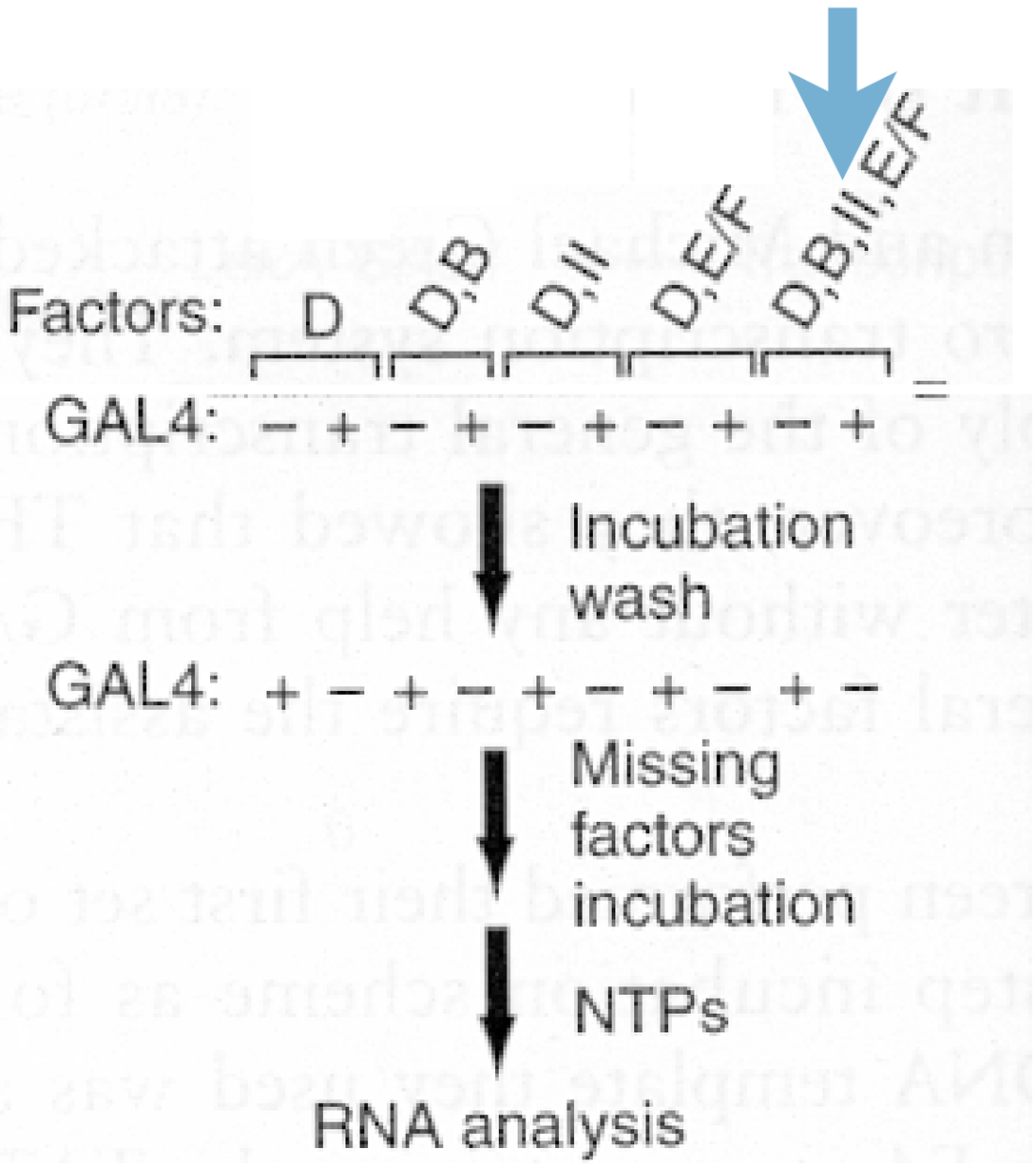
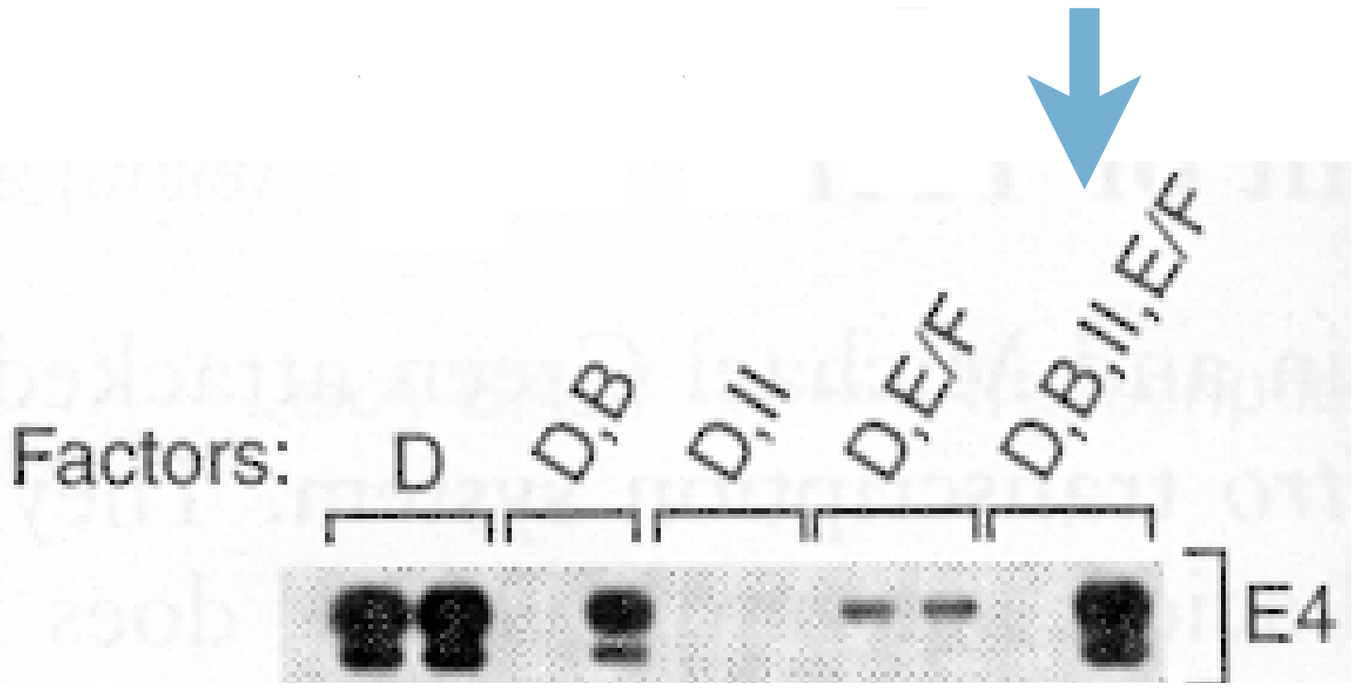
Another groups asks with what does Gal4-VP16 interact?

* DNA attached to agarose beads
* Now add components in steps
* Ask if the recruitment of a component requires that Gal4-VP16 be present.

