S1.Discuss and distinguish the functional roles of the maternal effect genes, gap genes, pair-rule genes, and segment-polarity genes in *Drosophila.*

Answer: These genes are involved in pattern formation of the *Drosophila* embryo. The asymmetric distribution of maternal effect gene products in the oocyte establishes the antero-posterior and dorso-ventral axes. These gene products also control the expression of the gap genes, which are expressed as broad bands in certain regions of the embryo. The overlapping expression of maternal effect genes and gap genes controls the pair-rule genes, which are expressed in alternating stripes. A stripe corresponds to a parasegment. Within each parasegment, the expression of segment-polarity genes defines an anterior and posterior compartment. With regard to morphology, an anterior compartment of one parasegment and the posterior compartment of an adjacent parasegment will form a segment of the fly.

S2. With regard to genes affecting development, what are the phenotypic effects of gain-of-function mutations versus loss-of-function mutations?

Answer: Gain-of-function mutations cause a gene to be expressed in the wrong place, at the wrong time, or in an abnormal way. When they are expressed in the wrong place, that region may develop into an inappropriate structure. For example, when *Antp* is abnormally expressed in an anterior segment, this segment develops legs in place of antennae. When gain-of-function mutations cause a gene to be expressed at the wrong time, this can also disrupt the development process. Gain-of-function heterochronic alleles cause cell lineages to be reiterated and thereby alter the course of development. By comparison, loss-of-function mutations result in a defect in the expression of a gene. This usually will disrupt the developmental process, because the cells in the region where the gene is supposed to be expressed will not be directed to develop along the correct pathway.

S3. Mutations in genes that control the early stages of development are often lethal (e.g., see fig. 23.6*b*). To circumvent this problem, developmental geneticists may try to isolate *temperature-sensitive developmental mutants* or *ts alleles.* If an embryo carries a *ts* allele, it will develop correctly at the permissive temperature (e.g., 25°C) but will fail to develop if incubated at the nonpermissive temperature (e.g., 30°C). In most cases, *ts* alleles are missense mutations that slightly alter the amino acid sequence of a protein, causing a change in its structure that prevents it from working properly at the nonpermissive temperature. *Ts* alleles are particularly useful because they can provide insight regarding the stage of development when the protein is necessary. Researchers can take groups of embryos that carry a *ts* allele and expose them to the permissive and nonpermissive temperature at different stages of development. In the experiment described next, embryos were divided into five groups and exposed to the permissive or nonpermissive temperature at different times after fertilization.

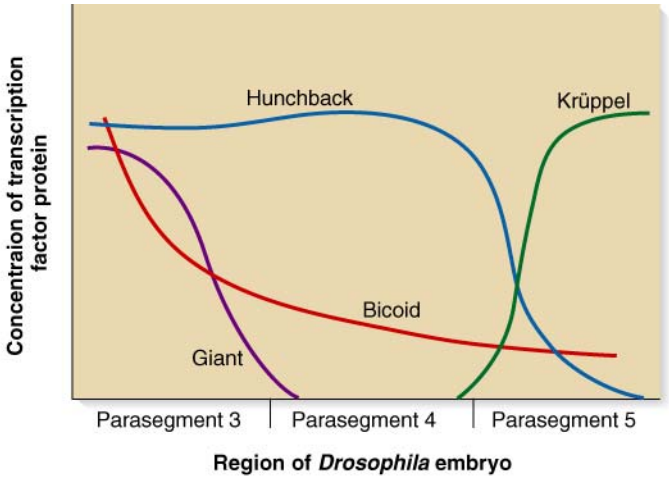
Time After Fertilization (hours)	**Group:**	**1**	**2**	**3**	**4**	**5**
0–1		25°C	25°C	25°C	25°C	25°C
1–2		25°C	30°C	25°C	25°C	25°C
2–3		25°C	25°C	30°C	25°C	25°C
3–4		25°C	25°C	25°C	30°C	25°C
4–5		25°C	25°C	25°C	25°C	30°C
5–6		25°C	25°C	25°C	25°C	25°C
SURVIVAL:		Yes	Yes	Yes	No	Yes

Explain these results.

