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On dorsal/ventral-specific genes in the iris during lens regeneration

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This paper is dedicated to the memory of a friend and mentor Dr. Victor Mitashov

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Abstract. Recent experiments on gene expression during lens regeneration in adult newts have revealed that both the regeneration-competent dorsal iris and the regeneration-incompetent ventral iris are quite active in expressing important regulatory genes. In this paper we outline some of the issues pertaining to this dorsal/ventral specificity and identity. (Part of a Multi-author Review)

Keywords. Newt, regeneration, dorsal/ventral iris, bone morphogenetic proteins (BMPs).

The dorsal iris pigment epithelium is the source of cells that contribute to lens regeneration after lentectomy in the adult newt. One of the interesting features of this type of regeneration is that the same cells from the ventral iris do not participate in the regeneration process, obviously to avoid the formation of two lenses [1, 2]. It has been thought, therefore, that the specific activation of 'regeneration-inducing' factors occurs in the dorsal iris only. Recently, it was possible to coax the ventral iris to transdifferentiate and form a lens [3]. The responsible factors were involved in bone morphogenetic protein (BMP) signaling inhibition (Fig. 1). In particular, we found that inhibition of the BMP action induced the ventral iris to transdifferentiate to lens. Also, *six-3* overexpression along with retinoic acid treatment was able to elicit a lens from the ventral iris as well. When expression of key genes involved in the actions of these factors was studied, it was found that they were expressed in the ventral iris as well (Fig. 2). We examined the expression of *six-3, pax-6* and *BMPR1A*. We found that these genes were expressed in both dorsal and ventral intact iris and that upon lentectomy there was a gradual increase in *six-3* expression in the dorsal iris. We have also expanded expression studies using a microarray of several hundred complementary DNAs with the same conclusions [4]. So, if these genes are expressed in both, what gives an 'identity' of dorsal or ventral to the iris?

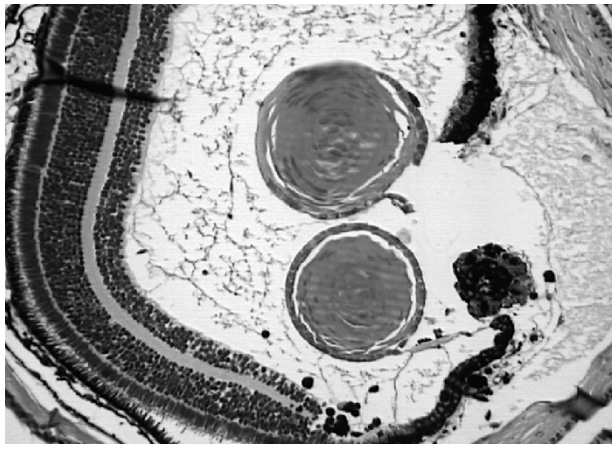

Figure 1. Induction of lens regeneration in a ventral iris explant (bottom lens) treated with the bone morphogenetic protein (BMP) inhibitor chordin. The host-regenerating lens from the dorsal iris is on the top (from [3]).

