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Dose-dependent *Smad1*, *Smad5* and *Smad8* signaling in the early mouse embryo

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Abstract

Three closely related mammalian R-Smads, namely *Smad1*, *Smad5* and *Smad8*, are activated by BMP receptors. Here we have taken a genetic approach to further dissect their possibly unique and/or shared roles during early mouse development. A *Smad8.LacZ* reporter allele was created to visualize *Smad8* expression domains. *Smad8* is initially expressed only in the visceral yolk sac (VYS) endoderm and shows a highly restricted pattern of expression in the embryo proper at later stages. In addition, *Smad8* conditional and null alleles were engineered. All alleles clearly demonstrate that adult *Smad8* homozygous mutants are viable and fertile. To elucidate gene dosage effects, we manipulated expression ratios of the three BMP R-Smads. *Smad8* homozygotes also lacking one copy of *Smad1* or *Smad5* did not exhibit overt phenotypes, and the tissue disturbances seen in *Smad1* or *Smad5* null embryos were not exacerbated in the absence of *Smad8*. However, we discovered a profound genetic interaction between *Smad1* and *Smad5*. Thus, as for *Smad1* and *Smad5* mutant embryos, $Smad1^{+/-}$:$Smad5^{+/-}$ double heterozygotes die by E10.5 and display defects in allantois morphogenesis, cardiac looping and primordial germ cell (PGC) specification. These experiments demonstrate for the first time that *Smad1* and *Smad5* function cooperatively to govern BMP target gene expression in the early mammalian embryo.

Keywords: BMP signaling; *Smad1*; *Smad5*; *Smad8*; Early mouse development; Heart morphogenesis; Genetics

Introduction

Members of the TGFβ family of secreted growth factors regulate key processes during postimplantation mammalian development including embryonic axis patterning, organogenesis and specification of the germ line (reviewed in Chang et al., 2002; Massague et al., 2005). The ligands can be broadly divided into two groups, namely, the BMPs and activin/TGFβ/nodals in accordance with biological and structural criteria (reviewed in Massague and Chen, 2000; Massague and Wotton, 2000; Massague et al., 2000). Despite considerable ligand diversity, signal transduction is controlled by only a few Smad transcription factors that are activated by cell surface receptor kinases and are highly conserved across the animal kingdom including vertebrates, insects and nematodes.

A key question is whether R-Smads have unique and/or partially overlapping functions in the early embryo. It is well known that *Smad2* and *Smad3*, acting downstream of TGFβ/activin/nodal ligands, differ in their abilities to activate or repress selected target genes (Chou et al., 2003; Labbe et al., 1998; Massague et al., 2005; Seoane et al., 2004). Mice lacking *Smad2* or *Smad3* display quite different phenotypes. Loss of *Smad2* results in embryonic lethality shortly after implantation (Heyer et al., 1999; Waldrip et al., 1998), whereas *Smad3*-deficient animals are viable and fertile (Datto et al., 1999; Zhu et al., 1998). Nonetheless, our recent experiments reveal that *Smad3* coding sequences can functionally substitute for *Smad2* during mouse development (Dunn et al., 2005). These results demonstrate that the strikingly different phenotypes are not due to divergent functional activities but rather result from the unique *Smad2* expression domain in the extra-embryonic visceral endoderm (VE), essential for inducing the anterior visceral endoderm (AVE) signaling center that patterns the early embryo (Brennan et al., 2001).

Three mammalian R-Smads, namely *Smad1*, *Smad5* and *Smad8*, are activated by BMP receptors. *Smad1* and *Smad5* have been shown to play essential roles in the early mouse embryo. *Smad5* mutants display multiple embryonic and extra-

