

# Induction into the Hall of Fame: tracing the lineage of Spemann's organizer

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The grafting experiments of Spemann and Mangold have been a textbook classic for years, but as with many conclusions from experimental embryology, the idea that the dorsal lip of the blastopore 'organized' the early patterning of the embryo has sometimes come under question. In their 1983 paper in *JEEM*, Smith and Slack extended these classical experiments in newts to the now-standard amphibian model *Xenopus laevis*. By using injected lineage tracers, they distinguished the fates of graft and host, and showed unambiguously that the organizer is responsible for neural induction and that it dorsalizes the mesoderm.

## Introduction

How do vertebrate embryos generate a dorsal neural plate, notochord and somites on one side, and ventral blood and gut on the other? Two extreme possibilities are that: (1) the egg is endowed with determinants for each tissue that segregate into different regions during cleavage (a so-called mosaic mode of development); or (2) asymmetries build up progressively, and that devoted signaling centers organize the rest of the embryo by cell-cell interactions. According to our current understanding of amphibian embryogenesis, the egg starts with localized determinants, and after some cytoplasmic rearrangement, these determinants dictate the identities of the dorsal and ventral mesoderm, as well as the ectoderm and endoderm. But once these asymmetries are established, induction – a process by which a cell or tissue directs the development of a neighboring tissue or cell – takes over. In *Xenopus*, for example, it is known that the dorsal mesoderm is an organizing center, and that from the late blastula stage onwards, the tissues of the tadpole are elaborated by cell-to-cell signaling.

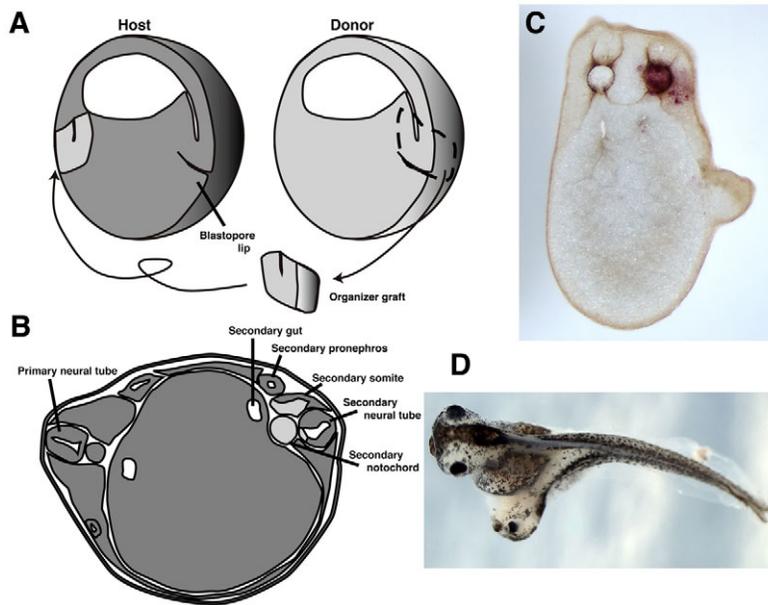
The organizer graft is a famous and influential experiment because it showed that one part of the embryo is endowed with special signaling properties that dictate the patterning of the neurulating embryo. In the early part of the 20th century, and prior to the organizer experiment, work by Hans Spemann and his colleagues had suggested that much of the amphibian embryo was regulative, such that if a piece of tissue was grafted from a donor embryo to a different location in a new host embryo, then the graft would develop according to its new surroundings. However, experiments by Warren Harmon Lewis, and later by Spemann, showed that the dorsal or upper lip of the blastopore was an exception to this general rule: in grafting experiments, it would not adopt a new fate (reviewed by Sander and Faessler, 2001). This led Spemann to address the extent to which this 'determined' fate was exclusive to the graft or determined by some response of the host.

