

UNIT 3: CONTROL OF GENE EXPRESSION AND ANIMAL DEVELOPMENT

Readings: Chapter 18 (pages 351-373)
Chapter 16 (pages 320-323)
Chapter 20 (pages 412-414)
Chapter 21
"Morphogens and Homeotic Genes" at the end of this unit
Chapter 47

To Do This Unit:

1. Skim the key concepts and objectives below.
2. Read the text assignment.
3. Examine the demonstration materials, both online and in the Study Center.
4. Study the slides on the course website, using the notes at the end of this unit to guide your study.
5. Write out the answers to the objectives, using the text and demonstration materials.
6. Take an examination.

KEY CONCEPTS AND OBJECTIVES:

After you have studied the material in this unit you should understand the following concepts and you should be able to carry out the objectives listed for each.

It is very "expensive" for the cell to synthesize enzymes when they are not needed, so various control mechanisms act to turn on or turn off specific genes.

1. Using Fig. 18.2 (p. 352) explain the two ways in which bacteria can control their metabolic pathways. Of the two, which would produce the more rapid response? Why?

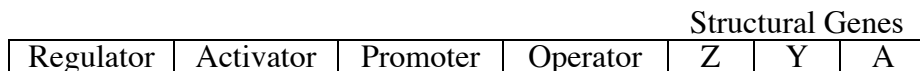
2. Using Fig. 18.3 (p. 353), explain what an operator is and describe the *trp* operon. In doing so, be able to answer the following questions:
 - a) How many genes does the transcription unit code for? Is there one mRNA molecule produced by transcription of these genes or many smaller ones? How does the cell correctly translate the coding information for each polypeptide?

b) State the roles of the promoter and regulatory genes. Relative to the operator, where are each of these genes located? Are they near the operator or far from it?

c) What is a repressor protein and what does it do? Is the *trp* repressor active or inactive when synthesized? What is a corepressor and what does it do?

d) How does a repressible operon control gene transcription? What happens when there is no tryptophan in the cell? What happens when there is an abundance of tryptophan in the cell?

3. a) Using the information in Fig. 18.4 (p. 354), explain what an operon is and show how the expression of the *lac* operon of *E. coli* is regulated according to the operon model. This simplified diagram of the *lac* operon may help:



Identify and explain the roles of the inducer, the operator, the promoter, the repressor protein (is it active or inactive when synthesized?), the regulatory gene, and the structural genes in prokaryotic cells. Explain how the presence of lactose induces the operon.

b) How is a repressible operon (*trp*) different from an inducible operon (*lac*)? Focus on the activity of the repressor protein. Why are both considered examples of negative control? See **demo**.

c) What is the advantage to the bacteria of having their DNA organized in operons?

4. Using the information in the text (p. 355) and in the **demo**, differentiate between positive regulation and negative regulation of prokaryotic gene transcription. Can both types of control operate at the same time?

5. Give one example of positive control in bacteria and explain how it works in *E. coli* (Fig. 18.5, p. 355).

a) In the drawing below of *lac* operon indicate where the RNA polymerase binds and where the active CAP binds. Why is positive control important in the *lac* operon (see **demo**)¹?

Structural Genes						
Regulator	Activator	Promoter	Operator	Z	Y	A

¹ It turns out that the promoter for the *lac* operon is weak (i.e., not close to the consensus sequence) so the RNA polymerase does not bind strongly to the promoter and transcription of the structural genes occurs only at low levels. When active CAP is present, however, it facilitates the binding of RNA polymerase and transcription is rapid.

b) complete the chart below to understand positive vs. negative gene regulation:

Environmental Conditions	Operon		Level of transcription of <i>lac</i> operon (none, low, high)
	CAP Bound	Repressor Bound	
glucose, no lactose			
glucose, lactose			
no glucose, no lactose			
no glucose, lactose			

Each gene codes for only one kind of messenger RNA; regulators determine if and when each gene will be transcribed into its particular mRNA. Environmental influences can act by helping to turn on or to turn off the transcriptional activity of the various genes or by influencing the synthesis and activity of enzymes.

