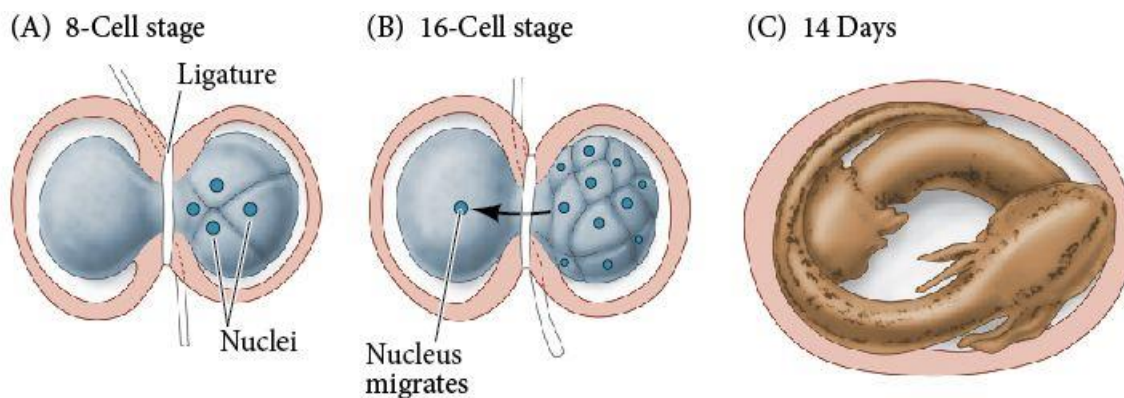


Autonomous Specification versus Inductive Interactions

The experiment that began the Spemann laboratory's research program was performed in 1903, when Spemann demonstrated that early newt blastomeres have identical nuclei, each capable of producing an entire larva. His procedure was ingenious: Shortly after fertilizing a newt egg, Spemann used a baby's hair (taken from his infant daughter) to "lasso" the zygote in the plane of the first cleavage. He then partially constricted the egg, causing all the nuclear divisions to remain on one side of the constriction. Eventually—often as late as the 16-cell stage—a nucleus would escape across the constriction into the non-nucleated side. Cleavage then began on this side too, whereupon Spemann tightened the lasso until the two halves were completely separated. Twin larvae developed, one slightly more advanced than the other (Figure 1). Spemann concluded from this experiment that early amphibian nuclei were genetically identical and that each cell was capable of giving rise to an entire organism.



After H. Spemann. 1928. *Zeitschrift für wissenschaftliche Zoologie* v132. Wilhelm Engelmann: Leipzig.

Figure 1 Spemann's demonstration of nuclear equivalence in newt cleavage. (A) When the fertilized egg of the newt *Triturus taeniatus* was constricted by a ligature, the nucleus was restricted to one half of the embryo. The cleavage on that side of the embryo reached the 8-cell stage, while the other side remained undivided. (B) At the 16-cell stage, a single nucleus entered the as-yet undivided half, and the ligature was further constricted to complete the separation of the two halves. (C) After 14 days, each side had developed into a normal embryo.

However, when Spemann performed a similar experiment with the constriction still longitudinal but perpendicular to the plane of the first cleavage (i.e., separating the future dorsal and ventral regions rather than the right and left sides), he obtained a different result altogether. The nuclei continued to divide on both sides of the constriction, but only one side—the future dorsal side of the embryo—gave rise to a normal larva. The other side produced an unorganized tissue mass of ventral cells, which Spemann called the *Bauchstück* ("belly piece"). This tissue mass was a ball of epidermal cells (ectoderm) containing blood cells and mesenchyme (mesoderm) and gut cells (endoderm), but it contained no dorsal structures such as nervous system, notochord, or somites.

