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Axis formation in Mouse
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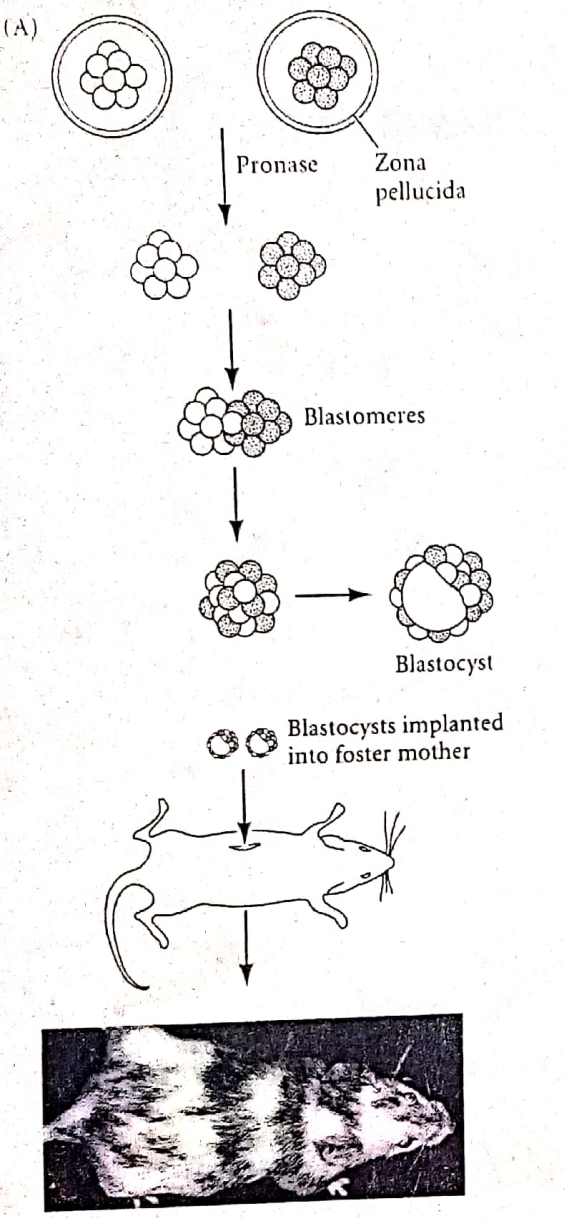


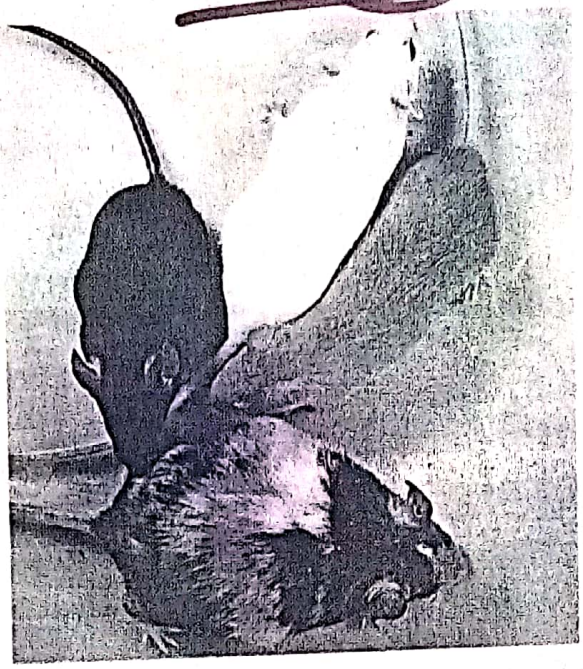


Figure 11.33
Production of chimeric mice. (A) The experimental procedures used to produce chimeric mice. Early 8-cell embryos of genetically distinct mice (here, with coat color differences) are isolated from mouse oviducts and brought together after their zonae are removed by proteolytic enzymes. The cells form a composite blastocyst, which is implanted into the uterus of a foster mother. The photograph shows one of the actual chimeric mice produced in this manner. (B) An adult female chimeric mouse (bottom) produced from the fusion of three 4-cell embryos: one from two white-furred parents, one from two black-furred parents, and one from two brown-furred parents. The resulting mouse has coat colors from all three embryos. Moreover, each embryo contributed germ line cells, as is evidenced by the three colors of offspring (above) produced when this chimeric female was mated with recessive (white-furred) males. (A, photograph courtesy of B. Mintz; B from Markert and Petters 1978, photograph courtesy of C. Markert.)

Vertebrate Axis Formation

Mammalian Anterior-Posterior Axis Formation

Two signaling centers

The mammalian embryo appears to have two signaling centers: one in the node ("the organizer") and one in the **anterior visceral endoderm** (Figure 11.34A; Beddington et al. 1994). The node appears to be responsible for the creation of all of the body, and these two signaling centers work together to form the forebrain (Bachiller et al. 2000). Both the mouse node and the anterior visceral endoderm express many of the genes known to be expressed in the chick and frog organizer tissue. The node produces Chordin and Noggin (which the anterior visceral endoderm does not), while the anterior visceral endoderm expresses several genes that are necessary for head formation. These include the genes for transcription factors Hesx-1, Lim-1, and Otx-2, as well as the gene for the paracrine factor Cerberus. The anterior visceral endoderm is established before the node, and the primitive streak always forms on the side of the epiblast *opposite* this anterior site. Homozygosity for mutant alleles of any of the above-mentioned head-specific organizing genes produces mice lacking forebrains (Figure 11.35; Thomas and Beddington 1996; Beddington and Robertson 1999). While knockouts of either *chordin* or *noggin* do not affect development, mice missing both sets of genes develop a body lacking forebrain, nose, and facial structures.

