Spemann-Mangold Organizer [1]


In the first three decades of the twentieth century, Hans Spemann [9] experimented and led graduate students in conducting experiments with South African clawed frog (Xenopus laevis [13]) and newt embryos (Triturus taeniatus and Triturus cristatus [15]). Spemann also developed the microtools needed for early experimental embryology [16], namely glass needles [17] and micropipettes. To make a glass needle, Spemann held a glass rod over a burner and pulled it apart so that it became incredibly thin in the middle. The thin needle-like part of the rod was broken off, and then placed over a smaller burner called a micro-burner, another one of Spemann's inventions. When heated and drawn a second time, the needle had an even finer point that allowed experimental embryologists to take embryos out of the jelly membranes in which they were ensconced. Additionally, Spemann created micropipettes that relied on the suction created by a piece of rubber covering the top of the hollow, thin glass rod. The rubber could be depressed by the thumb of the user to create a minute amount of suction and was useful for transplantation experiments. Experimental embryologists used micropipettes to remove cells from developing gastrulas, and transplant the cells to new sites.

Prior to the Spemann-Mangold organizer [6] experiment, Spemann had focused on constricting salamander [18] eggs at the blastopore [19] lip by tying single strands of his baby son's hair around the tiny eggs. Spemann observed that when the hair tightly constricted the eggs at the dorsal end, across the blastopore [19] lip, two embryos developed. Spemann also tested the degree to which constricting the embryo with hair led to different levels of conjoinment. For example, when Spemann constricted the eggs only a little, two heads formed, but if he pulled the hair strand tighter, the embryos developed separate heads and sets of forelimbs. However, when Spemann conducted the same experiment but separated the dorsal end from the ventral end by constriction, only the dorsal end developed, while the other half was left in a vegetative state.

Building on those initial experiments, Spemann investigated how cell fates were determined during embryogenesis [20]. Spemann was particularly interested in exploring the mechanism of neural plate [21] induction [11]. The neural plate [21] is the embryonic structure that gives rise to
the central nervous system [7] during development. To explore neural plate [21] induction [11], Spemann first performed a transplant experiment that was nearly identical to the later organizer [6] experiment. Spemann transplanted the blastopore [19] lip from one newt gastrula [22] into another, and noticed a second notochord [23] that developed at the site of transplantation. However, the newts were of the same species and it was difficult to determine whether the host tissue or transplanted tissue was acting to create the second nervous system.

Based on the intra-species transplantation experiments, Spemann hypothesized that the cells at the blastopore [19] lip were composed of ectoderm [24]. The rationale was that the transplanted cells appeared to assimilate with the ectoderm [24] of the host’s cells to form neural structures. Spemann also believed the cells at the blastopore [19] lip became determined in their fate first, and that fixed determination [25] then spread outward from that blastopore [19] lip across the ectoderm [24]. Spemann was mistaken, however, as the organizer [6] experiment would later demonstrate. Cells at the blastopore [19] lip are not ectoderm [24], but mesoderm [26]. The mesoderm [26] cells of the blastopore lip invaginate over the course of gastrulation [27] and are subjacent to the ectoderm [24].

Spemann revised his previous hypothesis after conducting transplantation experiments between different newt species of the same genus. The eggs from one newt species were pigmented (Triturus taeniatus), while eggs from the second species of newt were unpigmented (Triturus cristatus [19]), allowing for visual identification of which tissues gave rise to features observed. The transplantation of ectoderm [24] cells from one species (Triturus cristatus [19]) at a distance from the blastopore [19] of a second newt’s gastrula [22] (Triturus taeniatus) revealed that the transplanted tissue was progressively pulled towards the blastopore [19] lip. Spemann realized that the cells at the lip of the blastopore [19] were invaginating inward. Thus the ectoderm [24] undergoing neural differentiation [28] would be sitting atop mesoderm [26] that formerly comprised the blastopore [19] lip. The blastopore [19] lip was therefore composed of pre-mesodermal cells that initiated the invagination of surrounding cells in a process called gastrulation [27], which gives rise to the three embryonic germ layers [29] (ectoderm [24], mesoderm [26], and endoderm [30]).

In the spring of 1921, Spemann assigned his graduate student, Mangold, with the task of conducting a cross-species transplant of blastopore [19] lips between different newt species. The tissue color was different between species, allowing Mangold to see whether the features that developed were from transplanted or host tissue. Mangold used the microtools developed by Spemann to excise the blastopore [19] lip of the unpigmented Triturus cristatus [19] egg [31], and transplant it under the ectoderm [24] of a pigmented Triturus taeniatus newt egg [31]. The transplanted blastopore [19] lip differentiated into a notochord [23] and somites [32], while the ectoderm [24] of the host tissue that was sitting above the transplanted mesoderm [26] differentiated into a neural plate [21]. The neural plate [21] then went on to form neural arches and a completely separate central nervous system [7]. The ultimate result was what appeared to be two embryos conjoined at the gut.

