INDUCTION, COMPETENCE and MORPHOGENETIC GRADIENT

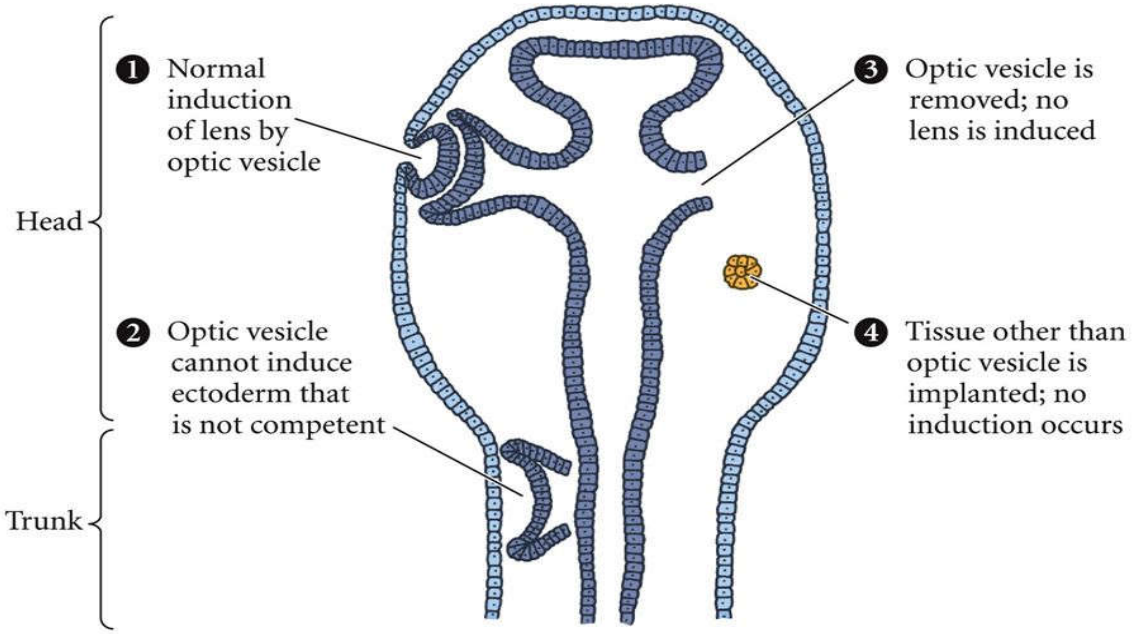
- **Induction and competence:**

From the earliest phases of development through the adult, cell differentiation and behavior (such as adhesion, migration, and cell division) are regulated by signals from one cell being received by another cell. Indeed, these interactions (which are often reciprocal) are what allow organs to be constructed. The development of the vertebrate eye is a classic example generally used to describe the modus operandi of tissue organization via intercellular interactions. In the vertebrate eye, light is transmitted through the transparent corneal tissue and focused by the lens tissue (the diameter of which is controlled by muscle tissue), eventually impinging on the tissue of the neural retina. The precise arrangement of tissues in the eye cannot be disturbed without impairing its function. Such coordination in the construction of organs is accomplished by one group of cells changing the behavior of an adjacent set of cells, thereby causing them to change their shape, mitotic rate, or cell fate. **This kind of interaction at close range between two or more cells or tissues of different histories and properties is called induction**. There are at least two components in every inductive interaction. The first component is **the inducer**: the tissue that produces a signal (or Signals) that changes the cellular behavior of the other tissue. Often, this signal is a secreted protein called a paracrine factor. Paracrine factors are proteins made by a cell or a group of cells that alter the behavior or differentiation of adjacent ceils, In contrast to endocrine factors (i.e" hormones), which move through the blood and exert their impacts on cells and tissues far away, paracrine factors are secreted into the extracellular space and influence their close neighbors, (The Branchless protein, is such. a factor). The second component, the **responder,** is the tissue being induced, Cells of the responding tissue must have both a receptor protein for the inducing factor (the receptor for Branchless is the Breathless protein) and the ability to respond to the signal.

