Expresión de la información genética en eucariotas

transcripción

► regulación de la transcripción

ver cap. 15 Alberts: receptores y señales

Alberts – Molecular Biology Of The Cell. 5th.Ed. pdf

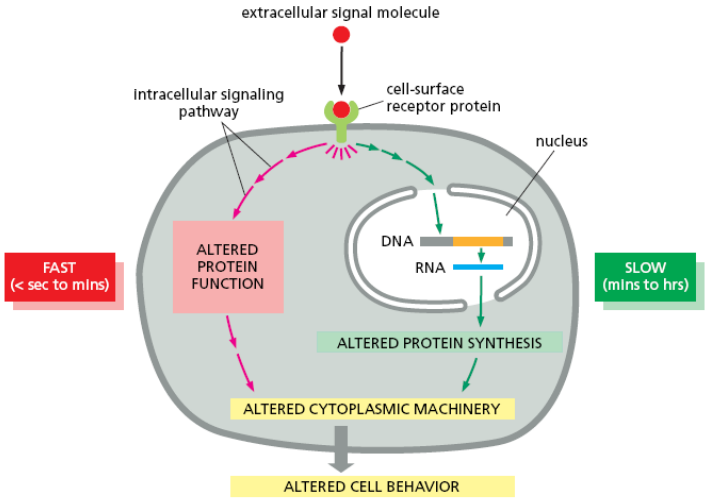
Víctor Romanowski, 2013

Regulación hormonal:

Hormonas y factores de crecimiento hidrosolubles
Receptores de membrana
(transducción de señales, forforilación, activación, traslocación al núcleo…)

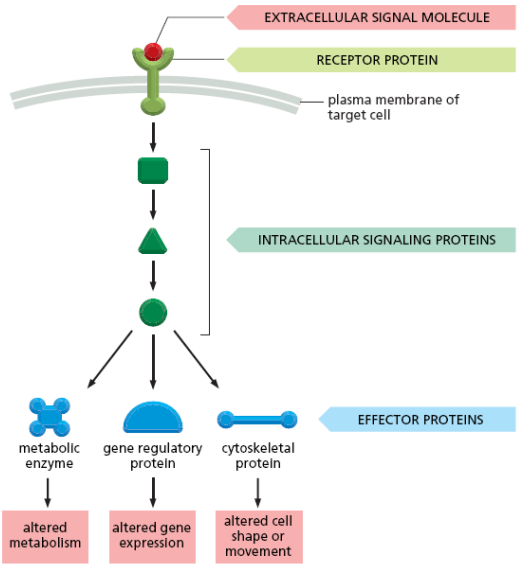
Hormonas liposolubles
Receptores intracelulares
(factores de transcripción localizados en citosol o en el núcleo, traslocación al núcleo y/o activación…)

Respuesta a estímulos ambientales



Señal ►►►transcripción ► expresión regulada ► respuesta… fenotipo…

Respuesta a estímulos ambientales



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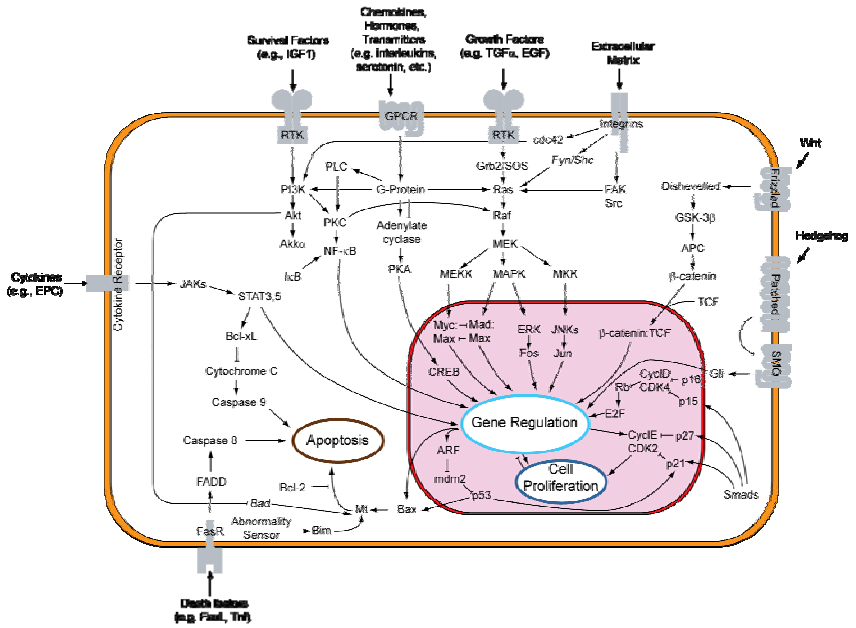
Respuesta a estímulos ambientales



