BI 8 LECTURE 16

DEVELOPMENTAL GENE REGULATION: INTERSECTION WITH EPIGENETICS AND GENE NETWORKS

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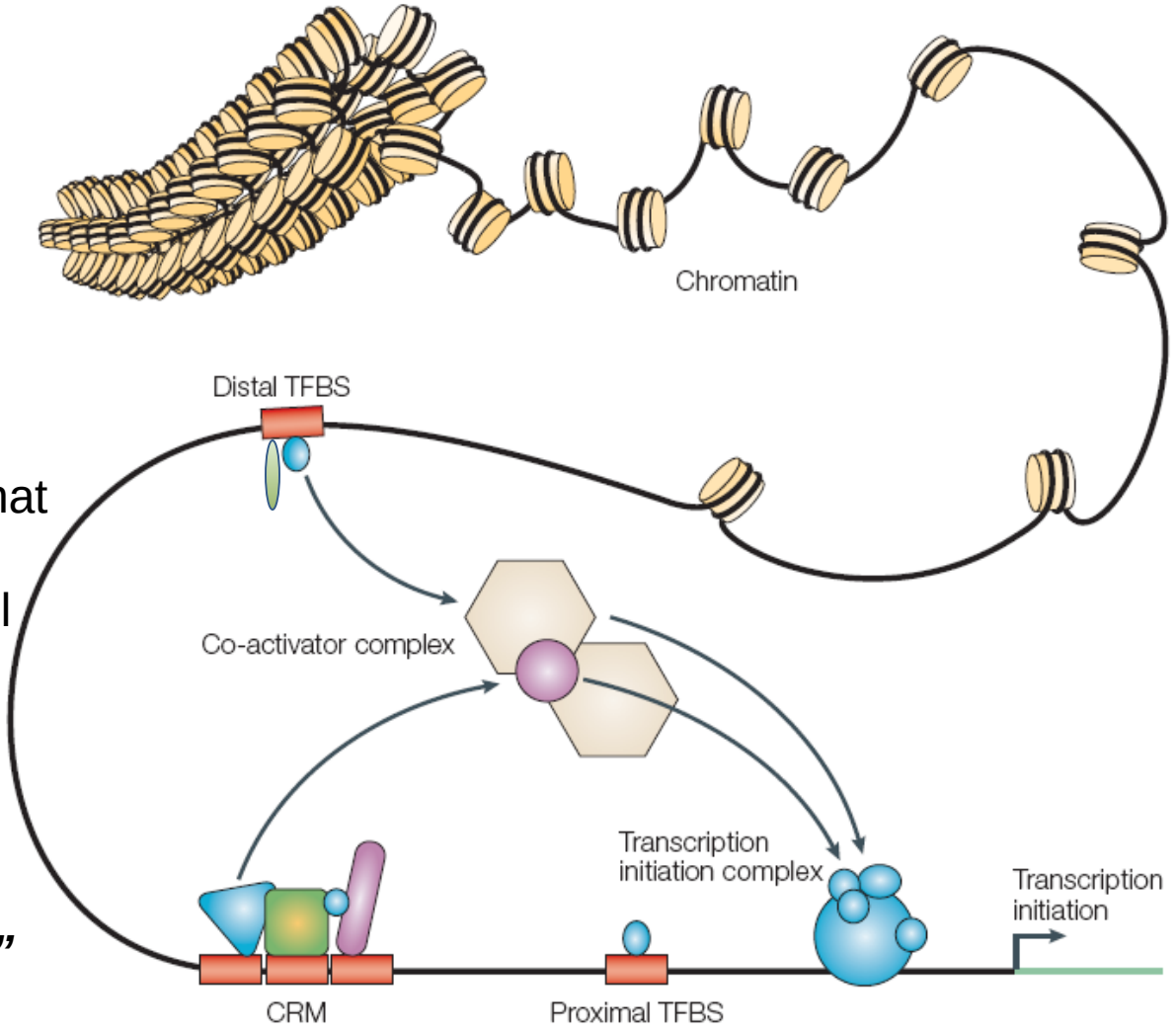
Take home lessons so far about eukaryotic gene regulation: transcription factor binding

- Transcription factors (TFs) work in ***groups*** by binding to closely spaced sites in the DNA… most DNA is not bound.
- Functionally active clusters = “enhancers” (for positive regulation) or “cis-regulatory elements”
- The DNA sequences of these regions are what bring a particular combination of factors together due to TF ***recognition specificity***
- Neighbor-neighbor interactions with other factors, protein to protein, can also help binding
- Factors can be necessary even if they are not sufficient:
  - Part of a consortium that carries out “AND” logic at a cis-reg element
  - Necessary but not sufficient for binding *in vivo*
  - Or: sufficient for their own binding, but not sufficient to cause transcriptional effect until other factors “arrive”

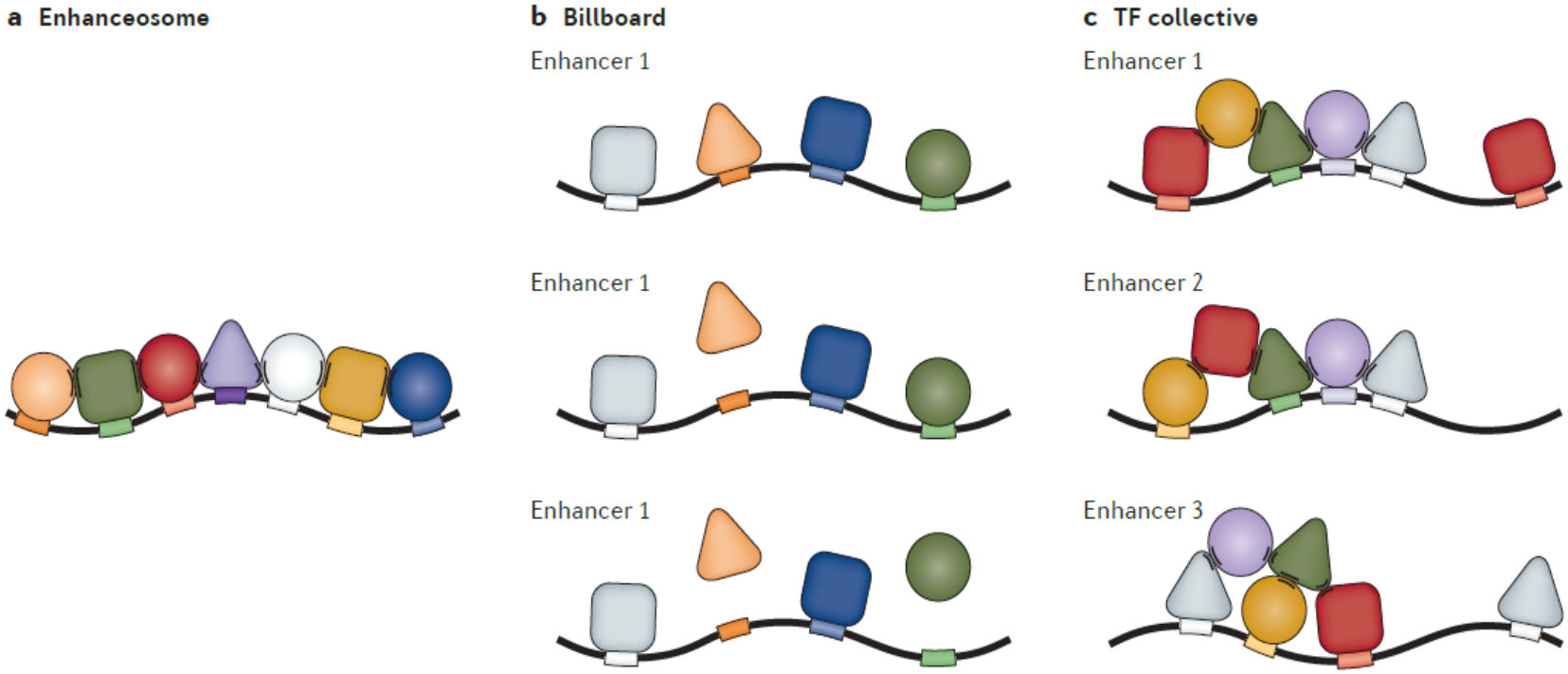
Overview of transcriptional regulation in metazoans

Transcription factors bind in clusters at cis-regulatory elements that can be far from the promoters they control

Each cis-regulatory element has its own rule for how many bound transcription factors are "enough"



Exactly ***how*** does enhancer binding site combination set requirement of TFs needed for gene expression? 3 models



	a Enhanceosome	b Billboard	c TF collective
Protein DNA	• Highly cooperative DNA binding • DNA sequence acts as scaffold	• Cooperative and additive binding	• Cooperative DNA binding • Both DNA and protein may act as scaffold
Protein interface	• Fixed (formed from a higher-order TF–DNA complex)	• Variable	• Variable
Motif	• Fixed motif composition (sites for all factors must be there) • Fixed motif positioning (grammar)	• Fixed motif composition • Flexible motif grammar	• Flexible motif composition (as different TFs directly bind to DNA) • Flexible motif grammar
Output	• Unitary (requires the integrated activity of all TFs)	• Only requires a subset of factors to be active	• Collective (requires most TFs but not clear if it requires all TFs)

(from Spitz & Furlong, 2012; after Arnosti et al 2005)

Using coactivators to create combinatorial bridges

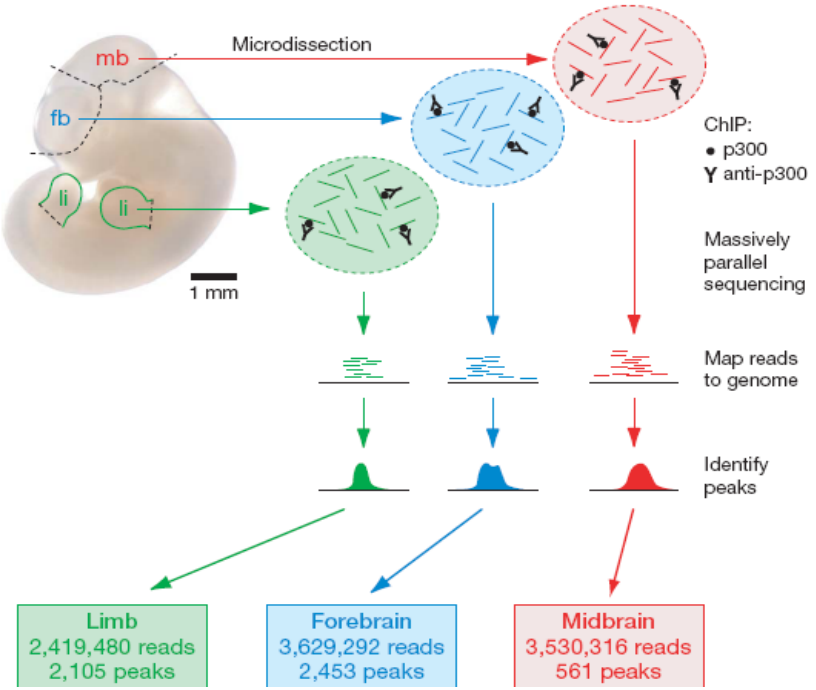
- Partner interaction sensing mechanisms
  - Direct (e.g. Ets1 and CBFα2)… but this is not the only way
  - Via alterations in histone packing architecture (for next time)
  - **Via bridging factors/ coactivators**
  - Activity as transcriptional activator (& usefulness in txf "team") can depend on ability to recruit coactivators

