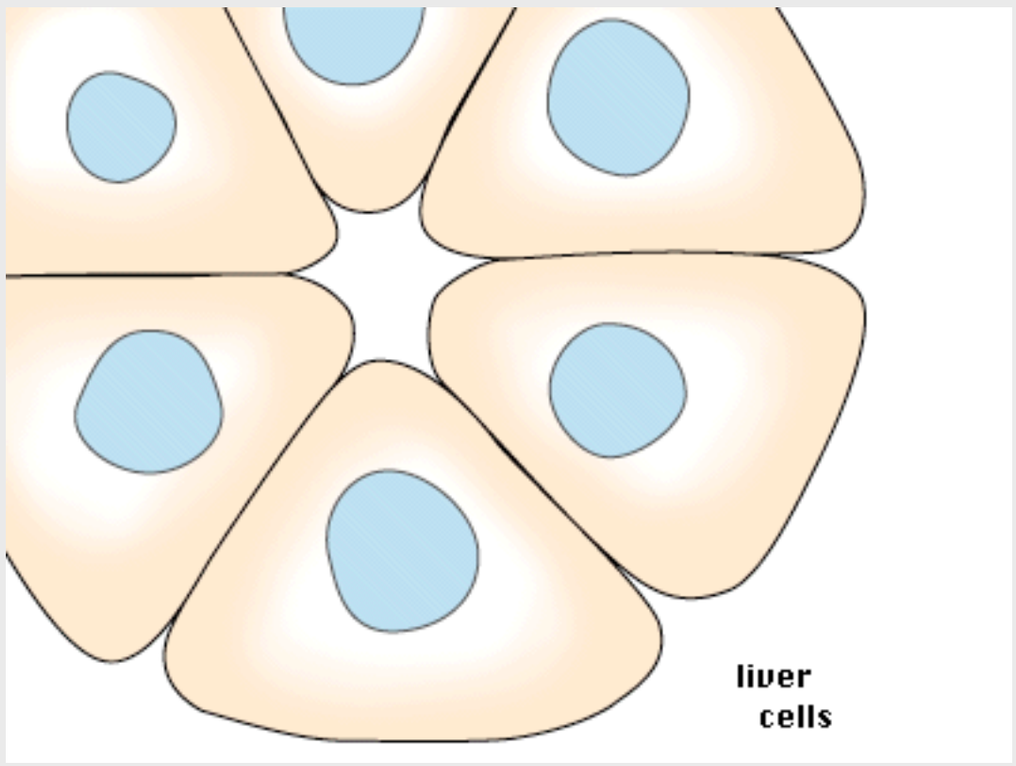
Transcription Activators in Eukaryotes

Chapter 12

Techniques

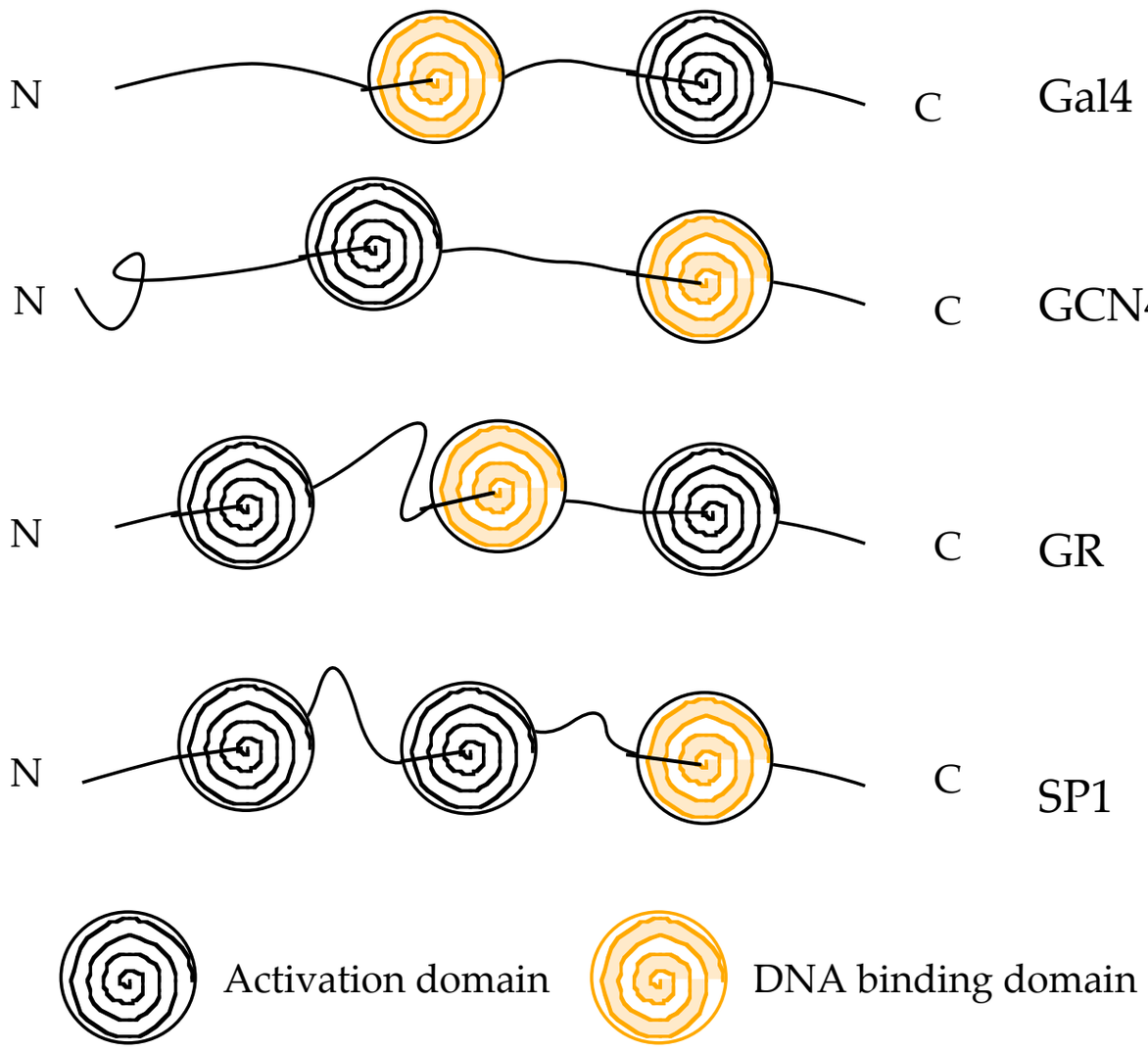
* Run-off transcription
* Primer extension
* Quantitative S1 mapping


liver
cells

Modular

- Protein domain is an independingly folding region.
  - DNA binding domain
  - Transcription-activation domain
  - Dimerization domain
    most do not bind without dimerization
    - homodimers, heterodimers

homodimers, heterodimers, tetramers (rare)
some bind small molecules (eg. steroid hormones).



From Molecular Cell Biology 3rd edition, Lodish et al Scientific American Books 1995

