Eukaryotic Transcription title

Regulation of Transcription

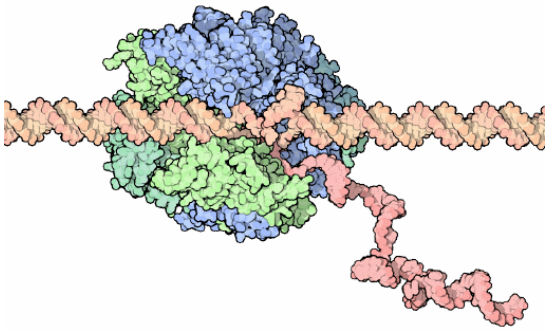
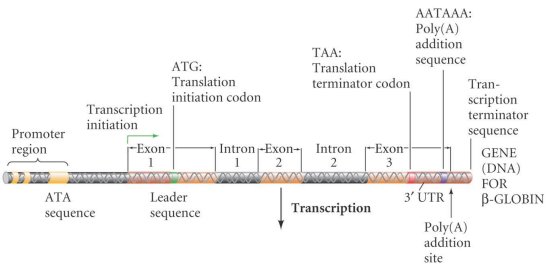


Figure 5.3(1) ***Steps involved in the Production of β-globin and Hemoglobin***



Eukaryotic Transcription: RNA Polymerases

3 Eukaryotic RNA Polymerases:

RNA Pol II - Transcribes all mRNAs, must be versatile

RNA Pol I, RNA Pol III - Transcribe tRNA, rRNA, other RNA genes

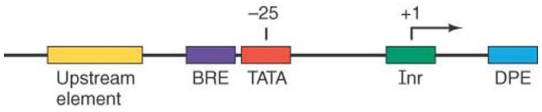
General Transcription Factors -

work in all cells, aiding RNA Pols I, II and III to form basal transcription complex

named for the RNA Pol they work with; e.g., TFIID works with RNA Pol II.

Specific Transcription Factors - work in some cells or tissues, at specific stages of development

Class II Promoters



Promoters elements:

Core:

TATA box Inr - Initiator

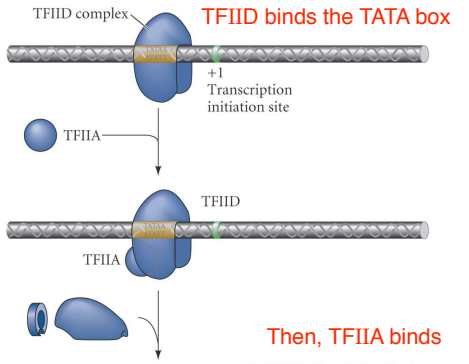
BRE - TFIIB Recognition Element

DPE - Downstream Promoter Element

Upstream elements (various), e.g., CAAT box

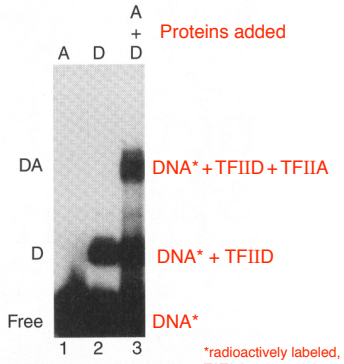
Enhancers

Figure 5.4(1) ***Formation of the Active Eukaryotic Transcription Initiation Complex***



How do we know?

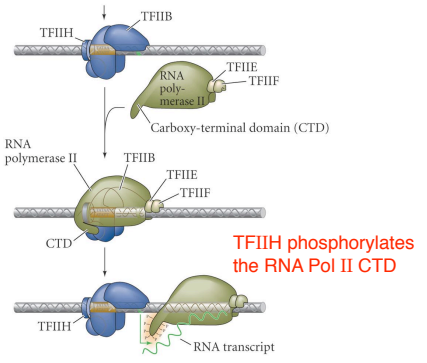
TFIID binds first, then TFIIA



Gel-shift expt

*radioactively labeled, TATA-box containing

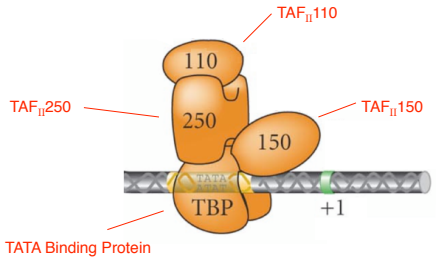
Figure 5.4(2) ***Formation of the Active Eukaryotic Transcription Initiation Complex***



