Gene regulation in eukaryotes

Combinatorial control of gene expression

Unit II, Paper Zoo 401A

By

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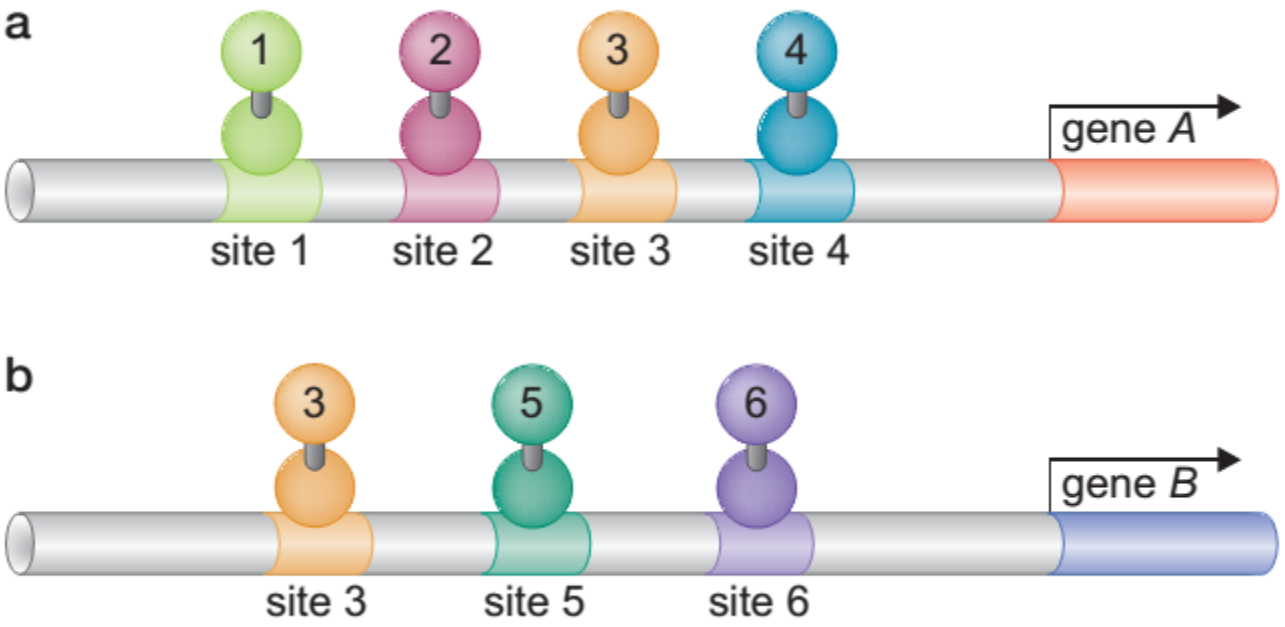
Distinct features of gene regulation in eukaryotes

- Access to promoters restricted by chromatin structures.
- Positive gene regulation
- Involve multiprotein regulatory networks
- Transcription separated from translation

Combinatorial control of gene expresion



Two genes are shown, each controlled by multiple signals—four in the case of gene *A* (a); three in the case of gene *B* (b). Each signal is communicated to a gene by one regulatory protein. Regulatory protein 3 acts at both genes, in combination with different additional regulators in the two cases.

- Control of gene expression achieved by combinations of regulators, at least one of which is common to the different cell types

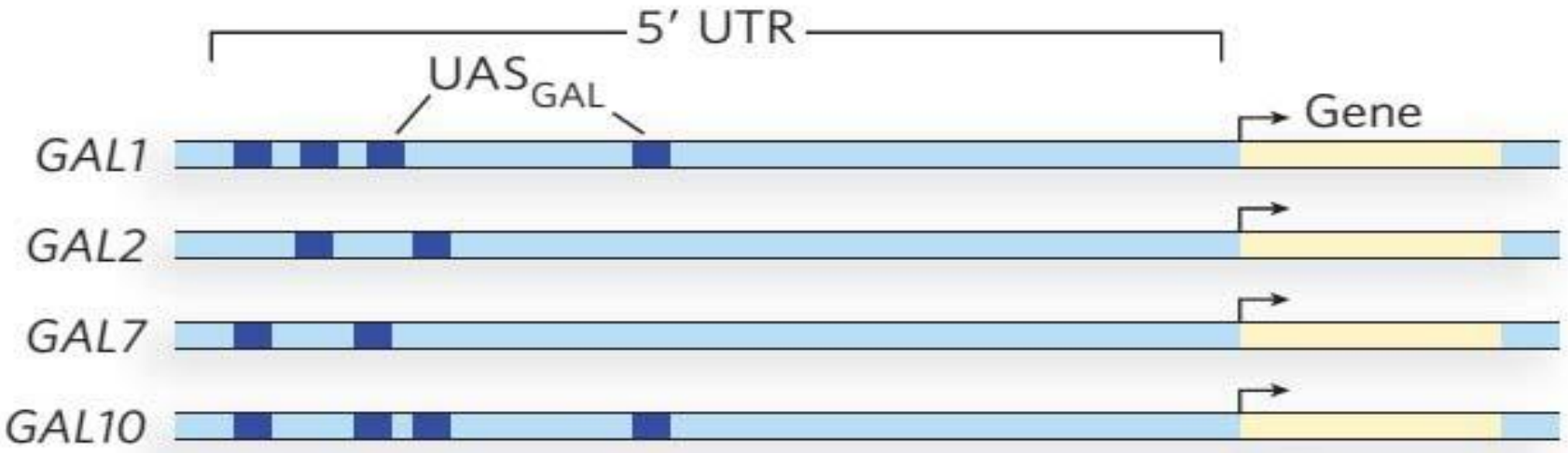
Combinatorial control of gene expression ?

- It is the regulation of different sets of target genes by the association of the same transcription factor with different binding partners.
- Here control of gene expression is achieved by combination of regulators, at least one of which is common to the different cell types.

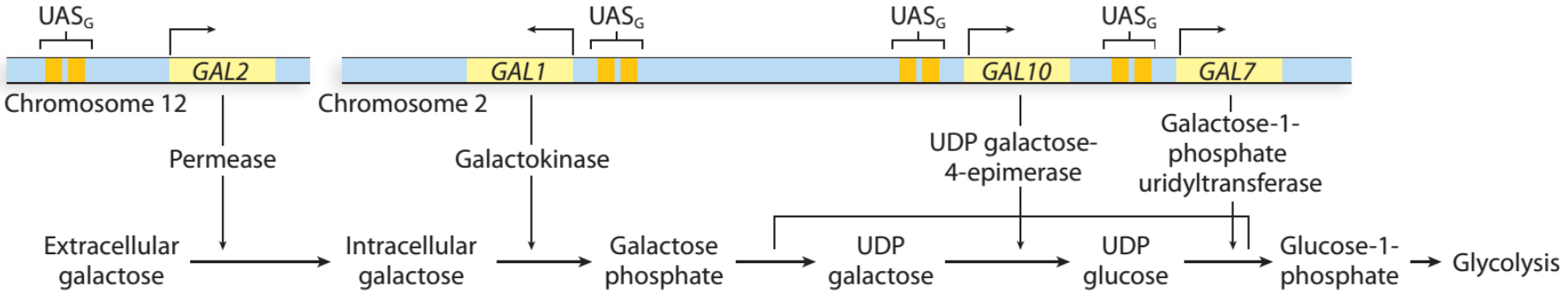
examples

- Control of *Gal* (galactose metabolizing) gene expression in yeast
- Yeast mating type switching regulation
- Transcription regulation by formation of heterodimers in transcription factors
- Control of *eve* gene (for even-skipped protein) expression in fruit fly