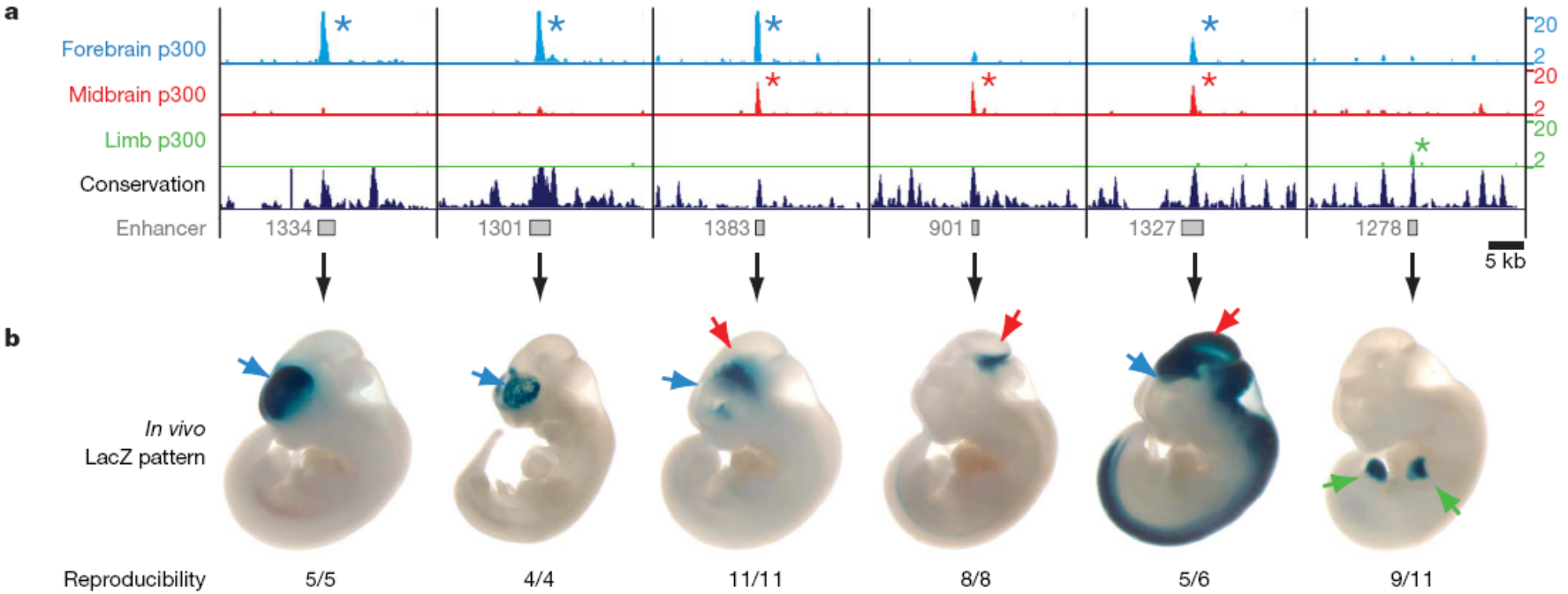
- General purpose coactivators
  - p300 and CBP
  - Recruitment of histone modification machinery (to be continued!!)
- Dedicated cell type-defining coactivator
  - CIITA
  - Creates "teamwork"

Profile of p300 binding genome-wide can locate sites of active enhancers

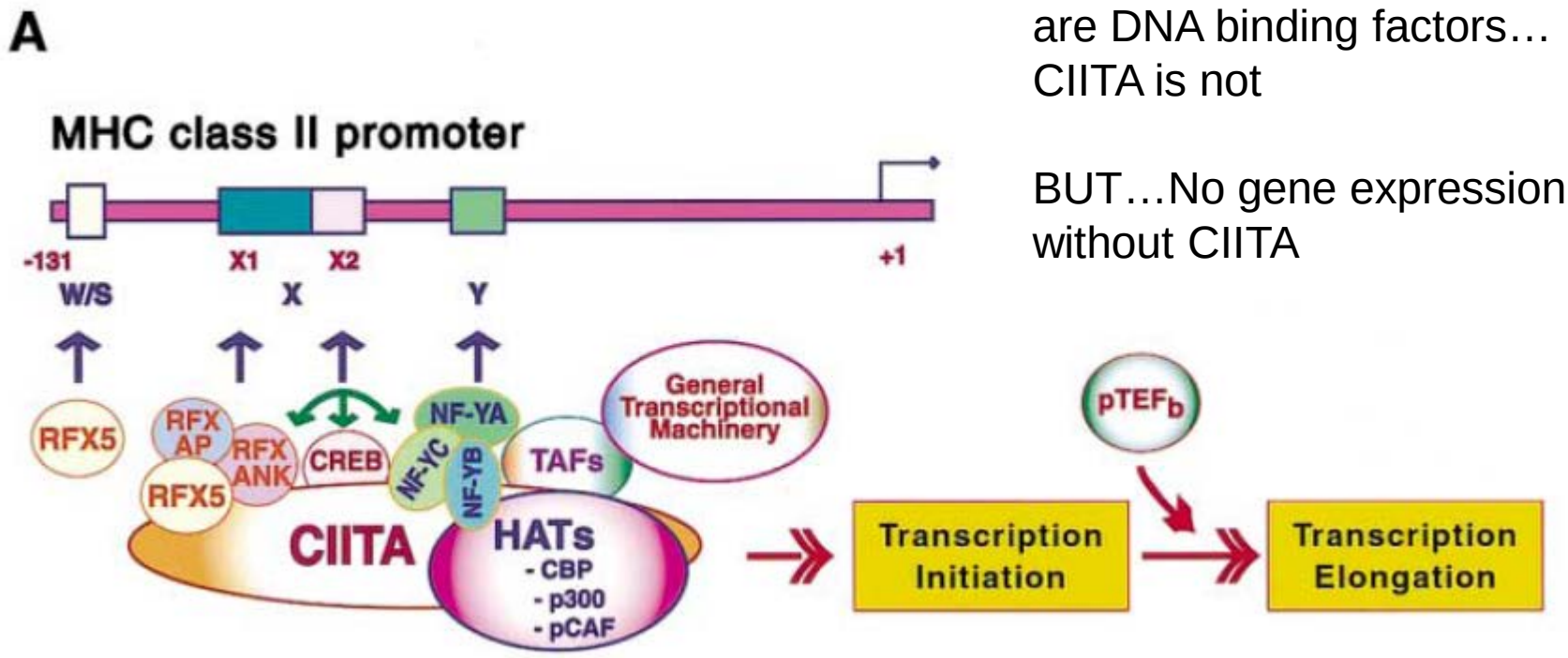
Crosslink chromatin, use antibodies vs. p300 to precipitate bound DNA, and sequence

(Visel, … Pennacchio 2009, *Nature)*





The “master cis-regulatory motif” for a large group of antigen presentation genes is a cluster of sites for “friends of CIITA”



Rfx5, RfxAP, CREB & NF-Y are DNA binding factors… CIITA is not

BUT…No gene expression without CIITA

Ting, Trowsdale, Cell, Vol. 109, S21–S33, April, 2002

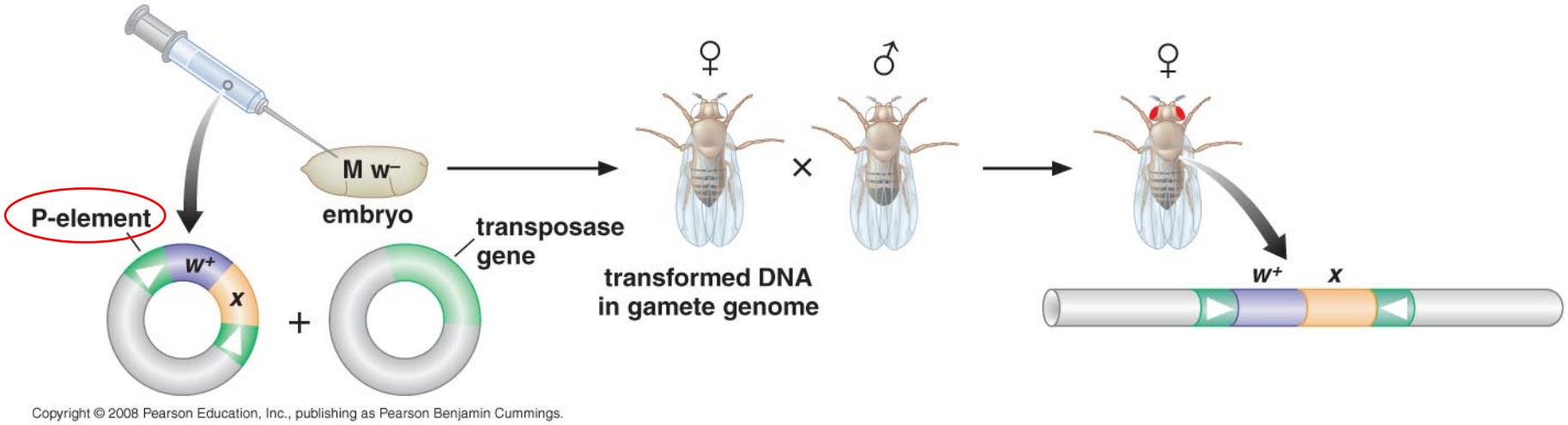
To dissect mechanisms involved in developmental gene regulation, need experimental systems to compare trans-acting factor impacts on cis-reg element targets in multiple cell types from a single organism

- Transfection is always the strategy
- Cis-effects and trans-effects are always compared

  …but somehow these interactions need to be viewed in different types of cells, with different developmental histories, in parallel

- ➢ Go beyond “regular” transfection techniques – transient or stable transfection of cell lines – to make transgenic animals

Specialized transgenic production in Drosophila takes advantage of a transposase that is activated during mating: rapidly get whole, developed animals carrying gene of interest



Drosophila naturally have a transposable element – a repeated DNA sequence that can encode its own recombination enzyme – and integrates randomly into the chromosomal DNA. Can be made into a vector to insert your gene of interest, X….

Detect the flies with X in the genome by screening for linked gene “W+”.

Techniques exist to *replace* normal DNA sequences in mouse genome precisely with desired mutants

Key: having a cell type that has the developmental potential of a fertilized egg, but grows in culture:

ES cells

ES cells re-introduced into mouse embryos enter germline, make whole normal mice

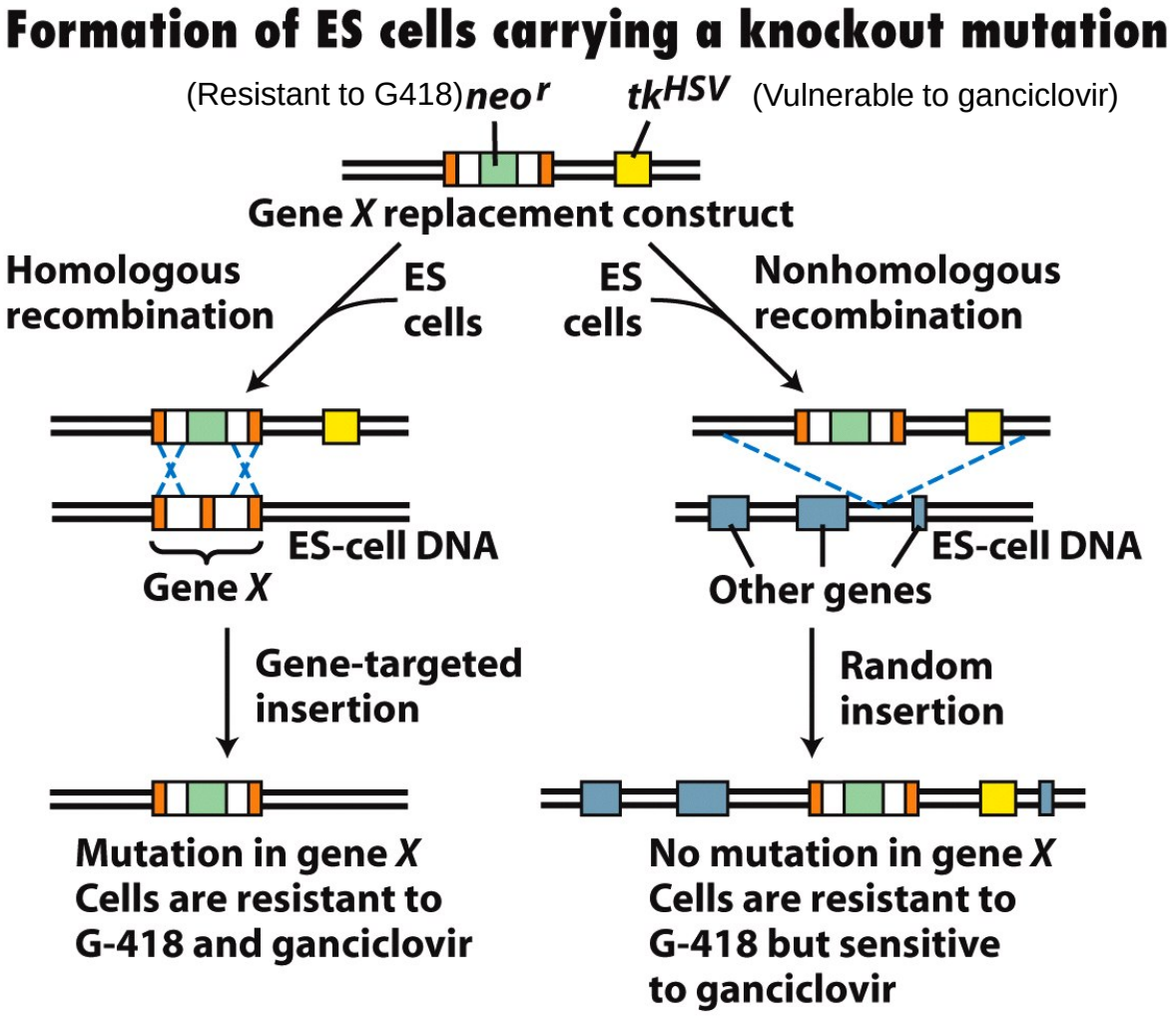


Figure 5-40a
Molecular Cell Biology, Sixth Edition

WANTED

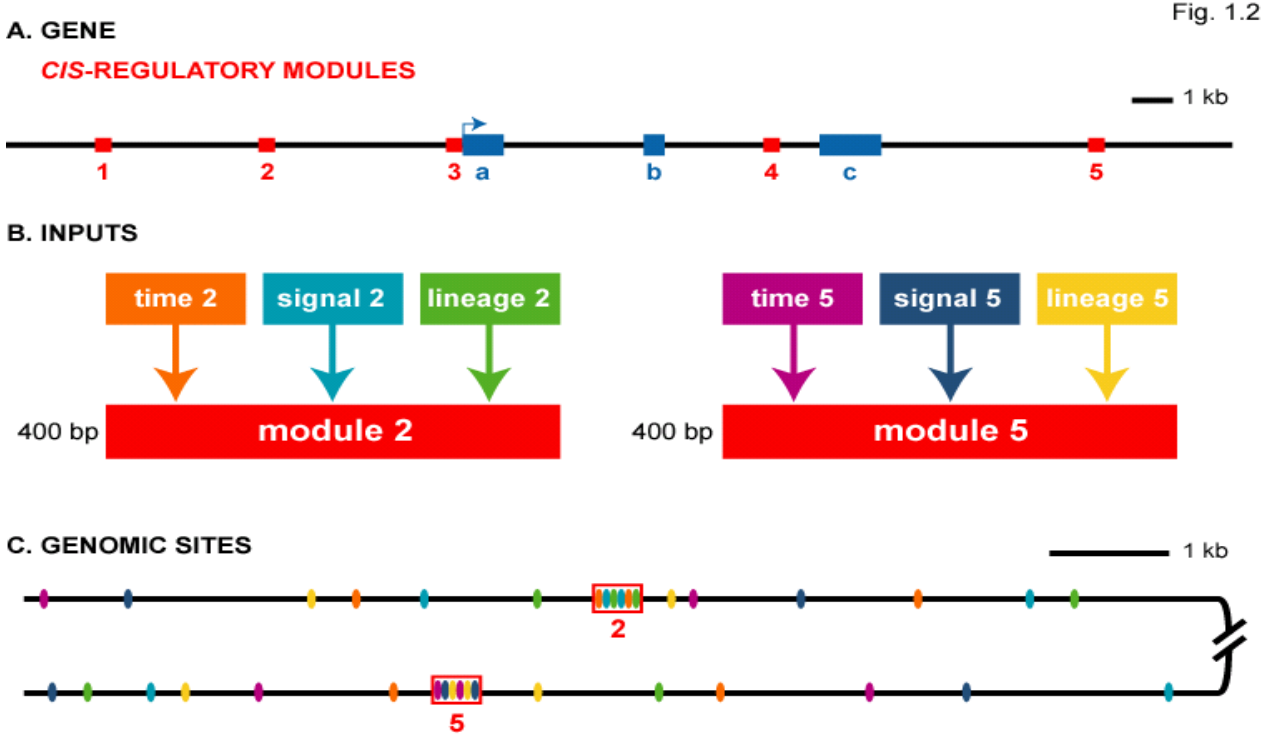
UNWANTED, DISCARDED

Developmental gene regulation

- Development is not a steady state: irreversible forward drive
- Development: ordered increase in complexity
- Outcomes based on “memory” of prior events: cumulative divergences between cells in regulatory states based on history
- The whole genome has to have its expression correctly regulated in time and in anatomical space

Target sites in specific cis-regulatory "enhancer" modules determine specific combinations of transcription factors that can collaborate to regulate a gene ***in a given context***…

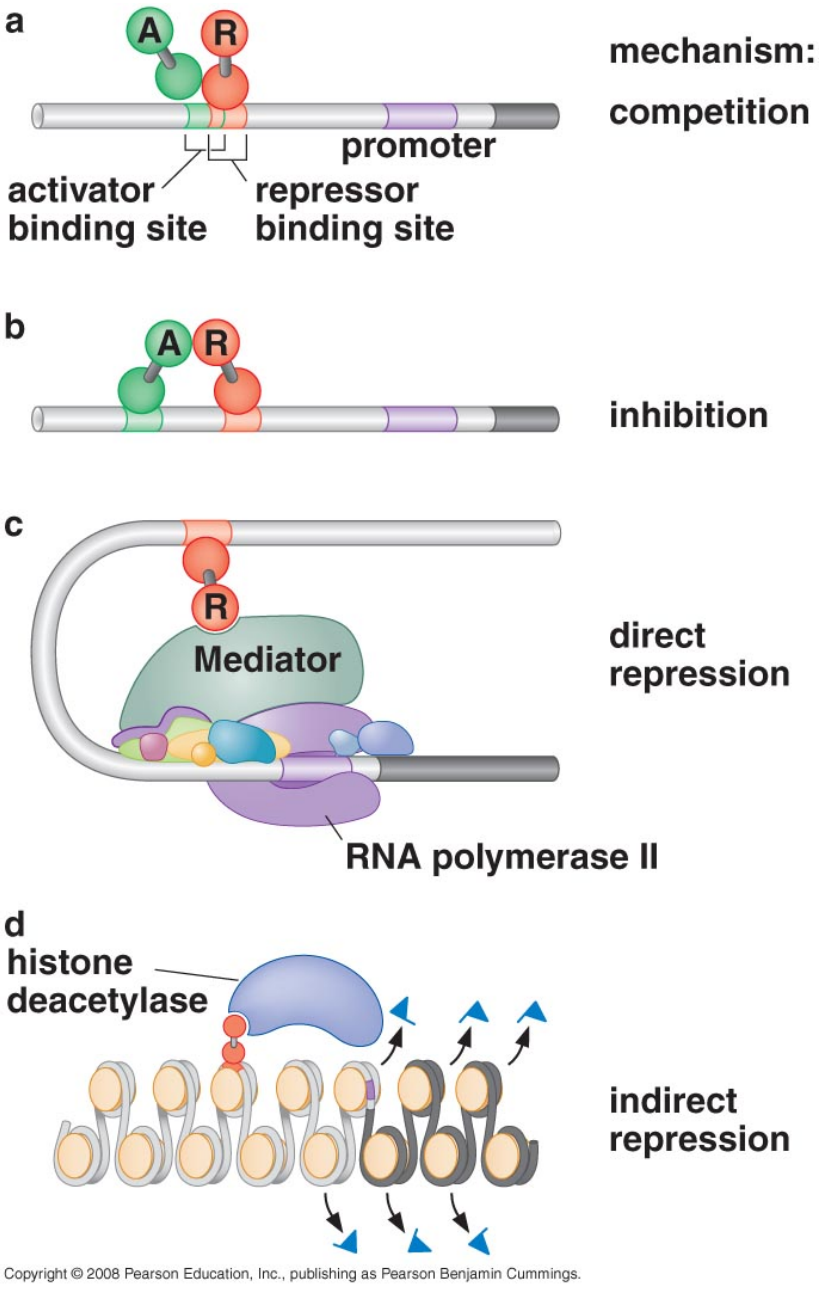
Fig. 1.2



Several mechanisms enable transcription factors to repress in eukaryotic cells

Most usually *enhancer-specific*

Contrast with prokaryotes! Not just blocking polymerase II progression



Handling of repression/ negative regulation is key difference between prokaryotes and multicellular eukaryotes

- Prokaryotes can do repression by simple blockade
- Multicellular eukaryotes have three kinds of negative regulation in cell lineage
  - Silencing (like prokaryotes)
  - Temporary withdrawal of activators
  - Conditional antagonism of activators: not here, not now, but open for activation via other cis-regulatory elements
- Repression mechanisms 2 & 3 cannot work by simple polymerase blockade
  - Block activator TF binding to enhancers
  - Control coactivator access & enhancer looping to promoter

Gene regulation in complex organisms is not simply additive

Transcription factors binding to combinations of positive and negative regulatory sites create ***new*** patterns of target gene expression ***distinct*** from those of input transcription factors themselves

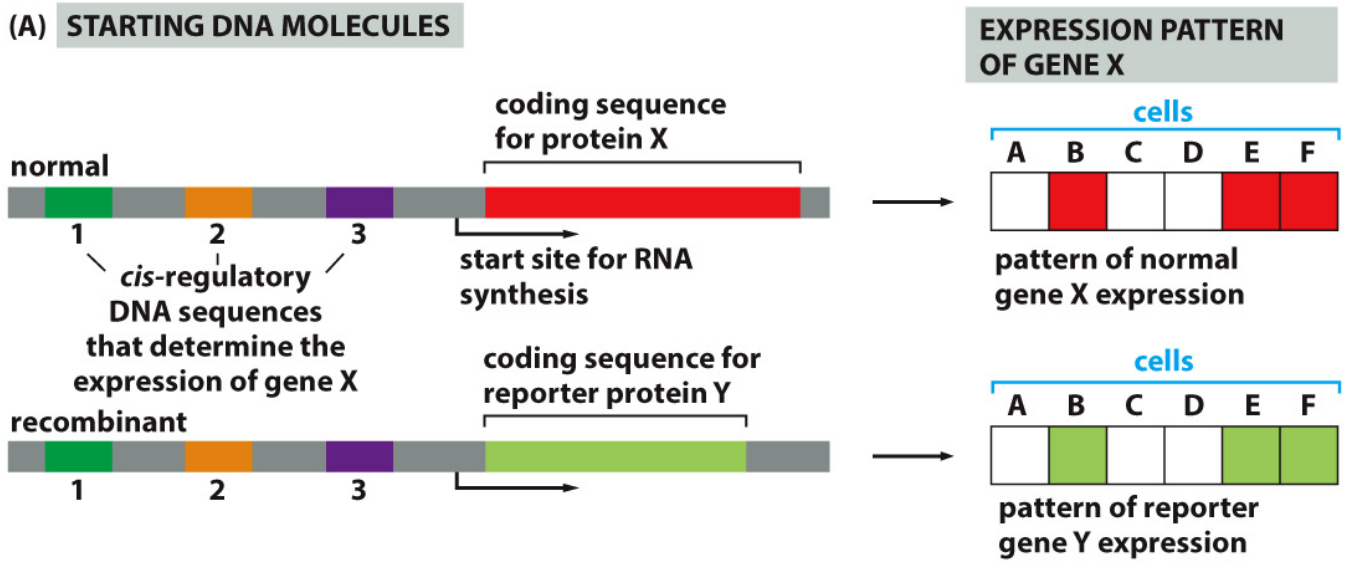


Figure 8-60 *Molecular Biology of the Cell* (© Garland Science 2015)