The ability to respond to a specific inductive signal is called competence (Waddington 1940). Competence is not a passive state, but an actively acquired condition. For example, in the developing chick and mammalian eye, the Pax6 protein appears to be important in making the ectoderm competent to respond to the inductive signal from the optic vesicle. Pax6 expression is

seen in the head ectoderm, which can respond to the optic vesicle by forming lenses, and it is not seen in other regions of the surface ectoderm (Li *et al*. 1994). Moreover, the importance of Pax6 as a **competence factor** was demonstrated by recombination experiments using embryonic rat eye tissue (Fujiwara *et al.* 1994). The homozygous Pax6-mutant rat has a phenotype similar to the homozygous Pax6-mutant mouse, lacking eyes and nose. It has been shown that part of this phenotype is due to the failure of lens induction. But which is the defective component—the optic vesicle or the surface ectoderm? When head ectoderm from Pax6-mutant rat embryos was combined with a wild-type optic vesicle, no lenses were formed. However, when the head ectoderm from wild-type rat embryos was combined with a Pax6-mutant optic vesicle, lenses formed normally. Therefore, Pax6 is needed for the surface ectoderm to respond to the inductive signal from the optic vesicle. The inducing tissue does not need it. It is not known how Pax6 becomes expressed in the anterior ectoderm of the embryo, although it is thought that its expression is induced by the anterior regions of the neural plate. Competence to respond to the optic vesicle inducer can be conferred on ectodermal tissue by incubating it next to anterior neural plate tissue (Henry and Grainger 1990; Li et al. 1994; Zygar et al. 1998). Even if receptor proteins are present, not every tissue type is competent to respond to an inducer's signal. For instance, if the optic vesicle (the presumptive retina) of a *Xenopus laevis* embryo is placed in an ectopic location underneath the head ectoderm (i.e., in a different part of the head from where the frog's optic vesicle normally occurs), it will induce that ectoderm to form lens tissue. Only the optic vesicle appears to be able to do this; therefore, it is an inducer. However, if the optic vesicle is placed beneath ectoderm in the flank or abdomen of the same organism, that ectoderm will not be able to form lens tissue. Only head ectoderm is competent to respond to the signals from the optic vesicle by producing a lens.

Figure 3.13 Ectodermal competence and the ability to respond to the optic vesicle inducer in *Xenopus*

