

## Transcription Activators & Repressors

- Basal Transcription HAPPENS!
- Activators increase transcription while Repressors decrease transcription
- Activators can also be Repressors
- Both bind *directly* to DNA (Co-activators and Co-repressors bind to other proteins)
- Sometimes Activators bind indirectly to DNA (ER binds AP-1 element which binds DNA)

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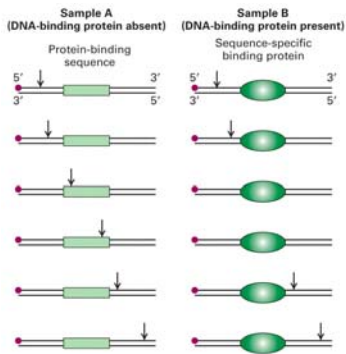
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DNase I cuts the DNA at every arrow point.

What will the gel look like with and without bound protein?




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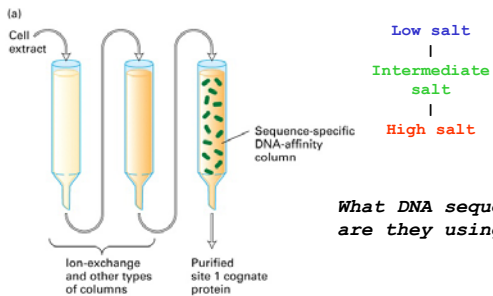
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Fig 11-13

## Transcription Factor Purification

Happens after mutational analysis




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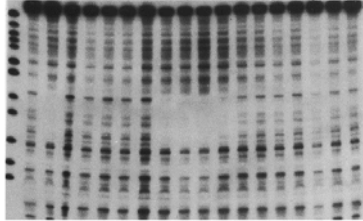
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## Transcription Factor Purification

(b)  
MNE O FT 1 6 7 8 9 10 11 12 13 14 15 16 18 20 22 Fraction



Dnase I footprint

Fractions separated by chromatography assayed for ability to bind DNA site

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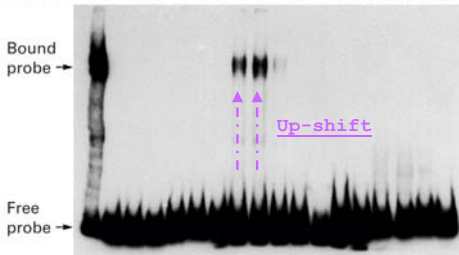
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## Gel Shift Assay

Fraction ON 1 2 3 4 5 6 7 8 9 10 11 12 14 16 18 20 22



Commonly used molecular biology assay.  
Radioactively labelled DNA Probe "shifts"

Fig 11-14

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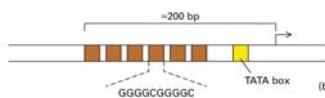
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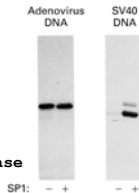
## In vitro Transcription Activation Assay

(a) SP1-binding sites in SV40 genome



Still lots of activity.  
How measured?

(b) SP1 transcription-activating assay



Transcription factor isolated previously by fractionation

(a) SV40 - 6 copies of promoter upstream

(b) Activity test:

HeLa cell extract - incl Pol II  
and transcription factors

Adenovirus - no promoter; no increase

SV40 - has promoter; big increase

Fig 11-15

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# In vivo Transcription Activation Assay

- A. Two plasmids:
  - (1) transcription factor
  - (2) binding site & reporter gene
- B. Host cell transfection
- C. Measure mRNA levels
- D. What is a good control?

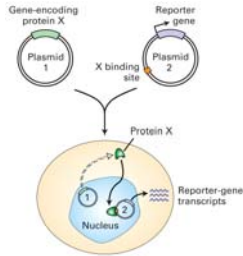


Fig 11-16

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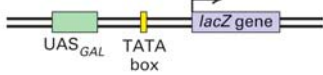
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# Yeast Gal4 Domains

**Question:** What components of transcription activator (Gal4) are important and what do they do?

(a) Reporter-gene construct



- DNA construct** with lacZ gene and added TATA box
- UAS<sub>GAL</sub>** Regulatory element with several Gal4 binding sites
- Introduce** WT and mutant Gal4 to yeast (Gal4- cells)
- Assay **β-galactosidase** activity in yeast
- Activity high** if mutants contain functional Gal4 (see next slide)

Fig 11-17

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# Yeast Gal4 Domains

**Localize**  
**DBD**  
**&**  
**AD**

(b) Wild-type and mutant GAL4 proteins	Binding to UAS <sub>GAL</sub>	β-galactosidase activity
Wild-type: 1-74 (DNA-binding domain), 738-823 (Activation domain)	+	+++
50-881	-	-
848-881	+	+++
823-881	+	+++
N- and C-terminal deletion mutants: 792-881	+	++
755-881	+	+
692-881	+	-
74-881	+	-
Internal deletion mutants: 74-684-881	+	+++
74-738-881	+	+++
74-768-881	+	++

