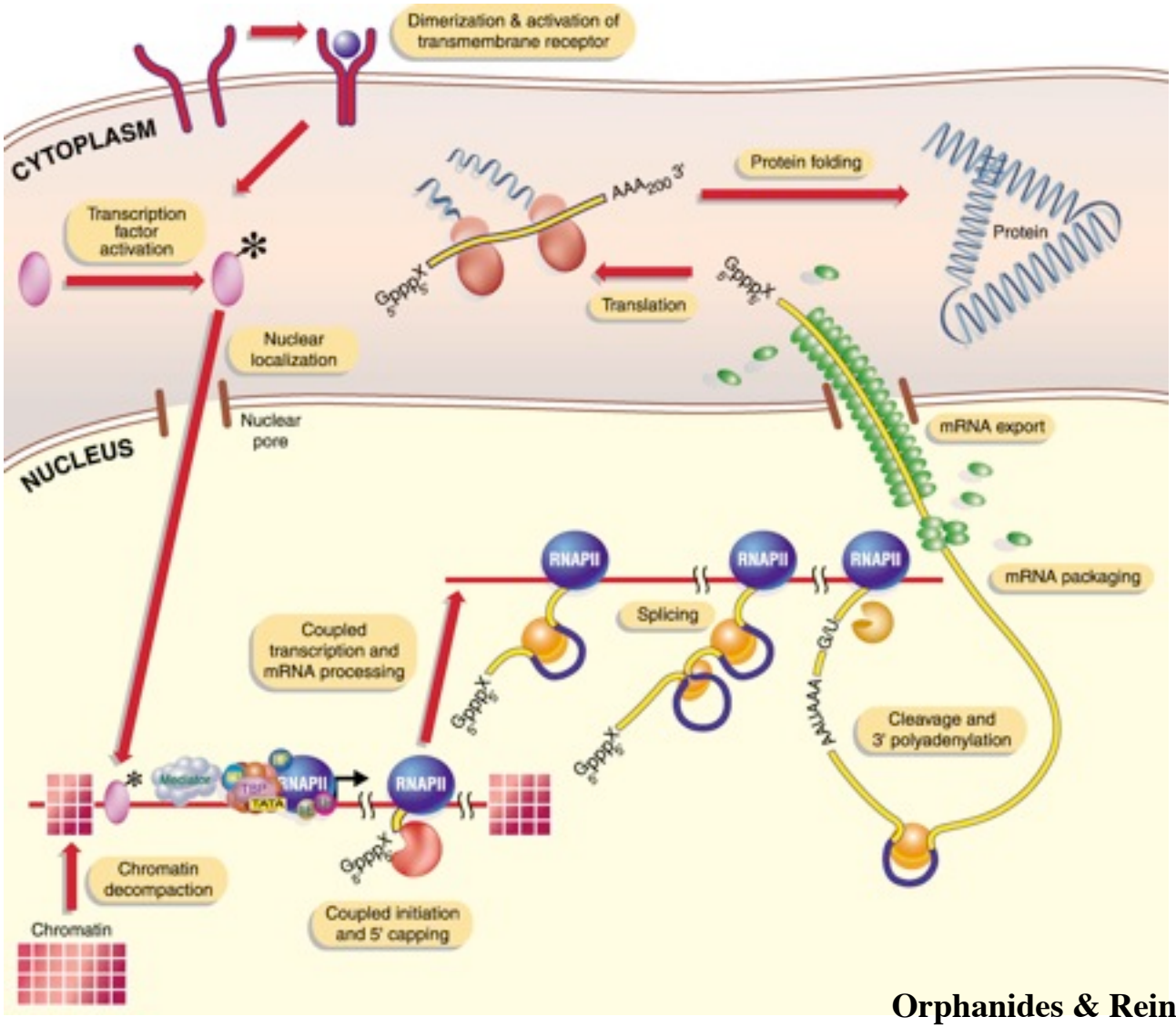
Visión contemporánea (unificada) de la expresión génica



Orphanides & Reinberg, 2002

Contexto cromosómico de los genes.

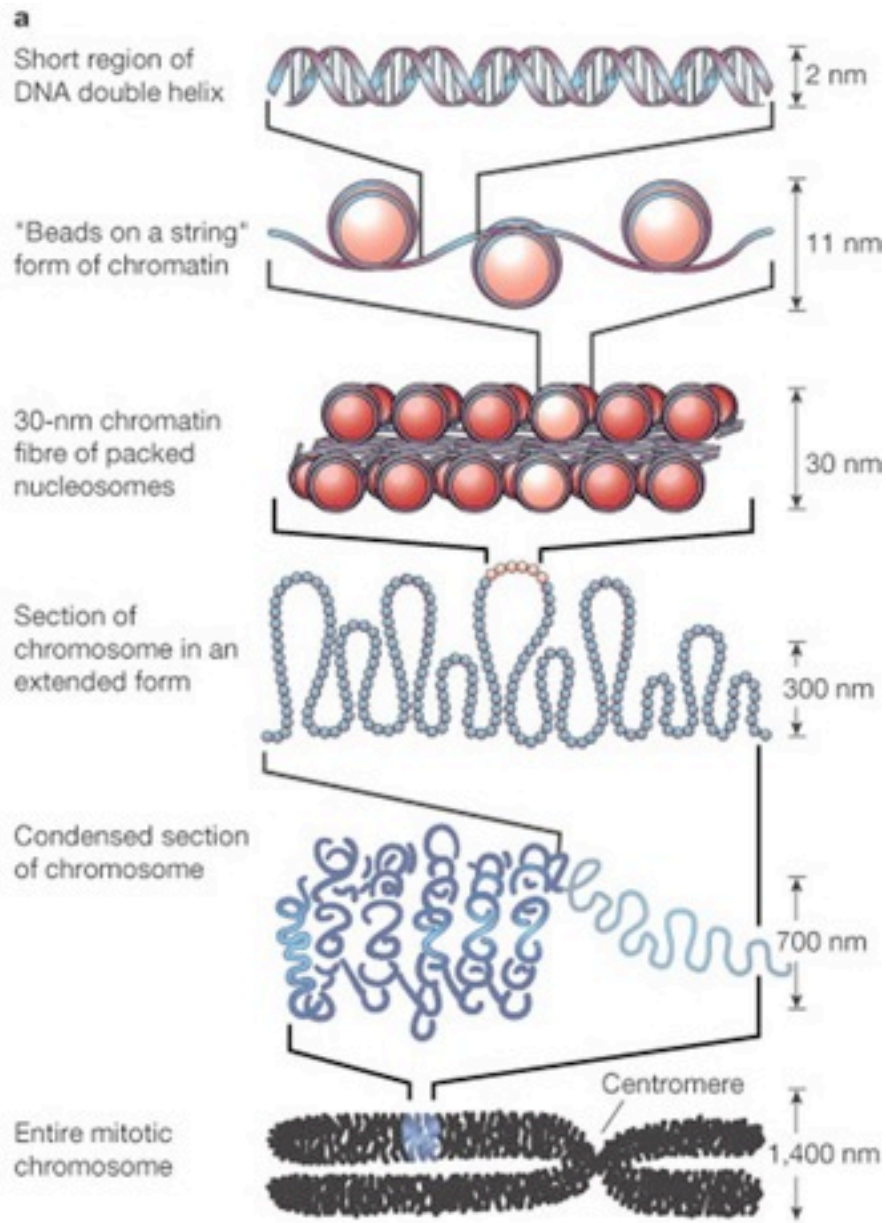


Figure 1 Packaging DNA. a, The organization of DNA within the chromatin structure. The lowest level of organization is the nucleosome, in which two superhelical turns of DNA (a total of 165 base pairs) are wound around the outside of a histone octamer. Nucleosomes are connected to one another by short stretches of linker DNA. At the next level of organization the string of nucleosomes is folded into a fibre about 30 nm in diameter, and these fibres are then further folded into higher-order structures. At levels of structure beyond the nucleosome the details of folding are still uncertain. (Redrawn from ref. 41, with permission).

Figure 19.14 The most exposed positions on DNA recur with a periodicity that reflects the structure of the double helix. (For clarity, sites are shown for only one strand.)

