CHAPTER 16 DEVELOPMENT: DIFFERENTIAL GENE EXPRESSION

I The Processes of Development

- Development is a series of progressive changes in shape, form and function that occurs during an organisms life cycle. *See. Figure 16.1.*
- Earliest stage is called embryonic.
  - Some embryos are contained in the starchy matrix of a seed.
  - Some are in eggs.
- Embryos typically acquire food from stores provided by or directly from a parent.

A. Development consists of growth, differentiation, and morphogenesis

- Growth occurs by cell division and/or expansion.
- Mitotic cell divisions increase cell number.
- Plants use cell elongation more to increase size, while animal embryos use cell division to increase cell number but the size of the organism often changes only slightly during the embryonic period.
- Differentiation is the process that distinguishes cells from each other in terms of structure and function.
  - Humans typically have 100^{14} cells of 200 functionally distinct cell types.
  - Cells of the embryo have fewer functional types.
  - The original cell, the fertilized egg, is the source of them all.
  - Cells change from general types, which can become other types, to specific types, which have fixed functions and forms.
  - Morphogenesis is the shaping of the whole organism.
  - Morphogenesis results first from the setting up of general coordinates such as dorsal-ventral and anterior-posterior.
  - Simple form is established and structures in each region, following the same approach, define and then refine.
  - In plants, movement of cells is limited, because cell walls adhere and restrict movement.
  - Animal cells can move, and this movement is of predominant importance during morphogenesis.

B. As development proceeds, cells become more specialized

- Moving a section of cells from a region of a frog embryo to another region causes the cells to differentiate appropriate to the new not old location.
- *See Figure 16.2.*
- This is evidence for the effect of location on cell differentiation.
- Cells do not generally maintain this developmental plasticity.
- Cells become restricted during development, which limits what kind of cell they become.
- These cells are said to be determined.

C. Determination precedes differentiation

- Cells become *determined* before they become *differentiated*.

    - This is as if molecular decisions have been made prior to their consequences.

II The Role of Differential Gene Expression in Cell Differentiation

- Differentiation results from differential gene expression.
- This means differential regulation of transcription, posttranscriptional events and translation.
- The fertilized egg is a *totipotent* cell, able to give rise to all other types of the organism.
- As the cells divide, become determined and then differentiate, they loose totipotency.

A. Differentiation usually does not include an irreversible change in the genome

- Generally, mature cells contain all the genetic information found in the zygote.
    - There are a few exceptions: red blood cells lose their nucleus as they mature.
    - Tracheid cells of plants die before they can become functional water transporting structures.
- It is currently believed by many scientists that mature *cells* from plants can be de-differentiated while those of animals cannot.
    - The differentiation of the nucleus, though, seems to be reversible.
    - Carrot cells can be tricked into forming whole new plants.
    - *See Figure 16.3.*
    - The nucleus from a frog somatic cell can be used to replace a zygote's nucleus.
    - Even a rhesus monkey has been successfully produced using nuclear transplantation.
    - *See Figure 16.5.*
    - Cells from an adult ewe (a female sheep) have served as a source of donor nuclei for cloning.
    - Dr Wilmut used the nucleus of starved udder cell, which was arrested in G_1, and generated a lamb named Dolly.
        - *See Figure 16.4.*
        - The udder cell was from transgenic (genetically modified) ewe that produced alpha -1- antitrpsin drug in her milk.
        - This drug is used to treat people with cystic fibrosis.
    - An example of mammalian cells that occasionally de-differentiated to form an unspecialized type of cell are primordial germ cells.
    - These form teratocarcinoma tumors, some with hair, teeth or kidney tubes.

B. Stem cells can be induced to differentiate by environmental signals

- Stem cells are undifferentiated, dividing cells that are found even in adults.
- A few examples are those found in bone marrow, skin, intestine and testes.
- They have limited ability to differentiate.