6. a) Using Fig. 16.21 (pp. 320-321), explain the general organization of the eukaryotic chromosome and give the general roles of the nucleosomes and histone proteins. Differentiate between euchromatin and heterochromatin.

b) How does the organization of the eukaryotic genome differ from that of prokaryotes? Complete the following chart to summarize the differences. Consider the number of chromosomes, whether or not they are linear or circular, presence and absence of exons and introns, and the presence and absence of operons.

	Prokaryotes	Eukaryotes
number of chromosomes?		
generally haploid or diploid?		
linear or circular chromosomes?		
introns & exons present?		
operons present?		

c) What percentage of the eukaryotic genome is not translated? _____
 What does the rest of the DNA consist of? See p. 434

How much of the prokaryotic genome is transcribed and translated? _____
 See **demo**.

d) Explain what is meant by a multigene family, give an example, and explain how they might have arisen. How might transposition be involved?

Normally all cells of a multicellular organism are genetically identical; control mechanisms determine which of the cell's inherited instructions will be acted upon and which will not.

In all organisms the expression of certain genes is most commonly regulated at the level of transcription. In eukaryotes, most control is positive; transcription factors recognize and bind to the promoter sequence. In turn, they bind the activators and help the RNA polymerase to bind and position it at the right starting point.

7. Discuss the following aspects of control of gene expression in eukaryotes.
- a) State the role of histone acetylation in the regulation of gene transcription see pp. 357-358).
- b) Explain the role that DNA methylation plays in modifying chromatin. How is methylation passed on from cell to cell? How is DNA methylation related to euchromatin/heterochromatin? What is genomic imprinting?
- c) Using Figs. 18.8-18.9 (pp. 359-360), explain the role of activators and enhancers in controlling gene transcription in eukaryotes. Where are the regions located with respect to the gene to be transcribed? What role do transcription factors play? Would a eukaryotic gene be transcribed if no transcription factors were present? Is the control of gene transcription in eukaryotes primarily positive or negative control?

d) Summarize the differences between prokaryotic and eukaryotic gene control by completing the following chart:

	Prokaryotes	Eukaryotes
presence of nucleosomes		
genes organized in operons		
control primarily positive or negative		
role of transcription factors		
role of enhancers		
levels of control (1 or 2 vs. 3 or more)		
need for mRNA processing		

e) Explain what is meant by post-transcriptional control of gene expression and give three examples.

f) Use Fig. 18.6 (p. 357) to summarize the various steps in which gene expression can be controlled in eukaryotes.

All the cells in a multicellular organism except the germ cells have genomic equivalence; cells become different because of differences in gene expression.

8. Define the terms differentiation and morphogenesis and explain how each process contributes to embryonic development. See pp. 366-367.

As development of a multicellular organism proceeds, the individual cells become more and more committed to one particular course of differentiation. However, in some organisms, differentiation may be reversible.

9. Is adult cellular differentiation ever reversible? Can cells (nuclei) resume totipotency? See pp. 412-414 and the **demo** for examples.

Differentiation is the process by which a cell undergoes a series of changes to become a specialized cell type. It is a matter of progressive determination; development is gradually restricted to one of the many initially possible pathways.

10. Distinguish between the processes of determination and differentiation. Using MyoD as an example and Fig. 18.16 (p. 369), explain the mechanisms of determination and differentiation in the development of muscle cells.
11. The cytoplasm of the unfertilized egg is responsible for many of the initial characteristics of the cells in an early embryo. Describe how the cytoplasm of the egg impacts early development of the zygote.

12. Using Fig. 18.19 (p. 372) in your text and the section “Morphogens and Homeotic Genes” at the end of this unit, describe the process of pattern formation in *Drosophila*. In your answer,
- Explain the role that maternal effect genes (e.g., *bicoid*) have on determining the body axis and pattern formation.
 - Describe the role of morphogens and positional information in pattern formation in the embryo, using as an example segmentation in *Drosophila*. What are morphogens? How do they exert their effect on target cells? How does this relate to the process of determination? What is their significance in orchestrating development?
 - Briefly explain the developmental cascade involved in the development of the segmentation pattern in *Drosophila*. (See especially pp. 371-373.)
 - Explain the function of homeotic genes and discuss their general role in development.
 - Explain what the homeobox is, what the sequences code for, and its relationship to homeotic genes. Is the homeobox found only in developmental genes? (See pp. 445-446.)