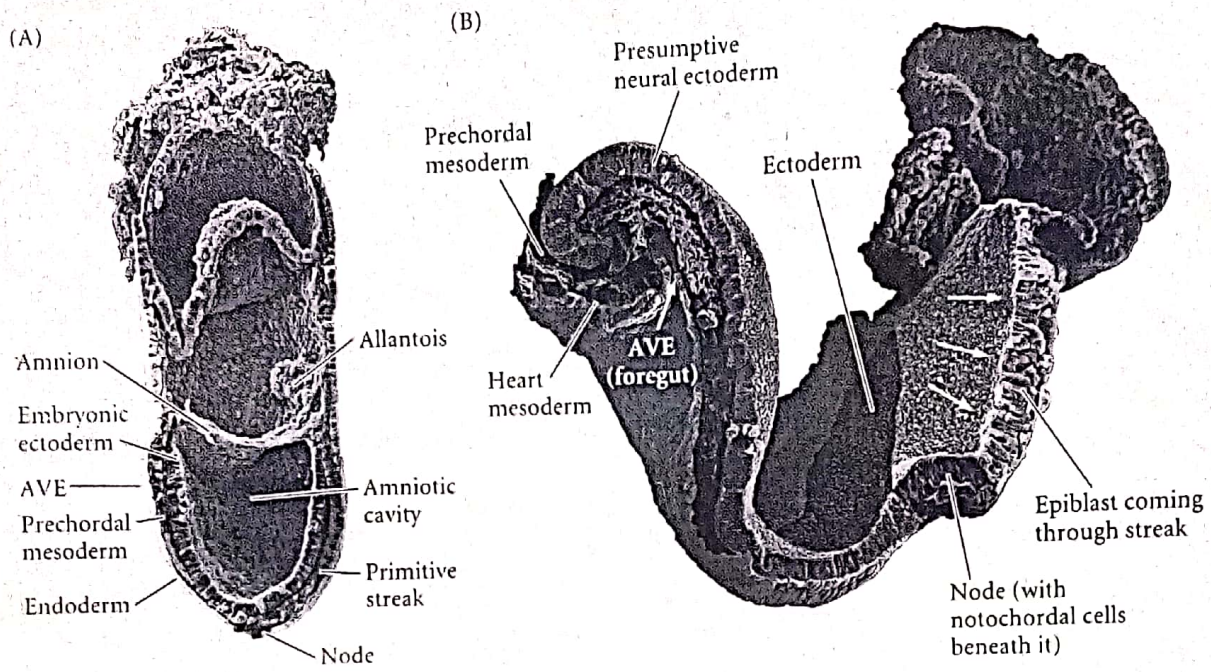


Figure 11.34
The two signaling centers of the mammalian embryo. (A) In the day 7 mouse embryo, the dorsal surface of the epiblast (embryonic ectoderm) is in contact with the amnionic cavity. The ventral surface of the epiblast contacts the newly formed mesoderm. In this cuplike arrangement, the endoderm covers the surface of the embryo. The node is at the bottom of the cup, and it has generated chordamesoderm. The two signaling centers, the node and the anterior visceral endoderm, are located on opposite sides of the cup. Eventually, the notochord will link them. The caudal side of the embryo is marked by the presence of the allantois. (B) By embryonic day 8, the anterior visceral endoderm lines the foregut, and the prechordal mesoderm is now in contact with the forebrain ectoderm. The node is now farther caudal, due largely to the rapid growth of the anterior portion of the embryo. The cells in the midline of the epiblast migrate through the primitive streak (white arrows). (Photographs courtesy of K. Sulik.)

WEBSITE **11.10 Gastrulation in the mouse.** The mouse gastrula is shaped like a cup and has a more complicated structure than the human gastrula. The extraembryonic tissues of the mouse appear to be critical in establishing the position of the node and anterior visceral endoderm signaling centers.

Patterning the anterior-posterior axis: The Hox code hypothesis

Once gastrulation begins, anterior-posterior polarity in all vertebrates becomes specified by the expression of **Hox genes**. These genes are homologous to the homeotic gene complex (Hom-C) of the fruit fly (see Chapter 9). The *Drosophila* homeotic gene complex on chromosome 3 contains the Antennapedia and bithorax clusters of homeotic genes (see Figure 9.28), and can be seen as a single functional unit. (Indeed, in other insects, such as the flour beetle *Tribolium*, it is a single unit.) The Hom-C genes are arranged in the same general order as their expression pattern along the anterior-posterior axis, the most 3′ gene (*labial*) being required for producing the most anterior structures, and the most 5′ gene (*AbdB*) specifying the development of the posterior abdomen. Mouse and human genomes contain four copies of the Hox complex per haploid set, located on four different chromosomes (*Hoxa* through *Hoxd* in the mouse, *HOXA* through *HOXD* in humans: Boncinelli et al. 1988; McGinnis and Krumlauf 1992; Scott 1992). Not only are the same general types of homeotic genes found in both flies and mammals, but the order of these genes on their respective chromosomes is

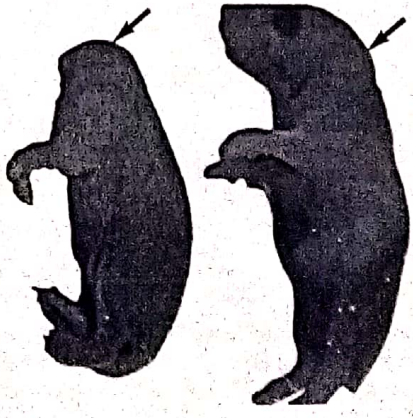


Figure 11.35
Headless phenotype of the *Lim-1* knockout mouse. A *Lim-1*-deficient mouse is shown next to a wild-type littermate. The ear pinnae (arrows) are the most anterior structures seen in these mutants. (From Shawlot and Behringer 1995, photograph courtesy of the authors.)