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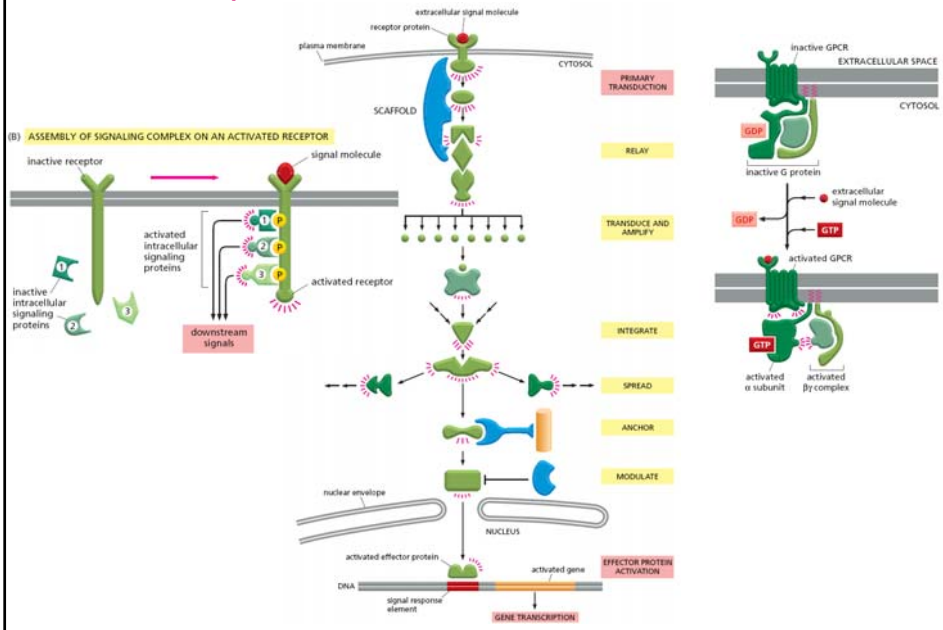

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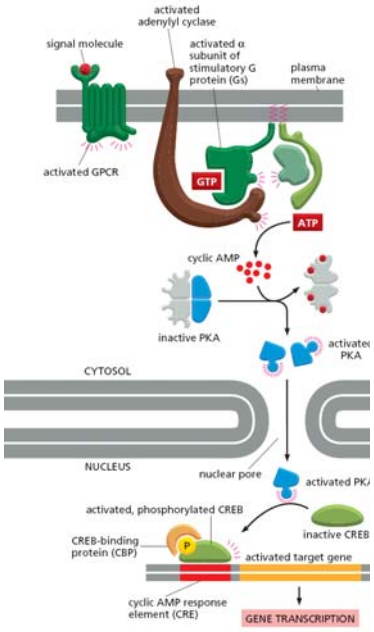
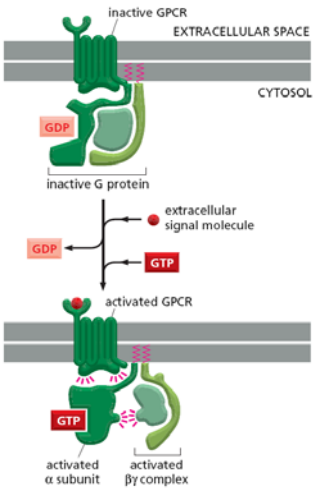
Respuesta a estímulos ambientales





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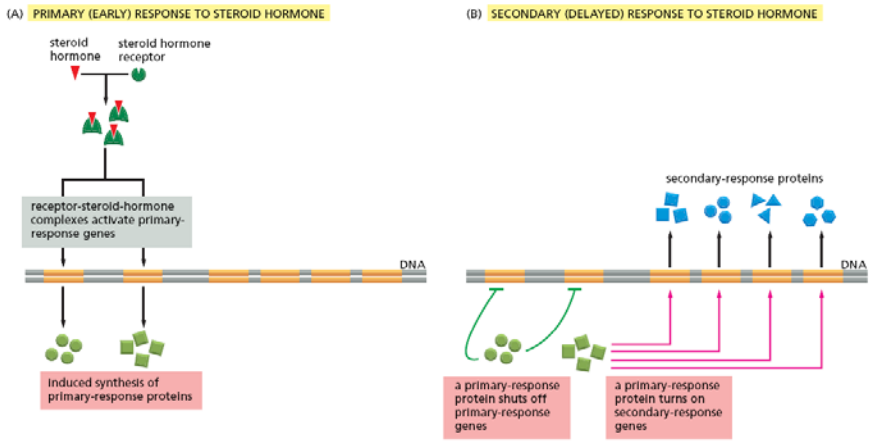

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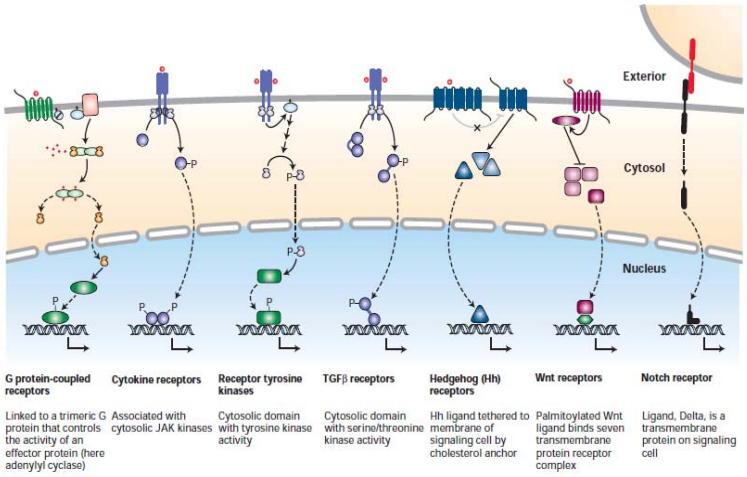
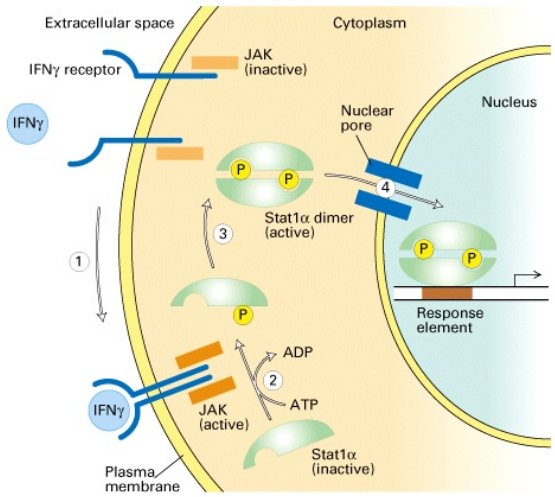
Respuesta a estímulos ambientales