13. a) Define the term induction (see Fig. 18.15b, p. 367).

b) Give 2 examples of the role that apoptosis plays in animal development. See pp. 223-225.

In animals, the developmental processes of cell division, cell growth, cell differentiation, and morphogenetic movements convert the fertilized egg into the mature organism.

14. a) Compare and contrast the process of fertilization in sea urchins and in mammals as summarized in Figs. 47.3 (p. 1023) and 47.5 (p. 1025).

b) Why doesn't more than one sperm fertilize the egg?

Embryonic development begins with cleavage, a series of mitotic divisions whereby the enormous amount of egg cytoplasm in the zygote is divided into numerous smaller, nucleated cells.

15. The single diploid cell, the zygote is now ready to develop into a multicellular organism. Using the online slides or Figs. 47.6 (p. 1025) and 47.8 (pp. 1027), describe the process of cleavage in a sea urchin, frog, and chick, answering the following questions:

a) How do cell size and number of cells change as a result of cleavage? Does the embryo increase in size during cleavage?

b) How is it possible for the zygote to undergo such extremely rapid cell division? How much gene transcription and protein synthesis occurs during cleavage? When did it occur?

c) What is the effect of the amount of yolk in an egg on the early patterns of cleavage in the sea urchin, frog, and chick? How much yolk does the human egg have? (See **demo**.)

d) Be able to point out the zygote and morula (from Latin for mulberry, whose shape it vaguely resembles) in the diagrams or in the slides of the sea star or frog.

e) Observe the information on human development in the **demo**. Which pattern of cleavage does human development most closely resemble?

f) What is the embryo called at the end of cleavage?

Gastrulation is the process of highly integrated cell and tissue movements in which the cells of the blastula are dramatically rearranged into the three primary germ layers.

16. Using the slides or Figs. 47.9-47.11 (pp. 1028-1030), describe the process of gastrulation in sea urchin, frog, and chick, answering the following:

a) Identify the blastula and blastocoel in the diagrams or in the online slides of the sea star, frog, or chick embryos. What is meant by the terms "animal pole" and "vegetal pole?"

b) What movements lead to the formation of the gastrula in sea urchin, frog, and chick? Why are the patterns of gastrulation different in the three representative organisms?

c) Identify the gastrula with its blastopore and archenteron in the above diagrams or in the slides of the sea star and frog embryos. What is the fate of the blastopore and archenteron in these animals?

d) How many primary germ layers are present in the late gastrula?

Neurulation is the process in which a flat layer of ectodermal cells is transformed into a hollow tube. It is an early stage in organogenesis during which the organs begin to develop.

17. Describe the movement of cells during neurulation (part of early organogenesis) in frogs and chicks by answering the following:

a) What is the notochord and how is it formed? How is it involved in the development of the CNS? (See **demo**.)

b) What is a neural fold and how is it formed? Identify neural folds in the diagrams or in the slides of the frog or chick embryos and Figs. 47.12-47.13a (pp. 1031-1032).

c) What structures does the neural tube give rise to?

d) What are the neural crest cells and where are they formed? What do they give rise to? (See **demo**.)

e) Explain what somites are, where and how they form (see Figs. 47.12-47.13, pp. 1031-1032 and the **demo**), and what they give rise to in the adult vertebrate.

18. a) After completing objectives 15-17, you should be able to point out the blastula, gastrula, neurula, ectoderm, endoderm, mesoderm, archenteron, blastopore, and neural folds in Figs. 47.9-47.13 (pp. 1028-1032), the online slides, or models in the **demo**.

b) Complete the following chart to summarize the similarities and differences in the processes of cleavage, gastrulation, and neurulation in the sea urchin, frog, and chick. (Note: The **Demo** has helpful information.)