Why did these two experiments have such different results? One possibility was that when the egg was divided perpendicular to the first cleavage plane, some *cytoplasmic* substance was not equally

distributed into the two halves. Fortunately, the salamander egg was a good organism to test that hypothesis. As we saw earlier in Chapter 12 of the textbook (see Figure 12.3), there are dramatic movements in the cytoplasm following the fertilization of amphibian eggs, and in some amphibians these movements expose a gray, crescent-shaped area of cytoplasm in the region directly opposite the point of sperm entry. The first cleavage plane normally splits this gray crescent equally between the two blastomeres (see Figure 12.2D). If these cells are then separated, two complete larvae develop (Figure 2A). However, should this cleavage plane be aberrant (either in the rare natural event or in an experiment), the gray crescent material passes into only one of the two blastomeres. Spemann's work revealed that when two blastomeres are separated such that only one of the two cells contains the crescent, only the blastomere containing the gray crescent develops normally (Figure 2B).

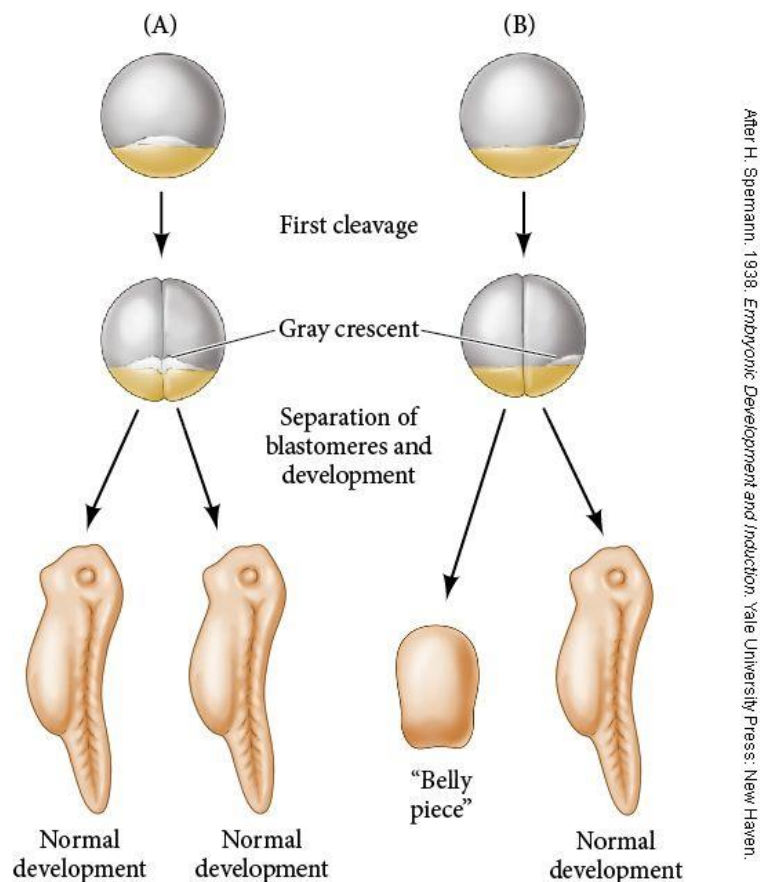


Figure 2 Asymmetry in the amphibian egg. (A) When the egg is divided along the plane of first cleavage into two blastomeres, each of which gets half of the gray crescent, each experimentally separated cell develops into a normal embryo. (B) When only one of the two blastomeres receives the entire gray crescent, it alone forms a normal embryo. The other blastomere produces a mass of unorganized tissue lacking dorsal structures.

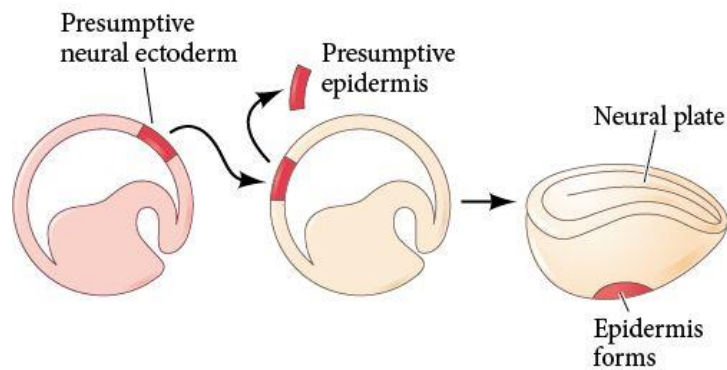
It appeared, then, that *something in the region of the gray crescent was essential for proper embryonic development*. But how did it function? What role did it play in normal development? The most important clue came from fate maps, which showed that the gray crescent region gives rise to those cells that form the dorsal lip of the blastopore. These dorsal lip cells are committed to invaginate into the blastula, initiating gastrulation and the formation of the head endomesoderm and notochord. Because all future amphibian development depends on the interaction of cells that are rearranged during gastrulation, Spemann speculated that the importance of the gray crescent