- An interesting example of a stem cell becoming a different type of stem cell in response to altered environmental signals has recently been discovered in mice. Brain cells, which usually differentiate into nerve cells, were transplanted to bone marrow, where they became bone marrow stem cells.
- Stem cells with greatest totipotency are the cells of the inner cell mass of early embryos.
- If inner-cell-mass cells are injected into a blastocyst of another, an individual with two different types of cells develops.
- Embryonic stem cells can be induced to differentiate using certain signal molecules.
- A derivative of vitamin A causes them to form nerve cells.
- Possibly, in the future, manipulation of embryonic cells in culture will make new important disease treatments possible. *See Figure 16.6.*

C. Genes are differentially expressed in cell differentiation

- β-globin is synthesized in blood cells but the DNA coding for it can be found in other cells.
- There is differential gene expression between the 200 different cell types.
- How are the differences established?
    - Myoblast, precursors to muscle cells have been studied for expression of muscle specific genes.
    - *MyoD1* is the first gene switched on.
    - It codes for a helix-loop-helix type DNA binding protein.
    - It binds to the promoters of muscle-specific genes switching them on.
    - It also binds to its own promoter, keeping itself on, similar to a memory circuit.

III The Role of Polarity in Cell Determination

- Two general mechanisms for producing signals used for cellular programming of differential gene expression are cytoplasmic segregation and induction.
- In cytoplasmic segregation, factors within eggs, zygotes, or precursor cells are partitioned, unequally.
- These differences influence the differentiation of the cells.
- Induction involves one cell type influencing another.
- A factor is actively produced and secreted by a certain type of cells to induce change in another type.
- Polarity is obvious in development.
    - Polarity includes the establishment of the most basic ones.
    - Anterior-posterior; dorsal-ventral.
- In some organisms, polar distribution of materials in the egg changes and orients relative to the location of the sperm-egg fusion.
    - Cells receive heterogeneous cytoplasms, which influences differentiation.
    - Sea urchin eggs have polarity at the 8-cell stage.
        - If an embryo is divided into two parts, left and right halves, normal development of dwarfed larvae occurs.

    - If cut in upper and lower halves, the upper cannot form a larva and the lower is misshapen.
    - *See Figure 16.7.*
- Experiments have established the existence of cytoplasmic determinants in some types of organism, which are unequally distributed in the egg.

IV The Role of Embryonic Induction in Cell Determination

A. Tissues direct the development of their neighbors by secreting inducers

- An early discovery in development was the induction of cells to form lens cells in frogs.
- The forebrain bulges out at both sides of the future head region forming optic vesicles.
- These cells expand until they reach the cells at the surface.
- Surface tissue thickens, forming the placodes of the two lenses.
- These bend inward at their margins, constrict and pinch to form a structure that then differentiates into lenses.
- If growing optic vesicles is cut away, no lenses form.
- If an impermeable barrier is placed between, still no lenses form.
- The induction of lenses is two ways.
    - The developing lenses influence the size of the optical cups.
    - *See Figure 16.8.*

B. Single cells can induce changes in their neighbors

- The roundworm, *Caenorhabditis elegans*, also called a nematode has been studied extensively during development.
- Egg to larva takes just 8 hours at 25^{o}C.
- Development can be observed easily because of the transparency of the embryo.
- The source of the larva's 959 somatic cells from the original cell, the zygote, has been determined.
- It has been proven that one cell, called an anchor cell, induces the vulva to form. *See Figure 16.9.*
    - If the anchor cell is destroyed, no vulva forms.
    - This prevents eggs from being laid.
    - Its own progeny consumes the larva.
- The anchor cell controls the fates of six cells on the ventral surface through two molecular switches.
    - Each of the six cells have three possible fates.
        - It might become a primary vulval precursor.
        - It might become a secondary vulval precursor.
        - It might become a simple part of the worm's surface.
- Cells that get enough inducer signals become vulval precursors.
- Those further away from the anchor get fewer signals and become epidermis.
- The sequentially first signal sets the track of the cells.