Hox gene

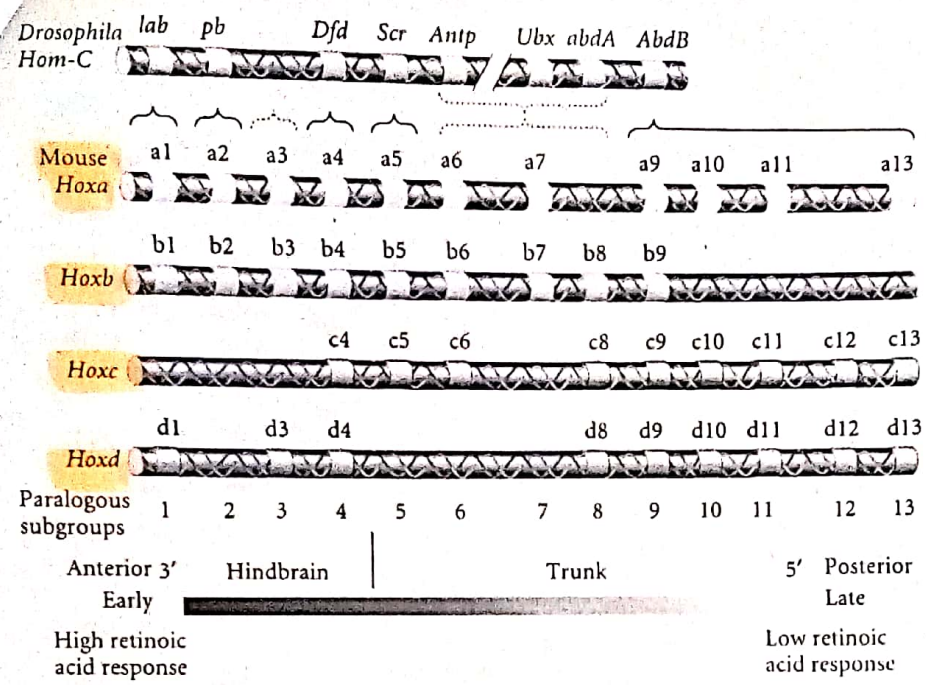
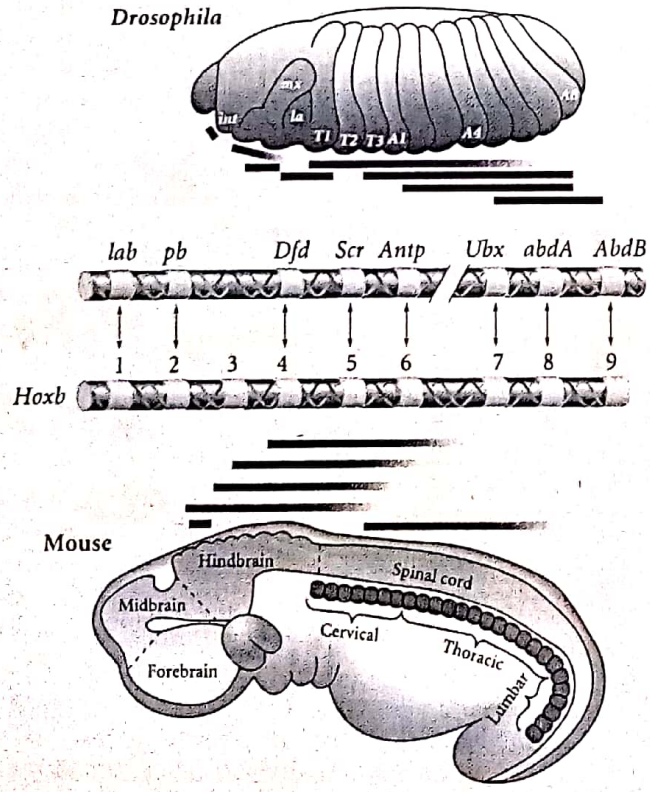


Figure 11.36
Evolutionary conservation of homeotic gene organization and transcriptional expression in fruit flies and mice. (A) Similarity between the *Hom-C* cluster on *Drosophila* chromosome 3 and the four Hox gene clusters in the mouse genome. The shaded regions show particularly strong structural similarities between species, and one can see that the order of the genes on the chromosomes has been conserved. Those genes at the 5′ end (since all mouse Hox genes are transcribed in the same direction) are those that are expressed more posteriorly, are expressed later, and can be induced only by high doses of retinoic acid. Genes having similar structures, the same relative positions on each of the four chromosomes, and similar expression patterns belong to the same paralogous group. (B) Comparison of the transcription patterns of the *Hom-C* and *Hoxb* genes of *Drosophila* (at 10 hours) and mice (at 12 days), respectively. The homologous human genes are called (capitalized) HOX genes. (A after Krumlauf 1993; B after McGinnis and Krumlauf 1992.)

