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The novel *C. elegans* gene *sop-3* modulates Wnt signaling to regulate Hox gene expression

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SUMMARY

We describe the properties of a new gene, *sop-3*, that is required for the regulated expression of a *C. elegans* Hox gene, *egl-5*, in a postembryonic neuroectodermal cell lineage. Regulated expression of *egl-5* in this cell lineage is necessary for development of the sensory rays of the male tail. *sop-3* encodes a predicted novel protein of 1475 amino acids without clear homologs in other organisms. However, the sequence contains motifs consisting of homopolymeric runs of amino acids found in several other transcriptional regulators, some of which also act in Hox gene regulatory pathways. The genetic properties of *sop-3* are very similar to those of *sop-1*, which encodes a component of the transcriptional Mediator complex, and mutations in the two genes are synthetic lethal. This suggests that SOP-3 may act at the level of the Mediator complex in regulating transcription initiation. In a *sop-3* loss-of-function background, *egl-5* is expressed ectopically in lineage branches that normally do not express this gene. Such expression is dependent on the Hox gene *mab-5*, as it is in branches where *egl-5* is normally expressed. Ectopic *egl-5* expression is also dependent on the Wnt pathway. Thus, *sop-3* contributes to the combinatorial control of *egl-5* by blocking *egl-5* activation by MAB-5 and the Wnt pathway in inappropriate lineage branches.

Key words: Transcription, Homeobox gene, Wnt signaling, Regulation, *C. elegans*, *sop-3*

INTRODUCTION

Cell fate specification during multicellular development occurs by a progressive series of changes in cell transcriptional states. Through such programs of gene transcriptional regulation, differentiated cells of defined types are generated at specific sites in the body. In the transcriptional switching events that constitute the steps of these developmental programs, gene promoters integrate multiple developmental signals in responding to conditions that may occur in unique combinations in individual cells. What the number and nature of these combinatorial inputs is, and how their combined actions determine transcriptional output, are major questions in developmental biology.

Hox transcription factors constitute one class of transcriptional inputs whose role appears to be to convey positional cell identity (Gellon and McGinnis, 1998). Hox proteins interact with target promoters in combination with the co-factors *extradenticle* and *homothorax*, which can function to bring action of the Hox transcription factor under the control of extracellular signals (Mann and Abu-Shaar, 1996; Reickhof et al., 1997). Several additional factors have been identified in *Drosophila* that contribute to the regulation of Hox target promoters in ways that are less well understood: *lines* (*lin*), which encodes a novel protein, is required for Hox gene *Abdominal-B* (*Abd-B*) to specify the posterior spiracles and the eighth abdominal denticle belt (Castelli-Gair, 1998; Hatini et al., 2000); *teashirt* (*tsh*), which encodes a zinc-finger protein, modulates the function of Hox gene *Sex combs reduced* (*Scr*) in the establishment of the identities of the prothoracic and labial segments (Fasano et al., 1991; de Zulueta et al., 1994); and *cap-n-collar B* (*cncB*), which encodes a bZIP protein, plays a similar role in specifying the identities of two *Deformed* (*Dfd*) expression domains (Mohler et al., 1995; McGinnis et al., 1998). In vertebrates, the retinoic acid receptor heterodimer, RAR/RXR, is a co-regulator of Hox target promoters (Marshall et al., 1996).

Regulatory transcription factors such as Hox proteins and other combinatorial transcription factors exert their effects by influencing one or more of the steps of an assembly pathway that must be traversed before an actively transcribing transcription complex forms on a promoter. These steps include initiation of chromatin opening, chromatin remodeling and histone acetylation, recruitment of holoenzyme to the promoter, and release of the polymerase from a potentially inactive preinitiation complex (Struhl, 1999; Kornberg and Lorch, 1999; Bjorklund et al., 1999). One function of this multistep assembly process is no doubt to ensure fidelity in bringing transcription under the combined control of multiple developmental signals (Cosma et al., 1999).

Postembryonic development of the rays of the *Caenorhabditis elegans* adult male tail (Fig. 1A) provides an opportunity for studying these issues in the context of a particular developmental transcriptional program (Emmons,

1999). Rays, sensory organs used in mating, develop from postembryonic cell lineages generated by three bilateral pairs of neuroectodermal blast cells (Fig. 2A). Each ray is clonally derived through a stereotyped cell sublineage from a ray precursor cell, and each is grossly similar, constituted of two sensory neurons and a support cell. Yet each ray also has unique characteristics, such as whether or not one of its sensory neurons expresses the neurotransmitters dopamine or serotonin, and the position where it forms in the epidermis. These ray differences are a result in part of a regulated pattern of expression of two Hox genes, *mab-5* and *egl-5* (Fig. 2A). Mutations that affect expression of these genes in the ray lineages can be identified by their effects on ray morphology. In particular, changes in the levels of MAB-5 and EGL-5 result in homeotic transformations of ray morphological identities that often result in ray fusions (Chow and Emmons, 1994).

MAB-5 and EGL-5 are each expressed in specific subsets of the rays and are required for expression of the unique characteristics of those rays (Chow and Emmons, 1994; Salser and Kenyon, 1996; Ferreira et al., 1999). In the transcriptional program that leads to this expression pattern, *mab-5* activates *egl-5* expression, but it does so only in a subset of the lineage branches in which MAB-5 is present (Ferreira et al., 1999). Furthermore, MAB-5 is present in the lineage before *egl-5* is activated, and is present in the hermaphrodite where *egl-5* is not expressed. Thus, *egl-5* is regulated by multiple factors so that it is expressed in a cell lineage that contains MAB-5 protein, but only in certain branches of this lineage, only at a specific time and only in one sex. We wish to determine the identities of the additional factors that bring about this expression pattern and resolve how they contribute to regulation of the *egl-5* promoter.

Here, we describe a new gene, *sop-3*, that contributes to the regulated pattern of *egl-5* expression. *sop-3* was identified as a genetic suppressor influencing development of the rays. We previously described another gene, *sop-1*, identified in the same suppressor screen, which encodes a component of the transcriptional Mediator complex (Zhang and Emmons, 2000). Mutations in both *sop-1* and *sop-3* suppress a mutation in a cis regulatory element of the *C. elegans caudal* homolog, *pal-1*. This mutation prevents expression of *pal-1* in the progenitor cell of rays 2-6 and thus results in absence of these rays (Hunter et al., 1999; Zhang and Emmons, 2000). In both *sop-1* and *sop-3* loss-of-function backgrounds, the crippled *pal-1* gene is expressed under the influence of the Wnt pathway. In addition to its effect on *pal-1* expression, loss of *sop-3* function also results in mis-regulation of *egl-5* in subsequent steps of the cell lineage. In particular, *egl-5* is expressed in inappropriate lineage branches and this expression is stimulated by the Wnt pathway. Such inappropriate expression remains under the control of *mab-5*. Thus, SOP-3 appears to be one of the combinatorial inputs that together with *mab-5* determines the regulated pattern of *egl-5* expression. The similarity in genetic properties of *sop-3* and *sop-1* suggests that *sop-3* acts at the level of the assembly or activation of a transcription complex at the *egl-5* promoter.

