(Reifenstein, feminización testic, Síndrome Kennedy)

Resistencia a Glucoc.

Resistencia a h. Tiroidea (dominante)

VDDR II (recesiva)

RECEPTOR DE ANDRÓGENOS

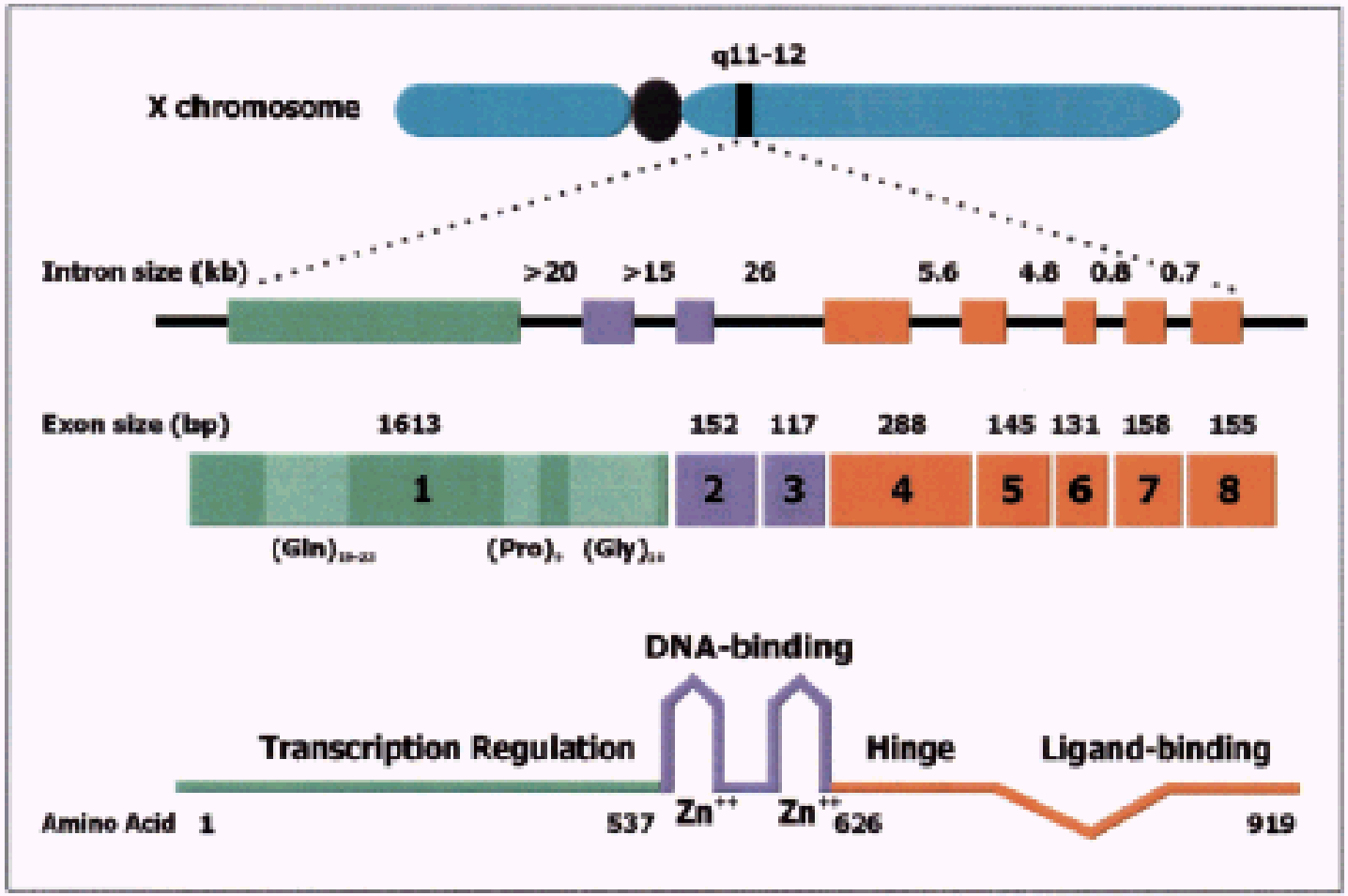
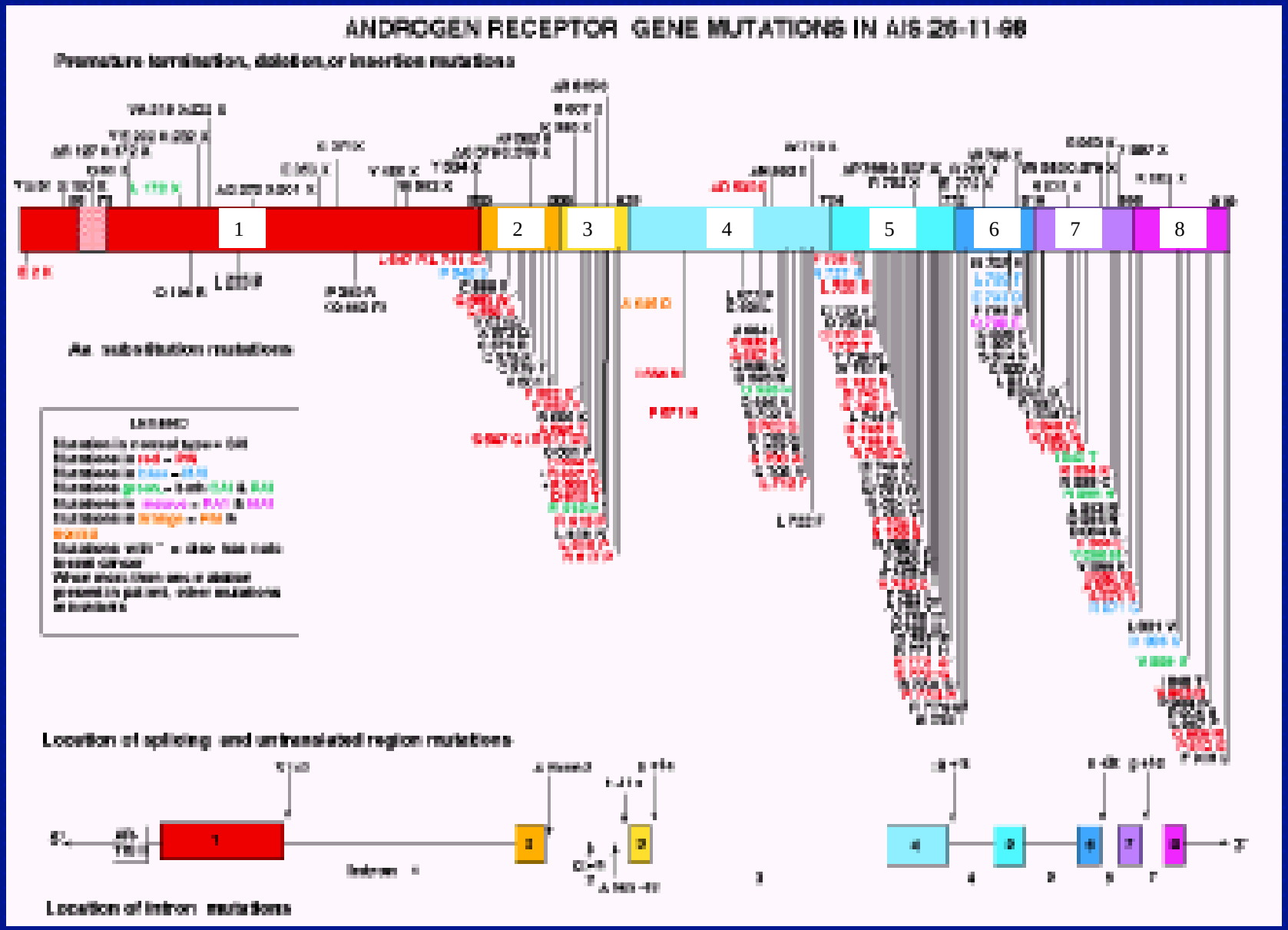
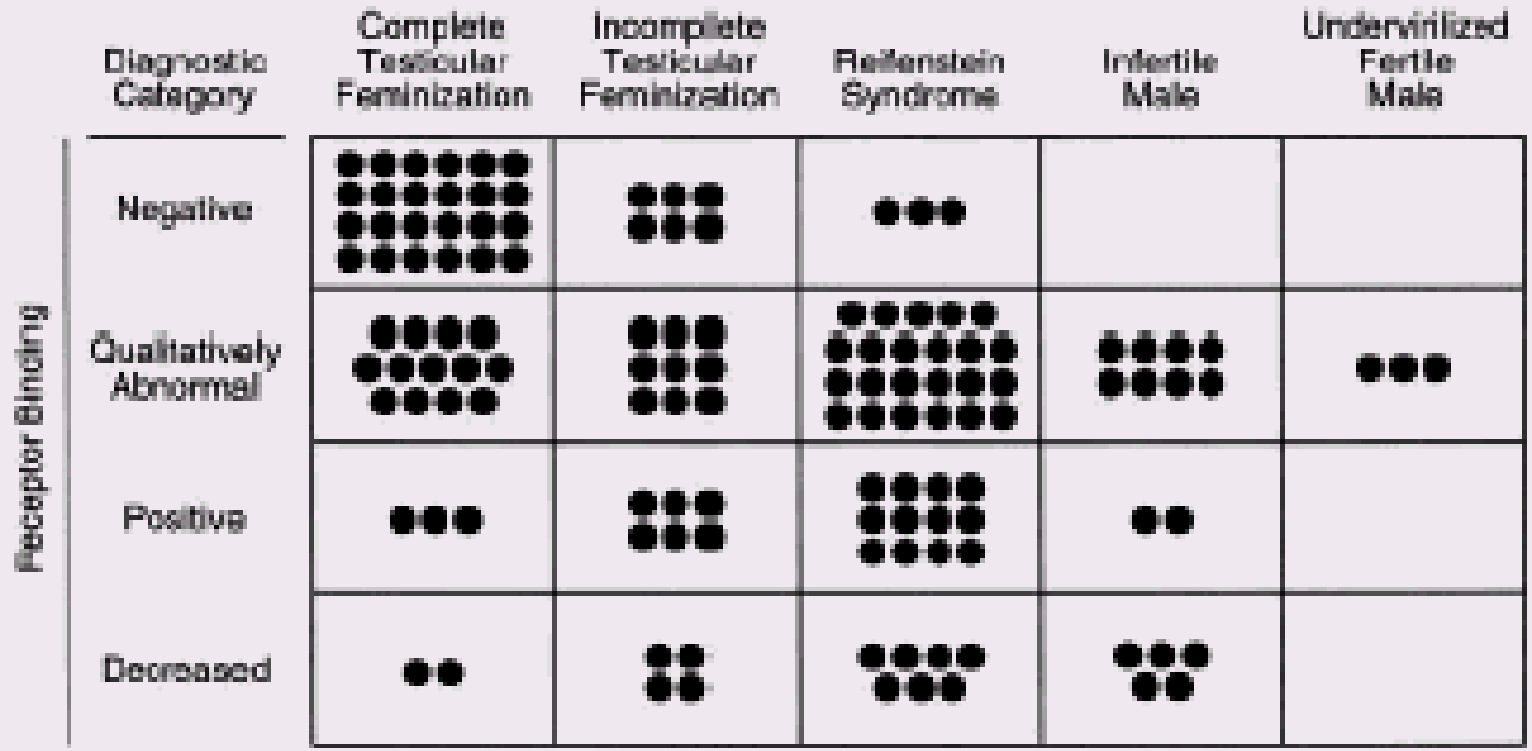