The techniques used to discover the Spemann-Mangold organizer had limitations. The technique for transplanting the organizer involved surgery at a cellular scale, and it demanded great precision. When possible, transplanting was difficult, and many things could not be transferred under the surface of the ectodermic layer because it is only one cell thick in many places. The fact that many materials could not be transplanted kept scientists from testing the inductive capacity of other cellular materials on the embryo. The Einsteck method circumvented that limitation. Spemann and Otto Mangold, Hilde Mangold’s husband, developed the technique. While each claimed credit for the novel technique, the exact origin of its invention is unknown. Spemann proposed that both may have discussed the idea conversationally, and that each investigator felt justified in claiming credit for the idea.

The Einsteck method consists of using the glass and hair microsurgical tools developed by Spemann to plant material inside of the blastocoels of a developing embryo in either the blastula or early gastrula stage. This technique insures that material need not fuse with or adhere to the ectodermic layer. It simply passes through the ectoderm into the cavity beneath, where it can affect the embryo. The insertion wound also heals quickly, leaving the foreign material within the developing embryo to affect change.

Spemann’s co-researcher, Hanns Bruno Geinitz transplanted a Spemann-Mangold organizer into developing blastocoels using the Einsteck method, which induced embryos like the ones obtained in Spemann and Mangold’s original experiments. Geinitz expanded on the original organizer experiment by transferring organizers from frogs and toads into salamander gastrulae. Geinitz called this type of transfer xenoplastic because it involved transplant of cells from a different genus, as opposed to heteroplastic, which involved cross-species transfers like those used by Spemann and Mangold.

Although it was initially proposed that the Spemann-Mangold organizer induced differentiation of the central nervous system, more recent mechanistic examinations have revealed more complicated genetic interactions. Embryologists have determined that the presence of the Spemann-Mangold organizer impedes signaling to the overlying ectoderm. Instead of becoming skin cells, the organizer-affected ectoderm cells become central nervous system tissue. In 1992, Richard M. Harland and William C. Smith at the University of California at Berkeley in Berkeley, California discovered a protein integral to cellular induction at the Spemann-Mangold Organizer. As the protein was essential for neural development and eventually the formation of the head, the scientists called it noggin.

Since that discovery, the collective work of many experimental embryologists has revealed patterns of genetic interactions. The developing blastula secretes bone morphogenetic protein-4 (BMP-4) from the ventral side of the embryo, opposite the organizer. BMP-4 diffuses throughout the blastocoel and induces skin cells where it binds to ectodermal cells. However, the organizer blocks BMP-4 from binding to the surrounding ectoderm by secreting the proteins chordin and noggin. Chordin and noggin bind to BMP-4 in the organizer-affected area to prevent BMP-4 from binding to ectoderm receptors. Instead of becoming skin cells, the ectodermal cells in the area of the organizer take the default path of becoming central nervous system tissues.

Edward De Robertis, the first scientist to isolate the Homeobox gene in 1984 and the co-discoverer of the protein chordin in 1994, published a review of the changing conception.
of the organizer, ?Spemann?s Organizer and Self-Regulation in Amphibian Embryos.? In the review, De Robertis covers much of the recent research examining the role of RNA and gene signaling in embryonic induction. De Robertis also explains the tendency of embryonic cells to differentiate into neural plate cells when exposed to organizing material observed in many experiments that followed Spemann?s original publications.

The amphibious Spemann-Mangold organizer has developmental analogues in both fish (embryonic shield) and bird (Hensen?s node) embryos that are responsible for body plan arrangement. Spemann and Mangold?s work with induction and organization in developing amphibian embryos culminated in Spemann being awarded the Nobel Prize in Medicine in 1935. The Nobel Prize is not posthumously awarded, and Mangold had died prior to Spemann's award. Embryologists continue to study the Spemann-Mangold organizer and induction as they relate to body plans and nervous system development in amphibians.

Sources

The Spemann-Mangold organizer, also known as the Spemann organizer, is a cluster of cells in the developing embryo of an amphibian that induces development of the central nervous system. Hilde Mangold was a PhD candidate who conducted the organizer experiment in 1921 under the direction of her graduate advisor, Hans Spemann, at the University of Freiburg in Freiburg, German. The discovery of the Spemann-Mangold organizer introduced the concept of induction in embryonic development. Now integral to the field of developmental biology, induction is the process by which the identity of certain cells influences the developmental fate of surrounding cells. Spemann received the Nobel Prize in Medicine in 1935 for his work in describing the process of induction in amphibians. The Spemann-Mangold organizer drew the attention of embryologists, and it spurred numerous experiments on the nature of induction in many types of developing embryos.