TFIIH phosphorylates the RNA Pol II CTD

Structure of TFIID

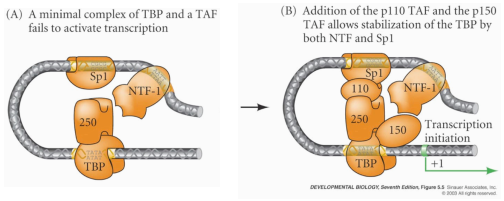
TFIID is a complex of TBP and various TAFs



A simple version of TFIID

Figure 5.5 ***Model of TAF Stabilization of TBP***

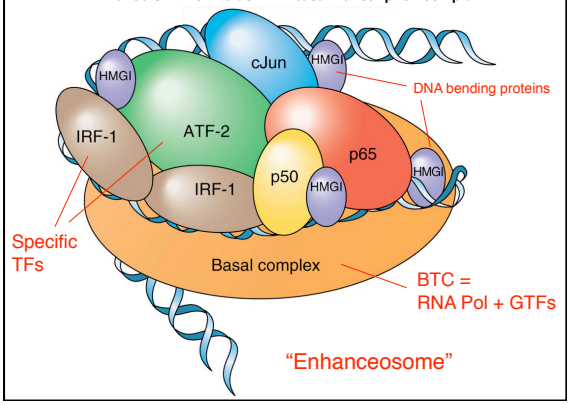
General TFs can function by interacting with specific TFs



TAFs in TFIID interact with specific TFs bound to enhancers

DNA looping allows long-distance effects by specific TFs

Enhancers + TFs interact with basal transcription complex



Eukaryotic Transcription: Other Regulators

Other regulators of Eukaryotic transcription

Players so far: RNA Pol, General TFs, Specific TFs

Mediator

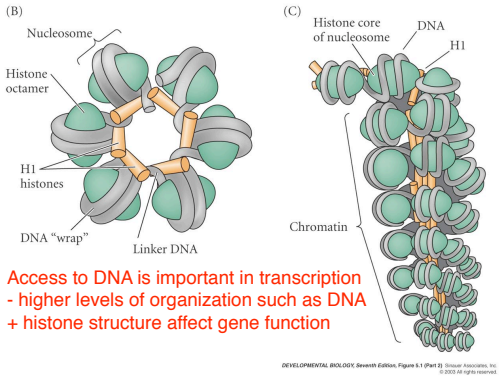
Chromatin structure modifiers

helicases

histone modifiers

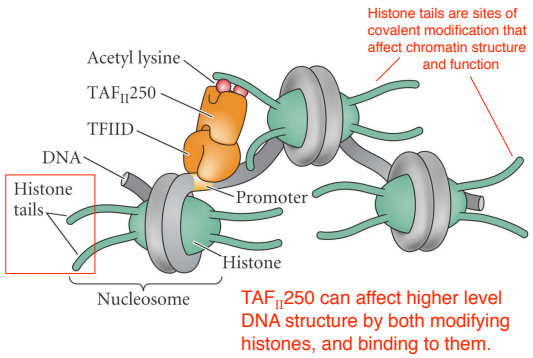
(e.g., histone acetyltransferases)

Figure 5.1(2) ***Nucleosome and Chromatin Structure***



Access to DNA is important in transcription
- higher levels of organization such as DNA + histone structure affect gene function