MATERIALS AND METHODS

Strains

The maintenance of nematode strains, mutagenesis and genetic analysis were handled according to standard procedures (Brenner, 1974). Nematodes were grown at 20ºC unless otherwise noted. Most strains carried the *him-5(e1490)* mutation, which gives a high frequency of males in selfing populations. The following alleles were used in this work. LGI: *dpy-5(e61)*, *lin-17(n677)* and *sur-2(ku9)*. LGIII: *pal-1(e2091)*, *pal-1(ct224)*, *mab-5(e1239)*, *egl-5(n945)* and *egl-5(u202)*. LGIV: *egl-20(n585)*. LGV: *him-5(e1490)*. LGX: *bar-1(ga80)*, *sop-1(bx92)* and *nIs118(cat-2::gfp*, *lin-15*) (kindly provided by Hillel Schwartz). The linkage group of *bxIs13(egl-5::gfp*, *lin-15*) and *bxIs14(pkd-2::gfp*, *pha-1*) is undetermined. *pal-1(ct224)* is a putative null allele that deletes part of exon 1 and the remainder of the *pal-1* gene (Hunter et al., 1999).

Rearrangement: *sDp3*, a duplication that covers the left portion of chromosome III, including *pal-1*.

sDp3 and *egl-20* strains were grown at 25ºC.

Isolation, characterization and mapping of *bx96*

bx96 was isolated as a suppressor of the V6 ray loss phenotype of *pal-1(e2091)*. F_2 or F_3 males of ethyl methanesulfonate-treated *pal-1(e2091)* hermaphrodites were screened for the presence of V6 rays. To recover the mutation that restored the V6 rays, sibling hermaphrodites were cloned. 19 mutations that defined more than 10 genes were obtained from about 4000 genomes screened. Four of these 19 lie in the gene *sop-1* (Zhang and Emmons, 2000); the remaining are being characterized.

bx96 was mapped on linkage group I. Three-factor mapping indicated that *sop-3(bx96)* is very close to *lin-17*: 51 out of 52 Dpy nonLin recombinants from *dpy-5* + *lin-17/* + *sop-3(bx96)* + cross carried *sop-3(bx96)*.

sop-3(bx96) has a maternal effect: 95% of Dpy males from + + / *sop-3 dpy-5; pal-1(e2091)* hermaphrodites have Pal phenotype (*n*=200), although most Dpy males should be homozygous for *sop-3(bx96)*.

The cloning of *sop-3*

sop-3 was mapped between *lin-17* and *pop-1*. YAC Y71F9B contains the sequence between cosmid F32B5 (containing the *lin-17* gene) and W03D8 (containing *pop-1*). Co-injection of two overlapping PCR fragments (fragments 1 and 2, Fig. 3), but not the fragments individually, from this YAC rescued *sop-3(bx96)*. Fragment 1 corresponds to the sequence from 54101 to 71198 of Y71F9B; fragment 2 corresponds to the sequence from 65797 to 81865 of Y71F9B. The DNAs were injected into *sop-3(bx96); pal-1(e2091)* hermaphrodites at a concentration of 20-50 ng/μl. pRF4, which carries the dominant *rol-6(su1006)* marker, was co-injected at a concentration of 100-200 ng/μl. F_2 Rol males were scored for the presence of V6 rays. Dissecting these two fragments further, we found that one 16 kb PCR fragment, corresponding to the sequence from 58679 to 74891 of Y71F9B, contained *sop-3* function.

A cDNA expressed sequence tag from Dr Y. Kohara, yk533h12, was sequenced. RT-PCR was performed and the products sequenced to get the reminder of *sop-3* mRNA. *sop-3* is SL-1 trans-spliced, suggesting that the cDNA is full length. The primers for the RT-PCR were SL1, 5′GGTTTAATTACCCAAGTTTGAG3′; and Y71F9B, 5′(69698) CCAGTTTGTGTGAATACCGGC 3′(69718).

sop-3 RNAi

RNAi experiments were performed as described by Fire et al. (Fire et al., 1998). The RNA was synthesized using MEGAscript T3 and T7 kit (Ambion). 200 ng/μl dsRNA was injected. The eggs from injected animals are collected from 4 to 48 hours postinjection.

The efficiency of dsRNA in causing the duplication of V6-rays and suppression of *pal-1(e2091)* was strongly dependent on which part of the gene was used as template for synthesizing RNA. RNA from close to either C-terminal or N-terminal end was much less efficient than RNA from central regions. In this paper, the DNA fragment used as template for synthesizing RNA corresponds to Y71F9B 66012-65569, which covers the ninth *sop-3* exon.

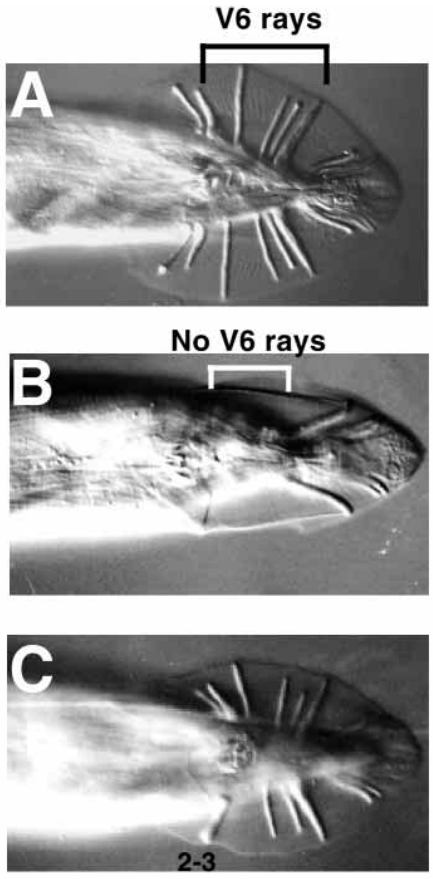

Fig. 1. *sop-3(bx96)* suppresses the Pal phenotype of *pal-1(e2091)*. (A) Adult male tail, ventral view, showing the nine pairs of wild-type rays (Nomarski photomicrograph). (B) Mutant male tail in *pal-1(e2091)*. Rays 2-6 are absent and replaced by longitudinal cuticular ridges called alae (not visible). (C) Suppressed male tails. Rays are mostly normal but exhibit a low level of defects: rays 2 and 3 are fused on one side shown here.

The construction of *sop-3* reporter genes

Reporter gene EM#300 was constructed by joining the 9.3 kb *Bam*HI-digested *sop-3*-rescuing fragment (from Y71F9B 74891 to 65620), which contains 507 nucleotides 5′ of the putative AUG start codon and the first 9 exons, to the 4.5 kb *Hin*cII-*Bam*HI fragment of pPD95.67, which contains *gfp* and *unc-54* 3′ UTR (see Fire laboratory vector kit). The transgenic line, *bxEx73*, was obtained by co-injection of EM#300 with pBR1, carrying the *pha-1* gene, into *pha-1* hermaphrodites. A reporter gene consisting of the entire *sop-3* gene with a *gfp* insertion was constructed as follows. A plasmid (EM#301) was made by inserting the 1 kb *Hin*dIII fragment from pPD103.87 into the *Hin*dIII site of a second plasmid (EM#302), which contained the segment 65587 to 58766 of Y71F9B. The 9.3 kb *Spl*I-digested *sop-3*-rescuing fragment (from 74891 to 65575) was gel-purified and ligated to *Spl*I-digested EM#301. The transgenic line, *bxEx74*, was obtained by co-injection of the ligation mixture together with pBR1 into *pha-1* hermaphrodites.

RESULTS

sop-3(bx96) is a *pal-1(e2091)* suppressor

During wild-type male development, the *C. elegans caudal* homolog *pal-1* acts cell autonomously in seam cell V6 to activate the transcriptional program leading to postembryonic ray development (see Fig. 2A) (Waring and Kenyon, 1990; Waring and Kenyon, 1991; Hunter et al., 1999). In V6, *pal-1* activates the *Antennapedia* homolog *mab-5*, which is expressed continuously throughout most of the postembryonic V6 cell lineage (Salser and Kenyon, 1996). *mab-5* in turn activates the *Abdominal-B* homolog *egl-5* in a single lineage branch during L2, and the bHLH gene *lin-32* during L3 (C. Zhao, PhD thesis, Albert Einstein College of Medicine, 1995; Ferreira et al., 1999). Proper expression of this transcription factor cascade is required for wild-type ray development (Fig. 1A) (Chisholm, 1991; Zhao and Emmons, 1995; Chow and Emmons, 1995; Salser and Kenyon, 1996; Ferreira et al., 1999; Hunter et al., 1999).

