

Week 9**Study Questions from the textbook:**

6th Edition: Chapter 19- 19.6, 19.7, 19.15, 19.17

OR

7th Edition: Chapter 18- 18.6 18.7, 18.15, 18.17

19.6/18.6 How would you distinguish between an enhancer and a promoter?

ANS: An enhancer can be located upstream, downstream, or within a gene and it functions independently of its orientation. A promoter is almost always immediately upstream of a gene and it functions only in one direction with respect to the gene.

19.7/18.7 Tropomyosins are proteins that mediate the interaction of actin and troponin, two proteins involved in muscle contractions. In higher animals, tropomyosins exist as a family of closely related proteins that share some amino acid sequences but differ in others. Explain how these proteins could be created from the transcript of a single gene.

ANS: By alternate splicing of the transcript.

19.15/18.15 A particular transcription factor binds to enhancers in 40 different genes. Predict the phenotype of individuals homozygous for a frameshift mutation in the coding sequence of the gene that specifies this transcription factor.

ANS: The mutation is likely to be lethal in homozygous condition because the transcription factor controls so many different genes and a frameshift mutation in the coding sequence will almost certainly destroy the transcription factor's function.

19.17/18.17 The RNA from the *Drosophila Sex-lethal (Sxl)* gene is alternately spliced. In males, the sequence of the mRNA derived from the primary transcript contains all eight exons of the *Sxl* gene. In females, the mRNA contains only seven of the exons because during splicing exon 3 is removed from the primary transcript along with its flanking introns. The coding region in the female's mRNA is therefore shorter than it is in the male's mRNA. However, the protein encoded by the female's mRNA is longer than the one encoded by the male's mRNA. How might you explain this paradox?

ANS: Exon 3 contains an in-frame stop codon. Thus, the protein translated from the *Sxl* mRNA in males will be shorter than the protein translated from the shorter *Sxl* mRNA in females.

Additional questions on Chapter 19 material:

*1. What is the effect of chromatin state on gene expression? What effect does a) chromatin remodeling, b) histone modification (will go over in lecture- your book doesn't call it this, but this is what it is referring to when it discusses modifications of amino acids on histones by HATs and Kinases, first full paragraph of p.547), and c) DNA methylation have on transcription?

ANS: Generally, more open chromatin is associated with higher rates of transcription, whereas more closed or condensed chromatin is associated with lower or no transcription. Chromatin is made up of nucleosomes, and nucleosomes are made up of DNA wrapped around histone proteins (8 histone proteins, 2 each of H2a, H2b, H3, and H4). All of these mechanisms affecting chromatin state (or whatever DNA methylation is doing—we don't entirely understand yet!) described below are *epigenetic*, they affect transcription without changing the DNA sequence.

a) Chromatin remodeling is one mechanism that can change the chromatin composition of regulatory regions. Nucleosomes can be rearranged or repositioned by protein complexes to either open up the region to transcription (such as the case with the SWI/SNF complex) or to keep the region covered and unavailable to transcription (ISWI complex). **b)** Histone modifications can also alter chromatin state, by either opening or closing chromatin. The acetylation of histones, whereby histone acetyltransferases (HATs) add acetyl groups to particular amino acids on histone “tails” can open chromatin and thus promote transcription. The methylation of certain amino acids on histone tails (mentioned in passing in your book) by histone methyltransferases (HMTs) can close chromatin, reducing or shutting down transcription. There are enzymes that can remove these added groups from histone tails (deacetylation, demethylation), reversing the process. Additional chemical modifications to histone tails are known, such as the addition of phosphate groups by Kinases, and in real life, all of these modifications are found in various combinations, which we haven't entirely worked out what they all mean for chromatin state and transcription. **c)** Chemical modification of DNA nucleotides themselves is important for regulating genes, especially in mammals. Methyl groups can be added to cytosines found next to guanines (on the same strand, see your book for the structure). Methylated DNA is associated with transcriptional repression. How this is accomplished is not entirely understood, but methylated regions of DNA tend to have nucleosomes with different histone components and chemical modifications. DNA methylation is the mechanism of imprinting.