	Sea Urchin	Frog	Chick
Amount of yolk in egg			
Cleavage			
Gastrulation			
Neurulation			

c) Name the three primary germ layers and what each gives rise to. Indicate which primary cell layer gives rise to each of the following adult structures or tissues (see Fig. 47.14, p. 1032):

	Primary germ layer		Primary germ layer
fingernails		anus	
hair		skin (epithelial portion)	
brain		gonad	
lining of digestive tract		bone	
notochord		blood	
nerve cord		liver	
lungs		kidney	
muscle		bladder	

19. Using Figs. 47.15-47.16 (pp. 1033-1034) contrast the development of the extraembryonic membranes in a chick and human embryo. Which of the following structures are present in both: amnion, chorion, yolk sac, allantois, shell, placenta? What is the function of each of these structures?

All the cells of a single organism arise by repeated division of the fertilized egg and are genetically identical. Which genes are active, and hence which potentialities are expressed, is determined in part by the non-uniform distribution of cytoplasmic substances in dividing cells.

20. Using diagrams such as Fig. 47.23 (p. 1040) and the **demo**, explain how the polarity of an egg cell and the location of the plane of cleavage influence development. Distinguish between determinate and indeterminate cleavage (see **demo**). Which type of cleavage results in the formation of totipotent cells? Which type of cleavage, determinate or indeterminate, do vertebrates (including humans) have? ...mollusks?

As cells and tissues become more differentiated, they alter the environment of other cells near them through the chemicals they secrete; these changes in the cellular environment profoundly affect gene activity.

21. a) Describe the process of embryonic induction, using as an example the role of the grey crescent, dorsal lip of the blastopore, and chordamesoderm in a salamander (Fig. 47.24, p. 1041) in inducing gastrulation and the formation of the central nervous system. The **demo** will help you meet this objective.
- b) Briefly describe the role that induction, pattern formation, positional information, and organizer regions play in the development of the vertebrate limb.

Below are summary questions relating to important concepts in this unit. The TA may use these questions in his or her oral test or you may see one of them as an essay question on the final exam. Take a few moments now to formulate your answers.

Differentiate among inducible and repressible enzymes and describe the Jacob-Monod operon model for substrate induction. Include in your answer the role of the inducer, operator, regulator, promoter, repressor protein, and structural genes. Explain how end-product corepression differs from substrate induction.

Trace the early embryonic development of an animal from a single fertilized cell to a complex multicellular animal at the neurula stage. Indicate the fate of the various germ layers in the adult.

Explain the various factors in embryonic development that play a role in making different cells have different characteristics, despite their identical genetic content.

MORPHOGENS AND HOMEOTIC GENES

Morphogens are proteins secreted from a specific point in the embryo, the concentration of which induces the surrounding cells in a region to develop in a particular way, the exact developmental fate depending on the local concentration of morphogen. Morphogens either are transcription factors themselves or lead to the activation of a transcription factor in the localized region. The concentration gradient of these morphogens "tells" cells their position, and hence what their developmental fate is. The best example of morphogens used for positional information comes from the segmentation patterns of *Drosophila*. *Drosophila* development is interesting because the segmentation patterns can be physically observed during development, and the relatively undifferentiated egg develops into a complex larva with clear anterior and posterior ends and well-differentiated individual segments (see Fig. 18.17, p. 370). The segments are established by the gradients of morphogens present at specific points during development.

The gene *bicoid* codes for a morphogen that establishes the anterior end of the egg. Another gene *nanos* codes for a morphogen that establishes the posterior end of the egg. *Bicoid* and *nanos* are *maternal effect genes* because these genes are active in the fly ovaries and code for proteins and mRNA's that affect the egg and zygote. The overlapping of these two morphogen gradients differentially activates other genes called *gap genes*. Morphogens from the gap genes divide the embryo into four broad segments. Mutations in these genes cause "gaps" in the animal's segmentation.

Next, *pair rule genes* further subdivide the broad regions into segments. *Segment polarity genes* maintain specific structures within each segment. As you can see in Fig. 1, there is a hierarchy of gene regulation that controls pattern formation. Morphogens from the maternal effect genes regulate the gap genes, gap gene morphogens interact to control the expression of the pair rule genes, and so on. The pair rule and gap gene morphogens also interact to control the expression of *homeotic genes*. The **homeotic genes** specify the types of appendages and other structures that each segment will form, but do not affect the number, polarity, or size of the segments. Mutations in these genes cause one body part to develop the phenotype of another. Most of these homeotic genes are located in two regions of chromosome 3 in *Drosophila*. The first region is the **Antennapedia complex** that specifies the head region. The other region is the **bithorax complex** that contains genes that code for the third thoracic segment and abdominal segments.