protein domain independently folds

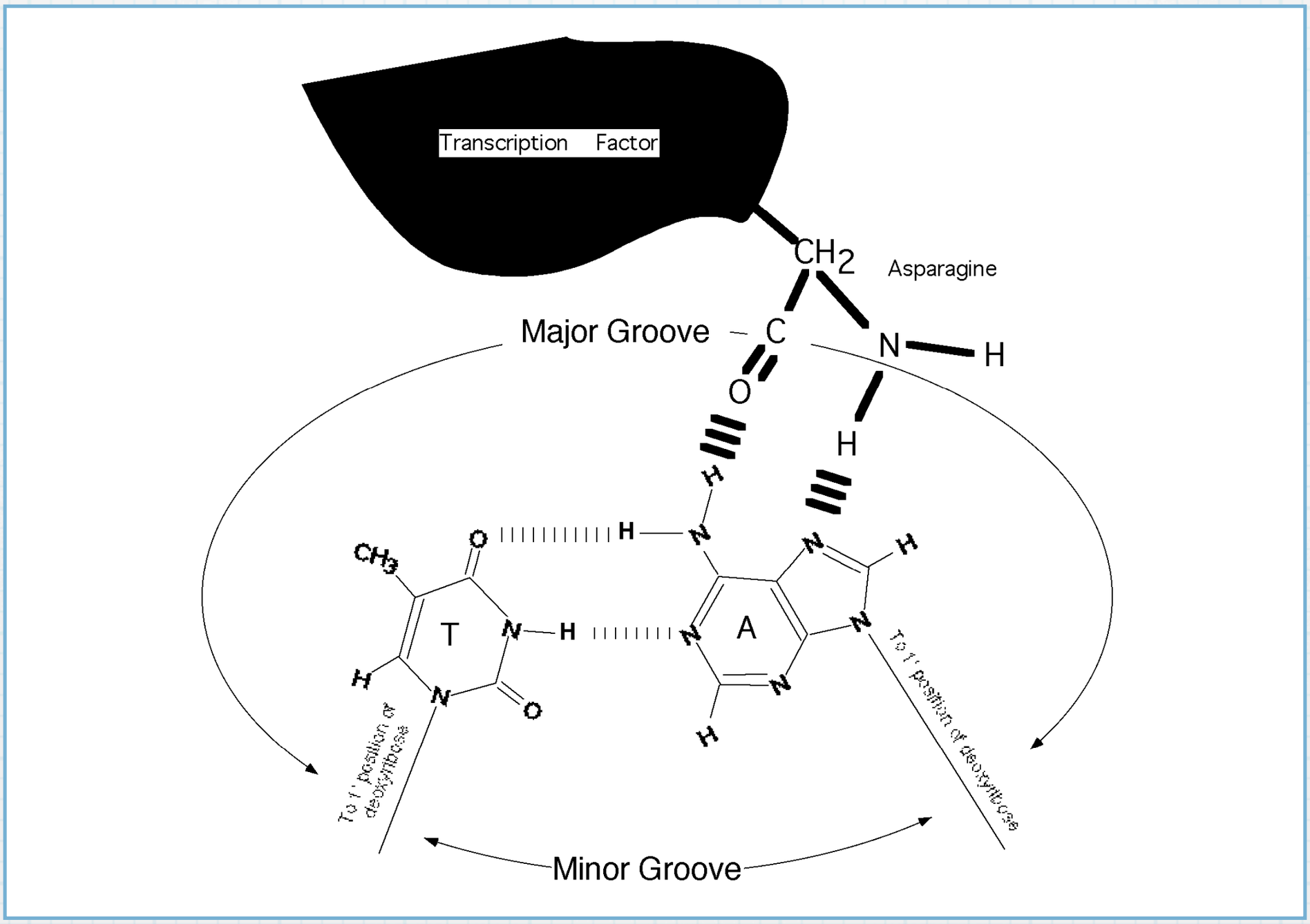
It has been estimated that 8-10% of the proteins coded in the genome are specifically for regulating the transcription of other proteins, yielding more than 1000 in Drosophila alone (Tupler et al., 2001; Adams et al., 2000). (Reference: Moon Draper Thesis, University of Texas at Austin 2005).

Activation domains

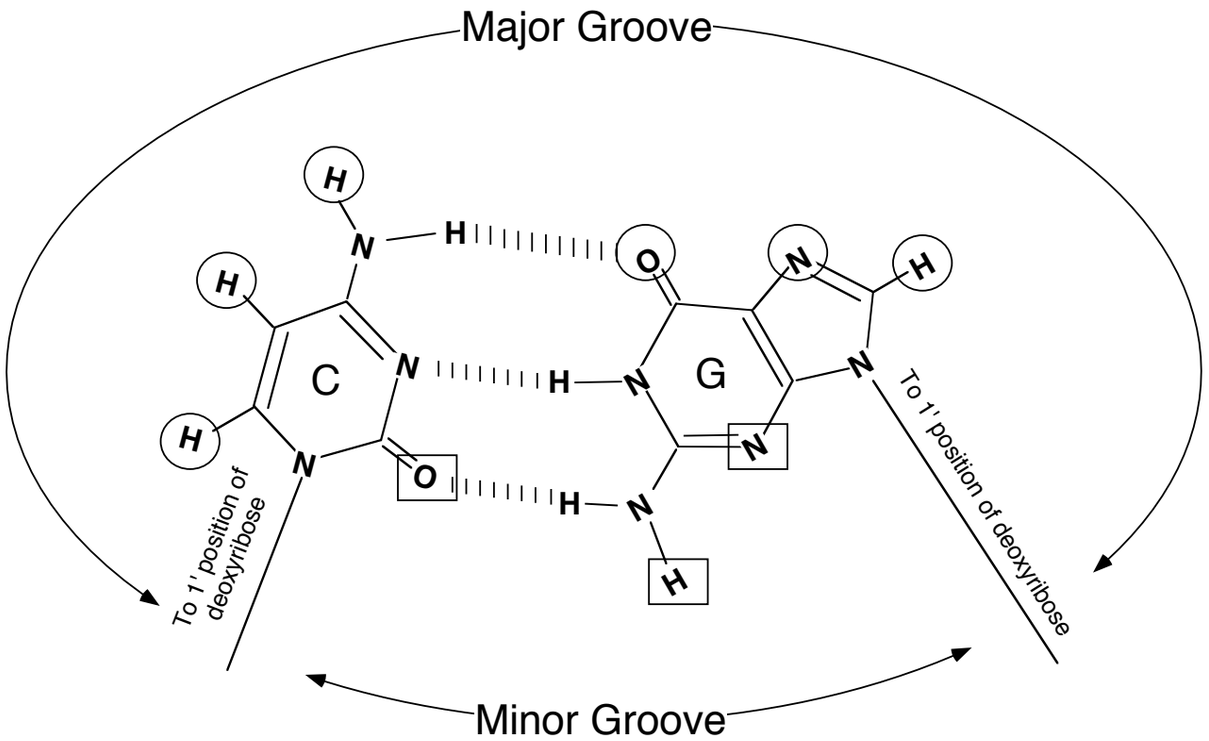
* acidic domains - acidic Gal4
* Glutamine rich - Sp1 ~25% glutamine
* Proline-rich - CTF is >20% proline

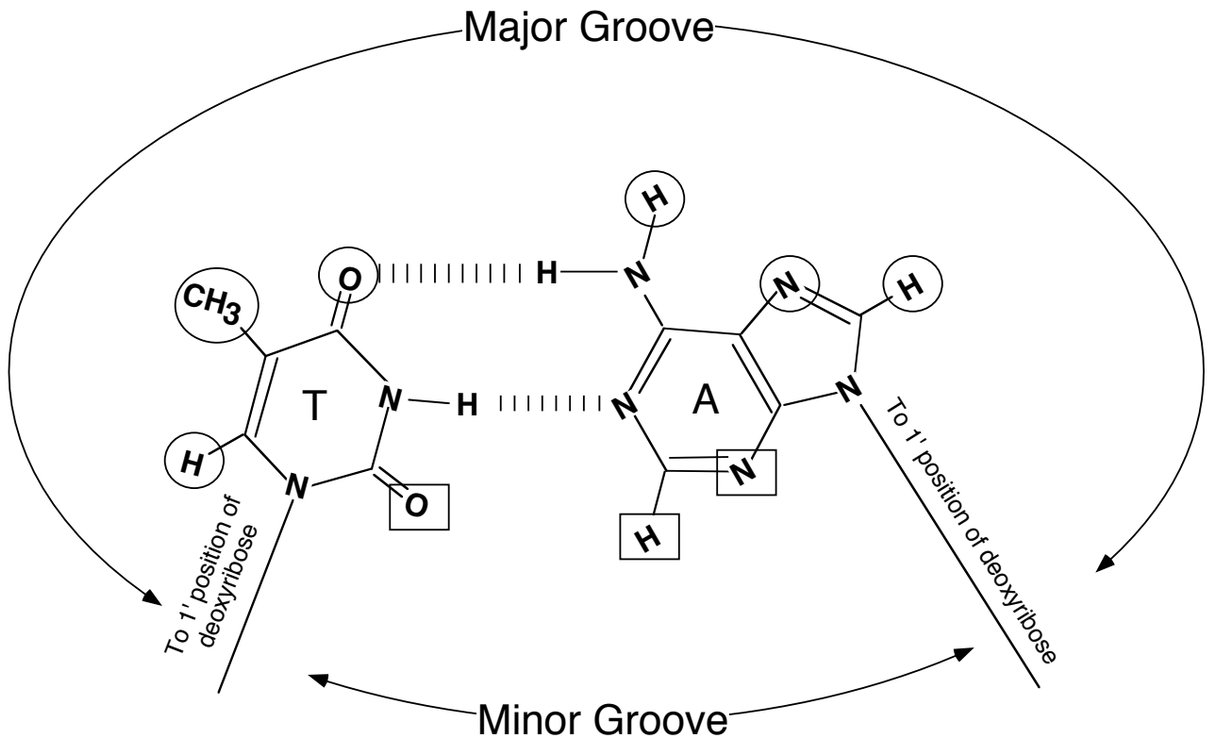
http://www.sanger.ac.uk/cgi-bin/Pfam/getacc?PF00859
CTF/NF-I binding sites are present in viral and cellular promoters and in the origin of DNA replication of Adenovirus type 2.