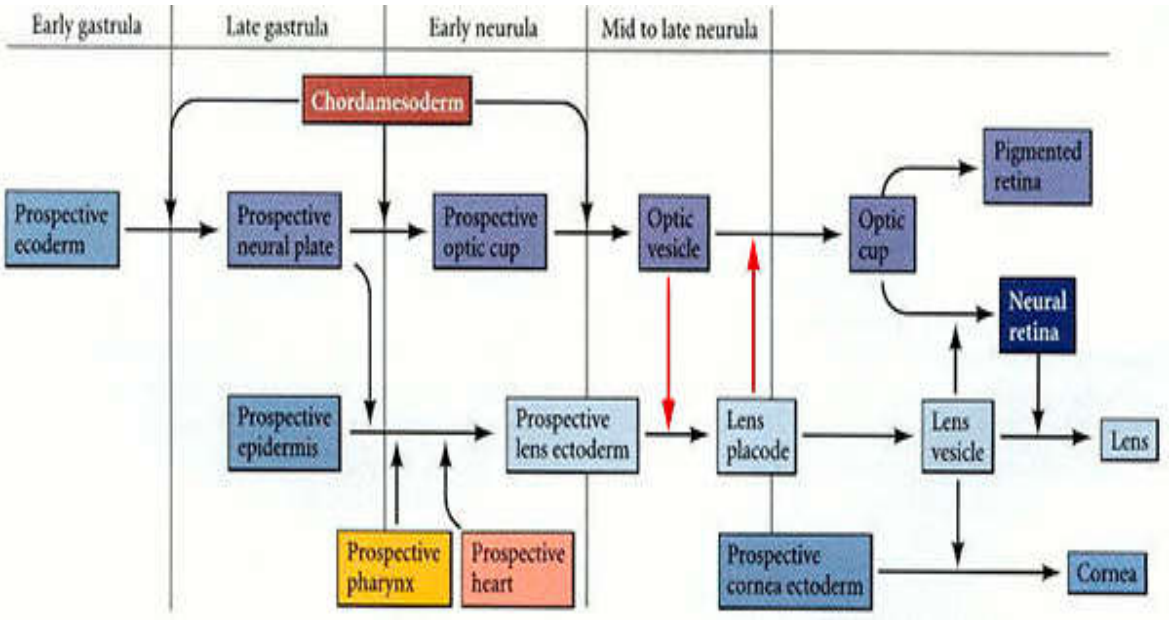


DEVELOPMENTAL BIOLOGY, 9e, Figure 3.13

- **Cascades of induction:**

Reciprocal and sequential inductive events: Another feature of induction is the reciprocal nature of many inductive interactions. To continue the above example, once the lens has produced, it induces other tissues. One of these responding tissues is the optic vesicle itself; thus the inducer becomes the induced. Under the influence of factors secreted by the lens, the optic vesicle becomes the optic cup and the wall of the optic cup differentiates into two layers, the pigmented retina and the neural retina. Such interactions are called reciprocal inductions. At the same time, the lens is inducing the ectoderm above it to become the cornea. Like the lens-forming ectoderm, the cornea-forming ectoderm has achieved a particular competence to respond to inductive signals, in this case the Signals from the lens (Meier 1977; Thut *et al.* 2001). Under the influence of the lens, the corneal ectoderm cells become columnar and secrete multiple layers of collagen. Mesenchymal cells from the neural crest use this collagen matrix to enter the area and secrete a set of proteins (including the enzyme hyaluronidase) that further differentiate the cornea. A third

Signal, the hormone thyroxin, dehydrates the tissue and makes it transparent (Bard 1990). Thus, there are sequential inductive events, and multiple causes for each induction.



Instructive and permissive interactions

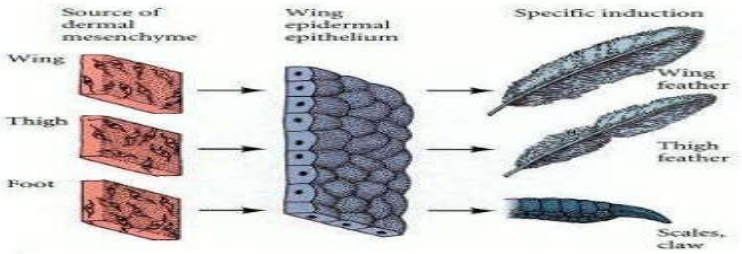
Howard Holtzer (1968) distinguished two major modes of inductive interaction. In instructive interaction, a signal from the inducing cell is necessary for initiating new gene expression in the responding cell. Without the inducing cell, the responding cell is not capable to differentiate in that particular way. For example, when the optic vesicle is experimentally placed under a new region of the head ectoderm and causes that region of the ectoderm to form a lens that is an instructive interaction. The second type of inductive interaction is permissive interaction. Here, the responding tissue has already been specified, and needs only an environment that allows the expression of these traits. For instance, many tissues need a solid substrate containing fibronectin or laminin in order to develop. The fibronectin or laminin does not alter the type of cell that is produced, but it enables what has already been determined to be expressed.

Regional specificity of induction

Using the induction of cutaneous (skin) structures as our examples, we will look at the properties of epithelial-mesenchymal interactions. The first of these properties is the regional specificity of induction. Skin is composed of two main tissues: an outer epidermis (an epithelial tissue derived from ectoderm), and a dermis (a mesenchymal tissue derived from mesoderm). The chick epidermis secretes proteins that signal the underlying dermal cells to form condensations, and the condensed dermal mesenchyme responds by secreting factors that cause the epidermis to form regionally specific cutaneous structures (Nohno et a1. 1995; Ting-Berreth and Chuong 1996). These structures can be the broad feathers of the wing, the narrow feathers of the thigh, or the scales and claws of the feet.

Regional specificity of induction

The first property of epithelial-mesenchyma interaction is regional specificity of induction. Skin is composed of two main tissues an outer epidermis an epithelial tissue derived from ectoderm and a dermis a mesenchymal tissue derived from mesoderm. mesenchyme is responsible for the regional specificity of induction in the competent epidermal epithelium. The same type of epithelium develops cutaneous structures according to the region from which the mesenchyme was taken.
Here, the mesenchyme plays an instructive role.



- Wing dermal mesenchyma and wing epidermal epithelium give rise to wing epithelium.
- Thigh dermal mesenchyma and wing epiderml epithelium give rise to thigh feather.
- Foot dermal mesenchyma and wing epidermal epithelium give rise to scales and claws.

Regionally specific inductions can generate different structures from the same tissue.

Conclusion

Interaction among different cells occurs through paracrine and juxtacrine induction.Formation of heart and lens are secondary embryonic induction whereas tissue interaction that that generate neural tube is known as primary embryonic induction.