The expression of *pal-1* in V6 requires a V6-specific *cis* regulatory element located in the last *pal-1* intron. The regulatory mutation *pal-1(e2091)* contains a point mutation within this putative enhancer that prevents V6 expression (Hunter et al., 1999; Zhang and Emmons, 2000). As a result, *mab-5* and its downstream targets are not activated and the V6 cell lineage is transformed to one resembling an anterior seam cell lineage (Waring and Kenyon, 1990; Hunter et al., 1999); instead of rays 2-6, V6 generates longitudinal cuticular ridges, termed alae, normally found along the body only anterior of the ray domain (Pal phenotype, for posterior alae) (Fig. 1B). To identify genes governing expression of the ray transcription factor cascade, we performed a genetic screen for suppressors of the Pal phenotype of *pal-1(e2091)* (Zhang and Emmons, 2000). One maternal effect recessive suppressor mutation mapping to LG I defines the gene *sop-3* (suppressor of *pal-1*). In *sop-3(bx96); pal-1(e2091)* mutants, 85% of V6 lineages produced 5 normal rays, compared with 5% in *pal-1(e2091)* mutants (Fig. 1C and Table 1, lines 1 and 2).

sop-3(bx96) suppresses the *pal-1(e2091)* mutation by reactivating *pal-1* expression in V6. We demonstrated this by showing that *sop-3(bx96)* could not suppress ray loss in a *pal-1* mutant containing a deletion of *pal-1* coding sequences, *pal-1(ct224)*. Because of the embryonic lethality of *pal-1(ct224)*, it was necessary to carry out this test in genetic mosaics. We examined the percentage of males with Pal phenotype segregated by a strain of genotype *sop-3(bx96)*; *pal-1(ct224)*; *him-5(e1490); sDp3*. *sDp3* is a free duplication carrying a wild-type allele of *pal-1* that is not completely stable, being lost from the V6 lineage at a frequency of about 3% (Waring and Kenyon, 1991). Thus 3.1% of males segregated from *pal-1(ct224)*; *him-5(e1490); sDp3* are Pal (*n*=420) (Table 1, line 3). If *sop-3(bx96)* could suppress the *pal-1* deletion allele, then the Pal phenotype of *pal-1(ct224)* mosaics would be suppressed and most sides which lost *sDp3* would produce V6

Table 1. Suppression of *pal-1(e2091)* by *sop-3* mutants

	percent sides with 5 or more V6 rays	total sides
1. *pal-1(e2091)*	5	232
2. *sop-3(bx96)*; *pal-1(e2091)*	85	1968
3. *pal-1(ct224)*; *sDP3*	96.9	420
4. *sop-3(bx96)*; *pal-1(ct224)*; *sDP3*	96.4	220
5. *sop-3(bx96)*; *mab-5(e1239) pal-1(e2091)*	0*	482
6. *sop-3(RNAi)*; *pal-1(e2091)*	82	216
7. *sop-3(bx96)*; *pal-1(e2091)*; *bar-1(ga80)*	42	554
8. *sop-3(bx96)*; *pal-1(e2091)*; *egl-20(n585)*	78	512

*1.2% of male sides have one ray.