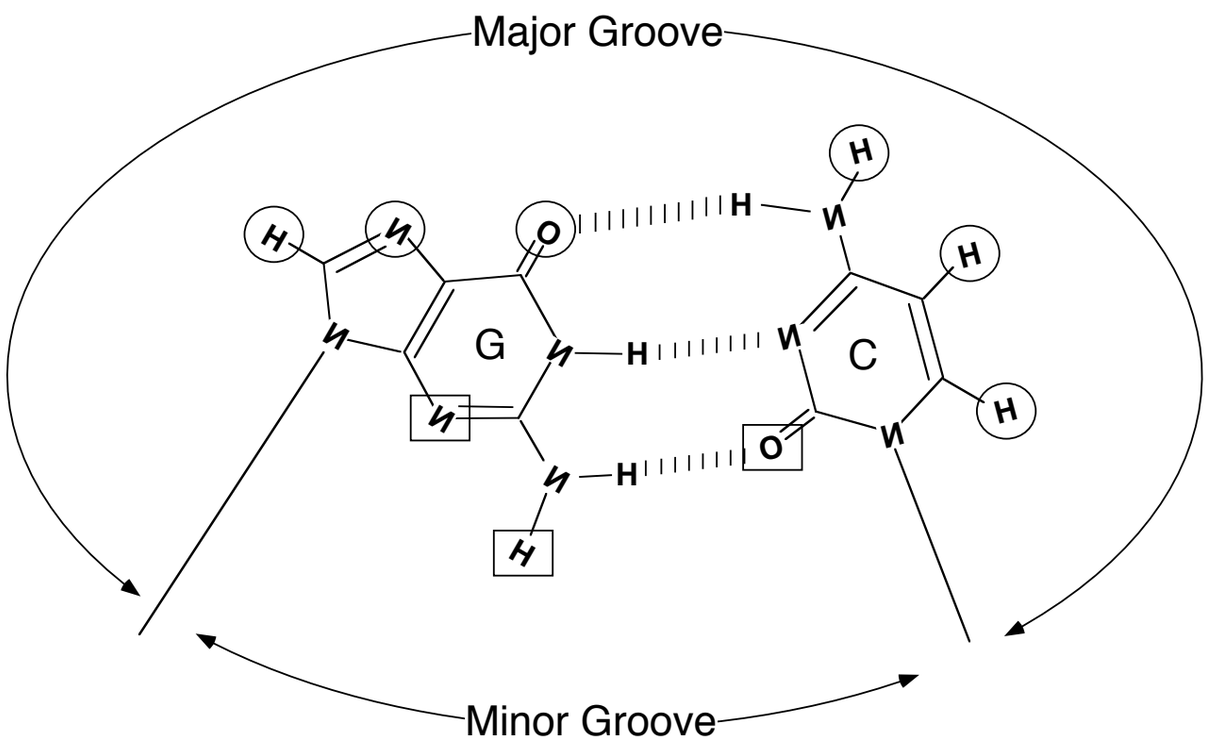
Review. Most TF use major groove.

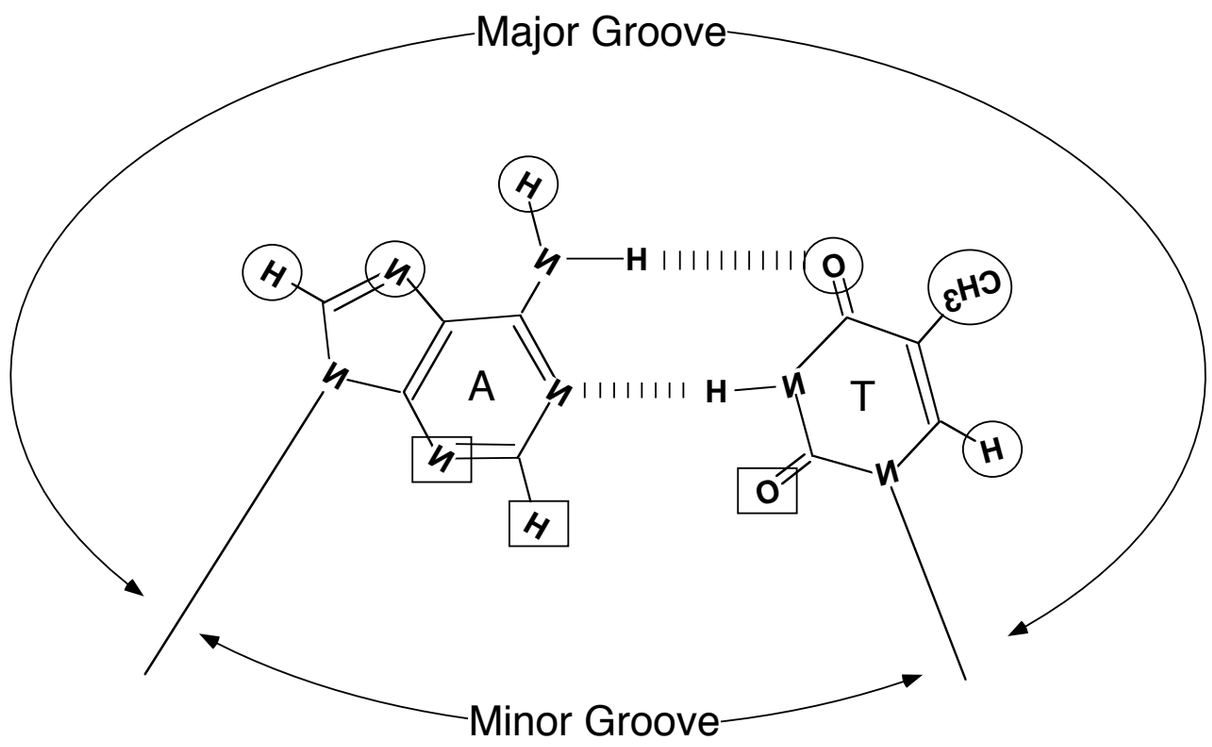


Most TF use major groove.







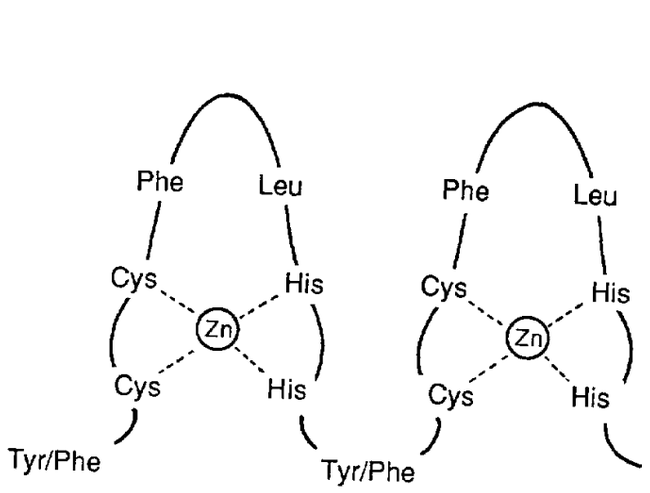


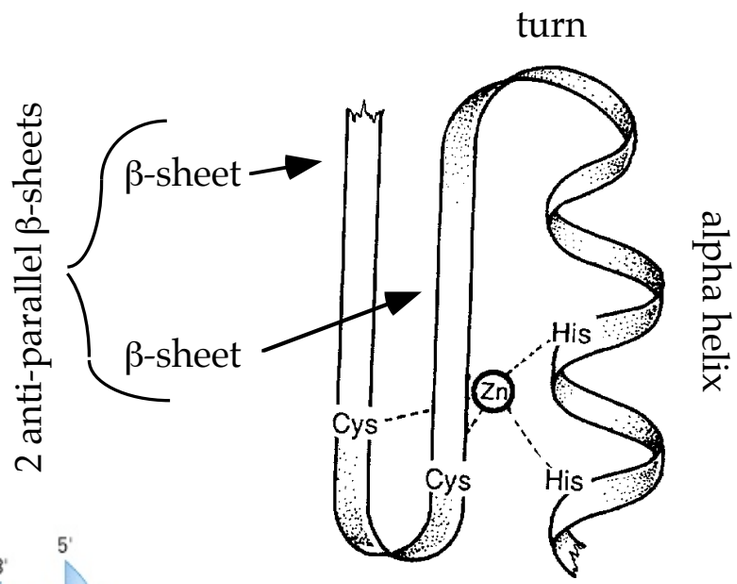
DNA binding domains

* Zinc containing domains <- eukaryotic
* Homeodomains <- eukaryotic
* bZIP bHLH <- eukaryotic

Zinc Fingers

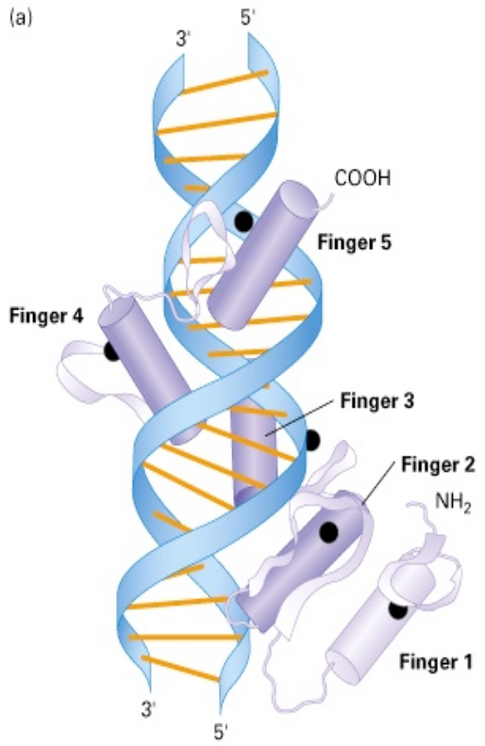
C2H2 Zinc Finger





EXCEPTION

Don't need to dimerize.



