Available online at www.sciencedirect.com
ScienceDirect
Developmental Biology 305 (2007) 714–725
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

Genomes & Developmental Control

Transcriptional repressor and activator activities of SMA-9 contribute differentially to BMP-related signaling outputs

Jun Liang [1], Ling Yu, Jianghua Yin, Cathy Savage-Dunn *

Department of Biology, Queens College, and Biochemistry PhD Program, the Graduate School and University Center, the City University of New York, 65-30 Kissena Blvd., Flushing, NY 11367, USA

Received for publication 1 September 2006; revised 9 January 2007; accepted 27 February 2007
Available online 3 March 2007

Abstract

In the nematode *Caenorhabditis elegans*, the BMP-related growth factor DBL-1 regulates body size and male tail morphogenesis via a conserved receptor/Smad signaling pathway. Smads are transcription factors, but rely on transcription cofactors for appropriate regulation of target genes in response to TGF-β- and BMP-related signals. In the DBL-1 pathway, *sma-9* encodes multiple zinc finger transcription factors homologous to *Drosophila* Schnurri, which functions in Dpp/BMP signaling. We have studied the molecular functions of SMA-9 as a model for transcription cofactor-dependent regulation of gene expression. Using SMA-9 fusions to known transcriptional activators and repressors, we demonstrate that SMA-9 acts primarily as a transcriptional repressor in body size regulation in vivo. In contrast, both activator and repressor functions contribute to male tail patterning. We further show that different SMA-9 regions have intrinsic repressor and activator activities using a yeast transcription assay. We use microarray analysis to identify transcriptional target genes in body size regulation. Consistent with the importance of repression in mediating body size regulation, we find more repressed genes than activated genes in this pool. Finally, we identify five transcriptional targets with body size and/or male tail patterning phenotypes, including transcription factors related to *Runx* and *fos* and signaling molecules related to hedgehog and patched. Our results thus suggest that SMA-9 products function differentially as transcriptional repressors and activators in DBL-1/BMP pathway regulated body size and male tail morphogenesis.

Keywords: *sma-9*; BMP; Schnurri; Transcriptional repressor; Transcriptional activator; DNA microarray; Gene regulation; Body size

Introduction

Cell signaling pathways can elicit appropriate responses either by direct modification of cellular components, such as the cytoskeleton, or by the modulation of cell transcription profiles. Smads are signal transducers for transforming growth factor-beta (TGF-β) superfamily ligands that function by regulating gene transcription. Since DNA binding sites for Smads show low complexity and affinity, transcriptional cofactors are often required for target gene regulation (Shi et al., 1998; Massague, 1998; Derynck and Zhang, 2003). Smad complexes activate or repress gene transcription depending on whether they associate with transcriptional activators or repressors. We have previously identified a gene, *sma-9*, that encodes a putative transcription cofactor in the DBL-1 signaling pathway in the nematode *Caenorhabditis elegans*. SMA-9 protein products show motifs consistent with a role in transcriptional regulation, including a Gln-rich domain, seven zinc finger motifs, an acidic residue-rich domain and nuclear localization signals (Liang et al., 2003). Furthermore, SMA-9 is homologous to *Drosophila* Schnurri (Shn), which has been proposed to function as a transcription factor in Dpp signaling. Three vertebrate homologs of Schnurri have also been identified. These bind the κB-binding site and function in T cell development (Takagi et al., 2001; Oukka et al., 2002; Wu, 2002). Like *sma-9*, human *Shn-1* and *Shn-3* genes undergo alternative splicing (Muchardt et al., 1992; Hicar et al., 2001). Notably, *Shn-1* and *Shn-2* have recently been demonstrated to play a role in BMP-dependent transcriptional regu-

* Corresponding author. Fax: +1 718 997 3445.
E-mail address: cathy.savagedunn@qc.cuny.edu (C. Savage-Dunn).
[1] Current address: Department of Developmental Hematopoiesis, Cell Biology Program, Memorial Sloan-Kettering Cancer Institute, New York, NY 10021, USA.

0012-1606/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2007.02.038

lation of mesodermal patterning and adipogenesis, respectively (Yao et al., 2006; Jin et al., 2006). In this study, we have addressed the molecular mechanisms of SMA-9 function in BMP-related signaling in *C. elegans.*

C. elegans DBL-1 (*D*pp and *B*MP-*l*ike) is a member of the TGF-β superfamily closely related to *Drosophila* Dpp and vertebrate BMP-2/BMP-4 (Suzuki et al., 1999). The DBL-1 signaling pathway regulates body size and patterning of male-specific copulatory structures (Savage-Dunn, 2005). DBL-1 pathway loss of function (*lf*) mutants have small body size in both hermaphrodites and males (Sma phenotype). In addition, the mutant male has abnormal male tail sensory ray fusions and crumpled spicules (Mab phenotype) (Savage et al., 1996). Wild-type male tails have an acellular structure called the fan, which is formed from the cuticle matrix (Sulston et al., 1980). Within the fan, there are two sets of nine sensory rays distinguished by their specific positions and shapes. DBL-1 pathway (*lf*) mutant males have frequent ray fusions of rays 4–5, 6–7, and 8–9. Furthermore, in these mutants, the pair of copulatory spicules necessary for mating are crumpled with 100% penetrance. When *dbl-1* is overexpressed in a wild type background, the animal has a long body size (Lon phenotype) and the male contains ray 3–4 fusions (Suzuki et al., 1999). *sma-9* functions as a downstream component of the DBL-1 pathway. Like other DBL-1 pathway components, *sma-9*(*lf*) mutants have small body size and abnormal male tails. Phenotypic analysis shows that *sma-9* regulates body size development specifically in early larval stages and regulates morphogenesis of only sensory rays 8 and 9 (Liang et al., 2003). In addition to these roles in body size and male tail patterning, *sma-9* has recently been reported to regulate mesodermal cell lineages, a role in which *sma-9* antagonizes DBL-1 pathway function (Foehr et al., 2006).

