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REVIEWS

Building the Body, Building the Brain

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Abstract | The body plan of any organism presents a daunting engineering challenge. A single fertilized egg divides multiple times to give rise to a ball of seemingly identical cells which eventually achieves the phenomenal task of forming limbs, heart, eyes and brain. What breaks the symmetry such that particular structures are formed in specific regions, each distinct in its organization, appearance, and function? In this review we focus on the fundamental role of "signaling centers'' in the process of symmetry breaking and patterning of specific structures- the limb, spinal cord and the telencephalon. We will examine the roles of key molecules that are secreted from each signaling center, and bring out how their mutual regulation and interactions in the surrounding tissue result in patterning of different structures.

1 Introduction

Signaling centers are transient groups of cells that appear at key locations during development. These sites are often at tissue boundaries or where one type of tissue is juxtaposed to another type. A signaling center is a source of "*morphogens*," molecules that act to induce patterning in adjacent tissues. As a result of diffusion, the concentration of the morphogen(s) decreases with distance creating a morphogen gradient. Cells in the responding tissue acquire distinct identities because of different concentrations of the morphogen. A signaling center can induce an entire patterned structure adjacent to it and is therefore called an "organizer." We will bring out how multiple organizers interact to create the complexity of the body and the brain via a common set of mechanisms. In the vertebrate limb, spinal cord, and forebrain, members of four signaling molecule families, Wnt, Bmp, Fgf, and Shh, interact to induce different aspects of patterning in the surrounding tissue. These signaling molecules also regulate each other's expression via mutual induction or repression. A complex choreography of these four players creates the diverse cell types seen in the body and in the brain.

2 Building the Hand—Digit Patterning in Vertebrates

The vertebrate hand is a good example of a patterned structure. The hand/limb/wing arises from an undifferentiated mass called the 'limb bud' (Fig. 1). As the limb develops, skeletal and other elements are patterned such that digits form at the distal end in a specific, invariant orientation. The cells in the limb bud acquire positional information in the form of gradients of signaling molecules which are then translated into forming the different fingers.

The chick wing/limb provides an easily accessible system to test the role of organizer tissues in inducing pattern formation. Seminal experiments in 1968 demonstrated that a small region of the posterior portion of the limb bud was capable of acting as an organizer.[24] This region, called the Zone of Polarizing Activity (ZPA) was capable of inducing a new antero-posterior axis causing a mirror-image duplication of the limb (Fig. 2). The ZPA is remarkably conserved across vertebrates. In 1993, the morphogen secreted by the ZPA was identified as *Sonic Hedgehog* (*Shh*),[22] a gene homologous to the Drosophila *hedgehog* (hh). Functionally, Shh is capable of recapitulating ZPA activity in that a bead soaked with Shh has the same effect as an additional ZPA transplanted ectopically into the anterior region of the limb bud.[27] Shh therefore plays a pivotal role in limb-patterning along the anterio-posterior axes.

The expression of Shh in the ZPA needs to be maintained, and this is achieved via an FGF dependent positive feed-back loop.[15] In the