All are in the major groove

Examples are TFIIIA & zif268

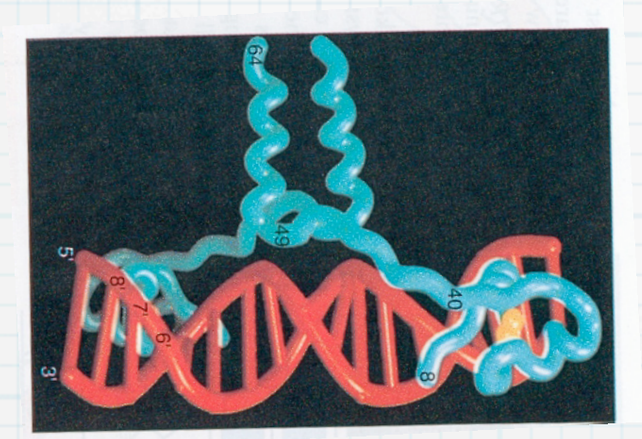
Fig 12.2 and 12.3

Zinc Fingers

Basic residues on 'outside' of alpha helix. These are used to interact/read/bind the DNA.
B-strands interact with DNA backbone and position the alpha helix so that it can read the DNA.
Abt 23 AA.
Characterized by the sequence CX2-4C....HX2-4. <-- consensus may be incorrect. Need to verify.

C6 Zinc containing TF

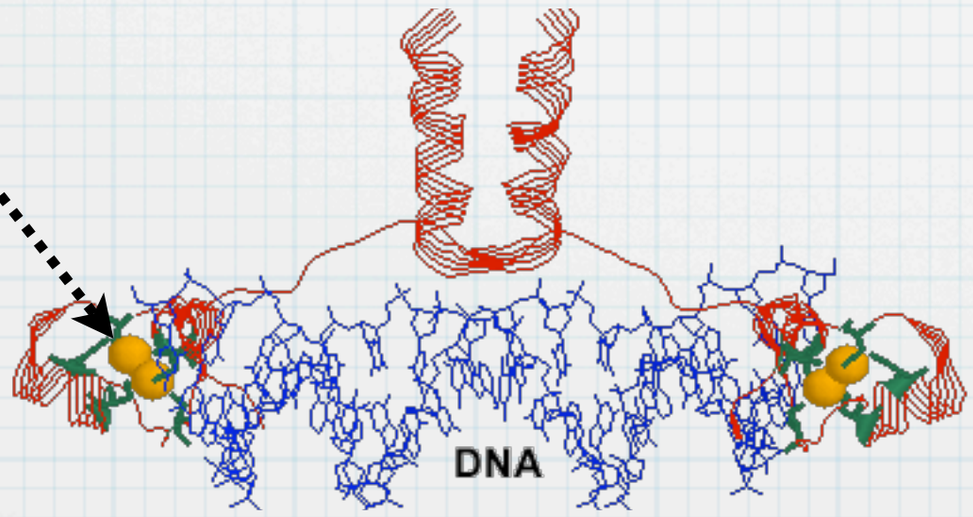
Dimer



alpha helix dimerization domain

N-terminus DNA binding domain with two Zns

2 zinc atoms



example Gal4

consensus sequence
$CX_2CX_6CX_{5\text{-}6}CX_2CX_6C$

Zinc Fingers

consensus sequence CX2CX6CX5-6CX2CX6C
Top pictures are from your textbook.
Bottom one is from http://www.web-books.com/MoBio/Free/Ch4F2.htm#4F3

Gal4

Nuclear Receptor C4 Zn finger

* Nuclear receptors
* Hormones diffuse through the cell membrane enter the nucleus and bind these receptors.
* The enhancers that they bind are sometimes called Hormone Response Elements.
* Each one binds different small molecules eg. Androgens, estrogens, progesterone, glucocorticoids, vitamin D, thyroid hormone, retinoic acid. Ligand binding domain is separate from DNA binding domain.

“C4 zinc finger: Its consensus sequence is CX2CX13CX2CX14-15CX5CX9CX2C. The first four cysteine residues bind to a zinc ion and the last four cysteine residues bind to another zinc ion (Figure 4-F-2).” Reference is http://www.web-books.com/MoBio/Free/Ch4F2.htm#4F3
DNA and ligand binding domains are separate.

Nuclear Receptor C4 Zn finger

- All are ligand-dependent activators of transcription.
- How do the activate transcription?
  - Bind a co-activator complex.
    - Includes CBP/p300
      - Acetylates histones.

Some info from Genes VIII Lewin pg 646.
“C4 zinc finger: Its consensus sequence is CX2CX13CX2CX14-15CX5CX9CX2C. The first four cysteine residues bind to a zinc ion and the last four cysteine residues bind to another zinc ion (Figure 4-F-2).” Reference is http://www.web-books.com/MoBio/Free/Ch4F2.htm#4F3
DNA and ligand binding domains are separate.

Nuclear Receptors

* Three general types

Type I Hormonal

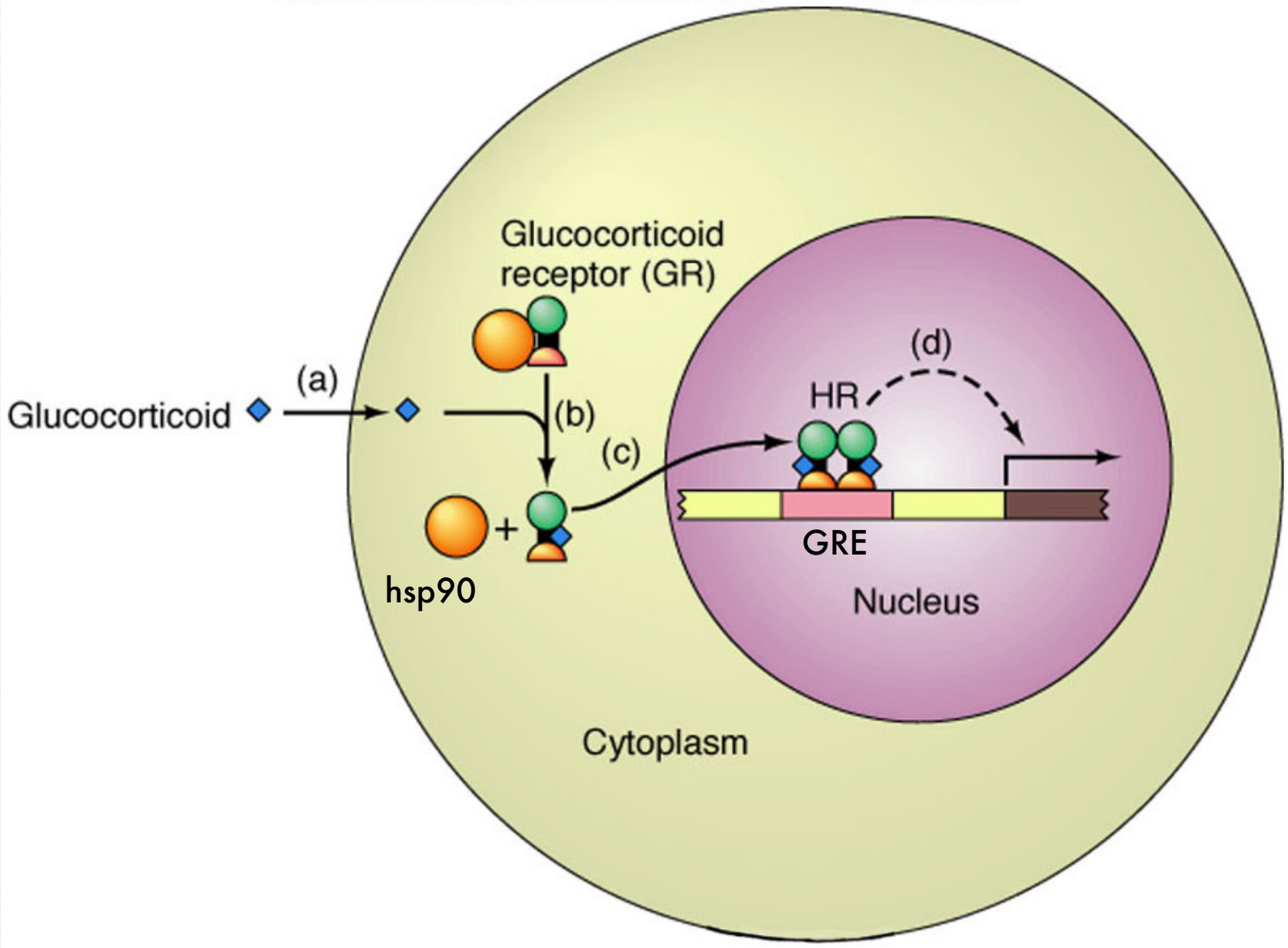
* glucocorticoid receptor
* androgen receptor
* mineralcorticoid receptor
* progesterone receptor

Binds as a dimer.