- The cells closest become primary vulval cell precursors and these cells themselves then influence the two neighboring cells through another inducer to become *secondary* vulval precursors.
- Inducers cause differential gene expression.
- The primary inducer in nematode vulval differentiation is homologous to the mammalian growth factor, epidermal growth factor (EGF).
- This nematode factor, called LIN-S, binds a receptor on the surface of the cell.
- This activates a protein kinase cascade that includes the GTPase protein ras and MAP kinases. *(See Figure 15.11.)*

V The Role of Pattern Formation in Organ Development

Pattern formation, the spatial organization of a tissue or organism, is absolutely linked to morphogenesis.

A. Some cells are programmed to die

- Apoptosis is programmed cell death.
    - It is caused by the activation of suicide genes.
    - *C. elegans* produces precisely 1,090 cells.
    - Of these, 131 cells are programmed to die.
    - The genes *ced-4* and *ced-3* appear to control this process.
    - A third gene, *ced-9,* codes for an inhibitor of *ced-4*.
- In human embryos, webs exist between fingers and toes. *See Figure 16.10.*
    - Between day 41 and 56, the cells of the webbing die.
    - The enzyme, caspase, stimulates apoptosis and is homologous to *ced-3.*
    - The protein in humans, that inhibit apoptosis, which is like *ced-9,* is *bcl-2*.
    - One form of cancer, follicular large-cell lymphoma is caused by *bcl-2*, which inhibits appropriate cell death.

B. Plants have organ identity genes

- Some plants have flowers, and many flowers have four different organs: sepals, petals, stamens and carpels.
- These organs occur in whorls, which are groups of each organ arranged around a central axis.
- These whorls are formed from meristem cells,
- A plant species, *Arabidopsis thaliana*, called the mouse-ear cress, has been studied.
- This plant is small, has a relatively small genome (80 X 10^6), produces many seeds (1000) and develops rapidly for a plant (6 weeks).
    - Normal *Arabidopsis*; have four whorls of organs.
    - Mutants have incorrect organs in whorls.
    - Such plants are called homeotic mutants.
    - *See Figure 16.11.*
    - The system involves genes that act as molecular switches.
        - Three different organ identity genes exist.

      - Each of the four types of whorls express certain ones: whorl 1 expresses A; 2 expresses A and B; 3 expresses B and C; and, whorl 4 expresses C.
    - The products of these genes are transcription factors that form dimers (two subunits).
      - In whorl 1, A gene products form AA dimers.
      - In whorl 2, A and B genes products form Aa, AB and BB dimers.
      - This is called combinatorial gene regulation
    - A gene called *leafy* controls the transcription of the *ABC* genes. *(See Figure 16.12)*
  - Research that increases the understanding of flowering could increase food production.

C. Plants and animals use positional information

  - Certain cells in both plants and animals seem to "know" where they are within the organism
  - This is called positional information.
  - The position of certain cells in plants can influence oxygen and CO_2 concentrations they experience.
  - There are signal proteins that are secreted from cells at opposite positions setting up gradients which can be interpreted.
    - These molecules are called morphogens.
    - Chick wings develop from small round buds.
    - The cells must organize to shape properly.
    - Positional information is provided by morphogens.
      - The first morphogen determines the proximal-distal axis of the wing.
      - The second determines the anterior-posterior, for example, thumb to little finger.
      - The third determines the dorsal-ventral (palm to knuckles) axis.

VI The Role of Differential Gene Expression in Establishing Body Segmentation

- *Drosophila* is another important developmental biologists' model organism.
- *Drosophila* segments are clearly different from one another.
  - The head is several fused segments.
  - There are three different thoracic segments.
  - There are eight abdominal segments.
  - There is a terminal, posterior segment.

A. Maternal effect genes determine polarity

  - In *Drosophila,* unequal distribution of morphogens helps establish the basic coordinates.
  - Nurse cells that support the egg influence this.
  - It is their influence and genes from the egg's mothers that influence the establishment of gradient.
  - These determine dorsal-ventral and anterior-posterior axes of the embryo.