One of the most dramatic consequences of these homeotic genes is the formation of imaginal discs. In order to fully understand the specificity of these homeotic genes, a little background on imaginal discs is necessary. In the development of the fly, there is a dramatic transformation between the larval and adult stages. In *Drosophila*, the embryo develops into the first-instar larva, then molts to become the second-instar larva, to the third-instar larva, and then forms a pupa. During pupal stage the larva is transformed into an adult: most of the old larva is destroyed while new adult organs develop from a group of undifferentiated cells called **imaginal discs**. In *Drosophila*, there are 10 pairs of imaginal discs and a genital disc (which controls the development of the reproductive structures.) The imaginal discs, which eventually construct the adult fly, are controlled by the **homeotic genes**. Though the mechanism by which this is accomplished is not clearly understood, what is currently known is that if homeotic genes are expressed in the wrong imaginal disc, the disc becomes respecified to become another structure. For example, if a homeotic gene specific for leg is expressed in the eye-antenna disc, what results is a leg growing out from where an antenna would have grown (see Fig. 18.18, p. 371).

Each homeotic gene includes a 180 nucleotide sequence called a **homeobox**. The homeobox encodes a 60 amino acid domain (homeodomain) in the transcription factor protein that is the product of the homeotic gene. The homeodomain is known to be the region that actually binds to the DNA in the regulatory region of a gene activated by that transcription factor. The variation in homeobox sequences (and therefore transcription factors) is responsible for the specificity of the particular homeotic gene, that is, it determines which particular genes that produce the characteristics of a given segment will be activated. Thus, for example, the homeotic gene normally active in the head segment in which antennae are produced codes for a transcription factor that will activate those genes involved in the production of antennae, but not genes that will lead to production of (for example) legs or wings.

It is significant that the homeobox region is found (with variations) in genes of distantly related species. Thus a similar type of sequence exists in developmental control genes (called Hox genes) found in the mouse. Since the homeobox sequence codes for the DNA-binding region of the transcription factor, it is not surprising that this region is found in so many organisms. It shows that there are relatively few molecular shapes that allow proteins to bind to DNA and thus turn on genes, and that the genes that code for such proteins, once evolved, have been conserved by evolution. The discovery of such common properties of genes controlling development provides hope that much of what we learn about *Drosophila* development can be applied to mammals, particularly humans.

