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# The secreted EGF-Discoidin factor xDel1 is essential for dorsal development of the *Xenopus* embryo

Akiko Arakawa [a,c,1], Mami Matsuo-Takasaki [a,1], Akira Takai [a,b], Hidehiko Inomata [a], Michiru Matsumura [a], Makoto Ikeya [a], Kenzo Takahashi [c], Yoshiki Miyachi [c], Noriaki Sasai [a], Yoshiki Sasai [a,b,*]

[a] *Organogenesis and Neurogenesis Group, Center for Developmental Biology, RIKEN, 2-2-3 Minatojima-minamimachi, Chuo, Kobe 650-0047, Japan*
[b] *Department of Medical Embryology, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan*
[c] *Department of Dermatology, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan*

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**Abstract**

We show here that a secreted EGF-Discoidin-domain protein, *Xenopus* Del1 (xDel1), is an essential factor for dorsal development in the early *Xenopus* embryo. Knockdown of the xDel1 function causes obvious ventralization of the embryo. Conversely, overexpression of *xDel1* expands dorsal-marker expression and suppresses ventral-marker expression in the gastrula embryo. Forced expression of *xDel1* dorsalizes ventral marginal zone explants, whereas it weakly induces neural differentiation but not mesodermal differentiation in animal caps. The dorsalizing activity of xDel1 is dependent on the Discoidin domains and not on the RGD motif (which is implicated in its angiogenic activity) or EGF repeats. Luciferase assays show that *xDel1* attenuates BMP-signaling reporter activity by interfering with the pathway downstream of the BMP receptor. Thus, xDel1 functions as a unique extracellular regulatory factor of DV patterning in early vertebrate embryogenesis.


*Keywords:* DV axis; Secreted factor; Del1; Discoidin; BMP; *Xenopus*

## Introduction

In early vertebrate development, the embryo is patterned along two main axes: the dorsal–ventral (DV) and rostral–caudal (RC) axes. In *Xenopus*, the DV polarity is established following sperm entry and cortical rotation by the dorsally dominant activation of canonical Wnt signaling (Moon and Kimelman, 1998; Heasman, 2006). With regard to mesoendodermal patterning, the DV and RC axes are closely associated until the onset of gastrulation, when the two are separated by the rostral migration of head mesoendodermal tissues from the dorsal marginal zone (DMZ) (Keller, 2005).

Dorsal determination in the early *Xenopus* embryo is controlled by at least three signaling pathways in a stage-specific manner. Canonical Wnt signaling acts during the early cleavage stages as described above, and it also plays an inductive role for dorsal gene expression (e.g., *Siamois* and *Chordin*) during the late blastula and early gastrula stages (Carnac et al., 1996; Heasman, 2006). Nodal/Activin signals promote dorsal mesodermal differentiation during the mid- to late-blastula stages in a dose-dependent fashion (Osada and Wright, 1999). Nodal/Activin signaling is unique in that it can induce mesodermal tissues directly from uncommitted animal cap cells (Smith, 1995). BMP antagonists, such as Chordin and Noggin, are considered to function from the late blastula stages onwards by inhibiting the ventralizing activity of the BMP signals (Sasai et al., 1995; Sasai and De Robertis, 1997; Piccolo et al., 1996; Zimmerman et al., 1996). Although BMP inhibition plays a role in DV patterning at relatively late stages (late blastula ~ early gastrula), a recent report using a combination of MOs has demonstrated that BMP antagonists are indispensable for the development of dorsal structures (Khokha et al., 2005). BMP signaling controls DV patterning via an intricate system

* Corresponding author. Organogenesis and Neurogenesis Group, Center for Developmental Biology, RIKEN, 2-2-3 Minatojima-minamimachi, Chuo, Kobe 650-0047, Japan. Fax: +81 78 306 1854.
*E-mail address:* sasaicdb@mub.biglobe.ne.jp (Y. Sasai).
[1] These authors contributed equally to this work.