Polypeptide hormones signal phosphorylation of some transcription factors



Model of IFNγ-mediated gene activation by phosphorylation and dimerization of Stat1α

Regulación hormonal

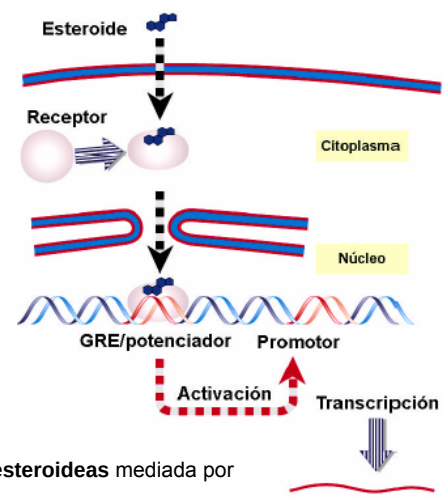
Es frecuente que **un mismo factor de transcripción controle a un gran número de genes**. Esto se debe a la existencia de elementos de respuesta para ese factor en los promotores o potenciadores de esos genes.

Ejemplos:

•HSE: elemento de respuesta a choque térmico

•**GRE**: elemento de respuesta a glucocorticoides

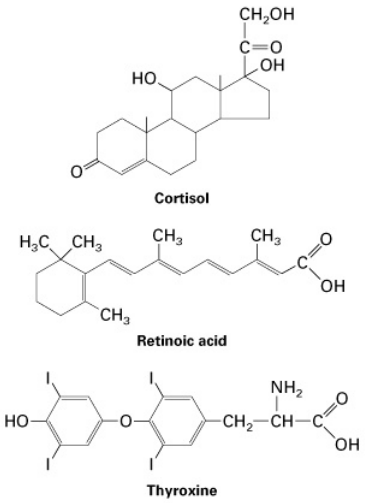
•SRE: elemento de respuesta al suero



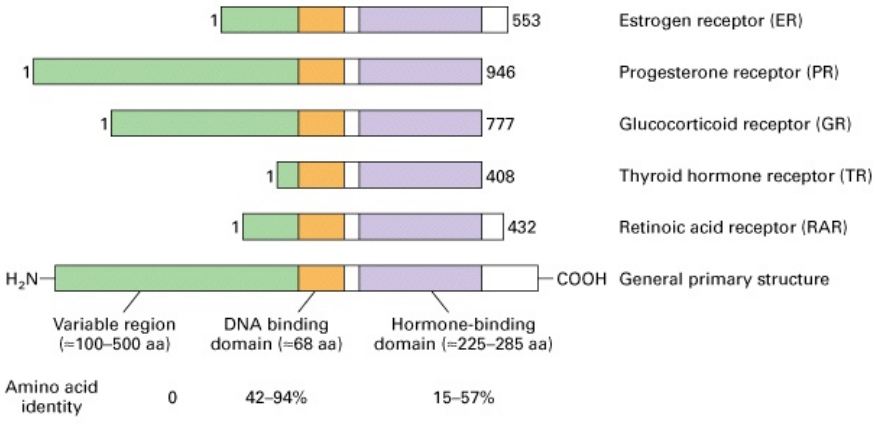
Respuesta a **hormonas esteroideas** mediada por elementos GRE.

Estas hormonas se sintetizan en respuesta a un gran variedad de actividades neuroendocrinas y controlan crecimiento, desarrollo de tejidos y homeostasis corporal. Todas tienen un modo de acción similar: **se unen a un receptor citoplásmico haciendo que este se una al DNA y active la transcripción**

Lipid-soluble hormones control the activities of nuclear receptors



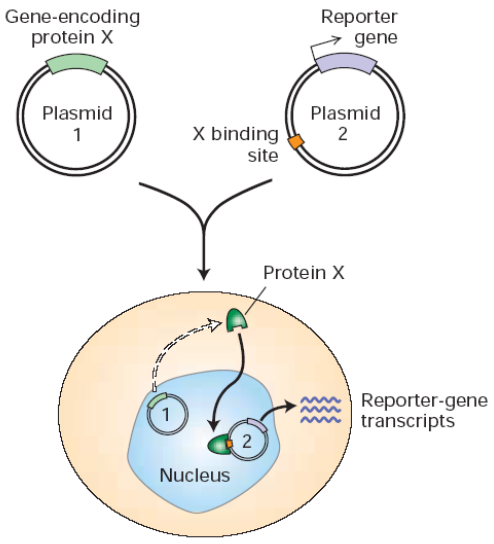
Domain structure of nuclear receptors



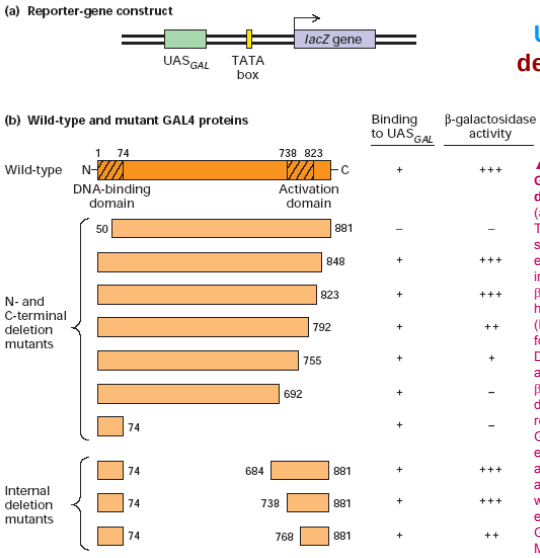
▲ EXPERIMENTAL FIGURE 11-16

In vivo transfection assay measures transcription activity to evaluate proteins believed to be transcription factors.