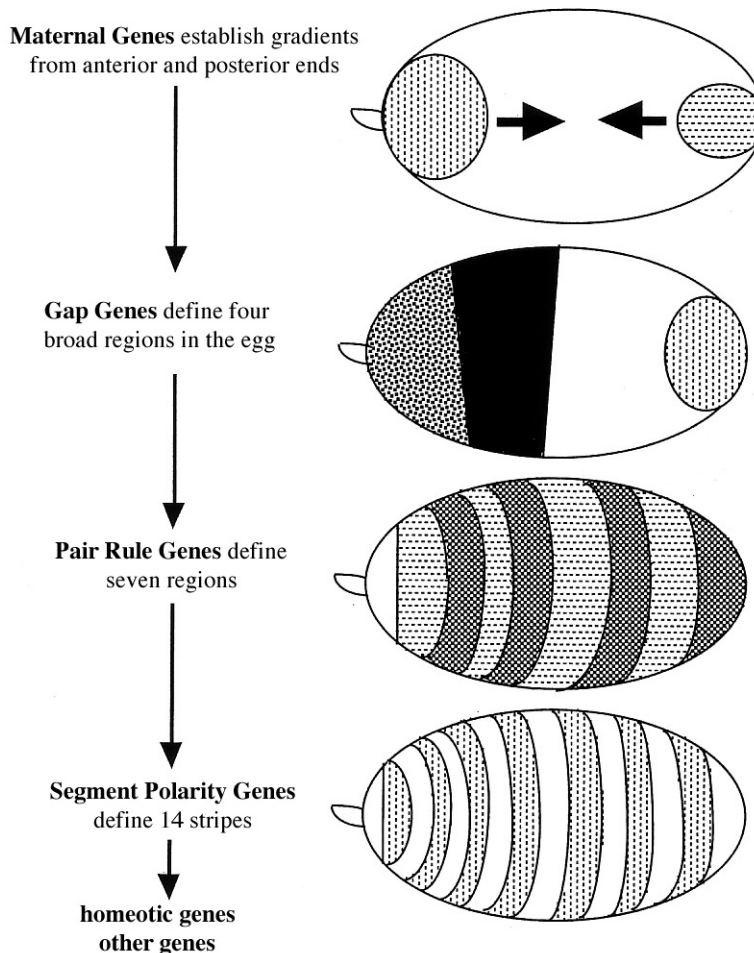


Fig. 1. Segmentation genes act sequentially on smaller and smaller regions of the embryo.

DEMONSTRATION SLIDES

Slides 1-17 show the embryology of the sea star (starfish). If you are confused about any of the terms used, consult the diagrams and photographs of starfish and sea urchin development that are in the demonstration.

Slide 1: Sea star primary oocyte. During meiosis I both the nucleus and the nucleolus of the oocyte increase greatly in size. The nucleolus becomes very conspicuous; it is the prominent dark red circle located within the large light-colored nucleus. Why is it so prominent?

Slide 2: After the sperm penetrates the egg, the egg will complete meiosis II. Note the tiny polar body (center top) formed during this process.

Slide 3: The zygote begins cleavage; here we see the 2-cell stage. Is the first cleavage in the sea star determinate or indeterminate? Cleavage produces a large number of small cells from the original zygote by rapid cell division. The two functions of cleavage are: (1) to increase the surface to volume ratio of the individual cells (why?) and (2) to segregate different factors in the zygote cytoplasm into different cells; these factors determine how the various cells will develop later.

Slide 4: 4-celled stage.

Slide 5: 8-celled stage.

Slide 6: 16-celled stage. This grapelike cluster of cells is called the morula. Notice that the morula is little, if any, larger than the single cell from which it is derived.

Slide 7: 32-celled stage.

Slide 8: 64-celled stage.

Slide 9: Early blastula. As cleavage continues, the newly formed cells begin to secrete a fluid into the center of the mass of cells. The cells then come to be arranged in a sphere surrounding a fluid-filled cavity called the blastocoel. The embryo at this stage is termed a blastula. The process of cleavage ends when the blastula is formed.

Slide 10: Late blastula. The blastula cells form cilia and the blastula becomes motile as the cilia beat in a coordinated fashion. Notice that the cells at the bottom of the blastula (the vegetal pole) are larger than those at the top.

Slide 11: Gastrulation begins. A small depression or invagination starts to form on the surface of the blastula at the vegetal pole.

Slide 12: As gastrulation proceeds, more and more cells move to the point of invagination and then fold inward; the invagination becomes larger and larger.

Slide 13: The embryo is now called a gastrula; it is a two-layered cup. The new cavity is called the archenteron; it will become the digestive tract. The opening into the archenteron, the point where invagination began, is known as the blastopore. The outer layer of cells is the ectoderm, the invaginated layer is the endoderm.

Slide 14: Late gastrula. In the sea star, certain cells (which can be seen here and in Slide 15 in the blastocoel) bud off the endoderm. These cells are mesenchyme cells and will give rise to the mesoderm.

Slides 15-17 show the cells of the embryo undergo differentiation to form a ciliated free-swimming bilaterally symmetrical larva (see Fig. 47.9, p. 1028). The digestive tract formed from the archenteron is clearly seen in Slide 17. The larva remains as a bipinnaria for many months, differentiates into a brachiolaria and then settles down on the bottom and undergoes metamorphosis to form the sedentary adult form.

Slides 18-32 depict the early embryology of the frog. Frog eggs which contain more yolk than those of sea stars but much less than those of most birds, serve as examples of eggs with an intermediate amount of yolk. The amount of yolk influences cleavage and gastrulation. In looking at these slides, notice how cleavage and gastrulation in the frog compares with that seen in the sea star.

Slide 18: A frog in amplexus (mating clasp). The male mounts the female's back and grasps her thorax with his forelegs. As the female extrudes her eggs, the male discharges sperm to fertilize them.