Primer extension

Lin & Green

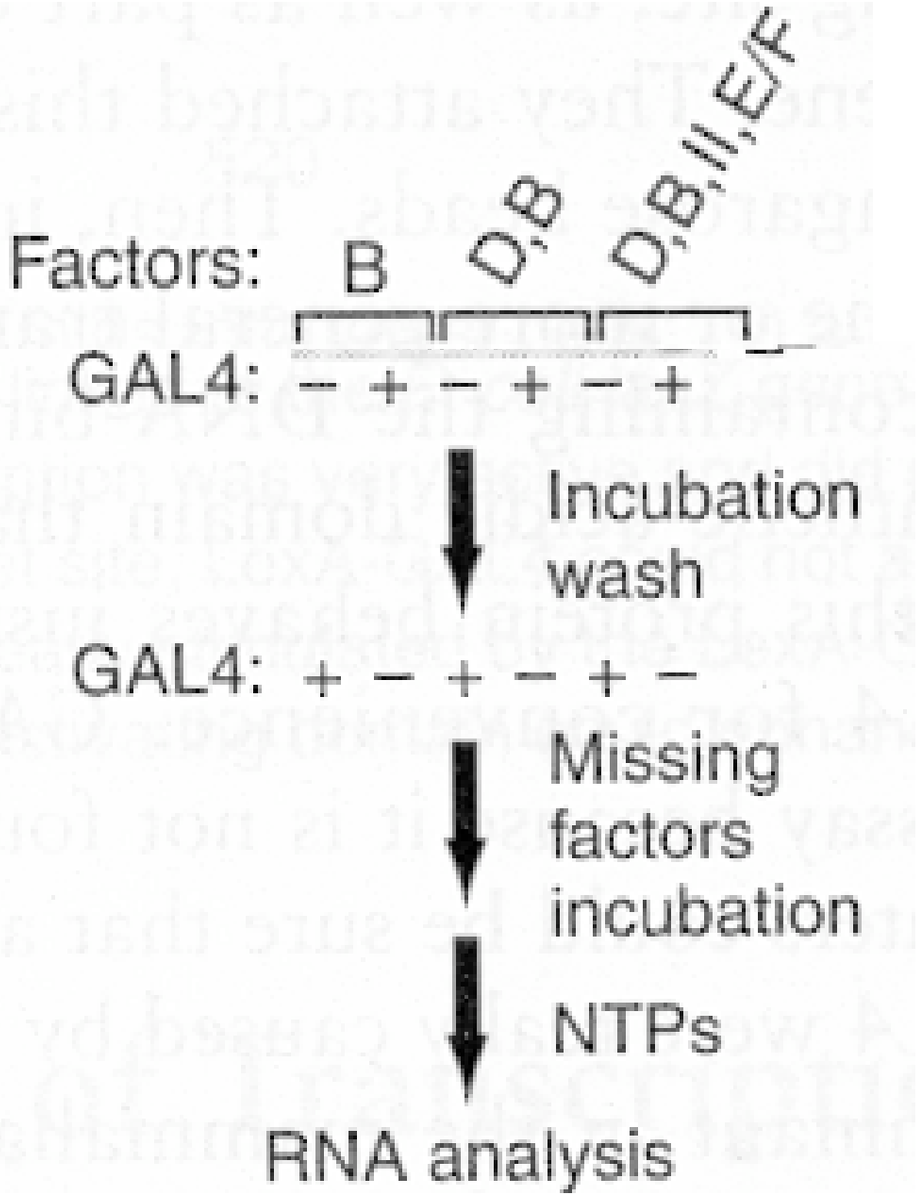


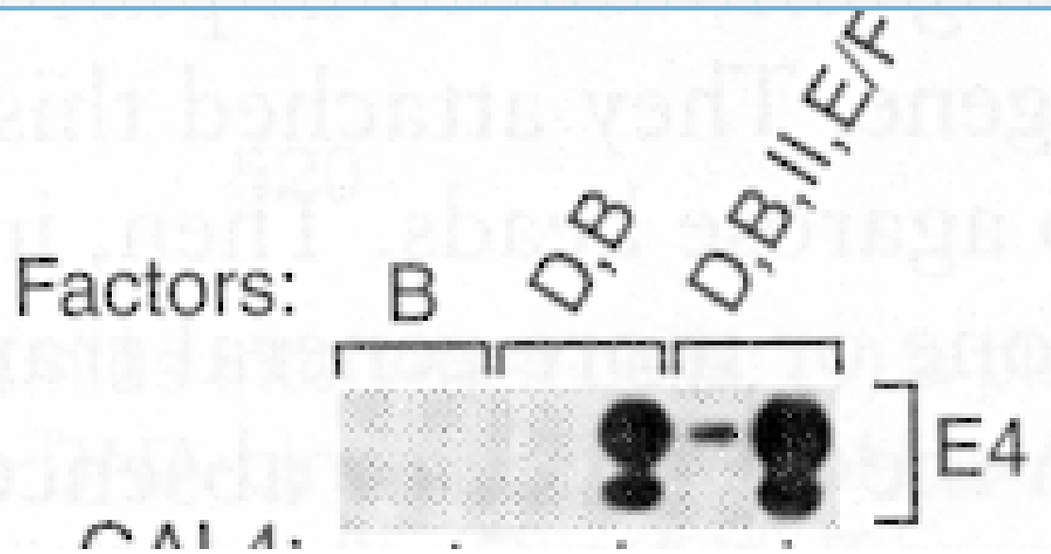


Conclusion: Gal4 acts through TFIIB. Gal4 helps to recruit TFIIB.

Also here we see that TFIID can stabiliy bind on its own.

TFIIB does not bind in the absence of TFIID





TFIIB cannot bind without TFIID.

Exp by Lin & Green

Nuclear extract is passed over an affinity column made using acidic domain of a transcription factor (VP16).

Now test what has stuck to the column by leaving out transcription factors one at a time.

Determine what the column provides and then you know what the activation domain strongly binds.

Now test the flow through to see what is missing.

Supplement

Which TF complements the flow through?

