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Organizing the DV axis during planarian regeneration

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During regeneration, lost structures are rebuilt and perfectly integrated within the remaining non-injured tissues. This fascinating process captured the attention of one of the founders of modern genetics, T.H. Morgan. He was particularly interested in understanding regeneration in freshwater planarians, which can regenerate a whole animal from a small piece of their bodies. He performed numerous experiments to understand how polarity is re-established such that an anterior-facing wound regenerates a head whereas a posterior-facing wound regenerates a tail. However, it has not been until more than 100 years later that the molecules required to determine axial polarity have been identified. Several studies have now shown that the Wnt/β-catenin and Hedgehog pathways are required for anteroposterior axis specification, whereas the establishment of the planarian dorsoventral (DV) axis relies on the Bone Morphogenetic Protein (BMP) pathway. Two recent papers have now uncovered additional conserved (anti-dorsalizing morphogenetic protein) and novel (noggin-like genes) elements that regulate planarian DV axis regeneration. Here, we summarize those results and present new data and hypotheses to explain the role that noggin-like genes might play.

Key words: planarian, dorsoventral axis, BMP, noggin, noggin-like, ADMP, nervous system, regeneration, patterning, neurogenesis

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Similarly to its function during invertebrate embryonic development, the BMP pathway is required to specify and maintain dorsal identity during planarian regeneration and homeostasis.[1-3] Thus, loss-of-function of the ligand *bmp* or the intracellular elements *smad1* or *smad4-1* results in partial ventralization; an ectopic central nervous system (CNS) and a mouth opening differentiate dorsally, expression of some dorsal markers disappears and dorsal epithelial cilia adopt a ventral-like pattern.[1-4] Silencing of the BMP pathway does not result in fully ventralized animals, however, as new DV boundaries appear dorsally[1,2] and some dorsal structures and cell types such as the eyes and the *nanos*-positive presumptive male primordial germ cells[5-7] do not disappear but rather differentiate deeper inside the mesenchyme.[1,2] The resulting phenotype appears to involve two ventral sides connected by an intermediate dorsal region. On the other hand, the silencing of planarian *noggins*, well-known antagonists of BMP signaling, yields complementary dorsalizing phenotypes in which ectopic outgrowths expressing dorsal markers and differentiating eyes eventually develop in the anterior ventral region.[4]

Although in vertebrates BMP signaling promotes ventral fates, during Xenopus development both dorsal and ventral signaling centers serve as sources of BMPs and their modulators (reviewed in ref. 8–10). For instance, the ligand ADMP (anti-dorsalizing morphogenetic protein, a member of the BMP family) and antagonists such as Chordin and Noggin are secreted by the dorsal center, whereas BMP4 and BMP7, and other antagonists are secreted ventrally. Together, these proteins configure a complex self-regulatory circuit that restricts BMPs and ADMP signaling to the ventral region of the embryo.[8,9,11] Thus, the dorsal side has the lowest BMP signaling and the ventral side the highest.

Recently, our group and others have reported that a BMP/ADMP regulatory circuit directs re-establishment of DV polarity in invertebrates.[4,12] As expected,

