CHAPTER 21
THE GENETIC BASIS OF DEVELOPMENT

OUTLINE

I. From Single Cell to Multicellular Organism
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   B. Researchers study development in model organisms to identify general principles: *science as a process*

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   C. Different cell types make different proteins, usually as a result of transcriptional regulation
   D. Transcriptional regulation is directed by maternal molecules in the cytoplasm and signals from other cells

III. Genetic and Cellular Mechanisms of Pattern Formation
   A. Genetic analysis of development in *Drosophila* reveals how genes control development: *an overview*
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   F. Neighboring cells instruct other cells to form particular structures: cell signaling and induction in the nematode
   G. Plant development depends on cell signaling and transcriptional regulation: *science as a process*

OBJECTIVES

After reading the chapter and attending lecture, students should be able to do the following:

1. Distinguish between the patterns of morphogesis in plants and in animals.
2. List the animals used as models for developmental biology research and provide a rationale for their choice.
3. Describe how genomic equivalence was determined for plants and animals.
4. Describe what kinds of changes occur to the genome during differentiation.
5. Describe the general processes by which "Dolly" was cloned.
6. Describe the molecular basis of determination.
7. Describe the two sources of information that instruct a cell to express genes at the appropriate time.
8. Describe how *Drosophila* were used to explain basic aspects of pattern formation (axis formation and segmentation).

9. Describe how homeotic genes serve to identify parts of the developing organism.
10. Provide evidence of the conservation of homeobox sequences.
11. Describe how the study of nematodes contributed to the general understanding of embryonic induction.
12. Describe how apoptosis functions in normal and abnormal development.
13. Describe how the study of tomatoes has contributed to the understanding of flower development.
14. Describe how the study of *Arabidopsis* has contributed to the understanding of organ identity in plants.

KEY TERMS

differentiation	determination	maternal effect genes	segment-polarity genes
morphogenesis	cytoplasmic determinants	egg-polarity genes	homeotic genes
apical meristems	pattern formation	morphogens	homeobox
model organism	induction	segmentation genes	apoptosis
cell lineage	positional information	gap genes	chimeras
totipotent	embryonic lethals	pair-rule genes	organ-identity genes

LECTURE NOTES

The study of how a single cell develops into a multicellular organism and the functional maintenance of the developed structures is one of the most intriguing aspects of biology. The complete instructions to execute the developmental program of an organism are encoded in its genes. This chapter discusses how control of the spatial and temporal expression of genes contributes to the development of a multicellular organism.

I. From Single Cell to Multicellular Organism

A. Embryonic development involves cell division, morphogenesis, and cell differentiation

A multicellular organism develops from a fertilized egg through three processes: cell division, cell differentiation, and morphogenesis (see Campbell, Figure 21.1).

- Cell division increases cell number.
- During cell *differentiation*, the cells become specialized in structure and function.
- Through a host of processes, collectively referred to as *morphogenesis*, the overall shape of the organism is established.

During development, these three processes overlap in time.

- Initial aspects of morphogenesis during early development establish the basic body plan (e.g., which end of an animal will give rise to the head).
- Cell division and differentiation as well as selective cell death are important components of morphogenesis.

Animals and plants differ in their developmental programs (see Campbell, Figure 21.2).

- In animals, movement of cells and tissues are involved in the development of physical form.
- Growth in plants is not limited to embryonic and juvenile periods as it is in animals. The root and shoot tips of plants possess perpetual embryonic tissues, known as *apical meristems*, that are responsible for the continuous growth of new organs.

B. Researchers study development in model organisms to identify general principles: *science as a process*

Researchers use *model organisms* to facilitate to discovery of fundamental developmental processes. Model organisms are chosen because they possess features that make the study easier to conduct; the criteria used to select an organism include the following:

- Large eggs (easy to manipulate and observe)
- Readily observable embryos
- Short generation times
- Small genomes
- Preexisting knowledge of organism's genes and life history

Frogs were used widely as models in early studies of development, but they actually have relatively complex genetics. As a result, most current research is conducted on the following organisms because of their unique characteristics (see Campbell, Figure 21.3):

- The fruit fly, *Drosophila melanogaster:* easily grown in the lab, short generation time, embryos grow outside of the mother's body
- The nematode, *Caenorhabditis elegans:* easily grown in lab, transparent body composed of only a few cell types that always arise in the same way, short generation time, hermaphroditic

⇒ Researchers have been able to construct the complete *cell lineage* of *C. elegans*, or the ancestry of every cell in the adult body (see Campbell, Figure 21.4).

