MECHANISMS OF DEVELOPMENT 126 (2009) 925–941

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/modo

MECHANISMS OF DEVELOPMENT

Review

Spemann's organizer and the self-regulation of embryonic fields

E.M. De Robertis *

Howard Hughes Medical Institute, Department of Biological Chemistry, University of California, Los Angeles, CA 90095-1662, USA

ARTICLE INFO

Article history:
Received 8 July 2009
Received in revised form
26 August 2009
Accepted 28 August 2009
Available online 4 September 2009

Keywords:
Morphogenetic fields
Embryonic induction
Dorsal–ventral patterning
BMP
Chordin
Crossveinless-2
Tolloid
Sizzled
Hox genes
Urbilateria

ABSTRACT

Embryos and developing organs have the remarkable ability of self-regenerating after experimental manipulations. In the *Xenopus* blastula half-embryos can regenerate the missing part, producing identical twins. Studies on the molecular nature of Spemann's organizer have revealed that self-regulation results from the battle between two signaling centers under reciprocal transcriptional control. Long-range communication between the dorsal and ventral sides is mediated by the action of growth factor antagonists – such as the BMP antagonist Chordin – that regulate the flow of BMPs within the embryonic morphogenetic field. BMPs secreted by the dorsal Spemann organizer tissue are released by metalloproteinases of the Tolloid family, which cleave Chordin at a distance of where they were produced. The dorsal center secretes Chordin, Noggin, BMP2 and ADMP. The ventral center of the embryo secretes BMP4, BMP7, Sizzled, Crossveinless-2 and Tolloid-related. Crossveinless-2 binds Chordin/BMP complexes, facilitating their flow towards the ventral side, where BMPs are released by Tolloid allowing peak BMP signaling. Self-regulation occurs because transcription of ventral genes is induced by BMP while transcription of dorsal genes is repressed by BMP signals. This assures that for each action of Spemann's organizer there is a reaction in the ventral side of the embryo. Because both dorsal and ventral centers express proteins of similar biochemical activities, they can compensate for each other. A novel biochemical pathway of extracellular growth factor signaling regulation has emerged from these studies in *Xenopus*. This remarkable dorsal–ventral positional information network has been conserved in evolution and is ancestral to all bilateral animals.

1. Introduction

Within the organism cells do not lead individual lives as they do in a tissue culture Petri dish. They proliferate, differentiate and die as part of groups of hundreds or thousands of cells called morphogenetic fields. Embryology has shown that cells within a field can communicate with each other over long distances, self-regulating pattern to generate the most perfect form possible after experimental perturbations. The molecular mechanisms of cell–cell communication within morphogenetic fields are key to understanding the development and homeostasis of animal tissues and organs, and are the topic of this review. As we will see, the flow of growth factors and their antagonists within the embryonic field is a fundamental property of self-regulating patterning systems.

* Tel.: +1 310 206 1401; fax: +1 310 206 2008.
E-mail address: ederobertis@mednet.ucla.edu.
0925-4773/$ - see front matter
doi:10.1016/j.mod.2009.08.004

1.1. *Self-regulation*

Self-regulation has captured the interest of biologists since the very beginning of experimental embryology. In 1891 Hans Driesch separated the first two cells and in 1936 Hörstadius succeeded in separating the first four cells of a sea urchin embryo (Hörstadius, 1973). As shown in Fig. 1, each cell was able to form a complete sea urchin larva. This tendency of the embryo to form the whole constitutes one of the deepest mysteries in developmental biology.

Hans Spemann investigated self-regulation in amphibian embryos gently constricted by fine loops from the hair of his newborn daughter, and was able to generate twins (reviewed in Spemann, 1938). Much later, I realized it is sufficient to bisect a *Xenopus* embryo at the blastula stage with a scalpel in order to generate identical twins (De Robertis, 2006) (Fig. 2). This simple procedure proved a very useful tool in the investigations discussed below. Twinning after experimental perturbation also takes place in insect embryos (Sander, 1976), and thus self-regulation is a universal phenomenon in animal development.