Fig 1. Genomic organization of the *AR* gene is shown. The genome spans more than 90 kb that includes the exonic organization shown in the second panel. Location of three codon repeat regions in the first exon that codes for the N-terminal domain is shown in the third panel. The diagram of the protein structure demonstrates how the exon organization translates into discrete functional regions of the receptor. Adapted from Quigley et al.[7]


ANDROGEN RECEPTOR GENE MUTATIONS IN AIS 26-11-98
Premature termination, deletion or insertion mutations
1
2
3
4
5
6
7
8
Aa substitution mutations
Location of splicing and untranslated region mutations
Location of intron mutations

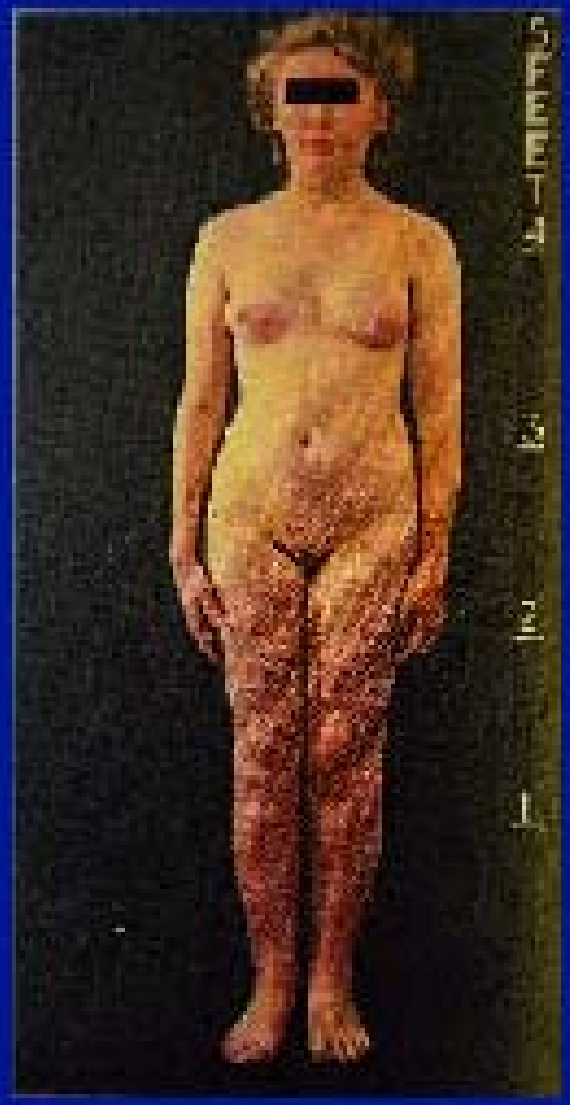
Defectos moleculares del receptor de andrógenos

183



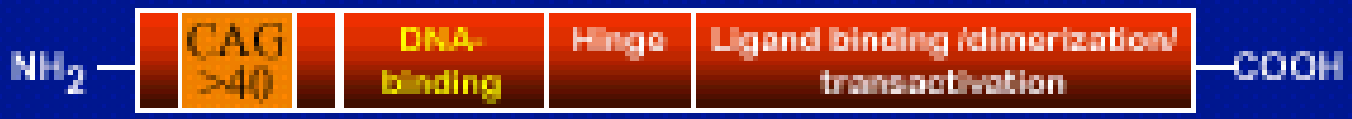
Rec Prog Horm Res 57: 181

Feminización testicular



XY

Atrofia muscular espino-bulbar



Clínica:

-Comienzo retrasado (30-50 A) de atrofia progresiva músculos espinales y bulbares

-Resistencia leve a los andrógenos.

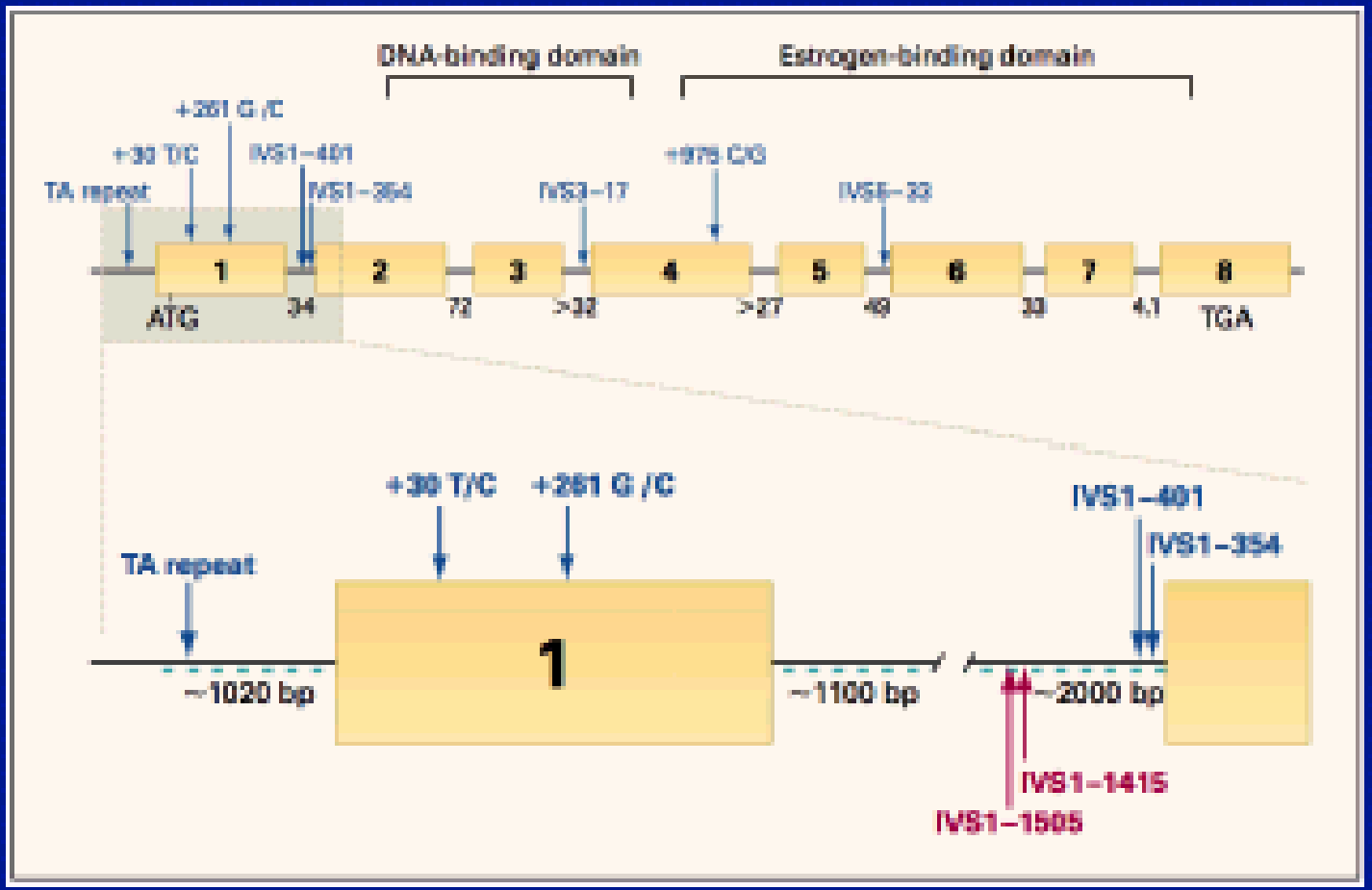
Etiología:

-AR con función alterada.