(B)



remarkably similar. In addition, the expression of these genes follows the same pattern: those mammalian genes homologous to the *Drosophila labial*, *proboscipedia*, and *deformed* genes are expressed anteriorly, while those genes that are homologous to the *Drosophila Abdominal-B* gene are expressed posteriorly. Another set of genes that controls the formation of the fly head (*orthodenticle* and *empty spiracles*) has homologues in the mouse that show expression in the midbrain and forebrain.

While Hox genes appear to specify the anterior-posterior axis throughout the vertebrates, we shall discuss mammals here, since the experimental evidence is particularly strong for this class. The mammalian Hox/HOX genes are numbered from 1 to 13, starting from that end of each complex that is expressed most anteriorly. Figure 11.36 shows the relationships between the *Drosophila* and mouse homeotic gene sets. The equivalent genes in each mouse complex (such as *Hoxa-1*, *Hoxb-1*, and *Hoxd-1*) are called a **paralogous group**. It is thought that the four mammalian Hox complexes were formed from chromosome duplications. Because there is not a one-to-one correspondence between the *Drosophila* Hom-C genes and the mouse Hox genes, it is likely that independent gene duplications have occurred since these two animal branches diverged (Hunt and Krumlauf 1992; see Chapter 22).

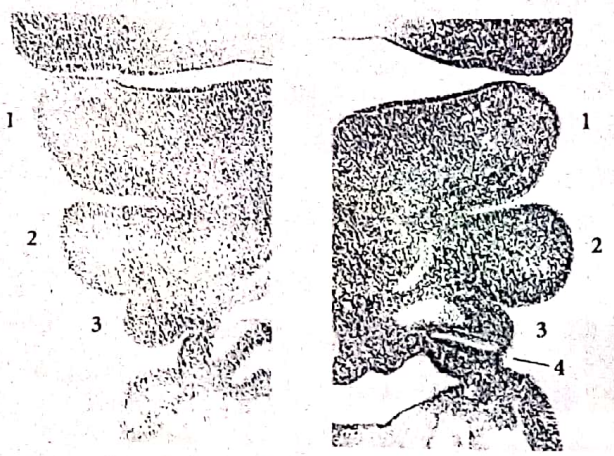


Figure 11.37
Deficient development of neural crest-derived pharyngeal arch and pouch structures in *Hoxa-3*-deficient mice. The arches are numbered. (Right) A 10.5-day embryo of a heterozygous *Hoxa-3* mouse (wild-type), showing normal development of pouch 3 (thymus), pouch 4 (parathyroid), and other structures. (Left) A homozygous mutant *Hoxa-3*-deficient mouse lacks the proper development of these structures. (From Chisaka and Capecchi 1991.)

Expression of Hox genes along the dorsal axis

Hox gene expression can be seen along the dorsal axis (in the neural tube, neural crest, paraxial mesoderm, and surface ectoderm) from the anterior boundary of the hindbrain through the tail. The different regions of the body from the midbrain through the tail are characterized by different constellations of Hox gene expression, and the pattern of Hox gene expression is thought to specify the different regions. In general, the genes of paralogous group 1 are expressed from the tip of the tail to the most anterior border of the hindbrain. Paralogue 2 genes are expressed throughout the spinal cord, but the anterior limit of expression stops two segments more caudally than that of the paralogue 1 genes (see Figure 11.36; Wilkinson et al. 1989; Keynes and Lumsden 1990). The higher-numbered Hox paralogues are expressed solely in the posterior regions of the neural tube, where they also form a "nested" set.

Experimental analysis of the Hox code

The expression patterns of the murine Hox genes suggest a code whereby certain combinations of Hox genes specify a particular region of the anterior-posterior axis (Hunt and Krumlauf 1991). Particular sets of paralogous genes provide segmental identity along the anterior-posterior axis of the body. Evidence for such a code comes from three sources:

- Gene targeting or "knockout" experiments (see Chapter 4), in which mice are constructed that lack both copies of one or more Hox genes
- Retinoic acid teratogenesis, in which mouse embryos exposed to retinoic acid show a different pattern of Hox gene expression along the anterior-posterior axis and abnormal differentiation of their axial structures
- Comparative anatomy, in which the types of vertebrae in different vertebrate species are correlated with the constellation of Hox gene expression

GENE TARGETING. When Chisaka and Capecchi (1991) knocked out the *Hoxa-3* gene from inbred mice, these homozygous mutants died soon after birth. Autopsies of these mice revealed that their neck cartilage was abnormally short and thick and that they had severely deficient or absent thymuses, thyroids, and parathyroid glands (Figure 11.37). The heart and major blood vessels were also malformed. Further analysis showed that the *number* and *migration* of the neural crest cells that normally form these structures were not affected. Rather, it appears that the *Hoxa-3* genes are responsible for specifying cranial neural crest cell fate and for enabling these cells to differentiate and proliferate into neck cartilage and the glands that form the fourth and sixth pharyngeal pouches (Manley and Capecchi 1995).

Knockout of the *Hoxa-2* gene also produces mice whose neural crest cells have been respecified, but the defects in these mice are anterior to those in the *Hoxa-3* knockouts. Cranial elements normally formed by the neural crest cells of the second pharyngeal arch (stapes, styloid bones) are missing and are replaced by duplicates of the structures of the first pharyngeal arch (incus, malleus, etc.) (Gendron-Maguire et al. 1993; Rijli et al. 1993). Thus, without certain Hox genes, some regionally specific organs along the anterior-posterior axis fail to form, or become respecified as other regions. Similarly, when the *Hoxc-8* gene is knocked out (Le Mouellic et al. 1992), several axial skeletal segments resemble more anterior segments, much like what is seen in *Drosophila* loss-of-function homeotic mutations. As can be seen in Figure 11.38, the first lumbar vertebra of the mouse has formed a rib—something characteristic of the thoracic vertebrae anterior to it.