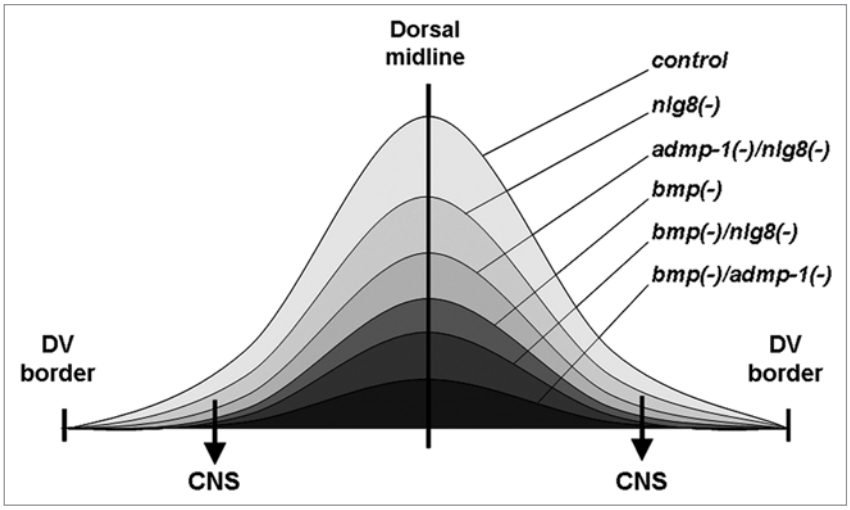

Figure 1. Graphical representation of a proposed gradient of BMP signaling in the dorsal region of the planarian and its hypothetical reduction after different RNAi knockdowns. After *nlg8* silencing the decrease in BMP signaling around the midline would not be sufficient to affect DV patterning. However, in the lateral regions, the reduction of BMP activity would eliminate the antineurogenic effect of the pathway and allow the differentiation of an ectopic CNS (arrows). Only after *bmp* silencing, alone or in combination, would the reduction in the level of BMP activity around the midline result in ventralized planarians.

planarian *bmp* and *admp-1* show complementary expression patterns along the dorsal and ventral midlines, respectively.[1,3,4,12,13] As in Xenopus, activation of BMP signaling inhibits *admp-1* expression whereas ADMP activity promotes *bmp* expression.[12] Further evidence for this BMP/ADMP circuit comes from the observation that co-silencing of *bmp* and *admp-1* strengthens *bmp* loss-of-function phenotypes.[4,12] Despite these similarities between planarians and Xenopus, simultaneous inhibition of *bmps* and *admp* homologs in planarians does not lead to the catastrophic loss of DV polarity seen in Xenopus,[11] suggesting that additional molecules may play a role in planarian DV patterning.[4,12]

The planarian BMP/ADMP circuit seems to be regulated by canonical antagonists of the noggin family as well as by novel noggin-like genes (nlg).[4,14] The unexpected activity of this family of novel regulatory elements adds a new step in the complex regulation of DV axis establishment. In contrast to *noggins*, the silencing of planarian *noggin-like gene 8* (*nlg8*) yields similar phenotypes to those obtained in *bmp* knockdowns, with ectopic dorsal CNS differentiation. Unlike *bmp* silencing, however, *nlg8* knockdown does not cause thickening, DV border duplication or disappearance of dorsal markers, and although cilia are reduced in the dorsal lateral regions, ventralization of the stereotypical pattern of dorsal cilia does not occur.[4] In addition, whereas *bmp* or *smad1* loss-of-function results in a posterior-to-anterior differentiation of ectopic dorsal nerve cords (connected to the ventral nerve cords at the tail end), after *nlg8* silencing the ectopic CNS appears preferentially in the anterior region and, in some cases, seems to connect with a dorsal expansion of the ventral cephalic ganglia.[1,4] Finally, although *nlg8* silencing produces essentially neural phenotypes, the fact that its co-silencing enhances both the dorsalization obtained after *noggins* loss-of-function and the ventralization produced by the inhibition of BMP signaling[4] suggests that this novel element may be involved in DV patterning. Therefore, *nlg8* appears to have a dorsalizing or ventralizing effect depending on the context, as has been shown for some antagonists of the BMP pathway in other organisms (reviewed in ref. 9).

In organisms as distant as Drosophila and Xenopus, the establishment of the DV axis and the specification of neural and ectodermal territories are closely related processes.[10,15] During early development, the BMP pathway acts as a potent antineurogenic factor and, consequently, neural territories are specified in the region of lowest BMP signaling. Accordingly, the overactivation or inhibition of the BMP pathway in Xenopus embryos ventralizes (expansion of the ectodermal territory) or dorsalizes (expansion and/or duplication of neural territories) those embryos, respectively.[11] Whereas the overexpression of planarian *nlg8* in Xenopus embryos results in the ventralization of the embryo and reduces the expression domain of the neural marker *sox2*, its silencing in planarians does not severely affect the DV axis but mainly promotes ectopic neural differentiation.[4] On this basis, we hypothesize that, in planarians, *nlg8* knockdowns reduce a threshold of BMP activity required for its antineurogenic role without further affecting DV patterning (**Fig. 1**).