A classic model: *eve* stripe regulation in the early *Drosophila* embryo

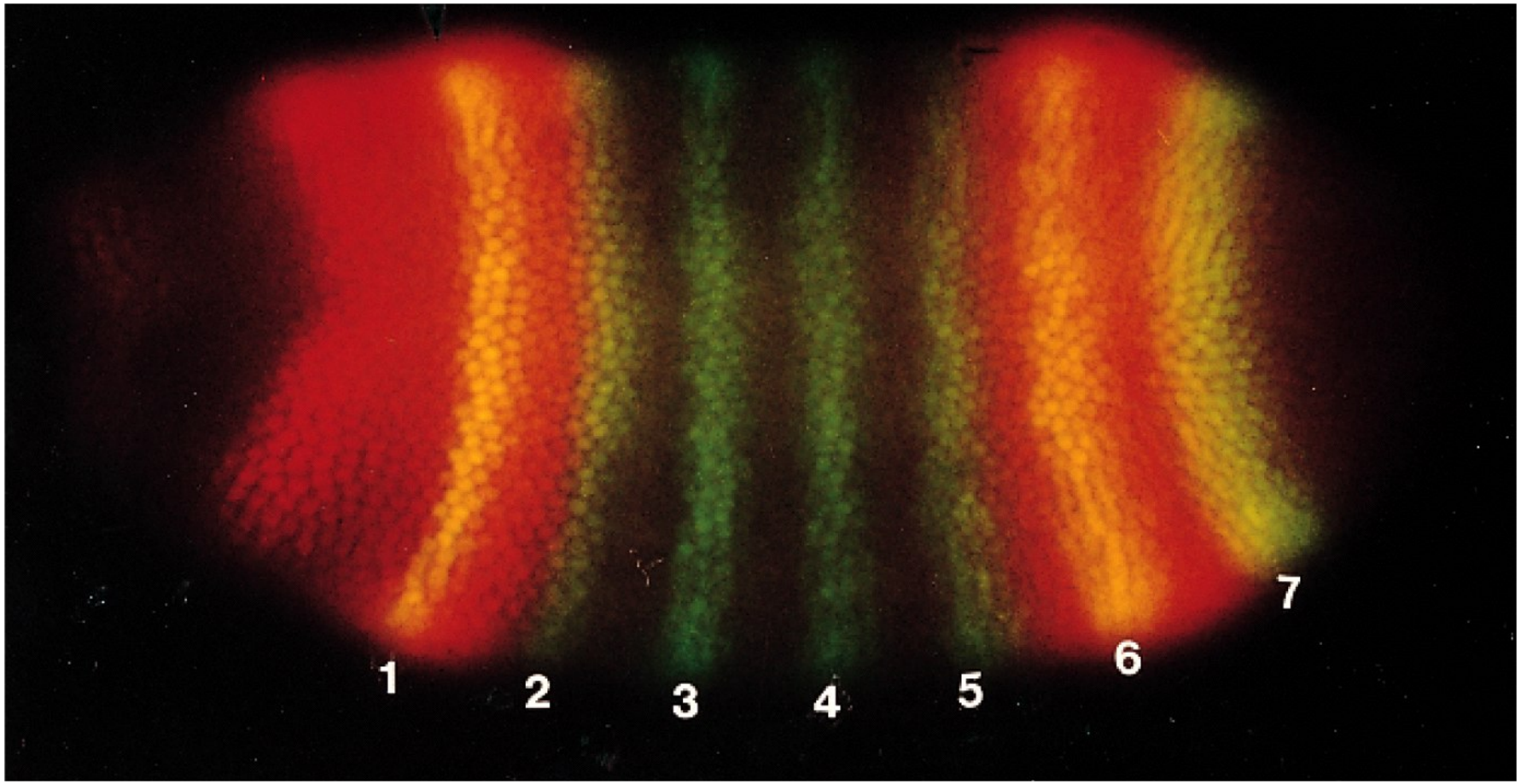


Figure 7-54 *Molecular Biology of the Cell* (© Garland Science 2008)

All *Eve* expression is controlled by a small number of transcription factors expressed in broad bands in earlier embryo

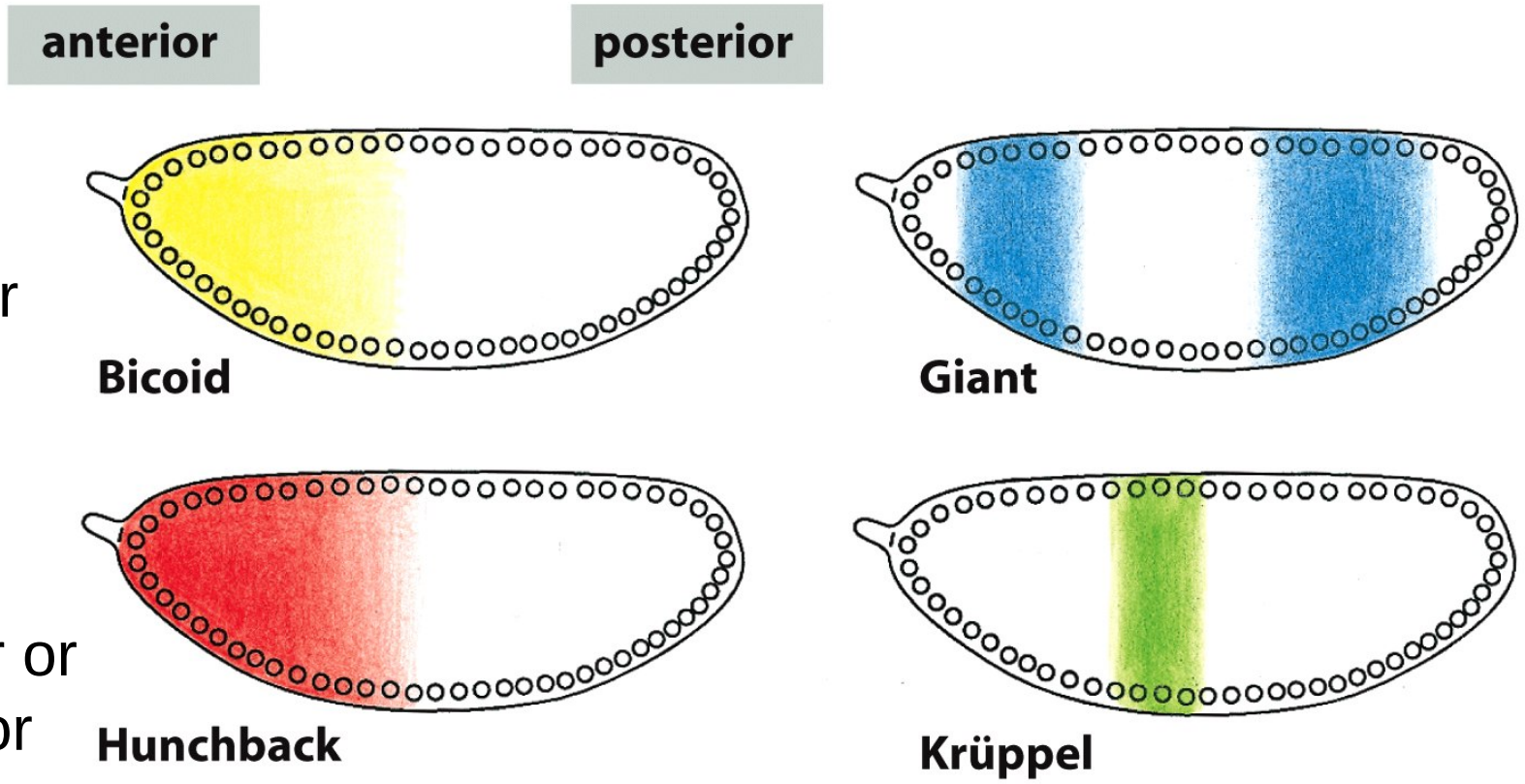
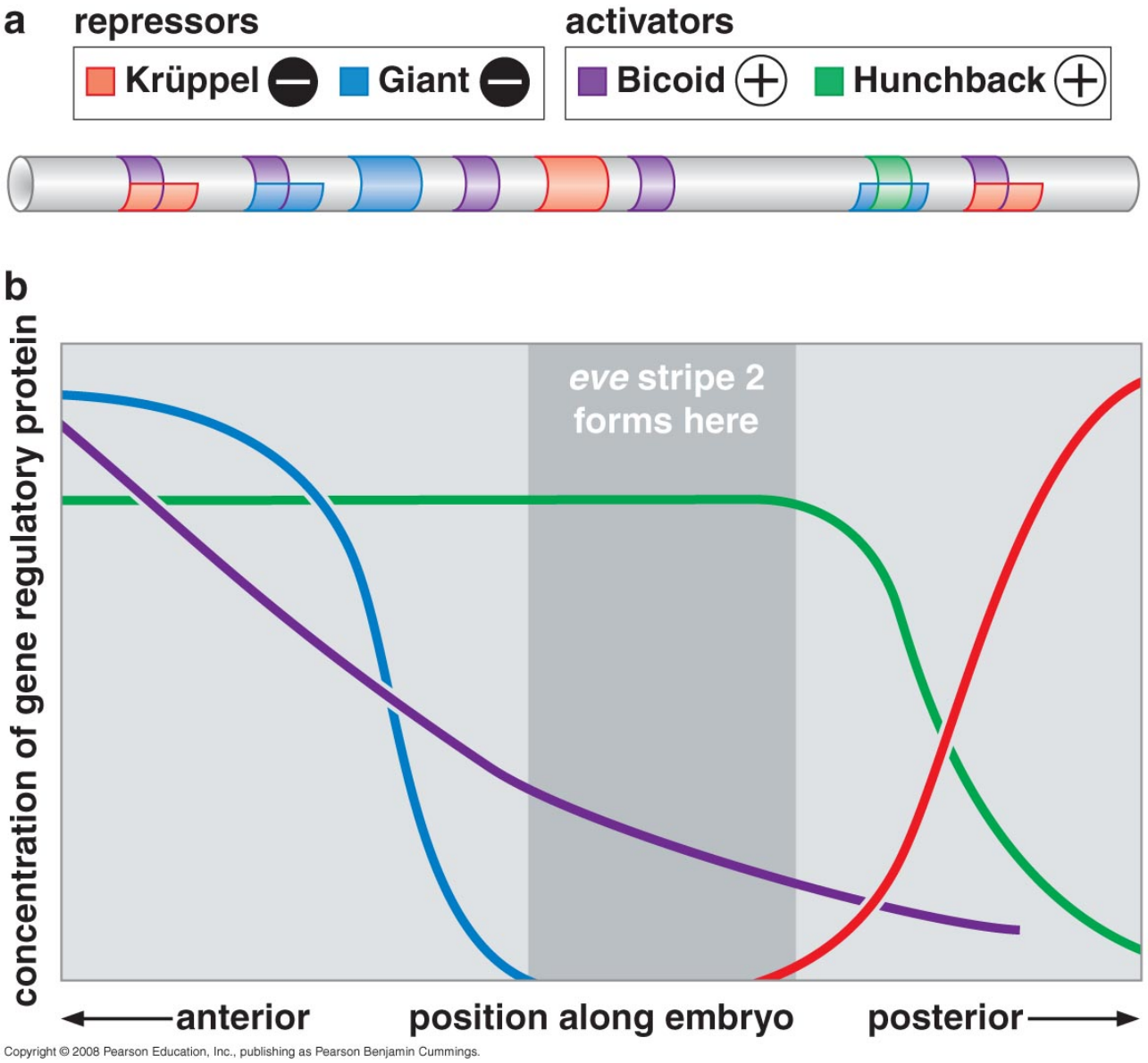