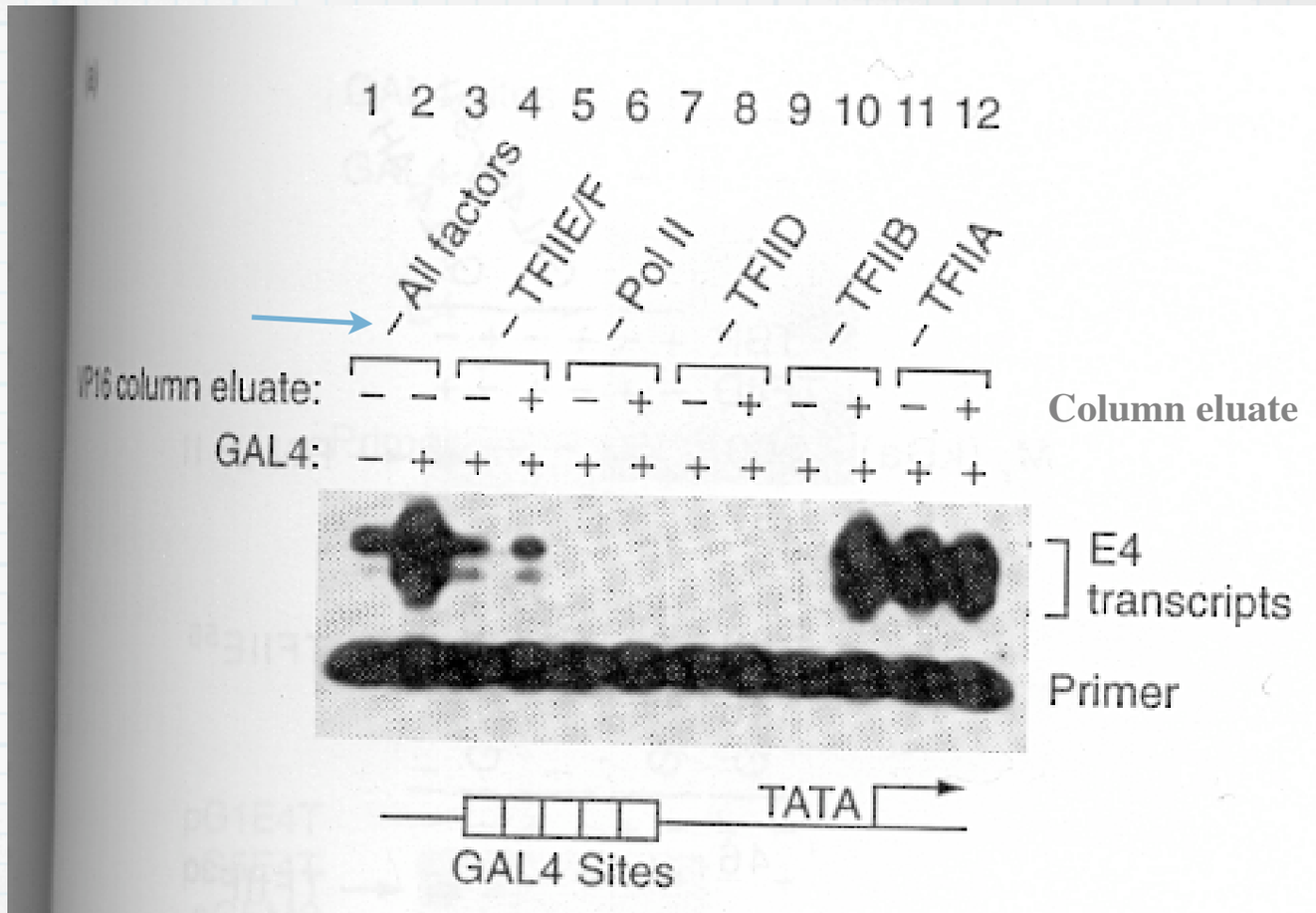
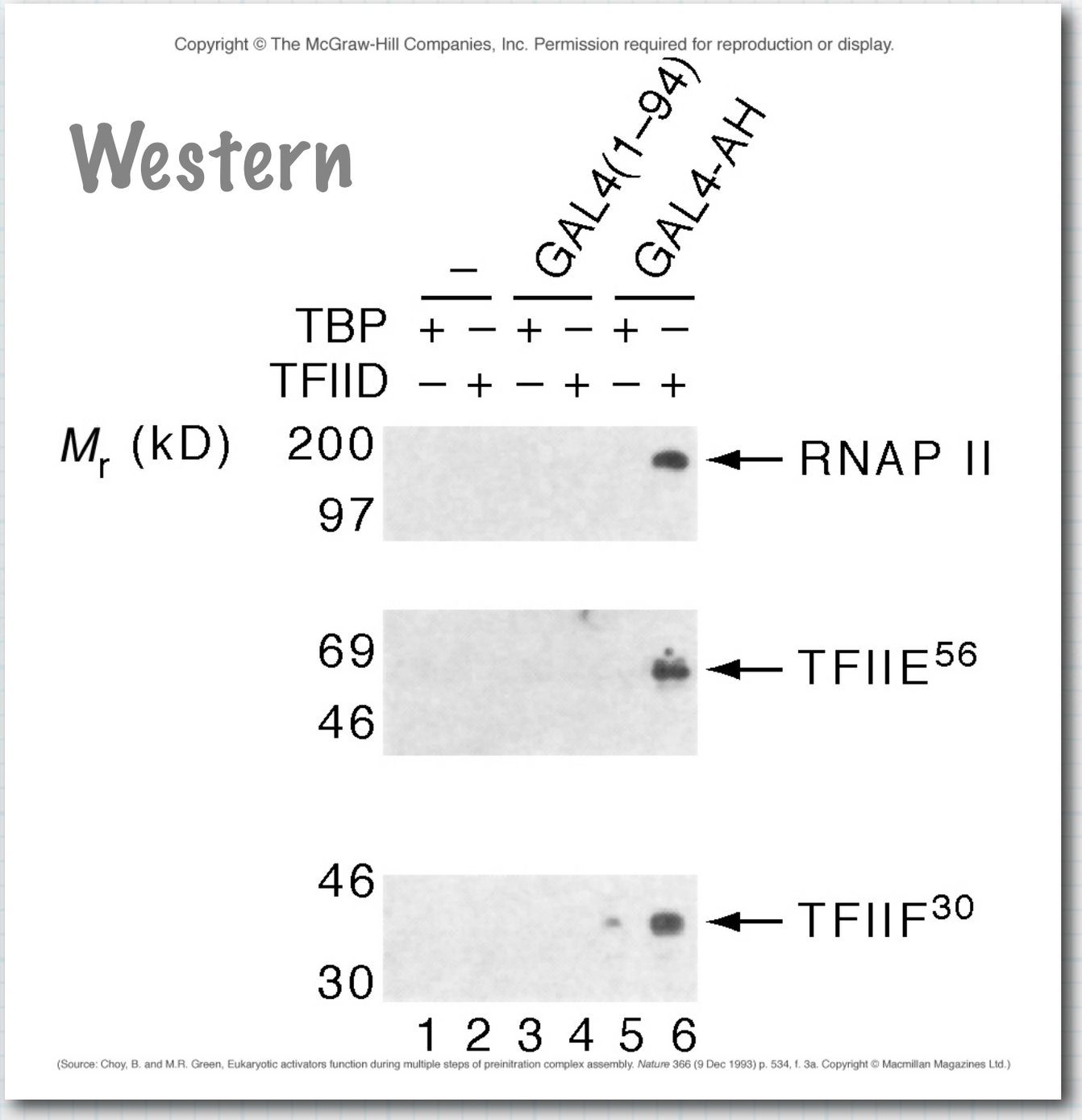


Figure 12.22 **TFIIB interacts directly with an acidic activation** domain. Lin and Green made an affinity column with the acidic

TAF's are needed to proceed beyond TFIIB step

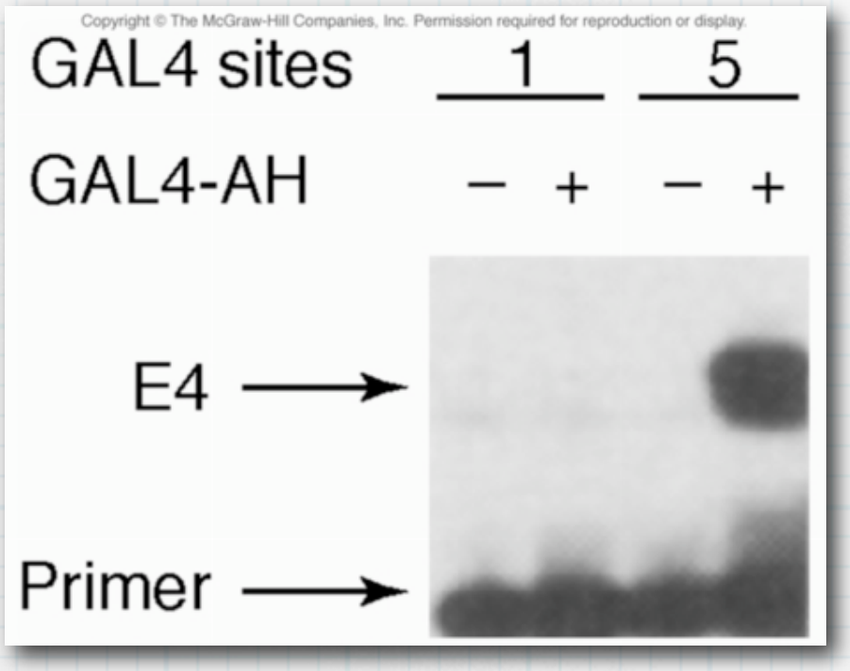


(Source: Choy, B. and M.R. Green, Eukaryotic activators function during multiple steps of preinitration complex assembly. *Nature* 366 (9 Dec 1993) p. 534, f. 3a. Copyright © Macmillan Magazines Ltd.)

