over, they use a similar strategy and even similar molecules. In the 16-cell sea urchin embryo, a group of cells called the micromeres inherit a set of transcription factors from the egg cytoplasm. These transcription factors cause the micromeres to develop *autonomously* into the larval skeleton. These transcription factors also activate the genes for paracrine factors and juxtacrine factors that are employed by the micromeres to *conditionally* specify the cells around them. In the late blastula frog embryo, the cells located opposite the point of sperm entry inherit a set of transcription factors from the egg cytoplasm as well. These cells are autonomously instructed to form the head endodermal cells. They are also autonomously specified to produce and secrete paracrine factors that will conditionally induce the cells around them to form the brain. So the head endoderm is specified autonomously, whereas the brain is specified conditionally, in part, by the head endoderm.

Those embryos (especially vertebrates) wherein most of the early blastomeres are conditionally specified have traditionally been called *regulative embryos*. But as we become more cognizant of the manner in which both autonomous and conditional specification are used in each embryo, the notions of "mosaic" and "regulative" development are appearing less tenable. Indeed, attempts to get rid of these distinctions were begun by no less an embryologist than Edmund B. Wilson (1894, 1904). Wilson (a student of the above-mentioned W. K. Brooks) was one of the first scientists to theorize that chromosomes in the nucleus put forth cell-specifying factors into the cytoplasm. "Mosaic embryos," he wrote, received these factors from the cytoplasm of the egg during cleavage stages, while the nuclei of "regulative embryos" were instructed by other cells to produce these factors later in development.

Morphogen Gradients and Cell Specification

Throughout this book, we will see many instances of cell fate specification that involve morphogen gradients. A **morphogen** (Greek, "form-giver") is a diffusable biochemical molecule that can determine the fate of a cell by its concentration.* 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 (Hörstadius 1939; Toivonen and Saxén 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 either 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 (**Figure II.9**). The specification of the multipotential cells in this tissue is accom-

*Although there is overlap in the terminology, a *morphogen* specifies cells in quantitative manner, while a *morphogenetic determinant* specifies cells in a qualitative manner. Morphogens are analog; morphogenetic determinants are digital.