Figure 7-53 *Molecular Biology of the Cell* (© Garland Science 2008)

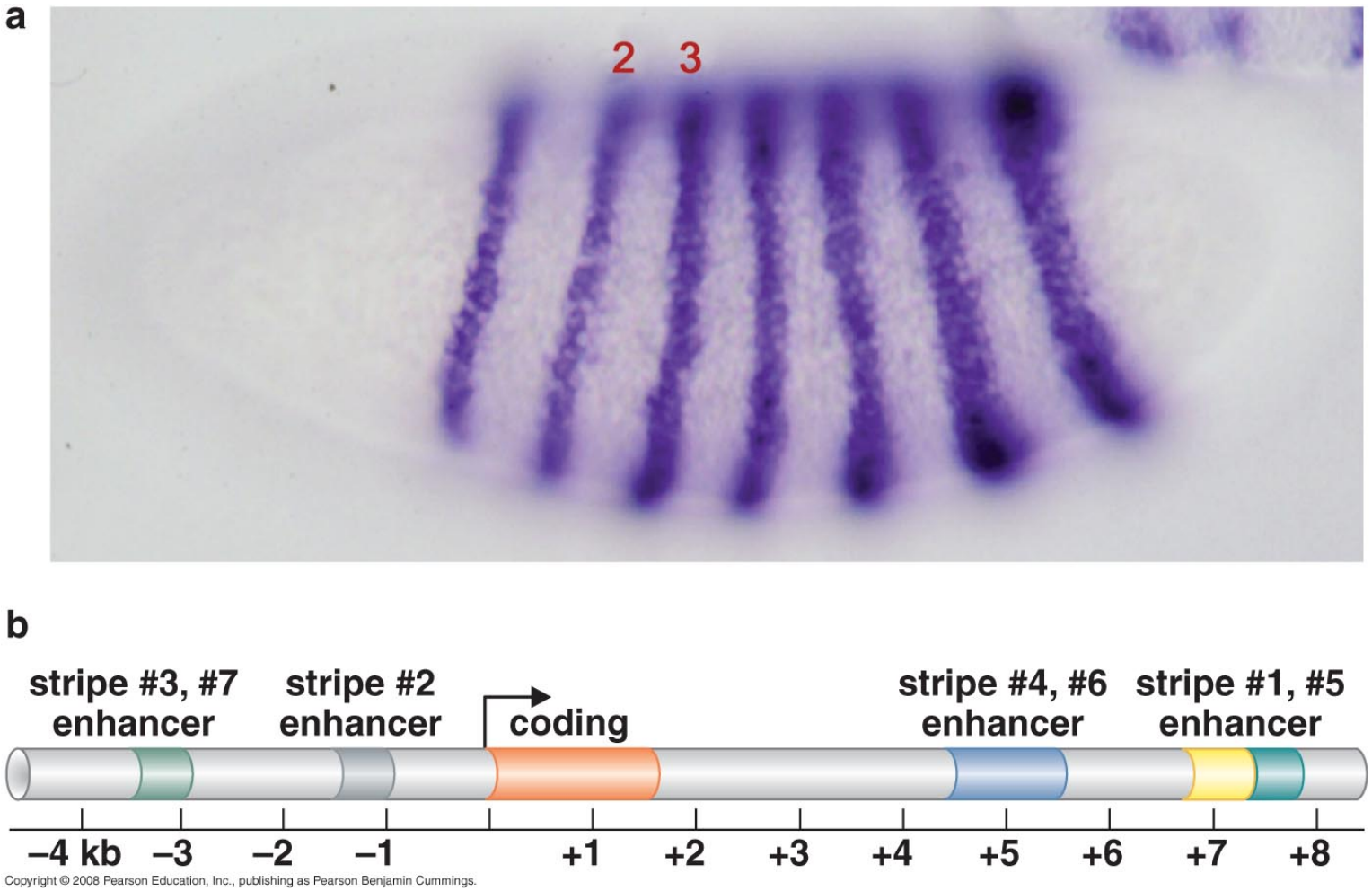
Combinatorial transcriptional control = logic statement

Binding site combination constrains activity =>
IF(B) and (H) andNOT (K) andNOT (G)

As long as gene is using stripe 2 enhancer

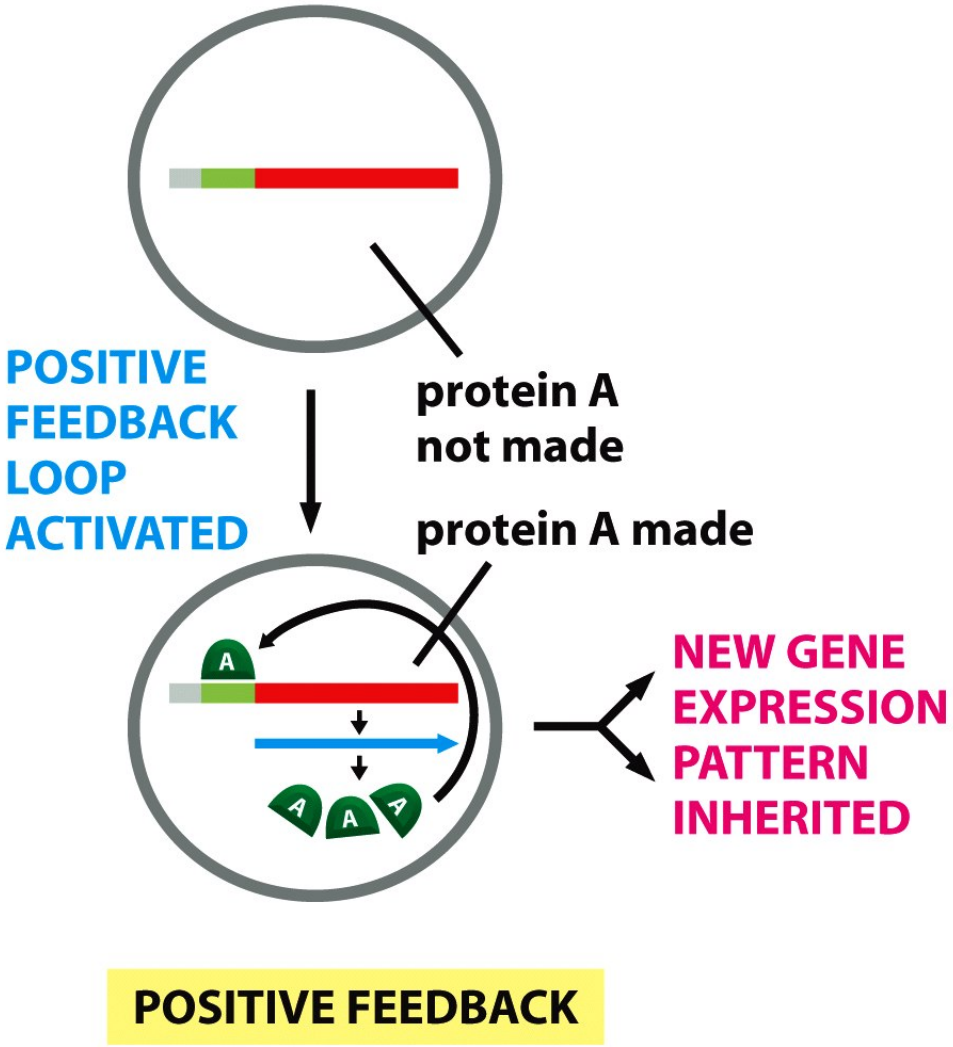


For *Eve*, different cis-regulatory elements promote activity in different regions of Drosophila body



How do different cell types become different from each other?

- Problem of development: transformation of precursor into one/several different types of cells
- Physiological responses (e.g. NF-κB activation) can be reversible; development ***cannot*** be
- How do you cause ***one-way*** changes in gene expression… that do not go back to starting point?
- Key: among genes turned on, encode transcription factors
- These change the "regulatory state" of the cells
- This makes new genes accessible for activation

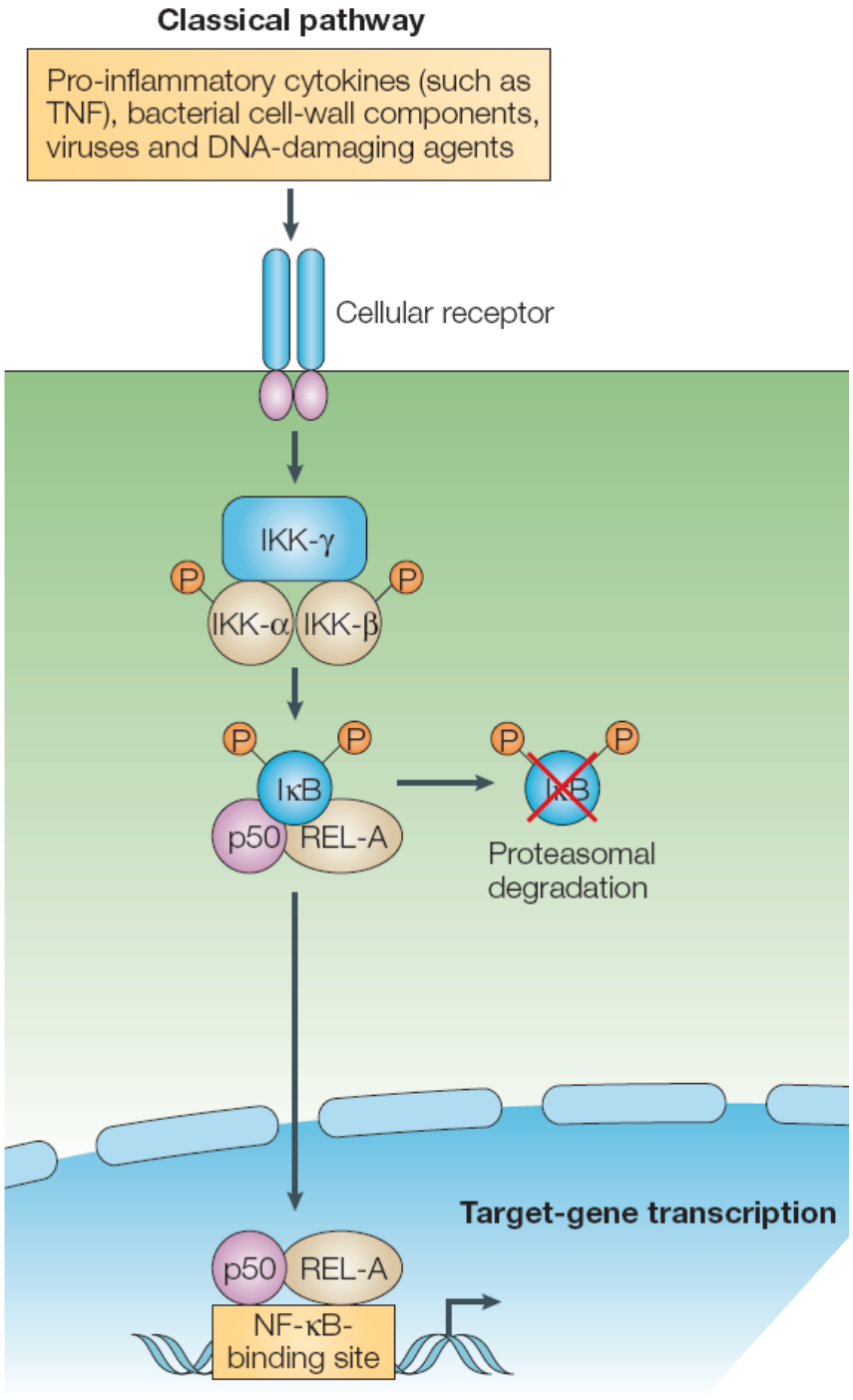


Multiple methods for cells to remember prior regulatory states: active propagation of transcription factor action across cell cycles

as some of these regulated genes code for the transcription factors themselves, this pattern also can be stable…

gene network mechanism

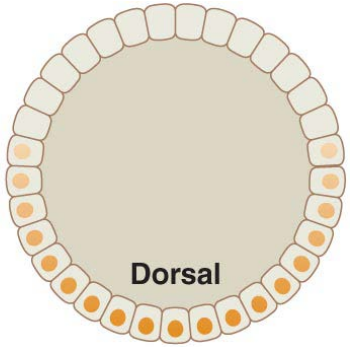
Figure 7-86a *Molecular Biology of the Cell* (© Garland Science 2008)