- The zebrafish, *Danio rerio*: small and easy to breed in the lab, transparent embryo, rapid embryonic development, smaller genome size than that of mice
- The mouse, *Mus musculus*: long used as a vertebrate model, much is known about its genes; gene manipulations and gene "knock out" technologies are available; however, complex genetics and large genome
- The plant, *Arabidopsis thaliana:* easily grown in culture, small genome, cells take up foreign DNA

II. Differential Gene Expression

Differences among the cells of a multicellular organism arise from different patterns of gene expression and not from differences in the genomes of the cells.

A. Different types of cells in an organism have the same DNA

Nearly all of the cells of an organism have the same genes (genomic equivalence). What happens to these genes as the cells differentiate?

1. Totipotency in plants

Genomic equivalence among the cells of plants was demonstrated by experiments in which entire individuals developed from differentiated somatic cells (see Campbell, Figure 21.5).

- The observation that somatic cells can dedifferentiate and then give rise to all of the various cells of a new individual demonstrates that differentiation does involve irreversible changes to the genome.
- Cells that retain the ability of the zygote to give rise to all the specialized cells of a mature organism are called *totipotent.*

2. Nuclear transplantation in animals

Because the cells of animals will not often divide in culture, scientists have adopted alternative approaches to examine genomic equivalence in animals (see Campbell, Figure 21.6).

- By transplanting the nuclei of differentiated cells into enucleated egg cells of frogs, Briggs and King determined that the genome within the transplanted nuclei could support development; however, normal development was inversely related to the age of donor embryos.

Such transplantation studies lead to the following conclusions:

- Nuclei do change in some ways during differentiation.
- Changes do not occur to the sequence of DNA, but rather, in chromatin structure.

  ⇒ The age-related relationship in developmental potential of frog nuclei is related to development-related changes in chromatin structure.

Mammals have been successfully cloned form nuclei and cells of early embryos.

- An adult sheep, "Dolly," was cloned by Ian Wilmut and colleagues in Scotland by transplanting the nucleus of a dedifferentiated mammary cell from one sheep into an unfertilized, enucleated egg of another sheep.

B. Different cell types make different proteins, usually as a result of transcription regulation

As cells differentiate, they become obviously different in structure and function.

- The earliest changes are subtle, manifested only at the molecular level; such changes, known as *determination*, irreversibly commit the cell to its final fate.
- The result of determination is the presence of tissue-specific proteins (e.g., crystallins of the vertebrate lens, muscle-specific forms of actin and myosin) characteristic of a cell's structure and function.

The complement of proteins that a cell makes results from the pattern of gene expression in the differentiating cell; a pattern that is, for the most part, regulated at the level of transcription.

Transcription regulation of gene expression during development is exemplified in muscle cell determination (see Campbell, Figure 21.8).

Researchers tested the hypothesis that certain muscle-specific regulatory genes were active in myoblasts in the following way:

- By using reverse transcriptase, a *cDNA* library of genes was generated from RNA isolated from cultured myoblasts. (These cDNAs were intron-lacking versions of the genes that normally occur in myoblasts.)
- The cDNAs were ligated into bioengineered plasmids that contained a promoter that would turn on any kind of gene.
- The plasmids were then inserted into embryonic precursor cells to determine if differentiation into myoblasts and muscle cells would occur.

Researchers determined that the molecular basis of muscle cell determination is the transcription (and translation) of critical muscle-determination genes (a type of "master regulatory gene"). One of these muscle-determination genes is called *myoD*.

- The protein product of *myoD*, called MyoD, is a transcription factor that binds to control elements of DNA, and in turn, enhances the expression of other muscle-specific transcription factors.
- The secondary transcription factors activate genes encoding muscle-protein.

C. Transcriptional regulation is directed by maternal molecules in the cytoplasm and signals from other cells

Explaining the molecular basis of determination of a single cell type, such as the role of *myoD* in muscle cell differentiation, is only part of how a multicellular organism arises.

Lingering questions about how such master regulatory genes themselves are turned on remain. The answers to such questions rest again on understanding control of differential gene expression during early development.

Two sources of information instruct a cell on which genes to express at a given time:

- Information in the cytoplasm of the unfertilized egg, in the form of RNA and protein, that is of maternal origin (*cytoplasmic determinants*) (see Campbell, Figure 21.9).
- Chemical signals produced by neighboring embryonic cells; such signals, through a process known as *induction*, influence the growth and differentiation of adjacent cells.

III. Genetic and Cellular Mechanisms of Pattern Formation

Cytoplasmic determinants and inductive signals contribute to morphogenesis by modeling *pattern formation*, the spatial organization of tissues and organs characteristic of a mature organism. In plants, pattern formation occurs continuously; in animals, pattern formation is usually restricted to embryos and juveniles.

A. Genetic analysis of *Drosophila* reveals how genes control development: *an overview*

By studying *Drosophila*, researchers have identified how specific molecules influence position and direct differentiation.