-Acumulación de una proteína tóxica ? (solo en hombres)

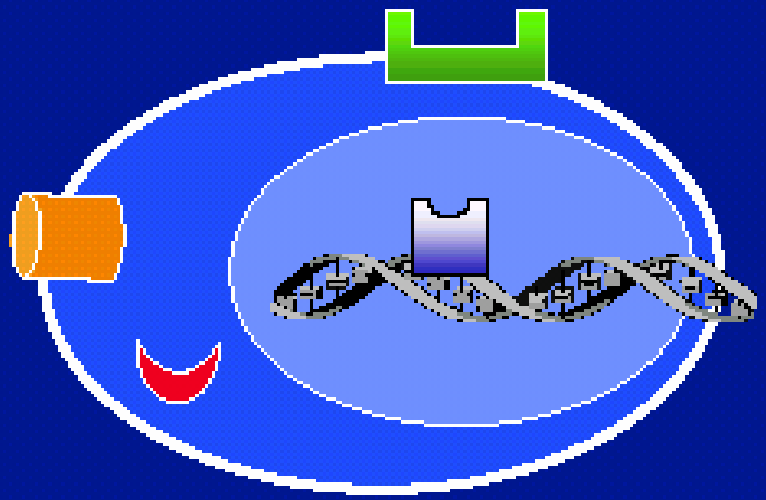
Expansión de reticiones de Gln en la región N-terminal del dominio de transactivación

Polimorfismos del ER en humanos



BLANCOS MOLECULARES PARA FARMACOTERAPIA

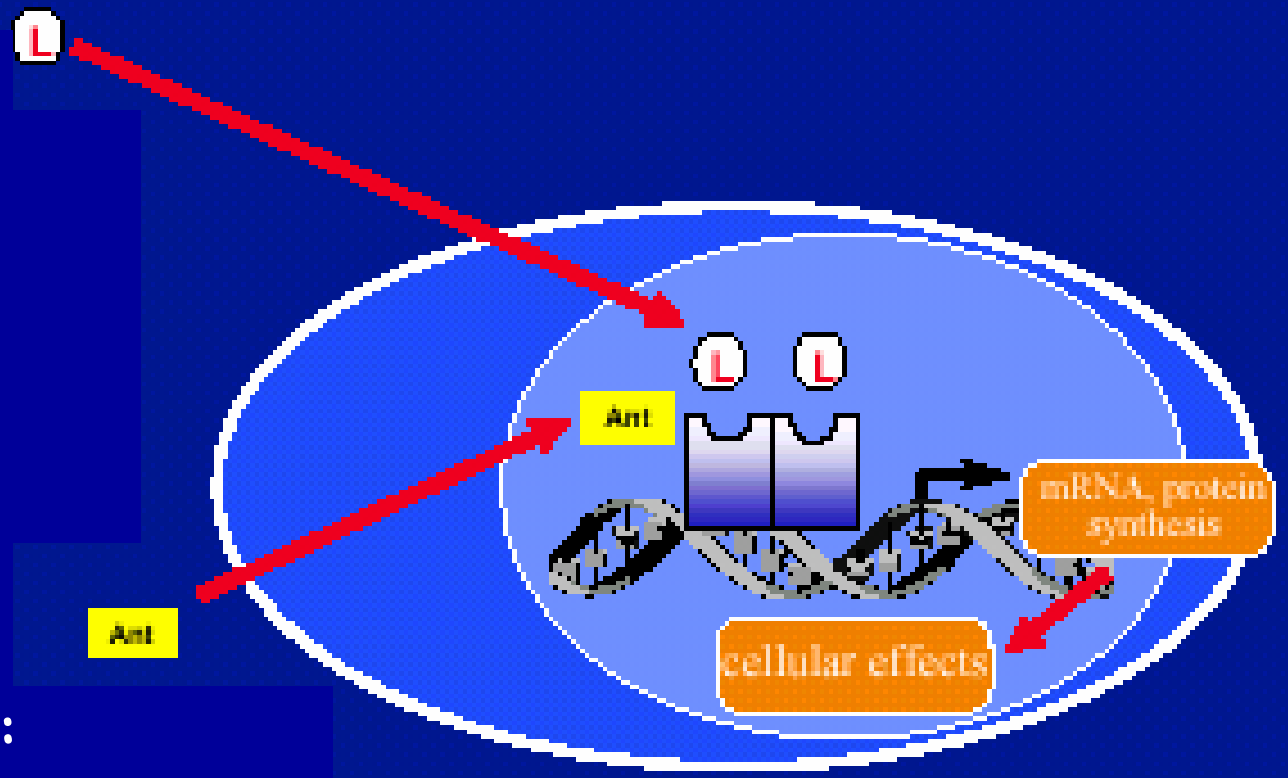
- membrane receptors 50%
- enzymes 20%
- hormones, growth actors 15%
- ion channels 5%
- nuclear receptors **2%**
- other 5%



RECEPTORES NUCLEARES COMO BLANCOS PARA FARMACOTERAPIA

AGONISTAS:
T3, Prednisona,
E2,
Progesterona,
Testosterona,
Vitamina D,
Raloxifeno,
tamoxifeno,
Glitazonas, etc.

ANTAGONISTAS:
Flutamida, ciproterona acetato,
RU486, raloxifeno, tamoxifeno



Las progestinas son modificaciones sintéticas de la progesterona.

Se utilizan para:

- Pastillas anticonceptivas.
- Terapia de reemplazo hormonal (**HRT**) para reducir síntomas de la menopausia.
- Tratamiento de mujeres jóvenes con ciclos menstruales alterados.
- Prevención de nacimientos prematuros.

Ej:

- Norgestrel
- Levonorgestrel
- Norethindrone

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.