Combinatorial control of Gal gene expression in yeast



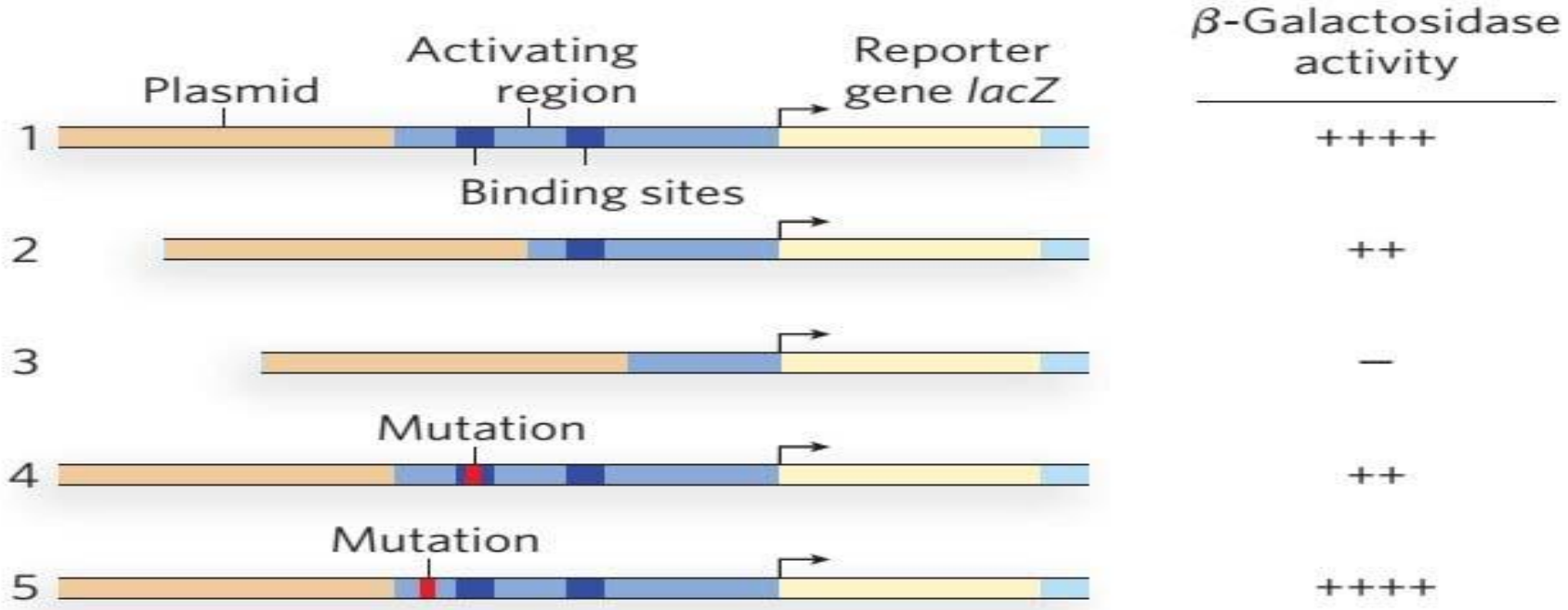
A comparison of the upstream sequences of the yeast *GAL* genes showed that they have common sequences, the UAS$_{GAL}$ sites, each 17 bp long (blue).



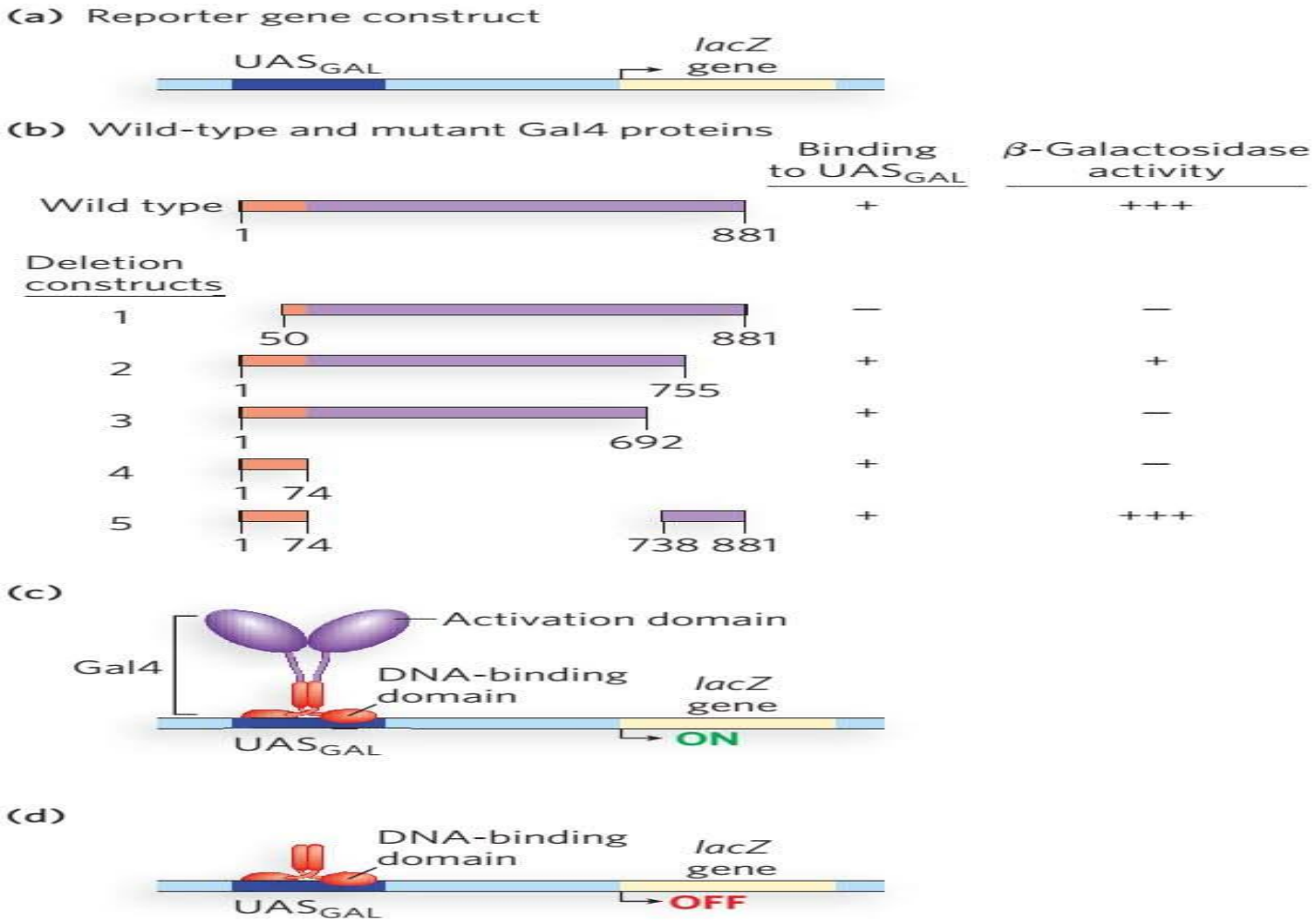
Galactose utilization in *S. cerevisiae*. Galactose utilization requires the action of products of each of four galactose-utilization (*GAL*) genes.

Yeast *GAL* gene

The GAL1 promoter. The promoters of the *GAL* genes of yeast each contain an upstream activator sequence (UAS), composed of one or more UAS_{GAL} sites. Each 17 bp UAS_{GAL} sequence is a binding site for transcription activator Gal4p. The UAS of the *GAL1* gene has four UAS_{GAL} sites.