1. The life cycle of *Drosophila*

Fruit flies and other arthropods are segmented into three major body parts: head, thorax, and abdomen.

The cytoplasmic determinants provide positional information.

- In unfertilized eggs, the placement of the anterior-posterior and dorsal-ventral axes is determined
- After fertilization, orientation of body segments and development of associated structures is initiated.

The developmental stages of *Drosophila* are shown in Campbell, Figure 21.10. Note that by division 13, the basic body plan, including body axes and segmentation, has been determined.

2. Genetic analysis of early development in *Drosophila*: *science as a process*

By using mutants, E.B. Lewis in the 1940s demonstrated that genes somehow direct development.

In the 1970s, Nusslein-Volhard and Wieschaus (who were awarded a Nobel prize in 1995), studied pattern formation, specifically, the basis of segmentation at the molecular level.

- Their research was fraught with many challenges (see Campbell Methods box on *Drosophila* development):
  - ⇒ Segmentation may be influenced a large number of genes (out of a possible 12,000).
  - ⇒ Mutations affecting segmentation would be lethal to embryos (*embryonic lethals*).
  - ⇒ Because maternally-derived cytoplasmic determinants affected segmentation, the scope of their analysis would have to include maternal genes as well as embryonic genes.
- Eventually, they identified some 1200 genes that were essential for development, of which 120 played a role in segmentation.
- Various cytoplasmic determinants were found to control the expression of segmentation genes.

B. Gradients of maternal molecules in the early embryo control axis formation

Cytoplasmic determinants are encoded by maternal genes called *maternal effect genes* (or sometimes *egg-polarity genes* because of the effects of their products on orientation/polarity).

- One set of genes helps establish the anterior-posterior axis of the embryo.
- A second set of genes is involved with the dorsal-ventral axis of the embryo.

The means by which maternal effect genes influence pattern formation is exemplified by the *bicoid* gene.

- A mother missing the *bicoid* gene gives rise to an embryo that lacks the front half of its body.
  - ⇒ The phenotype of the offspring suggests that the *bicoid* gene is essential for development of the anterior end of the fly, possibly because the gene product, a cytoplasmic determinant, is required at the anterior end.
  - ⇒ The requirement for the appropriate distribution of cytoplasmic determinants is a special version of the gradient hypothesis developed over 100 years ago. It maintained that gradients of substances, or *morphogens*, were required to establish the axes of the embryo.

Recent research indicates that the *bicoid* product is a morphogen that affects head-end development.

- *Bicoid* mRNA is concentrated at the anterior end of unfertilized eggs produced by wild-type mothers. After fertilization, the mRNA is translated and forms a gradient of *bicoid* protein within the embryo.
- Injection of *bicoid* mRNA into early embryos results in the development of anterior structures at the injection sites.

The factors involved with posterior end development, as well as with the development of anterior and posterior surfaces, also have been identified.

C. A cascade of gene activations sets up the segmentation pattern of *Drosophila*: a closer look

The *bicoid* protein and other morphogens are transcription factors that regulate the transcription of selected genes of the embryo.

- The gradients of the morphogens are responsible for the pattern of regional differences in the expression of *segmentation genes* (the genes that control segmentation following the establishment of the major body axes).

The sequential activation of three sets of segmentation genes are responsible for refinement of the body plan; in order of activation, the gene sets are as follows (se Campbell, Figure 21.12):

- Products of *map genes* influence basic subdivision along the anterior-posterior axis.
- *Pair-rule* genes control the pairing of segments.
- *Segment-polarity genes* serve to direct anterior-posterior orientation within each segment.

The products of the segmentation genes operate in numerous ways:

- Many are transcription factors that enhance the expression of the segmentation gene next in the sequence.
- Others are components of signaling pathways, including signal molecules used in the cell-cell communication and the membrane receptors that recognize them.

D. Homeotic genes direct the identity of body parts

Continued morphogenesis, including the appropriate placement of appendages, requires identification of specific regions of the body. The identity of segments is conveyed through master regulatory genes called *homeotic genes*.

- Homeotic genes encode for transcription factors that influence the genes responsible for specific structures.
  - ⇒ For example, homeotic proteins produced in cells of a particular thoracic segment lead to leg development.
  - ⇒ Homeotic mutations replace structures characteristic of one part of an animal with structures normally found at some other location (see Campbell, Figure 21.13).
- Scientists are in the process of identifying the genes activated by homeotic genes.

E. Homeobox genes have been highly conserved in evolution

The homeotic genes of Drosophila all contain a 180-nucleotide sequence called the *homeobox*. (For this reason, all genes that contain the homeobox are referred to as *Hox* genes.)