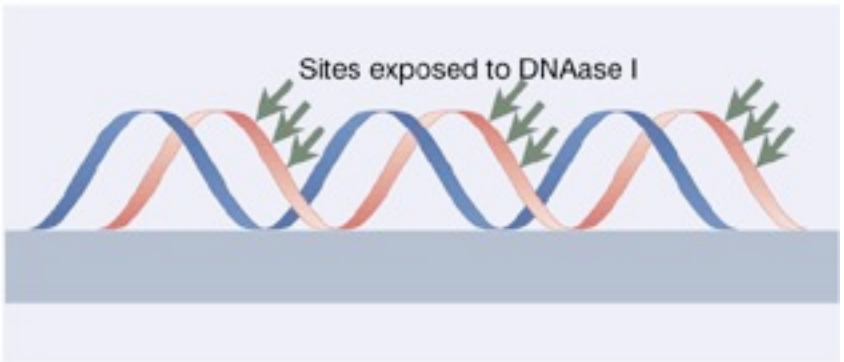
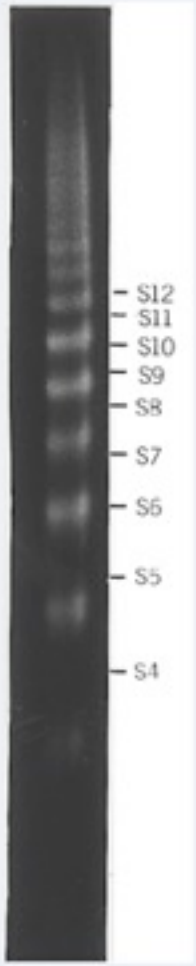
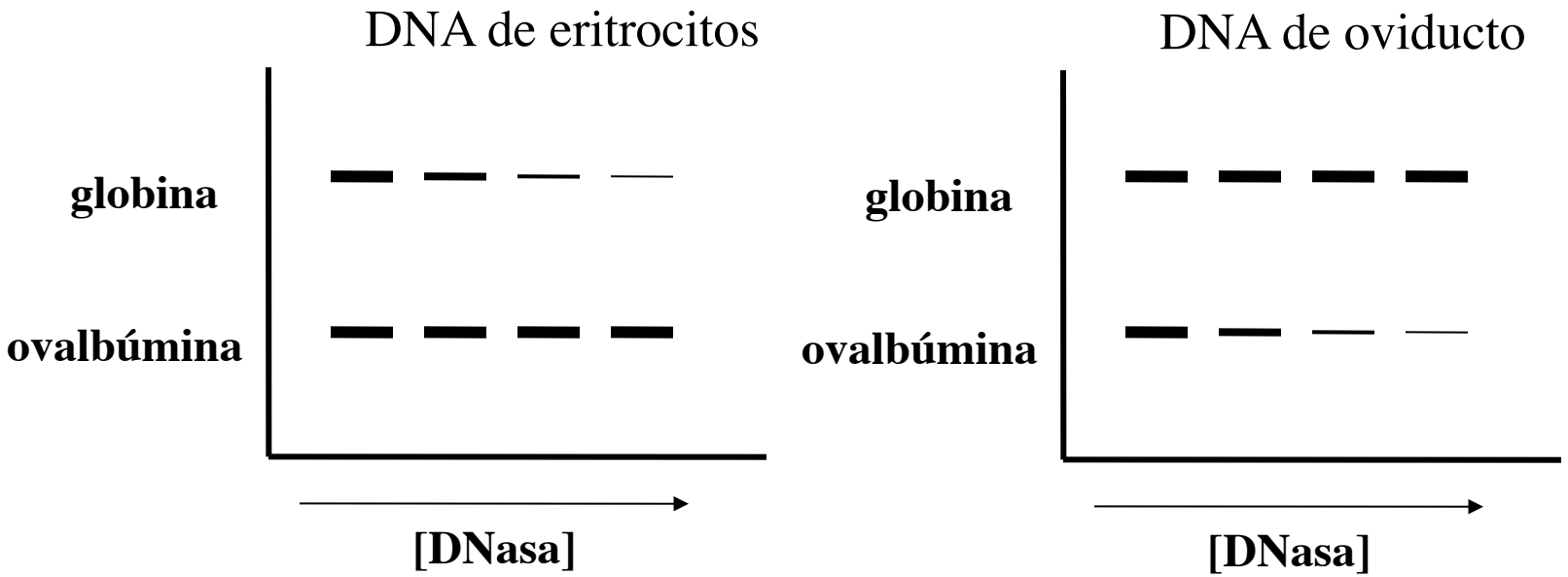


Figure 19.12 Sites for nicking lie at regular intervals along core DNA, as seen in a DNAase I digest of nuclei. Photograph kindly provided by Leonard Lutter.



Accesibilidad diferencial del DNA según actividad génica

Southern Blot con DNA genómico de pollo

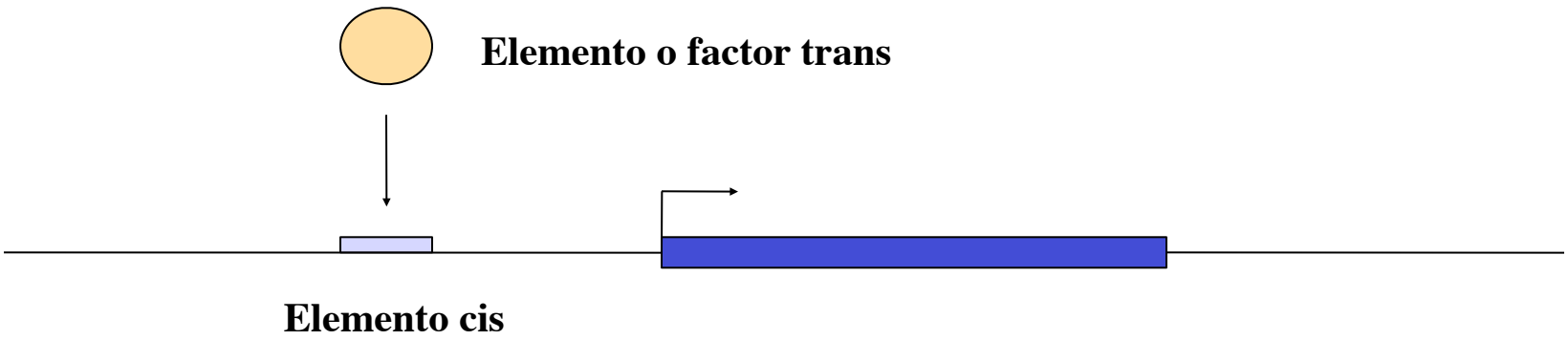


→ Hipersensibilidad de genes a DNasa es tejido específica

→ Locus Control Region (LCR) regulan accesibilidad; modulares

Resistencia a DNasa dada por varios factores, compactación de cromatina y modificaciones covalentes, p. ej., metilación en dinucleótidos C-p-G. Análogos de Citidina (5-aza-citidina) liberan de represión al impedir metilación.

Transcripción: regulada a nivel de iniciación. Mecanismo se basa en interacciones DNA-Proteína.



Elementos <u>cis</u>: permanentes, aunque de accesibilidad variable.

- → Promotor (distancia fija)
- → Enhancer (modular y variable)

Elementos <u>trans</u>: regulados (disponibilidad y abundancia)

- → Basales
- → Señal dependientes

Genes clase II (RNA Pol II)

Son todos los mRNAs citoplásmicos.
Sufren procesamiento: metil-7-G (cap)
splicing o procesamiento
poliadenilación

Elementos cis reguladores:

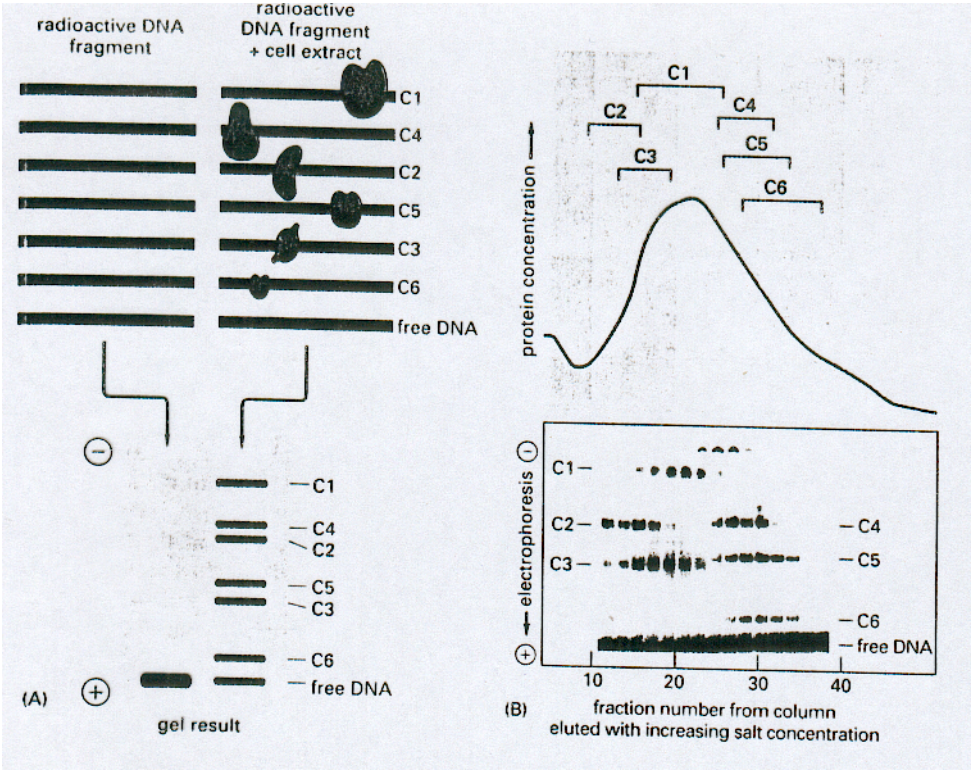
•Se estudian generando constructos por DNA recombinante, "Promoter Bashing" y "Linker Scanning Mutagenesis".