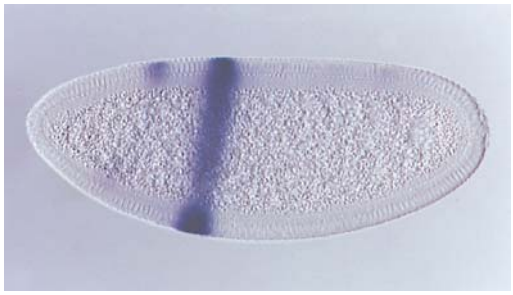
Answer: By varying the temperature during different stages of development, researchers can pinpoint the stage when the function of the protein encoded by this *ts* allele is critical. As shown, embryos fail to survive if they are subjected to the nonpermissive temperature between 3 and 4 hours after fertilization, but they do survive if subjected to the nonpermissive temperature at other times of development. These results indicate that this protein plays a crucial role at the 3–4-hour stage of development.

S4. An intriguing question in developmental genetics is, How can a particular gene, such as *even-skipped,* be expressed in a multiple banding pattern as seen in figure 23.9? Another way of asking this question is, How is the positional information within the broad bands of the gap genes able to be deciphered in a way that causes the pair-rule genes to be expressed in this alternating banding pattern? The answer lies in a complex mechanism of genetic regulation. Certain pair-rule genes have several **stripe-specific enhancers** that are controlled by multiple transcription factors. A stripe-specific enhancer is typically a short segment of DNA, 300 to 500 bp in length, that contains binding sequences that are recognized by several different transcription factors. This term is a bit misleading since a stripe-specific enhancer is a regulatory region that contains both enhancer and silencer elements.

As an example, Michael Levine and his colleagues have investigated stripe-specific enhancers that are located near the promoter of the *even-skipped* gene. A segment of DNA, termed the stripe 2 enhancer, controls the expression of the *even-skipped* gene; this enhancer is responsible for the expression of the *even-skipped* gene in stripe 2, which corresponds to parasegment 3 of the embryo. The stripe 2 enhancer is a segment of DNA that contains binding sites for four transcription factors that are the products of the *Krüppel, bicoid, hunchback,* and *giant* genes. The Hunchback and Bicoid transcription factors bind to this enhancer and activate the transcription of the *even-skipped* gene. In contrast, the transcription factors encoded by the *Krüppel* and *giant* genes bind to the stripe 2 enhancer and repress transcription. The figure shown next describes the concentrations of these four transcription factor proteins in the region of parasegment 3 (i.e., stripe 2) in the *Drosophila* embryo.



To study stripe-specific enhancers, researchers have constructed artificial genes in which the enhancer is linked to a reporter gene; the expression of the reporter gene is easy to detect. The next figure shows the results of an experiment in which an artificial gene was made by putting the stripe 2 enhancer next to the ß-galactosidase gene. This artificial gene was introduced into *Drosophila* and then embryos containing this gene were analyzed for ß-galactosidase activity. If a region of the embryo is expressing ß-galactosidase, the region will stain darkly because ß-galactosidase converts a colorless compound into a dark blue compound.



Explain these results.

Answer: As shown in the first figure to this problem, the concentrations of the Hunchback and Bicoid transcription factors are relatively high in the region of the embryo corresponding to stripe 2 (which is parasegment 3). The levels of Krüppel and Giant are very low in this region. Therefore, the high levels of activators and low levels of repressors cause the *even-skipped* gene to be transcribed. In this experiment, ß-galactosidase was made only in stripe 2 (i.e., parasegment 3). These results show that the stripe-2-specific enhancer controls gene expression only in parasegment 3. Because we know that the *even-skipped* gene is expressed as several alternating stripes (as seen in fig. 23.9), the *even-skipped* gene must contain other stripe-specific enhancers that allow it to be expressed in these other alternating parasegments.