Irrespective of the effect of *nlg8* silencing on the level of BMP signaling, *nlg8* or *bmp* silencing always generates an ectopic dorsal CNS in a position mirroring that of the ventral nervous system.[1-4] Throughout the evolution of the Platyhelminthes, there seems to have been a reduction in the number of nerve cords, as some groups present several pairs of nerve cords both ventrally and dorsally.[16] Therefore, an evolutionary explanation for the position of the ectopic CNS could be that only specific dorsal regions are competent to differentiate such tissues. Such a possibility is also consistent with the observation that strictly asexual planarians have two dorsal rows of cells expressing the germ cell marker *nanos* in a pattern reminiscent of the position of the testis in sexual animals.[5-7]

Remarkably, noggin-like genes are evolutionarily conserved and can be found from cnidarians to vertebrates.[4] In agreement with the functional studies in planarians, the overexpression of the Xenopus *noggin-like* mRNA into Xenopus embryos phenocopies *bmp* overexpression and produces ventralized embryos. However, unexpectedly, this ventralization does not occur due to increased SMAD1/5/8 phosphorylation (a readout of BMP signaling activation).[4] Thus, the explanation for the opposing functions of noggins and noggin-likes remains unclear.[4] One area that deserves further attention is the correlation between structure and function.

Noggin-like genes lack a heparin-binding domain in their N-terminal region and bear an amino acidic insertion within the conserved noggin domain.[14] Interestingly, the removal of the amino-acid insertion from Xenopus nlg yields dorsalized embryos.[4] These results indicate a relevant function for the insertion, perhaps via an effect on binding to BMP or other factors involved in DV patterning (**Fig. 2**). Alternatively, the absence of a heparin-binding domain in noggin-like genes could increase their capacity to diffuse through the extracellular matrix.

Finally, these data suggest that the phenotypes obtained after silencing or overexpression of *nlg* could be explained as the result of either noggin-like modulating BMP activity through a pSMAD1/5/8-independent mechanism or an effect on pathways other than BMP. In order to clarify the mechanism of action of noggin-like and its exact relationship with BMP signaling, biochemical studies will be required to asses the interactions between this novel family and the other elements of the pathway.

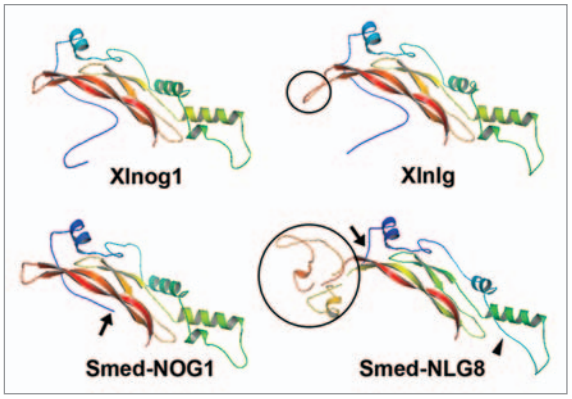

Figure 2. Comparison of the predicted protein structures of noggin and noggin-like proteins. Homology-based protein structures of Xenopus noggin1 (Xlnog1), planarian NOGGIN1 (Smed-NOG1), Xenopus noggin-like (Xlnlg) and planarian NOGGIN-LIKE 8 (Smed-NLG8) constructed by the Swissmodel program[17] and further modified using PyMOL software (DeLano Scientific LLC, http://pymol.sourceforge.net/) are shown. The structure of planarian NOGGIN shows a high degree of similarity to the Xenopus noggin1 homolog, although its BMP-binding domain is shorter (arrow). With the exception of the insertion (black circles), the predicted structures of the noggin-like homologs resemble those of noggins. Although the length of the amino-acid insertion varies between species,[4] its presence could probably modify the binding capacities of noggin-like homologs. Note that a helix is absent in Smed-NLG8 (arrowhead). Accession numbers: AAT91717, ABV04323, NP_001089147, ACO06233.

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