•Se usan genes reporteros y sistemas de expresión *in vitro* e *in vivo*

Estudio de factores trans:

Mobility shift assay (gel retardation)

- Secuencia específica
- Debe poder competirse la unión
- Permite definir distribución temporal y espacial
- Control de especificidad por mutaciones



Purificación en columnas de afinidad

Columnas de afinidad o rastreo de genotecas de exp.

Clonamiento...

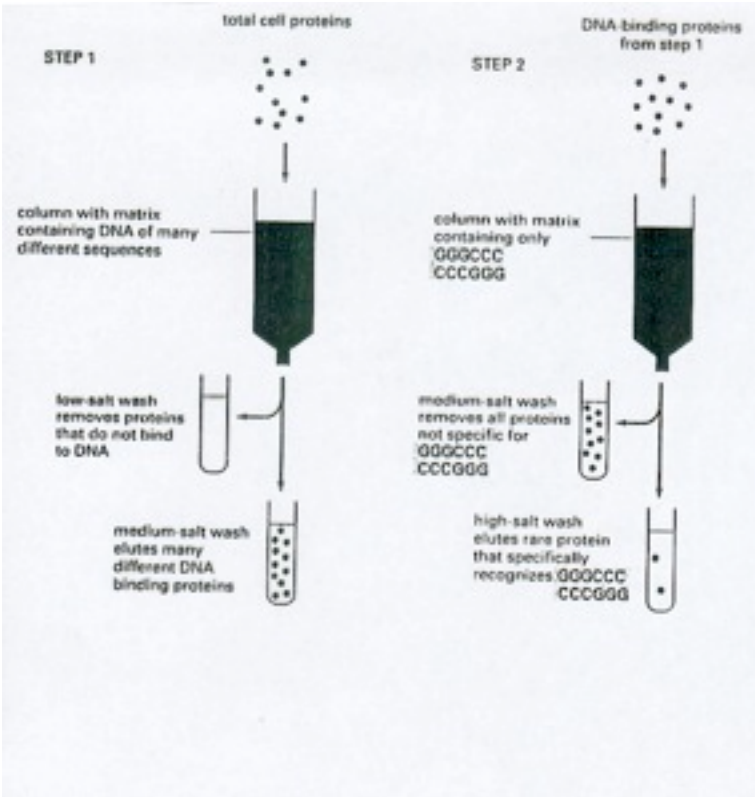


Figure 9-23 DNA affinity chromatography. In the first step all the proteins that can bind DNA are separated from the remainder of the cellular proteins on a column containing a huge number of different DNA sequences. Most sequence-specific DNA-binding proteins have a weak (nonspecific) affinity for bulk DNA and are therefore retained on the column. This affinity is due largely to ionic attractions, and the proteins can be washed off the DNA by a solution that contains a moderate concentration of salt. In the second step the mixture of DNA-binding proteins is passed through a column that contains only DNA of a particular sequence. Typically, all the DN binding proteins will stick to t column, the great majority by nonspecific interactions. Thes again eluted by solutions of m salt concentration, leaving on column only those proteins (t one or only a few) that bind specifically and therefore very to the particular DNA sequenc These remaining proteins can eluted from the column by sol containing a very high concen of salt.

Tjian lab. 1986

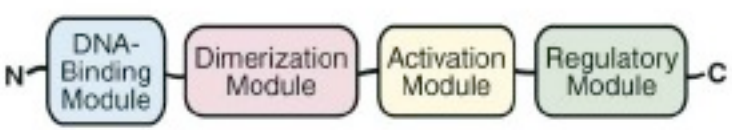
Table 9–1 Some Gene Regulatory Proteins and the DNA Sequences That They Recognize

	Name	DNA Sequence Recognized*
Bacteria	lac repressor	AATTGTGAGCGGATAACAATT TTAACACTCGCCTATTGTTAA
	CAP	TGTGAGTTAGCTCACT ACACTCAATCGAGTGA
	lambda repressor	TATCACCGCCAGAGGTA ATAGTGGCGGTCTCCAT
Yeast	GAL4	CGGAGGACTGTCCTCCG GCCTCCTGACAGGAGGC
	MAT α2	CATGTAATT GTACATTAA
	GCN4	ATGACTCAT TACTGAGTA
Drosophila	Krüppel	AACGGGTTAA TTGCCCAATT
	bicoid	GGGATTAGA CCCTAATCT
Mammals	Sp1	GGGCGG CCCGCC
	Oct-1	ATGCAAAT TACGTTTA
	GATA-1	TGATAG ACTATC

*Each protein in this table can recognize a set of closely related DNA sequences; for convenience, only one recognition sequence is given for each protein.

Figure 9–8 The bending of DNA induced by the binding of the catabolite activator protein (CAP). CAP is a gene regulatory protein from *E. coli*. In the absence of the bound protein, this DNA helix is straight.

Sequence-specific Transcription Factors Are Modular



Dedos de zinc

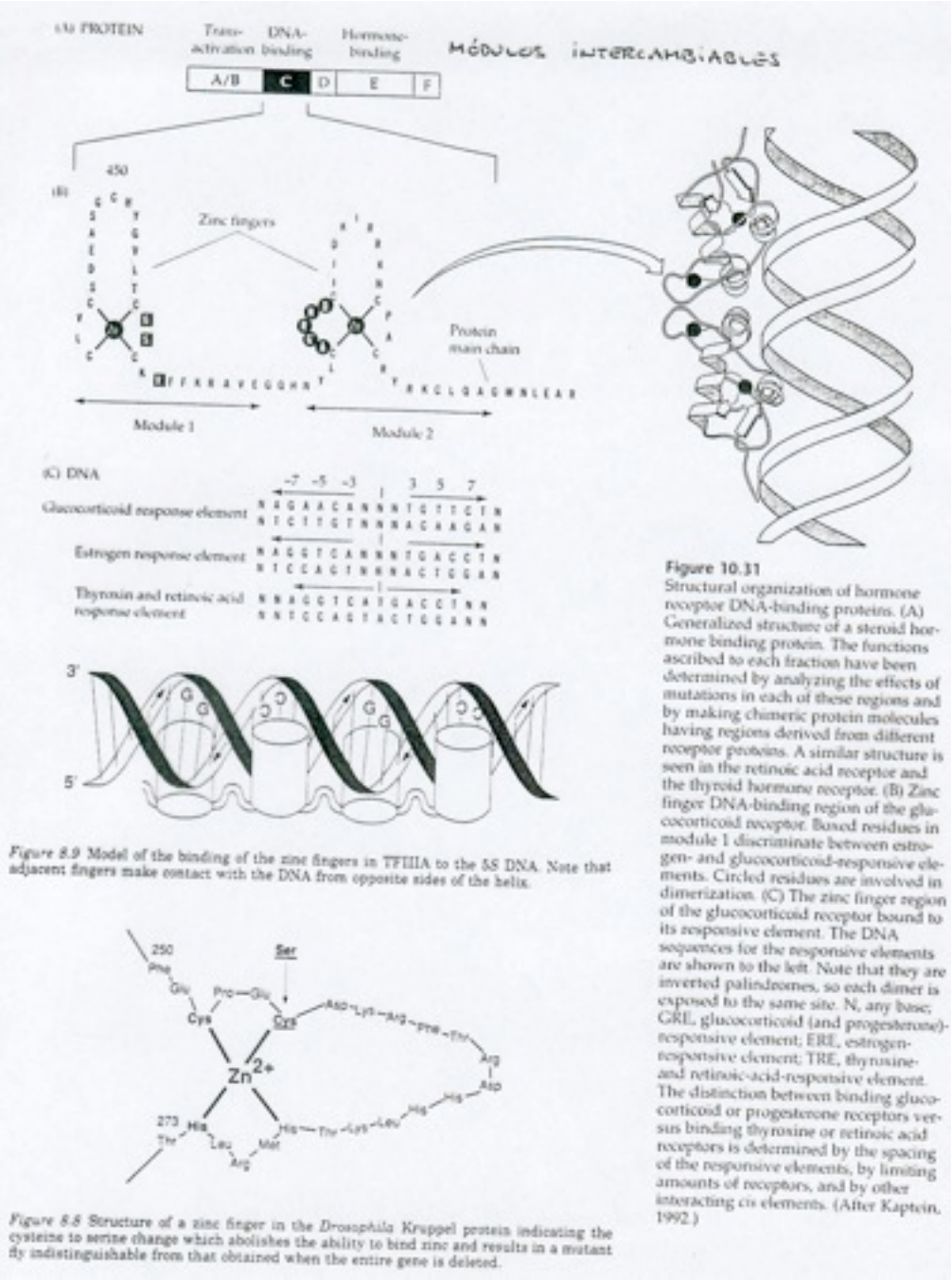


Figure 8.9 Model of the binding of the zinc fingers in TFIIIA to the 5S DNA. Note that adjacent fingers make contact with the DNA from opposite sides of the helix.