The assay system requires two plasmids. One plasmid contains the gene encoding the putative transcription factor (protein X). The second plasmid contains a reporter gene (e.g., *lacZ*) and one or more binding sites for protein X. Both plasmids are simultaneously introduced into cells that lack the gene encoding protein X. The production of reporter-gene RNA transcripts is measured; alternatively, the activity of the encoded protein can be assayed. If reporter-gene transcription is greater in the presence of the X-encoding plasmid, then the protein is an activator; if transcription is less, then it is a repressor. By use of plasmids encoding a mutated or rearranged transcription factor, important domains of the protein can be identified.

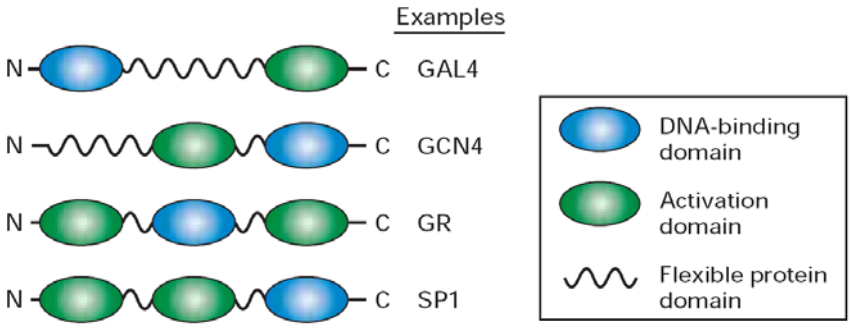


Deletion mutants of the GAL4 gene in yeast with a UAS*GAL* reporter-gene construct demonstrate the separate functional domains in an activator.



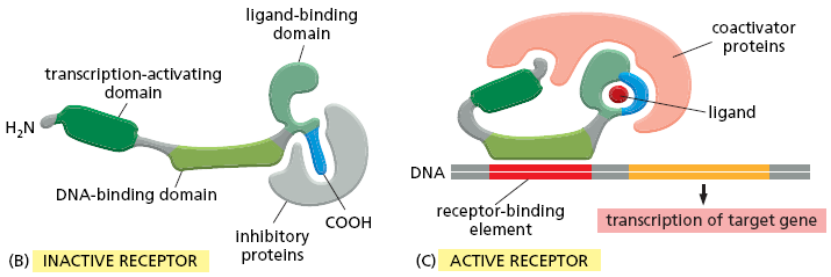
▲ EXPERIMENTAL FIGURE 11-17 Deletion mutants of the GAL4 gene in yeast with a UAS*GAL* reporter-gene construct demonstrate the separate functional domains in an activator. (a) Diagram of DNA construct containing a *lacZ* reporter gene and TATA box ligated to UAS*GAL*, a regulatory element that contains several GAL4-binding sites. The reporter-gene construct and DNA encoding wild-type or mutant (deleted) GAL4 were simultaneously introduced into mutant (*gal4*) yeast cells, and the activity of β-galactosidase expressed from *lacZ* was assayed. Activity will be high if the introduced *GAL4* DNA encodes a functional protein. (b) Schematic diagrams of wild-type GAL4 and various mutant forms. Small numbers refer to positions in the wild-type sequence. Deletion of 50 amino acids from the N-terminal end destroyed the ability of GAL4 to bind to UAS*GAL* and to stimulate expression of β-galactosidase from the reporter gene. Proteins with extensive deletions from the C-terminal end still bound to UAS*GAL*. These results localize the DNA-binding domain to the N-terminal end of GAL4. The ability to activate β-galactosidase expression was not entirely eliminated unless somewhere between 126–189 or more amino acids were deleted from the C-terminal end. Thus the activation domain lies in the C-terminal region of GAL4. Proteins with internal deletions (*bottom*) also were able to stimulate expression of -galactosidase, indicating that the central region of GAL4 is not crucial for its function in this assay. [See J. Ma and M. Ptashne, 1987, *Cell* **48**:847; I. A. Hope and K. Struhl, 1986, *Cell* **46**:885; and R. Brent and M. Ptashne, 1985, *Cell* **43**:729.]

modular structure of eukaryotic transcription activators



Schematic diagrams illustrating the modular structure of eukaryotic transcription activators. These transcription factors may contain more than one **activation domain** (**AD**) but rarely contain more than one **DNA-binding domain** (**DBD**).
GAL4 and GCN4 are yeast transcription activators. The glucocorticoid receptor (GR) promotes transcription of target genes when certain hormones are bound to the C-terminal activation domain. SP1 binds to GC-rich promoter elements in a large number of mammalian genes.

nuclear receptors-transcription factors



Response elements are DNA sites that bind several major nuclear receptors

(a) GRE
5′ AGAACA(N)$_3$ TGTTCT 3′
3′ TCTTGT(N)$_3$ ACAAGA 5′

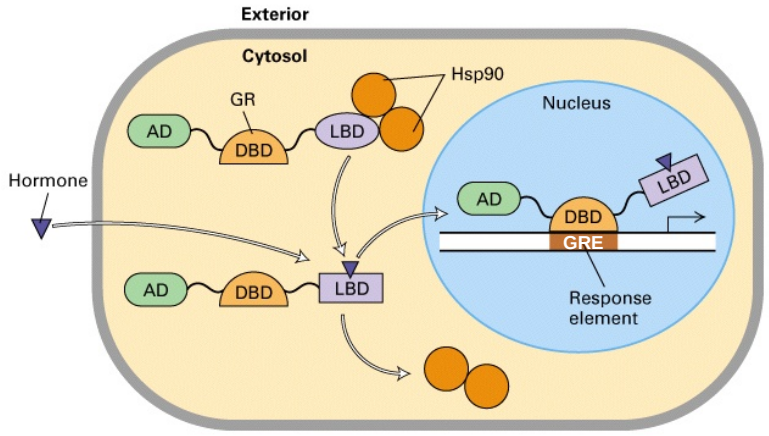
(b) ERE
5′ AGGTCA(N)$_3$ TGACCT 3′
3′ TCCAGT(N)$_3$ ACTGGA 5′