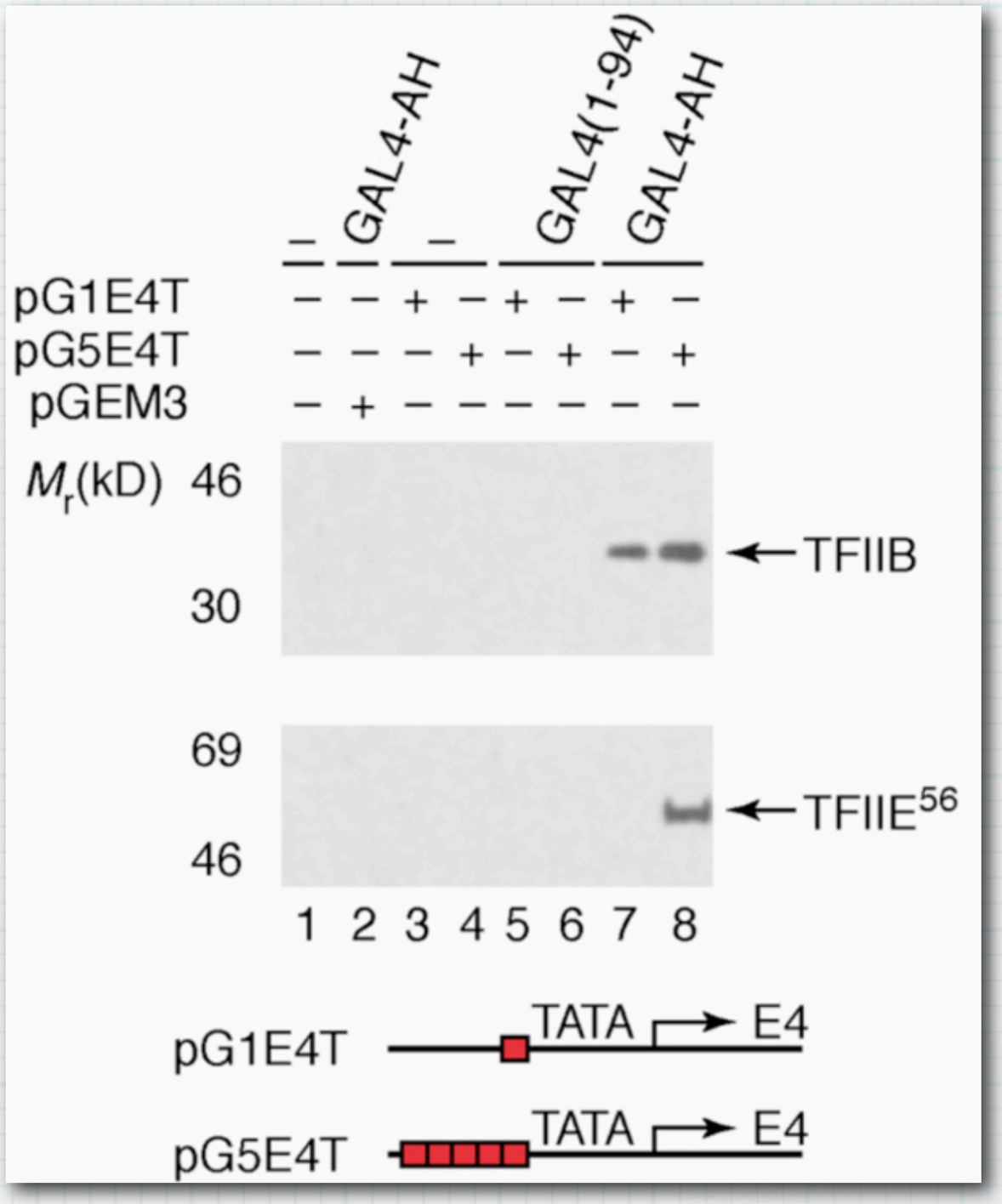
Recruit or Rearrange
&
Gal4 + TFIID bind other general transcription factors COOPERATIVELY.
Weaver 3rd ed. Page 357 Figure 12.19. Immunoblots
Which mix can coax other TF's to assemble?
TFIID but not TBP cooperates with Gal4 to recruit downstream general transc factors.
Gal4 does more than interact with TFIIB. It helps other TF's bind as well.
Interaction must be through TAFs.

Cooperativity

Transcription



anchored DNA + Western



But what does this mean? Previous results did not show us evidence that Enhancers present in multiple copies. Function?
5X Gal4 UAS is about 50X more than one Gal4 UAS.
Cooperativity! At the TFIIB step or another?
Anchored DNA see what binds it by Immunoblot.

Questino why does the 1X + lane seem blank?

* Gal4 has to be there when the preinitation complex forms.
* Gal4 must be present when TFIIB enters into the complex.
* TFIIB interacts with acidic domains.
* TFIID cooperates with Gal4 to recruit other transcription factors.
* Gal4 binds cooperatively.
* Acts to stabilize the initiation complex.

Types of activation domains

* Acidic glob
* Glutamine Rich
* Proline Rich

Collaboration

Why dimers?

* The affinity of binding between a protein and DNA varies with the square of the free energy of binding.
* Free energy depends on the number of contacts.
* Doubling the contacts with a dimer quadruples the affinity between the protein and the DNA.

Not very satisfying because Why not trimers?

Jun Fos

* proto-oncogenes
* Two different bZip transcription factors
* AP-1 = Jun dimers
* Jun and Fos heterodimers bind enhancer tighter than AP-1.
* Enhancer that they bind is called TPA response element

TPA = a phorbol ester that stimulates cell division. One thing that happens is that AP-1 binds the TPA response element.