2. What factors affect RNA stability? What effect is the stability of mRNA likely to have on protein levels?

ANS: RNA stability affects the number of mRNA molecules available for translation at any one time. The stability of an RNA molecule can be affected by the Poly(A) tail, the sequence of the 3' untranslated region (3'UTR) preceding the Poly(A)tail, chemical factors such as hormones, and the presence of targeted miRNAs or siRNAs. mRNA transcripts can be translated as long as they are intact, so longer-lived mRNAs have the potential to produce more proteins.

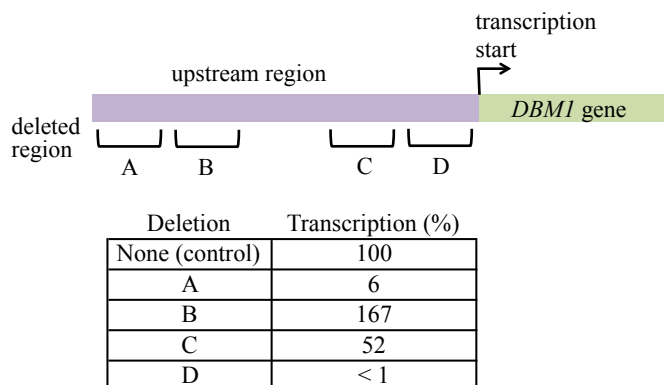
3. Summarize the RNAi pathway, as pictured in Figure 19.8. What goes into this pathway (what is the starting material), and what is the result? How does RNAi ultimately affect phenotypes?

ANS: Double-stranded RNA is processed by Dicer into small double-stranded interfering RNA, which are ~21-28 base pairs long. This double-stranded small interfering RNA assembles with proteins, and the double-stranded RNA is unwound or cleaved to leave a single-stranded small RNA molecule, associated with the proteins, forming the RNA-Induced Silencing Complex (RISC). The RISC targets a sequence in a messenger RNA that is complementary to the single-stranded small interfering RNA in the RISC. The small interfering RNA in the RISC basepairs with its target messenger RNA. The target mRNA is either cleaved and subsequently degraded, sequestered, or in other ways prevented from being translated.

The input for this pathway is double-stranded RNAs that are complementary to sequences within the target mRNAs. Broadly, miRNAs are usually short sequences, encoded in the genome, and transcribed by RNA Pol II, and form double-stranded RNA molecules through the formation of hairpins. miRNAs are more likely to pair imperfectly with their target mRNAs, and the translation of the mRNA is suppressed or the mRNA is sequestered. Generally, siRNAs can come from different sources, such as viral RNA, transposons, transgenes. siRNAs tend to have perfect complementarity to their target mRNAs, and cleave their mRNA targets so that they are subsequently degraded.

RNAi affects phenotypes by affecting protein level. Removing mRNAs from the transcript pool prevents them from being translated into polypeptides. This is post-transcriptional regulation.

*4. The consequences of four deletions from the region upstream of the yeast gene *DBM1* are studied to determine the effect on transcription. The normal rate of transcription, determined from study of transcription of genes that do not have upstream deletions, is defined as 100%. The location of each deletion and the effects of deletions on *DBM1* transcription are shown below:



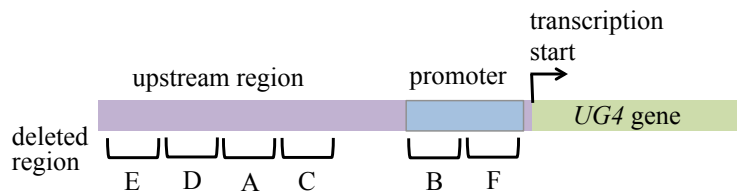
a) Which mutation(s) affect an enhancer sequence? Explain your reasoning.

b) Which mutations(s) affect a silencer sequence? Explain your reasoning.

c) Which mutation(s) affect the promoter? Explain your reasoning.