To tackle this definitively, Spemann and his student Hilde Pröscholdt (later Hilde Mangold) used newts with differently pigmented eggs to track the contributions of host and graft in their organizer experiments (see Fig. 1) (Spemann and Mangold, 1924; Spemann and Mangold, 2001). The use of the differences in pigmentation provided the crucial marking which revealed that the secondary neural plate was induced from the host tissue and did not self-differentiate from the graft, as both Spemann and Lewis had erroneously concluded from their earlier trials that lacked a tracer. The clarity as well as the limitations of using natural pigmentation to mark donor and host tissue can be seen in photographs of some of the original organizer grafts (Sander and Faessler, 2001). In addition to the limitations of the pigment as a clear lineage tracer, the experiments by Spemann and Mangold were few in number, and not all of them gave the clean results reproduced in the textbooks. In light of the limitations of these classical experiments, there was a need, therefore, to examine the activities of the organizer in greater numbers of embryos and with more attention paid to precisely where the graft was taken from and to the results obtained. This was indeed what Jim Smith and Jonathan Slack achieved in their 1983 paper in the *Journal of Experimental Embryology and Morphology (JEEM)* (Smith and Slack, 1983).

## New lineage tracers

Most classical lineage tracing studies had used vital dyes, such as Nile Blue, that could be applied to the outside of tissues and would stain subcellular structures, such as yolk platelets. Although these tracers provided a great deal of information about lineage that is still relied upon today, the possibility that the dye might leach from a graft diminished the value of such lineage tracers in transplantation experiments. In the 1970s, new injectable lineage tracers were introduced that were deployed in repeats of classical experiments, such as those by Spemann and Mangold. However, not everyone reproduced these experiments' findings; indeed, Marcus Jacobson concluded that amphibian development was considerably more mosaic, and criticized Spemann and Mangold's earlier conclusions (Jacobson, 1982). Thus, in the early 1980s, the stage was set for more authoritative repeats of the organizer experiments to be performed using the new and improved lineage tracers.

To be useful, a lineage tracer must be cell autonomous, so that the fate of adjacent cells is not conflated with that of the marked cell. The tracer must also be non-toxic and developmentally neutral. Horseradish peroxidase (HRP) had been used through the 1970s as a retrograde tracer that was taken up by neurons, then transported back to cell bodies; subsequent staining for the enzyme revealed the fine structure of these neurons. In 1976, Kenneth Muller and Jack McMahan (Muller and McMahan, 1976) used the direct injection of HRP into the large neurons of leech ganglia to describe the fine structure of these cells; from there, David Weisblat and Gunther Stent (Weisblat et al., 1978) extended the use of HRP injection to trace the progeny of the early blastomeres of the leech embryo. Other tracers quickly followed, including the fixable fluorescent dextran developed by Bob Gimlich and Jochen Braun (Gimlich and Braun, 1985); this fluorescent tracer was used by Gimlich and Jonathan Cooke (Gimlich and Cooke, 1983) for a series of experiments that, like Smith and Slack's work, reinforced the idea that the organizer acted through induction.



**Fig. 1. Organizer grafts result in induction of a secondary axis.**

(A) Schematic of the organizer graft created by Spemann and Mangold, using a light-gray newt donor (*Triturus cristatus*) grafted into a dark-gray host (*Triturus taeniatus*). The gastrulae are shown in hemisection for illustrative purposes only (dorsal is towards the right, and the dimensions of these embryos are more *Xenopus*-like than *Triturus*-like). (B) The famous result of an optimal grafting experiment (Spemann and Mangold, 1924), showing a section through the trunk of a twinned embryo. The light-gray graft has contributed to the notochord, medial somite and floor plate of the secondary axis. The graft has an induced neural tube, somites, a pronephros and a secondary archenteron cavity. (C, D) Contemporary organizer grafts from Andrea E. Wills (UC Berkeley, CA, USA). (C) The section shows a rafted organizer labeled with *lacZ* mRNA and stained with Red-Gal; the section is taken through the trunk of a stage 28 *Xenopus laevis* embryo, where the axial tissues are also stained with Tor70 antibody. (D) Twinned *Xenopus* embryo, resulting from an organizer graft carried out at stage 10.

