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Bmp signaling promotes intermediate mesoderm gene expression in a dose-dependent, cell-autonomous and translation-dependent manner

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Abstract

The intermediate mesoderm lies between the somites and the lateral plate and is the source of all kidney tissue in the developing vertebrate embryo. While bone morphogenetic protein (Bmp) signaling is known to regulate mesodermal cell type determination along the medio-lateral axis, its role in intermediate mesoderm formation has not been well characterized. The current study finds that low and high levels of Bmp ligand are both necessary and sufficient to activate intermediate and lateral mesodermal gene expression, respectively, both in vivo and in vitro. Dose-dependent activation of intermediate and lateral mesodermal genes by Bmp signaling is cell-autonomous, as demonstrated by electroporation of the avian embryo with constitutively active Bmp receptors driven by promoters of varying strengths. In explant cultures, Bmp activation of Odd-skipped related 1 (Odd-1), the earliest known gene expressed in the intermediate mesoderm, is blocked by cyclohexamide, indicating that the activation of Odd-1 by Bmp signaling is translation-dependent. The data from this study are integrated with that of other studies to generate a model for the role of Bmp signaling in trunk mesodermal patterning in which low levels of Bmp activate intermediate mesoderm gene expression by inhibition of repressors present in medial mesoderm, whereas high levels of Bmp repress both medial and intermediate mesoderm gene expression and activate lateral plate genes.
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Introduction

Secreted growth factors of the bone morphogenic protein (Bmp) family establish concentration gradients that contribute to patterning along the embryonic medio-lateral axis (in other embryos, such as *Drosophila* and *Xenopus*, this axis is "dorso-ventral"). In several contexts in both *Drosophila* (Holley and Ferguson, 1997; Podos and Ferguson, 1999; Rusch and Levine, 1996) and vertebrates (Harland and Gerhart, 1997; Hogan, 1996; Niehrs et al., 2000), the level of Bmp signaling along the medio-lateral axis correlates with cell fate. In vertebrate, mesoderm high levels of Bmp signal have been found to promote formation of lateral structures such as blood; intermediate levels to promote intermediate structures such as kidney; and lower levels are associated with muscle and notochord development (Dosch et al., 1997; Jones et al., 1996).

Two specific mechanisms have been proposed to explain dose-dependent activation of target genes by Bmp. One proposed mechanism is that components of the Bmp pathway interact with regulatory regions of Bmp-responsive genes in a concentration-dependent manner. In the case of *Drosophila* Decapentaplegic (Dpp, the *Drosophila* Bmp-2/4 homolog), enhancer elements can have differential binding affinity for the Dpp transducer Mothers against dpp (Mad) (Wharton et al., 2004): high-dose responders have low affinity Mad binding sites and are transcribed only in response to high concentrations of Mad, whereas low-dose responders have high affinity Mad binding sites and are transcribed in response to both low and high concentrations of Mad. Alternatively, in the *Drosophila* wing and ectoderm, autonomous expression of some low-dose Bmp responders does not require Mad. Instead, in the absence of Dpp signal, the protein Brinker represses transcription of these genes (Campbell and Tomlinson, 1999; Jazwinska

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et al., 1999a; Minami et al., 1999). Low doses of Dpp facilitate Smad-dependent silencing of Brinker (Jazwinska et al., 1999a,b; Pyrowolakis et al., 2004) and indirect activation of the low-dose responders.

In vertebrates, Bmp responders such as Msx-1/2, Id-1/2/3 and others can be directly activated by Bmp signaling in vitro (Hollnagel et al., 1999), and Smad binding sites in the Msx-2 enhancer are necessary for its expression (Brugger et al., 2004). However, it is unclear how differential doses of Bmp signaling activate tissue-specific genes during vertebrate mesodermal patterning in vivo. We sought to address this issue by studying the formation of the avian intermediate mesoderm (IM).

The IM is a strip of tissue, located between the developing somites and lateral plate, and which is the source of all kidney tissue in the body (Sainio and Raatikainen-Ahokas, 1999). Shortly after gastrulation, the transcription factors Odd-1 (previously known as Osr-1) (So and Danielian, 1999) (Fig. 1A), Pax-2 (Dressler et al., 1990) (Fig. 1B) and Lim-1 (Fujii et al., 1994) (Fig. 1C) are expressed in the developing IM. Reports in *Xenopus* and zebrafish have shown that Bmp signaling is necessary for the expression of Lim-1 and Pax-2 (Kishimoto et al., 1997; Mullins et al., 1996), and the presence of nuclear localized phosphorylated Smad-1 protein in the IM demonstrates that it is an area of active Bmp signaling (Faure et al., 2002).

In this report, we demonstrate that low, but not high doses of Bmp signal activate transcription of Lim-1, Pax-2 and Odd-1 in a cell-autonomous, but translation-dependent manner. Combining our findings with those of others, we propose a two-part model to explain how low-dose Bmp response genes are activated specifically in the intermediate mesoderm. First, low-doses of Bmp signal promote transcription of Odd-1, Lim-1 and Pax-2 in the IM by inhibiting a repressive activity that is present in somitic mesoderm. Second, high doses of Bmp signal activate additional inhibitors in the lateral plate, which restrict the expression of Odd-1, Pax-2 and Lim-1 to the intermediate mesoderm.