The function of UAS_{GAL} sequences was confirmed in reporter gene assays in which promoter activity was determined by the activity of β-galactosidase (produced by the bacterial *lacZ* gene). As shown in these five assays (1 is the wild-type), deletion or mutation of UAS_{GAL} elements, but not other areas close to the promoter, resulted in decreased promoter activity (β-galactosidase level).

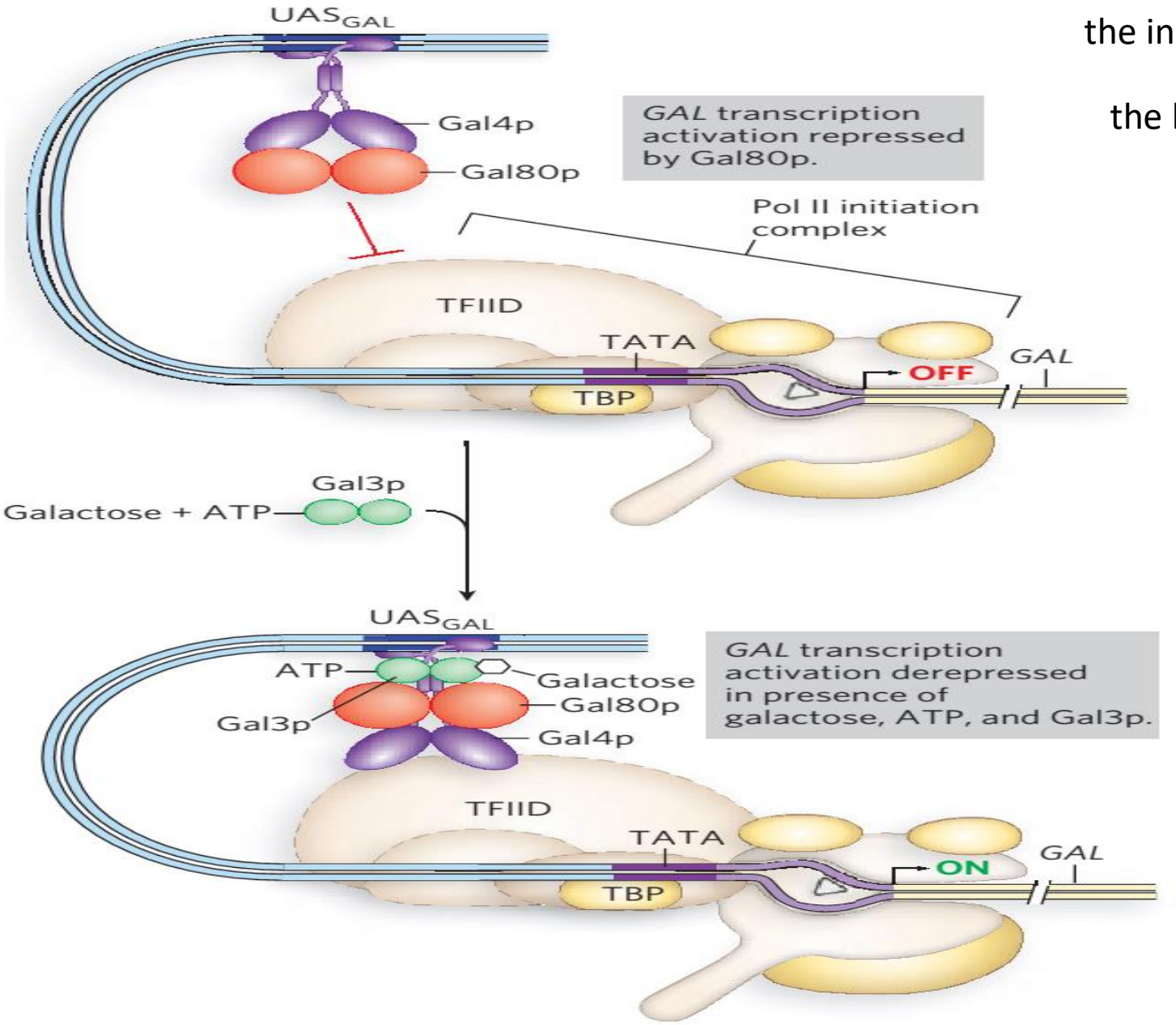


(a) The reporter gene construct used for deletion analysis of Gal4p. Only constructs with functional Gal4p will bind UAS$_{GAL}$ and drive expression of the reporter gene (*lacZ*). (b) Deletion analysis of Gal4p. Two activities were measured: in vitro DNA binding (indicated by + or — in the first column on the right) and in vivo transcriptional activation of the reporter gene construct (second column). (c) In this model of the Gal4 protein, derived from the deletion analysis, Gal4p has separable DNA-binding and transcription-activation domains joined by a flexible linker. (d) The DNA-binding domain, expressed alone, will bind DNA but will not activate transcription.