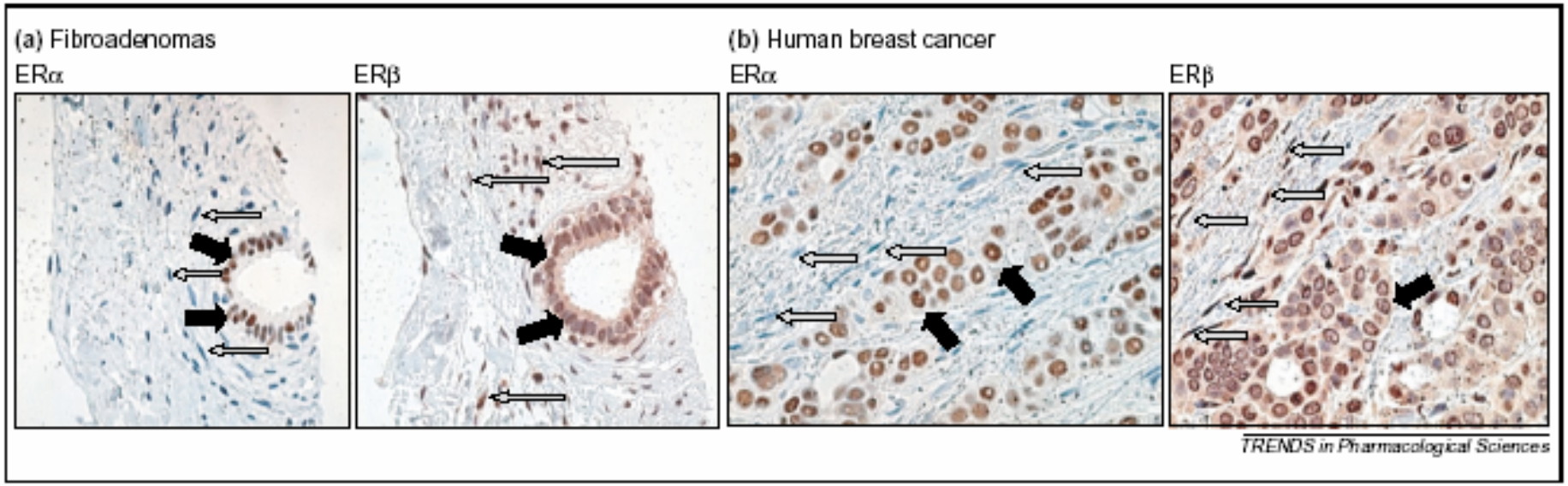
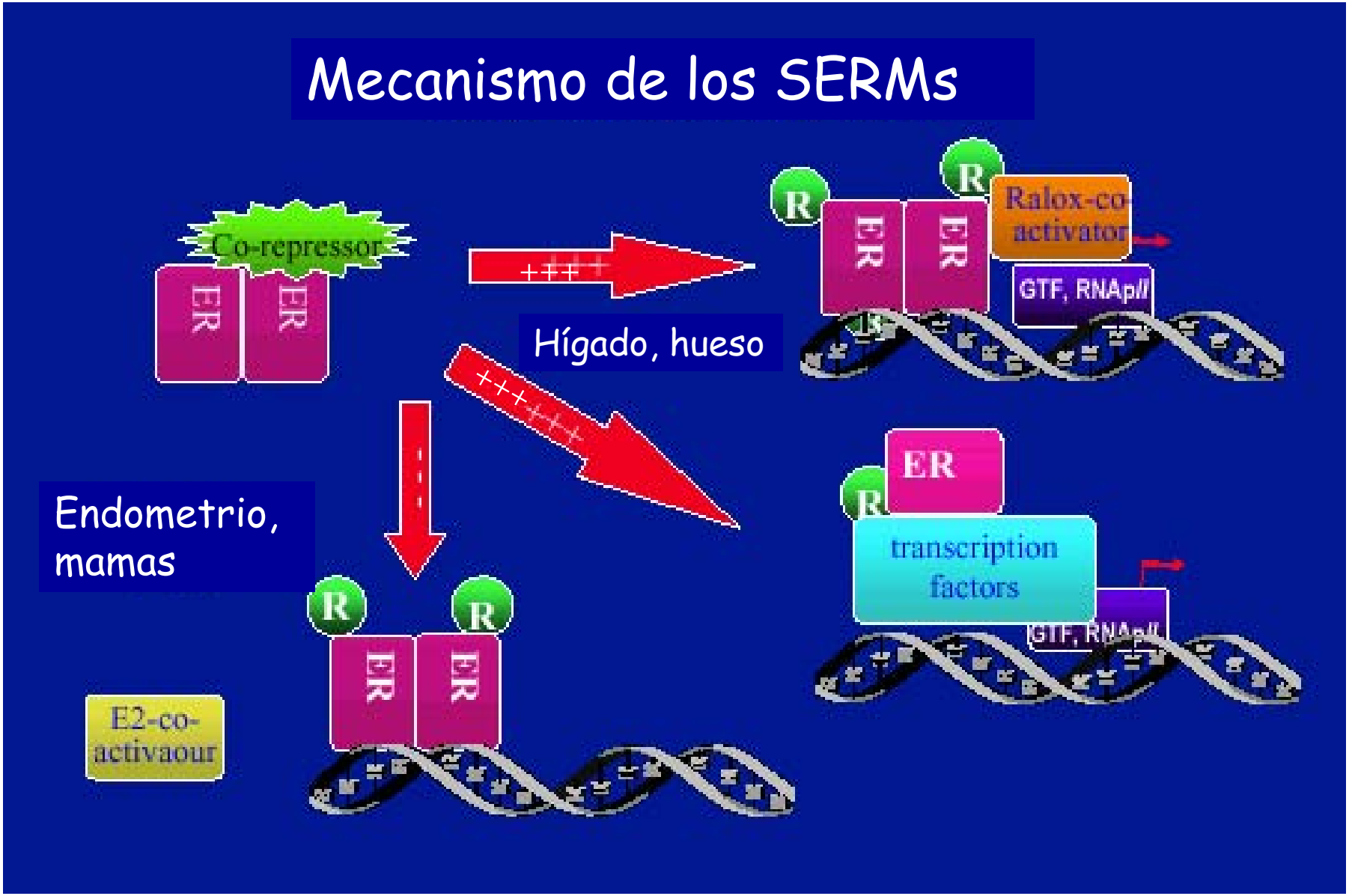


Fig. 1. **(a)** Estrogen receptor (ER) expression in fibroadenomas. ERα expression (brown) is exclusively epithelial (black arrows) with no stromal expression (grey arrows), whereas ERβ is expressed in both epithelial and stromal cells. **(b)** ER expression in human breast cancer. Note the intense staining 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 monoclonal 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].