In *Drosophila*, early reports attributed to Shn roles as both a transcriptional activator and a transcriptional repressor (Dai et al., 2000; Marty et al., 2000; Torres-Vazquez et al., 2001). Some studies have suggested that the principle role of Shn is to repress expression of *brinker* (*brk*), a transcriptional repressor of Dpp target genes (Marty et al., 2000; Muller et al., 2003). However, *brk* is not a conserved gene, and to date, no *brk* homolog has been found in *C. elegans* or other genomes. More recently, Pyrowolakis et al. (2004) reported that transcriptional repression of *brk* is dependent on a short *cis*-acting silencer element (SE). The SE has high affinity for the Smads Mad and Medea, which subsequently recruit the transcriptional repressor Shn. The authors found that many Dpp pathway target genes contain this sequence, which suggests a *brk*-independent transcriptional repressor activity of Shn. However, whether Shn contributes to the transcriptional repression of these genes has in most cases not been tested. In an elegant extension to this work, Yao et al. (2006) have reported that vertebrate *Shn-1* regulates the *Xenopus* BMP target gene *Xvent2* via a sequence homologous to the SE. In this system, Shn functions as a transcriptional activator rather than as a repressor. By exchanging *Drosophila* and *Xenopus* Shn genes in these experiments, they demonstrate that transcriptional activation or repression may be context-dependent rather than an intrinsic property of the protein.

None of these previous studies have determined whether transcriptional repressor or activator activities are sufficient to mediate Shn functions throughout development, rather than in the context of selected target genes, nor have studies addressed the intrinsic transcriptional activities of Shn proteins. To address the nature of the SMA-9 transcriptional activity, we have used transgenic and DNA microarray approaches. These approaches are facilitated in *C. elegans* relative to other systems, such as *Drosophila* and vertebrates, because DBL-1 pathway mutants are viable. We have created SMA-9 fusions to known transcriptional activator and repressor domains. The transcriptional repressor fusion partially rescued *sma-9* mutant phenotypes in all tissues tested, and caused a ligand overexpression phenotype in the male tail. The transcriptional activator fusion acted as a dominant negative in body size, but partially rescued the male tail phenotypes. Additionally, we have shown that regions of SMA-9 have intrinsic transcriptional repressor and activator activities. Finally, we have used DNA microarray analysis to identify target genes of *dbl-1* and *sma-9* involved in early larval development, when *sma-9* is active in regulating body growth. This analysis expands substantially on a previous smaller-scale screen of transcriptional target genes for this pathway using arrayed cDNAs (Mochii et al., 1999). In our microarray positives, there are more genes transcriptionally repressed than activated. We identified conserved and novel transcriptional target genes, including homologs of Runx and bZIP transcription factors. Notably, we demonstrate that *T27F2.4*, a *C. elegans* homolog of the mouse gene *fosB*, is a downstream negative regulator of DBL-1 pathway-regulated development. Taken together, these experiments suggest that *sma-9* provides transcriptional repressor activity to regulate body size, while contributing both repressor and activator activities for the regulation of male tail patterning.

Materials and methods

Strains

C. elegans strains were cultured using standard methods and grown at 20 °C (Brenner, 1974). In addition to strains generated in this work, the following were used: N2 (wild type), LG II: *sma-6(wk7)*; LG III: *sma-2(e297)*, *sma-3(wk30)*, *sma-4(e729)*; LG IV: *dbl-1(wk70)*; LG V: *him-5(e1490)*; LG X: *sma-9(wk55)*. Since *sma-9(wk55)* has the strongest loss of function phenotype, most of our work was done on this strain.

Molecular cloning

En and VP-16 fusions: 4.0 kb *sma-9* promoter region (Liang et al., 2003) was cloned in pBluescript SK+ at *Not*I and *Sac*II sites (pCS301); and then 3.6 kb *sma-9* C-terminal genomic sequence, from the first zinc finger region to the stop codon, was cloned into pCS301 at *Spe*I and *Kpn*I sites (pCS303); finally VP-16 (370 bp) or Engrailed (900 bp) transcription activity domain (from vector TCF-vp16 and TCF-Engrailed (gifts from Gary Struhl)) was inserted into pCS303 at *Not*I and *Spe*I sites (pCS304 – *VP-16-sma-9*; pCS305 – *en-sma-9*), respectively.

sma-9 ARD II and cDNA clones *yk1285a11* and pCS272 were cloned into yeast vector pSH 2-1, which give rise to pCS327, pCS326, and pCS330, respectively. These constructs were cotransfected with pLGΔ312S and pJK1621 (gifts from S. Kuchin) into yeast strain MCY 829. Yeast transcription activity was performed as described (Kuchin and Carlson, 2003). Heat-shock vector pPD49.83 was a kind gift from Dr. Liu. *yk1285a11* and *yk1237d01* was cloned into pPD49.83 at *Nhe*I and *Kpn*I.