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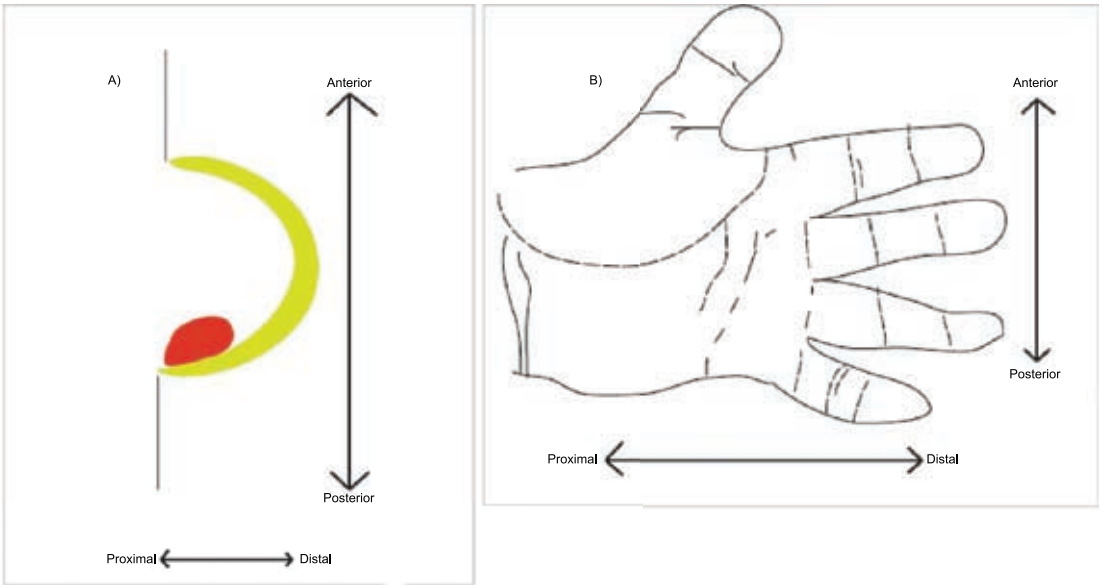

Figure 1: The vertebrate hand develops from the limb-bud and consists of different axes. A) A schematic of the developing limb bud with the signaling center AER (Apical Ectodermal Ridge) shown in green and the ZPA (Zone of Polarizing Activity) shown in red.

posterior limb bud tissue close to the ZPA, Shh induces direct or indirect expression of the Gli family of transcription factors. In particular, an activator version of Gli3 (Gli3a) is directly induced by Shh. High levels of Gli3a activity maintains expression of Gremlin in the posterior regions of the limb-bud.[20] Gremlin is a known inhibitor of Bmp activity. Inhibition of Bmp activity in the posterior mesenchyme in turn makes the adjacent cells of the posterior AER competent to activate the expression of Fgf4. Fgf4 is secreted and helps maintain Shh expression in the ZPA (Fig. 3). This feed-back loop operative between the AER and ZPA is however not maintained in the anterior regions of the limb bud, since Gli3a is not induced there. Instead, a repressor form of Gli3 (Gli3R) is active in these regions which represses Gremlin and thereby Fgf4 expression in the anterior AER.

Apart from Fgf4 secreted from the AER region, Wnt7a (a member of the Wnt family of morphogens), secreted from the ectoderm of the developing limb bud is also required for the maintenance of Shh expression.[19] When the source tissue of Wnt7a and Fgf4 i.e., dorsal limb ectoderm including the AER is removed, Shh expression and posterior skeletal development is affected. These can be rescued by applying Wnt7a and Fgf4 in combination to the remaining tissue.[19]

Maintenance of Shh expression is therefore under combinatorial control of Fgf4 and Wnt7a. Finally, Wnt signaling also plays a crucial and independent role in maintaining the AER and thereby ultimately aids in anterio-posterior patterning.[2,16]

3 Patterning the Spinal Cord

The spinal cord develops from the caudal portion of the neural tube. At each position along the dorsoventral axis of the spinal cord a group of distinct neurons arise, which perform specific functions (either sensory, motor or interneuron subtype). The progenitors of these neurons are patterned by opposing signals from the ventral floor plate, which secretes Shh[4] and the dorsal roof plate, which secretes Wnts and Bmps.[11,12]

Shh is secreted by the notochord and also the floor plate, and is both necessary and sufficient to induce a range of ventral fates in the neural tube (Fig. 4). Shh overexpression can ventralize the spinal cord,[4] and can specify distinct ventral interneuron classes and motor neurons in a concentration dependent manner.[5] Removal of key Shh signaling pathway molecule Smo (smoothened) leads to a complete absence of floor plate, majority of ventral interneurons and all the motor neurons.[26] As in the limb, a major role of Shh even in the spinal cord is to inhibit Gli3 from being processed into its repressive form. In compound mutants lacking both Gli3 as well as Shh (or Smo) a partial rescue of phenotypes is seen such that a majority of ventral interneurons and motor neurons were generated, though there were defects in positioning and segregation from one another.[13,21] This indicates