Genetic specificity of induction

The second property of epithelial-mesenchymal interactions is the genetic specificity of induction. Whereas the mesenchyme may instruct the epithelium as to what sets of genes to activate, the responding epithelium can comply with these instructions only so far as its genome permits. This property was discovered through experiments involving the transplantation of tissues from one species to another.

Paracrine Factors: The Inducer Molecules

How are the Signals between inducer and responder transmitted? While studying the mechanisms of induction that produce the kidney tubules and teeth, Grobstein (1956) and others (Saxen et a1. 1976; Slavkin and Bringas 1976) found that some inductive events could occur despite a filter separating the epithelial and mesenchymal cells. Other inductions, however, were blocked by the filter. The researchers therefore concluded that some of the inductive molecules were soluble factors that could pass through the small pores of the filter, and that other inductive events required physical contact between the epithelial and mesenchymal cells. When cell membrane proteins on one cell surface interact with receptor proteins on adjacent cell surfaces, these events are called juxtacrine interactions (since the cell membranes are juxtaposed). When proteins synthesized by one cell can diffuse over small distances to induce changes in neighboring cells, the event is called a paracrine interaction. This type of interaction is mediated by paracrine factors and their receptors. We will consider paracrine interactions first, returning to juxtacrine interactions. Whereas endocrine factors (hormones) travel through the blood to exert their effects, paracrine factors are secreted into the immediate spaces around the cell producing them. These proteins are the "inducing factors" of the classic experimental embryologists. There is considerable debate as to the distances at which paracrine factors can operate. The proteins Nodal and activin, for instance, can diffuse over many cell diameters and induce different sets of genes at different concentrations (Gurdon et a1. 1994, 1995). The Wnt, Vg1, and BMP4 proteins, however, probably work only on their adjacent neighbors (Jones et a1. 1996; Reilly and Melton

1996). These factors may induce the expression of other short-range factors from these neighbors, and a cascade of paracrine inductions can be initiated.

The Hedgehog family

The proteins of the Hedgehog family of paracrine factors are often used by the embryo to induce particular cell types and to create boundaries between tissues. Hedgehog proteins are processed such that only the amino-terminal two thirds of the molecule is secreted; once this takes place, the protein must become comp1exed with a molecule of cholesterol in order to function. Vertebrates have at least three homologues of the *Drosophila hedgehog gene: sonic hedgehog (shh), desert hedgehog (dhh), and indian hedgehog (ihh).* The **Desert hedgehog** protein is expressed in the **Sertoli cells of the testes**, and mice homozygous for a null allele of dhh exhibit defective spermatogenesis. **Indian hedgehog protein** is expressed in the **gut and cartilage** and is important in postnatal bone growth (Bitgood and McMahon 1995; Bitgood et a1. 1996). Sonic hedgehog has the greatest number of functions of the three vertebrate Hedgehog homologues. Among other important functions, this paracrine factor is responsible for assuring that motor neurons come only from the ventral portion of the neural tube, that a portion of each somite forms the vertebrae, that the feathers of the chick form in their proper places (see Figure 3.16), and that our pinkies are always our most posterior digits (see Chapter 14). Sonic hedgehog often works with other paracrine factors, such as Wnt and FGF proteins.

The Hedgehog pathway is extremely important in vertebrate limb development, neural differentiation, and facial morphogenesis (McMahon *et a1*. 2003). When mice were made homozygous for a mutant allele of sonic hedgehog, they had major limb and facial abnormalities. The midline of the face was severely reduced and a single eye formed in the center of the forehead, a condition known as cyclopia (Chiang *et al.* 1996). In later development, Sonic hedgehog is critical for feather formation in the chick embryo and hair formation in mammals (Harris *et al.* 2002; Michino *et a1*. 2003).