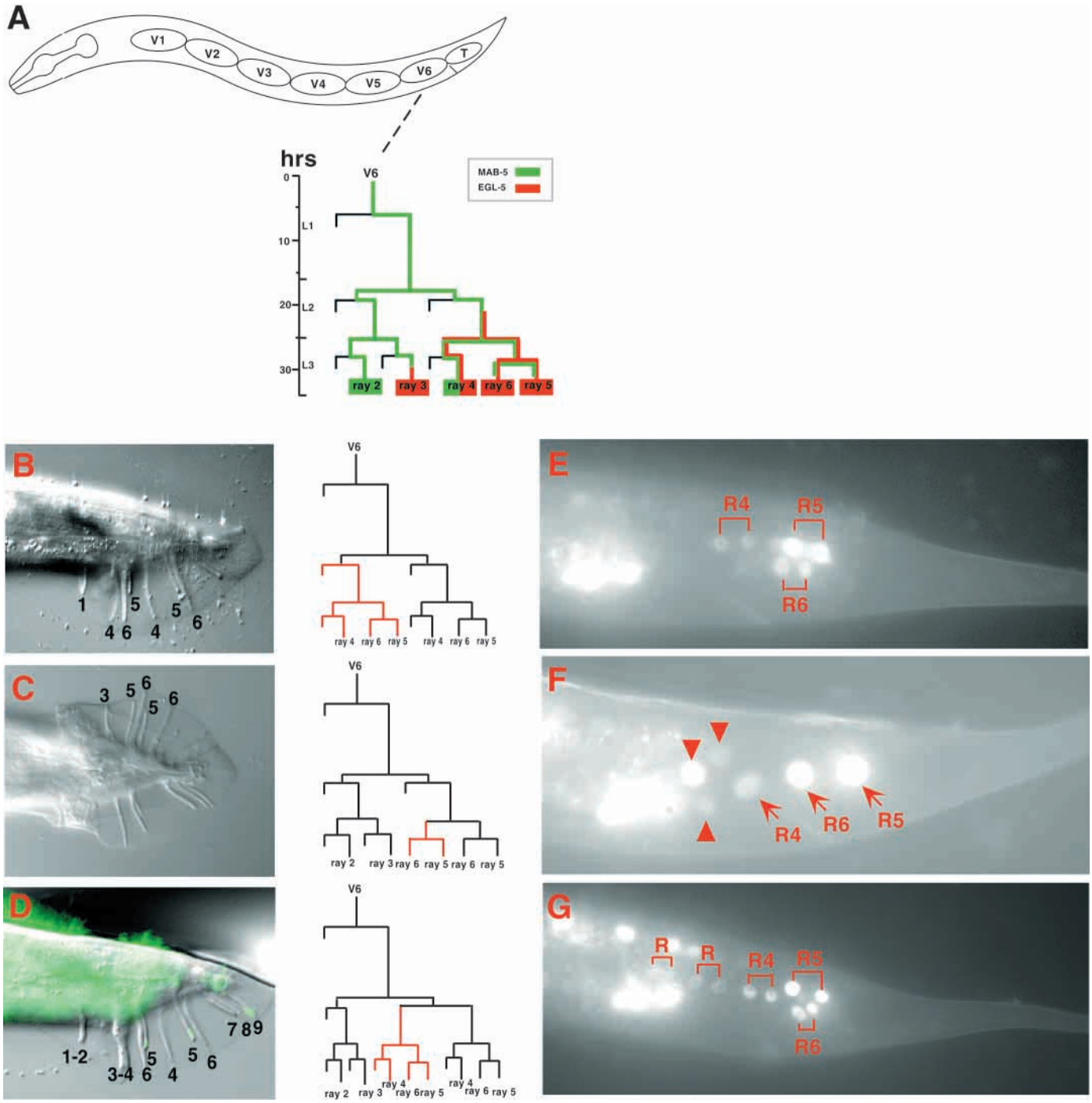

Fig. 2. Duplication of V6 rays and abnormal expression of *egl-5* in *sop-3(RNAi)* males. (A) Expression of the Hox proteins MAB-5 and EGL-5 in the wild-type V6 cell lineage. Hours of postembryonic development and larval stage are shown on the left-hand scale. Seam cells V5 and T give rise to rays 1 and 7-9, respectively. Boxes at the ends of the lineage branches represent the ray sublineages, which continue to express the proteins as shown. In *mab-5* and *egl-5* mutants, the morphogenetic identities and neurotransmitter expression profiles of rays 2-6 are altered. (B-D) Examples of *sop-3(RNAi)* males with ray duplications, together with postulated lineage transformations that can account for them. In D, a tyrosine hydroxylase::gfp (*cat-2::gfp*) reporter gene, normally expressed in the dopaminergic rays (rays 5, 7 and 9) is expressed in both of the rays that are identified as ray 5 by morphological criteria (lack of reporter expression in ray 7, as here, occurs at low frequency in this strain). (E-G) Expression of an *egl-5::gfp* reporter gene (*bxIs13*) in ray precursor cells (Rn cells), which generate the ray sublineage, or their progeny. (E) Wild type: expression of the reporter is visible in the daughters of R4, R5 and R6. (F) *sop-3(RNAi)*; there is strong expression in R4-R6 (arrows), and also in three additional cells (arrowheads). (G) *sop-3(RNAi)*: an animal showing expression in daughters of R4-R6 and daughters of two additional Rn cells.

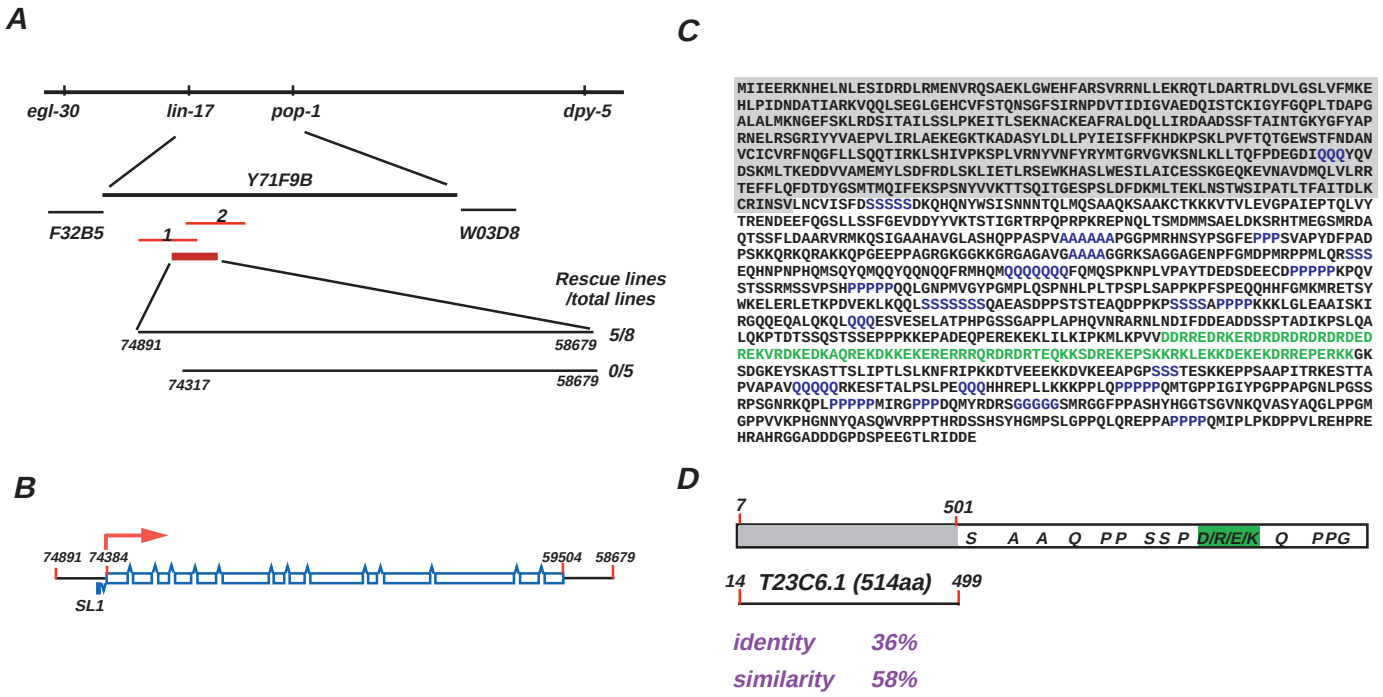

Fig. 3. Molecular structure of *sop-3* and its predicted gene product. (A) *sop-3* was mapped genetically to the region of LGI between cloned genes *lin-17* and *pop-1*, covered by the YAC Y71F9B. PCR was used to amplify the DNA of Y71F9B into several overlapping fragments, two or three of which were co-injected into *sop-3(bx96); pal-1(e2091)* hermaphrodites to test for their ability to restore the Pal phenotype (missing V6 rays). Fragments 1 and 2 (see Materials and Methods) rescued upon co-injection but not individually. Rescue was further localized to a minimal fragment extending between the Y71F9B nucleotides shown. (B) Structure of *sop-3*. Intron/exon structure was confirmed by the cDNA sequence from yk533h12 and the sequences of RT-PCR products. *sop-3* is SL-1 trans-spliced. The lengths of the introns are not to scale. The GenBank Accession Number for the nucleotide and amino acid sequence of the *sop-3* gene is AF308860. (C) The derived protein sequence of SOP-3. The Ser-rich, Ala-rich, Gln-rich, Pro-rich, Gly-rich regions are in purple and a highly charged domain is in green. The sequence that shows strong similarity to T23C6.1 is shaded. (D) Schematic diagram of protein structure of SOP-3. The shaded region in the N terminus is the region of similarity to T23C6.1. S, A, Q, P and G stand for the Ser-rich, Ala-rich, Gln-rich, Pro-rich, Gly-rich regions, respectively. D/K/E/R stands for the highly charged domain.

rays. However, we found that this was not the case: the frequency of Pal males was not reduced by introduction of *sop-3(bx96)* into *pal-1(ct224); him-5(e1490); sDp3*. 3.6% of V6 lineages produced no rays in *sop-3(bx96); pal-1(ct224); him-5(e1490); sDp3* mutants (n=220) (Table 1, line 4). This result shows that *pal-1*-coding sequences are necessary for expression of rays in a *sop-3(bx96)* mutant and implies that *sop-3(bx96)* suppresses *pal-1(e2091)* by restoring *pal-1* expression.

sop-3(bx96) affects the activity of additional components of the ray transcription factor cascade

In wild type, *pal-1* activates the ray transcriptional cascade by activating *mab-5* (Hunter et al., 1999). We therefore tested whether rays in *sop-3(bx96); pal-1(e2091)* required *mab-5*. Consistent with the conclusion that *sop-3(bx96)* suppresses *pal-1(e2091)* by reactivating *pal-1* expression, we found that V6 rays in *sop-3(bx96); pal-1(e2091)* mutants are dependent on *mab-5* activity. In *sop-3(bx96); pal-1(e2091) mab-5(e1239)* triple mutants, almost no V6 rays are present (Table 1, line 5).

This dependence of ray development on *mab-5*, however, was not as complete as in an otherwise wild-type background. Whereas in *mab-5(e1239)*, descendants of V6 generate alae instead of rays in 100% of animals, in *sop-3(bx96); mab-5(e1239)* double mutants, 14% of male sides lacked alae in the post-anal region, and these males generated an average of 1.5 V6 rays per suppressed side (Table 2, line 3). Generation of these *mab-5*-independent rays required the function of *egl-5* because in *sop-3(bx96); mab-5(e1239) egl-5(n945)* there were no V5 or V6 rays and alae extended into the tail region in all sides (Table 2, line 5). Therefore, it appears that *sop-3(bx96)* makes expression of *egl-5* at least partially independent of *mab-5*, and *egl-5* can apparently activate the ray developmental program. Whereas in wild type, expression of *egl-5* is completely dependent on *mab-5* gene function, in a *sop-3(bx96)* mutant, the stringency of this relationship is weakened. We conclude that in addition to altering the conditions for expression of *pal-1* in V6, *sop-3(bx96)* also affects the activity of one or more additional downstream components of the ray transcription factor cascade.

