ter macromere, creating the characteristic spiral pattern. Looking down on the embryo from the animal pole, the upper ends of the mitotic spindles appear to alternate clockwise and counterclockwise (see Figure 8.24). This arrangement causes alternate micromeres to form obliquely to the left and to the right of their macromeres.

At the third cleavage, the A macromere gives rise to two daughter cells, macromere 1A and micromere 1a. The B, C, and D cells behave similarly, producing the first quartet of micromeres. In most species, these micromeres are to the *right* of their macromeres (looking down on the animal pole). At the fourth cleavage, macromere 1A divides to form macromere 2A and micromere 2a, and micromere 1a divides to form two more micromeres, $1a^1$ and $1a^2$ (see Figure 8.23). The micromeres of this second quartet are to the *left* of the macromeres. Further cleavage yields blastomeres 3A and 3a from macromere 2A, and micromere $1a^2$ divides to produce cells $1a^{21}$ and $1a^{22}$. In normal development, the first-quartet micromeres form the head structures, while the second-quartet micromeres form the statocyst (balance organ) and shell. These fates are specified both by cytoplasmic localization and by induction (Clement 1967; Cather 1967; Render 1991; Sweet 1998).

The orientation of the cleavage plane to the left or to the right is controlled by cytoplasmic factors within the oocyte. This was discovered by analyzing mutations of snail coiling. Some snails have their coils opening to the right of their shells (**dextral coiling**), whereas the coils of other snails open to the left (**sinistral coiling**). Usually the direction of coiling is the same for all members of a given species, but occasional mutants are found (i.e., in a population of right-coiling snails, a few individuals will be found with coils that open on the left). Crampton (1894) analyzed the embryos of such aberrant snails and found that their early cleavage differed from the norm. The orientation of the cells after the second cleavage was different in the sinistrally coiling snails as a result of a different orientation of the mitotic apparatus (Figure 8.25). In some species (such as the pond snail *Physa*, an entirely sinistral species), the sinistrally coiling cleavage patterns are mirror-images of the dextrally coiling pattern of the right-handed species. In other instances (such as *Lymnaea*, where about 2 percent of the snails are lefties), sinistrality is the result of a two-step process: at each division, the initial cleavage is radial; however, as the cleavage furrow forms, the blastomeres shift to the left-hand spiral position (Shibazaki et al. 2004). In Figure 8.25, one can see that the position of the 4d blastomere (which is extremely important, as its progeny will form the mesodermal organs) is different in the two types of spiraling embryos.

FIGURE 8.25 Looking down on the animal pole of (A) left-coiling and (B) right-coiling snails. The origin of sinistral and dextral coiling can be traced to the orientation of the mitotic spindle at the second cleavage. Left- and right-coiling snails develop as mirror images of each other. (After Morgan 1927.)