1.2. *Morphogenetic fields*

Natural selection would not have generated self-regulation just in case an inquisitive developmental biologist came by to cut embryos up. Deeper causes must be in play, offering an evolutionary advantage to self-regulating embryos. The tendency to re-form the whole is also observed in later development. During early development (up to gastrulation), we speak about "primary morphogenetic field" regulation, but at later stages experimental embryology has demonstrated that most organs also start their development as "secondary self-regulating morphogenetic fields" (reviewed in Huxley and De Beer, 1934; De Robertis et al., 1991) (Fig. 3).

The concept of morphogenetic fields was proposed by the famous American embryologist Ross G. Harrison. Working at Yale on embryos of the American salamander *Amblystoma punctatum* (now renamed *Ambystoma maculatum*), Harrison showed that a circular region of lateral plate mesoderm would induce the development of forelimbs when transplanted into host embryos. When he cut this region in half, each half could induce a limb. Not a half-limb, but rather an entire limb (Harrison, 1918). Since this experiment a key question in developmental biology has been: How does this regeneration of pattern towards the whole come about?

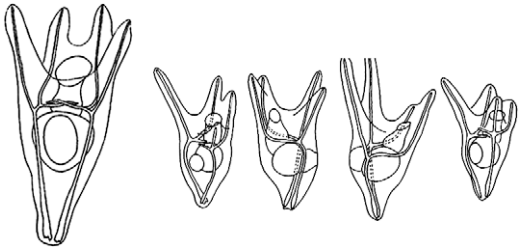

Fig. 1 – Separation of the first four blastomeres of a sea urchin embryo can give rise to four well-formed pluteus larvae. This powerful regulation was first reported by Hans Driesch in 1891, marking the beginning of experimental embryology. It now appears that the self-regulation of embryonic fragments had been reported even earlier, in 1869, by Ernst Haeckel in cnidarian embryos (Sánchez Alvarado, 2008). The experiment shown here is from Hörstadius and Wolsky, 1936, W. Roux. Arch. Entw. Mech. Org. 135, 69–113, reproduced with permission.

2. The organizer

2.1. *Hans Spemann, Hilde Mangold and the organizer*

The way forward in the analysis of self-regulation of pattern came from a transplantation experiment carried out by a graduate student at Freiburg University named Hilde Mangold. Under the direction of Hans Spemann, she grafted the dorsal blastopore lip, the region where gastrulation starts, from a weakly pigmented salamander gastrula to the ventral side of a more pigmented species. This allowed her to distinguish the cells contributed by the graft from those of the host embryo. The lineage-tracing technique used, named heteroplastic transplantation, had been invented by Ross Harrison, who used it to demonstrate that lateral line organ cells of the amphibian tadpole trunk and tail migrate from anterior (auditory) regions of the embryo (Harrison, 1903). Harrison was a close friend of Spemann, hence the use of this lineage-tracing method to follow the fate of dorsal lip grafts. During earlier salamander breeding seasons, Spemann had found that the dorsal lip of the blastopore was the only region of the embryo that did not adopt the fate of the surrounding cells when transplanted, but instead kept its own fate giving rise to dorsal tissues (Spemann, 1938).

Hilde Mangold found, and described in exquisite camera lucida drawings of histological sections, that the transplanted dorsal tissue gave rise mostly to notochord, while the neighboring cells from the host were induced to form a Siamese twin containing dorsal tissues such as somites and central nervous system (CNS) (Spemann and Mangold, 1924). This experiment provided the basis for our current view that embryonic development occurs through a succession of cell–cell inductions. Tragically, Hilde Mangold (née Pröscholdt) died shortly afterwards in a kitchen stove accident while warming milk for her recently born baby. She did not live to see her paper published.

Spemann named the inducing activity of the dorsal lip the "organizer", for it induced a well-formed Siamese twin. Fig. 4 shows a Spemann graft in which the transplanted tissue caused the primary embryonic field to become divided almost perfectly in two. This experiment became extremely well known because Spemann was awarded the Nobel Prize for Medicine or Physiology in 1935 for the discovery of embryonic induction by the organizer. However, the demise of Spemann's organizer was to follow soon afterwards, once the search for the chemical nature of the organizer inducing activity began.

2.2. *The demise of Spemann's organizer*

Spemann thought of the organizer in terms of physics, which was the dominant science of his time. From electro-