Loss of *sop-3* gene function causes mis-regulation of *egl-5*

sop-3 was cloned by identifying its genetic map location followed by complementation rescue experiments (see below). Isolation of the *sop-3* gene allowed us to test the loss-of-function mutant phenotype by RNAi experiments. *sop-3(RNAi)* in *pal-1(e2091)* resulted in suppression of the Pal phenotype, confirming identification of the gene and indicating that loss of *sop-3* gene function resulted in *pal-1* suppression (Table 1, line 6).

RNAi experiments revealed that *sop-3* is also required at additional steps in ray development. After microinjection of *sop-3* dsRNA into gonads of *him-5* hermaphrodites, adult self-progeny males had duplicated rays and fused rays (Fig. 2B-D; Table 2, line 1). Similar phenotypes were seen but at lower frequency in *sop-3(bx96)* (Fig. 1C; data not shown), suggesting that *sop-3(bx96)* may be a loss-of-function mutation but is unlikely to be a null allele.

Ray duplications and fusions suggested that expression and/or function of the Hox genes *mab-5* and *egl-5* were misregulated in the ray lineages. The most striking class of defects consisted of ray duplications. In the wild-type fan, ray identities, numbered from anterior to posterior, can be unambiguously determined by ray order, morphology and position (rays 1, 5 and 7 open on the dorsal surface of the fan; rays 2, 4 and 8 open on the ventral surface of the fan; ray 3 is thin and extends to the fan margin; ray 6 is thick at the base, extends nearly to the margin and lacks the characteristic ring-and-dot ending that marks the external openings of the other rays; and ray 9 extends to the fan margin). Using these criteria, we found that some *sop-3(RNAi)* males have such duplicated ray patterns as 1-4-5-6-4-5-6-7-8-9, 1-2-3-5-6-5-6-7-8-9, 1-2-3-4-5-6-4-5-6-7-8-9 (Fig. 2B-D). We injected *sop-3* dsRNA into a strain carrying a tyrosine hydroxylase reporter gene (*cat-2::gfp*) that is expressed in the dopaminergic neurons of rays 5, 7 and 9 (Lints and Emmons, 1999). The ectopic ray 5 in *sop-3(RNAi)* expressed this reporter, confirming duplication of ray 5 (Fig. 2D).

These duplicated ray patterns in *sop-3(RNAi)*, which involved only rays descended from V6 (rays 2-6), suggested that cell fate transformations had occurred during the V6 lineage. For example, the ray pattern 1-4-5-6-4-5-6-7-8-9, in which rays 2 and 3 are absent and rays 4-6 are duplicated, suggested that in this animal V6.pap had taken the fate of V6.ppp (Fig. 2B). Other duplication patterns suggested additional types of cell fate transformations among V6 descendants (Fig. 2C,D). In all such postulated transformations, an anterior cell assumed the fate of a more posterior cell, a transformation easily accounted for by mis-regulation of *egl-5*, in particular, its ectopic expression in an anterior lineage branch. For example, expression of *egl-5* normally occurs in the V6.ppp branch of the cell lineage but not in the V6.pap branch, and expression in the V6.ppp branch is necessary for this cell to generate three rays instead of two, and for ray 5 to express tyrosine hydroxylase (Chisholm, 1991; Ferreira et al., 1999; Lints and Emmons, 1999). V6.pap might take the V6.ppp fate if *egl-5* were inappropriately expressed in this cell, generating a duplication of rays 4-6.

To test the hypothesis that *egl-5* was expressed in additional cells in *sop-3(RNAi)*, we asked whether *egl-5* activity is required for the duplication of V6-rays. We injected *sop-3* dsRNA into an *egl-5* strain carrying a *pkd-2::gfp* reporter gene (Barr and Sternberg, 1999). This reporter is expressed in all rays and was used to overcome the difficulty of scoring the number of rays in an *egl-5*(*–)* background, where ray morphogenesis is largely blocked. We found that no extra rays are formed in *sop-3(RNAi); egl-5* mutants (0/142 sides had more than four V6 rays), indicating that *egl-5* activity is required for the duplicated V6-rays in *sop-3(RNAi)* males.

To test more directly for the mis-regulation of *egl-5*, we carried out *sop-3* RNAi experiments in a strain carrying an *egl-5*::*gfp* reporter. In wild type, this reporter is strongly expressed in ray precursor cells R5 and R6 and their progeny, weakly expressed in R4 and its progeny, and only rarely can be seen expressed very weakly in R3 and its progeny (Fig. 2E). After microinjection of dsRNA, expression was strong and consistent in R4-R6 and their progeny, and in additional ray precursor cells postulated to be anterior sister nuclei that never express *egl-5* in wild type (Fig. 2F,G). These results confirmed that *egl-5* was mis-expressed in the V6 lineage.

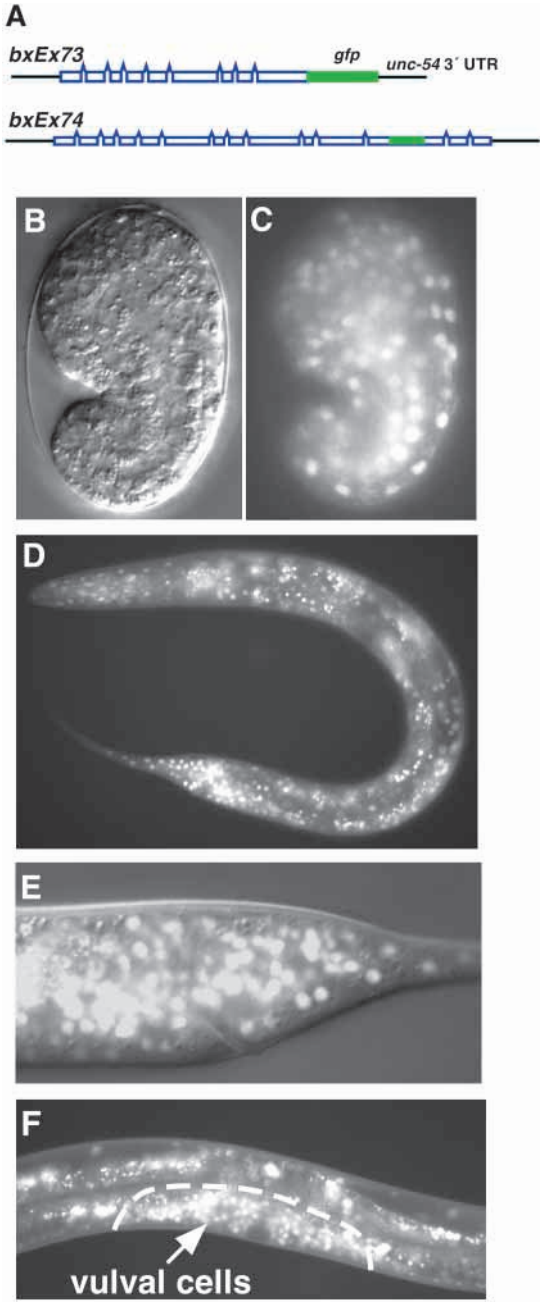

Fig. 4. *sop-3::gfp* reporter genes are expressed in many nuclei throughout development. (A) The structures of *sop-3::gfp* reporter genes. (B,C) Expression in comma stage embryo (B, Nomarski image; C, fluorescence image). (D) Expression in late L3 male. (E) Expression in ray precursor cells and many additional cells of an L3 male tail. (F) Expression in proliferating vulval cells of an L3 hermaphrodite. Large irregular fluorescence particles are gut autofluorescence.