Figure 8.8 Structure of a zinc finger in the *Drosophila* Kruppel protein indicating the cysteine to serine change which abolishes the ability to bind zinc and results in a mutant fly indistinguishable from that obtained when the entire gene is deleted.

Figure 10.31
Structural organization of hormone receptor DNA-binding proteins. (A) Generalized structure of a steroid hormone binding protein. The functions ascribed to each fraction have been determined by analyzing the effects of mutations in each of these regions and by making chimeric protein molecules having regions derived from different receptor proteins. A similar structure is seen in the retinoic acid receptor and the thyroid hormone receptor. (B) Zinc finger DNA-binding region of the glucocorticoid receptor. Based residues in module 1 discriminate between estrogen- and glucocorticoid-responsive elements. Circled residues are involved in dimerization. (C) The zinc finger region of the glucocorticoid receptor bound to its responsive element. The DNA sequences for the responsive elements are shown to the left. Note that they are inverted palindromes, so each dimer is exposed to the same site. N, any base; GRE, glucocorticoid (and progesterone)-responsive element; ERE, estrogen-responsive element; TRE, thyroxine- and retinoic-acid-responsive element. The distinction between binding glucocorticoid or progesterone receptors versus binding thyroxine or retinoic acid receptors is determined by the spacing of the responsive elements, by limiting amounts of receptors, and by other interacting *cis* elements. (After Kaptein, 1992.)

Cierre de Leucina

Basic Leucine Zipper Transcription Factors

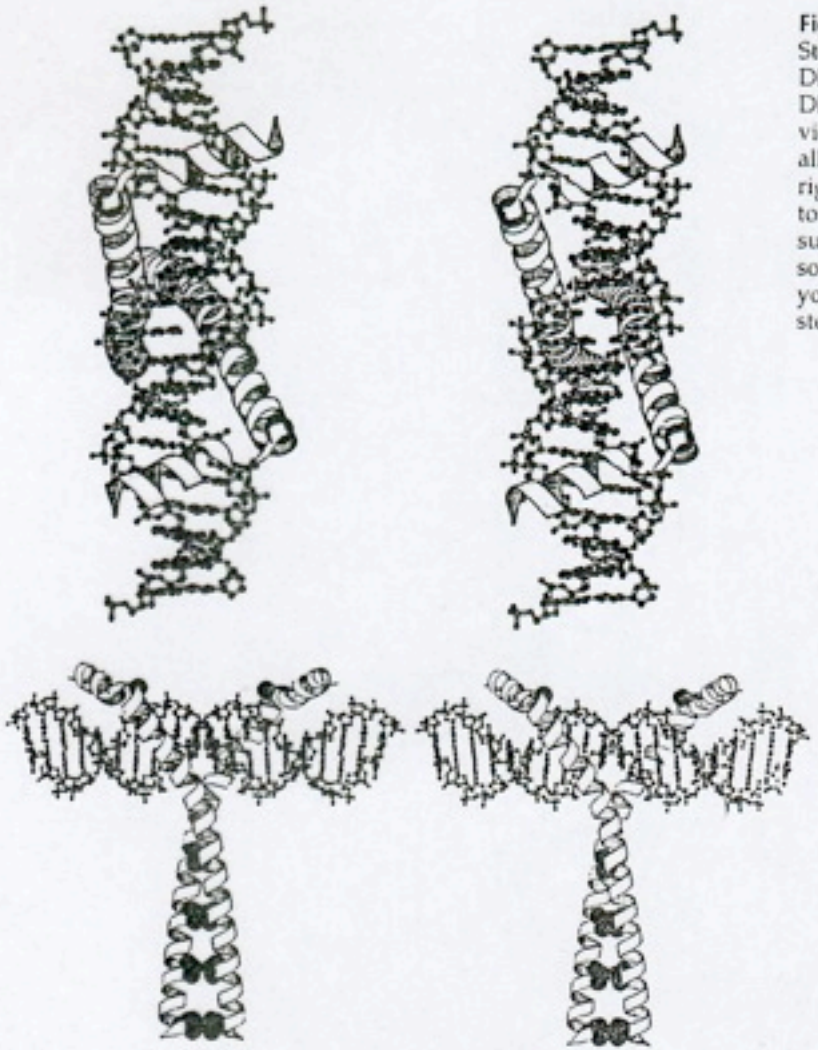


Figure 10.27
Stereoscopic representation of bZip protein C/EBP DNA-binding region interacting with 20 base pairs of DNA containing the CCAAT sequence. (Top) "Dorsal view," looking down at a DNA double helix and parallel to the leucine zipper. (Bottom) "Side view," at right angles to the upper diagram and perpendicular to the DNA axis. Leucine residues connecting the two subunits can be seen at the bottom, as can the "scissors grip" in the DNA. (If you aren't used to crossing your eyes to see the composite stereoimage, borrow a stereopticon.) (From Pathak and Sigler, 1992.)

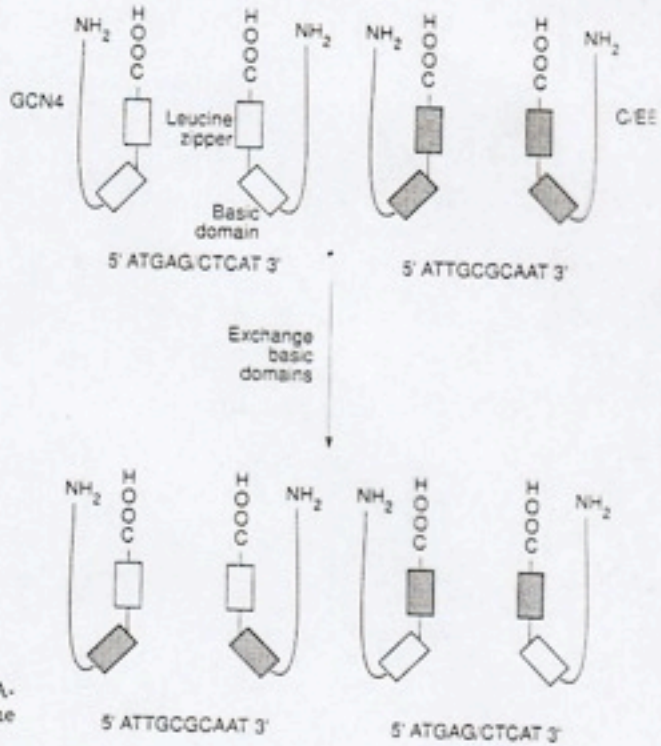
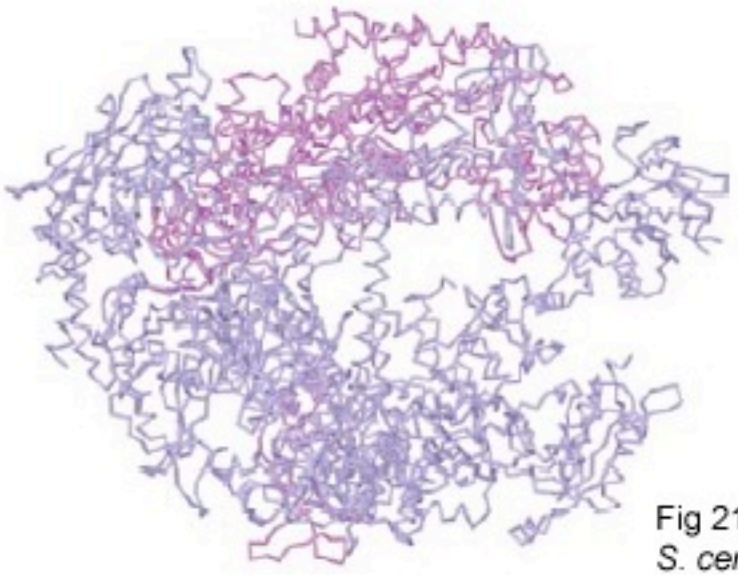


Figure 8.22 Effect of exchanging the basic domains of GCN4 and C/EBP on the DNA-binding specificity. Note that the DNA-binding specificity is determined by the origin of the basic domain and not that of the leucine zipper.