One can get severe axial transformations by knocking out two or more genes of a paralogous group. Mice homozygous for the *Hoxd-3* deletion have mild abnormalities of the first cervical vertebra (the atlas), while mice homozygous for the *Hoxa-3* deletion have no abnormality of this bone, though they have other malformations (see the discussion of this mutant above). When both sets of mutations a bred into the same mouse, both sets of problems become more severe. Mice with neither *Hoxa-3* nor *Hoxd-3* have no atlas bone at all, and the hyoid and thyroid cartilage is so reduced in size that there are holes in the skeleton (Condie and Capecchi 1994; Greer et al. 2000). It appears that there are interactions occurring between the products of the Hox genes, and that in some functions, one of the paralogues can replace the other.

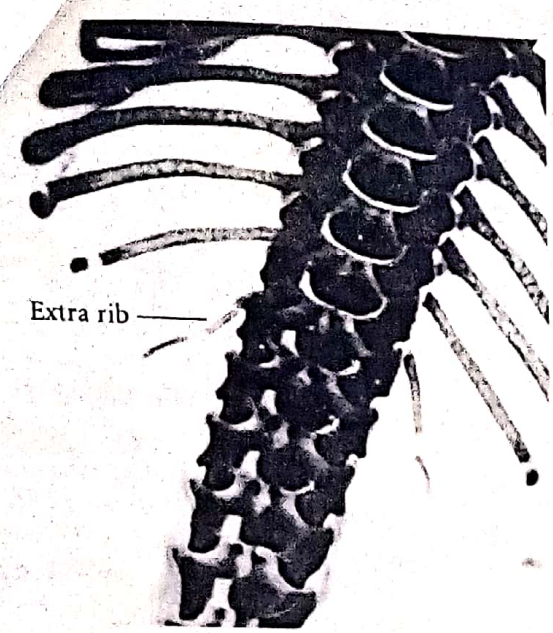


Figure 11.38
Partial transformation of the first lumbar vertebra into a thoracic vertebra by the knockout of the *Hoxc-8* gene. The first lumbar vertebra of this mouse has formed a rib—a structure normally formed only by the thoracic vertebrae anterior to it. (Photograph from Le Mouellic et al. 1992; courtesy of the authors.)

Thus, the evidence from gene knockouts supports the hypotheses that (1) different sets of Hox genes are necessary for the specification of any region of the anterior-posterior axis, (2) that the members of a paralogous group of Hox genes may be responsible for different subsets of organs within these regions, and (3) that the defects caused by knocking out particular Hox genes occur in the most anterior region of that gene's expression.

RETINOIC ACID TERATOGENESIS. Homeotic changes are also seen when mouse embryos are exposed to teratogenic doses of retinoic acid (RA). Exogenous retinoic acid given to mouse embryos in utero can cause certain Hox genes to become expressed in groups of cells that usually do not express them (Conlon and Rossant 1992; Kessel 1992). Moreover, the craniofacial abnormalities of mouse embryos exposed to teratogenic doses of RA (Figure 11.39) can be mimicked by causing the expression of *Hoxa-7* throughout the embryo (Balling et al. 1989). If high doses of RA can activate Hox genes in inappropriate locations along the anterior-posterior axis, and if the constellation of active Hox genes specifies the region of the anterior-posterior axis, then mice given RA in utero should show homeotic transformations manifested as rostralizing malformations occurring along that axis.

Kessel and Gruss (1991) found this to be the case. Wild-type mice have 7 cervical (neck) vertebrae, 13 thoracic (ribbed) vertebrae, and 6 lumbar (abdominal) vertebrae, in addition to the sacral and caudal (tail) vertebrae. In embryos exposed to RA on day 8 of gestation (during gastrulation), the first one or two lumbar vertebrae were transformed into thoracic (ribbed) vertebrae, while the first sacral vertebra often became a lumbar vertebra. In some cases, the entire posterior region of the mouse embryo failed to form (Figure 11.39E). These changes in structure were correlated with changes in the