Ectopic expression of *egl-5* in *sop-3(RNAi)* requires *mab-5*

Since expression of *egl-5* in the V6 lineage normally requires *mab-5* gene function (Ferreira et al., 1999), we asked whether the duplicated rays and mis-regulation of *egl-5* also required MAB-5 function. We injected *sop-3* dsRNA into a *mab-5(e1239)* strain and found that there were no duplicated rays (Table 2, line 4). Thus, the ectopic expression of *egl-5* in *sop-3(RNAi)* males is dependent on *mab-5* and is not due to the *mab-5*-independent activation of *egl-5*. However, as described above for *sop-3(bx96)*, alae are absent from 13% of sides and a few rays are generated in *sop-3(RNAi); mab-5(e1239)* mutants. This is consistent with the observation that 1 or 2 V6 descendants showed the expression of *egl-5* in about 15% of male sides when *sop-3* dsRNA was injected into a *mab-5(e1239)* strain carrying the *egl-5::gfp* reporter gene (data not shown). Thus, this *mab-5*-independent weak development of rays is also a *sop-3* loss-of-function phenotype.

In addition to ray duplications, ray fusions also suggested that MAB-5 and EGL-5 were expressed at inappropriate levels or were acting inappropriately in the later ray lineages. Ray fusion is thought to result when adjacent rays express the same morphological identity (Baird et al., 1991). Such fusions result if either *mab-5* or *egl-5* is expressed at abnormal levels in the ray lineages (Chow and Emmons, 1995; Salser and Kenyon, 1996). Ray fusions in *sop-3(RNAi)* suggest that *mab-5* and *egl-5* expression levels and/or activities are abnormal during the late L3 and early L4 larval stages – when ray cells are generated and their morphogenetic identities expressed.

We show below that *sop-3* is widely expressed throughout development, from embryogenesis onwards. It is strongly expressed in proliferating cells of the vulval cell lineages. Consistent with this, about 2% of hermaphrodites have a protruding vulva phenotype in *sop-3(RNAi)*. In view of the expression in many other cells, it is perhaps surprising that no additional strong phenotypes were observed after injection of *sop-3* dsRNA. We do not know whether this is because *sop-3* has no additional non-redundant functions, or because RNAi was ineffective in eliminating *sop-3* gene function.

sop-3 encodes a novel protein with sequence characteristics shared by several transcriptional regulators, including some that affect Hox gene function

sop-3 was cloned by placing it genetically between cloned genes *lin-17* and *pop-1* followed by complementation rescue (Fig. 3A). Gene identification was confirmed by showing that *sop-3(RNAi)* suppressed *pal-1(e2091)*. The predicted *sop-3* gene structure was verified by sequencing cDNA clones (Fig. 3B). We attempted to identify the *bx96* mutation by sequencing all of the exons and exon-intron boundaries and found no differences from wild type. Therefore, we believe *sop-3(bx96)* is a mutation in a *cis*-regulatory element.

sop-3 encodes a predicted protein of 1475 amino acids (Fig. 3C). The only related protein sequence found in available databases was the product of the conceptual *C. elegans* gene T23C6.1. T23C6.1 encodes a predicted protein of 522 amino acids that is 36% identical and 58% similar across its entire length to the N-terminal segment of SOP-3 (Fig. 3D). RNAi studies of T23C6.1 did not reveal any obvious phenotype (data not shown). In particular, dsRNA injection did not suppress the V6 ray-loss phenotype of *pal-1(e2091)*, and the phenotype obtained when dsRNA of *sop-3* and T23C6.1 were simultaneously injected was similar to that in *sop-3(RNAi)* alone.

Although database searches with SOP-3 identified no proteins other than T23C6.1 with overall sequence similarity, the C-terminal segment of SOP-3 contains a number of homopolymeric amino acid motifs found in several other transcription factors. These include proline-repeats, alanine-repeats, serine-repeats, glutamine-repeats and glycine-repeats (Fig. 3C,D). Homopolymeric tracts of Gln, Ser, Ala and Pro residues are present in the *Drosophila* proteins Cap-n-collar B (CncB), Teashirt (Tsh), Engrailed (En), Fushi tarazu (Ftz) and some Hox proteins (McGinnis et al., 1998; Fasano et al., 1991; Laughon et al., 1985). The presence of homopolymeric runs in Hox proteins and Hox protein modulators such as Tsh and CncB suggests that such motifs may perform a common function in transcriptional regulation. SOP-3 also contains a highly charged domain near the C terminus: 88% (79 out of 90) amino acids are charged (Asp, Lys, Arg or Glu) (Fig. 3C,D). Ala-rich, Pro-rich and highly charged domains have been associated with transcriptional regulatory functions, suggesting that SOP-3 might function as a transcription factor (Hanna-Rose and Hansen, 1996).

sop-3 is widely expressed throughout development

To determine where *sop-3* is expressed and to ask whether regulated expression of *sop-3* contributed to regulation of *egl-5* expression, we constructed two strains containing *sop-3::gfp* reporter transgenic arrays (Fig. 4A). One array, *bxEx73*, consists of the same 0.5 kb upstream region as the minimal

Table 2. Dependence of ray generation on *mab-5, egl-5* and *Wnt* pathway

	duplicated rays*	fused rays‡	alae posterior of anus	number of sides
1. *sop-3(RNAi)*	29	45	0	210
2. *mab-5(e1239)*	0	0	100	100
3. *sop-3(bx96); mab-5(e1239)*	0	0	86§	220
4. *sop-3(RNAi); mab-5(e1239)*	0	0	87§	302
5. *sop-3(bx96); mab-5(e1239) egl-5(n945)*	0	0	100	100
6. *sop-3(bx96); mab-5(e1239); bar-1(ga80)*	0	0	99.3	283
7. *sop-3(bx96); mab-5(e1239); egl-20(n585)*	0	0	86	326
8. *sop-3(RNAi); bar-1(ga80)*	0.6	1.4	0	355

Body of table gives the % of sides with the characteristic listed.
*% of sides with >5 V6 rays.
‡% of sides with fused rays, including those with >5 V6 rays.
§Sides without alae had 1-2 rays.

rescuing genomic fragment (Materials and Methods) plus the first 9 exons of *sop-3*, fused in-frame to *gfp* containing a nuclear localization signal and the 3′UTR of the *C. elegans* myosin gene *unc-54*. The other, *bxEx74*, is an in-frame insertion of *gfp* into the *sop-3*-rescuing fragment and contains the entire *sop-3* coding sequence, including all the introns plus 738 nucleotides 3′ of the predicted *sop-3* stop codon. Both reporters have the same expression pattern. During embryogenesis they are widely expressed in many or all cells (Fig. 4B,C). During larval stages, they are expressed in the seam cells, head neurons, ventral cord, male ray cells and other tail neurons (Fig. 4D,E). The reporter genes are also strongly expressed in proliferating cells; for example, they are expressed in the vulval precursor cells (Fig. 4F). In adult animals, the reporters are expressed mainly in neurons. For both reporters, GFP fluorescence was nuclear. In the case of *bxEx74*, which does not have an added nuclear localization signal, this suggests that SOP-3 is a nuclear protein. Nuclear localization of SOP-3 is consistent with the inference drawn from the SOP-3 sequence that the protein functions as a transcription factor.