RNA polymerase II

- Transcribes all protein-encoding genes and some small RNA encoding genes
- Protein-encoding RNA synthesized by RNA pol II is called **mRNA precursor** (or **hnRNA**)
- 20% to 40% of all cellular RNA synthesis
- General transcription factors interact directly with RNA pol II and control initiation at class II genes



Prentice Hall c2002

Fig 21.10 RNA polymerase II from *S. cerevisiae* (yeast)

Transcripción basal

GTFs:

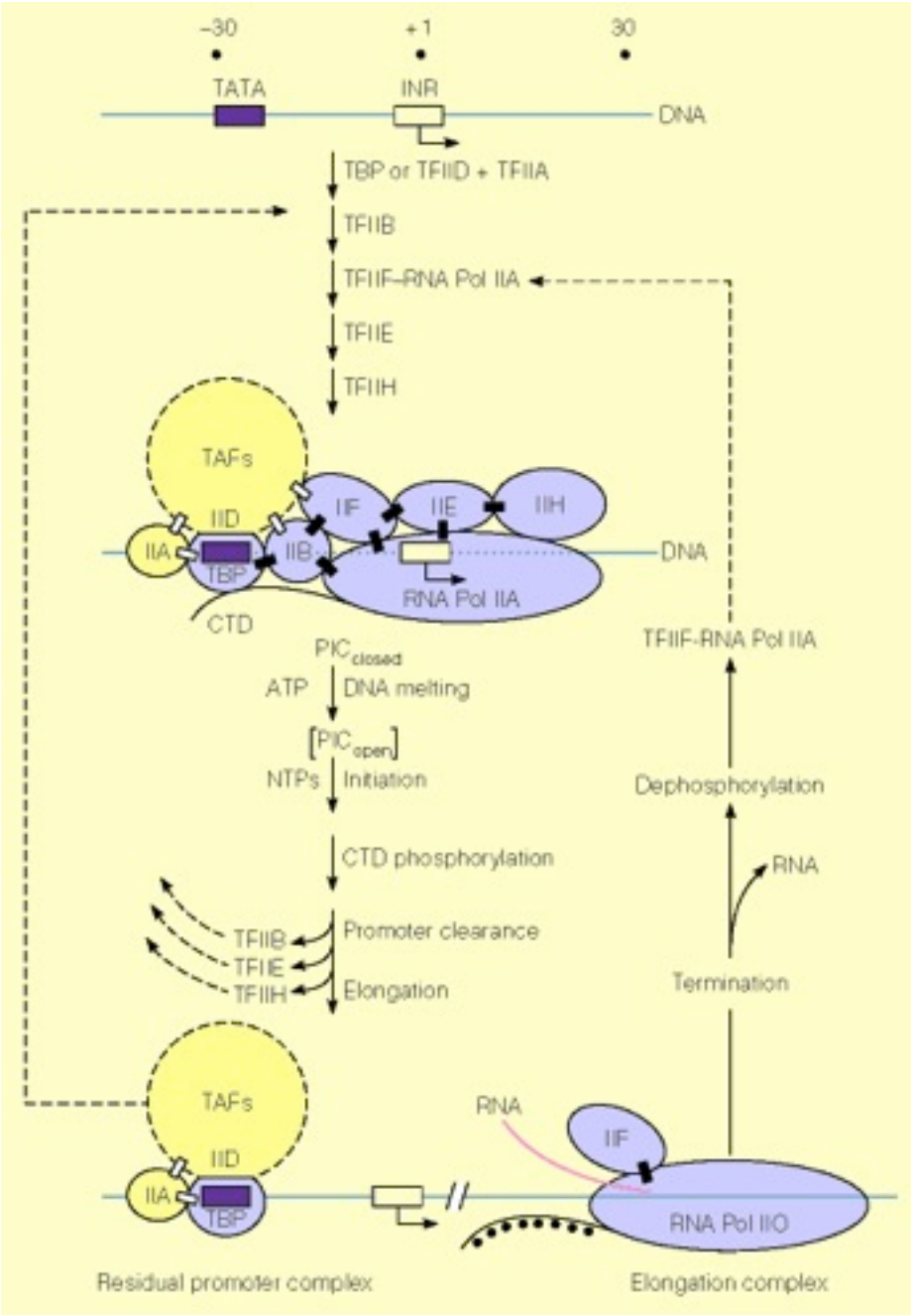
RNA Pol II (12 subunidades)
TFs, al menos 30 proteínas esenciales

Para Pol II se habla de TF II ⟶

Factor mas estudiado: TF IID
Se compone de TBP (TATA binding protein) y TAFs (TBP Associated Factors)

TF IIH: actividad quinasa que fosforila CTD (C terminal domain) de Pol II

Al menos dos modelos de formación del complejo de iniciación.



GTFs o TFIIs

Table 1. Polypeptide composition and known functions of general transcription factors.

Factor	Polypeptide composition	Native mass	Function	Equivalents
TBP	38 kD*	> 100 kD	Initiates complex assembly by binding TATA box Interacts with carboxyl-terminal repeat of RNAPII Interacts with many TAF polypeptides Contacts with acidic activators (VP16)	τ Factor d BTF-1 TFIID (TBP is part of a large protein complex occurring in *Drosophila* and HeLa cells)
TFIIA	34 kD 19 kD 14 kD	Unknown	Stabilizes IID/DNA interaction Removes repressor of basal transcription (Dr-2) from IID complex	STF
TFIIB	33 kD*	33 kD	Contacts with acidic activators (VP16) Binds to the DA complex	α, FA, Factor e
TFIIF	30 kD* 74 kD*	220 kD	Interacts with RNAPII Recruits RNAPII to promoter Affects efficiency of elongation	RAP30/RAP74 βγ, Factor 5, FC BTF4, Factor g
TFIIE	34 kD* 56 kD*	200 kD	Binds to DABPolF complex	ε, Factor a
TFIIH	90 kD 62 kD* 43 kD 41 kD 35 kD	230 kD	Binds to DABPolFE complex Associated carboxyl-terminal domain kinase	BTF2, δ† Factor b
TFIIJ	Unknown	Unknown	Binds to DABPolFEH complex	None defined

Individual citings for each human factor equivalent are not given (references in [1]). †Assumption based on properties reported for these factors. ‡cDNA isolated. RNAP, RNA polymerase; TBP, TATA-binding protein; TAF, TBP-associated factors.

II D ← TAFs TBP

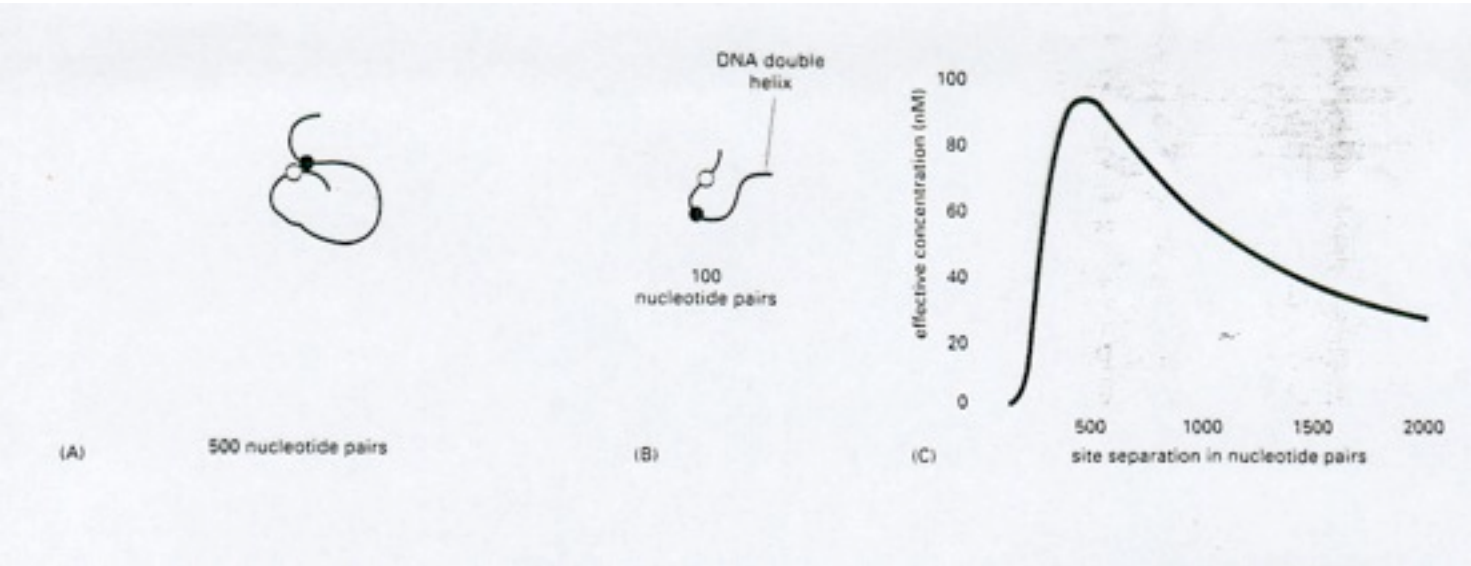
fosforila Pol II

←

Activación transcripcional:
factores transactivadores y elementos cis

- Acción a distancia
- Modulares (orientación y posición), distancias óptimas.
- Intercambiables: contexto independientes

Comportamiento teórico de elementos unidos a DNA:
efecto de concentración efectiva



