Homeotic genes are genes that control the pattern of body formation in embryonic development. Homeotic gene sequences contain an evolutionarily highly conserved region, called the homeobox, which directs development in organisms as diverse as fungi, plants, and animals. A homeobox is about 180 base pairs long and encodes transcription factors responsible for switching on gene expression. The Hox genes are a particular group of homeobox genes in animals. They are found in clusters and determine where particular body segments and limbs grow. Very disparate organisms share the same tool kit of genes, but regulate them differently. This means that large changes in morphology or function are attributable to changes in gene regulation, rather than the evolution of new genes, and natural selection associated with gene switches plays a major role in evolution.

### The Role of Hox Genes

Hox genes control the development of back and front parts of the body. The same genes (or homologous ones) are present in essentially all animals, including humans.

**Drosophila embryo**

<table>
<thead>
<tr>
<th>Head</th>
<th>Thorax</th>
<th>Abdomen</th>
</tr>
</thead>
</table>

The Hox genes are located on a single chromosome in Drosophila, and on four separate chromosomes in mice. The different shading indicates where in the body the genes are expressed.

### Shifting Hox Expression

Huge diversity in morphology in organisms within and across phyla could have arisen through small changes in the genes controlling development.

Differences in neck length in vertebrates provide a good example of how changes in gene expression can bring about changes in morphology. Different vertebrate species have different numbers of neck vertebrae (denoted by the black ovals on the diagram). The boundary between neck and trunk vertebrae is marked by expression of the Hox c6 gene (c6 denotes the sixth cervical or neck vertebra) in all cases, but the position varies in each animal relative to the overall body. The forelimb (arrow) arises at this boundary in all four-legged vertebrates. In snakes, the boundary is shifted forward to the base of the skull and no limbs develop. As a result of these differences in expression, mice have a short neck, geese a long neck, and snakes, no neck at all.

### The Evolution of Novel Forms

Even very small changes (mutations) in the Hox genes can have a profound effect on morphology. Such changes to the genes controlling development have almost certainly been important in the evolution of novel structures and body plans. Four principles underlie the role of developmental genes in the evolution of novel forms:

- **Evolution works with what is already present**: New structures are modifications of pre-existing structures.
- **Multifunctionality and redundancy**: Functional redundancy in any part of a multifunctional structure allows for specialization and division of labor through the development of two separate structures.
- **Example**: the diversity of appendages (including mouthparts) in arthropods.
- **Modularity**: Modular architecture in animals (arthropods, vertebrates) allows for the modification and specialization of individual body parts. Genetic switches allow changes in one part of a structure, independent of other parts.
Genetic Switches in Evolution

The \textit{Hox} genes are just part of the collection of genes that make up the genetic tool kit for animal development. The genes in the tool kit act as switches, shaping development by affecting how other genes are turned on or off. The distribution of genes in the tool kit indicates that it is ancient and was in place before the evolution of most types of animals. Differences in form arise through changes in genetic switches. One example is the evolution of eyespots on the wings of butterflies:

- The \textit{Distal-less} gene is one of the important \textbf{master body-building genes} in the genetic tool kit. Switches in the \textit{Distal-less} gene control expression in the embryo (E), larval legs (L), and wing (W) in flies and butterflies, but butterflies have also evolved an extra switch (S) to control eyespot development.

- Once spots evolved, changes in \textit{Distal-less} expression (through changes in the switch) produced more or fewer spots.

\begin{center}
\includegraphics[width=0.5\textwidth]{distal-less.png}
\end{center}

Changes in \textit{Distal-less} regulation were probably achieved by changing specific sequences of the \textit{Distal-less} gene eyespot switch. The result? Changes in eyespot size and number.

1. Briefly describe the role of homeobox genes in development:

2. (a) What does it mean when the homeobox genes are said to be highly conserved?

   \begin{itemize}
   \item The action of a tool kit protein depends on context: where particular cells are located at the time when the gene is switched on.
   \item Changes in the DNA sequence of a genetic switch can change the zone of gene expression without disrupting the function of the tool kit protein itself.
   \item The spectacular \textbf{eyespots} on butterfly wings (arrowed above) represent different degrees of a basic pattern, from virtually all eyespot elements expressed (\textit{Stichophthalma}) to very few (\textit{Taenaris}).
   \end{itemize}

(b) What does this tell you about the evolution and the importance of the homeobox sequences?