- **Morphogen Gradients and Cell Specification**

There are many instances of cell fate specification that involve morphogen gradients. A morphogen (Greek, "form-giver") is a signaling molecule that acts directly on cells (not through serial induction) to produce specific cellular responses dependent on morphogen concentration. During early development, morphogen gradients generate different cell types in distinct spatial order. Morphogens can be transcription factors produced within cells (as in the Drosophila embryos described in the following section). They can also be paracrine factors that are produced in one group of cells and then travel to another population of cells, specifying the target cells differentially according to the concentration of morphogen. Uncommitted cells exposed to high concentrations of the morphogen (nearest its source of production) are specified as one cell type; when the morphogen's concentration drops below a certain threshold, the cells are determined to another fate. When the concentration falls even lower, a cell of the same initial uncommitted type is specified in yet a third manner. Morphogen gradients provide a very important mechanism for conditional specification. The existence of morphogen gradients as a force in development and regeneration was predicted by Thomas Hunt Morgan (1905, 1906---before he became a geneticist), but it was many years before these gradient models were extended to explain how cells might be placed in specific positions along an embryonic axis (Horstadius 1939; Toivonen and Saxen 1955; Lawrence 1966; Stumpf 1966; Wolpert 1968, 1969). Lewis Wolpert illustrated such a gradient of positional information using the "French flag" analogy. Imagine a row of "flag cells," each of which is "multipotential"---capable of differentiating into a red, a white, or a blue cell. Then imagine a morphogen whose source is on the left-hand edge of the blue stripe and whose sink is at the other end of the flag, on the right-hand edge of the red stripe. A concentration gradient is thus formed, highest at one end of the "flag tissue" and lowest at the other. The specification of multipotential cells in this tissue is accomplished by threshold concentrations of the morphogen. Cells sensing the highest concentrations of morphogen become blue. Then there is a threshold of morphogen concentration below which cells become white. As the declining concentration of morphogen

falls below another threshold, the cells become red. According to such models (see Crick 1970), the morphogen diffuses from its site of synthesis (source) to its site of degradation (sink), its concentration dropping along the way. This drop in concentration can be due to simple diffusion, to the cells' binding the morphogen and thus using it up or to a combination of a source synthesizing the morphogen and an environment containing an enzyme that degrades it.

The "French Flag Model"

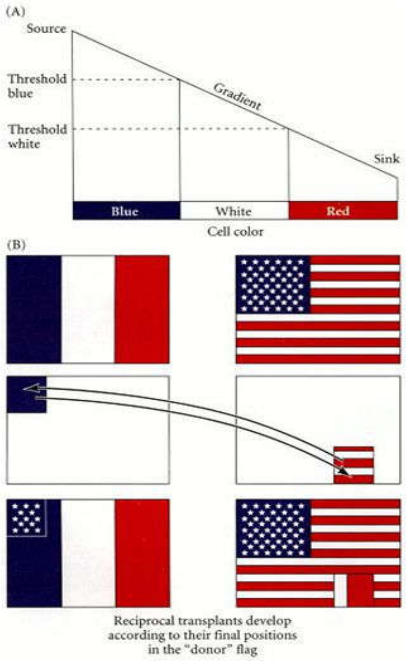


Figure 3.19. The French flag analogy for the operation of a gradient of positional information.

(A) In this model, positional information is delivered by a gradient of a diffusible morphogen from a source to a sink. The thresholds indicated on the left are cellular properties that enable the gradient to be interpreted. For example, cells becomes blue at one concentration of the morphogen, but as the concentration declines below a certain threshold, cells become white. Where the concentration falls below another threshold, cells become red. The result is a pattern of three colors.

(B) An important feature of this model is that a piece of tissue transplanted from one region of an embryo to another retains its identity (as to its origin), but differentiates according to its new positional instructions. This phenomenon is indicated schematically by reciprocal "grafts" between the flag of the United States of America and the French flag. (After Wolpert 1978.)

Examples of morphogen gradient:

Bicoid and *Hunchback* are the maternal effect genes that are most important for patterning of anterior parts (head and thorax) of the *Drosophila* embryo. *Nanos* and *Caudal* are maternal effect genes that are important in the formation of more posterior abdominal segments of the *Drosophila* embryo.

In embryos from *bicoid* mutant mothers, the head and thoracic structures are converted to the abdomen making the embryo with posterior structures on both ends, a lethal phenotype.