Brent & Ptashne, 1985

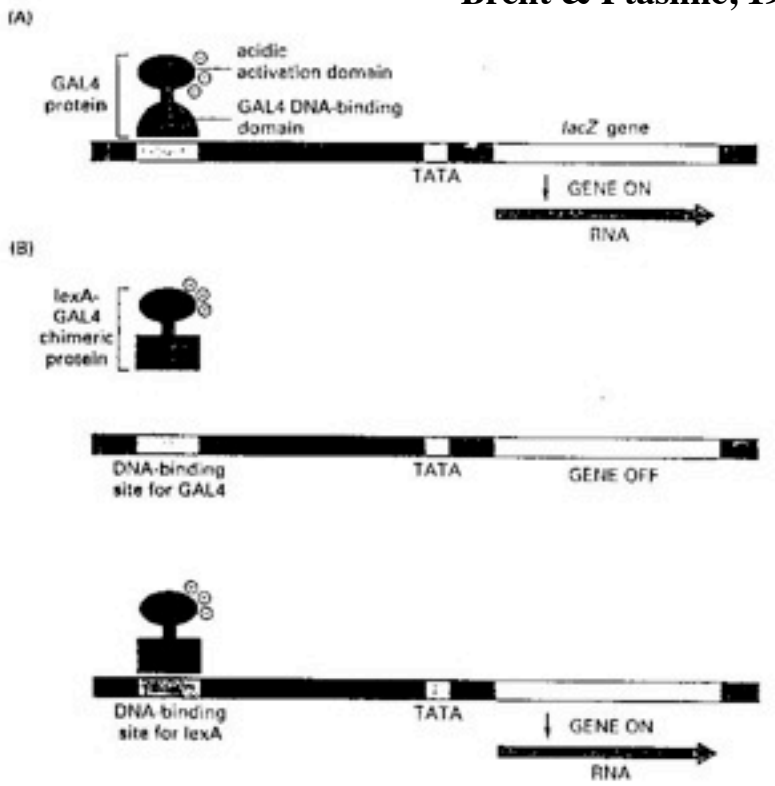


Figure 9–35 The modular structure of a gene activator protein. Outline of a domain-swap experiment that reveals the presence of independent DNA-binding and transcription-activating domains in the yeast gene activator protein GAL4. A functional activator can be reconstituted from the carboxyl-terminal portion of the GAL4 protein if it is attached to the DNA-binding domain of a bacterial gene regulatory protein (the lexA protein) by gene fusion techniques. When the resulting bacterial-yeast hybrid protein is produced in yeast cells, it will activate transcription from yeast genes provided that the specific DNA-binding site for the bacterial protein has been inserted next to them. (A) The normal activation of gene transcription produced by the GAL4 protein. (B) The chimeric gene regulatory protein requires the lexA-protein DNA-binding site for its activity.

GAL4 is normally responsible for activating the transcription of yeast genes that code for the enzymes that convert galactose to glucose. For the experiments shown here, the control region for one of these genes was fused to the *E. coli lacZ* gene, which codes for the enzyme β-galactosidase (see Figure 9–29). β-galactosidase is very simple to detect biochemically and thus provides a convenient way to monitor the expression level specified by a gene control region; *lacZ* thus serves as a *reporter gene* (see p. 321).

Factores transactivadores:

- Dominio unión a DNA
- Dominio activación
- Modulares

- Mecanismo de acción en eucariontes debe estar conservado por intercambiabilidad

- No hay necesida de interacciones específicas ni cambios conformacionales como producto de las interacciones.

Experimento de "activator bypass" de Ptashne.

Cell, Vol. 81, 359–368, May 5, 1995, Copyright © 1995 by Cell Press

Contact with a Component of the Polymerase II Holoenzyme Suffices for Gene Activation

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Natasha Simkovich,* Susan Farrell,*
Pamela Reinagel,* Cynthia Bamdad,*‡
George Sigal,§ and Mark Ptashne*
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Summary

In yeast strains bearing the point mutation called *GAL11P* (for potentiator), certain GAL4 derivatives lacking any classical activating region work as strong activators. The *P* mutation confers upon GAL11, a component of the RNA polymerase II holoenzyme, the ability to interact with a portion of the dimerization region of GAL4. The region of GAL11 affected by the *P* mutation is evidently functionally inert in ordinary cells, suggesting that this mutation is of no functional significance beyond creating an artificial target for the GAL4 dimerization fragment. From these observations and further analyses of GAL11, we propose that a single activator–holoenzyme contact can trigger gene activation simply by recruiting the latter to DNA.

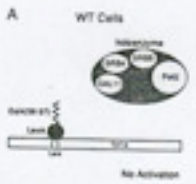

Figure 8. Potentiation by GAL11P and Activation by GAL11 Tethered to DNA

(A) A DNA-bound molecule fused to GAL4(58–97) does not activate transcription in *GAL11* wild-type cells. GAL11 and a subset of the other components that comprise RNA polymerase II holoenzyme are shown. Transcription factors TFIID and TFIIE, which are not part of the holoenzyme described by Koleske and Young (1994), are not shown.

(B) GAL4(58–97) works as an activating region in *GAL11P* cells because it specifically interacts with GAL11P and thereby recruits the RNA polymerase II holoenzyme complex to the promoter.

(C) A sequence-specific DNA-binding module fused to GAL11(or to GAL11P) recruits the holoenzyme to the promoter and activates transcription.

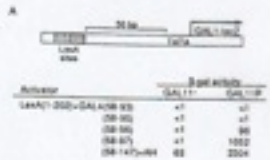

Figure 3. GAL4 Residues 58–97 Constitute an ... GAL11P Cells

(A) GAL4(58–97) fused to LexA activates in GAL... called fusion proteins were expressed from the ... low copy plasmid. GAL11 proteins were expressed ... promoter on low copy plasmids. The strain used ... is JPY42 and is *gal4⁻* and *gal11⁻*. The integrated reporter template has two LexA-binding sites positioned 50 bp upstream of the GAL1 TATA box.

(B) GAL4 residue 97 is the carboxyl border of the novel activating region. Deletion derivatives of GAL4 were expressed from the *ACT1* promoter on a low copy plasmid. *GAL11* alleles were those described in (A). The strain used in these experiments is JPY18 and is *gal4⁻* and *gal11⁻*. The integrated reporter template has the UAS_G (an element that includes the GAL4-binding sites) at its native position upstream of the GAL1 TATA box.

(C) Mutations in GAL4(58–97) impair the novel activating region. LexA(1–202)+GAL4(50–147)+AH, bearing either no mutations or the indicated amino acid substitutions, was expressed from the *ACT1* promoter on a low copy plasmid. *GAL11* alleles were those described in (A). The strain used in these experiments is JPY42.

Connecting a promoter-bound protein to TBP bypasses the need for a transcriptional activation domain

Sukalyan Chatterjee & Kevin Struhl*

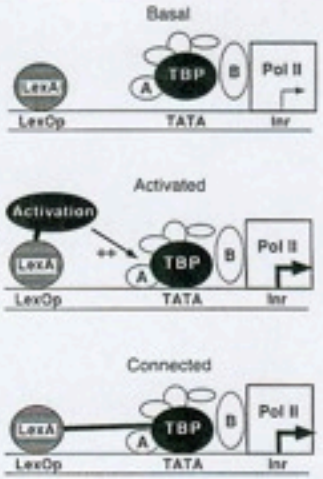

FIG. 2 Connecting a promoter-bound protein to TBP overrides the need for a transcriptional activation domain. Interactions of LexA hybrid proteins, TBP, TBP-associated factors (TAFs; shaded ovals), TFIIA, TFIIB and RNA polymerase II at a promoter containing a LexA operator, TATA element and initiator (Inr). In basal transcription (LexA alone), the interaction between TBP and the TATA element is limiting (indicated by space between the two components), thereby leading to low levels of mRNA (thin arrow). Activation domains increase the recruitment or stability of TBP to the TATA element, thereby stimulating transcription (thick arrow). The target of the activation domain is not specified in the illustration (see text for discussion). A similar increase in transcription occurs when LexA is directly connected (thick line) to TBP. See text for details and limitations of this model.

Stimulation of RNA polymerase II transcription initiation by recruitment of TBP *in vivo*

Medio AT - requiere His 3 exp.