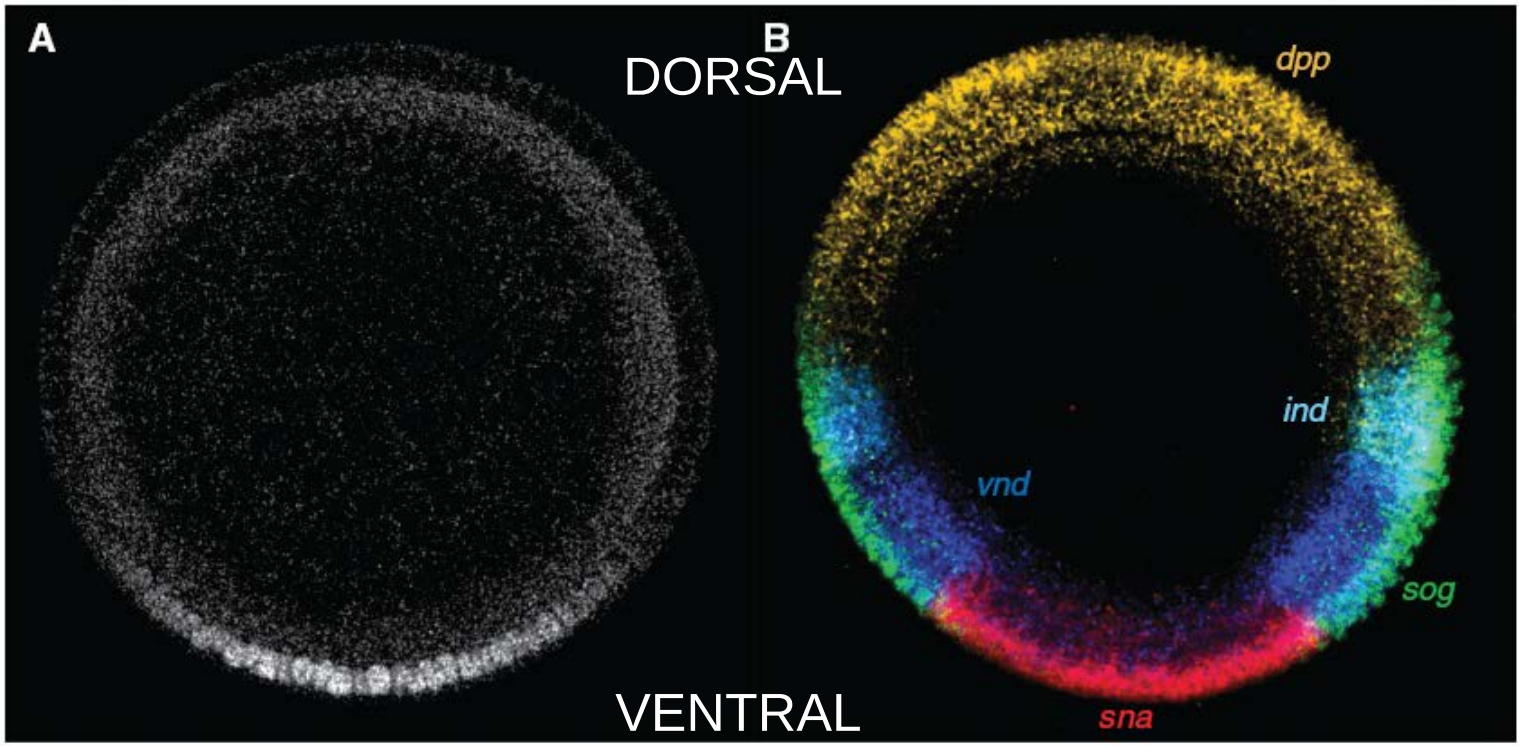
NF-κB is made long in advance of use, but held in cytoplasm by a tethering complex (IκB) until released to go to the nucleus by signaling

Cell exposure to cytokines, immune signals, bacteria, etc. cause breakdown of tethering complex

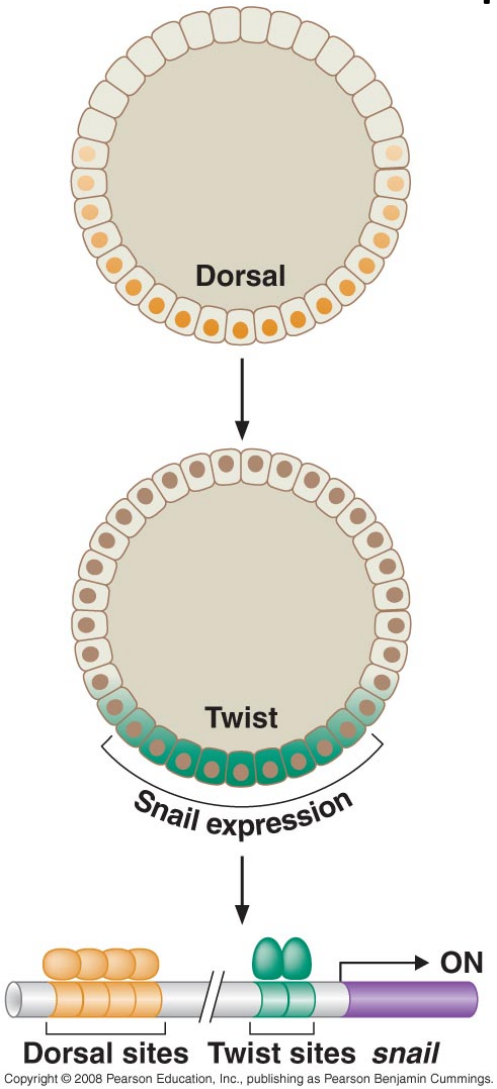
Dorsal-ventral axis of fly embryo is set up by graded activation of transcription factor “Dorsal” (fly NF-κB)



A different series of genes turns on in different, defined regions, dorsal to ventral: Patterns muscle, nervous system, skin

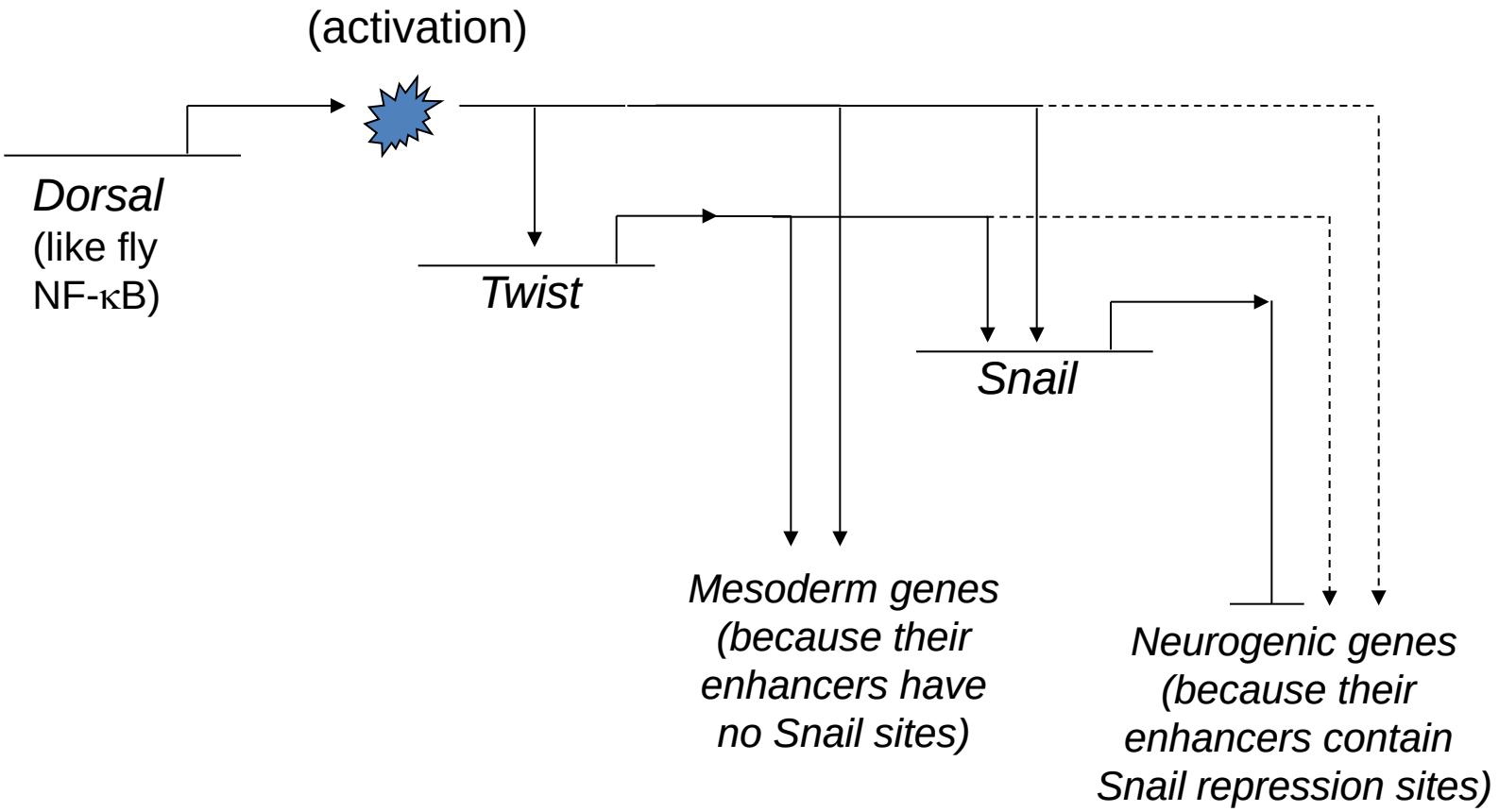


Dorsal-ventral axis is set up by transcription factor "Dorsal" in combination with its own target gene products, Twist and Snail (also TFs)



- Dorsal turns on Twist
- Twist is an activator
- It helps Dorsal turn on Snail
- Snail is a repressor
- This combination patterns most of dorsal/ventral gene expression in fly

Trans-acting factors in D/V patterning: two key players besides Dorsal itself are targets of Dorsal



This is a genetic network

Memory can be conveyed through activation of network

How does a cell use its history to create restrictions on TF action?

