A *C. elegans* mediator protein confers regulatory selectivity on lineage-specific expression of a transcription factor gene

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The *Caenorhabditis elegans caudal* homolog, *pal-1*, is required for neurogenesis in the male tail. We show that expression of *pal-1* in the postembryonic neuroblast cell V6 can be initiated by two alternate pathways. One pathway, acting in wild type, requires a regulatory element in the fifth *pal-1* intron. The other pathway, independent of this element, is normally repressed by the newly identified gene *sop-1*, which encodes a homolog of the mammalian Mediator complex protein TRAP230. In *sop-1* mutants, *pal-1* is activated by a pathway that is stimulated by *bar-1*/β-catenin, a component of the Wnt signal transduction pathway. The results support a physiological role of the Mediator complex in conveying regulatory signals to the transcriptional apparatus.

[*Key Words*: Transcription; homeobox gene; development; cell fate; regulation]

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The properties of differentiated cells are determined by the sets of transcription factors they express and lineage ancestry. The male rays in *Caenorhabditis elegans* are a model for studying how a transcription factor cascade in a postembryonic cell lineage leads to specification of a defined set of differentiated cells at a particular site in the adult body (Emmons 1999). The transcription factor cascade leading to rays involves several homologs of well-known cell-fate specifying regulatory transcription factors, notably the Hox transcription factors and a *caudal* homolog (Chow and Emmons 1994; Salser and Kenyon 1996; Hunter et al. 1999). Genes for these transcription factors are turned on and off at specific times during the late embryonic and postembryonic ray cell lineages, defining multiple alternate cell fates necessary for wild-type ray development (Salser and Kenyon 1996; Fereira et al. 1999; Hunter et al. 1999). Here we take advantage of a genetic approach to identify an element of the mechanism that governs the accurate expression of this cascade.

Regulation of gene expression occurs primarily through control of the several steps of the transcription initiation pathway (Kornberg and Lorch 1999; Struhl 1999). This pathway involves opening of the promoter region by chromatin modification and remodeling, followed by recruitment of the polymerase complex and relevant cofactor complexes, and finally initiation of elongation. Each of these steps is a potential target of regulatory factors. Recruitment of holoenzyme and communication of regulatory signals to the core polymerase are thought to occur in part via a multiprotein complex termed the Mediator complex, which includes the targets for several DNA-binding transcription factors (see Björklund et al. 1999). Like other metazoans, *C. elegans* contains one or more complexes containing homologs of yeast Mediator proteins (Kwon et al. 1999). The *C. elegans* genome encodes homologs of several additional components present in human complexes but not found in yeast (Ito et al. 1999).

Transcription factor cascades leading to rays can be considered to begin during late embryogenesis in three bilateral pairs of postembryonic neuroectoblasts. The ray transcriptional program in one of these pairs of neuroectoblasts, V6, begins with expression of *pal-1*, the *C. elegans* ortholog of the conserved homeodomain transcription factor *caudal* (Mlodzik and Gehring 1987; Waring and Kenyon 1991). Expression of *pal-1* in V6 in late embryogenesis results in the expression of *mab-5*, a *Hox* gene of the *Antennapedia* paralog family (Costa et al. 1988; Hunter et al. 1999). Later in the cell lineage, *mab-5* directly or indirectly turns on *egl-5* and *lin-32* (Wrischnik and Kenyon 1997; Ferreira et al. 1999). *egl-5* is a second gene of the *C. elegans Hox* family, most closely related to the *Abdominal-B* paralog family (Wang et al. 1993). *lin-32* is a bHLH transcription factor related to *Drosophila atonal* that is proneural in function, initiating the ray sublineage and neurogenesis (Zhao and Emmons 1995). *mab-5* and *egl-5*, in addition to regulating *lin-32*, define ray-specific properties, such as morphoge-

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netic identity and neurotransmitter expression (Chow and Emmons 1994; Salser and Kenyon 1996; Lints and Emmons 1999).

Accurate expression of this cascade is crucial to the development of the wild-type ray pattern. To define additional components governing its expression, we studied regulation of the first gene, the *caudal* homolog *pal-1*. We identified a *cis*-regulatory region within a *pal-1* intron that is necessary for expression of *pal-1* in V6. Taking an approach similar to that used previously in yeast to identify components of the transcriptional regulatory apparatus (Carlson 1997), we carried out a suppressor screen for mutations that restore ray development in a strain carrying a mutation in this *cis*-regulatory region. The screen resulted in the isolation of mutations that restored *pal-1* activity in V6.

This strategy allowed us to identify a Mediator component, encoded by the gene *sop-1* (suppressor of *pal-1*), which plays a role in determining which of two alternate activation pathways will express *pal-1*. SOP-1 corresponds to the *C. elegans* homolog of a large Mediator protein, TRAP230, identified by in vitro studies of human transcription complexes (Ito et al. 1999). Loss of SOP-1 function releases *pal-1* expression from the requirement for the *cis* regulatory region by allowing *pal-1* activation via a different pathway. This alternate pathway involves the function of β-catenin, a conserved DNA-binding component of the Wnt signal transduction pathway. Thus, as it does in yeast and in cell-free transcription systems, the Mediator complex in *C. elegans* appears to function in regulation of holoenzyme by DNA-binding factors, and to play a role in ensuring the stringency and specificity of their action. SOP-1/TRAP230 is not found in yeast. Thus its role may be in mediating developmental signals.