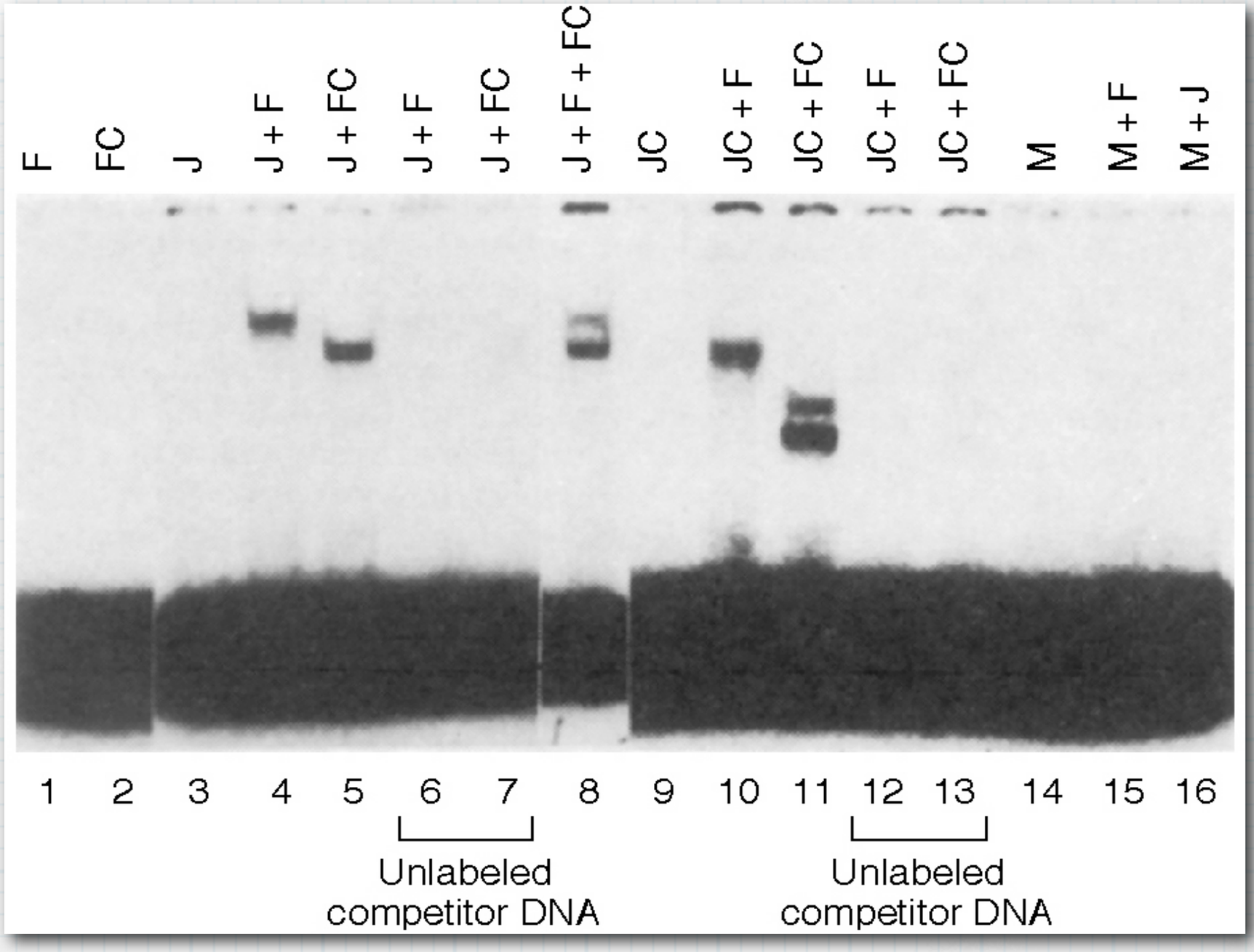
Jun Fos work best as heterodimers

DNA has AP-1 enhancer

low conc and Fosdimer nor Jundimer can bind

lane 8 Jun Fos form dimers

FC=Fos core
M=myc



Pg 337 Weaver 4th edition

Cooperatively in binding
M=myc

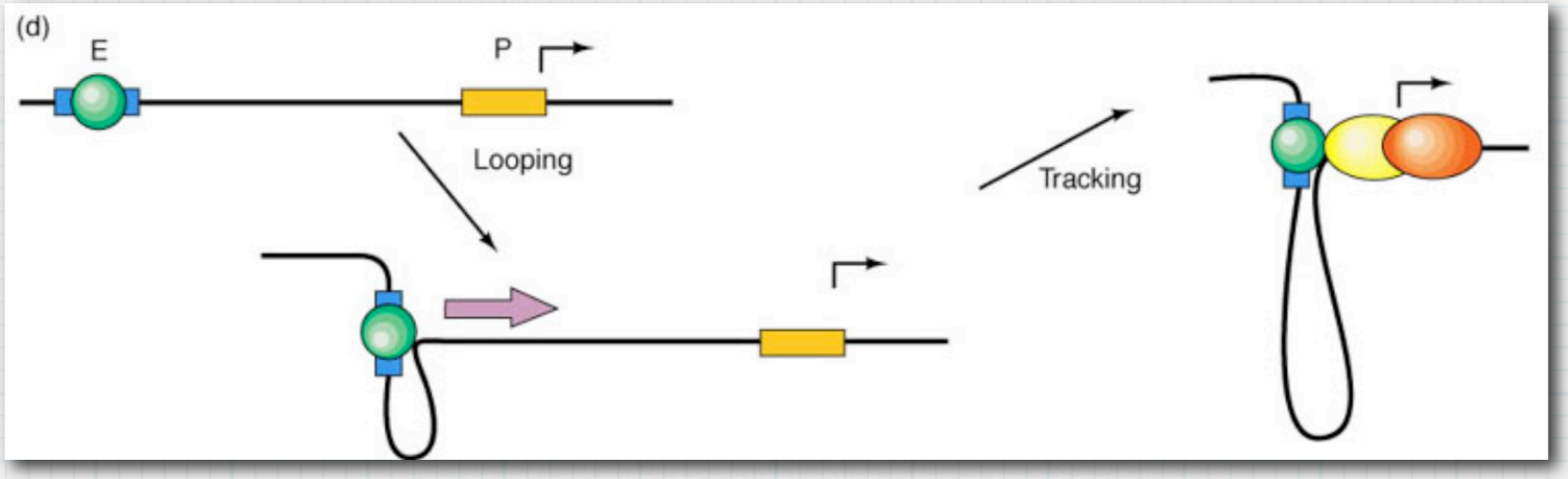
What is the purpose of cooperative binding?

Simulate a switch.

Simulate a switch.

Enhancers work at a distance

Figure 12.20E Data is consistent with looping model or loop + tracking model.

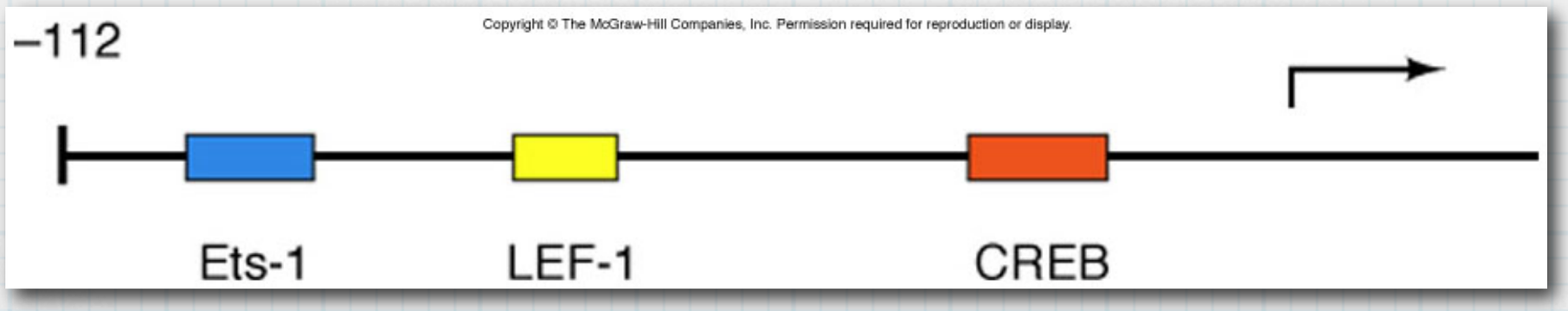


Protein:protien interaction is key.
Enhancer does not need to be on the same molecule but it must be able to interact.