Figure 5.10 ***TaF$_{II}$250 Can Function as a Histone Acetyltransferase***



We are going to organize our tour of TFs a bit differently

TABLE 5.1 Some major transcription factor families and subfamilies

Family	Representative transcription factors	Some functions
Homeodomain:		
Hox	Hoxa-1, Hoxb-2, etc.	Axis formation
POU	Pit-1, Unc-86, Oct-2	Pituitary development; neural fate
LIM	Lim-1, Forkhead	Head development
Pax	Pax1, 2, 3, 6, etc.	Neural specification; eye development
Basic helix-loop-helix (bHLH)	MyoD, MITF, daughterless	Muscle and nerve specification; *Drosophila* sex determination; pigmenttion
Basic leucine zipper (bZip)	C/EBP, AP1	Liver differentiation; fat cell specification
Zinc finger:		
Standard	WT1, Krüppel, Engrailed	Kidney, gonad, and macrophage development; *Drosophila* segmentation
Nuclear hormone receptors	Glucocorticoid receptor, estrogen receptor, testosterone receptor, retinoic acid receptors	Secondary sex determination; craniofacial development; limb development
Sry-Sox	Sry, SoxD, Sox2	Bend DNA; mammalian primary sex determination; ectoderm differentiation

TYPO

TABLE 5.1 Some major transcription factor families and subfamilies

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Basic leucine zipper (bZip)	C/EBP, AP1	Liver differentiation; fat cell specification
Zinc finger:		
Standard	WT1, Krüppel, Engrailed (Wrong!)	Kidney, gonad, and macrophage development; *Drosophila* segmentation
Nuclear hormone receptors	Glucocorticoid receptor, estrogen receptor, testosterone receptor, retinoic acid receptors	Secondary sex determination; craniofacial development; limb development
Sry-Sox	Sry, SoxD, Sox2	Bend DNA; mammalian primary sex determination; ectoderm differentiation

Inaccuracy?

TABLE 5.1 Some major transcription factor families and subfamilies

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Sry-Sox	Sry, SoxD, Sox2	Bend DNA; mammalian primary sex determination; ectoderm differentiation

MITF could go here, too

DEVELOPMENTAL BIOLOGY, Seventh Edition, Table 5.1 Sinauer Associates, Inc.

Transcription Factor protein structure

Domains of Transcription Factors:

DNA-binding

Trans-activating

Protein-protein interaction

Transcription Factors

Major Classes of Eukaryotic TFs

Homeodomain (HD)

Basic Helix-loop-helix (bHLH)

Basic Leucine Zipper (bZIP)

Standard Zinc Finger

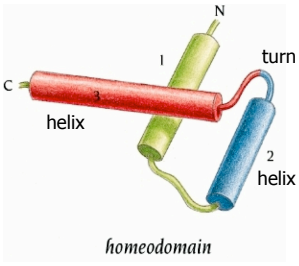
Nuclear Hormone Receptor (NHR)

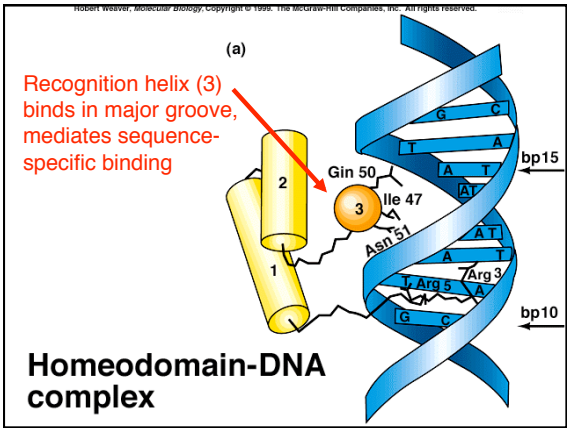
Other Zinc-binding

Transcription Factors: Homeodomain Proteins

Homeodomain TFs among the most common, widespread in animals

Homeodomain is a 60 aa DNA-binding domain of three α-helices; two form a "helix-turn-helix" motif.