3. Suggest why the \textit{Hox} genes are found in clusters (i.e. grouped tightly together):

4. Using an example, discuss how changes in gene expression can bring about changes in morphology:
The Timing of Development

Timing is extremely important in embryonic development if the body is to develop normally. Cells must differentiate into the correct cell at the correct time and in the correct place. This is achieved by cell signaling, either by producing a gradient of molecules through a section of the embryo or by signaling a specific cell that then signals other cells in sequence. Cell signals regulate specific transcription factors that in turn control the differential gene expression that shapes embryos. The time taken from the start of this embryonic induction to the response is between hours and days, while the time a cell can respond to an inducing signal is strictly limited. The state (readiness) of the responding tissue is also important in determining the timing of the response.

Development in Drosophila

Morphogens are signal molecules that govern the pattern of tissue development. Morphogens induce cells in different positions to adopt different fates by diffusing from an area of production through a field of cells.

![Morphogen concentration and cell fate graph](image)

**Morphogens** are signal molecules that govern the pattern of tissue development. Morphogens induce cells in different positions to adopt different fates by diffusing from an area of production through a field of cells.

**Development in Caenorhabditis elegans**

A second way of inducing change in embryonic cells is through **sequential induction**. A signal molecule reaches cell A, which responds by developing and producing signal B and so on.

![Sequential induction in C. elegans](image)

The nematode worm, *Caenorhabditis elegans*, is a **model organism** that is often used in developmental studies. The cells that form the vulva (ventral opening for copulation and egg laying) display sequential induction. Signals from the anchor cell induce a change in cell P6p. Cell P6p then signals cells P5p and P7p to develop.

**Apoptosis in C. elegans**

![Apoptosis in C. elegans graph](image)

1. (a) Describe the role of **morphogens**:

   (b) Explain the purpose of the **bicoid protein** in *Drosophila*:

2. Describe **sequential induction** in *C. elegans*:
Apoptosis: Programmed Cell Death

Apoptosis or programmed cell death (PCD) is a normal and necessary mechanism in multicellular organisms to trigger the death of a cell. Apoptosis has a number of crucial roles in the body, including the maintenance of adult cell numbers, and defense against damaged or dangerous cells, such as virus-infected cells and cells with DNA damage. Apoptosis also has a role in "sculpting" embryonic tissue during its development, e.g., in the formation of fingers and toes in a developing human embryo. Programmed cell death involves an orderly series of biochemical events that result in set changes in cell morphology and end in cell death. The process is carried out in such a way as to safely dispose of cell remains and fragments. This is in contrast to another type of cell death, called necrosis, in which traumatic damage to the cell results in premature cell death and spillage of the cell contents. Apoptosis is tightly regulated by a balance between the factors that promote cell survival and those that trigger cell death. An imbalance between these regulating factors leads to defective apoptotic processes and is implicated in an extensive variety of diseases. For example, low rates of apoptosis result in uncontrolled proliferation of cells and cancers.

Stages in Apoptosis

Apoptosis is a normal cell suicide process in response to particular cell signals. It characterized by an overall compaction (shrinking) of the cell and its nucleus, and the orderly dissection of chromatin by endonucleases. Death is finalized by a rapid engulfment of the dying cell by phagocytosis. The cell contents remain membrane-bound and there is no inflammation.

1. The cell shrinks and loses contact with neighboring cells. The chromatin condenses and begins to degrade.

2. The nuclear membrane degrades. The cell loses volume. The chromatin clumps into chromatin bodies.


4. The nucleus collapses, but many membrane-bound organelles are not affected.

5. The nucleus breaks up into spheres and the DNA breaks up into small fragments.

6. The cell breaks into numerous apoptotic bodies, which are quickly resorbed by phagocytosis.

In humans, the mesoderm initially formed between the fingers and toes is removed by apoptosis. Forty-one days after fertilization (top left), the digits of the hands and feet are webbed, making them look like small paddles. Apoptosis selectively destroys this superfluous webbing, sculpting them into digits when can be seen later in development (top right).

Regulating Apoptosis

Apoptosis is a complicated and tightly controlled process, distinct from cell necrosis (uncontrolled cell death), when the cell contents are spilled. Apoptosis is regulated through both:

- Positive signals, which prevent apoptosis and allow a cell to function normally. They include:
  - interleukin-2
  - bcl-2 protein and growth factors

Interleukin-2 is a positive signal for cell survival. Like other signaling molecules, it binds to cell surface receptors to regulate metabolism.

Negative signals (death activators), which trigger the changes leading to cell death. They include:

- inducer signals generated from within the cell itself in response to stress, e.g., DNA damage or cell starvation.
- signalling proteins and peptides such as lymphotoxin.

1. The photograph (right) shows a condition called syndactyly. Explain what might have happened during development to result in this condition:

2. Describe one difference between apoptosis and necrosis: ________________

3. Describe two situations, other than digit formation in development, in which apoptosis plays a crucial role:

   (a) ________________

   (b) ________________