Results

A lineage-specific cis *regulatory element lies in a* pal-1 *intron*

The *C. elegans caudal* homolog *pal-1* plays an essential role to define cell fates in posterior cell lineages during both early and late embryogenesis (Hunter and Kenyon 1996). In the latter part of embryogenesis, *pal-1* is also expressed in a bilateral pair of seam cells, the V6 cells, that are descendants of the anterior blastomere of the two-cell embryo. Expression of *pal-1* in V6 results in generation of a cell lineage leading to the postembryonic development of male rays (Fig. 1A,B) (Waring and Kenyon 1990, 1991; Hunter et al. 1999).

The viable *pal-1(e2091)* mutation results in the specific loss of *pal-1* expression in V6, while leaving unperturbed *pal-1*'s essential embryonic functions (Hunter et al. 1999). Absence of *pal-1* in V6 results in failure to generate the V6 rays (rays 2–6) in the adult male (Fig. 1C). In *pal-1(e2091)*, descendant cells of V6 divide following a cell lineage similar to the lineages of anterior seam cells, and instead of rays, produce alae, cuticular ridges running along the sides of adult animals from the head

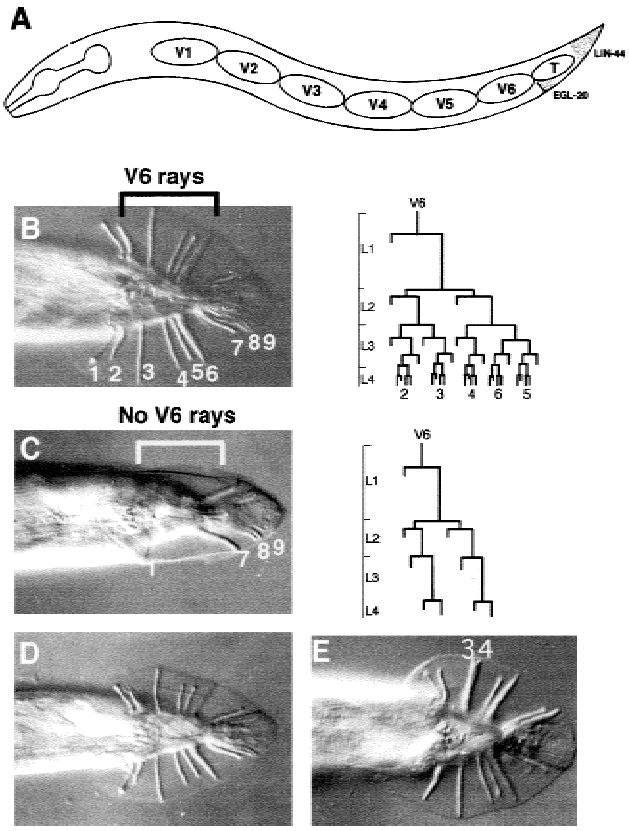

Figure 1. *sop-1* mutations suppress *pal-1(e2091)*. (*A*) Lateral view of an L1 larva showing the row of seam cells, V1–V6 and T, on one side. The Wnt ligands EGL-20 and LIN-44 are expressed, respectively, in the rectal epithelium and tail tip epithelium as shown (Herman et al. 1995; Whangbo and Kenyan 1999). (*B*) Wild-type adult male tail, ventral view, Nomarski photomicrograph. Rays 2–6 are derived from V6, which gives rise to the postembryonic cell lineage shown (larval stages are shown on the vertical scale) (Sulston and Horvitz 1977). (*C*) *pal-1(e2091)* mutant. V6 adopts a fate similar to anterior seam cells V1–V4 in wild type, producing alae, longitudinal cuticular ridges, instead of rays. Lineages of V5 and T, leading respectively to ray 1 and rays 7–9, are unaffected. Ray 1 usually fails to migrate posteriorly into the fan region and forms a papilla on the side of the body. (*D*) *pal-1(e2091); sop-1(bx92)*. V6 produces five normal rays. (*E*) In *pal-1(e2091); sop-1(bx107)* double mutants, 13.5% of sides have ray 3 and 4 fused (ray 4 appears to adopt a ray 3-like identity).

through most of the body (Pal phenotype, for posterior alae) (Fig. 1C; Waring and Kenyon 1990).