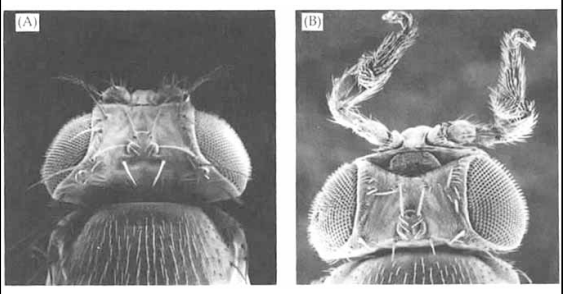


Transcription Factors: Homeodomain Proteins

The genes encoding the Homeodomain proteins were first discovered in *Drosophila* in the "homeotic" genes (whence comes the name).

Some Homeodomain genes are found in clusters, and pattern the A-P axis in all animals; these are called **Homeotic Complex (HOM-C)** genes

(A) Head of a Wild-type Fruit Fly.
(B) Head of a Fly with *Antennapedia* Mutation

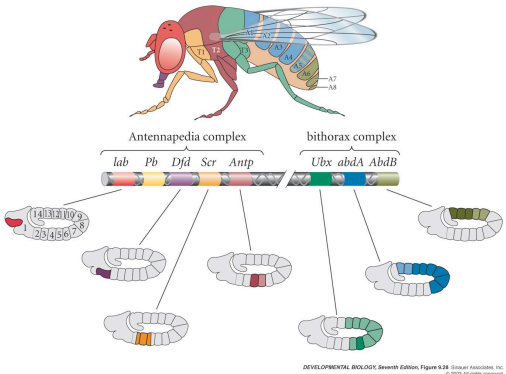


A Four-winged Fruit Fly with
Mutations in Ultrabithorax Gene Regulators

Wild type - 2 winged

Mutant - 4 winged

Homeotic Gene Complex in* Drosophila *(preview)



Transcription Factors: POU & Pax Homeodomain Proteins

There are many subclasses of Homeodomain TFs

The POU Homeodomain TFs are named for the first members of the class identified:

Pit-1: a mammalian pituitary-specific TF

Pit-1 regulates such genes as those encoding Growth Hormone, Prolactin, and Thyroid-stimulating Hormone

Oct1: a mammalian ubiquitous TF ("octamer binding")

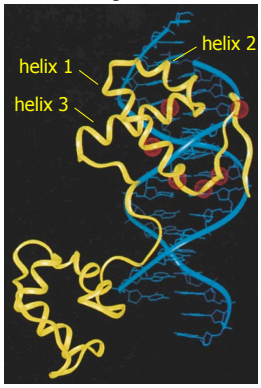
Oct2: a mammalian B-cell specific factor

Unc-86: a *C. elegans* TF determining neuronal fates

POU TFs have both a POU-homeodomain, and a POU-specific domain.

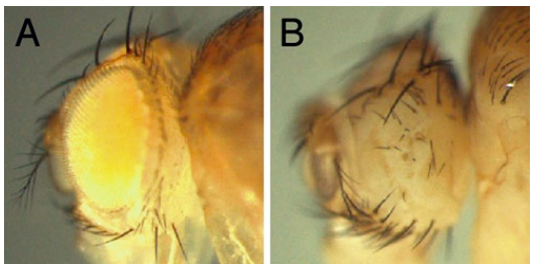
Another class includes the Paired HD TFs, such as the PAX genes