ANS: Enhancers are sequences located at a distance from the promoter, and they increase transcription of a gene above its basal level. Deletion of an enhancer will reduce transcription levels but typically will not eliminate expression. Mutants A, C, and D all reduce expression levels; however, mutant C removes sequences close to the transcription start site, so may be a promoter mutation or an enhancer mutation. Mutation A and possibly also C may be enhancer mutations. Mutation B is right next to the transcription start site, so is more likely to be a promoter mutation. **(b)** Silencers are sequences located at a distance from the promoter, and they decrease transcription of a gene. Deletion of a silencer will increase transcription over that observed in wild type. Mutant B has the only mutation that increases expression over wild-type levels. Mutant B affects a silencer sequence. This deletion results in a substantial increase in the level of transcription. **(c)** Promoters are sequences located (typically) just upstream of the transcription start site and are required for full-level transcription. Deletion of a promoter or part of a promoter will prevent or reduce transcription levels. Mutants C and D affect sequences proximal to the transcription start site, and both reduce expression levels. Both mutants C and D may be promoter mutations. Their location immediately upstream of the transcription start and the reduced levels of transcription from these mutants are consistent with promoter mutations.

5. The *UG4* gene is expressed in stem tissue and leaf tissue in *Arabidopsis thaliana*. To study mechanism regulating *UG4* expression, six small deletions of DNA sequence upstream of the transcription start site are made. The locations of the deletions and their effect on *UG4* expression are shown below:



Deletion	Transcription (%)	
	Stem	Leaf
None (control)	100	100
A	100	100
B	< 1	92
C	100	100
D	100	164
E	98	< 1
F	100	100

a) Explain the differential effects of deletions B and F on expression in the two tissues.

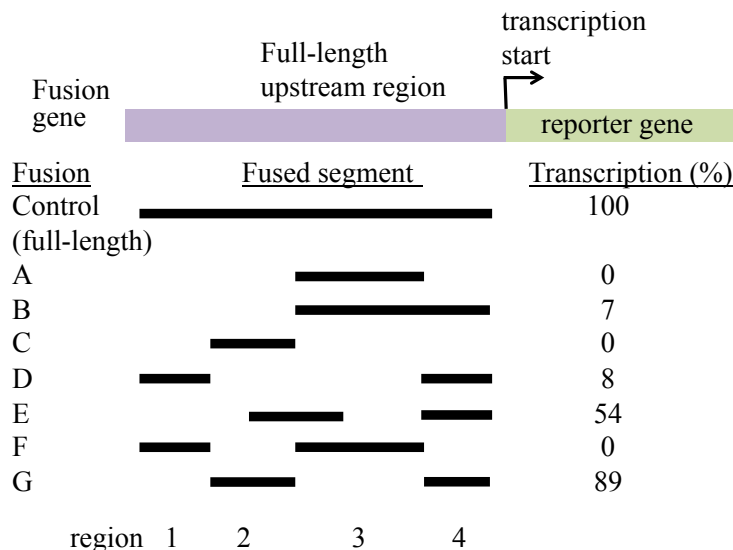
b) Why does deletion D raise *UG4* expression in leaf tissue but not in stem tissue?

c) Why does deletion E lower expression of *UG4* in leaf tissue but not stem tissue?

ANS: **(a)** The deletion in mutant F has no effect on *UG4* expression in either tissue, indicating that this region contains no sequences required for promoter action in those cells. Mutant B only mildly affects *UG4* transcription in leaves, but knocks it out almost entirely in stems. This indicates the use of different promoter sequences in the transcription of *UG4* in these tissues. **(b)** Mutation D results in greater than wild-type level expression in leaves but not stems. Mutations that increase expression typically

remove silencers; therefore, mutant D lacks a silencer sequence that regulates the level of transcription of *UG4* in leaf tissue but not in stem tissue. (c) Mutation E eliminates a region relatively far away from the transcription start site and prevents expression in leaves but has no effect in stems. Mutations in regions at a distance from the transcription start site that prevent expression typically affect an enhancer; therefore, mutation E deletes a required enhancer sequence for *UG4* transcription in leaf tissue but not in stem tissue.

6. A gene expressed in the long muscle of the mouse is identified, and the regulatory region upstream of the gene is isolated. Various segments of the upstream sequence are fused to a reporter gene, and each fusion is assayed to determine how efficiently it transcribes the gene. In the following diagram, the dark bars indicate the upstream segments that are present in each of 8 fusion genes. The transcriptional efficiency of each fusion is measured compared to the control, which is the full-length upstream region fused to the reporter gene.



a) Identify the upstream region (region 1, 2, 3, 4; indicated across the bottom) that contains the enhancer

b) Identify the upstream region that contains the promoter

c) Speculate about the reasons for the different transcription rates detected in fusions E and G.