B. Segmentation and homeotic genes act after the maternal effect genes

- Segmentation genes influence the number and boundaries of the body segments.
- The maternal effect gene products influence the axes of the embryo when there are 6000 nuclei existing in a common cytoplasm.
- After the nuclei are isolated into different cells, three other classes of segmentation genes act.
    - Gap genes organize large areas along the anterior-posterior axis.
    - Pair rule genes divide the embryo into units of two segments each.
    - Segment polarity genes determine the boundaries of anterior-posterior segments.

C. *Drosophila* development results from a transcriptionally controlled cascade

- Development is the result of a sequence of changes, each triggering the next.
- The egg begins with stored mRNA.
- The mRNA for Bicoid protein is located at the future anterior end of the *Drosophila* egg.
- After fertilization, the egg is laid and nuclear division occurs.
- The embryo consists of a multinucleated cell called a syncytium.
- Then, Bicoid protein is synthesized.
- The Bicoid protein diffuses away toward the posterior end.
- At the posterior, Nanos protein forms a gradient in the other direction.
- *See Figure 16.13.*
- These morphogens regulate the expression of the gap genes.
- Bicoid protein affects specific transcription.
- Nanos affects specific translation.
- High Bicoid at the anterior turns on *hunchback*, while simultaneously turning off *Krüppel*.
- Nanos at the posterior reduces *hunchback*.
- The gap genes control the expression of pair rule genes.
- The pair-rule gene products control the segmentation polarity genes.
- Each homeotic gene is expressed over a characteristic portion of the embryo.
- *See Figure 16.14.*

D. Homeotic mutation produce large-scale effects

- One mutant of a homeotic gene causes a leg to grow in the place of antennae. *(See Figure 16.15)*
- Another, *bithorax*, causes an extra pair of wings to grow.
- Homeotic genes provide functional identity to each segment.
- They are found in clusters.
- Two clusters, one for segments at the front of the fly called the *antennapedia* complex and another called *bithorax* complex.
- The genes within a complex are arranged in an order that is collinear to their expression in segments.
- There are DNA segments in common within the genes of both clusters.

E. Homeobox-containing genes encode transcription factors

- A 180-base-pair DNA sequence that is common to the homeotic genes is called the homeobox
- It codes for a 60-amino acid sequence called the homeodomain, which binds DNA.
- It has the helix-turn-helix motif.
- Each homeodomain recognizes a specific DNA sequence.
- Homeotic genes code for transcription factors.
- *See Figure 16.16.*

VII Evolution and Development

- *See Figure 16.17.*
- Homeobox sequences have been discovered in other organisms.
- From nematodes and plants to chickens and humans, the evidence is that homeobox and associated types of genes, are a conserved mechanism for controlling development.
- In mice, 38 genes in four clusters, each located on a different chromosome, control the development of specific regions of the mouse embryo.
- They are arranged in the same order on the chromosome as where they are expressed, from anterior to posterior, in the developing animal.
- The four clusters are assumed to have arisen via repeated duplications followed by a few mutations.
- The eye development of insects and mammals is controlled by a similar mechanism, even though their form and function is very different.
  - The gene *eyeless,* when mutant, results in eyelessness.
  - It has been found that the transcription factor coded for by *eyeless* binds to the over 1,500 different genes involved in eye formation.
  - The homolog of *eyeless* in vertebrates, *Pax6,* is also involved in eye formation.
  - A mutation in *Pax6* results in a disease called aniridia - the partial or total absence of the iris.
  - *Pax6* can be interchanged with *eyeless* and vise versa, and provide normal function.
- Signals for dorsal-ventral in arthropods and vertebrates are the same, but they have opposite meanings. *See Figure 16.18.*
  - Chordin determines dorsal in vertebrates, but its homolog Sog, specifies the ventral region of arthropods.
  - BMP4 is a ventral determiner in vertebrates, but its relative, Dpp, is a dorsal determiner in arthropods.