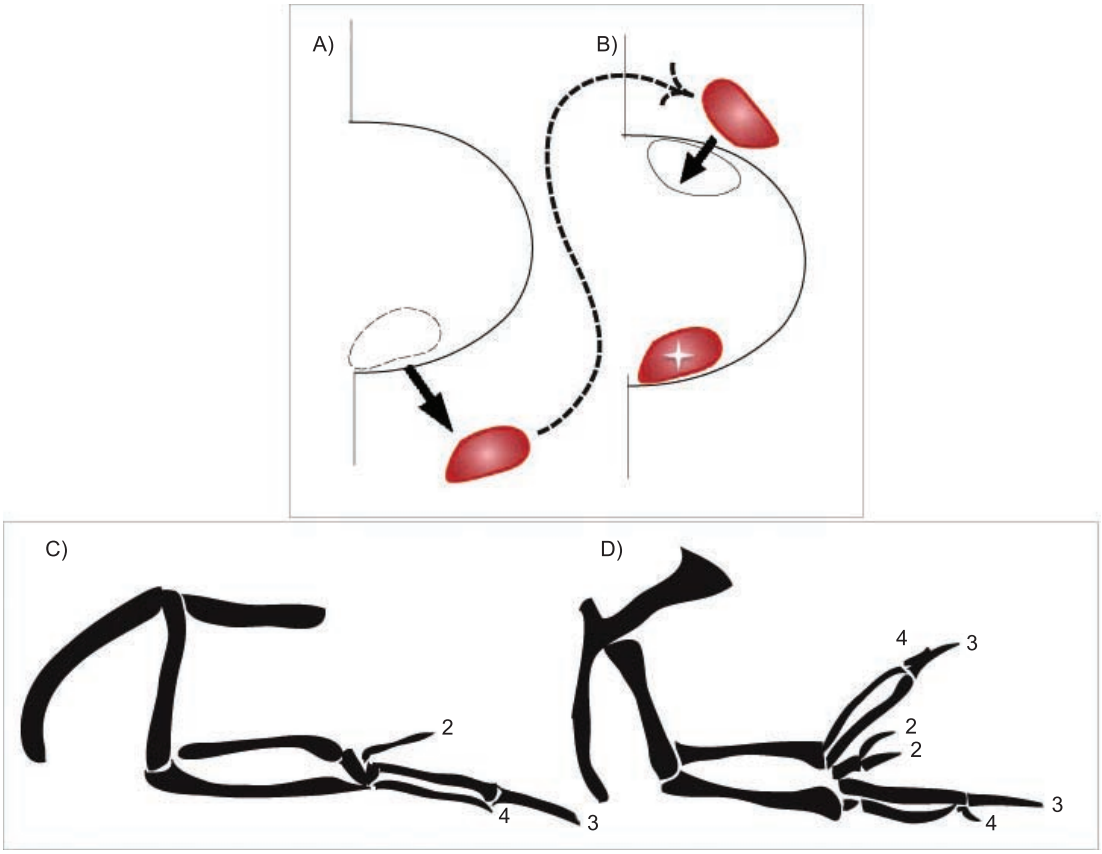

Figure 2: Transplantation experiments in chick prove function of ZPA as a signaling center. A) Schematic of a developing chick limb bud (donor) with the excised ZPA shown in red. The region of excision is outlined. B) The donor ZPA is transplanted into an ectopic location in a host limb-bud that has its own ZPA in the normal location (red, asterisk). C) The skeleton of a normal chick limb shown with the three digits numbered 2, 3 and 4 running from anterior to posterior. D) The skeleton of a chick limb in which an additional ZPA had been introduced into the anterior region of the limb during development showing mirror-duplication of digits. (C) and (D) have been drawn based on data from Saunders and Gasseling (1968).