In snails such as *Lymnaea*, the direction of snail shell coiling is controlled by a single pair of genes (Sturtevant 1923; Boycott et al. 1930). In *Lymnaea peregra*, rare mutants exhibiting sinistral coiling were found and mated with wild-type, dextrally coiling snails. These matings showed that the right-coiling allele, *D*, is dominant to the left-coiling allele, *d*. However, the direction of cleavage is determined not by the genotype of the developing snail, but by the genotype of the snail's mother. A *dd* female snail can produce only sinistrally coiling offspring, even if the offspring's genotype is *Dd*. A *Dd* individual will coil either left or right, depending on the genotype of its mother. Such matings produce a chart like this:
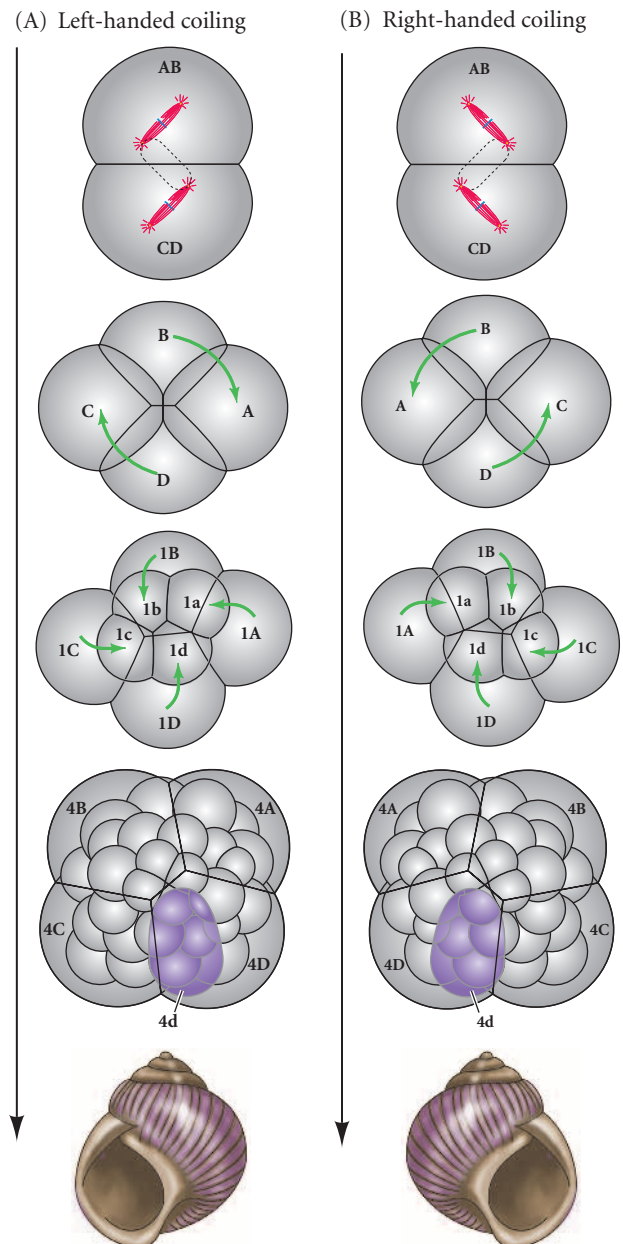

(A) Left-handed coiling   (B) Right-handed coiling

	Genotype	Phenotype
$DD\,♀ \times dd\,♂ \rightarrow$	Dd	All right-coiling
$DD\,♂ \times dd\,♀ \rightarrow$	Dd	All left-coiling
$Dd \times Dd \rightarrow$	$1DD{:}2Dd{:}1dd$	All right-coiling

The genetic factors involved in snail coiling are brought to the embryo by the oocyte cytoplasm. It is the genotype of the ovary in which the oocyte develops that determines which orientation cleavage will take. When Freeman and Lundelius (1982) injected a small amount of cytoplasm from dextrally coiling snails into the eggs of *dd* mothers, the resulting embryos coiled to the right. Cytoplasm from sinistrally coiling snails did not affect right-coiling embryos. These findings confirmed that the wild-type mothers were placing a factor into their eggs that was absent or defective in the *dd* mothers.

A fate map of *Ilyanassa obsoleta*

WEBSITE **8.3** **Alfred Sturtevant and the genetics of snail coiling.** By a masterful thought experiment, Sturtevant demonstrated the power of applying genetics to embryology. To do this, he brought Mendelian genetics into the study of snail coiling.

Joanne Render (1997) constructed a detailed fate map of the snail *Ilyanassa obsoleta* by injecting specific micromeres with large polymers conjugated to the fluorescent dye Lucifer Yellow. The fluorescence is maintained over the period of embryogenesis and can be seen in the larval tissue derived from the injected cells. The results of Render's map, given in Figure 8.26, showed that the second-quartet micromeres (2a–d) generally contribute to the shell-forming mantle, the velum, the mouth, and the heart. The third-quartet micromeres (3a–d) generate large regions of the foot, velum, esophagus, and heart. The 4d cell—the mesentoblast—contributes to the larval kidney, heart, retractor muscles, and intestine.