In the V6 lineage, both reporter genes were uniformly expressed throughout development in all lineage branches. Therefore regulated expression of *sop-3* does not account for the pattern of *egl-5* expression in this lineage. We conclude that *sop-3* is necessary for conveying regulatory information to the *egl-5* promoter, and that its activity is regulated post-transcriptionally and possibly post-translationally.

SOP-3 may function at the level of the transcriptional Mediator complex

Since the sequence and apparent nuclear localization of SOP-3 suggested that it might be a transcription factor, we tested for genetic interactions with other known components of the transcriptional apparatus. First, we investigated whether *sop-3(bx96)* interacted with mutations in *sop-1*. *sop-1* mutations have a phenotype nearly identical to *sop-3*. SOP-1 is the *C. elegans* homolog of TRAP230, a component of the human Mediator complex (Ito et al., 1999; Zhang and Emmons, 2000). Like *sop-3(bx96)*, loss-of-function mutations in *sop-1* are recessive, have maternal effects, and restore *pal-1* activity in V6 in *pal-1(e2091)* but not in *pal-1(0)* (Zhang and Emmons, 2000). We analyzed double mutants of *sop-3(bx96)* with a strong allele of *sop-1* and found that this combination is synthetic lethal. Whereas *sop-3* and *sop-1* mutations singly have little effect (<5%, n>1000) on viability, in *sop-3(bx96); sop-1(bx92)* double mutants, 50% of embryos do not hatch (n=2762) and 78% of hatching animals die at an early larval stage (n=1318). Since neither *sop-3(bx96)* nor *sop-1(bx92)* appear to be null alleles, this synthetic lethal phenotype, together with the nearly identical genetic properties of the two genes, suggest that *sop-3* and *sop-1* act in a single pathway. Because SOP-1 is a presumptive component of the Mediator complex, this conclusion implicates SOP-3 as a possible component of the Mediator complex as well, or as a transcriptional co-factor that interacts with the Mediator complex.

Further support for the hypothesis that *sop-3* functions at the level of the Mediator complex came from examining genetic interactions with a second Mediator component, SUR-2. SUR-2 is the *C. elegans* homolog of a protein of the human Mediator complex, hSur-2, that interacts with transcription factors targeted by the Ras/MAPK pathway (Boyer et al., 1999). Mutations in *sur-2* suppress the multivulva phenotype of an activated mutation of *Ras* and are synthetic lethal in combination with weak loss-of-function mutations in several genes acting in the Ras/MAPK signal transduction pathway (Singh and Han, 1995). A mutation in *sur-2* cannot suppress the Pal phenotype of *pal-1(e2091)* (data not shown), indicating that *sur-2* does not function in the same pathway as *sop-3* and *sop-1* in regulation of *pal-1*. Likewise, mutations in *sop-1* and *sop-3* do not suppress an activated-Ras pathway Muv mutation (Zhang and Emmons, 2000; data not shown). Therefore, in the ray and vulval pathways, *sop-1* and *sop-3* on the one hand and *sur-2* on the other have separate functions. Nevertheless, we found that a non-null mutation in *sur-2* causes synthetic lethality with *sop-3(bx96)*. In *sur-2(ku9) sop-3(bx96)* mutants, 38% of animals die as larvae. Consistent with the conclusion that *sop-3* and *sop-1* lie in the same pathway, a *sop-1* mutation is also synthetic lethal with *sur-2*. In *sur-2(ku9); sop-1(bx92)* mutants, 78% of animals die as larvae. These synthetic lethal phenotypes could be explained if the introduction of mutations simultaneously into multiple components of the Mediator complex has a cumulative effect, possibly disrupting the integrity or function of the complex. Alternatively, unlike in the ray and vulval pathways, elsewhere *sop-1*, *sop-3* and *sur-2* may all be required for expression of one or more essential functions, or their effects in separate pathways may be cumulatively lethal.

sop-3 regulates activity of the Wnt pathway

Previous studies have shown that the Wnt signaling pathway is capable of regulating the expression of *pal-1* and *mab-5* in V6 in some genetic backgrounds (Hunter et al., 1999; Zhang and Emmons, 2000). The Wnt pathway in the male seam has been defined by a gene for a β-catenin homolog, *bar-1*, a gene encoding a frizzled receptor, *lin-17*, and two genes encoding Wnt ligands, *egl-20* and *lin-44*. Mutations in *lin-17* and *lin-44* cause reversals in polarity or loss of polarity at many seam lineage cell divisions, resulting in severe disruption of tail development (Herman et al., 1995; Eisenmann et al., 1998; Maloof et al., 1999). These pleiotropic effects make testing the roles of *lin-17* and *lin-44* in ray development difficult.

We examined the role of *bar-1* and *egl-20* in misregulation of *pal-1* and *egl-5* in *sop-3(bx96)* and in *sop-3(RNAi)* mutants. The mutations we examined, *bar-1(ga80)* and *egl-20(n585)*, are thought to be a null and strong reduction-of-function mutation, respectively. These mutations singly have no effects on the rays, indicating these genes normally do not have essential roles in ray development. However, we found that *bar-1* activity is partially required for the V6 rays in *sop-3(bx96); pal-1(e2091)* mutants. Only 42% of V6 lineages produced rays in *sop-3(bx96); pal-1(e2091); bar-1(ga80)* triple mutants, compared with 85% in *sop-3(bx96); pal-1(e2091)* mutants (Table 1, line 7). V6 produces normal rays in *sop-3(bx96); bar-1(ga80)* double mutants, indicating that *sop-3(bx96)* does not make ray development sensitive to *bar-1* function in a *pal-1(+)* background. Thus, *bar-1* is partially required for suppression of *pal-1(e2091)* by *sop-3(bx96)*. A similar partial requirement for *bar-1* was found for suppression of *pal-1(e2091)* by *sop-1* mutations (Zhang and Emmons, 2000).

Unlike *bar-1*, *egl-20* activity does not stimulate ray development in *sop-3(bx96); pal-1(e2091)* mutants: 78% of V6 lineages produce 5 rays in *sop-3(bx96); pal-1(e2091); egl-20(n585)* mutants (Table 1, line 8). Lack of stimulation by *egl-20* could be because *bar-1* is activated in a ligand-independent manner, or because of redundancy of EGL-20 with LIN-44.

We showed above that *sop-3(bx96)* renders the activity of *egl-5* in the seam lineages partially independent of *mab-5*. This *mab-5*-independent activation of *egl-5* is also dependent on *bar-1* activity. In *sop-3(bx96); mab-5(e1239); bar-1(ga80)*, alae extended into the post-anal region in almost all animals (Table 2, line 6). However, as with *pal-1(e2091)* suppression, there was no effect of *egl-20(n585)* (Table 2, line 7).

Finally, we tested whether abnormal expression of Hox genes as evidenced by generation of duplicated rays and fused rays in *sop-3(RNAi)* required components of the Wnt pathway. We found that the frequency of duplicated rays and fused rays was significantly reduced by introduction of *bar-1(ga80)* (Table 2, line 8). Thus, all the effects of mutations in *sop-3* (i.e. *pal-1(e2091)* suppression, *mab-5*-independent activity of *egl-5*, and mis-expression of Hox genes) require or are stimulated by *bar-1*. This, together with the evidence that *sop-3* acts at the level of the Mediator complex, strongly suggests that SOP-3 plays a role in relaying signaling to the transcriptional apparatus through the Wnt pathway.