that specification of ventral interneurons is mediated by Shh by restricting activity of Gli3 repressor to more dorsal domains.[1,10]

Morphogens (Bmp and Wnt) secreted by the roof plate are also important for dorsal patterning. These morphogens act with Shh (secreted from the floorplate/notochord) in an antagonistic manner to specify dorso-ventral fates in the spinal cord. BMPs have been shown to have a limited effect on dorsal interneuron specification. There are three classes of dorsal interneurons, D1 through D3. In mice lacking the Bmp family member Gdf7 the D1A subclass of interneurons are not specified. It has been shown that Wnt molecules also act to specify dorsal interneuron populations. Overexpression of Wnt1[7] and Wnt3a[17] independently have been shown to expand the dorsal interneurons in the spinal cord. In *Wnt1* and *Wnt3a* double mutants, the specification of D1 and D2 interneurons is impaired with a compensatory expansion of D3 interneurons dorsally.[17]

In contrast to the genetic events involved in the anterio-posterior patterning of the limb, where Shh is involved in inducing Bmp inhibition, the latter in turn regulating Shh expression in the ZPA, Shh and Bmp do not regulate each other's expression domains or levels in the dorso-ventral patterning events of the spinal cord.

4 Telencephalic Patterning

The forebrain consists of the telencephalon, which gives rise to the cerebral cortex and basal ganglia, and the diencephalon which gives rise to the thalamus and hypothalamus. Even though the cerebral cortex is regarded as the most complex brain structure, and the seat of our highest cognitive and perceptual functions, early telencephalic patterning is controlled by mechanisms very similar to those seen in the limb bud and in the spinal cord.