Zinc Fingers

Book describes as Type I.
Move from cytoplasm to nucleus.
The GRE is 2 short half sites.
One Zn finger does DNA binding the other does dimerization!
The dimerization determines what the spacing between the half-sites should be. Homodimers recognize palindromic sites. (Genes VIII, Lewin pg 646)
The major hormones secreted by the adrenal cortex are cortisol, aldosterone and dehydroepandrosterone (13).....Aldosterone, the major mineralocorticoid of the body, stimulates sodium reabsorption and potassium excretion by the kidney. (Ref: Spinal Cord Medicine 2003).

Type I Hormonal



Binds as a dimer.

Zinc Fingers

Book describes as Type I.
Move from cytoplasm to nucleus.
The GRE is 2 short half sites.
One Zn finger does DNA binding the other does dimerization!
The dimerization determines what the spacing between the half-sites should be. Homodimers recognize palindromic sites. (Genes VIII, Lewin pg 646)

GR can activate or repress: “Furthermore, we show that GR acts both as a direct inhibitor of CREB binding protein (CBP)-associated HAT activity and also by recruiting HDAC2 to the p65-CBP HAT complex. “Glucocorticoid Receptor Recruitment of Histone Deacetylase 2 Inhibits Interleukin-1-Induced Histone H4 Acetylation on Lysines 8 and 12 Kazuhiro Ito, Peter J. Barnes, and Ian M. Adcock*

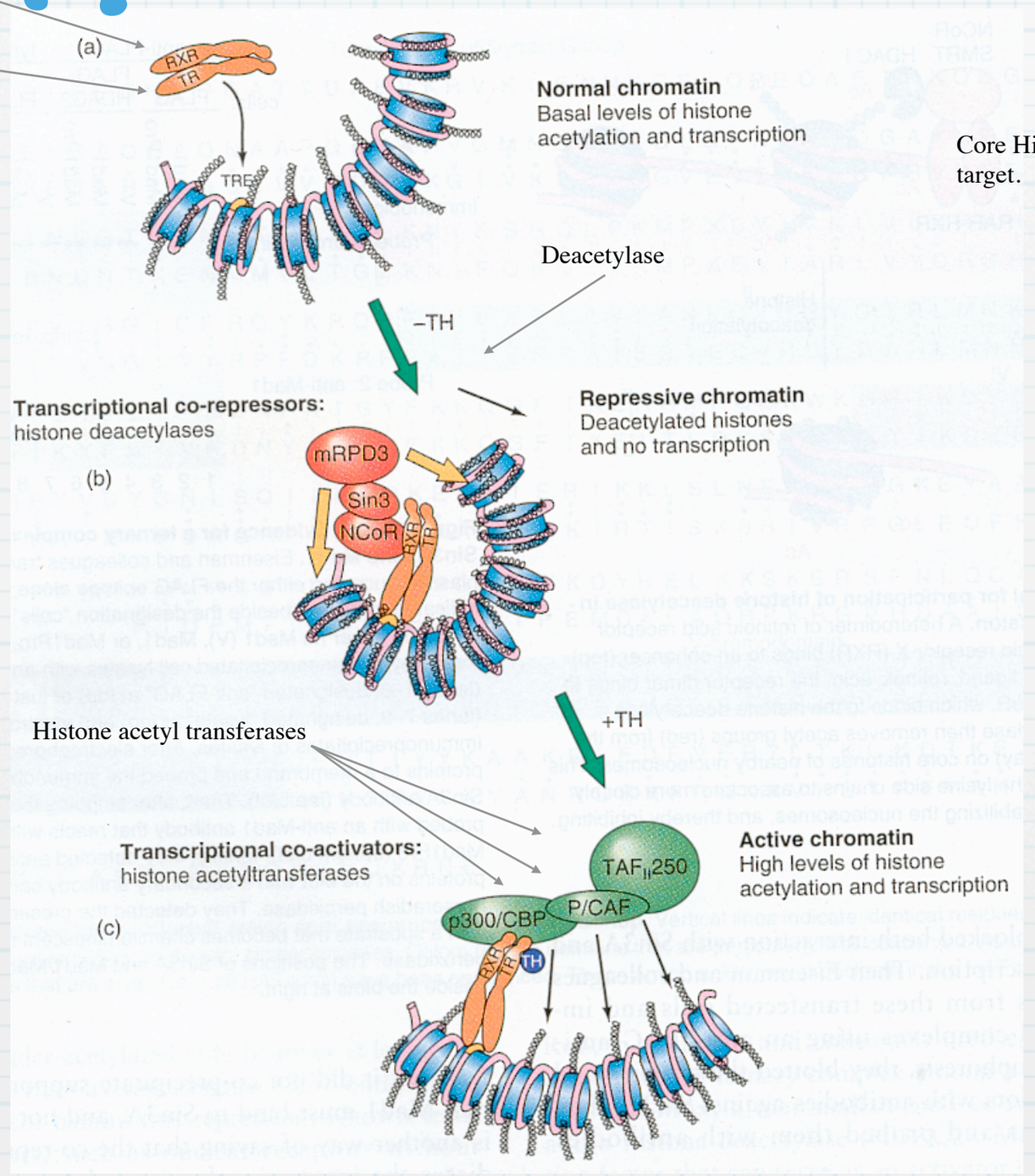
Type II Hormonal

retinoic acid receptor X

Thyroid hormone receptor heterodimer is active

GRE can be several kb up or downstream of the promoter.

Without thyroid hormone it is a repressor



Core Histones are the target.

thyroid hormone receptors & relatives.

Zinc Fingers

Book describes as Type II.
Stay in nucleus.
Retinoic Acid (Vitamen A) important for development in chicks of anterior posterior axis in embryogenesis.
These act as receptors in the same way that the lacI repressor is a receptor.

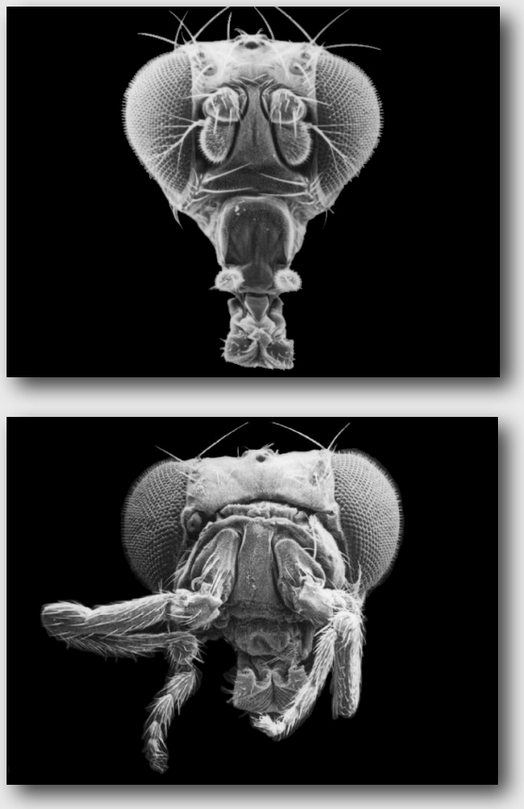
Type III

* Orphan Receptors - one whose ligand has not been found.

An orphan receptor is one whose ligand has not been found.

Homeotic Genes in Drosophila

These are transcription factors that act to determine the identify of specific tissue or body parts. Mis-expression can cause, during development, the incorrect body part or tissue to be produced in a particular position.



Homeodomain

Some appear to bind as monomers, some as homodimers, and some as heterodimers. Dimerization domains such as leucine zippers might be found with some of these.

Homeotic Genes in Drosophila

Found in almost all eukaryotes

The term homeodomain refers a 60 AA motif which is actually a specialized version of the HTH motif.