Figure 5.11 ***Pax6 Protein Binding to Its Enhancer Element in DNA***



Pax6
paired
h-t-h domain

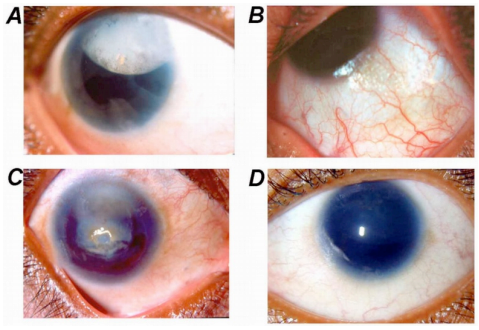
***Drosophila eyeless* mutant**



Wildtype*
*except eye color

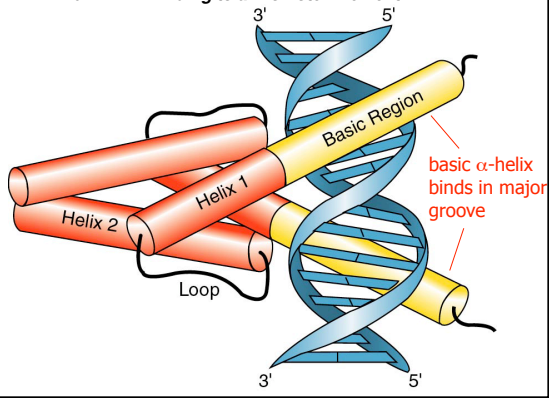
eyeless mutant
(Pax6 homolog)

Human aniridia defects (various Pax6 mutations)



Heterozygous for dominant Pax6 mutations (homozygous lethal)

bHLH TF *Binding to a Promoter Element in DNA*

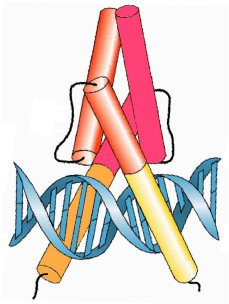


Transcription Factors: bHLH Proteins

Basic Helix-Loop-Helix TFs

bHLH TFs typically work as heterodimers:

1 ubiquitous bHLH +
1 cell-specific bHLH

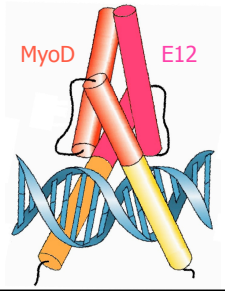


Transcription Factors: bHLH Proteins

Basic Helix-Loop-Helix TFs

Example: MyoD - myoblast determination factor -
is a muscle-specific bHLH, turns on muscle genes

MyoD works with ubiquitous
E12 or E47 HLHs

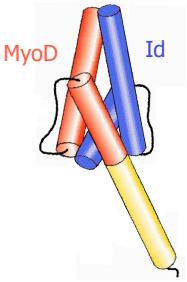


Transcription Factors: bHLH Proteins

Basic Helix-Loop-Helix TFs

Example: MyoD - myoblast determination factor
turns on muscle genes, including its own promoter
and other muscle-specifying genes

MyoD function blocked by
HLH lacking basic DNA-binding
domain:
Id (Inhibitor of differentiation).



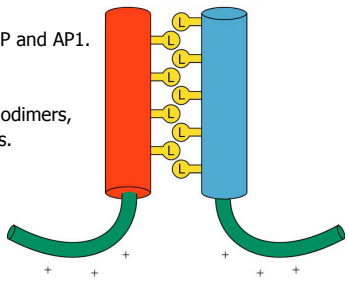
Transcription Factors: bZIP Proteins

Basic Leucine Zipper (bZIP) TFs

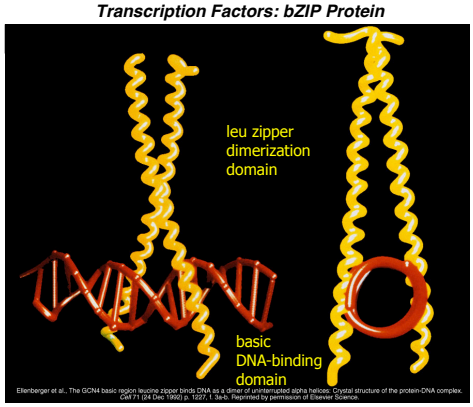
Also work as dimers, binding DNA in a similar fashion to
basic HLHs.

Examples include C/EBP and AP1.

Some function as homodimers,
others as heterodimers.