transcription activator Gal4p

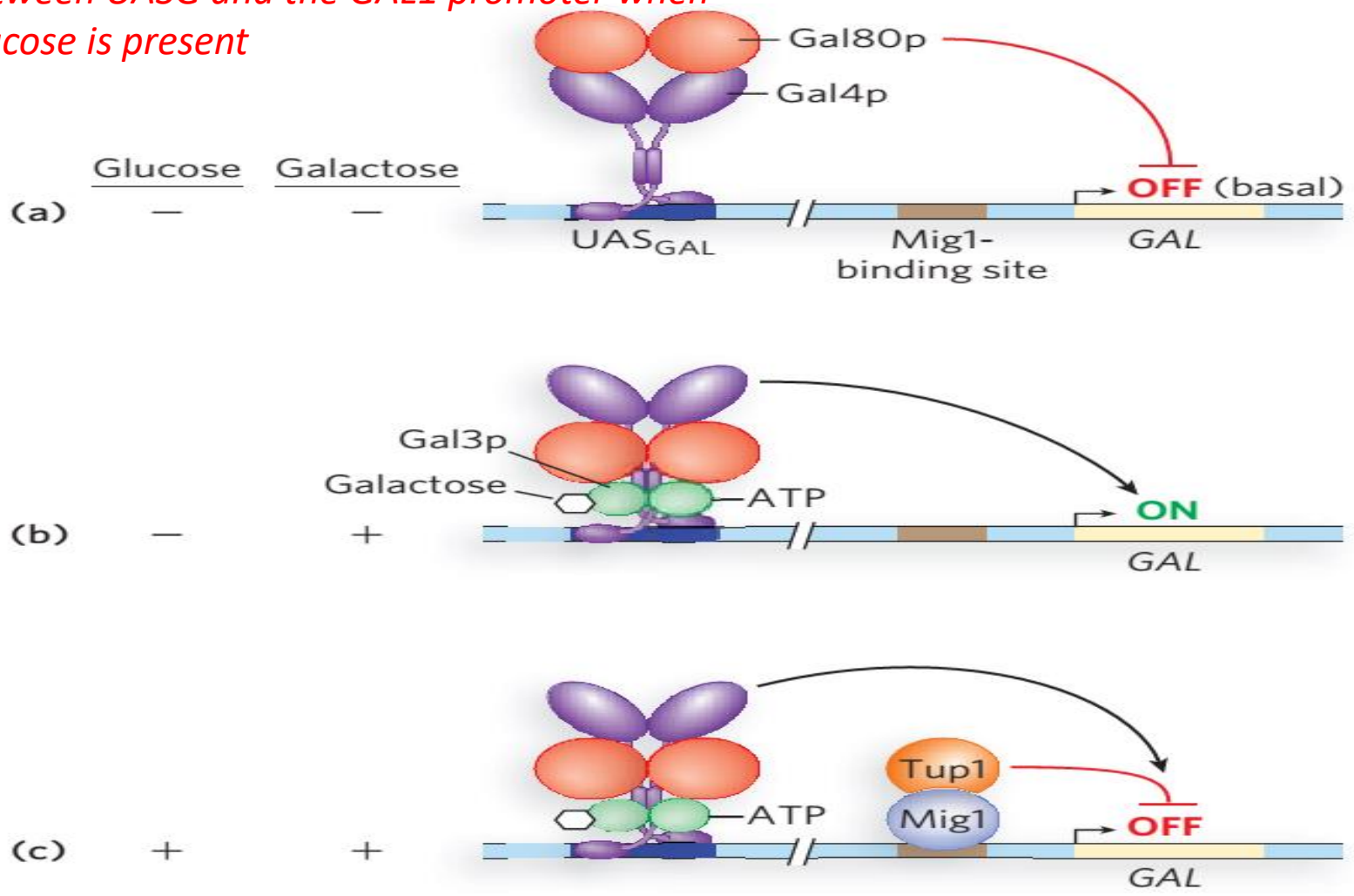
the inhibitor Gal80p

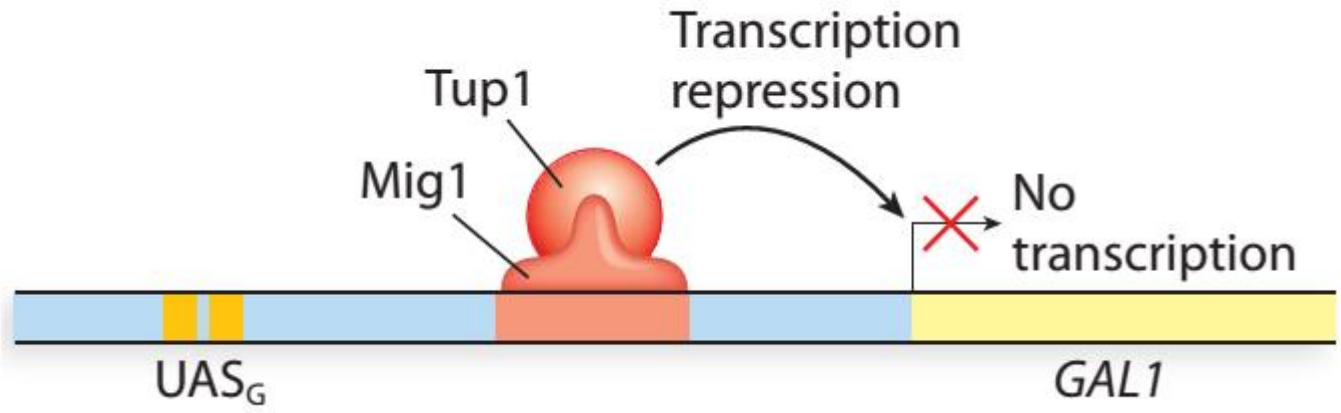
the ligand sensor Gal3p



Combinatorial control in global repression of yeast GAL genes.

Mig1 binds a silencer sequence located between UASG and the GAL1 promoter when glucose is present



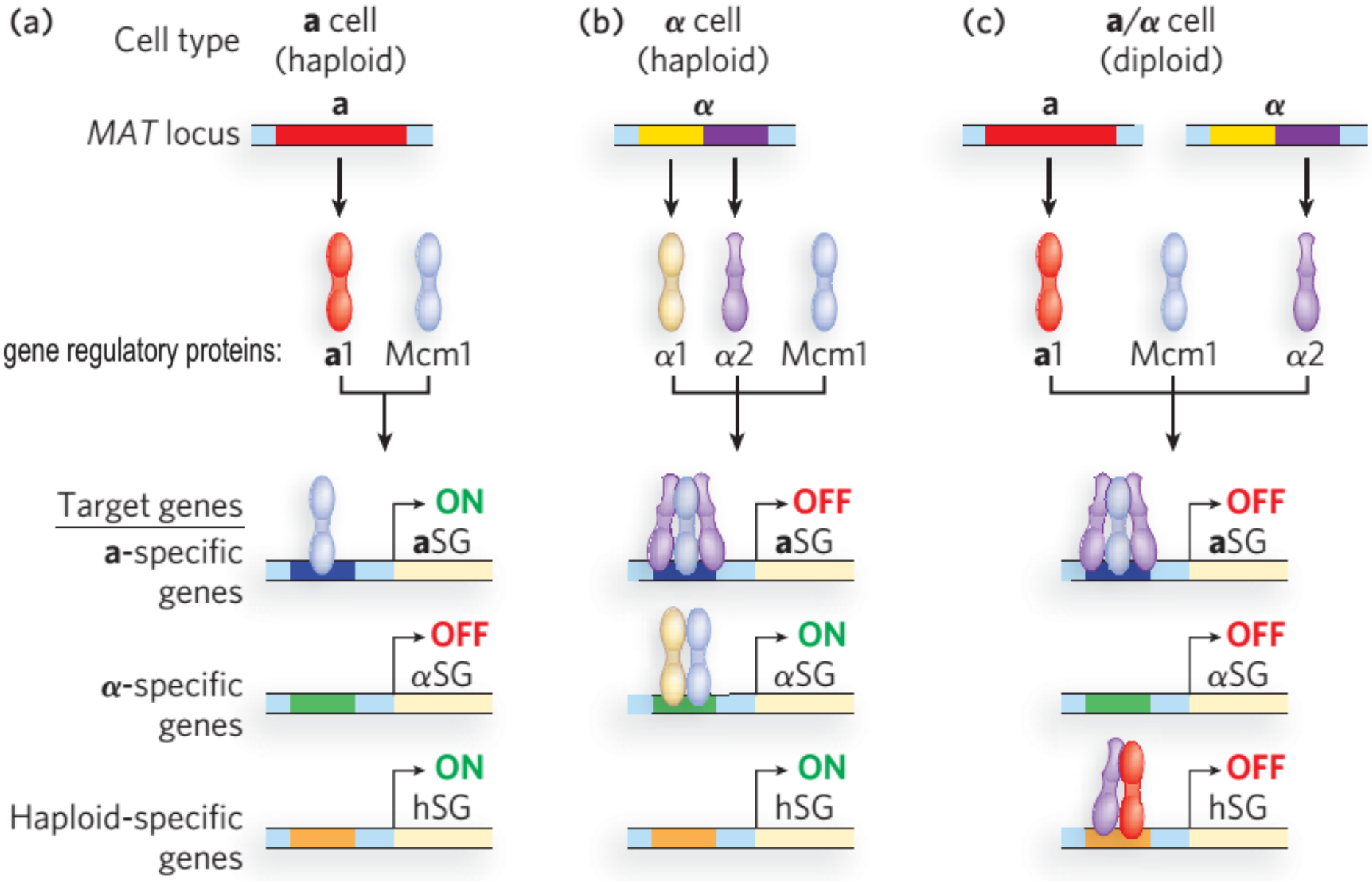


Transcription repression of the yeast *GAL1* gene. The proteins Mig1 and Tup1 bind to the Mig1 site to repress transcription when glucose is available in the growth medium.