Natacha Klages & Michel Strubin

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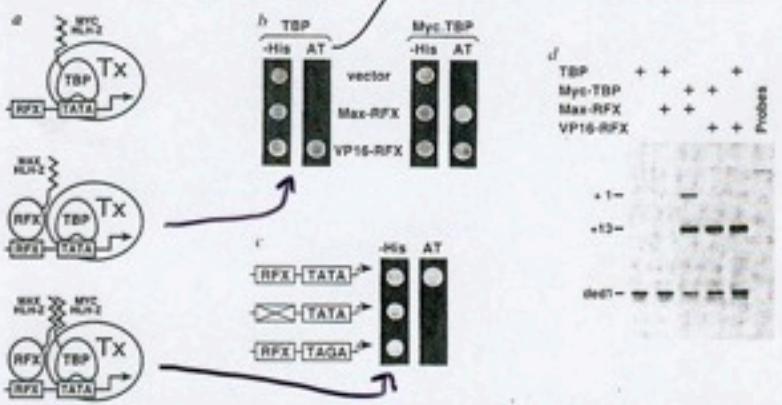

1 Recruitment of TBP to a target promoter stimulates RNA polymerase II transcription initiation in vivo. a, Schematic representation of strategy. MYC-HLH-Z and MAX-HLH-Z designate the helix-loop-helix-leucine-zipper dimerization motifs present in the human c-Myc oncoprotein and its partner Max, respectively[11]. Tx refers to the transcription machinery. b, Strains containing a *his3* allele with an upstream RFX-binding site and expressing the indicated TBP and RFX derivatives from plasmid DNAs were tested for growth on medium lacking histine (−His) and containing 10 mM aminotriazole (AT). Growth on AT requires induced levels of *his3* expression. c, Effect of deletion of the RFX-binding site, insertion of a point mutation in the *his3* TATA-box (TAGA), on the ability of cells expressing Myc–TBP and Max–RFX to grow on 40 mM AT. Quantitation of his3 RNA levels by S1 nuclease analysis. Strains whose growth phenotype shown in b were analysed for *his3* (+1 and +13 transcripts) and *ded1* RNAs. The level of *ded1* RNA was used as an internal control.

METHODS. All the proteins are expressed from CEN-ARS vectors[39] under control of yeast TBP regulatory sequences[14]. The Myc dimerization motif derived from pVP-Myc73 (ref. 11) was fused to the N terminus of TBP molecule 4 (ref. 14). Max–RFX contains the *EcoRI–SalI* fragment from pVP-Max72 (ref. 11) fused to the *AgeI* site found in the RFX1 coding region[13]. VP16–RFX results from the fusion of the VP16 activation domain derived from pMSVP16 D103 (ref. 21) to the ATG initiator of RFX. The yeast strains, derived from KY320 (ref. 22), contain *his3* alleles with a single native or mutated RFX-binding site and 50 bp of random DNA inserted at the unique *EcoRI* site in *his3*–Δ94 carrying the relevant TATA elements[23]. Phenotypes were analysed by spotting 10^4 cells on minimal medium lacking histidine or containing AT. S1 nuclease protection experiments were done as described[24], except that the *ded1* radiolabelled oligonucleotide was diluted five-fold before hybridization.

Conclusiones del modelo de reclutamiento de Ptashne:

Los activadores poseen afinidad por algún componente de la maquinaria basal.

No se requiere un cambio conformacional de la maquinaria basal (o de RNA Pol II) para lograr la iniciación; basta reclutar los componentes.

Las interacciones son arbitrarias y solo la fuerza de unión (energía) influye en el grado de activación.

Lo que ocurre *in vivo* es el aumento de la concentración de los activadores en la vecindad del promotor y el consiguiente aumento de la concentración de los componentes de la maquinaria basal.

La maquinaria basal (Pol II et al) no se une al promotor espontaneamente.

Efecto de "squelching".

La transcripción no ocurre sobre DNA desnudo

In vitro:

1º	2º	Tx
Histonas	GTFs	+
GTFs	Histonas	+++++

- **La activación transcripcional en eucariontes requiere mas pasos que el simple reclutamiento.**

- **Aunque el estado basal es represivo, existen muchos mecanismos de represión específicos.**

Cell, Vol. 98, 1–4, July 9, 1999, Copyright ©1999 by Cell Press

Fundamentally Different Logic of Gene Regulation in Eukaryotes and Prokaryotes

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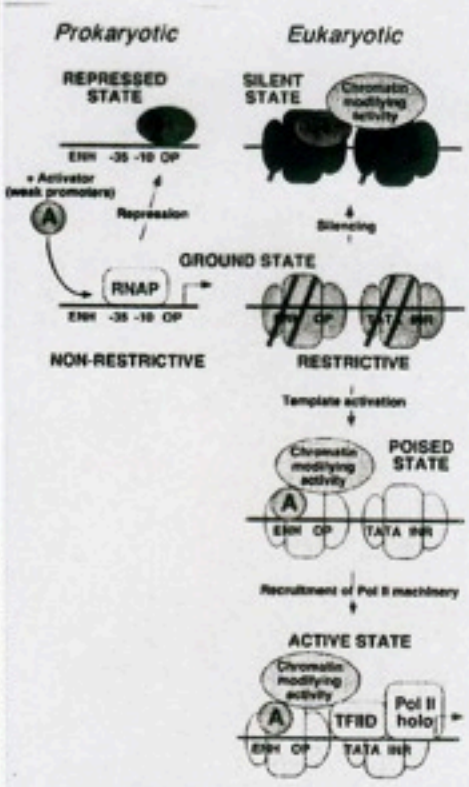


Figure 1. Transcriptional States in Prokaryotes and Eukaryotes

Activators (A) and repressors (R) interact respectively with enhancer (ENH) or operator (OP) sequences and affect transcription by prokaryotic RNA polymerase (RNAP) or the eukaryotic Pol II machinery (TFIID + Pol II holoenzyme). In eukaryotes, recruitment of chromatin modifying activities by activators or repressors leads to altered chromatin structure (depicted by color or DNA within nucleosomes). See text for details.

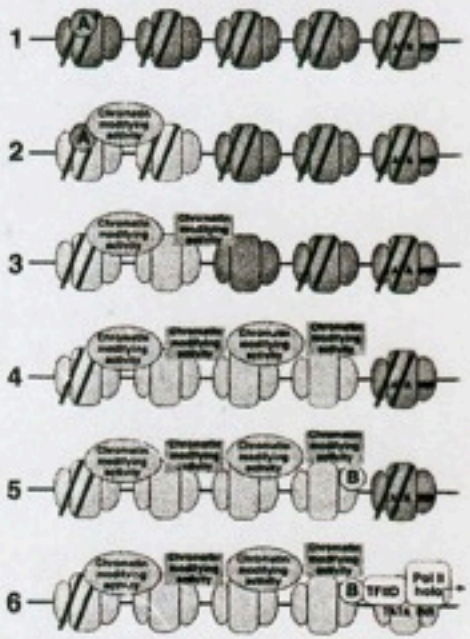
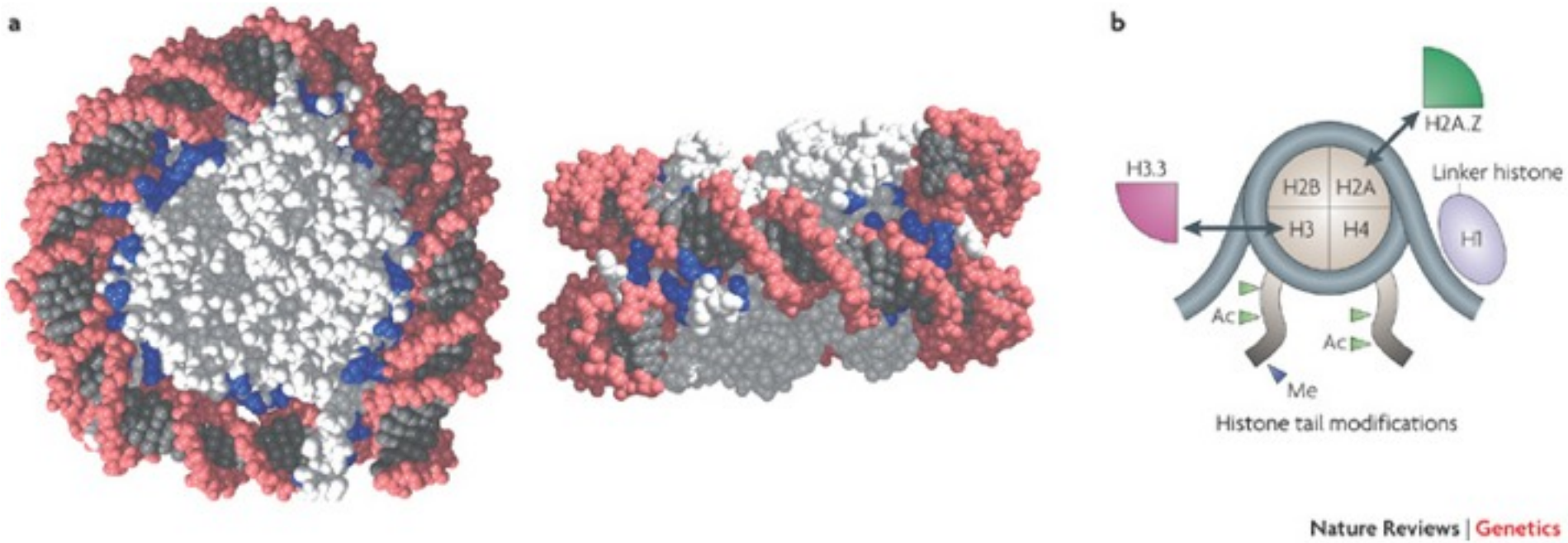