ANS: **a)** The region just upstream of the transcription start site, region 4, is clearly necessary for expression (all fusions containing region 4 have some expression), so this region is likely to contain the promoter. For the enhancer, we are looking for another region that is necessary for high levels of transcription. We observe higher levels of expression in the fusions that also contain part of region 2 in addition to region 4, so it is likely that region 2 contains the enhancer. **b)** All fusions that contain region 4 have some expression, and this region is closest to the transcription start, so this region is a likely candidate to contain the promoter. However, this region is not sufficient for high levels of transcription. In fusions B and D, region 4 is included, but the additional inclusions of regions 3 (fusion B) or region 1 (fusion D) are not sufficient for very high levels of transcription. **c)** fusion E contains most but not all of region 2 in addition to region 4, whereas fusion G contains all of region 2 and region 4. It is likely that the part of region 2 that fusion E does not have is responsible for some of the function of the enhancer

located in this region. The other alternative is that the position of the enhancer sequences in region 2 might be better located in fusion G relative to the promoter, though it is difficult to tell from this drawing if this would be true.

6th Edition: Chapter 20- 20.6, 20.8, 20.12

OR

7th Edition: Chapter 22 ([online](#))- 22.6, 22.8, 20.12

20.6/22.6 Like *dorsal*, *bicoid* is a strict maternal-effect gene in *Drosophila*; that is, it has no zygotic expression. Recessive mutations in *bicoid* (*bcd*) cause embryonic death by preventing the formation of anterior structures. Predict the phenotypes of (a) *bcd/bcd* animals produced by mating heterozygous males and females; (b) *bcd/bcd* animals produced by mating *bcd/bcd* females with *bcd/+* males; (c) *bcd/+* animals produced by mating *bcd/bcd* females with *bcd/+* males; (d) *bcd/bcd* animals produced by mating *bcd/+* females with *bcd/bcd* males; (e) *bcd/+* animals produced by mating *bcd/+* females with *bcd/bcd* males.

ANS: (a) Wild-type; (b) embryonic lethal; (c) embryonic lethal; (d) wild-type; (e) wild-type.

20.8/22.8 In *Drosophila*, recessive mutations in the dorsal-ventral axis gene *dorsal* (*dl*) cause a dorsalized phenotype in embryos produced by *dl/dl* mothers; that is, no ventral structures develop. Predict the phenotype of embryos produced by females homozygous for a recessive mutation in the anterior-posterior axis gene *nanos*.

ANS: Some structures fail to develop in the posterior portion of the embryo.

20.12/22.12 What events lead to a high concentration of hunchback protein in the anterior of *Drosophila* embryos?

ANS: The *hunchback* mRNA is translated into protein only in the anterior region of the developing embryo. This RNA is supplied to the egg by the nurse cells and it is also synthesized after fertilization by transcription of the *hunchback* gene. This zygotic transcription is stimulated by a transcription factor encoded by maternally supplied *bicoid* mRNA, which is located in the anterior of the egg. Thus, *hunchback* mRNA is concentrated in the anterior of the embryo. In addition, the *hunchback* mRNA that is located in the posterior of the embryo is bound by nanos protein, and then degraded. The nanos protein is concentrated in the posterior of the embryo because maternally supplied *nanos* mRNA is preferentially localized there.

Additional questions on Chapter 20/22 material:

1. What is a morphogen? How does Bicoid (as discussed in the book) fit the definition of a morphogen?

ANS: A morphogen is a substance that can control developmental fates in a concentration-dependent manner (i.e. different concentrations determine different fates). For this reason, morphogens are usually distributed in gradients.

Bicoid is a critical determinant of anterior (head) structures in embryogenesis. In oogenesis, *bicoid* mRNA is deposited at the anterior end of the oocyte. As embryonic development begins, *bicoid* mRNA diffuses from the anterior end outward, and it is translated into a gradient of Bicoid protein as this diffusion occurs, with high concentrations at the anterior of the embryo, and lower concentrations toward the posterior. Bicoid acts as a transcription factor to control gene expression of later expressed genes, such as gap or pair-rule genes, and it does so in a concentration dependent manner. In areas where Bicoid concentration is high, in the anterior of the embryo, it will be able to act strongly to regulate transcription of later genes. Bicoid activates different downstream genes in parts of the embryo where Bicoid concentrations differ.

