Spemann-Mangold Organizer

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, Germany. 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.

In the first three decades of the twentieth century, Hans Spemann experimented and led graduate students in conducting experiments with South African clawed frog embryos (Xenopus laevis) and newt embryos (Triturus taeniatus and Triturus cristatus). Spemann also developed the microtools needed for early experimental embryology, namely glass needles 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 experiment, Spemann had focused on constricting salamander eggs at the blastopore 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 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. Spemann was particularly interested in exploring the mechanism of neural plate induction. The neural plate is the embryonic structure that gives rise to the central nervous system during development. To explore neural plate induction, Spemann first performed a transplant experiment that was nearly identical to the later organizer experiment. Spemann transplanted the blastopore lip from one newt gastrula into another, and noticed a second notochord 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 lip were composed of ectoderm. The rationale was that the transplanted cells appeared to assimilate with the ectoderm of the host's cells to form neural structures. Spemann also believed

the cells at the blastopore lip became determined in their fate first, and that fixed determination then spread outward from that blastopore lip across the ectoderm. Spemann was mistaken, however, as the organizer experiment would later demonstrate. Cells at the blastopore lip are not ectoderm, but mesoderm. The mesoderm cells of the blatopore lip invaginate over the course of gastrulation and are subjacent to the ectoderm.

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), allowing for visual identification of which tissues gave rise to features observed. The transplantation of ectoderm cells from one species (Triturus cristatus) at a distance from the blastopore of a second newt's gastrula (Triturus taeniatus) revealed that the transplanted tissue was progressively pulled towards the blastopore lip. Spemann realized that the cells at the lip of the blastopore were invaginating inward. Thus the ectoderm undergoing neural differentiation would be sitting atop mesoderm that formerly comprised the blastopore lip. The blastopore lip was therefore composed of pre-mesodoermal cells that initiated the invagination of surrounding cells in a process called gastrulation, which gives rise to the three embryonic germ layers (ectoderm, mesoderm, and endoderm).

In the spring of 1921, Spemann assigned his graduate student, Mangold, with the task of conducting a cross-species transplant of blastopore 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 lip of the unpigmented Triturus cristatus egg, and transplant it under the ectoderm of a pigmented Triturus taeniatus newt egg. The transplanted blastopore lip differentiated into a notochord and somites, while the ectoderm of the host tissue that was sitting above the transplanted mesoderm differentiated into a neural plate. The neural plate then went on to form neural arches and a completely separate central nervous system. The ultimate result was what appeared to be two embryos conjoined at the gut.

The importance of the organizer experiment is the discovery that a part of the mesoderm influences the ectoderm as the ectoderm differentiates into central nervous system tissue. Spemann and Mangold published their finding of the amphibious organizer in 1924 in the article, "Über Induktion von Embryonalanlagen durch Implantation artfremder Organisatoren (Induction of Embryonic Primordia by Implantation of Organizers from a Different Species)".

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

1. Amundson, Ron. The Changing Role of the Embryo in Evolutionary Thought. Cambridge: Cambridge University Press, 2005.
2. Balinsky, Boris Ivan. An Introduction to Embryology. Philadelphia: W. B. Saunders, 1970.
3. De Robertis, Edward M. "Spemann's Organizer and Self-Regulation in Amphibian Embryos," Nature Reviews Molecular Cell Biology 7 (2006): 296–302.
4. Fulton, Chandler, and Attila O. Klein. Explorations in Developmental Biology. Cambridge: Harvard University Press, 1976.
5. Hamburger, Viktor. The Heritage of Experimental Embryology, Hans Spemann and the Organizer. New York: Oxford University Press, 1988.
6. Holtfreter, Johannes. "Neural Induction in Explants which have Passed through a Sublethal Cytolysis." In Explorations in Developmental Biology, ed. Chandler Fulton and Attila O. Klein. Cambridge: Harvard University Press, 1976. Abridged and originally published in Journal of Experimental Zoology 106 (1947): 197–222.
7. Kimball, John W. "Organizing the Embryo: The Central Nervous System," Biology Pages, last modified May 17, 2011. http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/S/Spemann.html (Accessed November 28, 2011).
8. Maienschein, Jane. Whose View of Life? Cambridge, MA: Harvard University Press, 2003.

9. Sasai, Yoshiki, Bin Lu, Herbert Steinbeisser, Douglas Geissert, Linda K. Gont, Edward M. De Robertis. “Xenopus Chordin: A Novel Dorsalizing Factor Activated by Organizer-Specific Homeobox Genes,” Cell 79 (1994): 779–90.
10. “Spemann, Hans.” In Hutchinson Dictionary of Scientific Biography, ed. Roy Porter, 566–69. Oxford: Helicon Publishing, 2005.
11. Smith, William C., and Richard M. Harland. “Expression Cloning of Noggin, a New Dorsalizing Factor Localized to the Spemann Organizer in Xenopus Embryos.” Cell 70 (1992): 829–40.
12. Spemann, Hans, and Hilde Mangold. “Induction of Embryonic Primordia by Implantation of Organizers from a Different Species,” The International Journal of Developmental Biology 45 (2001): 13–38. Originally published in Archiv für Mikroskopische Anatomie und Entwicklungsmechanik, 100 (1924): 599–63.
13. Waddington, Conrad Hal, Joseph Needham, and Dorothy M. Needham. “Physico-Chemical Experiments on the Amphibian Organizer.” In Explorations in Developmental Biology, ed. Chandler Fulton and Attila O. Klein. Cambridge: Harvard University Press, 1976. Originally published in Nature 132 (1933): 239.
14. Zimmerman, Lyle B., Jose M. De Jesus-Escobar, and Richard M. Harland. “The Spemann Organizer Signal Noggin Binds and Inactivates Bone Morphogenetic Protein-4.” Cell 86 (1996): 599–606.