(c) VDRE
5′ AGGTCA(N)$_3$ AGGTCA 3′
3′ TCCAGT(N)$_3$ TCCAGT 5′

(d) TRE
5′ AGGTCA(N)$_4$ AGGTCA 3′
3′ TCCAGT(N)$_4$ TCCAGT 5′

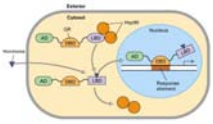
(e) RARE
5′ AGGTCA(N)$_5$ AGGTCA 3′
3′ TCCAGT(N)$_5$ TCCAGT 5′

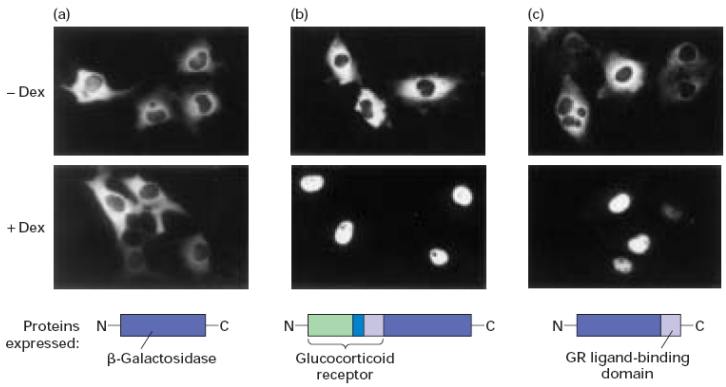
Model of hormone-dependent gene activation by the glucocorticoid receptor (GR)



GRE 5′ AGAACA(N)$_3$ TGTTCT 3′
3′ TCTTGT(N)$_3$ ACAAGA 5′

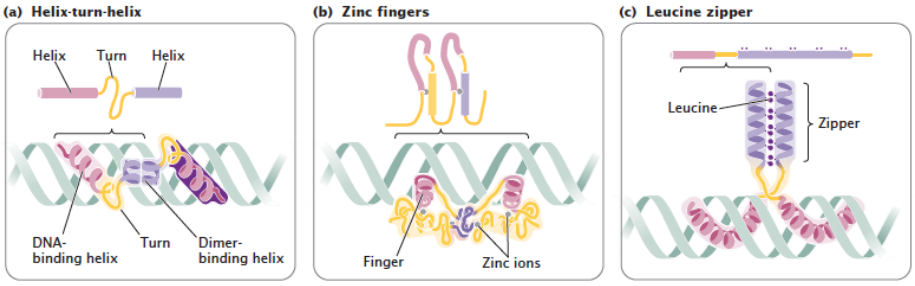
Experimento que demuestra la traslocación del GR al núcleo





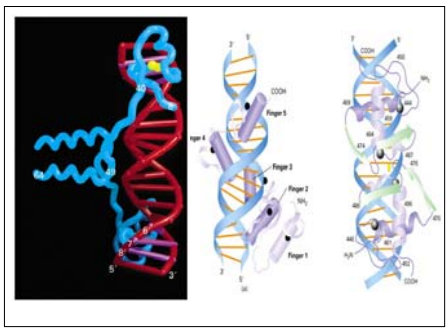
▲ EXPERIMENTAL FIGURE 11-43 Fusion proteins from expression vectors demonstrate that the hormone-binding domain of the glucocorticoid receptor (GR) mediates translocation to the nucleus in the presence of hormone. Cultured animal cells were transfected with expression vectors encoding the proteins diagrammed at the bottom. Immunofluorescence with a labeled antibody specific for β-galactosidase was used to detect the expressed proteins in transfected cells. (a) In cells that expressed β-galactosidase alone, the enzyme was localized to the cytoplasm in the presence and absence of the glucocorticoid hormone dexamethasone (Dex). (b) In cells that expressed a fusion protein consisting of β-galactosidase and the entire glucocorticoid receptor (GR), the fusion protein was present in the cytoplasm in the absence of hormone but was transported to the nucleus in the presence of hormone. (c) Cells that expressed a fusion protein composed of β-galactosidase and just the GR ligand-binding domain (light purple) also exhibited hormone-dependent transport of the fusion protein to the nucleus. [From D. Picard and K. R. Yamamoto, 1987, *EMBO J.* **6**:3333; courtesy of the authors.]

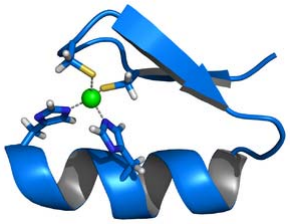
Los factores de transcripción se suelen clasificar según el tipo de dominio de fijación al DNA que contienen

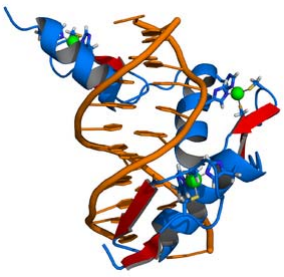


Los factores de transcripción se suelen clasificar según el tipo de dominio de fijación al DNA que contienen

Proteínas con "dedos de Zn"