DISCUSSION

Mode of *sop-3* action

Tightly regulated expression of the *C. elegans* Hox genes *mab-5* and *egl-5* is essential for the development and patterning of the male tail rays. Generation of ray 6 and specification of the identities of rays 3-6 requires that EGL-5 expression be initiated in two branches of the postembryonic V6 seam cell lineage. EGL-5 expression requires MAB-5. However, MAB-5 is expressed not only in lineage branches where it activates *egl-5*, but also in additional branches of the lineage, as well as at times earlier than the initiation of *egl-5* expression. Thus, additional genes are required to bring about the regulated pattern of EGL-5 expression. We have shown that one of these genes is the novel gene *sop-3*. Wild-type *sop-3* function is required to prevent the activation of *egl-5* in inappropriate lineage branches by MAB-5 and the Wnt pathway.

The structure of the SOP-3 protein, its nuclear localization, the similar mutant phenotypes of *sop-3* and *sop-1* (which encodes a putative component of the Mediator complex), and the synthetic lethal interaction of *sop-3* and *sop-1* all point to a relatively direct role of SOP-3 in regulating transcription initiation. As we showed earlier for *sop-1* (Zhang and Emmons, 2000), *sop-3* loss-of-function mutations suppress the enhancer mutation *pal-1(e2091)* by allowing activity of an alternative pathway for *pal-1* activation in V6. This alternative pathway somehow involves the Wnt pathway, because the degree of *pal-1* suppression is strongly decreased, although not eliminated, by mutation in the β-catenin gene *bar-1*. Mutations in *sop-1* that truncate a C-terminal Q-rich domain allow activation of this alternative pathway. One possibility is that SOP-3 interacts directly with SOP-1, possibly with the Q-rich domain, in bringing about repression of the alternative gene activation pathway. However, other models involving less direct modes of SOP-3 action are also possible.

Earlier studies had shown that *mab-5*, although it is expressed nearly ubiquitously throughout the V6 cell lineage, acts in only a subset of lineage branches to activate *egl-5* expression (Salser and Kenyon, 1996; Ferreira et al., 1999). This could be because its action is blocked in other lineage branches, potentiated in particular lineage branches, or both of these mechanisms could be operating. We show here that *mab-5* action in some lineage branches appears to be blocked by *sop-3*, because in a *sop-3(lf)* background, ray duplications and ectopic expression of an *egl-5* reporter gene require *mab-5* gene function. The selective action of *sop-3* in blocking *mab-5* only in certain lineage branches is not due to regulated expression of *sop-3*, based on the uniform expression of two *sop-3* reporter genes in all V6 descendants. Rather, it appears that SOP-3 activity may be regulated in a lineage-specific manner. Alternatively, its inhibitory activity may be ubiquitous, but blocked or bypassed by another pathway activated in branches of the lineage where *egl-5* is activated. This would be analogous to *pal-1* activation in V6, which normally occurs independently of Wnt signaling by a pathway that is apparently not subject to the inhibitory effects of SOP-1 and SOP-3.

Where SOP-3 blocks MAB-5 activity, it could do so directly as a Hox protein modulator by interfering with MAB-5 binding to target promoters (presumptively, here, the *egl-5* promoter) or by interfering with MAB-5 activity in promoting transcription once it is bound. Alternatively, SOP-3 could act indirectly. For example, it could potentiate a repressor that blocked gene activation by MAB-5, or it could block one or more additional components required together with MAB-5 for transcription initiation. For example, it might block the actions of the *extradenticle* (*ceh-20*) or *homothorax* (*unc-62*) homologs, both of which are required for normal ray development (Y. Teng and S. W. E., unpublished observations). We do not know in general how the effects of multiple factors 'add up' in activation of transcription initiation, and it is possible that SOP-3 blocks a necessary combinatorial pathway that is independent of MAB-5.

We have shown that ectopic gene activation in a *sop-3(lf)* background is stimulated by *bar-1*. BAR-1, a β-catenin homolog, is thought to activate gene expression through its interaction with the DNA-binding protein POP-1, a member of the Tcf/LEF family of HMG proteins (Korswagen et al., 2000). POP-1 is differentially expressed in anterior versus posterior daughters at many, and possibly all, anterior/posterior cell divisions during *C. elegans* development, including those occurring in the postembryonic cell lineages of the seam (Lin et al., 1998). Activation of ray development and ectopic expression of *egl-5* by *bar-1* in a *sop-3(lf)* background suggests that SOP-3 prevents a putative BAR-1/POP-1 factor from activating gene expression. In *Drosophila* and vertebrates, Tcf/LEF factors promote gene expression, through interaction with β-catenin, or alternatively inhibit gene expression, through interaction with the general co-repressor Groucho (Cavallo et al., 1998; Roose et al., 1998). This raises the possibility that SOP-3 regulates the activity of POP-1 target genes by promoting recruitment or activation of the *C. elegans* Groucho homolog, UNC-37. Indeed, we found significant genetic interactions between both *sop-3* and *unc-37* and *sop-1* and *unc-37*. Double mutants containing *sop-3(bx96)* or

sop-1(bx92) together with the viable missense mutation *unc-37(e262)* (Pflugrad et al., 1997) are synthetic lethal (H. Z. and S. W. E., unpublished observations). This finding implicates a role of SOP-3 and SOP-1 in regulatory pathways that also involve UNC-37.

A possible family of transcriptional regulators

SOP-3 characteristics exhibit a number of intriguing parallels with those of several known transcriptional regulators. This group of similar proteins includes the products of *cncB*, *tsh*, *lin*, and *mastermind(mam)* of *Drosophila*, and *lag-3* of *C. elegans* (McGinnis et al., 1998; Fasano et al., 1991; Hatini et al., 2000; Petcherski and Kimble, 2000). All of these proteins have several or all of following characteristics: (1) they are nuclear proteins involved in regulation of transcription; (2) they contain homopolymeric runs of amino acids, usually involving Q, S, A, P and G; (3) they are involved in Hox gene regulatory pathways; and (4) they act by modulating the Wnt pathway. Strikingly, though they act in pathways involving well-conserved components, none of these proteins has clear orthologs in other organisms. *cncB*, *tsh* and *lin*, like *sop-3*, are all involved in Hox gene regulatory pathways, affecting the outcome of Hox gene action (McGinnis et al., 1998; Castelli-Gair, 1998; de Zulueta et al., 1994). CncB and Tsh have recognizable DNA-binding domains (a basic leucine zipper domain in CncB, a Zn-finger domain in Tsh) whereas SOP-3 and Lin do not. SOP-3, Tsh and Lin all affect the action of the Wnt pathway (Gallet et al., 1998; Gallet et al., 1999; Hatini et al., 2000). Tsh modulates Wnt signaling by direct binding to the β-catenin homolog Armadillo (Gallet et al., 1998 Gallet et al., 1999). Alone among these proteins, Lin does not contain homopolymeric runs of amino acids. LAG-3 and Mam differ from the other proteins in being involved in the LIN-12/Notch pathway rather than in Hox gene regulatory pathways, and their structures include a greater number and length of homopolymeric runs of Q residues (Petchershi and Kimble, 2000; Smoller et al., 1990).

LAG-3 provides an attractive model for the function of this putative family of transcriptional regulators. It has recently been shown to participate in a ternary complex between the ankyrin-repeat-containing intracellular domain of the LIN-12 receptor, which translocates to the nucleus upon signaling, and the target DNA-binding factor of this pathway, LAG-1 (Petchershi and Kimble, 2000). A possible similar role for SOP-3 as constituent of a multi-protein complex that includes POP-1, BAR-1, UNC-37 and the Mediator component SOP-1 may be a reasonable premise for further investigation. Why such a mode of action would allow for unusual evolutionary variability of protein sequence is not obvious, since the interactions involved are between evolutionarily conserved components. Possibly the mechanism allows for the function of proteins of mixed and variable functional domains that act in the nature of linker proteins. Such a mechanism might provide an important point of evolutionarily flexibility that can lead to variation in the regulatory interactions involved in the combinatorial control of gene expression.

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