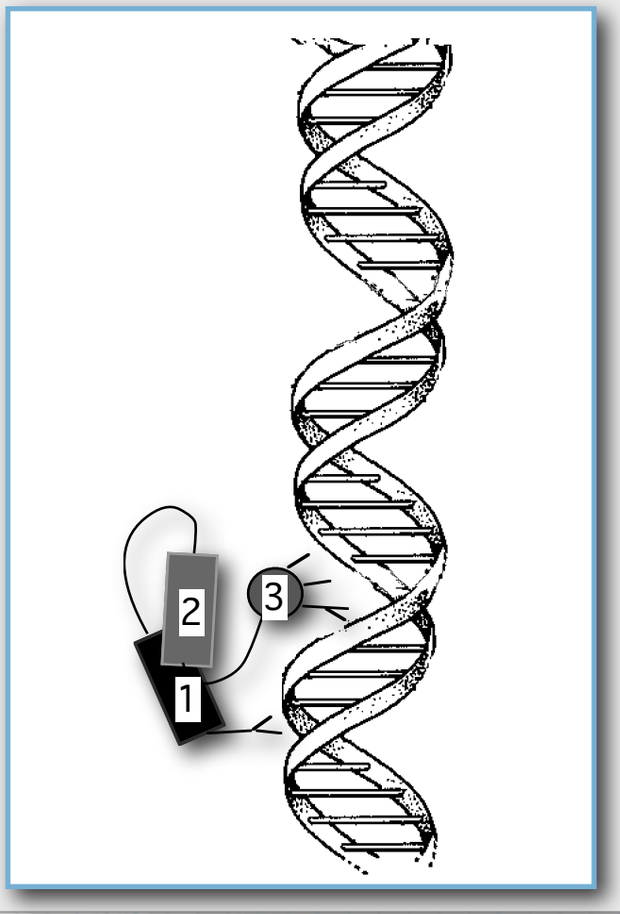
3 helixes. #2 & #3 like are related to the bacterial HTH motif
Hydrophobic interactionss hold each helix in position relative to its neighbor.

Usually found near the C-terminus

N terminal part inserts into minor groove

Some bind as monomers, some dimers, some both

Some are repressors, some are activators



Homeodomain

These are transcription factors that act to determine the identify of specific tissue or body parts. Mis-expression can cause, during development, the incorrect body part or tissue to be produced in a particular position.

The term homeodomain refers a 60 AA motif which is actually a specialized version of the HTH motif.

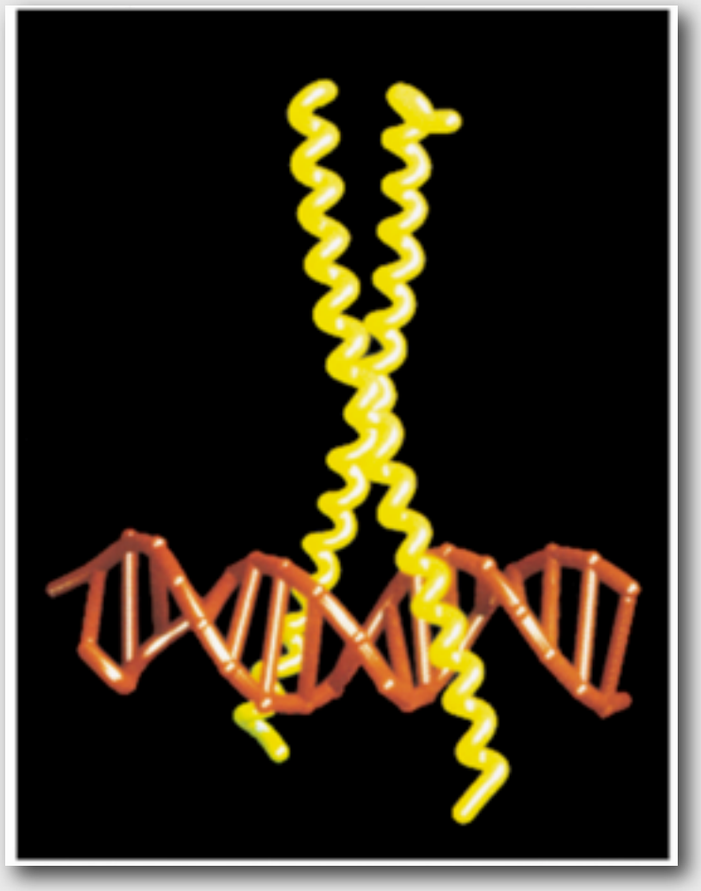
Three protein helixes are involved here. Helix #2 and #3 represent the HTH part of the protein.

Hydrophobic interactshold each helix in position relative to its neihbor.

Homeodomain proteins bind as a monomer (Prokaryotic HTH binds as dimers)

Basic Zipper
bZip

- 2 proteins
- each with a half zipper
  - amphipathic alpha helix
- **Binding and dimerization functions are combined in the same domain.**
- Examples: CREB, Jun, Fos



Targets for homodimer binding are inverted repeats with no separation.
Examples are
CREB ATF/CREB family of basic leucine zippers binds a DNA motif called the cAMP response element which is abbreviated CRE.
jun & fos
jun forms homo or heterodimers
fos forms only heterodimers
together called AP1 and bind AP1 sites 10X better than jun homodimers.
Lewin Genes VIII pg 652