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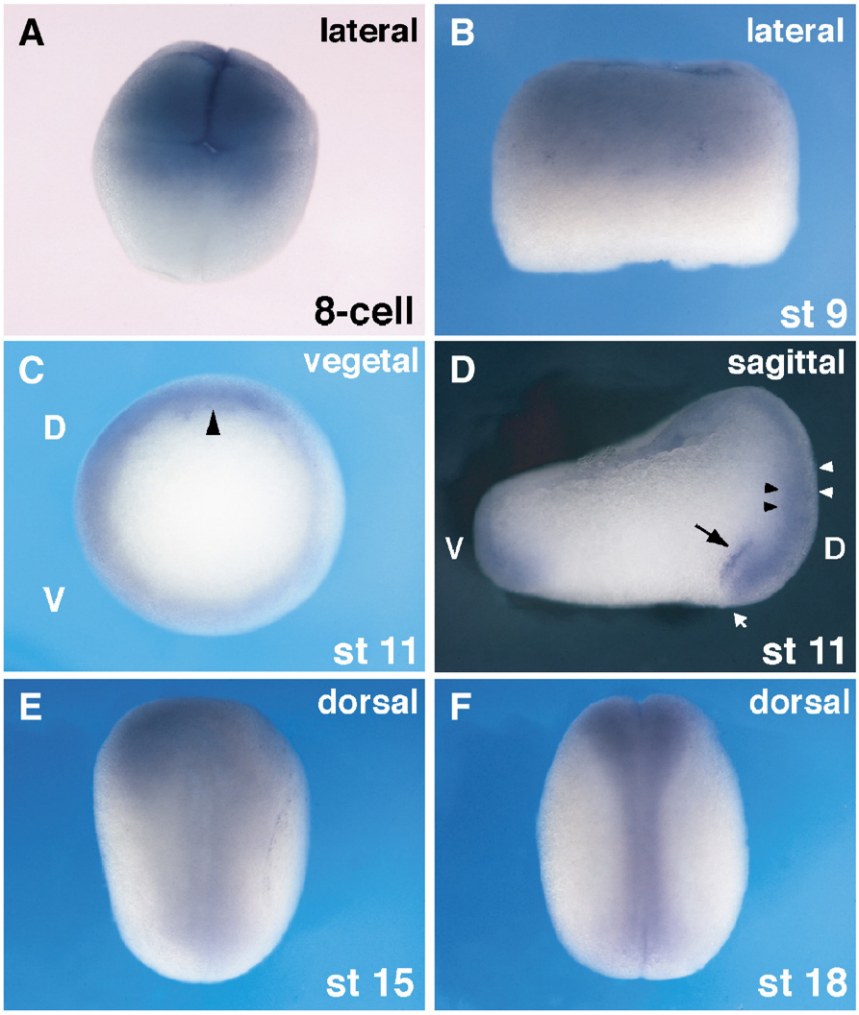


Fig. 1. Spatial and temporal pattern of *xDel1* expression analyzed by whole-mount in situ hybridization. (A) The 8-cell stage, lateral view. (B) Stage 9, lateral view. (C) Stage 11, vegetal view. Arrowhead, dorsal lip. (D) Stage 11, sagittal section across the dorsal lip (white arrow). Black arrow, bottle cells. Open and closed arrowheads, dorsal ectoderm and mesoderm, respectively. (E) Mid-neurula stage, dorsal view. (F) Late-neurula stage, dorsal view.

(Hogan, 1996), which involves multiple ligands, receptors, antagonists, modulators of antagonists (De Robertis et al., 2000), promoting factors (Little and Mullins, 2004; Rentzsch et al., 2006) and extracellular matrix proteins (Ohta et al., 2004; Moreno et al., 2005). However, how these signals are orchestrated to form a signaling network leading to the clear embryonic DV pattern remains elusive.

In this study, we introduce an extracellular protein, Del1 (Developmentally regulated endothelial cell locus 1), as a new signaling factor for dorsal determination in *Xenopus*. *Del1* was first isolated as an embryonic endothelial-specific gene in a mouse enhancer-trap study (Hidai et al., 1998). *Del1* encodes a secreted protein with two or three Notch-like EGF repeats (depending on the splicing form), an RGD motif, and two Discoidin domains (Supplementary Fig. S1), and is structurally related to *SED1*, which is involved in sperm–egg binding (Ensslin and Shur, 2003). Secreted Del1 protein associates with extracellular matrices such as the basement membrane (Hidai et al., 1998). Although Del1 promotes angiogenesis and the remodeling of blood vessels (Penta et al., 1999), little is known about its role in early embryogenesis. In this report, we show that xDel is essential for DV patterning in the *Xenopus* embryo. Both gain- and loss-of-function studies indicate that xDel promotes dorsalization of the mesoderm in the gastrula embryo. We also show that this dorsalizing effect does not depend on the RGD motif, which is essential for the angiogenic activity, but on the Discoidin domains.

## Materials and methods

### *Isolation of xDel1, plasmid construction and in vitro transcription*

A partial chick *Del1* cDNA was isolated in a signal-sequence-trap screen using a retrovirus-mediated expression system, SST–REX (Kojima and Kitamura, 1999) of an HH9-11 chick head cDNA library. For the isolation of the frog counterpart, we designed primers according to a database sequence (AY491055), amplified full-length *xDel1* cDNAs from *Xenopus* neurula mRNA by RT–PCR, sequenced and subcloned them into *pCS2+* vector. During this process, we noticed that *xDel1* cDNAs have two alternative splicing variants, which encode two and three EGF repeats, respectively (*xDel1-E2* and *xDel1-E3*; Supplementary Fig. S1; both variants were expressed during early development). Injection of *xDel1-E2*