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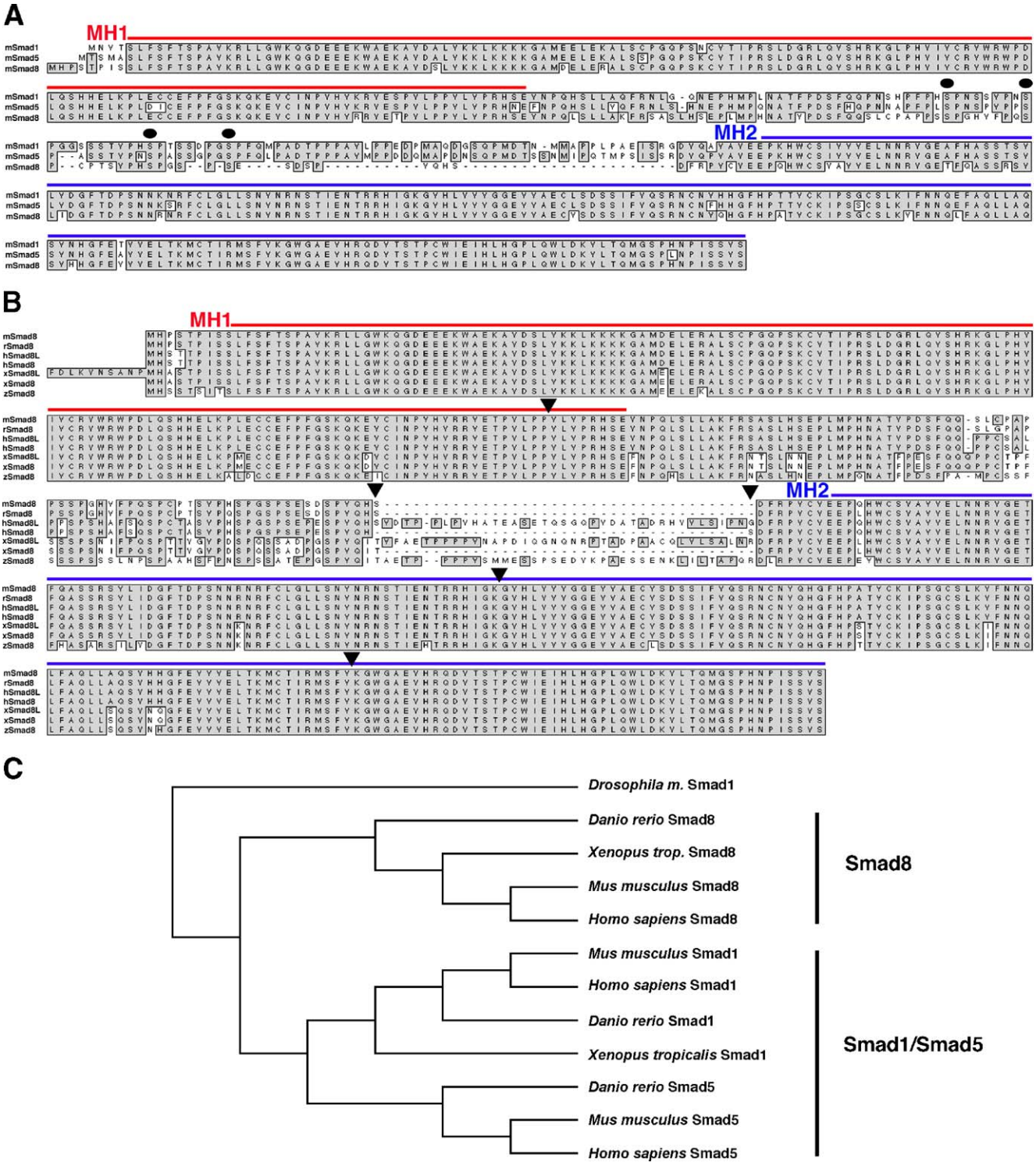

Fig. 1. *Smad1*, *Smad5* and *Smad8* sequence alignments. (A) Comparison of the *Smad1*, *Smad5* and *Smad8* amino acid sequences in mouse. Boxes show the conserved amino acids. The dots indicate serine residues within Erk-consensus motifs (PXSP). The presumptive boundaries of the MH1and MH2 domains are indicated. (B) The mouse *Smad8* (*mSmad8*) sequence is aligned with those of rat (r), human (h), frog (x) and zebrafish (z). Human and *Xenopus* longer isoforms are indicated as hSmad8L and xSmad8L, respectively. Boxes show conserved amino acids and exon boundaries are denoted by the arrow heads. Sequence alignments were performed using the Macvector software package (Accelrys.com). (C) A phylogenetic tree of BMP R-Smads shows early branching of *Smad8* from *Smad1* and *5* genes. Fruitfly (*Drosophila melanogaster*), zebrafish (*Danio rerio*), frog (*Xenopus tropicalis*), mouse (*Mus musculus*) and human (*Homo sapiens*). Phylogenetic alignment was generated using Macvector (Accelrys.com).