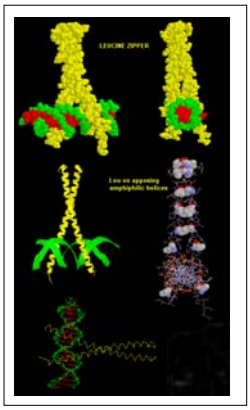




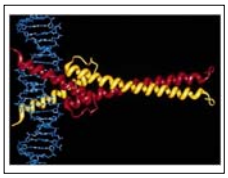
TFIIB, GAL4

Los factores de transcripción se suelen clasificar según el tipo de dominio de fijación al DNA que contienen

Proteína con **cremallera de leucina**



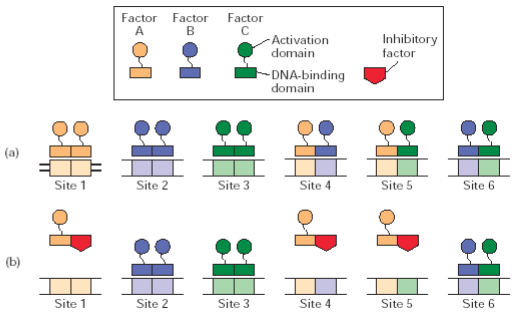
Proteína con **hélice-bucle-hélice**

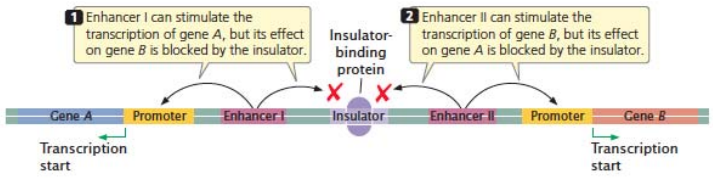


Proteínas de **homeodominio**

c-fos and c-jun (the AP1 transcription factor), important regulators of normal development, as well as myc family members including myc, max, and mdx1. If they are overproduced or mutated in a vital area, they may generate cancer. These proteins interact with the DNA as dimers (homo- or hetero-) and are also called basic zipper proteins (bZips).

▶ FIGURE 11-23 Combinatorial possibilities due to formation of heterodimeric transcription factors. (a) In the hypothetical example shown, transcription factors A, B, and C can all interact with one another, permitting the three factors to bind to six different DNA sequences (sites 1–6) and creating six combinations of activation domains. Each composite binding site is divided into two half-sites, and each heterodimeric factor contains the activation domains of its two constituent monomers. (b) Expression of an inhibitory factor (green) that interacts only with factor A inhibits binding; hence, transcriptional activation at sites 1, 4, and 5 is inhibited, but activation at sites 2, 3, and 6 is unaffected.





17.8 An insulator blocks the action of an enhancer on a promoter when the insulator lies between the enhancer and the promoter.

La mayoría de los activadores eucarióticos forman dímeros

- Homo y heterodímeros

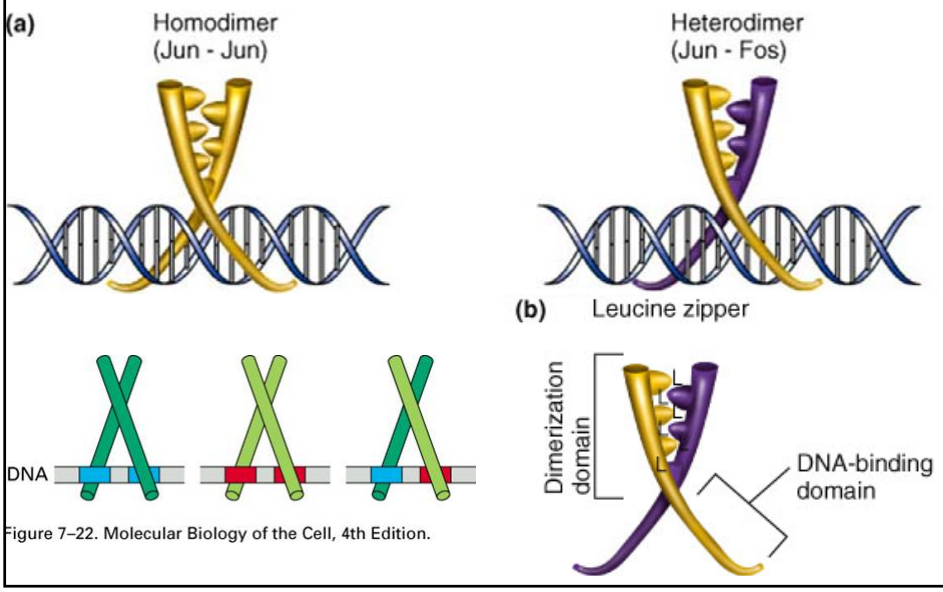
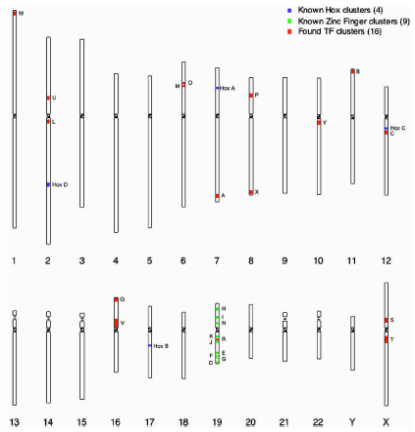


Figure 7–22. Molecular Biology of the Cell, 4th Edition.