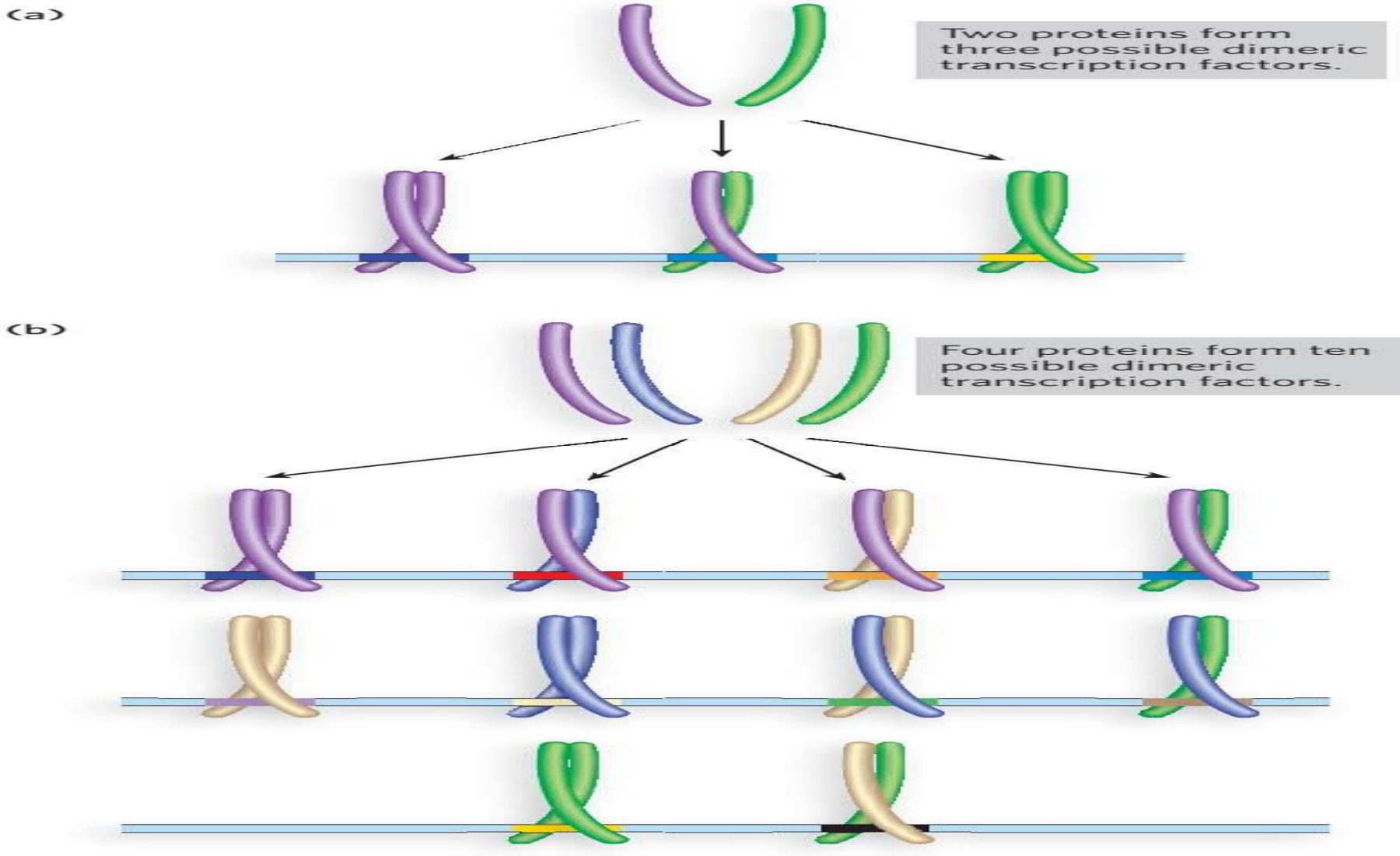
Combinatorial control of transcription causing yeast mating type switches

- e.g. *Saccharomyces cerevisiae*

mating types switching of yeast

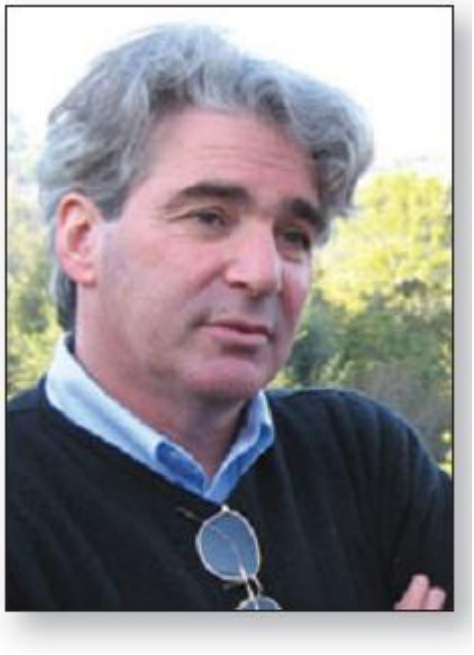


Combinatorial control by heterodimer formation



Combinatorial control by heterodimer formation. (a) Two regulatory proteins that form homodimers and a heterodimer could form 3 different structures, which could bind 3 different regulatory sites. (b) Four proteins have the potential to form 10 different structures and bind 10 regulatory sites. The possible combinations increase dramatically as the number of potential dimerization partners increases.

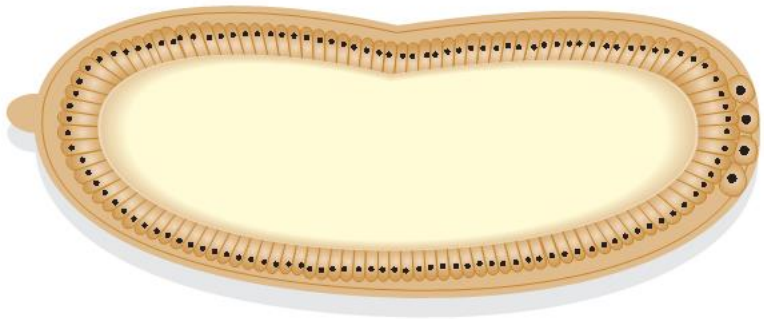
Combinatorial control of eve gene expression during body plan development in fruit fly *Drosophila melanogaster*