Transcription Factors: bZIP Protein



Ellenberger et al., The GCN4 basic region leucine zipper binds DNA as a dimer of uninterrupted alpha helices: Crystal structure of the protein-DNA complex. CAN 71 (24 Dec 1990) p. 1227. 1 tab. Reprinted by permission of Elsevier Science.

AP1 = Fos+Jun heterodimer

Gel shift expt with Fos, Jun heterodimer

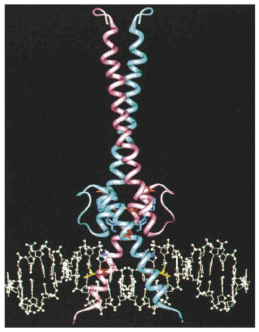
DNA bound by Fos/Jun (aka AP1)

In this expt, Fos or Jun homodimers do not bind the DNA

Labelled, unbound DNA

Figure 5.8 ***Three-Dimensional Model of the MITF Homodimer Binding to a Promoter Element in DNA***

MITF has both b-Zip and HLH-like domains



Leu-zipper

Helix-loop

Basic Helix

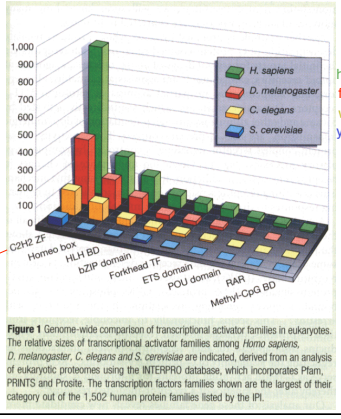
DEVELOPMENTAL BIOLOGY, Seventh Edition, Figure 5.8 Sinauer Associates, Inc.

Transcription Factors: Standard Zinc Finger Proteins

Standard Zinc Finger TFs

Very common: about 1% of all genes in completely sequenced eukaryotes have standard Zn fingers.

Whole genome comparisons show which transcription factor types are most common



C2H2 Zinc Fingers

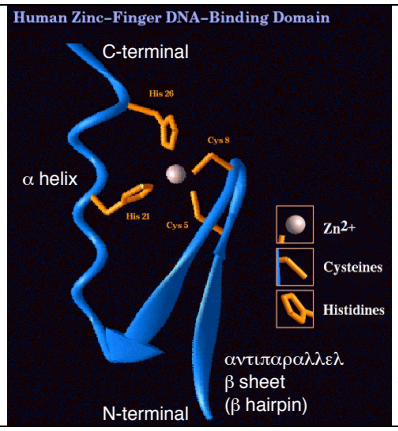
Figure 1 Genome-wide comparison of transcriptional activator families in eukaryotes. The relative sizes of transcriptional activator families among *Homo sapiens*, *D. melanogaster*, *C. elegans* and *S. cerevisiae* are indicated, derived from an analysis of eukaryotic proteomes using the INTERPRO database, which incorporates Pfam, PRINTS and Prosite. The transcription factors families shown are the largest of their category out of the 1,502 human protein families listed by the IPI.

Transcription Factors: Standard Zinc Finger Proteins

Standard Zinc Finger TFs

Very common: about 1% of all genes in completely sequenced eukaryotes have standard Zn fingers.

'Finger' is made of one alpha helix and an anti-parallel beta sheet held together at the base by a Zn^{++} ion conjugated by 2 Cys and 2 His amino acids ("C_2H_2 Zn finger")



Another Zn Finger view

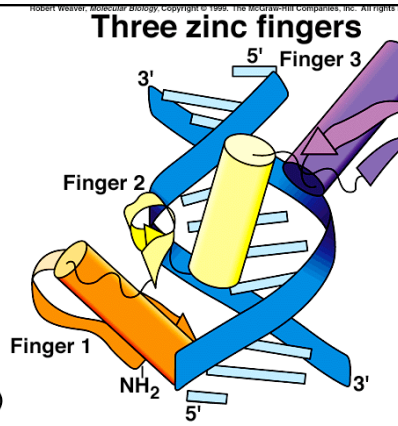
Transcription Factors: Standard Zinc Finger Proteins

Standard Zinc Finger TFs

Zn Finger inserts into major groove of DNA, TFs often have multiple fingers, with several side-by-side in the major groove.