material lies in its ability to initiate gastrulation, and that crucial changes in cell potency occur during gastrulation. In 1918, he performed experiments that showed both statements to be true. He found that the cells of the *early* gastrula were uncommitted, but that the fates of *late* gastrula cells were determined.

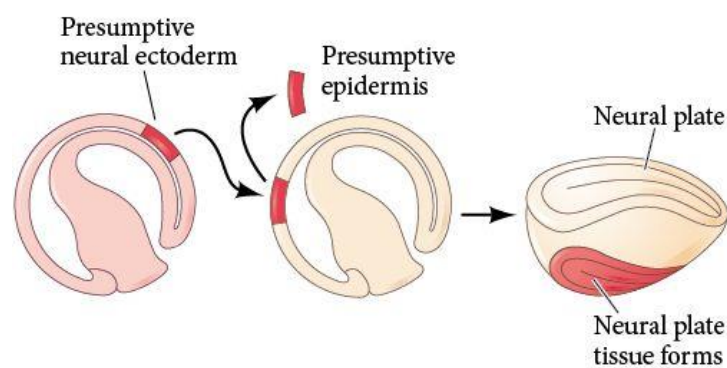
Spemann's demonstration involved exchanging tissues between the gastrulae of two species of newts whose embryos were differently pigmented—the darkly pigmented *Triturus taeniatus* and the nonpigmented *T. cristatus*. When a region of prospective epidermal cells from an early gastrula of one species was transplanted into an area in an early gastrula of the other species and placed in a region where neural tissue normally formed, the transplanted cells gave rise to neural tissue. When prospective neural tissue from early gastrulae was transplanted to the region fated to become belly skin, the neural tissue became epidermal (Figure 3; Table 1). Thus, cells of the early newt gastrula exhibit conditional (induction-dependent) specification: their ultimate fate depends on their location in the embryo.

However, when the same interspecies transplantation experiments were performed on *late* gastrulae, Spemann obtained completely different results. Rather than differentiating in accordance with their new location, the transplanted cells exhibited *autonomous* development. Their prospective fate was *determined*, and the cells developed independently of their new embryonic location. Specifically, prospective neural cells now developed into brain tissue even when placed in the region of prospective epidermis (Figure 3B), and prospective epidermis formed skin even in the region of the prospective neural tube. Within the time separating early and late gastrulation, the potencies of these groups of cells had become restricted to their eventual paths of differentiation. Something caused them to become committed to epidermal and neural fates. What was happening? See the section “The Work of Hans Spemann and Hilde Mangold: Primary embryonic induction” in Chapter 12.

(A) Transplantation in early gastrula



(B) Transplantation in late gastrula



After L. Saxén and S. Toivonen, 1962, *Primary Embryonic Induction*, Prentice-Hall, Englewood Cliffs, N.J.

Figure 3 Determination of ectoderm during newt gastrulation. Presumptive neural ectoderm from one newt embryo is transplanted into a region in another embryo that normally becomes epidermis. (A) When the tissues are transferred between early gastrulae, the presumptive neural tissue develops into epidermis, and only one neural plate is seen. (B) When the same experiment is performed using late-gastrula tissues, the presumptive neural cells form neural tissue, thereby causing two neural plates to form on the host.

TABLE 1 Results of tissue transplantation during early- and late-stage newt gastrulae

Donor region	Host region	Differentiation of donor tissue	Conclusion
EARLY GASTRULA			
Prospective neurons	Prospective epidermis	Epidermis	Conditional development
Prospective epidermis	Prospective neurons	Neurons	Conditional development
LATE GASTRULA			
Prospective neurons	Prospective epidermis	Neurons	Autonomous development
Prospective epidermis	Prospective neurons	Epidermis	Autonomous development