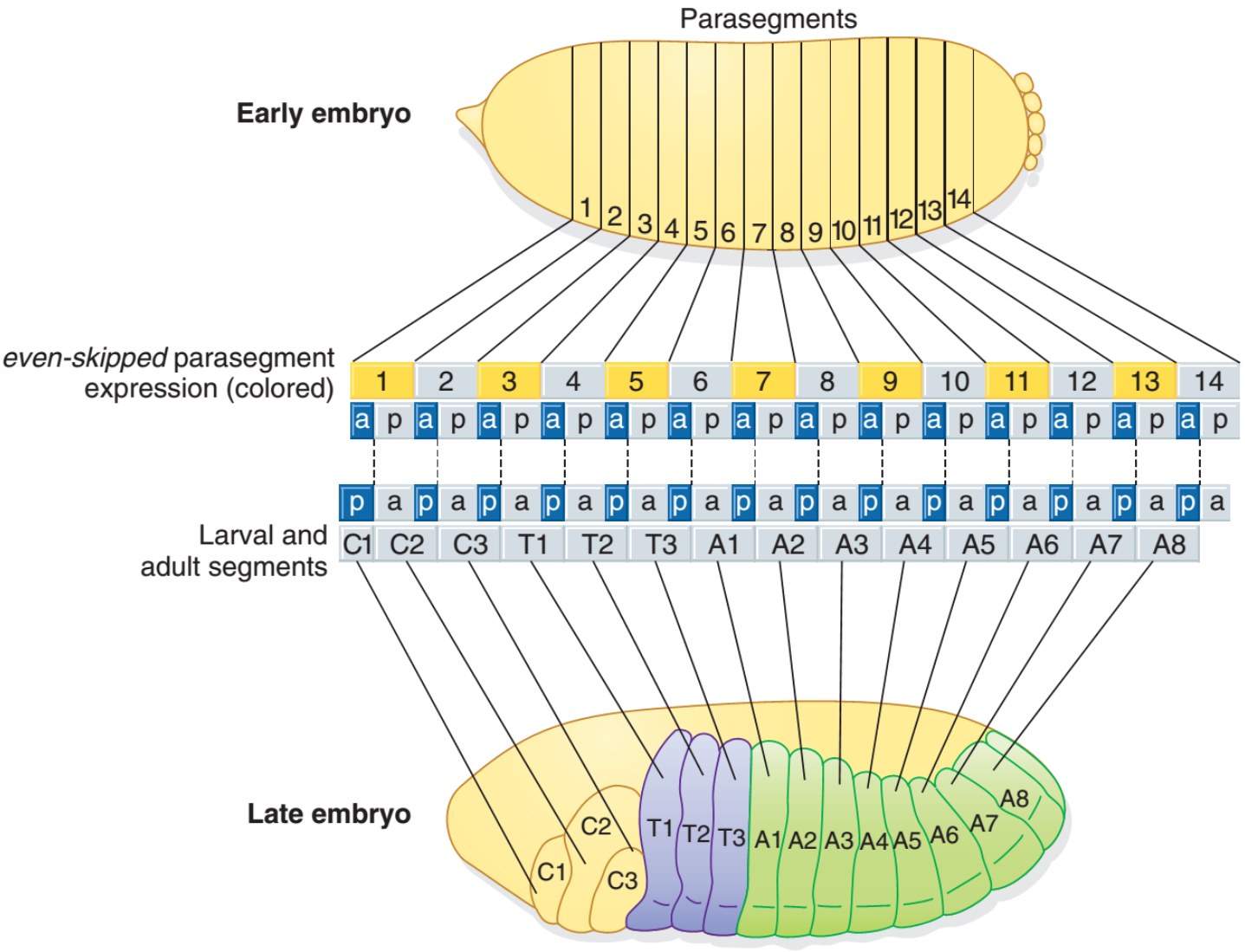
Michael Levine

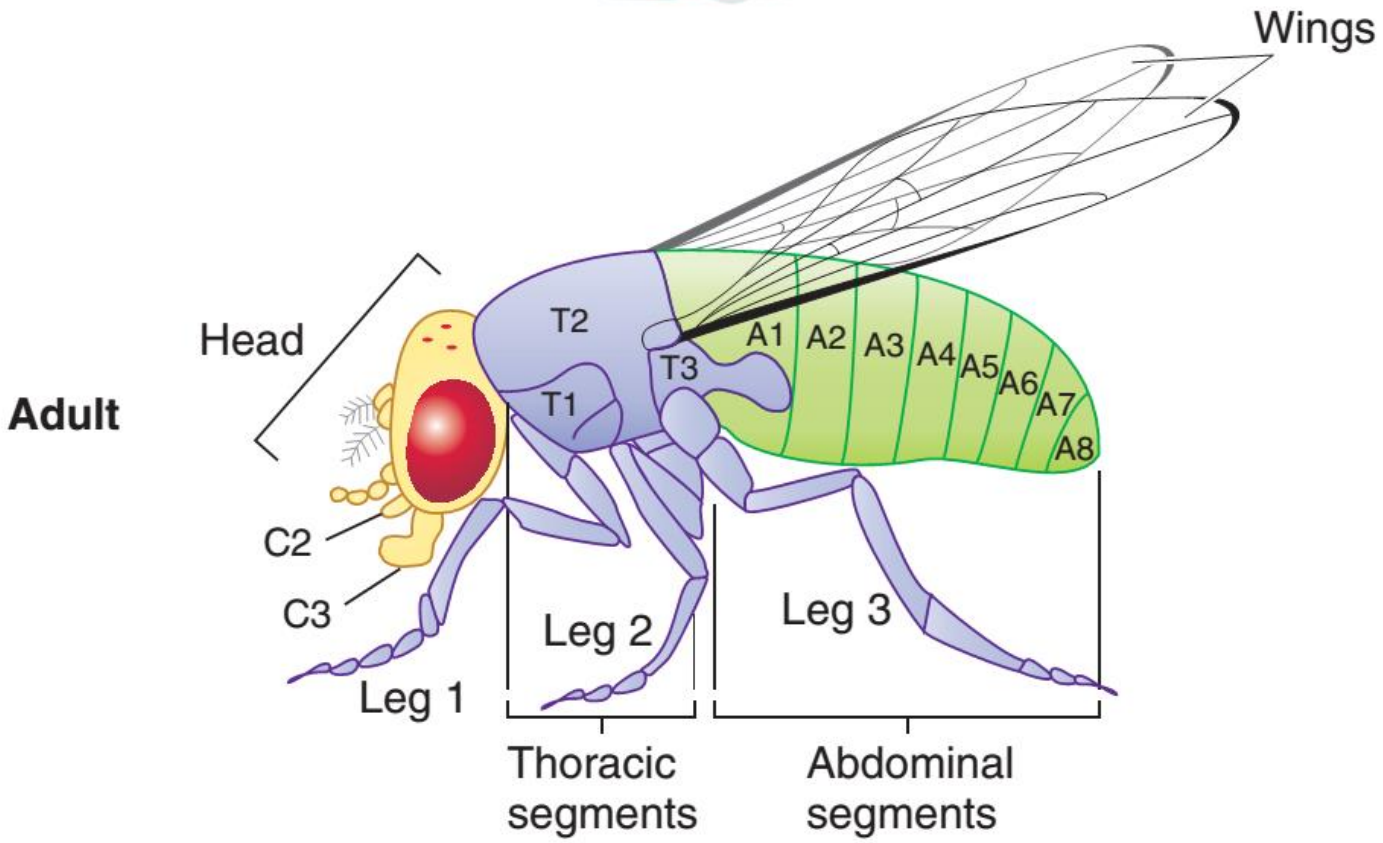
[Source: Courtesy of Michael Levine.]

a) **Syncytial blastoderm. The embryo at this stage is one large cell with many nuclei at the periphery all in a common cytoplasm**