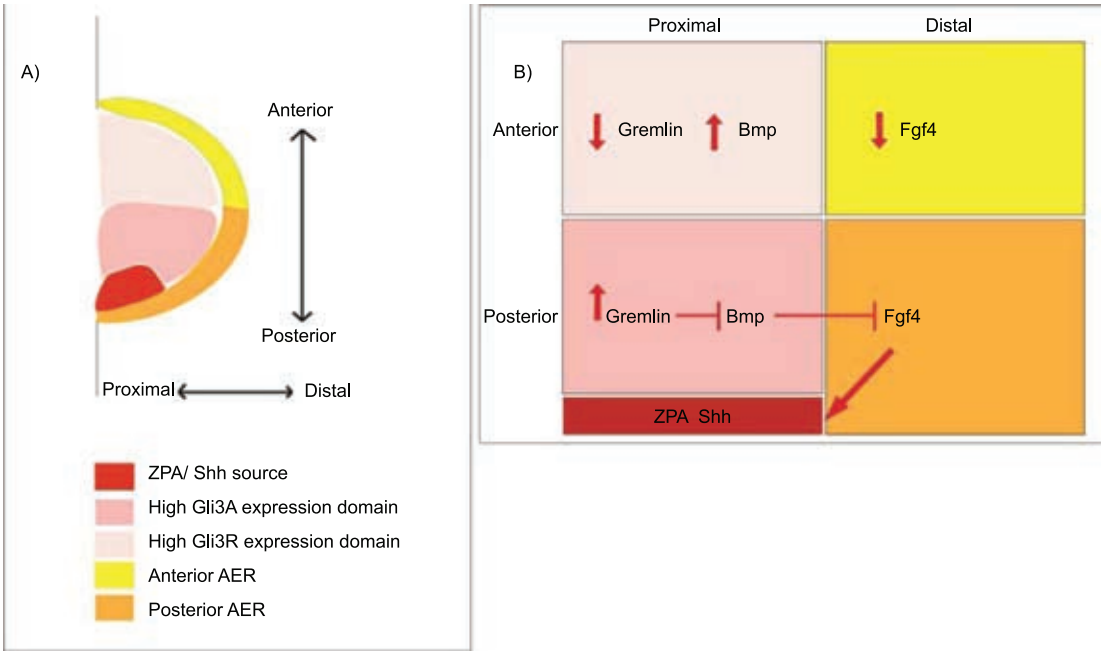

Figure 3: A feed-back loop involving Shh, Gremlin, Bmp and Fgf is operative between the ZPA and AER to maintain Shh expression in the ZPA. A) Schematic of a chick limb/wing bud of a 3 day old chick with Gli3a expression near the ZPA and Gli3R expression distant from it. B) As a consequence of Gli3a and Gli3R in different regions of the tissue, Gremlin, Bmp and Fgf4 are differentially regulated, resulting in Shh being reinforced in the posterior region where the ZPA lies.

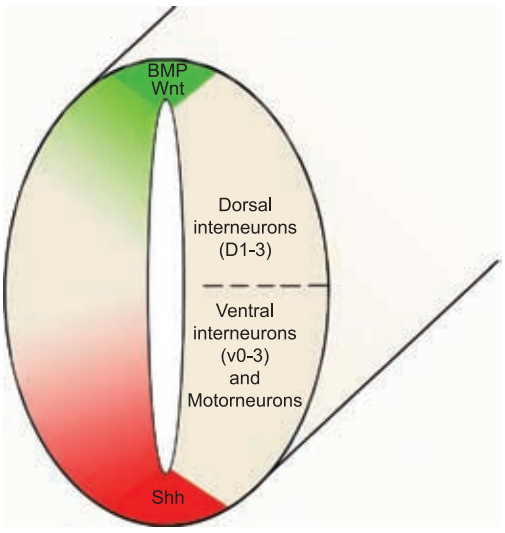

Figure 4: Antagonistic interaction between Shh and Bmp/Wnt determines the specification of neuronal identity along the dorso-ventral axis in the spinal cord. Schematic of cross-section of developing vertebrate spinal cord with regions of Shh expression shown in red and that of Bmp and Wnt shown in green.

Shh controls ventral telencephalic patterning, similar to its role in the spinal cord. There is no notochord at the level of the forebrain, nor any specialized floorplate, but Shh is expressed in the mesenchyme that underlies the forebrain, and also in the ventral forebrain structures themselves (Fig. 5). This morphogen is necessary for the patterning of ventral structures, such that in its absence these structures do not form and there is a profound disruption of the midline, causing holoprosencephaly (fusion of the telencephalic hemispheres to form a single vesicle with a single fused eye).[3,23]

The cerebral cortex, comprising the neocortex and hippocampus, forms from the dorsal telencephalic neuroepithelium. Two crucial signaling centers pattern these structures. The cortical hem, a source of Wnt and Bmp family members, is required to induce the hippocampus and its individual fields. The rostral signaling center, a source of Fgf signaling, controls the area map of the neocortex.

The hem is a small region at the medial edge of the dorsal telencephalic neuroepithelium, that most likely forms as a result of the expression of transcription factor Lhx2 being turned off in this region.[6,14] Lhx2 functions to suppress hem