TFs en el genoma humano

Gene family	*Homo sapiens* Seed	Found	All
Zinc binding[a,b]	422	340	762
Homeobox	186	13	199
BHLH	92	25	117
β-Scaffold[a]	77	10	87
BZip	59	13	72
NHR	49	0	49
Trp cluster	38	8	46
Forkhead	36	4	40
Bromodomain[a]	14	3	17
T-box	16	1	17
Jumonji	6	7	13
E2F	9	1	10
Dwarfin	9	0	9
Paired box	9	0	9
Heat shock	6	2	8
Tubby[a]	5	2	7
AF-4[b]	7	0	7
RFX	6	0	6
Methyl-CpG-binding[a]	4	1	5
AP-2	4	0	4
TEA[a]	4	0	4
Pocket domain (Rb)[a]	3	0	3
GCM[c,d]	2	0	2
Other	214	14	228
Coactivators and corepressors	111	11	122
Structural	80	39	119
Total	1468	494	1962

Organism	Approximate number of genes (TFs/total)	Percent
S. cerevisiae	224/6569[a]	3.4%
C. elegans	824/19546[b]	4.2%
D. melanogaster	744/13525[c]	5.5%
H. sapiens (NCBI)	1962/24652[d]	8.0%
H. sapiens (Ensembl)	1962/21787[e]	9.0%



Factores de transcripción: dominios de interacción con DNA

- **Helix-turn-helix**
- **Zinc-fingers**
- **basic domain**
- **Helix-loop-helix**

Helix-Turn-Helix

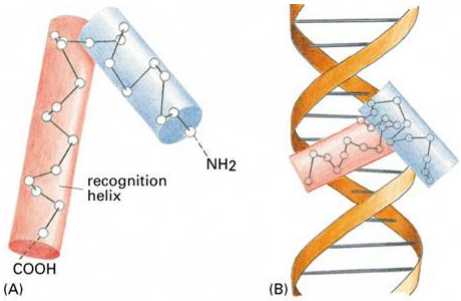
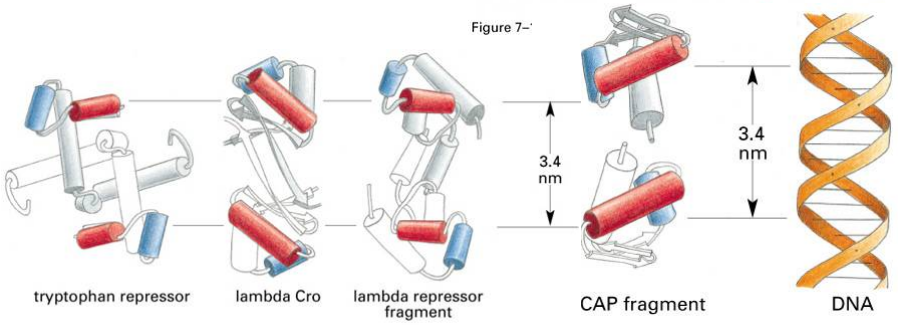
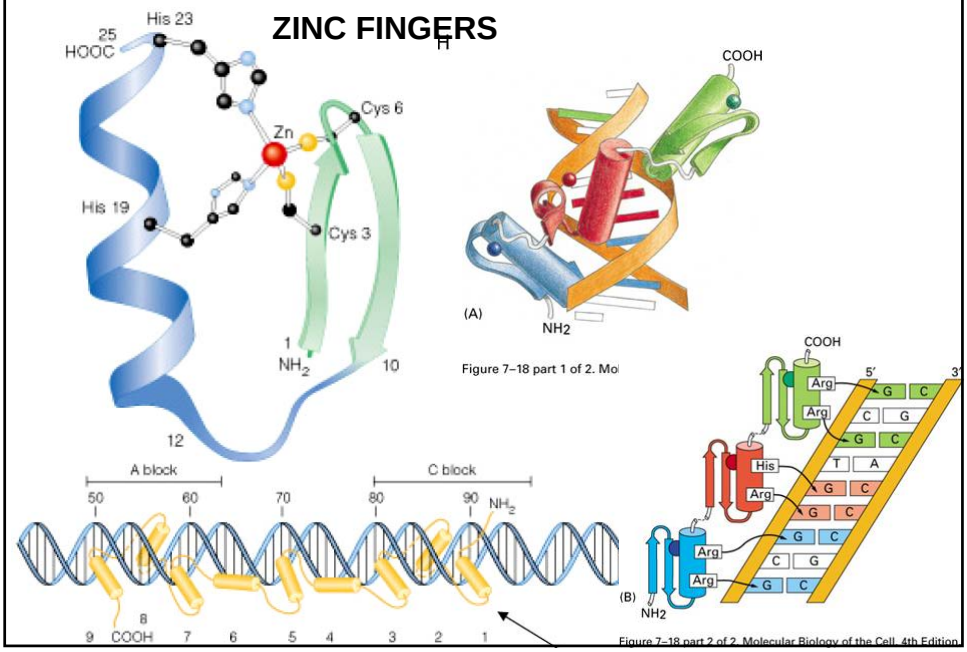


Figure 7–



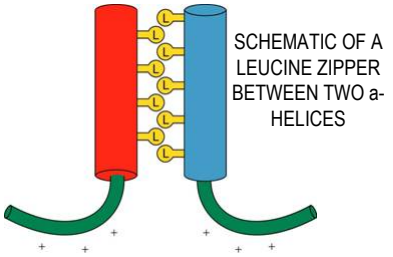
Factores de transcripción: dominios de interacción con DNA

ZINC FINGERS



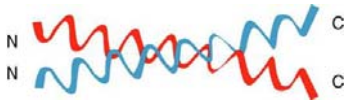
Factores de transcripción: dominios de interacción con DNA