Examples include:
TFIIIA (a General TF) - first discovered (*Xenopus*), has nine fingers, activates 5S rRNA genes

Drosophila gap genes: Hunchback, Krüppel



Transcription Factors: Nuclear Hormone Receptors

Nuclear Hormone Receptor (Zn binding) TFs

Also very common, but restricted to multicellular organisms -- not found in unicellular eukaryotes -- associated with cell-cell signaling.

Structure of 'finger' is completely different from Standard Zn finger.

'Fingers' are always in pairs, each held together by Zn^{++} typically conjugated by 4 Cys ("C_4"). Most bind DNA as dimers (so, 4 NHR-type Zn fingers all together).

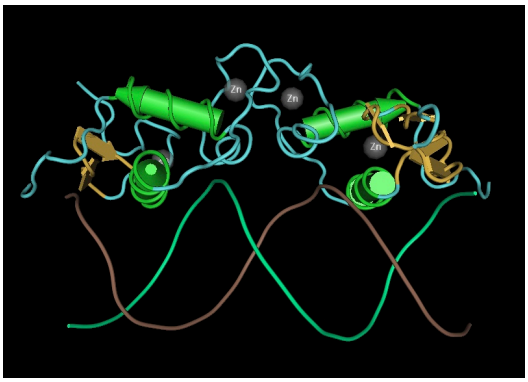
Examples include:

Steroid receptors: estrogen, testosterone, glucocorticoid receptors

Retinoic acid, thyroid hormone and vitamin D receptors

'Orphan' NHR TFs - many of these do not require ligands (those that don't are not actually receptors)

NHR dimer bound to DNA

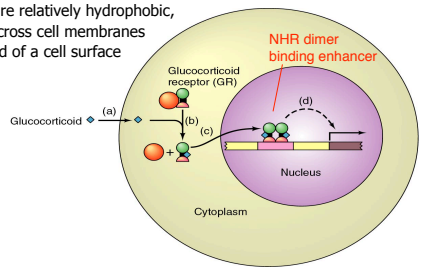


Transcription Factors: Nuclear Hormone Receptors

Nuclear Hormone Receptor TFs (continued)

NHR binds ligand in the cytoplasm, then enters the nucleus to act as a transcription factor (binds enhancers, activates genes)

NHR ligands are relatively hydrophobic, so can pass across cell membranes without the aid of a cell surface receptor.



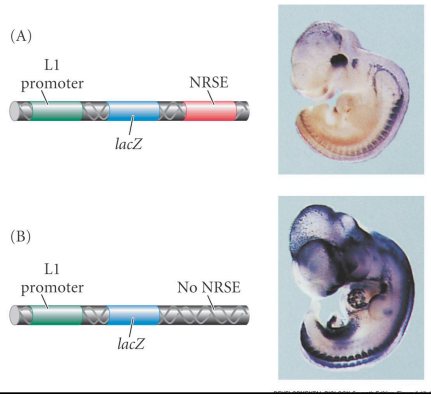
Transcription Factors: Inhibiting Transcription

Silencers and Inhibition of Transcription

Some TFs bind to 'enhancer' elements to keep genes OFF - such sequences are called 'Silencers

Example: Neural restrictive silencer element (NSRE) which is bound by a TF found in all non-neuronal cells.

Figure 5.17 ***Silencers***



Transcription Factors act synergistically

Strong gene expression usually requires binding several TFs to enhancers associated with the gene.

Example:

Regulation of **Prolactin gene** by **Pit-1** & **Estrogen Receptor**

in vitro test of prolactin gene transcription (cells in culture)

Basal transcription:	1X
+ Pit-1:	~80X
+ Estrogen/ER:	~5X
+ Pit-1 & Estr/ER:	~1400X

Effect was much greater than additive