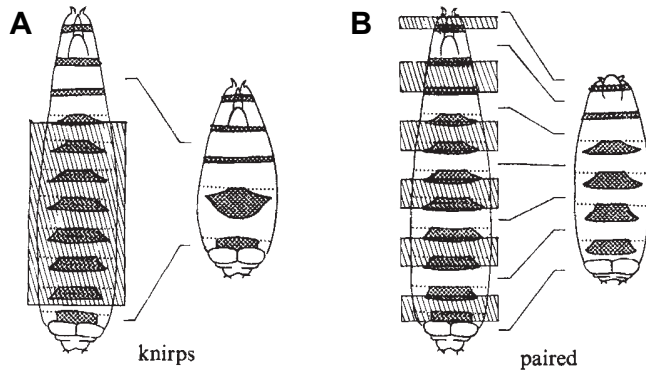
2. Given that maternal Bicoid activates the expression of *hunchback*, what would be the phenotypic consequence of adding extra copies of the *bicoid* gene to the mother, thus creating a female fly with three or four copies of the *bicoid* gene (compared to two in the wild-type)? *Hint: this was covered in lecture.* How would *hunchback* expression be altered? What about the expression of later gap and pair-rule genes?

ANS: Increased dosage of the *bicoid* gene will result in an increase in the amount of *bicoid* mRNA and therefore Bicoid protein. This increase would alter the gradient of Bicoid protein in the egg and, in turn, alter the expression of genes downstream of Bicoid function. For example, *hunchback* is activated by Bicoid, so *hunchback* expression also increases and the zone of expression is pushed further posterior in a fly with a higher Bicoid gene dosage. The entire body plan of the mutant flies would likely be altered, resulting in more anterior-like segment fates being adopted by what would normally be more posterior-like segments.

3. Describe the mutant phenotypes of gap gene, pair-rule gene, and segment-polarity gene mutants.

ANS: Mutations in the gap genes cause multiple contiguous body segments to be missing, this causes large “gaps” in the segment anatomy. Mutations in pair-rule genes produce larvae with every other segment missing, resulting mutants to have half the number of segments they should have. Mutations in segment-polarity mutants results in defects within the anterior or posterior of each of the 14 segments.

4. Below are two schematic drawings of mutant phenotypes from the 1980 paper by Nusslein-Volhard and Wieschaus (*Nature*, Vol. 287), where they characterized gap, pair-rule, and segment polarity genes. Which of these genes acts earlier in development? *Hint: look to your answer of the previous question, as well as the structure of the gene network.* How do you know?



On the right (in both A and B) is a drawing of the phenotype of a homozygous mutant for each gene (A, *patch*; B, *knirps*). On the left is a drawing showing what portion of the larva is missing in that mutant phenotype.

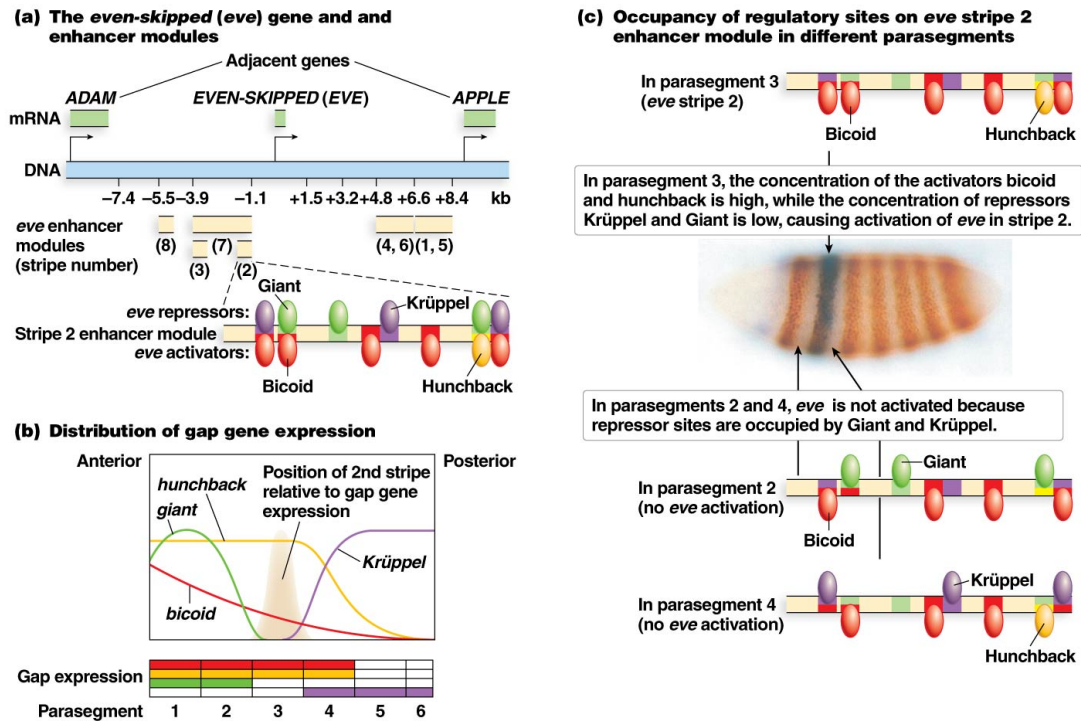
ANS: The gene on the left, *knirps*, acts earlier in development. From the mutant phenotypes, *knirps* is a gap gene, and *paired* is a pair-rule gene, and gap genes act earlier in development than do the pair-rule genes. The mutant phenotype of *knirps* affects a large number of contiguous segments, so it must act before all of the individual segments are specified. The other mutant phenotype, from the *paired* mutant, affects every other segment, so it must act later, at the stage when the segments are being specified.