DIMERIZATION MOTIFS: THE LEUCINE ZIPPER



SCHEMATIC OF A LEUCINE ZIPPER BETWEEN TWO a-HELICES

MODEL OF TWO α-HELICES JOINED BY A LEUCINE ZIPPER IN A PARALLEL COILED COIL STRUCTURE



WEAVER 2e: FIGS. 12.12, 12.13

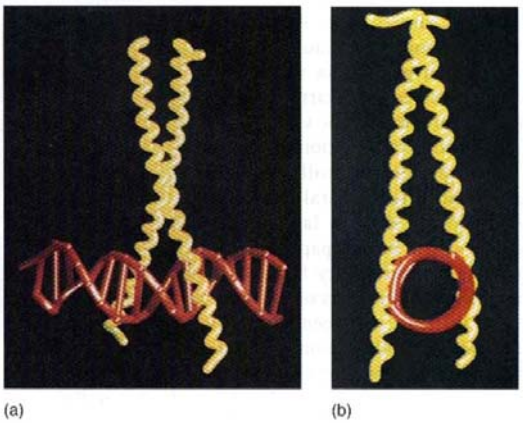
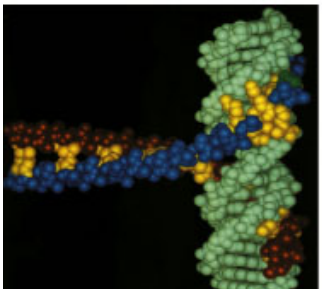


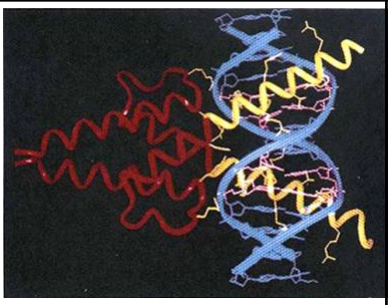
Figure 12.16 Crystal structure of the bZIP domain of GCN4 bound to its DNA target. The DNA (red) contains a DNA target for the bZIP domain (yellow). Notice the coiled coil nature of the interaction between the protein monomers, and the tong-like appearance of the protein grasping the DNA.

WEAVER: FIG. 12.11

Factores de transcripción: dominios de interacción con DNA

helix-loop-helix proteins bind to the DNA binding domains of enhancer elements





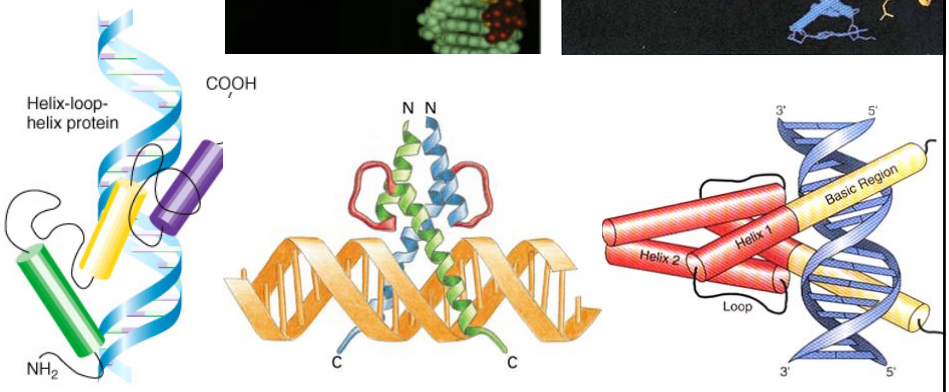
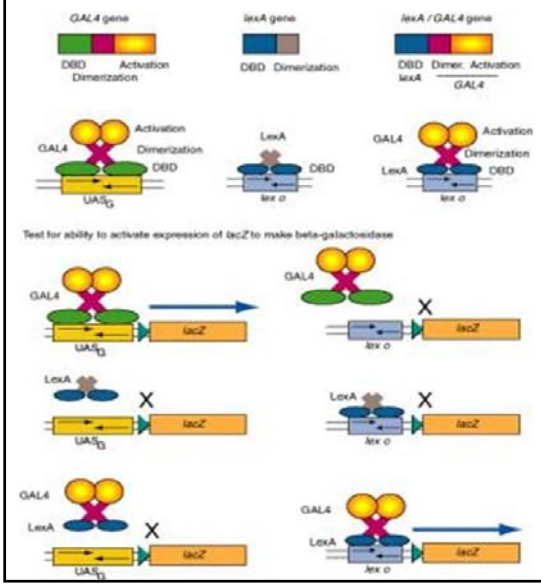


Figure 7–25. Molecular Biology of the Cell, 4th Edition.

Estructura modular de los factores de transcripción

-Poseen una estructura modular: dominio de unión a DNA, dominio transactivador



Domain swap experiments show DNA binding domains and activation domains are interchangable.

Replacement of the DNA binding domain with a different one will change the site at which the activator will act, but not affect its ability to activate a target promoter. In other words, the DNA binding domain can be altered without affecting the activation domain, and vice versa.

Actividad de un TF quimérico lexA-GAL4

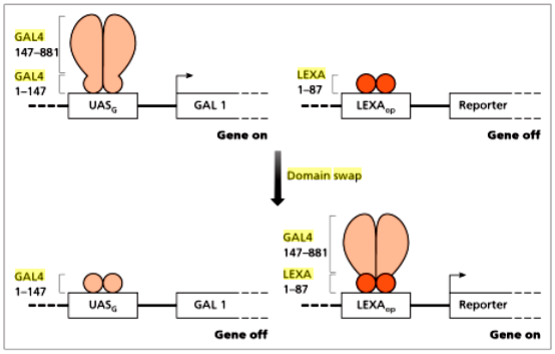


Fig. 5.4 A domain swap experiment. The isolated DNA-binding domains of Gal4 and LexA have little effect upon gene expression. However, either can stimulate transcription when linked to Gal4 residues 147–881, which include two activation regions. LexA$_{op}$ is the operator DNA sequence recognized by LexA.