Examine the experiment in Figure 12.22. Read associated text.

Architectural transcription factors

* DNA less than 300-400 bp behaves like a rod.
* Architectural TFs bend or unbend DNA.

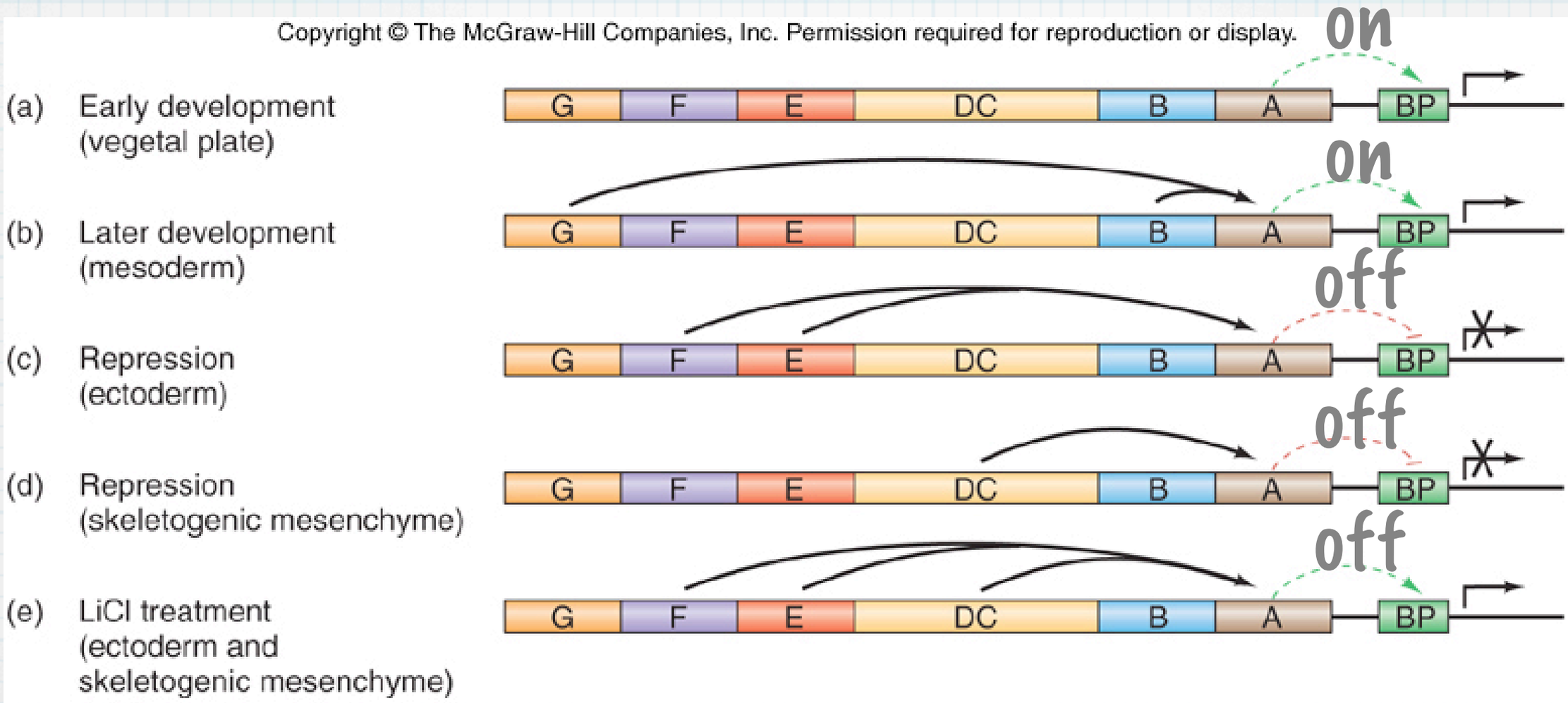


LEF-1 is lymphoid enhancer-binding factor.
TF is a HMG (high mobility group) protein. Causes 130 degree bending. Brings Ets-1 site closer to the promoter.
The 2nd example in your book is of one that unbends DNA and thereby makes the DNA ready to bind proteins.

* LEF-1 is lymphoid enhancer-binding factor.
* TF is a HMG (high mobility group) protein. Causes 130 degree bending. Brings Ets-1 site closer to the promoter.
* Others types can unbend the DNA.

Combinatorial code

Figure 12.25 4th edition page 342. Sea Urchin Endo 16 gene



BP identifies the core promoter

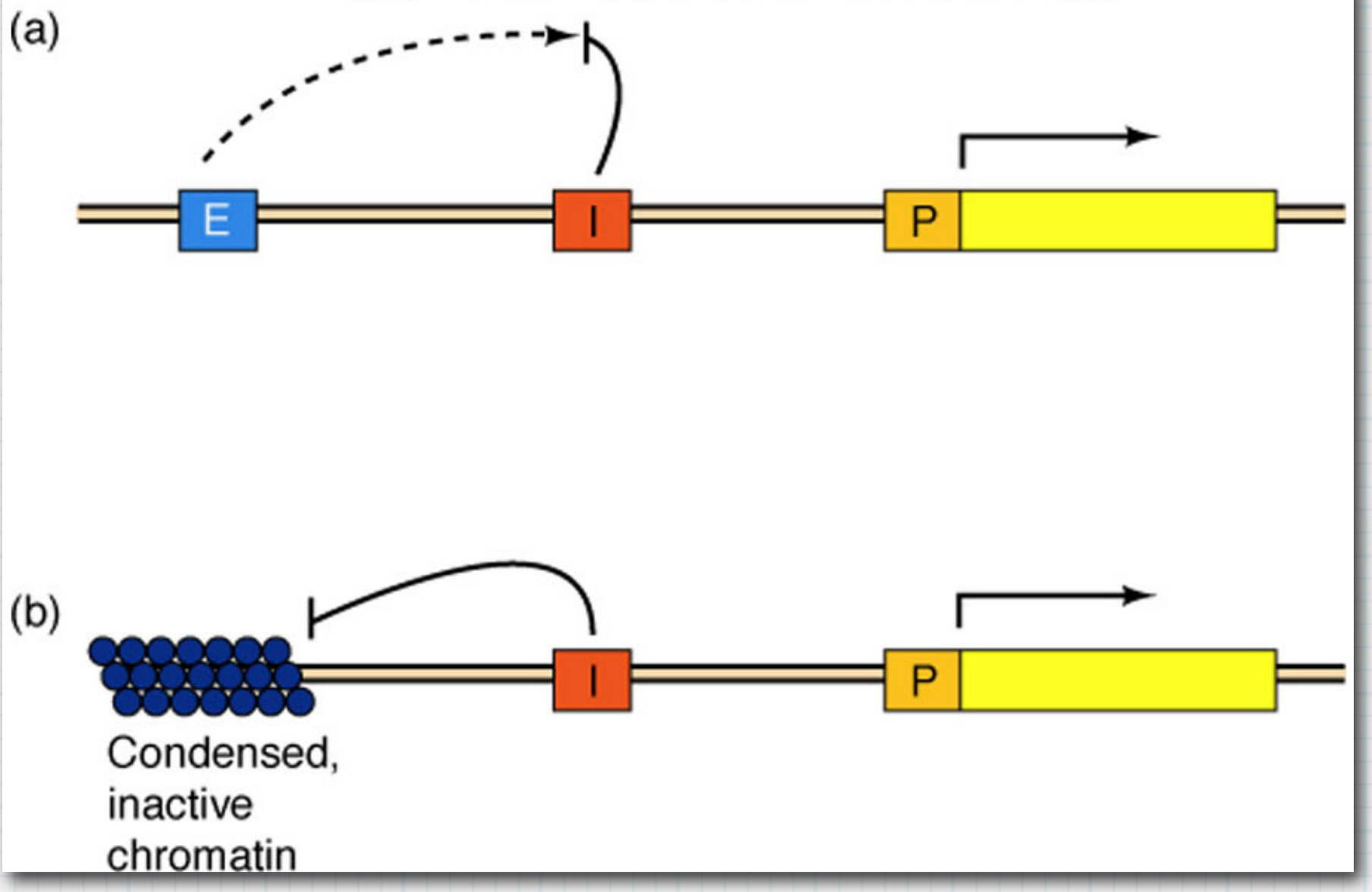
How do with simulate a switch? Cooperativity.