MODULADORES SELECTIVOS DE LA RESPUESTA A ESTRÓGENOS (SERMs)

	AGONISTA	ANTAGONISTA
E2	all tissues	
tamoxifene	endometrium, bone, lipids	breast
raloxifène	bone, lipids	breast, endometrium
ICI 164,384		all tissues

Mecanismo de los SERMs



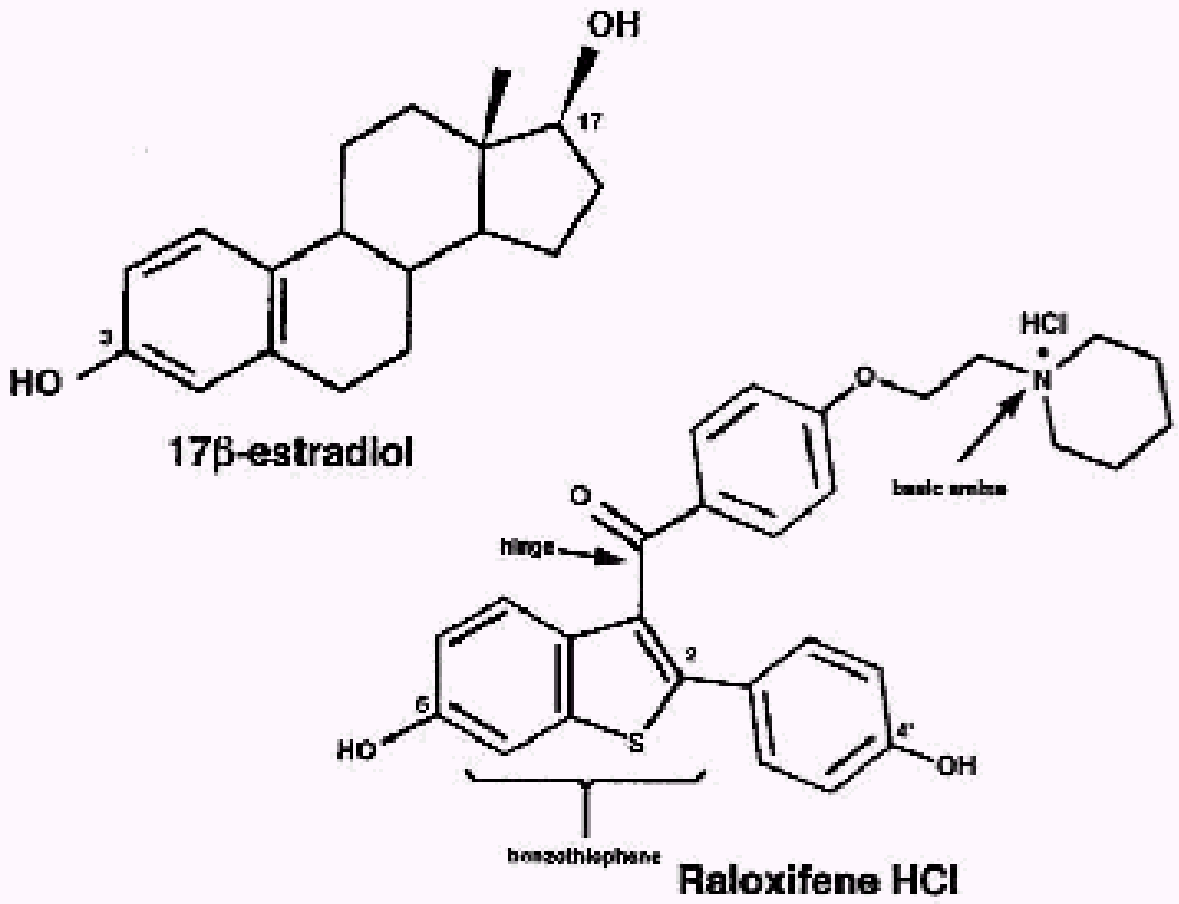
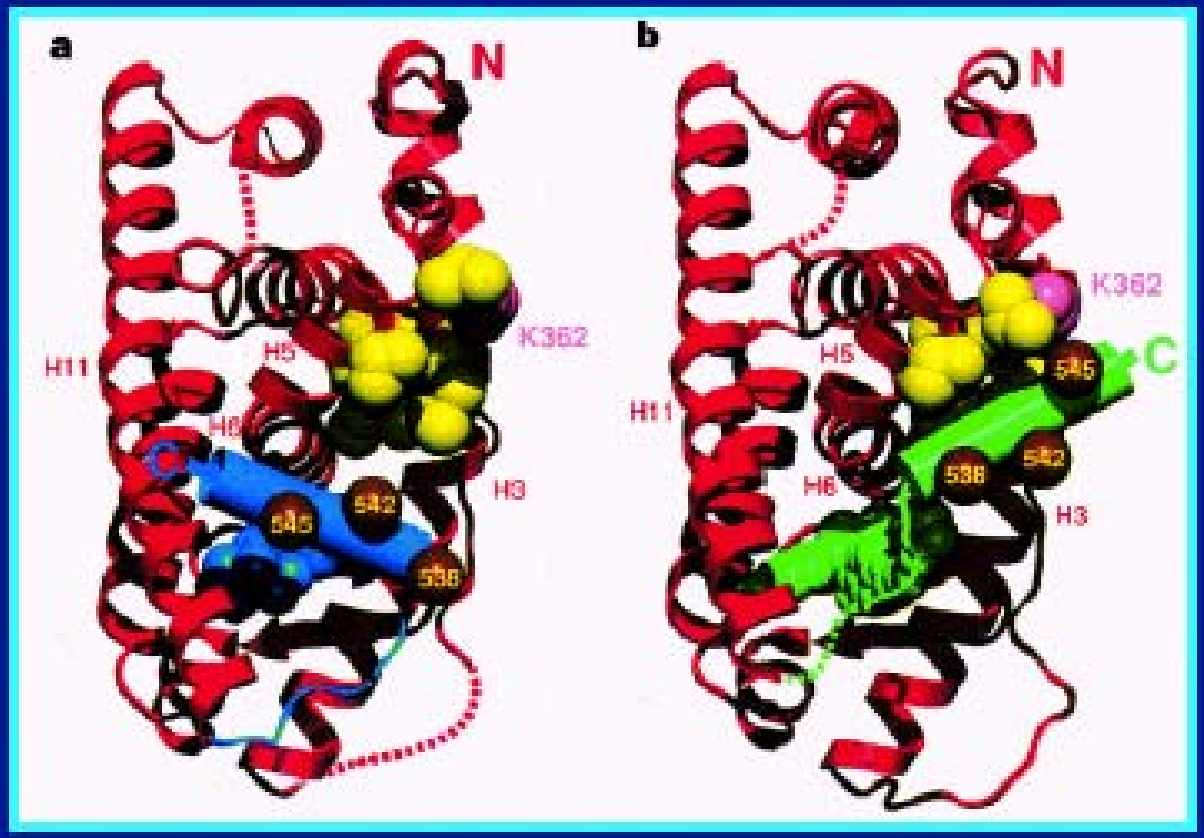


Figure. Chemical structures of raloxifene hydrochloride and 17β-estradiol.