Slide 19: Fertilized egg (center). When the egg is fertilized the jelly coat around the egg swells and the eggs stick together in a tapioca-like mass. The frog eggs have a dark pigment on the dorsal surface (animal pole).

Slide 20: 2-cell stage, looking down on the eggs from above.

Slide 21: 4-cell stage, top view. The first two cleavages are perpendicular to each other and cut through both the animal and vegetal poles.

Slide 22: 8-cell stage. The third cleavage is horizontal and is nearer the animal pole than the vegetal pole. The four cells at the animal end of the egg (seen here) are considerably smaller than the four at the vegetal pole, which contain more yolk.

Slide 23: 32+ cells. More cleavages occur in the animal hemisphere of the embryo than in the vegetal hemisphere as the blastula develops.

Slide 24: Early blastula.

Slide 25: Late blastula. Note that the blastula is about the same size as the fertilized egg; very little, if any, growth in size has occurred.

Slide 26: Early gastrula. Cells from the animal hemisphere move down around the yolk mass (yellowish white area) and then fold in at the edge of the yolk mass, initially forming a crescent-shaped blastopore. Identify the "dorsal lip" of the blastopore.

Slide 27: Late gastrula with yolk plug. The cells from the animal hemisphere move down over the yolk and the crescent-shaped blastopore is formed.

Slide 28: Late yolk plug. The crescent shaped blastopore (top) is converted into a circle (yolk plug; middle). See Fig. 47.10.3 (p. 1029) and the demo models.

- Slide 29: The embryo in the center is an early neurula, showing the neural folds which are derived from a sheet of ectodermal cells lying along the midline of the embryo. See Fig. 47.12 (p. 1031).
- Slide 30: Early neural groove. The sheet of ectodermal cells bends inward, forming a long groove extending most of the length of the embryo.
- Slide 31: Late neural groove. The dorsal folds that border this groove move towards each other and fuse, forming the neural tube.
- Slide 32: Neural tube. In time the neural tube differentiates into the spinal cord and brain. Notice that the embryo is beginning to elongate, establishing definite anterior and posterior ends.
- Slides 33-39 show gastrulation and later embryonic development in the chick embryo. Bird eggs contain so much yolk that no cleavage of the yolk is possible and cleavage and gastrulation are greatly modified.
- Slide 33: Primitive streak stage (16 hours). Cells from the upper layer of the blastoderm move inward along the longitudinal midline of the embryo to form the mesoderm (dark areas on either side of the primitive streak). The involution gives rise to the clearly visible streak seen in this slide. This is essentially an elongate blastopore. The slightly denser regions at the anterior end of the streak and on either side of the mesoderm are due to a thickening of the ectoderm. The thickened ectoderm will later become the neural plate.
- Slide 34: 21-hour chick. A headfold appears at the anterior end of the primitive streak; this is the first evidence of the site of the future head and foregut. The mesoderm (dark) lying on either side of the midline develops into block-like masses called somites. Two pairs of somites are seen here. At the anterior end of the mesoderm and internal to it on each side lies very darkly stained tissue, the neural folds. The formation of the neural plate and its subsequent folding to form the neural folds are the first indications of the differentiation of the central nervous system.
- Slide 35: 28-hour chick. The neural tube has formed, the brain has begun to differentiate, and the optic vesicles are developing as lateral outpockets from the brain. Additional somites have been formed. Two veins have formed about one-third of the way down the embryo (the upside down V).
- Slide 36: 33-hour chick. Here the two veins have joined to form the primitive heart that bulges out to the embryo's right.
- Slide 37: Chick heart at 35 hours. The large structure bulging out to the right is the ventricle of the heart. Blood is forming in blood islands outside of the embryo itself.
- Slide 38: 48-hour chick. The embryo has begun to twist to the right so the anterior region is lying on the chick's left side. The heart is clearly visible, and blood is circulating through the vessels. The eye with its lens has formed (dark circular area) and the primitive ear (lighter circular region behind the eye) is developing.
- Slide 39: 72-hour chick. The embryo now lies on its left side. The brain, eye, and ear continue to differentiate. The large "bump" in the brain is the midbrain, the portion anterior to it is the forebrain; posterior, the hindbrain. The posterior limb buds are starting to develop.