### Tracing normal *Xenopus* development

In their 1983 study, Smith and Slack decided to repeat the organizer graft experiments of Spemann and Mangold in *Xenopus laevis*, rather than in newts, using HRP as the lineage tracer. It had previously been established that HRP rapidly fills the cell it is injected into, so all of the progeny of the cell are labeled; at the same time, the tracer remains confined to that cell. Smith and Slack also established that cells do not take up HRP from the surrounding medium (where it might be released by dying cells). Thus, by all criteria, this tracer was ideal for the organizer grafting experiments they wanted to perform.

As a prelude to these experiments, it was important to know the normal fates of tissues in *Xenopus laevis* embryos. Therefore, they first used the tracer to monitor the normal fates of the dorsal and ventral marginal zones (the marginal zone is the region near the equator of the embryo, where the animal and vegetal hemispheres meet), by grafting pieces from HRP-filled embryos to the same (orthotopic) location of an unlabelled host. The results of these HRP grafting experiments supported an earlier analysis by Ray Keller in which vital dyes were used (Keller, 1976). Moreover, the clarity of the histochemical stain illustrated beautifully that the dorsal marginal zone populates a narrow strip of dorsal mesoderm – the prechordal plate and notochord – over the entire craniocaudal extent of the embryo, in addition to the anterior endoderm. Importantly for the experiments that followed, the dorsal marginal zone was not seen to contribute to the nervous system.

In contrast to the fate of the dorsal marginal zone, the small piece of orthotopically grafted ventral marginal zone spread considerably and populated the posterior lateral plate and endoderm. The latter point has been revisited lately, with some authors arguing that the prospective posterior fate of the ‘ventral’ marginal zone should prompt a different term to be used for this region of the embryo, and, together with the findings of other experiments, for the axes of the blastula to be renamed (reviewed by Lane and Sheets, 2006). However, there is little question that the dorsal marginal zone is both dorsally specified and dorsally fated, so there also remains a good rationale to adhere to the nomenclature used by Smith and Slack (reviewed by Harland, 2004). In any case, the main motivation of

Smith and Slack’s fate-mapping experiments was to rule out the possibility that a grafted dorsal marginal zone might contain any neural tissue, and, although they may not have provided a comprehensive fate map of the whole gastrula, this important point was resolved.

### Signaling from the organizer

Fate mapping aside, the most important experiments in the Smith and Slack *JEEM* paper addressed the signaling activities of the organizer, and the response of the ventral marginal zone to an organizer graft. Indeed, the results of the dorsal marginal zone graft showed that neural induction had occurred, such that the neural tube of the secondary axis was composed of host cells, and not of self-differentiating cells of the graft. Therefore, the neural tissue of the host’s secondary axis must have been derived from an inductive interaction. The results presented were extremely clear, and, together with those of Gimlich and Cooke, published in the same year (Gimlich and Cooke, 1983), reinforced the importance of the dorsal marginal zone as an organizing center that can recruit ectoderm into a secondary neural tube. The idea that the nervous system was already fully specified in the blastula (Jacobson, 1982) was effectively laid to rest.

After disposing of the controversy related to neural induction, the paper then focused on dorsalization of the mesoderm: the process that respecifies prospective ventral tissue, such as blood and mesenchyme, to more dorsal fates, such as muscle. This phenomenon had previously been recognized, but because so much attention had been devoted to neural induction, it had received less attention. Furthermore, experiments on mesoderm induction by Nieuwkoop had suggested that the pattern of the mesoderm was already induced by graded signals from the vegetal endoderm (Boterenbrood and Nieuwkoop, 1973). The ability of organizers, or indeed of chemicals (Yamada, 1950), to dorsalize mesoderm had been described, but one of the strengths of Smith and Slack’s paper is that it clearly states the distinction between the organizer’s role in dorsalizing the mesoderm and the process of mesoderm induction. Thus, the paper laid out a clear sequential signaling process: mesoderm induction in the blastula is followed by dorsalization of the

mesoderm by the organizer during gastrulation. These experiments laid the groundwork for the further dissection of dorsalization and its molecular basis.