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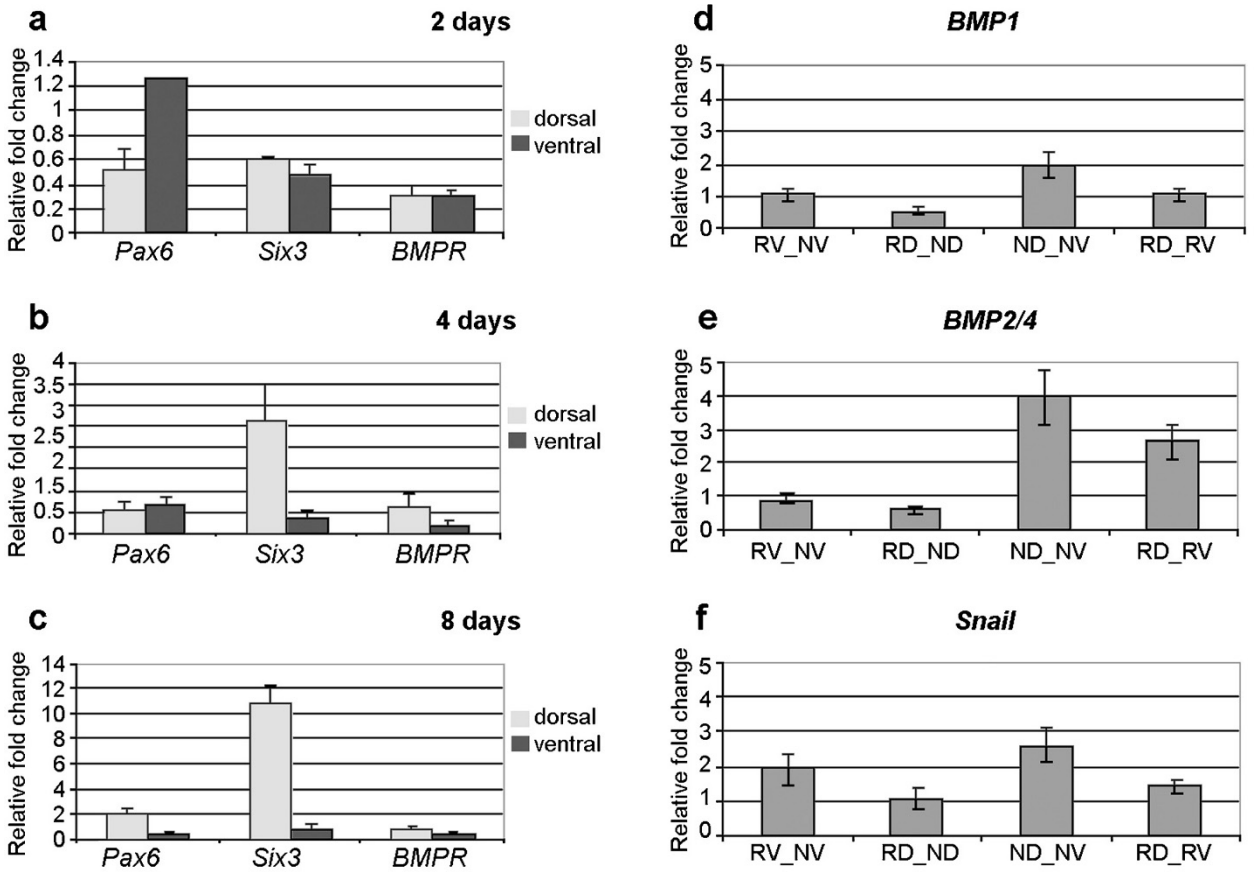

Figure 2. (*a–c*) Expression of *pax-6, six-3* and *BMPR-IA* during the initial stages of lens regeneration, 2, 4 and 8 days after lentectomy. Expression in both dorsal and ventral iris is compared with the levels of the intact iris. Gradual increase in the dorsal iris can be seen, especially for *six-3* (from [3]). (*d–f*) Comparison of expression levels for newt *BMP-1, BMP2/4* and *snail* in the dorsal and ventral iris either regenerating at day 8 after lentectomy or from intact newts. Comparisons are between regenerating over intact ventral iris (RV_NV), regenerating over intact dorsal iris (RD_ND), dorsal over ventral iris in intact newts (ND_NV) and regenerating dorsal over regenerating ventral iris (RD_RV). Note a pattern of *snail* up-regulation in the ventral iris during regeneration (RV versus NV). RD, regenerating dorsal; RV, regenerating ventral; ND, non-regenerating dorsal; NV, non-regenerating ventral.

Is it that elevation over established thresholds might be the key to lens regeneration induction? Interestingly, inhibition of the BMP pathway is also responsible for dorsalizing the embryo, while activation is a ventralizer [5–7]. In fact, it has been established that dorsal-ventral cell communication is controlled by a network of interacting secreted factors that can also antagonize each other [6]. For example, the dorsal part of the embryo secretes chordin and anti-dorsalizing morphogenetic protein (Admp), and the ventral part secretes, tolloid-related (BMP-1) and sizzled (Fig. 3). Since transdifferentiation in the iris of the newt eye seems to be regulated by these dorsal-ventral factors, it could be possible that the early patterning mechanisms of dorsal-ventral identity have been maintained in the adult newt eye and that this might provide the identity of the dorsal iris and a crucial difference (or advantage) over the ventral iris that might result in lens regeneration. Because of this and the obvious involvement of the BMP pathway in lens regeneration, we wanted to examine expression of some BMP signaling factors that are involved in the dorsal-ventral network to ascertain whether any of these genes would 'recapitulate' an expression pattern that will follow their pattern during embryogenesis. This network in *Drosophila* has nearly 90 genes involved in elaborate interactions [8].

To obtain some insight into this idea, expression of the newt ortholog of *decapentaplegic (dpp), BMP2/4* and *tolloid-related metalloproteinase (BMP-1)* was examined. BMP2/4 is an activator of ventral-specific genes during embryogenesis. The expression of *snail* was also evaluated. BMP-1 is ventral-specific; it inhibits chordin and thus allows release of BMPs, and this action is blocked by its antagonist, sizzled. *Snail* is activated by Dorsal and is a repressor of ventral neurogenic territory during development. For the readers' convenience, these genes in the frog and *Drosophila* contexts are highlighted in the networks presented in Figure 3. For *BMP2/4, BMP1* and *snail* a