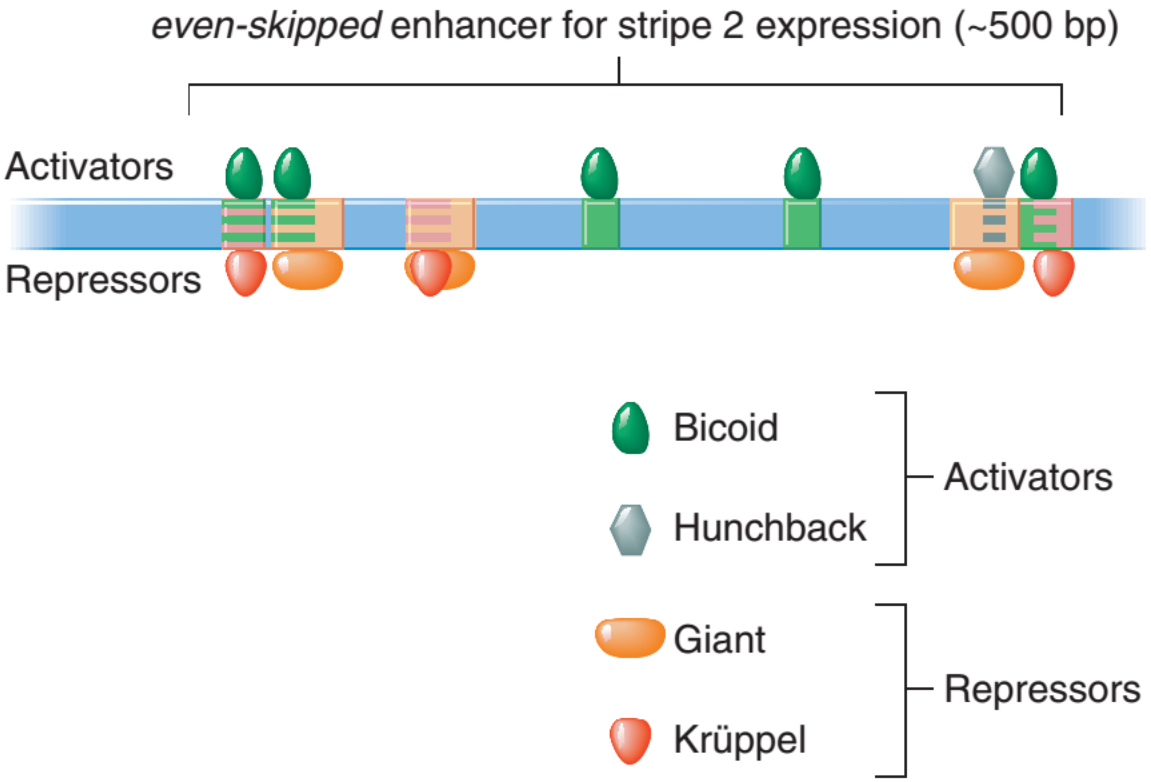
b) Parasegments and segments of the embryo, and the segments of the adult



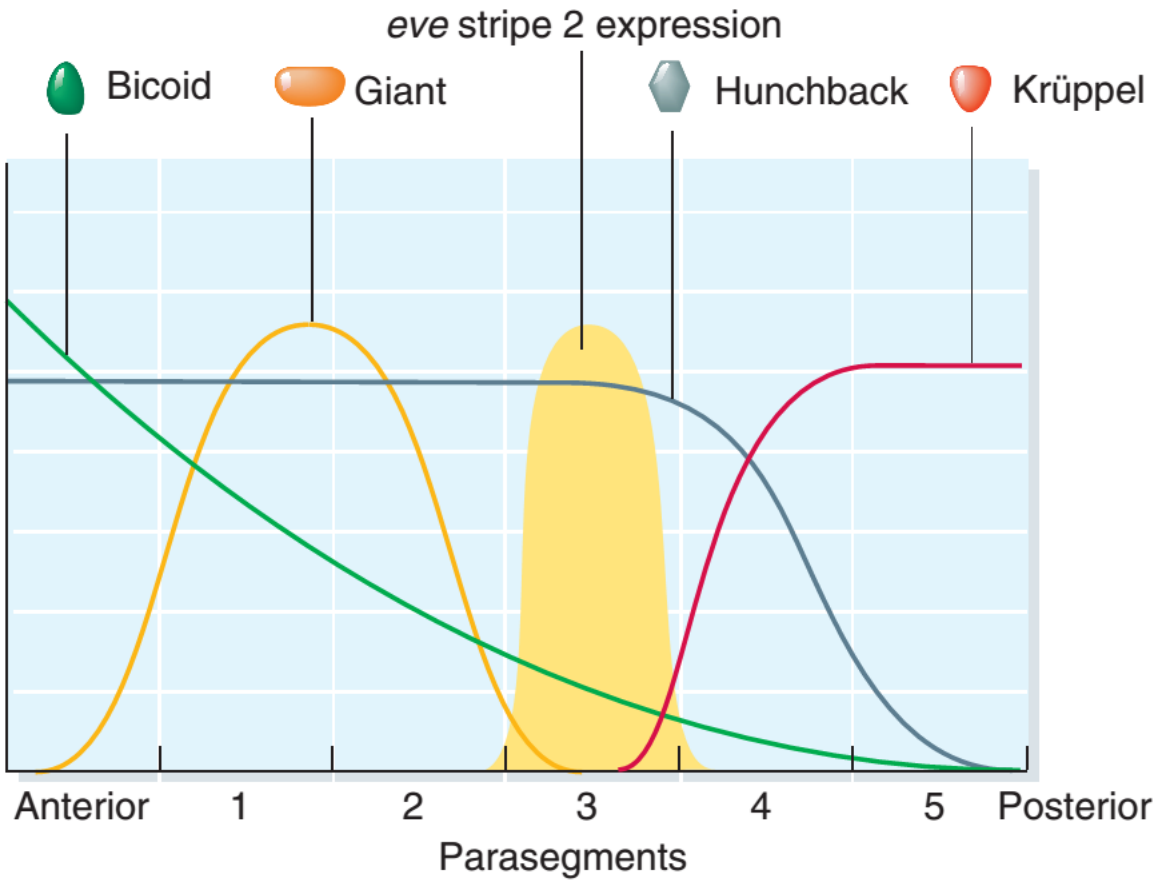

Wings
Head
Adult
T2
T3
T1
A1
A2
A3
A4
A5
A6
A7
A8
C2
C3
Leg 1
Leg 2
Leg 3
Thoracic segments
Abdominal segments