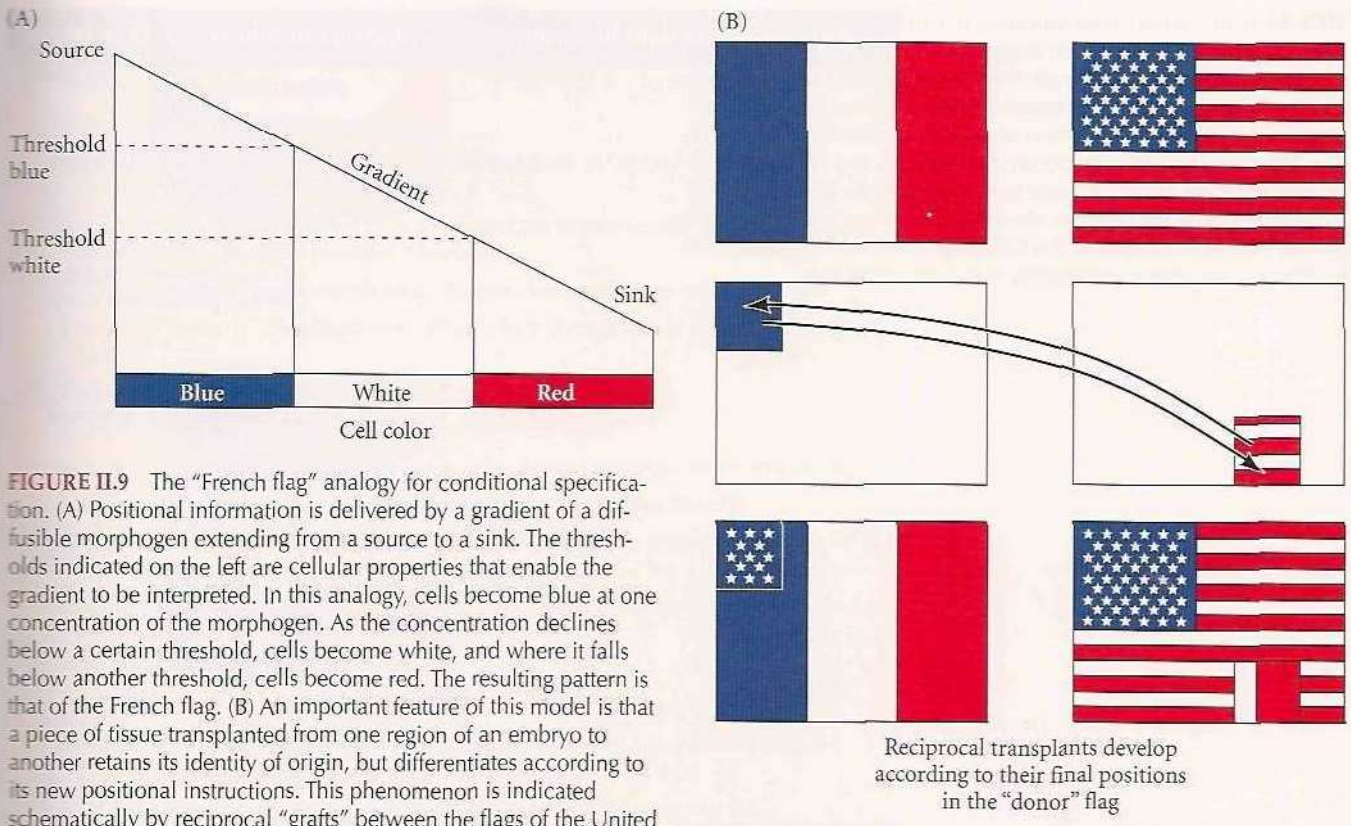

FIGURE II.9 The "French flag" analogy for conditional specification. (A) Positional information is delivered by a gradient of a diffusible morphogen extending from a source to a sink. The thresholds indicated on the left are cellular properties that enable the gradient to be interpreted. In this analogy, cells become blue at one concentration of the morphogen. As the concentration declines below a certain threshold, cells become white, and where it falls below another threshold, cells become red. The resulting pattern is that of the French flag. (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 of origin, but differentiates according to its new positional instructions. This phenomenon is indicated schematically by reciprocal "grafts" between the flags of the United States and France. (After Wolpert 1978.)

plished 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.

Later in the book we will see the concept of morphogen gradients used to model how regions of the vertebrate body axis are established by retinoic acid, Fgf8, and Wnts (Chapters 7 and 8); how the different regions of the mesoderm are specified by bone morphogenetic protein (BMP) from the lateral plate mesoderm (Chapters 11 and 12); and how vertebrate limbs and digits are specified by Sonic hedgehog (Chapter 13).

Syncytial Specification

In addition to autonomous and conditional specification, there is another strategy that uses elements of both. In early embryos of insects, nuclei divide within the egg; but the cell does not divide. In other words, many nuclei are formed within one common cytoplasm. A cytoplasm that contains many nuclei is called a **syncytium**, and the specification of presumptive cells within such a common cytoplasm is called **syncytial specification**. As in the other eggs we have mentioned, the insect egg cytoplasm is not uniform. Nuclei in the anterior part of the cell will be exposed to cyto-

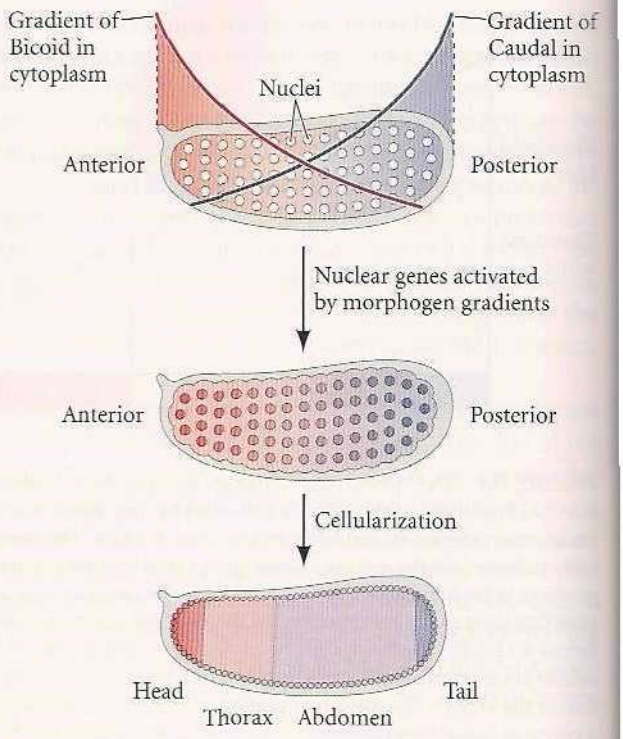

FIGURE II.10 Syncytial specification in *Drosophila melanogaster*. Anterior-posterior specification originates from morphogen gradients in the egg cytoplasm, specifically the morphogenetic transcription factors Bicoid and Caudal. The concentrations and ratios of these two proteins distinguish each position along the axis from any other position. When nuclear division occurs, the amounts and ratios of each morphogen differentially activate transcription of the various nuclear genes that specify the segment identities of the larval and the adult fly. (As we will see in Chapter 6, the Caudal gradient is itself constructed by interactions among constituents of the egg cytoplasm.)