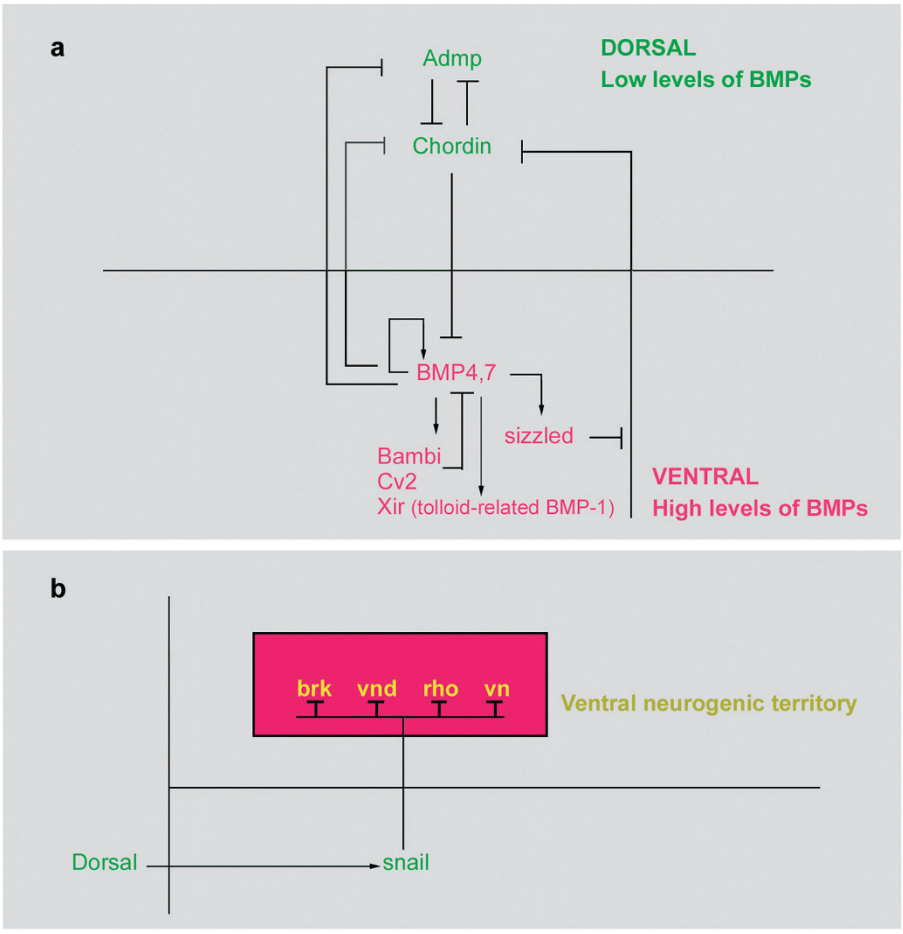

Figure 3. (*a*) A network which regulates dorsal-ventral cell communication in the frog embryo. The dorsal center secretes chordin and its antagonist Admp. Likewise, the ventral center secretes xolloid-related metalloproteinase (BMP-1) and its antagonist sizzled (adapted from [6]). (*b*) A part of the dorsal-ventral network in *Drosophila*, indicating control of snail by dorsal and inhibition of ventral neurogenic-specific genes by snail (adapted from [8]).

very similar pattern emerged when their expression was examined with QPCR (Fig. 2). The results show an interesting regulation during regeneration: While the levels are higher in the intact dorsal iris, during regeneration, this difference is smaller, something that might indicate downregulation in the regenerating dorsal iris, which will be consistent with 'dorsalization'. Notably, levels in the ventral iris were increased during regeneration, which is consistent with 'ventralization'.

From these results we can say that a dorsal-ventral identity in the adult newt iris is marked by expression of genes that do the same task during early development. This is quite interesting and might bear significance for the induction of lens regeneration and calls for significant expansion of this line of research. As has been quite elegantly demonstrated in several studies, this network is characterized by the presence of dorsalizing and ventralizing factors as well as by antagonist produced locally [6]. For example, in the developing frog embryo, the dorsal center secretes chordin, but also the anti-dorsalizing morphogenetic protein (Admp) (Fig. 3). It has been suggested that the diffusion rate of these activator-inhibitor pairs is different and that this might account for their specific action in establishing patterns. If such factors establish dorsal-ventral identity in the adult newt iris that is correlated with lens regeneration, their lack (or regulation) might account for the lack of lens regeneration in other species. This, of course, seems quite elaborate and complicated, but the fact that induction from incompetent iris is possible testifies to how important it is to pursue this line of research in order to understand a very basic biological phenomenon. A detailed analysis of expression of BMP inhibitors in the dorsal iris and comparison with the regeneration-incompetent relative salamander *Ambystoma mexicanum* might be quite informative in elucidating this important mechanism.

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