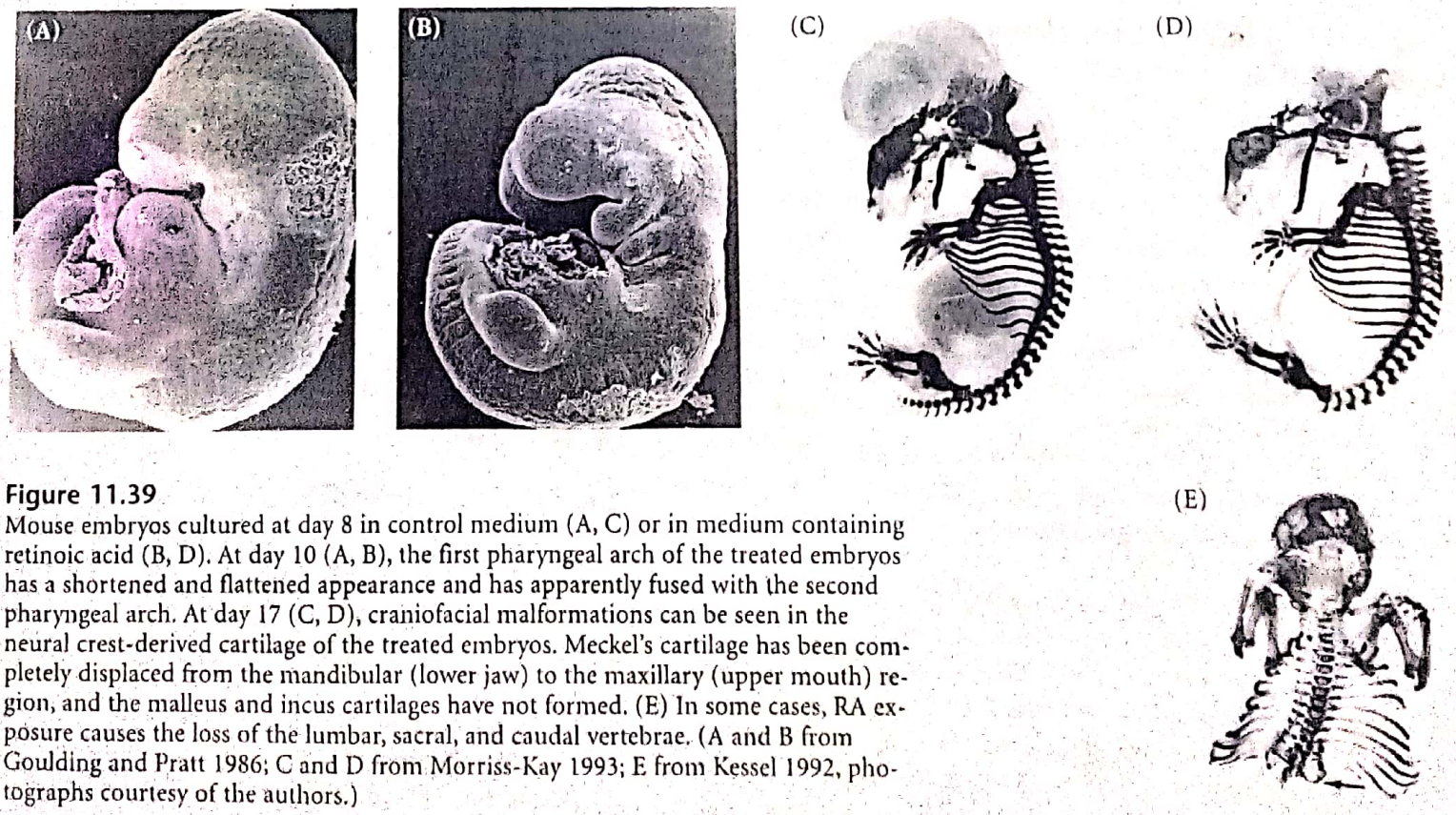


Figure 11.39
Mouse embryos cultured at day 8 in control medium (A, C) or in medium containing retinoic acid (B, D). At day 10 (A, B), the first pharyngeal arch of the treated embryos has a shortened and flattened appearance and has apparently fused with the second pharyngeal arch. At day 17 (C, D), craniofacial malformations can be seen in the neural crest-derived cartilage of the treated embryos. Meckel's cartilage has been completely displaced from the mandibular (lower jaw) to the maxillary (upper mouth) region, and the malleus and incus cartilages have not formed. (E) In some cases, RA exposure causes the loss of the lumbar, sacral, and caudal vertebrae. (A and B from Goulding and Pratt 1986; C and D from Morriss-Kay 1993; E from Kessel 1992, photographs courtesy of the authors.)