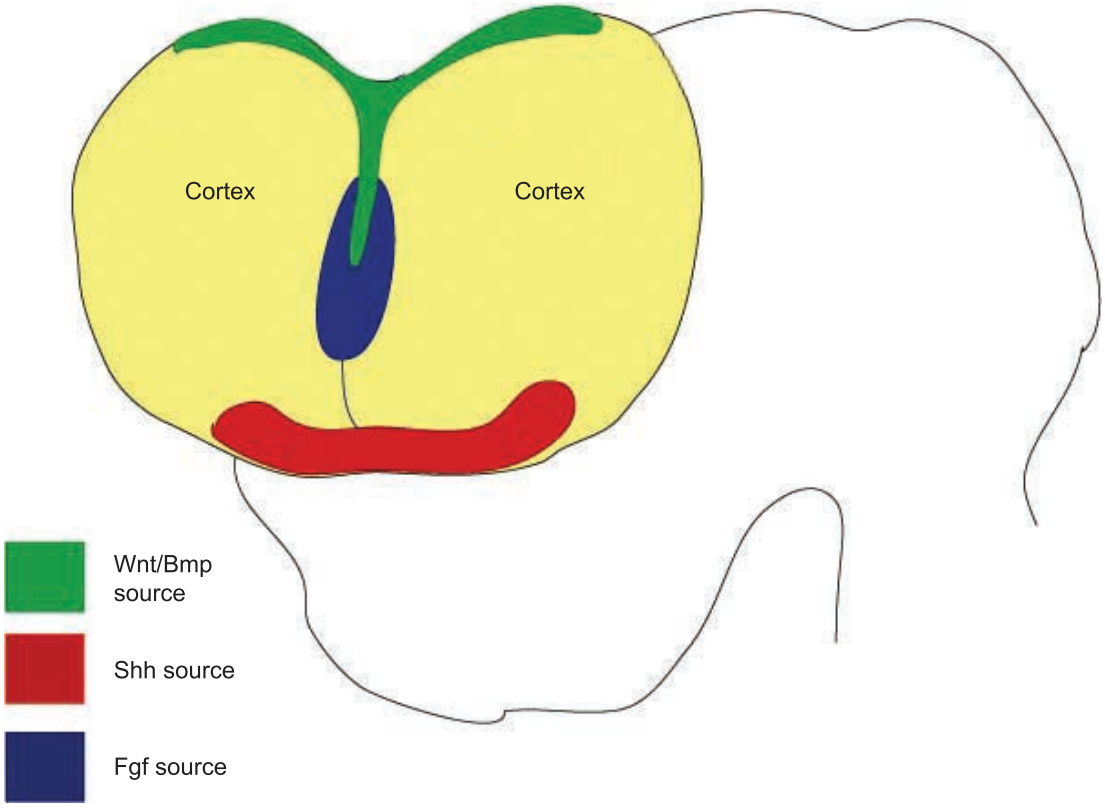

Figure 5: The developing telencephalon secretes Wnt, Fgfs and Bmps from telencephalic signaling centers. Schematic of a developing mouse brain at embryonic day 10.5 showing the three signaling centers expressing Shh, Fgf, and Wnt/Bmp family members.

fate, and when Lhx2 null patches are created in the dorsal telencephalon, ectopic hems are seen. Each ectopic hem induces the formation of a hippocampus adjacent to it, indicating that the hem functions as a hippocampal organizer.[14] The earliest Wnt gene expressed in the cortical hem, Wnt3a, is the most likely candidate for hippocampal field specification. In the Wnt3a knockout mouse, the hippocampus is largely missing.[9]

The rostral signaling center secretes Fgf family members of which Fgf8 plays a dominant role in creating the neocortical area map. When Fgf signaling is inhibited, the cortical area map shifts rostrally, and when it is augmented, the area map shifts caudally.[8] An ectopic caudal source of Fgf8 is capable of inducing a mirror image pattern of the cortical area map,[8] much like the mirror-image duplication of digits resulting from an ectopic source of shh in the limb bud (Fig. 2).

A beautiful feature of telencephalic patterning is that its three signaling centers cross-regulate each other. Shh inhibits Fgf8 and Bmp4 expression, which is consequently increased in the Shh mutant.[18] On the other hand, enhancing Bmp4 represses Shh and Fgf8.[18,25] Enhancing Fgf8 restricts the Wnt expression domain.[25] Therefore, the three telencephalic signaling centers maintain a balance of signaling molecules so that the complex pattern of the telencephalon may be generated.

In summary, patterning a complex structure involves controlling the size of the organizer and responding tissue, interactions between different organizers and their morphogens, and also bidirectional regulation of target molecules by the morphogens. It is interesting to note that the same group of signaling molecules are used throughout the developing embryo to pattern vastly different structures. Permutations and combinations of their interactions create vastly distinct genetic networks that create complexity from simple embryonic tissues.

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