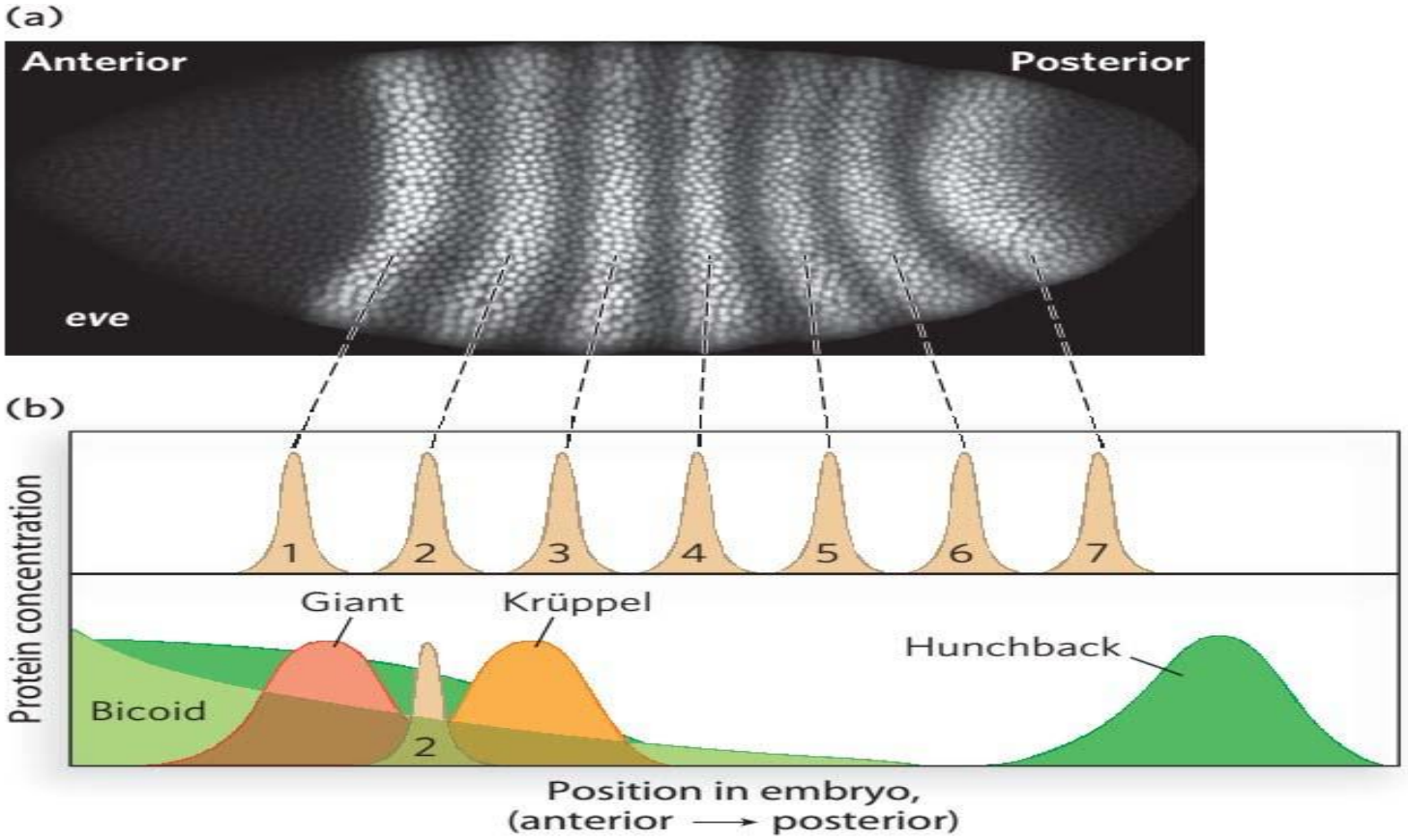
- Starting at the syncytial blastoderm stage, two pair rule genes, **eve** and **fushi-tarazu**, are each expressed in seven stripes that alternate with one another to produce a repeating series of stripes.
- The eve gene specifies odd parasegments (1, 3, and so on), while the fushi-tarazu gene specifies even parasegments.
- The **seven** eve stripes are controlled by five distinct enhancers of the eve gene, each about 500 bp long.
- Each enhancer contains binding sites for transcriptional activators and repressors and controls the expression of one or two stripes.
- Bicoid and Hunchback are transcription activators, while Giant and Krüppel are transcription repressors.

c) Activator (Bicoid, Hunchback) and repressor (Giant, Krüppel) binding sites in the *even-skipped* stripe 2 enhancer

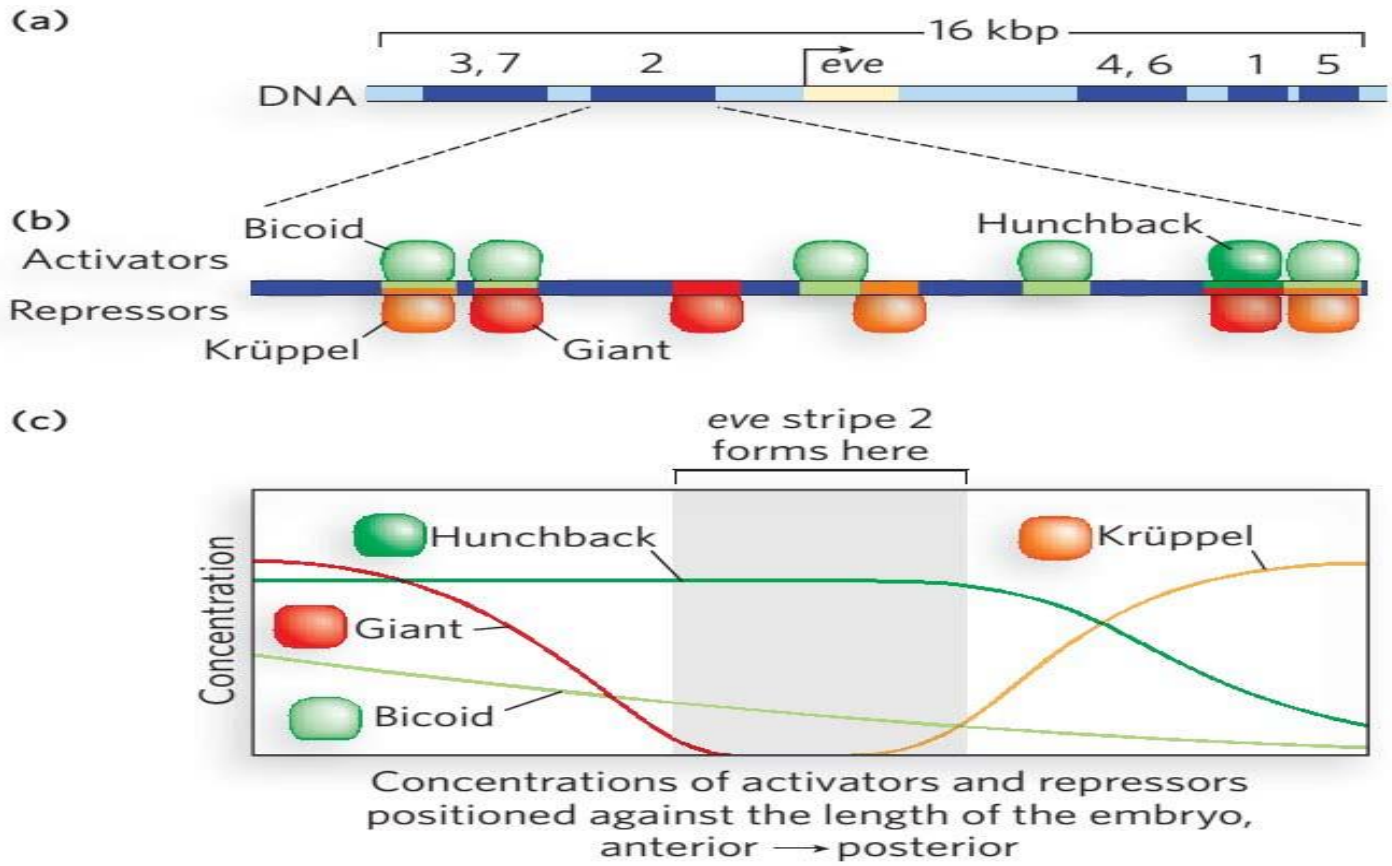


d) Gradients of the regulatory proteins Bicoid, Hunchback, Giant, and Krüppel along the anterior-posterior of the syncytial blastoderm





Combinatorial control of *eve* gene expression in fruit fly development. (a) This *Drosophila* embryo was stained with fluorescent antibodies that recognize the protein even-skipped (product of *eve*), showing its striped pattern of expression. (b) The graphs represent the relative levels and positions along the length of the embryo of even-skipped (top) and four transcription factors that regulate its expression (bottom). Specific combinations of transcription factors activate the *eve* gene. [Source: (a) Photo from A. V. Spirov and D. M. Holloway, *In Silico Biol.* 3:0009, 2003, Fig. 1. © 2002, Bioinformation Systems e.V.]



Five independently acting enhancers of the *eve* gene producing seven stripes of *eve* expression in the early embryo. (a) The *eve* gene and its upstream and downstream enhancers, any one of which can activate *eve* expression if bound by the correct combination of transcription factors. Numbers 1 through 7 indicate the stripe(s) activated by each enhancer. (b) The binding sites in the stripe 2 enhancer for the Bicoid and Hunchback activators and the Krüppel and Giant repressors. (c) Changes in concentration of the four transcription factors along the length of the embryo, in the region that expresses *eve* stripe 2.

Abbreviations used

- MAT- mating type locus
- UAS- upstream activator sequence