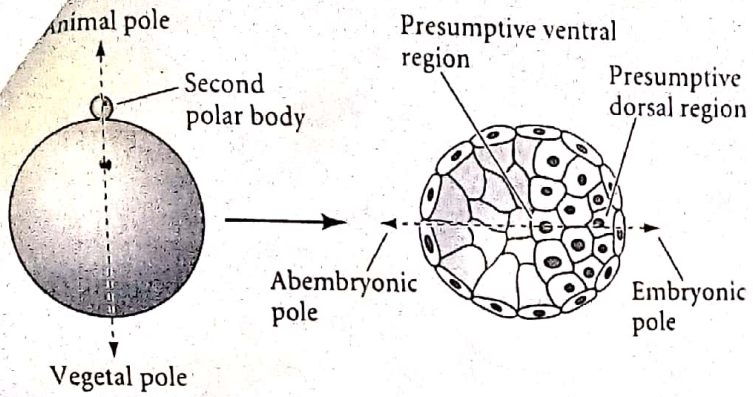


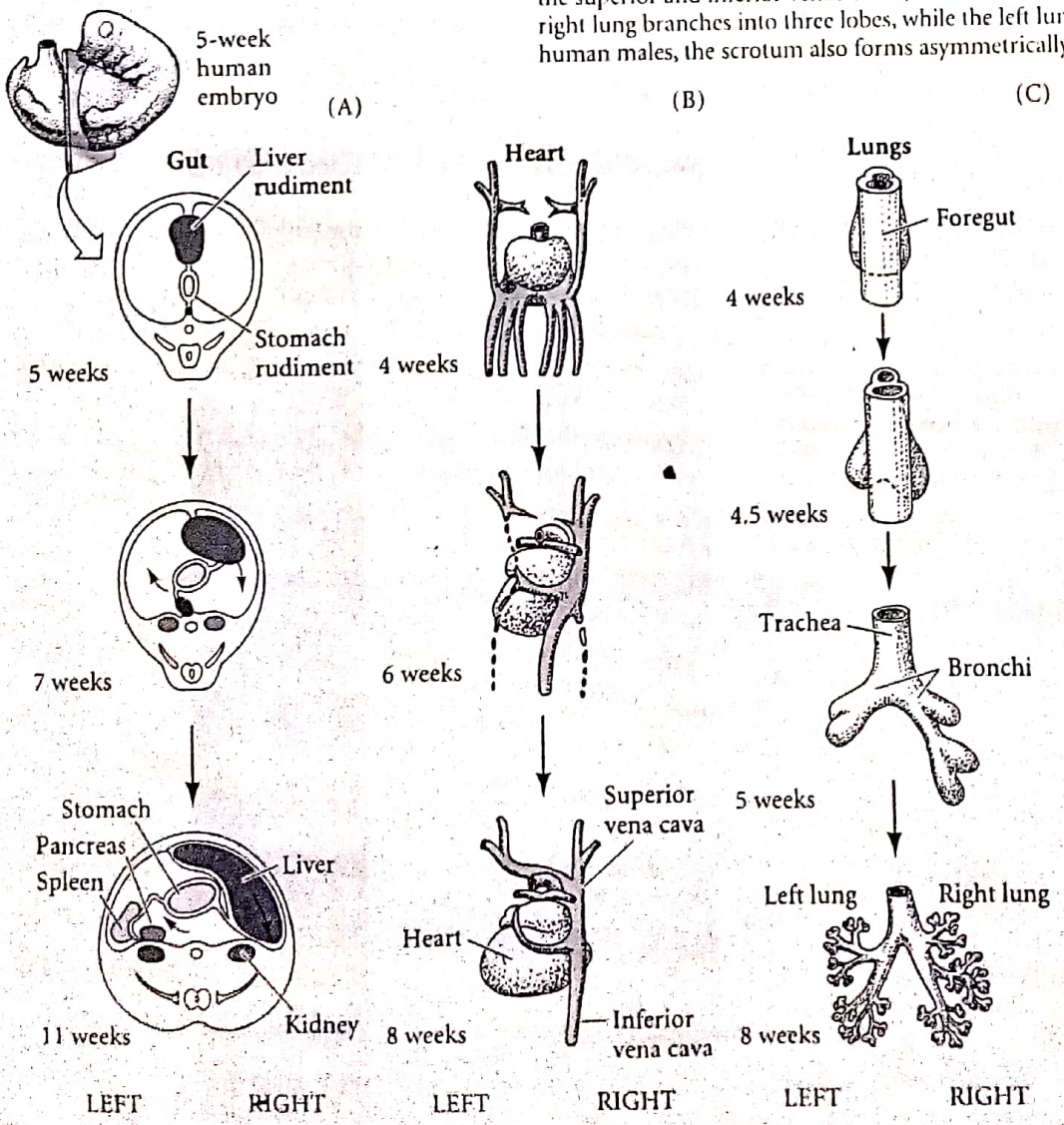
Figure 11.41
Relationship between the animal-vegetal axis of the egg and the embryonic-abembryonic axis of the blastocyst. The polar body marks the animal pole of the embryo. The dorsal-ventral axis of the embryo appears to form at right angles to the animal-vegetal axis of the egg.

The left-right axis

The mammalian body is not symmetrical. Although the heart begins its formation at the midline of the embryo, it moves to the left side of the chest cavity and loops to the right (Figure 11.42). The spleen is found solely on the left side of the abdomen, the major lobe of the liver forms on the right side of the abdomen, the large intestine loops right to left as it traverses the abdominal cavity, and the right lung has one more lobe than the left lung.

Mutations in mice have shown that there are two levels of regulating the left-right axis: a global level and an organ-specific level. Some genes, such as *situs inversus viscerum* (*iv*), randomizes the left-right axis for each asymmetrical organ (Hummel and Chapman 1959; Layton 1976). This means that the heart may loop to the left in one homozygous animal, but loop to the right in another (Figure 11.43). Moreover, the di-

Figure 11.42
Left-right asymmetry in the developing human. (A) Abdominal cross sections show that the originally symmetrical organ rudiments acquire asymmetric positions by week 11. The liver moves to the right and the spleen moves to the left. (B) Not only does the heart move to the left side of the body, but the originally symmetrical veins of the heart regress differentially to form the superior and inferior venae cavae, which connect only to the right side of the heart. (C) The right lung branches into three lobes, while the left lung (near the heart) forms only two lobes. In human males, the scrotum also forms asymmetrically. (After Kosaki and Casey 1998.)