Figure 2. Hypothetical Intermediate States of Eukaryotic Transcription for a Given Promoter

Activator A binds to unmodified nucleosomal templates, leading to successive recruitment of chromatin modifying activities and the creation of an extended domain of active chromatin structure that permits binding of activator B and subsequent recruitment of the Pol II machinery. Binding of activator A is transient, but the recruited chromatin modifying activities remain stably associated for extended times. The existence, order, and stability of the indicated states can be developmentally regulated, but will vary according to the specific promoter.


a
b
H2A.Z
H3.3
H2B
H2A
H3
H4
Linker histone
H1
Ac
Ac
Me
Histone tail modifications
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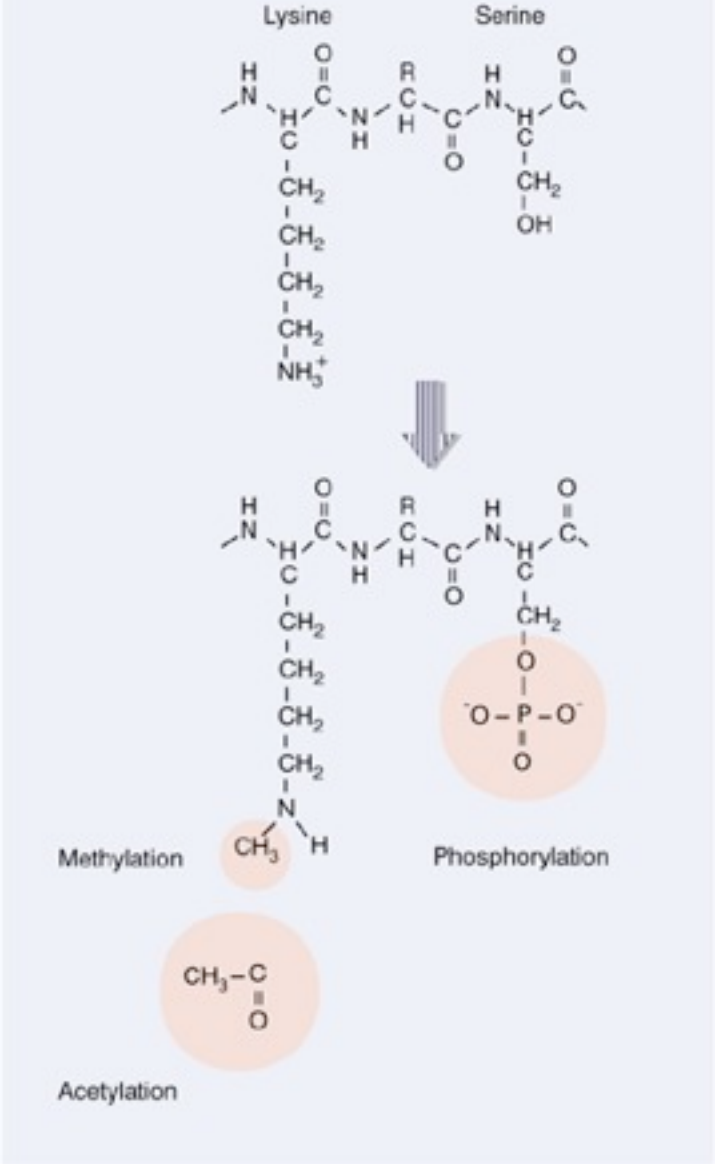
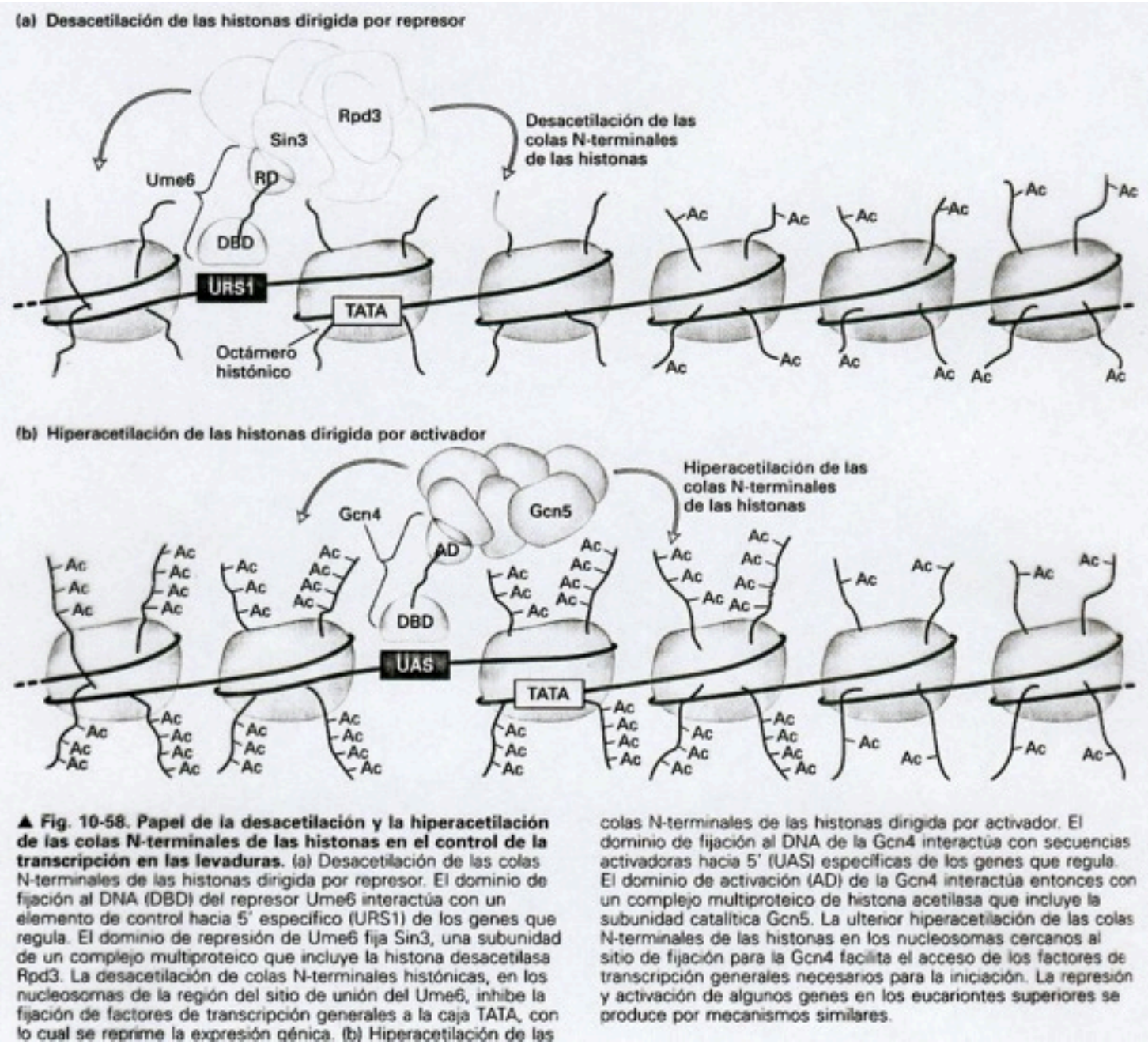
Modificaciones de histonas

Figure 19.25 Acetylation of lysine or phosphorylation of serine reduces the overall positive charge of a protein.





▲ **Fig. 10-58. Papel de la desacetilación y la hiperacetilación de las colas N-terminales de las histonas en el control de la transcripción en las levaduras.** (a) Desacetilación de las colas N-terminales de las histonas dirigida por represor. El dominio de fijación al DNA (DBD) del represor Ume6 interactúa con un elemento de control hacia 5' específico (URS1) de los genes que regula. El dominio de represión de Ume6 fija Sin3, una subunidad de un complejo multiproteico que incluye la histona desacetilasa Rpd3. La desacetilación de colas N-terminales histónicas, en los nucleosomas de la región del sitio de unión del Ume6, inhibe la fijación de factores de transcripción generales a la caja TATA, con lo cual se reprime la expresión génica. (b) Hiperacetilación de las colas N-terminales de las histonas dirigida por activador. El dominio de fijación al DNA de la Gcn4 interactúa con secuencias activadoras hacia 5' (UAS) específicas de los genes que regula. El dominio de activación (AD) de la Gcn4 interactúa entonces con un complejo multiproteico de histona acetilasa que incluye la subunidad catalítica Gcn5. La ulterior hiperacetilación de las colas N-terminales de las histonas en los nucleosomas cercanos al sitio de fijación para la Gcn4 facilita el acceso de los factores de transcripción generales necesarios para la iniciación. La represión y activación de algunos genes en los eucariontes superiores se produce por mecanismos similares.