plasmic transcription factors that are not present in the posterior part of the cell, and vice versa. The interactions of nuclei and transcription factors, which eventually result in cell specification, take place in a common cytoplasm.

Each nucleus in *Drosophila* is given positional information (i.e., whether that nucleus is to become part of the anterior, posterior, or midsection of the body) by transcription factors acting as morphogens. These transcription factors are made in specific sites in the embryo, diffuse over long distances, and form concentration gradients where the highest concentration is at the point of synthesis and gets lower as the morphogen diffuses away from its source and degrades over time. The concentration of specific morphogens at any particular site tells the nuclei where they are in relation to the source of the morphogens. As we will detail in Chapter 6, the anteriormost portion of the *Drosophila* embryo produces a morphogen called Bicoid with a concentration that is highest in the anterior and declines toward the posterior. The posteriormost portion of the egg forms a posterior-to-anterior gradient of the morphogen Caudal. Thus, the long axis of the *Drosophila* egg is spanned by opposing morphogen gradients—Bicoid coming from the anterior, and Caudal from the posterior (**Figure II.10**).

Bicoid and Caudal are both transcription factors, and different concentrations and ratios of Bicoid and Caudal proteins activate different sets of genes in the syncytial nuclei. Those nuclei in regions containing high amounts of Bicoid and little Caudal are instructed to activate those genes necessary for producing the head. Nuclei in regions with slightly less Bicoid and a small amount of Caudal are instructed to activate genes that generate the thorax. Nuclei in regions that have little or no Bicoid but plenty of Caudal are instructed to form the abdominal structures (Nüsslein-Volhard et al. 1987). Thus when the syncytial nuclei are eventually incorporated into cells, these cells will have their *general* fate specified. Afterward, the specific fate of each cell will become determined both autonomously (from the transcription factors acquired by the cell's nucleus from the egg cytoplasm) and conditionally (by interactions between the cell and its neighbors).

TABLE II.2 Modes of cell type specification and their characteristics

I.	**Autonomous specification**
	Predominates in most invertebrates.
	Specification by differential acquisition of certain cytoplasmic molecules present in the egg.
	Invariant cleavages produce the same lineages in each embryo of the species. Blastomere fates are generally invariant.
	Cell type specification precedes any large-scale embryonic cell migration.
	Produces "mosaic" development: cells cannot change fate if a blastomere is lost.
II.	**Conditional specification**
	Predominates in vertebrates and a few invertebrates.
	Specification by interactions between cells. Relative positions are important.
	Variable cleavages produce no invariant fate assignments to cells.
	Massive cell rearrangements and migrations precede or accompany specification.
	Capacity for "regulative" development; allows cells to acquire different functions.
III.	**Syncytial specification**
	Predominates in most insect classes.
	Specification of body regions by interactions between cytoplasmic regions prior to cellularization of the blastoderm.
	Variable cleavage produces no rigid cell fates for particular nuclei.
	After cellularization, both autonomous and conditional specification are seen.

Source: After Davidson 1991.

Summary

Each of the three major strategies of cell specification (summarized in Table II.2) offers a different way of providing an embryonic cell with a set of transcription factors that will activate specific genes and cause the cell to differentiate into a particular cell type. In autonomous specification, the transcription factors are already present in different regions of the egg cytoplasm. In conditional specification, the set of transcription factors is determined by paracrine and juxtacrine interactions between neighboring cells. In syncytial specification, there are interactions, not between cells, but between regions of the egg cytoplasm. These regional interactions give each nucleus a different ratio of particular transcription factors, and these ratios determine which genes are on and which are off.

The chapters that follow describe the early development of several organisms. In these chapters, we shall see how the mechanisms of fertilization, cleavage, and gastrulation use the three modes of specification to produce committed cell types and to organize the early embryo.