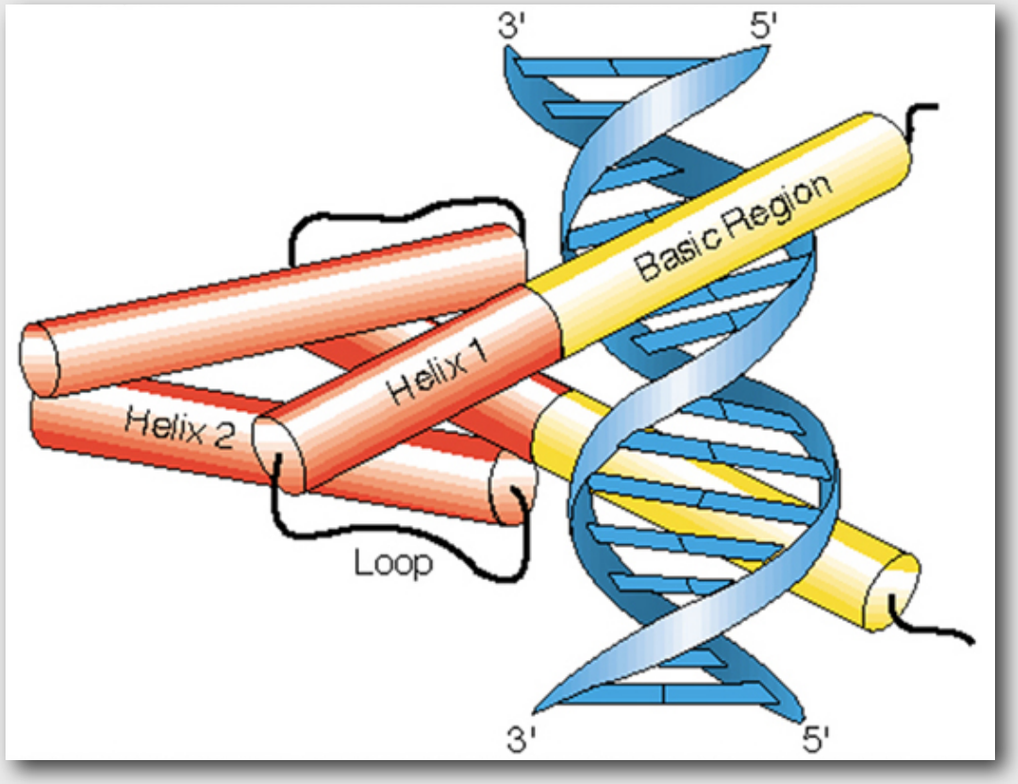
Basic Helix-loop-helix bHLH

Two amphipathic helices causes dimerization

* **Binding and dimerization functions are combined in the same domain.**

Helix 1 also contains basic amino acids

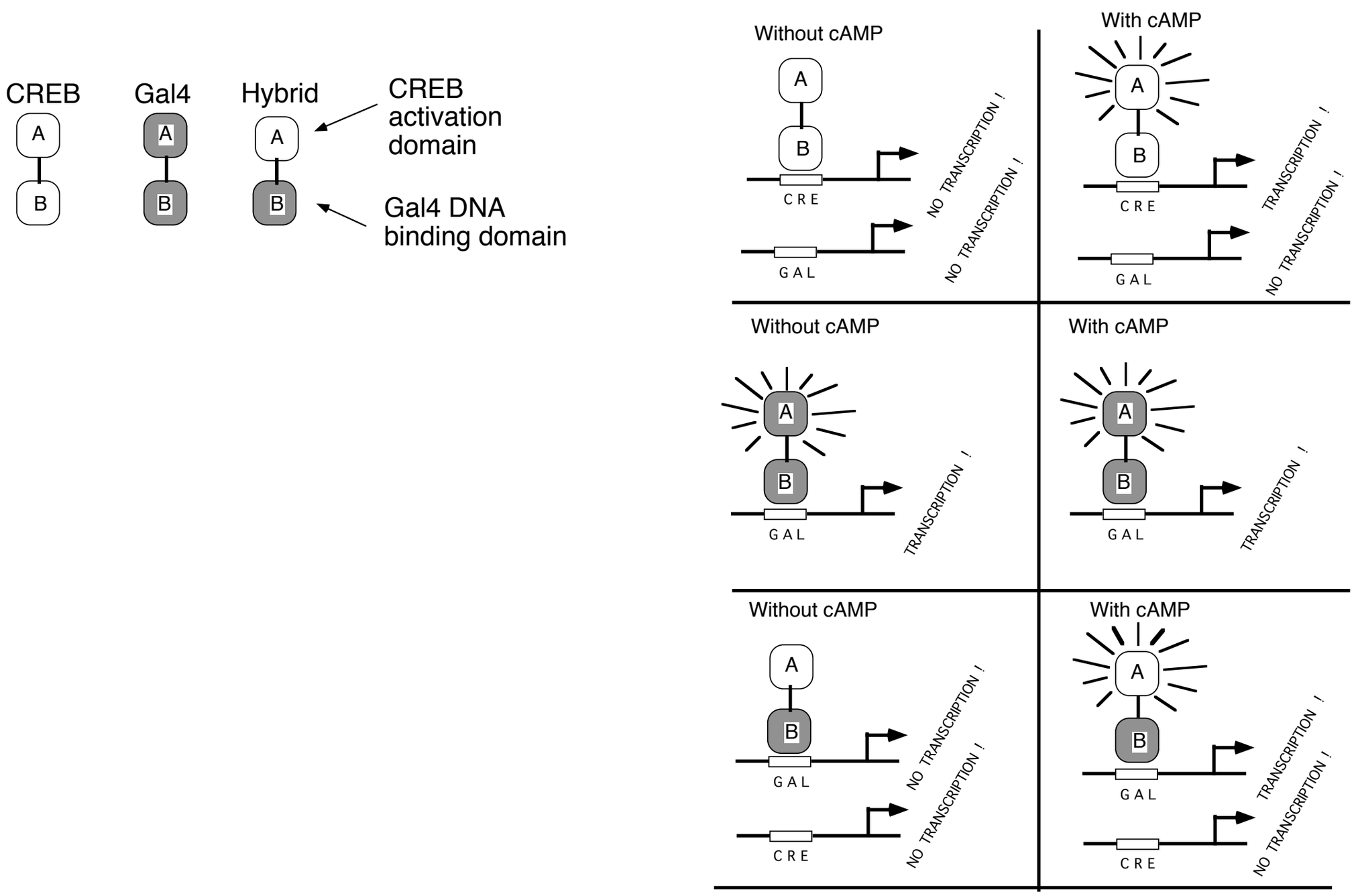
Homodimers & heterodimers form



MyoD, Myc and Max are examples

MyoD is an example
Figure is from page 351 of Weaver, 3rd edition.
Amphipathic helix has one hydrophilic face and one hydrophobic face.
“It has been estimated that 8-10% of the proteins coded in the genome are specifically for regulating the transcription of other proteins, yielding more than 1000 in Drosophila alone (Tupler et al., 2001; Adams et al., 2000). (Reference: Moon Draper Thesis, University of Texas at Austin 2005).”

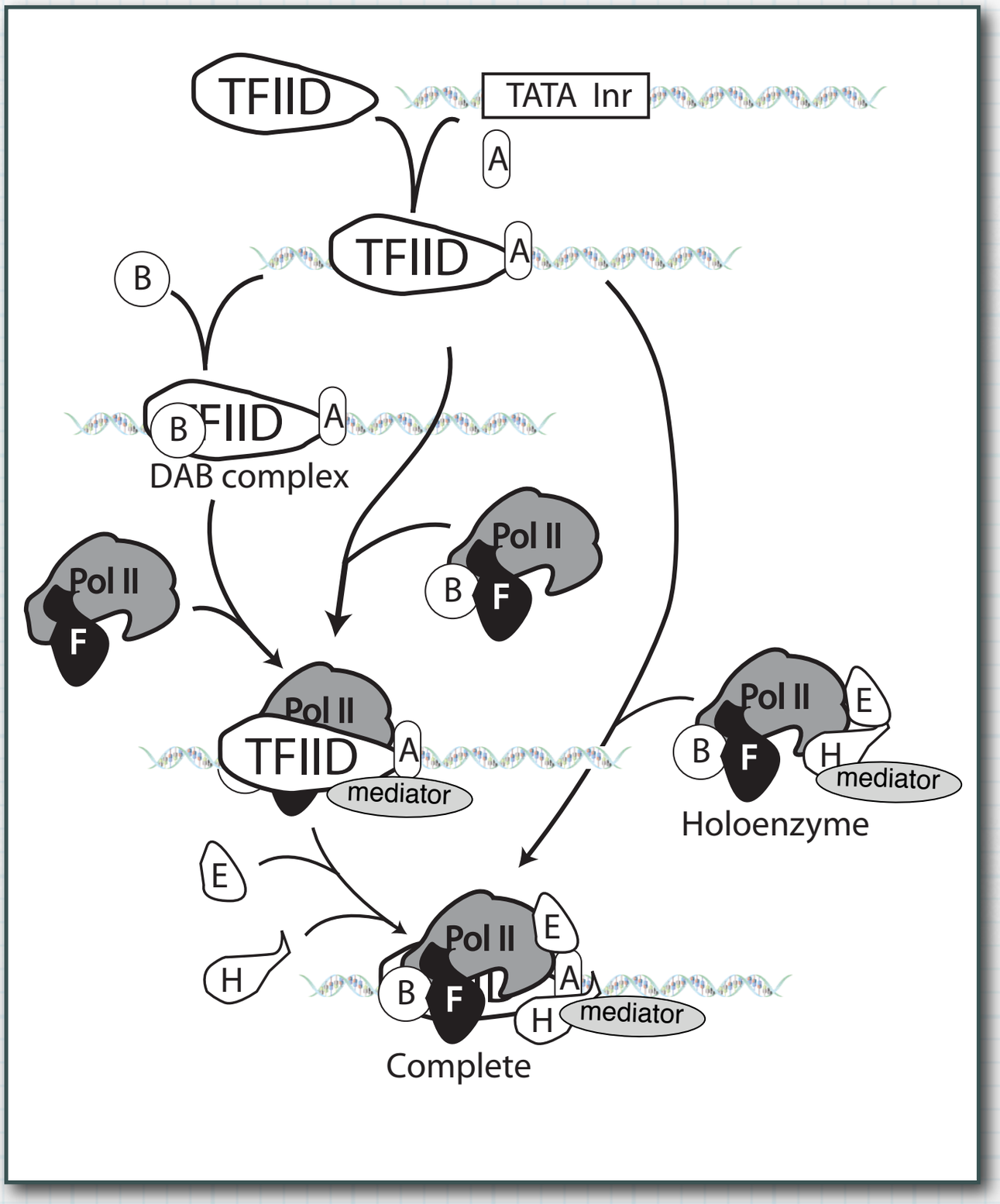
Independence



Weaver gives a different but comparable example in Fig 12.13 pg 331 4th edition.

Weaver gives a different example in Fig 12.13 pg 352 3rd edition.