Insulators

* Provides a barrier through which activation or repression cannot pass.
* Some also block the encroachment of condensed chromatin.
* Some do one, some the other.

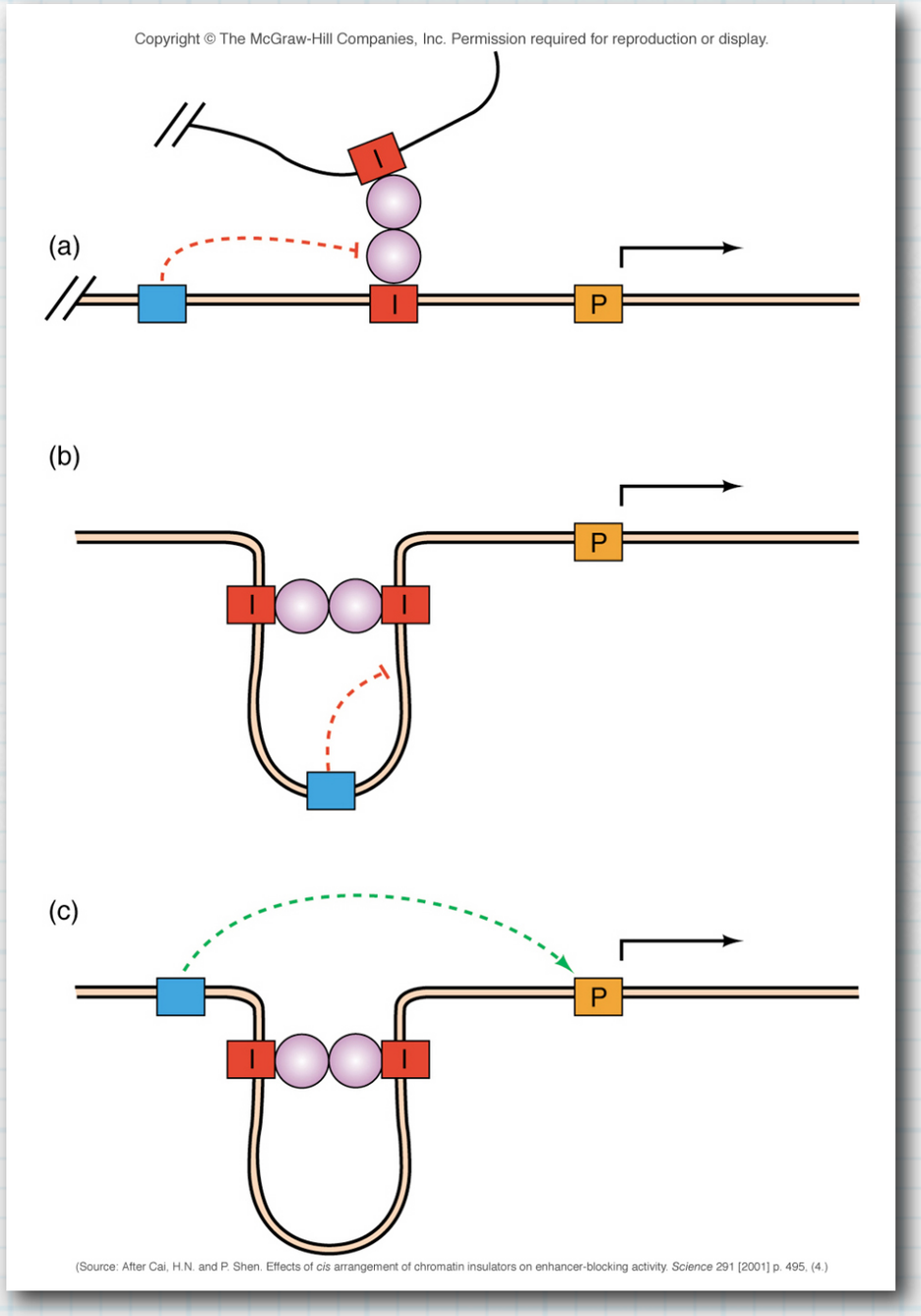
* What we know is based on very few examples.
* Probably many different types exist.
* There are probably many different mechanisms.

Do NOT act by silencing upstream DNA.