Materials and methods

Cloning of chick Odd-1

A portion of the mouse Odd-skipped related 1 gene (mOdd-1) (So and Danielian, 1999) corresponding to the coding part of the three zinc finger motifs was used to probe 5×10^5 plaques from an HH stage 11–14 chick embryo lambda ZapII Phage library (Nieto et al., 1994). Three plaques were identified, one of which contained a full-length coding sequence for a gene 81% identical to mOdd-1 at the amino acid level. This clone, which is named chick Odd-1 (cOdd-1), will be described in detail in a separate publication (R.G.J. and T.M.S., in preparation).

Expression plasmids

Constitutively active Alk3 and Alk6 (caALK3 and caAlk6) were obtained from L. Niswander (Zou et al., 1997) and subcloned into pCIG (Faure et al., 2002), which drives gene expression from a combination of chick beta actin enhancer and CMV promoter and which contains an Internal Ribosomal Entry Sequence (IRES) driving nuclear Green Fluorescence Protein. They were also subcloned into pCS2+, which drives gene expression from a CMV promoter/enhancer. pCAGGSdsRed was obtained from C. Cepko (Matsuda and Cepko, 2004).

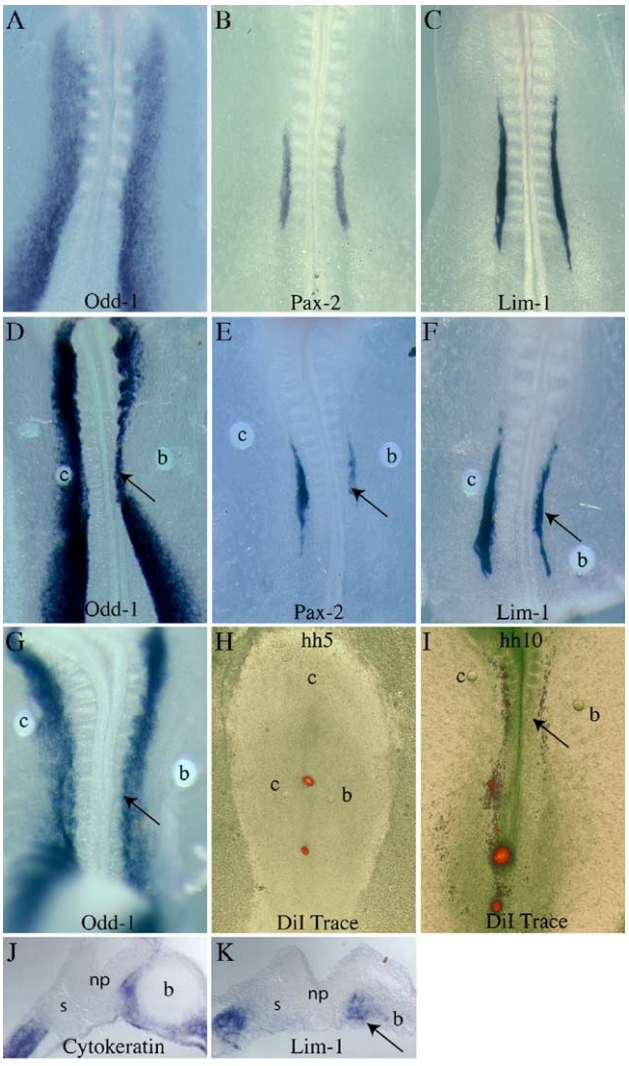

Fig. 1. Exogenous Bmp protein can induce IM gene expression in the paraxial mesoderm. (A–C) Expression of Odd-1 (A), Pax-2 (B) and Lim-1 (C) in stages 10–11 control embryos. (D–F) Effects of a heparin-acrylic bead soaked in recombinant human Bmp-2 (b) placed in the embryo at stages 5–6 and cultured until stages 10–11. A control bead (c) was placed on the opposite side. On the Bmp-treated side, expressions of Odd-1 (D), Pax-2 (E) and Lim-1 (F) are all moved towards the midline (arrows, D, E, F, G, K), into the region where paraxial genes are normally expressed. Note that in some regions expression of IM genes is also decreased on the treated side. (G–I) Bmp-2 affects the identity of paraxial mesoderm cells and not their migration. After labeling prospective paraxial mesoderm with DiI at HH stage 5, when it resided in the primitive streak (H), a Bmp-2 bead was placed in lateral plate, and embryos were cultured through stage 10 (I) when they were processed for expression of Odd-1 by in situ hybridization (G). Comparison of treated and control sides at stage 10 reveals that Odd-1 was expressed more medially on the Bmp-treated side (G), while migration patterns of the prospective paraxial mesoderm were unchanged (H, I). (J–K) Sections of Bmp-treated embryos. In Bmp-treated embryos, lateral plate (J, Cytokeratin) and intermediate mesoderm (K, Lim-1) gene expression moved towards the midline. np, neural plate; s, somite.

In situ hybridization

RNA probes were generated for chicken Odd-1 (this manuscript), Pax-2 (Burrill et al., 1997; Herbrand et al., 1998), Lim-1 (Tsuchida et al., 1994), Paraxis (Barnes et al., 1997), Tbx-6L (Knezevic et al., 1997) and cytokeratin (Tonegawa et al., 1997) using standard methods, as previously described (Schultheiss et al., 1995). Whole mount in situ hybridization was performed as previously described (Schultheiss et al., 1995).

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