Fig 11-17

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## Modular Activator Structure

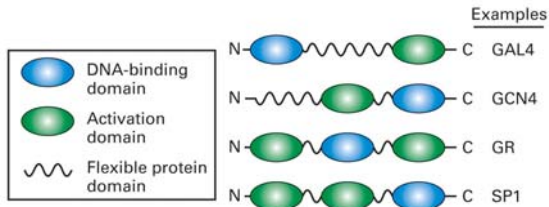


Fig 11-18

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## Common DNA Binding Domains (DBDs)

**Homeodomain** Found in homeotic genes  
Highly conserved 60aa region

**Winged-Helix** TFs in Development

**Leucine-Zipper** Hydrophobic Leucines required for dimerization every 7th position in C-terminus

**Helix-Loop-Helix** Similar to Leucine zipper except with a non-helical loop in-between

**Zinc-Finger** Folds around a central Zn<sup>2+</sup> ion (one of the most common DNA binding structures)

**Steroid receptors** -> Double Zinc finger motif

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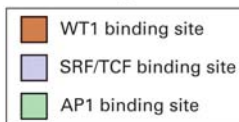
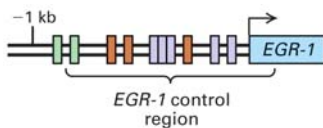
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## Example Control Region



- 1) WT1 **represses** EGR-1
- 2) SRF/TCF **activates** EGR-1
- 3) AP1 **activates** EGR-1
- 4) Sites do not overlap.

What does that say about WT1 repression?

Fig 11-19

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## Bacteriophage 434 Repressor

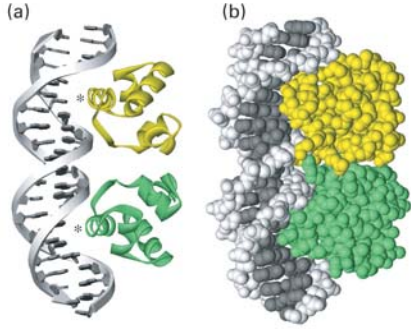


Fig 11-20

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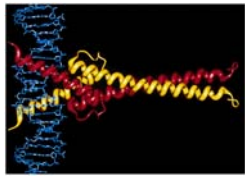
## DBD Models



Homeodomain



Leucine Zipper



Helix-Loop-Helix

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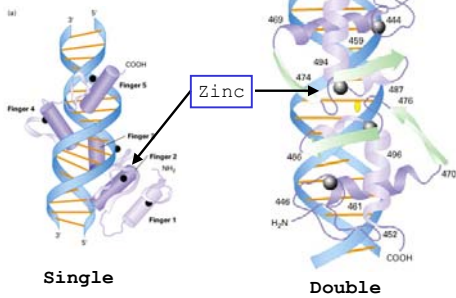
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## Zinc Finger Motif



## Transcription Factor Interactions

- TF's bind to "degenerate" sequences typically 6-10 bases in length. How could you predict them computationally?
- Heterodimeric TFs: two or more different monomers bind together
- In some hertermodimeric TFs, monomers bind to different DNA sequences
- ~ 2000 known TF's in human genome - lots of unique transcriptional control possible (incl. Cell specific)

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## TF Combinatorial Possibilities

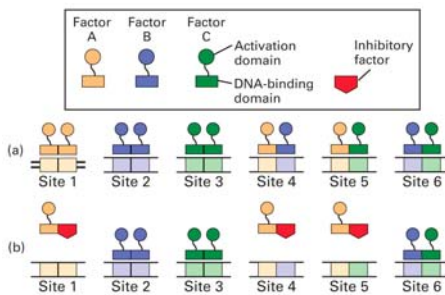


Fig 11-23

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## NFAT & API Cooperative Binding

IL-2 promoter has 2 weak binding sites

Protein-protein interactions stabilize complex with DNA

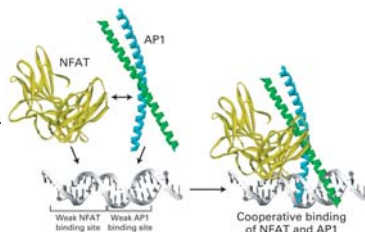


Fig 11-24

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## Multi-protein Complexes

- Enhancers range from 50-200bp in length
- Multiple TFs interact on enhancer
- Bound TFs often bend the DNA
- $\beta$ -interferon enhancer - four control elements that bind four TFs cooperatively

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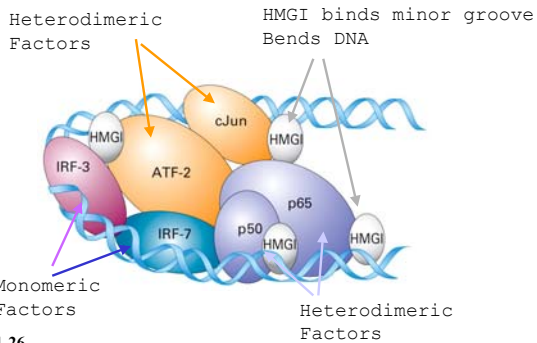
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## $\beta$ -interferon enhancer