One way that they are thought to work

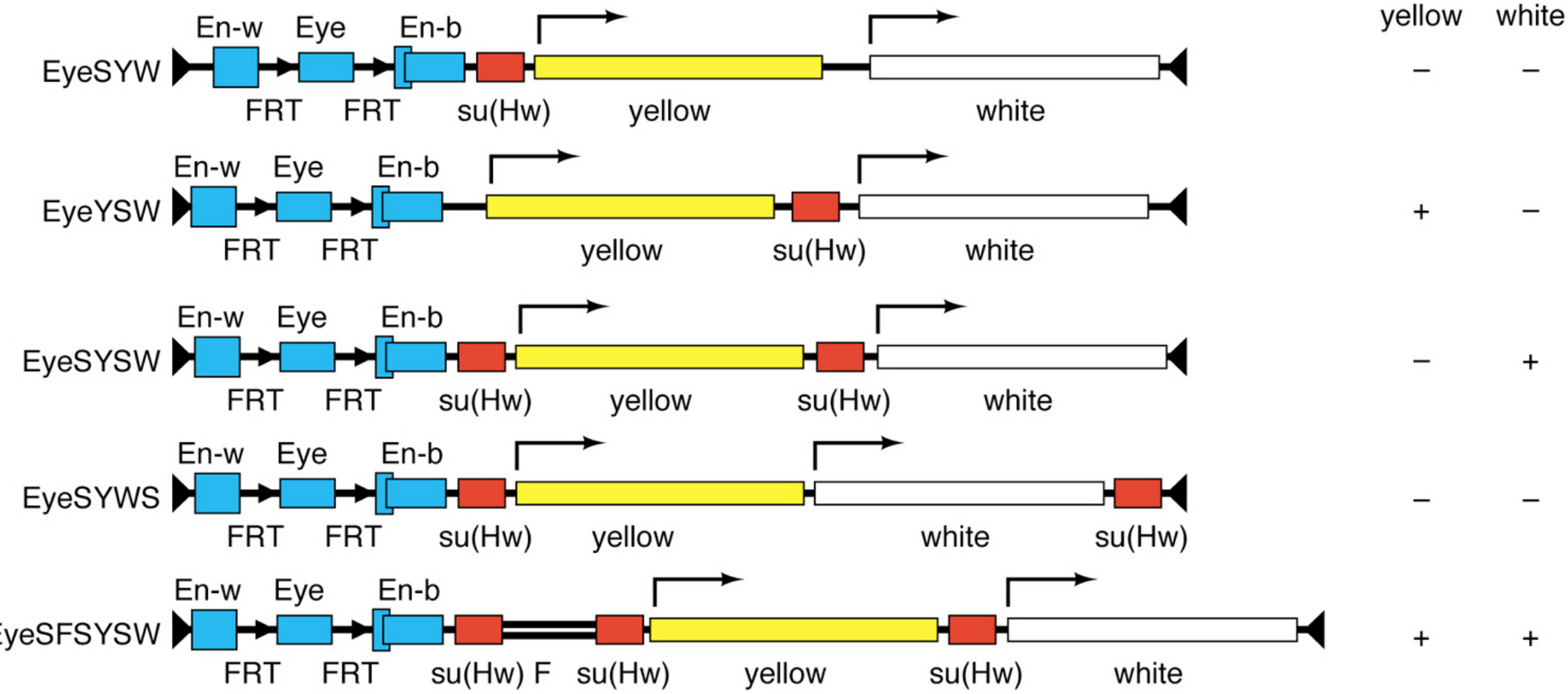
Haini Cai & Ping Shen



A) prevent
B) prevent
C) don’t prevent

Lab of Vincenzo Pirrotta

Figure 12.36



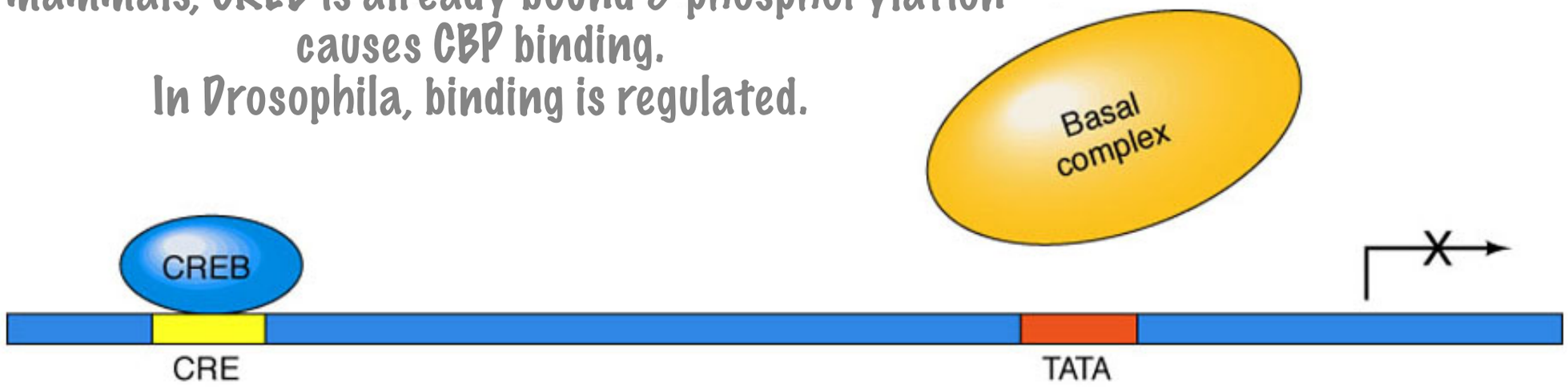
(Source: Muravyova, E., A. Golovnin, E. Gracheva, A. Parshikov, T. Belenkaya, V. Pirotta, and P. Georgiev. Loss of insulator activity by paired Su(Hu) chromatin insulators. *Science* 291 [2001] p. 497, f. 2. Reprinted with permission.)

Regulation of Transcription Factors

Regulation of Transcription Factors

* association with or disassociation from other proteins
* hormone binding
* phosphorylation
* ubiquitination
* sumoylation
* methylation
* acetylation
* more

In mammals, CREB is already bound & phosphorylation causes CBP binding.
In Drosophila, binding is regulated.

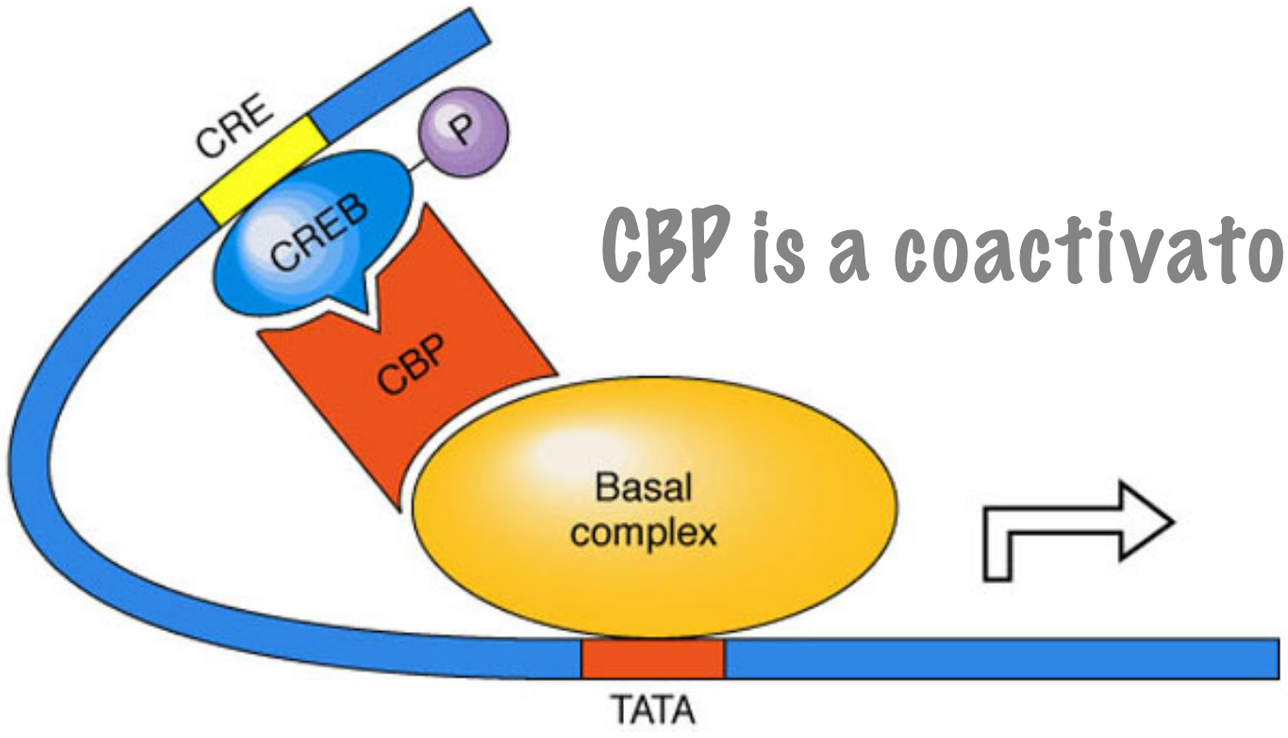


↑ cAMP

↑ PKA

↑ phosphorylation of CREB

PKA phosphorylates CREB



CBP is a coactivator

Other examples Mediator, CRSP (for Sp1).
CBP works with many genes.
A theme!

End