The polar lobe: Cell determination and axis formation

Molluscs provide some of the most impressive examples of both mosaic development—in which the blastomeres are specified autonomously—and of cytoplasmic localization, wherein morphogenetic determinants are placed in a specific region of the oocyte (see Chapter 3). Mosaic devel-

opment is widespread throughout the animal kingdom, especially among protostomes such as annelids, nematodes, and molluscs, all of which initiate gastrulation at the future anterior end after only a few cell divisions.

In molluscs, the mRNAs for some transcription factors and paracrine factors are placed in particular cells by associating with certain centrosomes (Figure 8.29; Lambert and
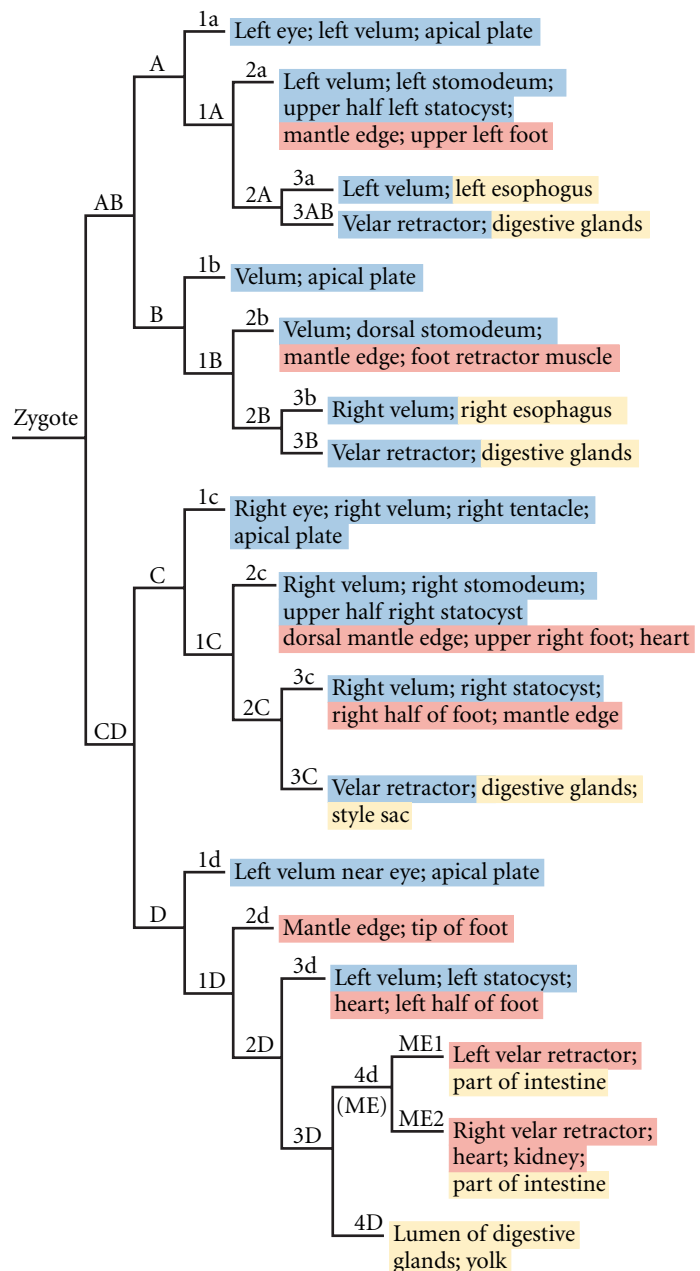

FIGURE 8.26 Fate map of *Ilyanassa obsoleta*. Beads containing Lucifer Yellow were injected into individual blastomeres at the 32-cell stage. When the embryos developed into larvae, their descendants could be identified by their fluorescence. (After Render 1997.)