5. Using the figure below, showing how the expression of the pair-rule gene *eve* stripe 2 is established by the coordinated activities of maternal and gap genes, answer the following questions. *NOTE: Remember in lecture when it was mentioned that sometimes the same sequence can work both as an enhancer (to promote gene expression) and a silencer (to repress gene expression)? This is one of those cases. This cis-regulatory region is called an “enhancer module” here, but it can bind both activating and repressing transcription factors.* Bicoid and Hunchback are activators of *eve* stripe 2, Giant and Kruppel are repressors. Parasegment is a term used to describe the 14 segments or zones of the embryo laid down in the segmentation process.

A) Using part “B” of the figure below, answer the following questions: Is the DNA sequence of the enhancer module different between parasegments? Do the activators and repressors present at each parasegment vary? Why does the gene expression of *eve* stripe 2 produced by this enhancer module differ between parasegments?

B) Consider the binding sites for gap proteins and Bicoid in the stripe 2 enhancer module. What sites are occupied in parasegments 2, 3, and 4, and how does this result in expression or no expression? Use part “C” of the figure as a guide.

C) Explain what you would expect to see happen to the expression pattern of *eve* stripe 2 in the parasegments if it is expressed in a *Kruppel* mutant background. What about a *giant* mutant background? What about a mutant background where both of the activators (*hunchback* and *bicoid*) are absent?



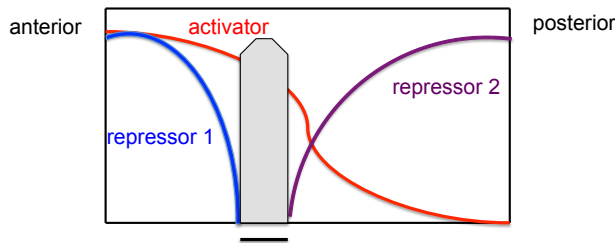
ANS: A) The DNA sequence is the same in all of the parasegments, with binding sites for the positive activators Hunchback and Bicoid and the negative regulators Giant and Krüppel. However, the activators and repressors present vary across the embryo, so there are different combinations of transcription factors present in different parasegments. The expression pattern produced by this enhancer differs depending on which combination of activators and repressors bind to the enhancer in each parasegment.

B) Giant, Hunchback, and Bicoid are bound to their sites in parasegment 2 (part C of the figure fails to show the Hunchback binding, this is an error, it clearly is present in parasegment 2 in part B of the figure); only Hunchback and Bicoid are bound to their sites in parasegment 3; and Krüppel, Hunchback, and Bicoid are all bound to their sites in parasegment 4. The binding of either Giant or Krüppel is sufficient to repress transcription; therefore, *eve* is transcribed only in parasegment 3.