Multiple methods exist for cells to remember prior regulatory states: the histone code-passive mechanism

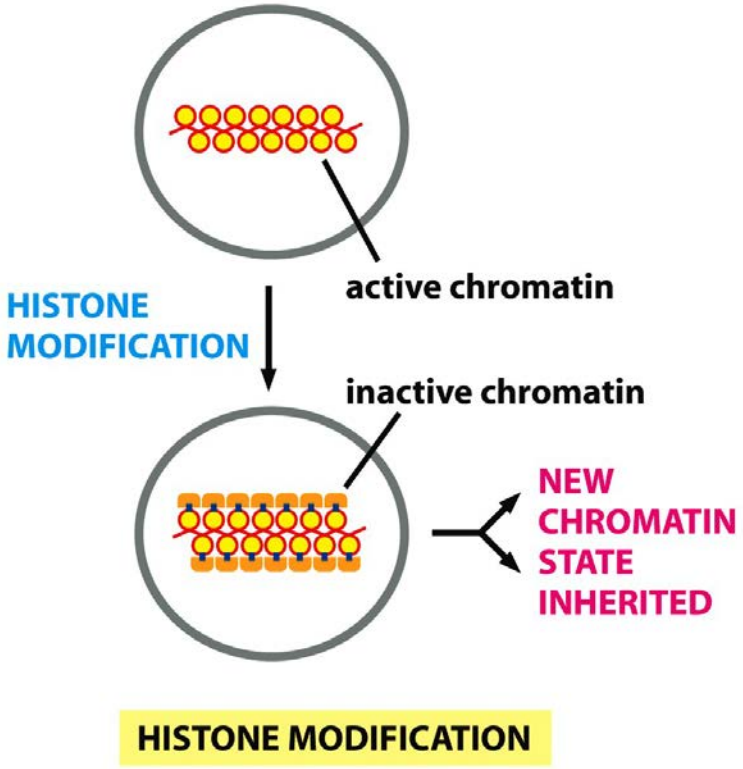
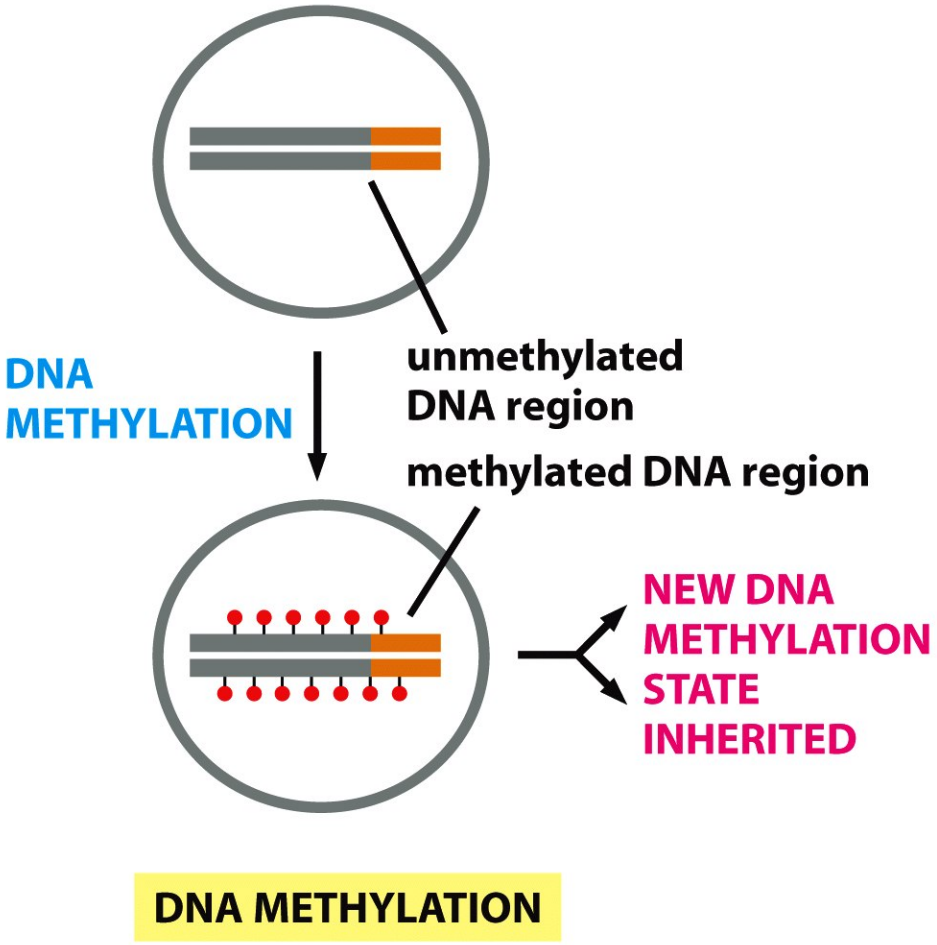


Figure 7-86b *Molecular Biology of the Cell* (© Garland Science 2008)

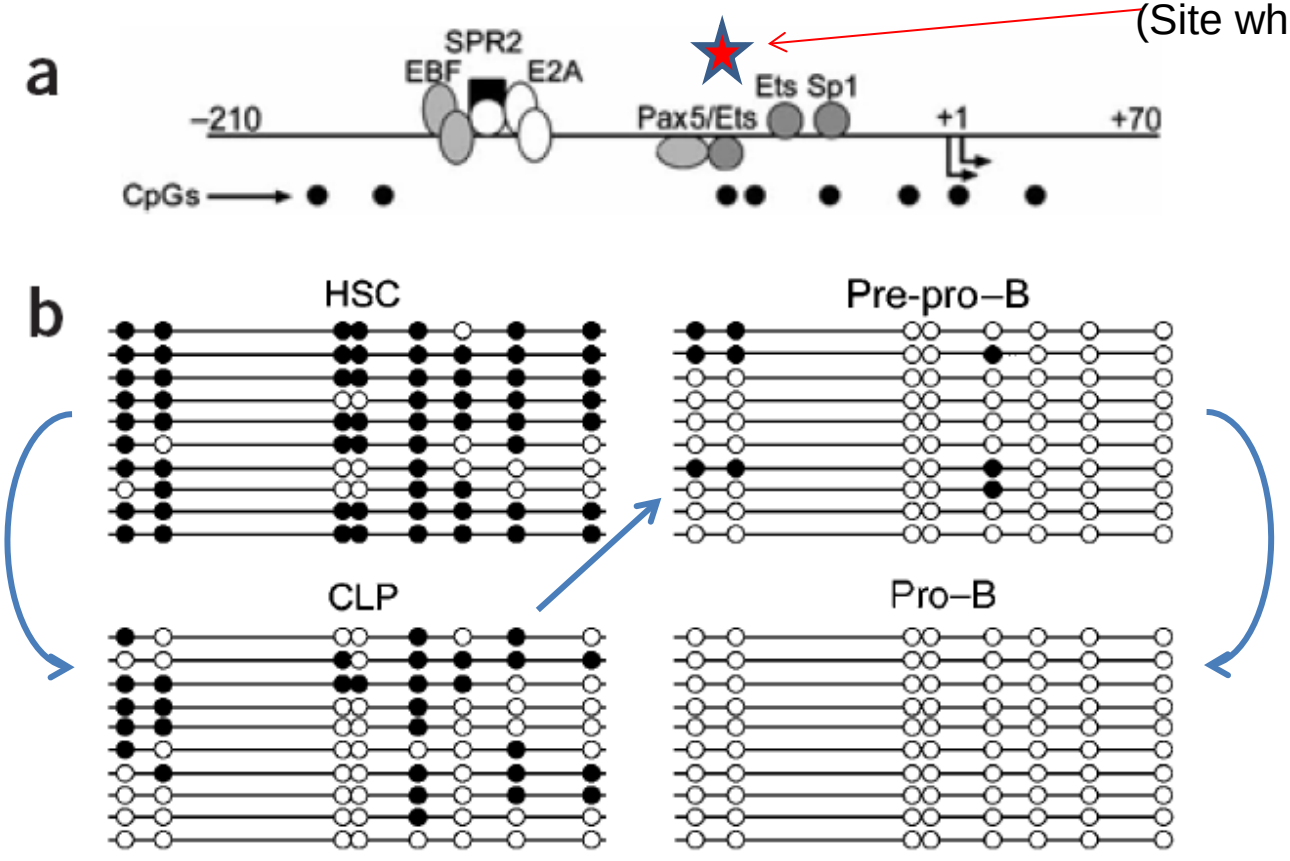


DNA methylation as another passive form of regulatory state stabilization memory:

not just affecting DNA access via chromatin configuration, but also affecting actual recognition sites for some transcription factors

Figure 7-86c *Molecular Biology of the Cell* (© Garland Science 2008)

A specific example: the B-cell specific *mb-1* gene



(Site where methylation blocks Ets1 binding)

Black dots: methylated CpG on a particular DNA strand
Open dots: demethylated CpG on a particular DNA

Many CpG sites are methylated in stem cells (HSC), but…

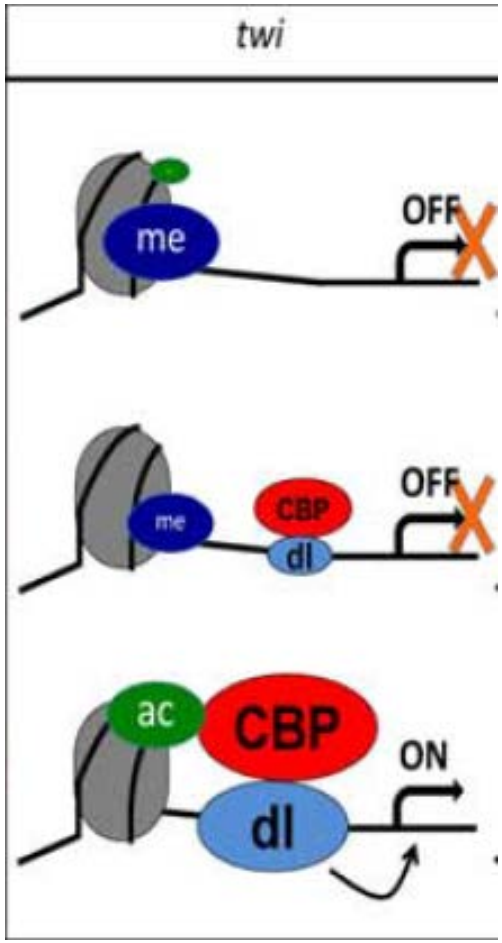
Methylation is ***removed*** as precursors enter the B-cell pathway

Process initiated by E2A/EBF1 binding

(Maier et al., 2004, Nat. Immunol.)