An activation domain can recruit general transcription factors

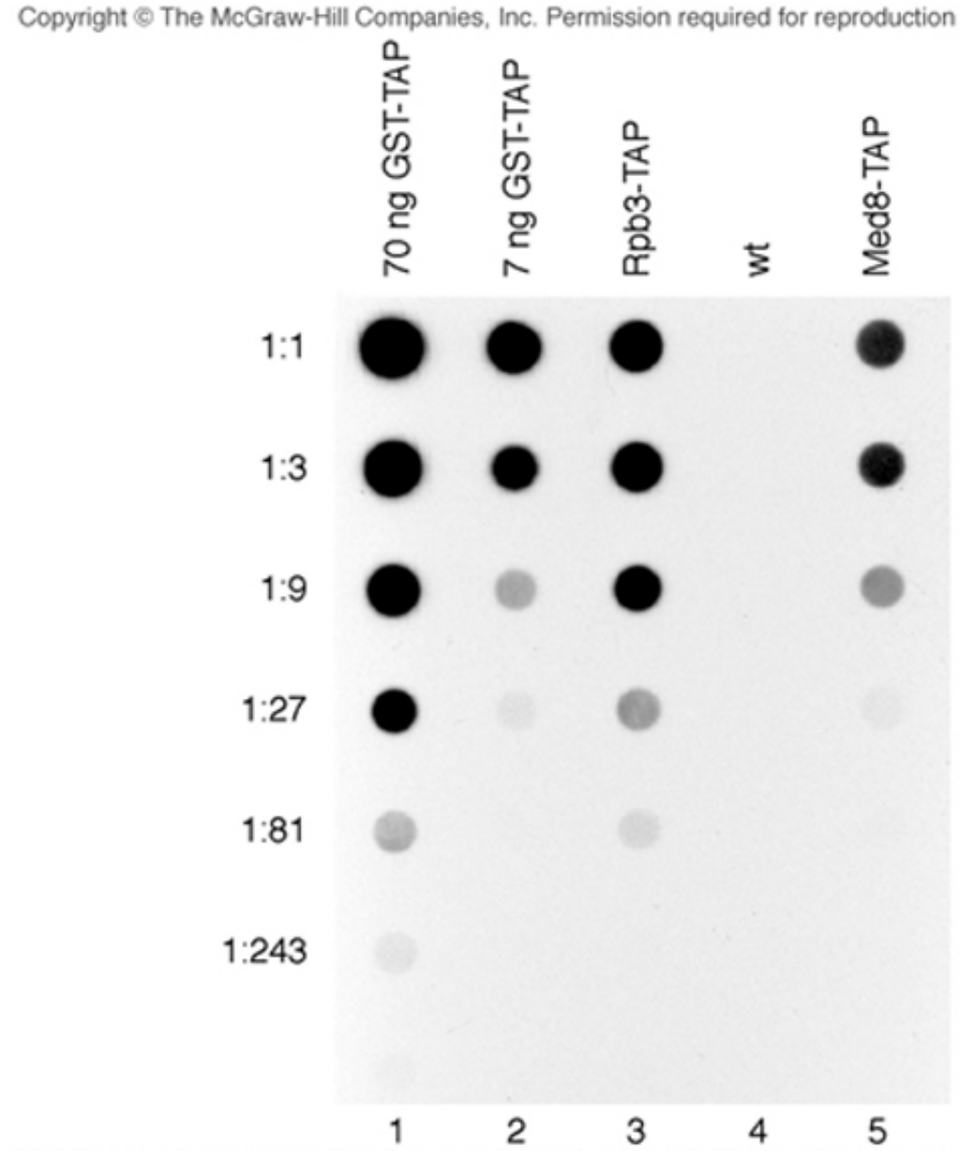


Step by step recruitment or holoenzyme recruitment?

* pg 333-335 Subtitle: Evidence of recruitment of the holoenzyme as a unit.
  * First they describe exp that suggest it is a unit. Then they describe exp that suggest it is not.
  * Other experiments indicate that it can happen both ways.

Figure 12.18

How does one quantify the components?



TAP tagged RNA polymerase II & Mediator

Dot blot cell extract

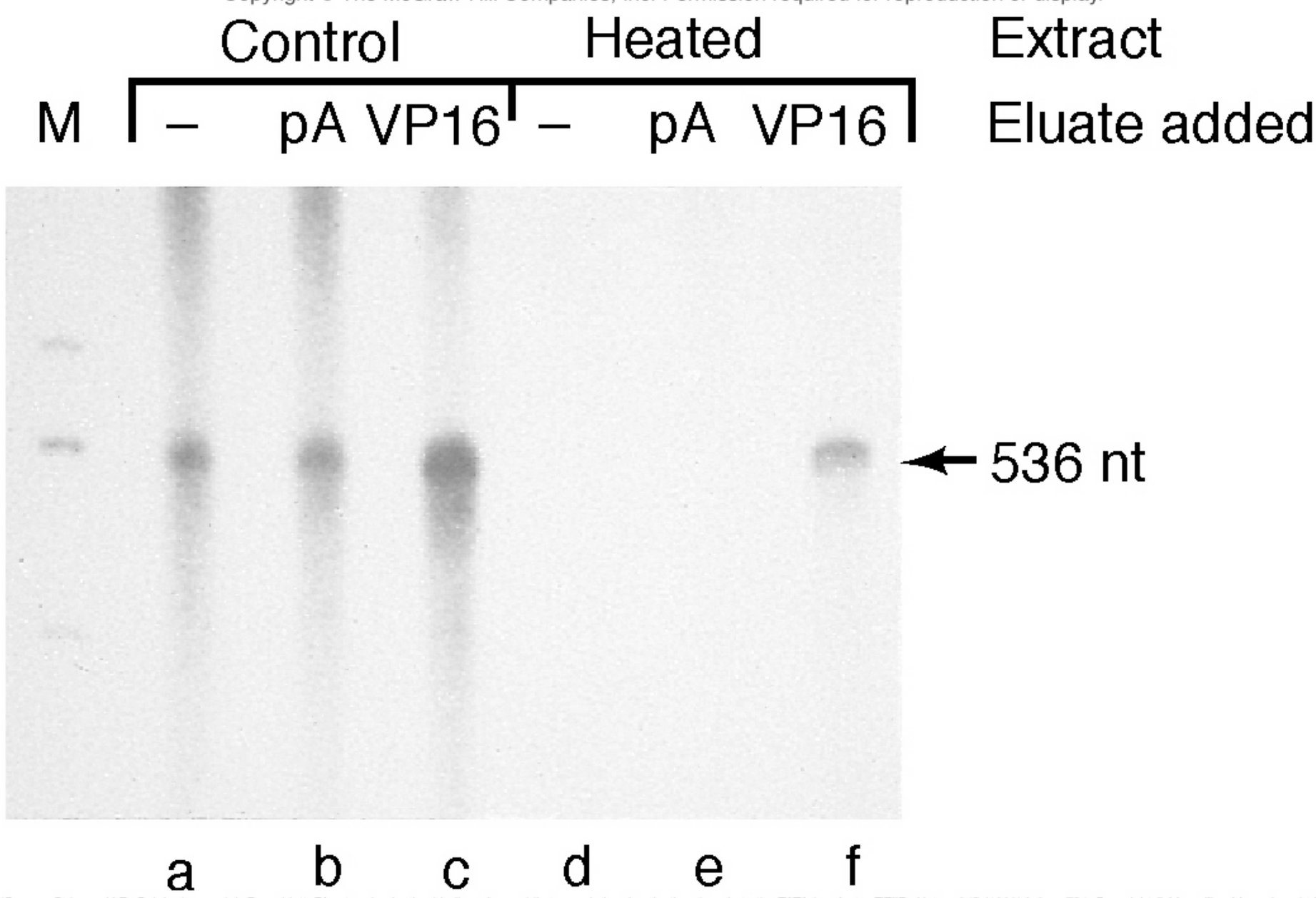
TAP has part of protein A IgG against peroxidase then add peroxidase and do a peroxidase enzymatic assay.

An activation domain can recruit general transcription factors

* TFIID
* TFIIB
* TFIIF
* TFIIH
* probably all of them are targets

Figure 12.15 Stringer et al.

TFIID



(Source: Stringer, K.F., C.J. Ingles, and J. Greenblatt, Direct and selective binding of an acidic transcriptional activation domain to the TATA-box factor TFIID. *Nature* 345 (1990) f. 2, p. 784. Copyright © Macmillan Magazines Ltd.)

VP16 activation domain fused to protein A. Protein A binds IgG.
IgG column to immobilize
HeLa nuclear extracts - Henrietta Lack's cervical cancer cells - 1951.
Read about them here: http://www.jhu.edu/~jhumag/0400web/01.html. <-very interesting.
One is heated in a way that breaks TFIID
Run off transcription of adenovirus late promoter
IgG-VP16 removed essential ingredient
Nature 345: 784-? 1990
Take home acidic VP16 binds TFIID

In the paper, we see that yeast TFIID can complement the depletion!

Kill TFIID in an extract. Supplement with stuff from column.
Specificity lies in what heat damages!
VP16 activation domain fused to protein A.
protein A binds IgG.
IgG column to immobilize
HeLa nuclear extracts
One is heated in a way that breaks TFIID
Run off transcription of adenovirus late promoter
IgG-VP16 removed essential ingredient
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Take home acidic VP16 binds TFIID

Run-off transcription

* VP16 activation domain fused to protein A. Protein A binds IgG. IgG column to immobilize
* Run off transcription of adenovirus late promoter is used to measure transcription
* HeLa nuclear extracts - Henrietta Lack's cervical cancer cells - 1951. Read about them here: http://www.jhu.edu/~jhumag/0400web/01.html. <-very interesting.
* Nuclear extract is passed through a IgG-VP16 column and some protein binds.
* Another sample is heated in a way that breaks TFIID. No transcription occurs.
* The stuff that bound the column complements the heat induced problem
* Take home acidic VP16 binds TFIID
* Nature 345: 784-? 1990. In the paper, we see that yeast TFIID can complement the depletion!

* With some promoters the acidic activation domain can act help stabilize TFIIB on the DNA.