C) In a *Krüppel* mutant, Hunchback and Bicoid are bound in parasegment 4 but Krüppel is not, resulting in expression of *eve* in both parasegments 3 and 4. In a *giant* mutant background, Hunchback and Bicoid are bound to their sites in parasegments 1, 2, and 3 but Giant would not be, resulting in *eve* expression in both parasegments 1, 2 and 3. In mutants lacking both of the activators, Bicoid and Hunchback, *eve* expression would be reduced or abolished in parasegment 3. In this mutant, there would be no repressive factors bound at this locus, but without activators bound, expression would likely be reduced or abolished.

6. The figure below represents the concentrations of activator and repressor transcription factors along the anterior-posterior (head to tail) axis in an embryo (see Figure above for a more specific example). A) Indicate on this figure (draw a stripe on the graph, or draw a line below the X axis) where the gene these activators and repressors are regulating would be expressed. B) What would happen to that expression pattern if repressor 2 was knocked out?

ANS:



A) The expression pattern of the gene these factors are regulating (Gene A) is shown in grey. The gene will be expressed where there is activator present, but no repressors present.

B) If repressor 2 were knocked out, then the posterior (rightmost) boundary of the Gene A expression pattern would no longer be repressed, so the area where expression occurs would expand toward the posterior (right).

7. What is the function of homeotic genes (Hox genes are the homeotic genes we discussed in class)? What phenotypes do mutant homeotic genes produce?

ANS: Homeotic genes determine segmental identities, the anatomical features to be developed in each segment. For example, the second thoracic segment in flies has a pair of wings, while the third thoracic segment has a pair of halteres, small balancing structures that help the fly fly. Hox genes are a category of homeotic genes we talked about in lecture, that contain homeobox DNA binding domains. Hox genes encode transcription factors, and the combinations of Hox genes expressed in each segment control developmental programs of gene expression resulting in the formation of the anatomical features of each segment. Hox genes are co-linear, that is they are expressed along the anterior-posterior axis in the same order as they are found on the chromosome. Homeotic genes are named for their mutant phenotypes. Homeotic mutants have one body part replaced by another body part, by transforming the identity of one segment into that of another segment. For examples, see the *Antennapedia* or *Ubx* mutant phenotypes (or the mouse Hox mutant) as described in lecture.

Study Questions from the literature:

Prabhakar et al. (2008):

*1. The HACNS1 region from all species studied produced expression in the eye, ear, and pharyngeal arches, whereas only the human HACNS1 region produced significant expression in the limb bud. Explain how gene expression can be turned on or off in a particular tissue.

ANS: The transcription factors present are going to vary between cell types or tissues. Thus, *cis*-regulatory elements (such as enhancers, silencers, promoters) will bind different transcription factors in different tissues.

For example, if in a particular cell type, an activating transcription factor can bind an enhancer element, then transcription can be activated. If in a different cell type, this activating transcription factor is not present, then transcription will not be activated.

Alternatively, in a particular cell type a repressing transcription factor is present and can bind to a silencer sequence, then transcription will not occur. If that repressing transcription factor is not present, then it will not prevent the activation of transcription.

We observe in many systems that different *cis*-regulatory sequences are used for regulation in different tissues. Therefore, like the example in your textbook, you might have an enhancer element in the DNA that binds only brain-specific transcription factors, so we would consider that a brain specific enhancer (even though that DNA sequence is present in all cells).

This is an especially interesting feature of gene regulation, as it allows for gain or loss of gene expression only in particular cell types or tissues, which we can observe over evolutionary time.

2. A later paper (Sumiyama & Saitou, 2011, *MBE*) provides evidence that the limb-specific expression pattern produced by the HACNS1 regulatory region is a loss of a *cis*-regulatory element that represses transcription (silencer) rather than a gain of a *cis*-regulatory element that promotes transcription (enhancer). Explain how the gain of an enhancer and the loss of a silencer can lead to the same expression pattern.

ANS: Both gaining an enhancer or losing a silencer can result in higher levels of transcription. Wild-type enhancer sequences are *cis*-regulatory elements that, when bound by transcription factors, can increase expression levels. Wild-type silencer elements are *cis*-regulatory elements which decrease or stop transcription when bound by repressing transcription factors. Thus mutating the regulatory DNA to either gain activating elements or lose repressive elements can potentially increase transcription levels.