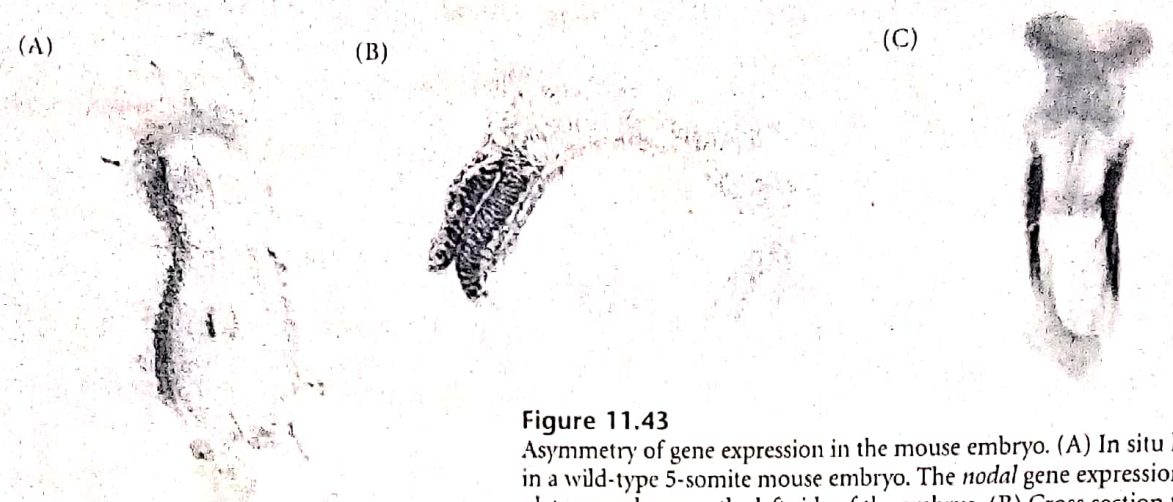


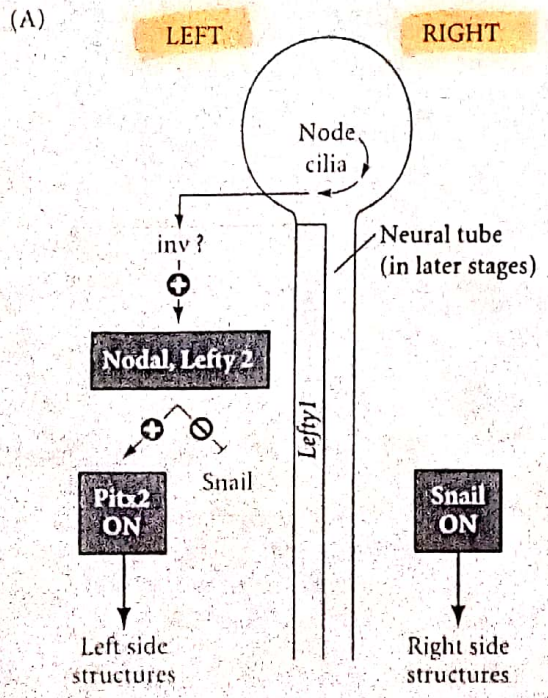
Figure 11.43
Asymmetry of gene expression in the mouse embryo. (A) In situ hybridization for *nodal* mRNA in a wild-type 5-somite mouse embryo. The *nodal* gene expression is confined to the lateral plate mesoderm on the left side of the embryo. (B) Cross section through the embryo at the same stage as (A). (C) In mice with the *situs inversus viscerum* (*iv*) mutation, *nodal* expression is seen in the lateral plate mesoderm on both sides of the embryo. The heart has an equal chance of looping to either side. (After Lowe et al. 1996; photographs courtesy of M. R. Kuehn.)

rection of heart looping is not coordinated with the placement of the spleen or the stomach. This can cause serious problems, even death. The second gene, *inversion of embryonic turning* (*inv*), causes a more global phenotype. Mice homozygous for an insertion mutation at this locus were found to have all their asymmetrical organs on the wrong side of the body (Yokoyama et al. 1993).* Since all the organs are reversed, this asymmetry does not have dire consequences for the mice.

*This gene was discovered accidentally when Yokoyama and colleagues (1993) made transgenic mice wherein the transgene (for the tyrosinase enzyme) was inserted randomly into the genome. In one instance, this gene inserted itself into a region of chromosome 4, knocking out the existing *inv* gene. The resulting homozygous mice had laterality defects.

Recently, several additional asymmetrically expressed genes have been discovered, and their influence on one another has enabled scientists to put them into a possible pathway. The end of this pathway—the activation of Nodal proteins and the Pitx2 transcription factor on the left side of the lateral plate mesoderm—appears to be the same as in frog and chick embryos, although the path leading to this point differs between the species (Figure 11.44; see Figure 11.17; Collignon et al. 1996; Lowe et al. 1996; Meno et al. 1996).

In frogs, the pathway begins with the placement of Vg1; in chicks it begins with the suppression of *sonic hedgehog* expression. In mammals, the distinction between left and right sides begins in the ciliary cells of the node (Figure 11.44B). The cilia cause the flow of fluid in the yolk sac cavity from right to left.



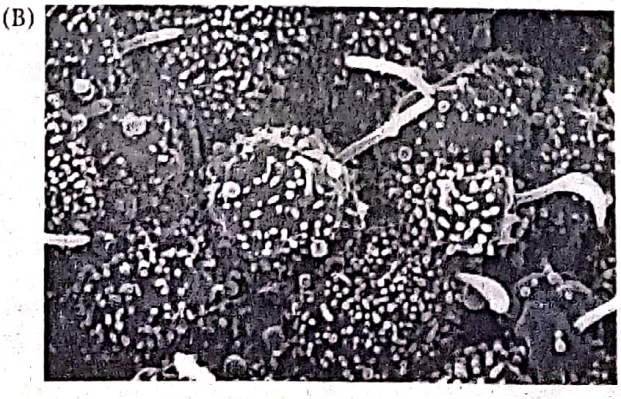


Figure 11.44
Situs formation in mammals. (A) Proposed pathway for left-right axis formation in the mouse. The leftward movement of cilia in the node activates some as yet unidentified factor (possibly the product of the *inv* gene). This product activates the *nodal* and *lefty2* genes. The diffusion of Nodal and Lefty2 proteins to the right-hand side is restricted by the product of the *Lefty1* gene which coats the bottom of the neural tube on the left side. Nodal activates *Pitx2*, the gene whose product activates left-sided properties in the various organs containing it. Either Nodal or Lefty2 (perhaps both) repress the *Snail* gene whose product is needed to instruct right-sidedness. (B) Ciliated cells of the mammalian node. This photograph is a close-up of the node seen in Figure 11.29A. (Photograph courtesy of K. Sulik and G. C. Schoenwolf.)