Sequences identical or very similar to the homeobox of *Drosophila* have been discovered in other invertebrates and vertebrates, as well as yeast and prokaryotes.

- Such sequence similarity suggests that the homeobox sequence emerged early during the evolution of life.
- Animal genes homologous to the homeotic genes of fruit flies have even kept their chromosomal arrangement (see Campbell, Figure 21.14)

Not all homeobox genes serve as homeotic genes, however, most homeobox genes are associated with some aspect of development. For example, in Drosophila, homeoboxes are present in homeotic genes, the bicoid gene, several of the segmentation genes, and in the master regulatory gene for eye development.

What is the role of the protein segment encoded by the homeobox sequence?

- The homeobox encodes for a 60-amino-acid-long homeodomain. Proteins containing homeodomains serve as transcription factors.
- The homeodomain influences protein-protein interactions critical to transcriptional regulation.

F. Neighboring cells instruct other cells to form particular structures: cell signaling and induction in the nematode

Communication between and among cells of the embryo is critical to the development of the organism. The signaling process helps to coordinate the appropriate spatial and temporal expression of genes.

1. Induction in vulval development

Research on the development of the opening (*vulva*) through which nematodes lay their eggs has provided much insight into cell signaling and induction of development. By studying mutants, scientists have identified a number of genes involved in vulval development (see Campbell, Figure 21.15).

The anchor cell releases an inducer that binds to vulval precursor cells. (This inducer is a growth factor that binds to a tyrosine kinase receptor; see Campbell, Figure 19.13a.)

- Initially, all precursor cells are the same. The cell that gives rise to the inner part of the vulva receives a higher concentration of inducer.
- The high concentration of inducer stimulates:
  - ⇒ Division and differentiation that lead to inner vulva formation
  - ⇒ The production of a second inducer
- The second inducer binds to the other precursor cells, stimulating them to form the outer vulva.

Vulva development illustrates several important developmental concepts not only in nematodes, but in animals generally.

- Sequential inductions control organ formation.
- The effect of an inducer can depend on its concentration.
- Inducers operate through signal systems similar to those in adult organisms.
- Induction results in the selective activation or inactivation of specific genes within the target cell.
- Genetics is useful to our understanding of the mechanisms that underlie development.

2. Programmed cell death (apoptosis)

The study of *C. elegans* also has revealed that normal pattern formation depends on selective, programmed cell death (*apoptosis*).

- Selective cell death occurs 131 times during normal development.
- Chemical signals initiate the activation of a cascade "suicide genes."
  - ⇒ Two key suicide genes are *ced-3* and *ced-4*; the protein products of these genes are continuously present in the cell in an inactive form.
  - ⇒ Control of apoptosis, then, depends not on transcription or translation, but on regulating protein activity (see Campbell, Figure 21.16)
- The cell is killed when enzymes are activated to hydrolyze DNA and protein.

Certain degenerative diseases and cancers may have their basis in faulty apoptotic mechanisms.

G. Plant development depends on cell signaling and transcriptional regulation.

Because the last common ancestor of plants and animals was a single-celled organism living millions of years ago, the developmental processes in these two phyla most likely evolved independently.

Despite the differences between plants and animals, some of the basic molecular, cellular, and genetic mechanisms of development are similar.

Clues to the details associated with plant development come from DNA technology, insights from animal research, and the study of the model plant *Arabidopsis*.

1. Cell signaling in flower development

Environmental cues (e.g., day length) initiate processes that convert ordinary shoot meristems to floral meristems. Such induction is exemplified with the development of tomato flowers (see Campbell, Figure 21.17).

- Tomato plants homozygous for a mutant allele, called fasciated (*f*), produce flowers with an abnormally large number of organs.
- Stems from mutant plants grafted onto wild-type plants resulted in new plants that were *chimeras*, organisms with a mix of genetically different cells.
- Some of the chimeras possessed floral meristems in which the three cell layers did not all arise from the same "parent."
- By tracing the sources of the meristem layers, it was determined that the number of organs per flower depended on genes in the L3 (innermost) cell layer.

2. Organ-identity genes in plants

Organ-identity genes determine the type of structure (e.g., petal) that will grow from a meristem. Most of the information on organ-identity genes comes from studies of *Arabidopsis*.

- Organ-identity genes are analogous to homeotic genes.
- Organ-identity genes are divided into three classes: A, B, and C.

- The simple model in Campbell, Figure 21.16 shows how three kinds of genes direct the formation of four type of organs.
- Organ-identity genes appear to be acting like master regulatory genes that control the transcription of other genes directly involved in plant morphogenesis.
  - ⇒ The organ-identity genes of plants do not contain the homeobox sequence
  - ⇒ A different sequence of about the same length is present; this sequence is also present in some transcription-factor genes of yeast and animals

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