Raloxifene



E2 raloxifene

Nature 389: 753f (1997)

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

Otras clases de SERMs: disruptores endocrinos

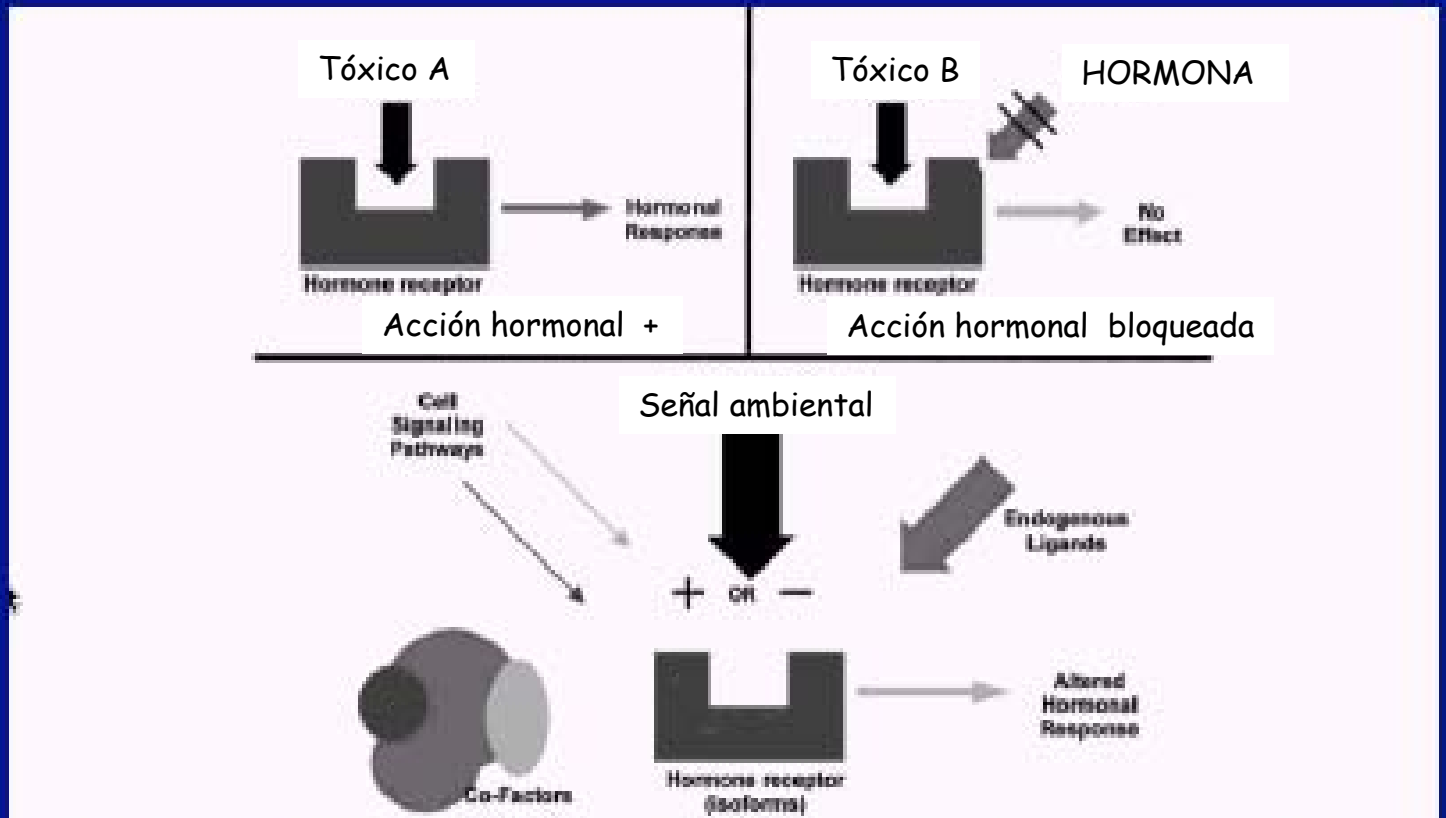


FIG. 8. Functional or receptor-based toxicology. The practice of using steroid hormone receptors to determine the hormonal or antihormonal activity of environmental chemicals is more than a decade old (49). The relatively simple concept presented then (*upper panels*), must now accommodate the advances 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).