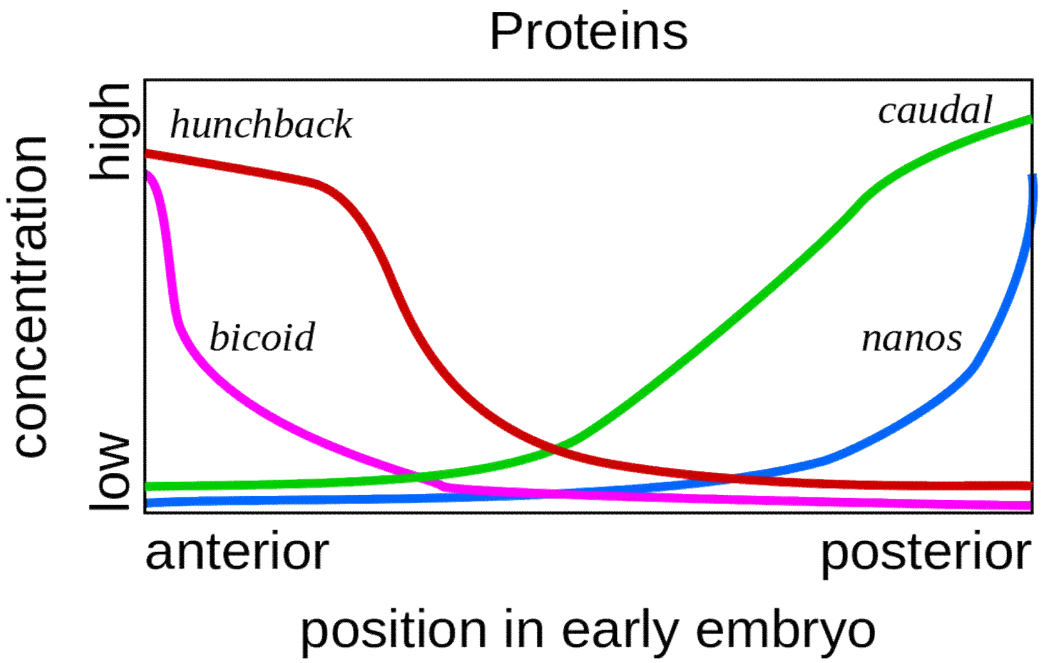
Cytoskeletal elements such as microtubules are polarized within the oocyte and can be used to allow the localization of mRNA molecules to specific parts of the cell. Maternally

synthesized *bicoid* mRNAs attach to microtubules and are concentrated at the anterior ends of forming *Drosophila* eggs. In unfertilized eggs, transcripts are still strictly localized at the tip, but immediately after fertilization, a small mRNA gradient is formed in the anterior 20% of the eggs. Another report documents a mRNA gradient up to 40%. *nanos* mRNA also attaches to a *Drosophila* egg's cytoskeleton but is concentrated at the posterior end of the egg. *hunchback* and *caudal* mRNAs lack special location control systems and are fairly evenly spread throughout the entire interior of the egg cells. It has been shown that the dsRNA-binding protein STAUFEN (STAU1) is responsible for guiding bicoid, nanos and other proteins, which play a role in forming the anterior-posterior axis, to the correct regions of the embryo to build gradients. When the mRNAs from the maternal effect genes are translated into proteins, a Bicoid protein concentration is formed at the anterior end of the egg. Nanos protein forms a gradient at the posterior end. The Bicoid protein blocks translation of *caudal* mRNA so Caudal protein is of lower concentration at the anterior part of the embryo and at higher concentration at the posterior part of the embryo. This is of opposite direction of the Bicoid protein. The caudal protein then activates later to turn genes on to form the posterior structures during the segmentation phase. Nanos protein creates a posterior-to-anterior slope and is a morphogen that helps in abdomen formation. Nanos protein, in complex with Pumilio protein, binds to the *hunchback* mRNA and blocks its translation in the posterior end of *Drosophila* embryos.

The Bicoid, Hunchback, and Caudal proteins are transcription factors. The Bicoid protein is a morphogen as well. The Nanos protein is a translational repressor protein. Bicoid has a DNA-binding homeodomain that binds both DNA and the *nanos* mRNA. Bicoid binds a specific RNA sequence in the 3' untranslated region, called the Bicoid 3'-UTR regulatory element, of *caudal* mRNA and blocks translation.

Hunchback protein levels in the early embryo are significantly augmented by new *hunchback* gene transcription and translation of the resulting zygotically produced mRNA. During early *Drosophila* embryogenesis, there are nuclear divisions without cell division. The many nuclei that are produced distribute themselves around the periphery of the cell cytoplasm. Gene expression in these nuclei is regulated by the Bicoid, Hunchback, and Caudal proteins. For example, Bicoid acts as a transcriptional activator of *hunchback* gene transcription. In order for development to continue, Hunchback is needed in an area that is declining in amount from

anterior to posterior. This is created by the Nanos protein whose existence is at a declining slope from posterior to anterior ends.



References:

1. ***Wolpert, 1978***
2. ***Gilbert, 2000***
3. ***Gilbert, 2010***
4. ***Russel &Peter, 2010***