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## Hormone Regulated Gene Expression

- Hormones: extracellular signals
- Small, lipid-soluble hormones: steroid hormones, retinoids and thyroid hormones
- Diffuse through plasma membrane and interact directly with TFs
- Significant conservation of hormone binding nuclear receptor amino acid sequence

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## Modular Design

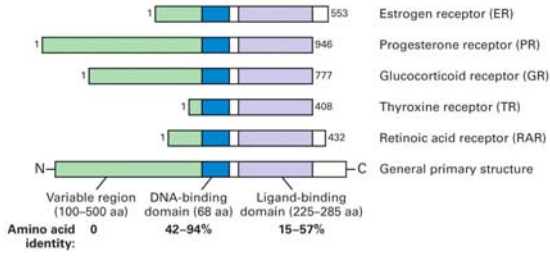


Fig 11-41

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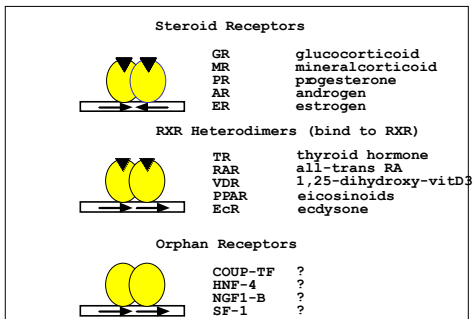
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## Nuclear Receptor Superfamily




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## Response Elements

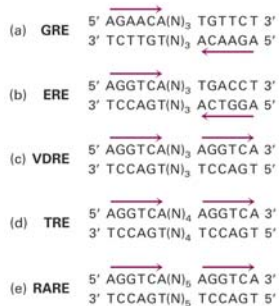


Fig 11-42

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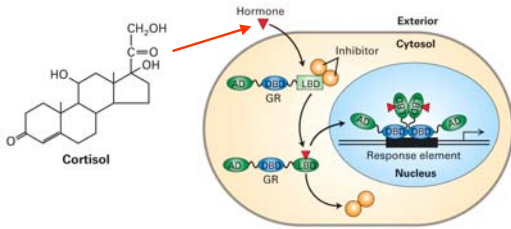
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# Hormone Activation



AD = Activation Domain (binds Co-activators)  
 DBD = DNA Binding Domain  
 LBD = Ligand Binding Domain

Fig 11-44

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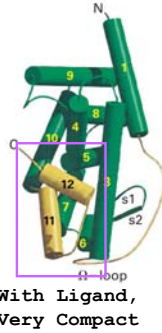
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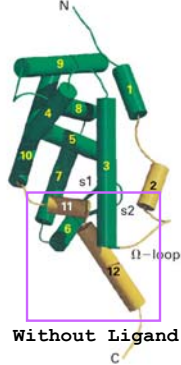
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(a) RAR $\gamma$  plus ligand



(b) RXR $\alpha$  without ligand




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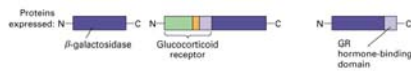
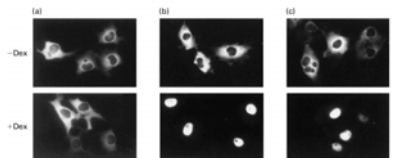
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# Hormone-Binding and the Nucleus



Used fluorescent labeled antibody for  $\beta$ -galactosidase      Dex = GR hormone

Fig 11-43

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## Steroid Receptor Antagonists

**Def:** Molecules that interfere with activity - especially binding of cofactors

**Tamoxifen** - used to treat breast cancer

**Raloxifen** - used to treat osteoporosis

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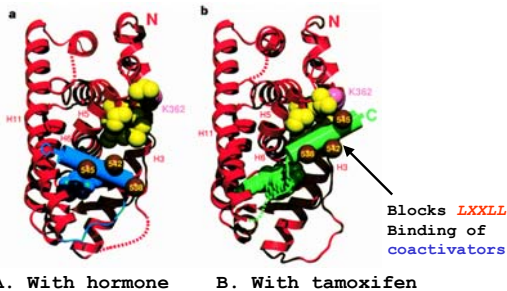
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## Ligand Binding Domain



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## Tamoxifen and Breast Cancer

- Tamoxifen blocks ER action by binding to LBD and rotating helix 12 to block hydrophobic cleft
  - Affects ER positive Breast cancer cells
  - Breast cancer cells can become resistant (drug selection)
  - **Hypothesis:** Overexpression of coactivators such as ACTR - also known as AIB (Amplified In Breast cancer) - cause tamoxifen to switch from antagonist to partial or weak agonist
- How might this happen?  
Why amplified in breast cancer?

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The  
Governator  
says,  
"Study  
hard or  
I'll raise  
fees even  
more."



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