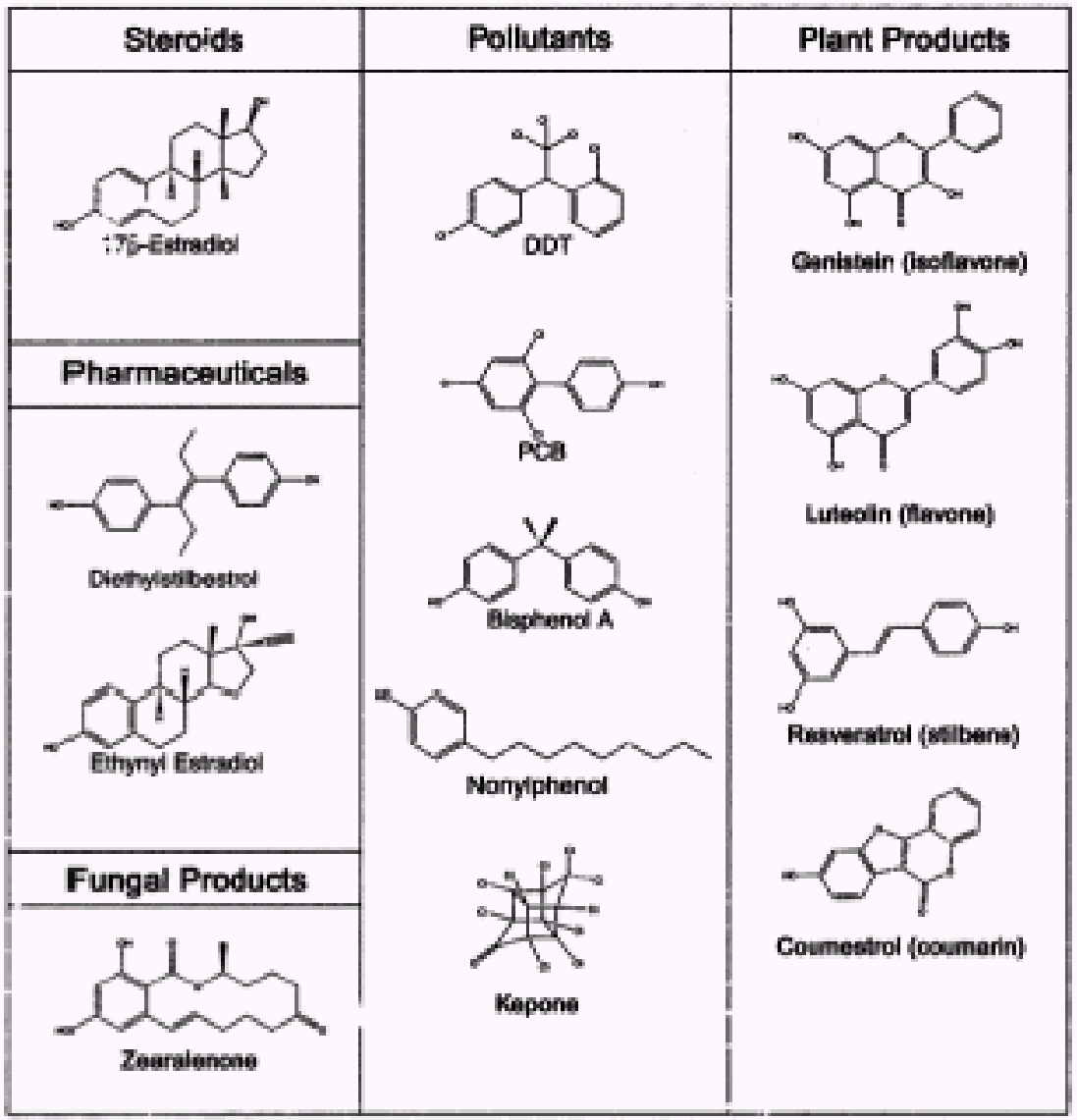


FIG. 6. Chemicals found in the environment reported to be estrogenic. This list is not comprehensive, but illustrates representative structures of estrogenic compounds from various sources. Information on these compounds is contained in the text.

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]	?

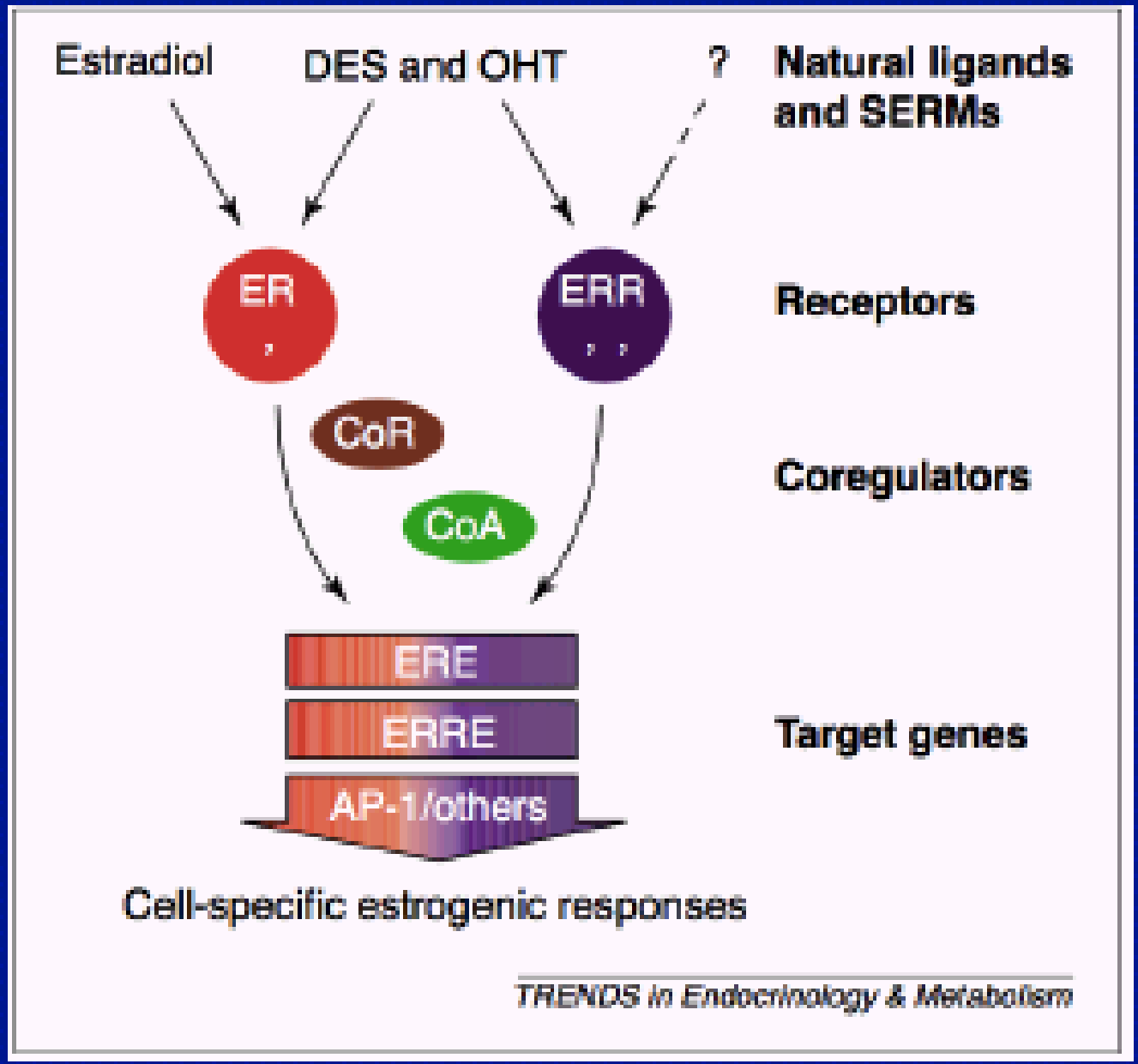
[a] See representative structures in Fig. 5.

[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).


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

Mecanismos posibles para la acción de los SERM

- Diferencia en afinidad U al receptor.
- Acción diferencial de los dominios de activación del receptor.
- Interacción del receptor con ERE diferentes.
- Interacción con diferentes co-activadores y co-represores.
- Efectos no genómicos.
- Patrones de expresión tejido específica de los receptores.

Muchas gracias por su atención !!!