Epigenetic marks "work" as predictors because they are ***results*** of local TF action… and then they also facilitate activity by later-arriving TFs

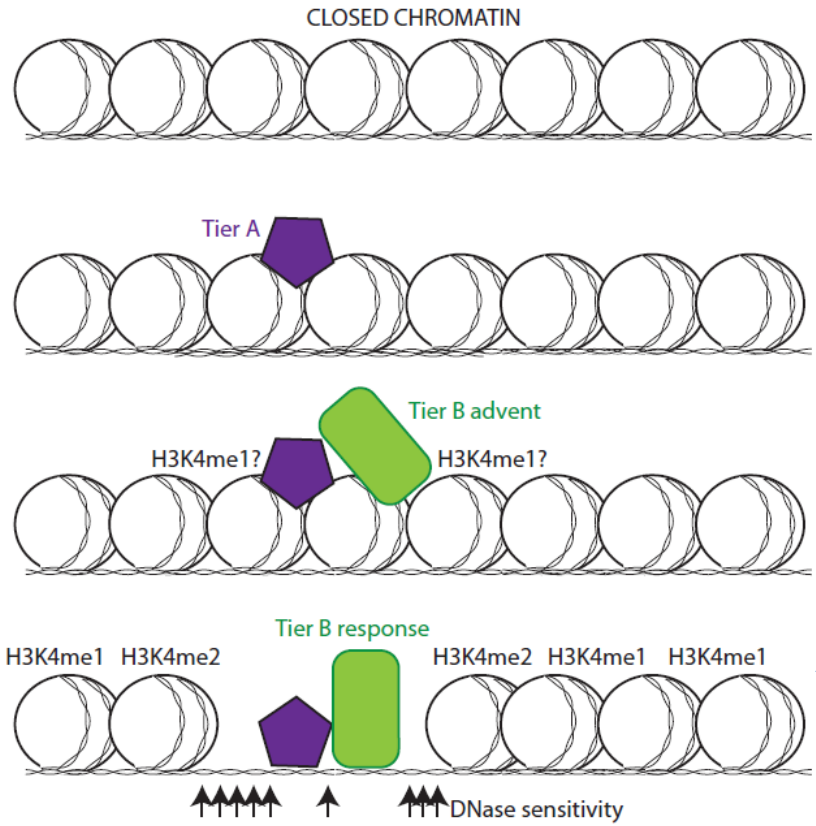
Amount of modifier recruited depends on TF binding and previous modification state



(Holmqvist…Manner-vik, PLoS Genet 2012, ***8:*** e1002769*)*

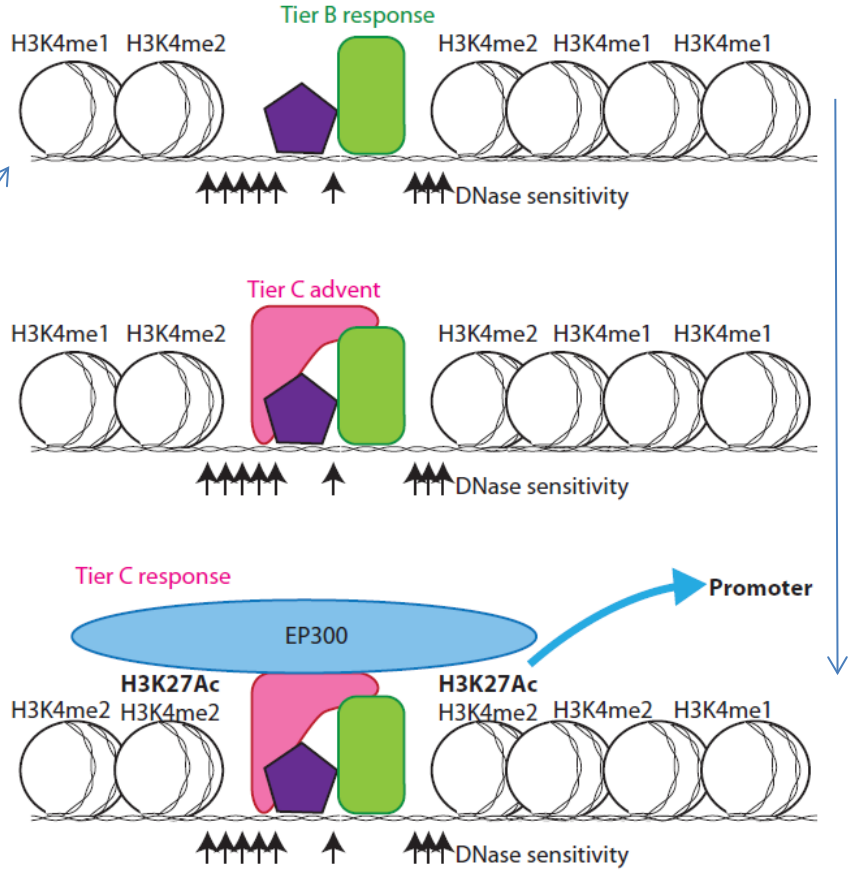
- Transcription factors can recruit MLL – histone H3K4 methyl transferases
- Transcription factors can recruit CBP or (E)P300 – histone H3K27 acetyl transferases
- Transcription factors can recruit Gcn5 (Kat2a) histone H3K9 or K14 acetyl transferases
- Transcription factors can recruit Utx or Jmjd3 histone H3K27 *demethylases:* remove repression

A stepwise interaction of transcription factors with chromatin can open & activate cis-reg elements



An open enhancer… but not yet activating a gene

Only now does the enhancer activate the target promoter



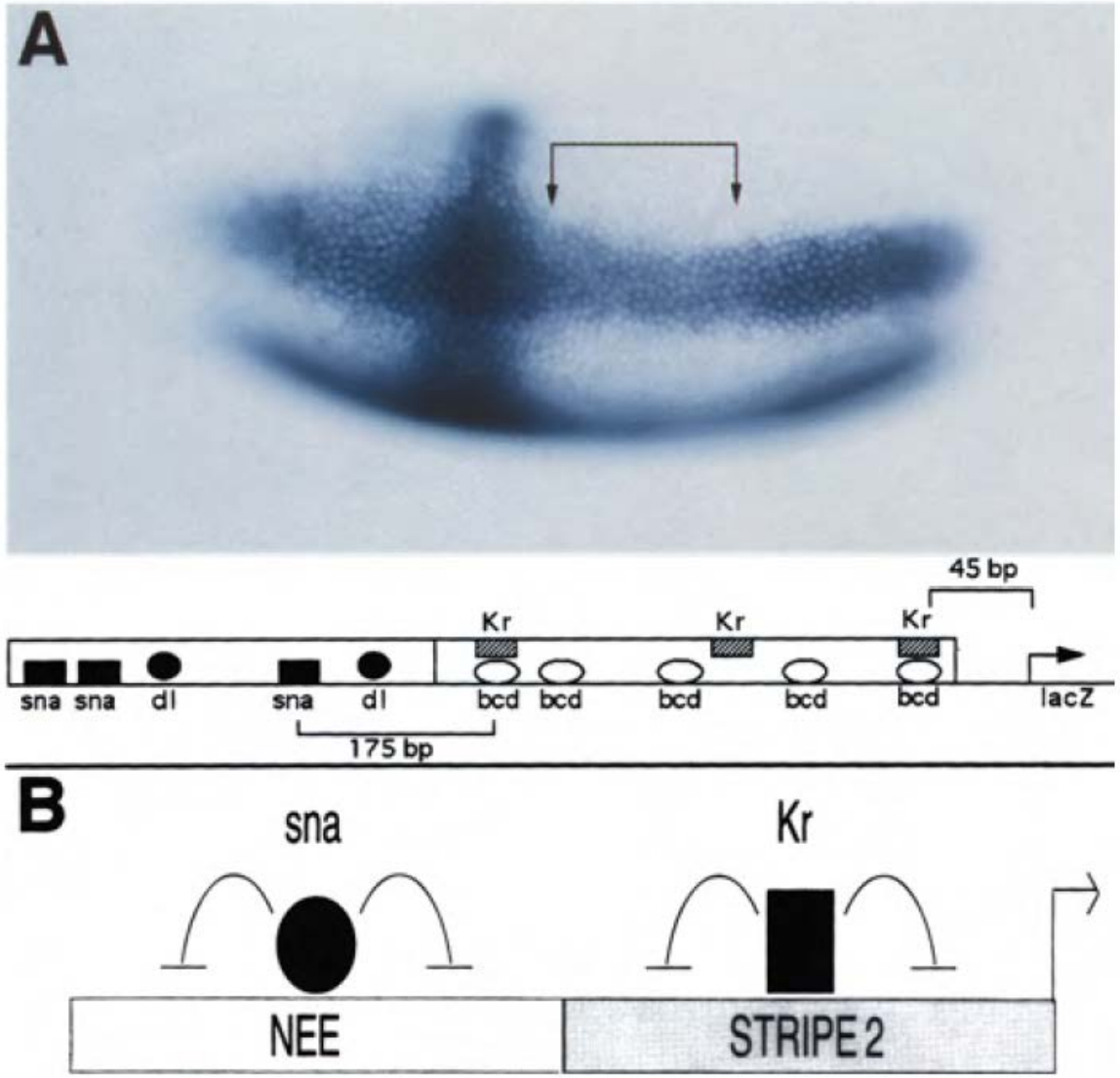
What are the possibilities for effects of multiple cis-regulatory elements for same gene?

- All local integration of effects of activators and repressors can occur within same cis-reg module: result is enhancer independence (like *Eve*)

- Dominant activity or repression by factors at one cis-element can overwhelm activity from another cis-element: "Silencing" (for repression) or "Locus Control" (for activation)

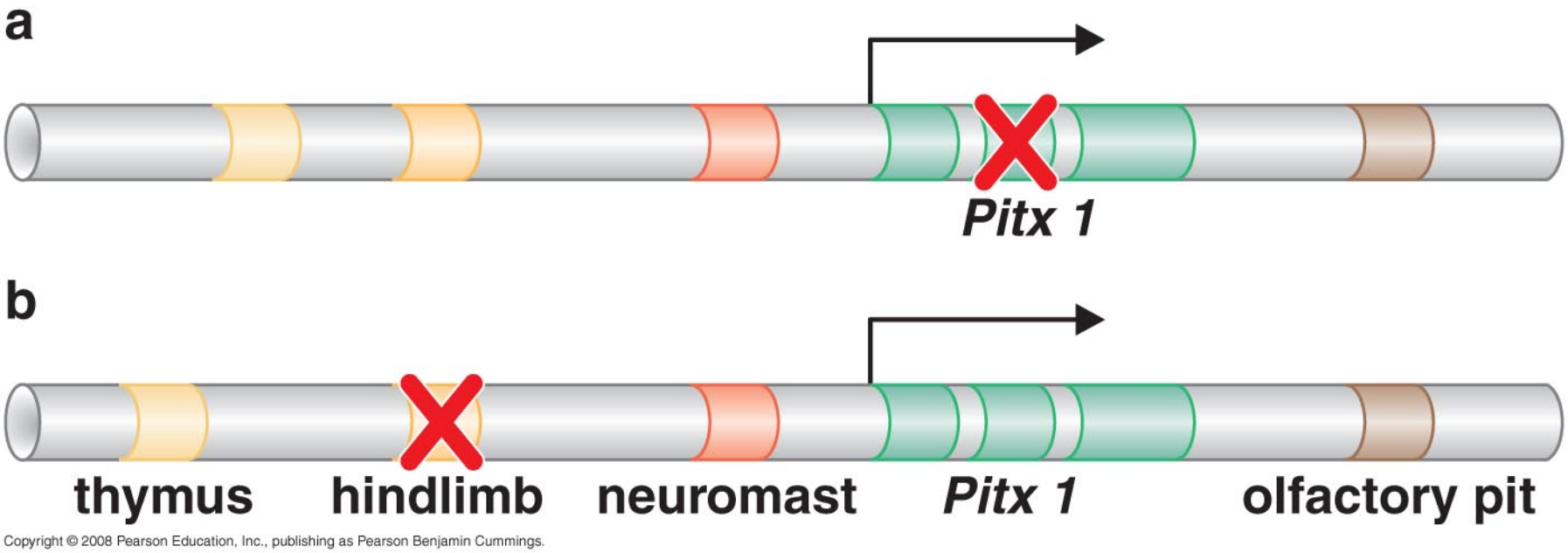
- Examples of both can be found

Two enhancers can act autonomously: not just additive domains of expression, but also ***ignoring*** each other's repressors



(Gray & Levine, 1996, Genes Dev)

Enhancer independence makes it possible for fish to evolve loss of hind fins… without losing key gene for use in other tissues



From work of D. M. Kingsley & colleagues… Pitx1 is a vital transcription factor in many organs, and only one of its jobs is to help a vertebrate “make” a hindlimb

Multi-purposing of genes in different tissues makes multiple enhancers vital for survival

- We have only about 25,000 genes… approximately same as Drosophila, C. elegans

- Our brains are much more complex
- Lots of gene re-use from one tissue to another
- Gene expression in one tissue needs to be regulated according to needs of *that tissue*

- Multiple enhancers with combinatorial logic in each are essential for health and evolutionary success