To address whether the ventral marginal zone has signaling activity that is analogous to that of the organizer, Smith and Slack implanted pieces of ventral marginal zone into a slit in the organizer region. The result of this manipulation was a split in the notochord, where the original notochord territory maintained its fate, while the ventral graft stayed in the middle without influencing the identity of surrounding tissue. In contrast to any ventralizing effect of the graft, the graft was itself dorsalized to develop into muscle. So the experimental embryology in the Smith and Slack paper tells us that, instead of dorsal and ventral marginal zones carrying equal weight, the signals from the organizer are dominant signals, and any signals from the ventral mesoderm are neither potent nor long range.

### Mesodermal pattern: graded action of mesoderm inducers or dorsalization by the organizer?

Shortly after Smith and Slack's 1983 *JEEM* study, Smith made the seminal finding that a soluble mesoderm inducer was made by a cell line, observations that were published as the very first paper in *JEEM*'s successor: *Development* (Smith, 1987). These and subsequent experiments showed that graded doses of the mesoderm inducer could induce progressively more dorsal structures from sensitive ectoderm. Therefore, the mesoderm inducer might, in principle, act as a classical morphogen, dictating different fates, such as notochord, muscle, kidney and blood, at different threshold concentrations. With the arrival of a molecular approach to studying mesoderm induction, and the possibility that mesoderm inducers act as morphogens, the phenomenon of mesoderm dorsalization, as supported only by experimental embryology, shifted into the background. However, despite the elegance of the idea that a mesoderm inducer might act as a morphogen to specify the pattern of the mesoderm, other experiments in experimental embryology argued that this mechanism was insufficient to account for mesoderm patterning. One of the clearest approaches was to assess the state of mesoderm immediately next to the organizer in the late blastula stage. The first approach used explants (Dale and Slack, 1987), and, in another study, two hemispheres cut at different angles from the dorsal midline were grafted together (Stewart and Gerhart, 1990). Both of these approaches showed that during the phase of mesoderm induction, the marginal zone adjacent to the organizer has not yet received signals to differentiate into muscle. Thus, the proposal that a graded mesoderm-inducing signal might induce muscle during the blastula stage was inadequate. These embryological 'loss-of-function' experiments showed that organizer signaling is necessary in normal development for patterning the mesoderm, and complemented the earlier 'gain-of-function' experiments, which showed that an organizer graft is sufficient to induce dorsal mesoderm in a secondary axis (Dale and Slack, 1987; Gimlich and Cooke, 1983; Smith and Slack, 1983; Stewart and Gerhart, 1990). In modern terms, we understand that the late blastula and gastrula-stage dorsalizing signals are molecular pathways that are distinct from those involved in mesoderm induction, and are mediated by dorsalizing molecules (Noggin, Chordin, Follistatin, Xnr3 and Cerberus) that antagonize the ventralizing bone morphogenetic proteins (BMPs). In this respect, a continuing relevance of Smith and Slack's 1983 paper is in the experimental embryology, which tells us that the source of BMP antagonists is dominant and presumably must produce a molar excess above the concentration of BMPs that are secreted from a ventral marginal zone graft.

### Conclusion

Needless to say, in the 25 years that have elapsed since the paper was published, we have reached a much more sophisticated understanding of the various molecular players that are active in patterning the *Xenopus* embryo. Grafting experiments are inherently somewhat variable in their results and limited in their implications; for example, it was only with the advent of molecular assays that it became clear that head induction was not a quantitative or temporal effect of the organizer, but rather due to a combination of different molecular signals (Glinka et al., 1997). In retrospect, it would have been useful to know more about how different types of organizer graft behaved with respect to the anterior extent of the secondary axis produced, but in the early 1980s, researchers' frustrations with the limitations of experimental embryology was driving many to the genetic and molecular approaches that still dominate developmental biology. However, it is also still important to know what the embryo tells us through well-designed 'cut and paste' experiments, so we still refer to the initial grafting experiments in *Xenopus* that extended the paradigm of the organizer that was established by Spemann and Mangold.

Thanks to John Gerhart and David Weisblat for discussing the historical background, and Andrea Wills and Jane Alfred for comments on the manuscript. R.M.H. is supported by the NIH.

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