To determine the basis for the loss of *pal-1* expression in V6, we sought to identify the *e2091* mutation. Earlier attempts to identify this mutation were unsuccessful, and showed that the nucleotide sequences of the exons and exon/intron boundaries were wild type (Hunter et al. 1999; data not shown). *e2091* was therefore presumed to affect a regulatory sequence. To localize the *e2091* mutation, we carried out complementation rescue experiments with chimeric genes constructed with segments derived from wild-type and *e2091* mutant DNA (Fig. 2A). We found that mutant DNA derived from the 5′

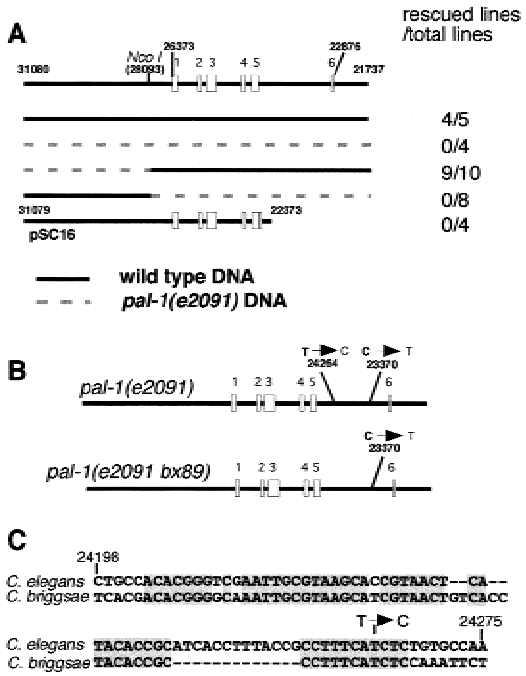

Figure 2. The *pal-1(e2091)* mutation is a point mutation within a conserved intronic sequence. (*A*) Complementation rescue experiments defining the gene segment containing the mutation. Two genomic DNA fragments were amplified from either wild type or *pal-1(e2091)* as shown, ligated at the *Nco*I site in either homologous or heterologous combinations, and injected into *pal-1(e2091); him-5* hermaphrodites. Only the 3′ fragment from *pal-1(e2091)* failed to support rescue. Nucleotide positions are in cosmid C38D4. Likewise, the genomic-cDNA hybrid construct pSC16, which lacks the last intron of *pal-1*, failed to support rescue. (*B*) The two sequence differences between *pal-1(e2091)* and wild type. The sequence of the *pal-1(e2091)* coding region, including all introns, plus 1 kb of 3′ UTR was determined. One of the two mutations was reverted in the intragenic revertant *pal-1(e2091 bx89)*, thus identifying T24264→C as the *pal-1(e2091)* mutation. (*C*) Alignment of part of the last intronic sequence of *C. elegans* and *C. briggsae pal-1*, showing the conserved region containing the *pal-1(e2091)* mutation.

flanking genomic region fused to wild-type coding sequence could rescue the ray defect in *pal-1(e2091)*, but that mutant DNA covering the exons, introns, and 3′ flanking genomic region driven by wild-type 5′ flanking sequence could not. We sequenced the downstream fragment from *pal-1(e2091)* and found two point mutations in the last intron (Fig. 2B), and no other changes.

In our screen for *pal-1(e2091)* suppressors, described below, we recovered a dominant suppressor mutation (*bx89*) that was linked tightly to *e2091*. We sequenced the last intron from this presumptive intragenic revertant and found that one of the two point mutations present in *pal-1(e2091)* was reverted to wild type (Fig. 2B). On this basis, we conclude that this mutation, T(24264)→C, is the *e2091* mutation.

A point mutation in an intron might block gene function for one of two reasons. It might act at the level of DNA and affect the function of a *cis*-acting transcriptional regulatory element, or it might act at the level of RNA and affect splicing or some other aspect of RNA function. According to the first model, the intron is necessary for gene expression, whereas under the second model, if the mutation blocks splicing, a gene lacking the intron altogether would be functional. To distinguish between these two models, we tested whether a *pal-1* transgene lacking the last intron could rescue *pal-1(e2091)*. pSC16 is a genomic DNA–cDNA hybrid constructed by joining genomic DNA up to the penultimate *pal-1* exon to cDNA sequence (L. Edgar and W.B. Wood, pers. comm.). pSC16 is capable of rescuing the *pal-1* null embryonic lethal phenotype (L. Edgar and W.B. Wood, pers. comm.), but we found that it cannot rescue the V6 ray loss phenotype of *pal-1(e2091)* (Fig. 2A). This result supports the identification of T(24264)→C as the *e2091* mutation and indicates that the last intron is necessary for gene function, suggesting that it contains a lineage-specific regulatory element required for expression of *pal-1* in V6. An alternative hypothesis is that a sequence within the intron acts at the level of RNA and is required for one or more steps in RNA processing or function other than simple removal of the intron. This possibility seems unlikely in view of the nature of the suppressor mutations discussed below, which affect a protein thought to act in transcription initiation.

If the last intron of *pal-1* contained an essential *cis*-regulatory element, we expected this element to be conserved in closely related nematodes. We therefore sequenced the corresponding intron of *pal-1* of *Caenorhabditis briggsae*. We found the *C. briggsae pal-1* homolog contained an intron of similar length (*C. briggsae*: 1469 nucleotides, *C. elegans*: 1446 nucleotides) at the same position within the coding region as found in *C. elegans*. The sequence of the *C. briggsae* intron was diverged from that of *C. elegans* with the exception of three conserved blocks of ~50 nucleotides. In one of these blocks, a 56-nucleotide region identical to *C. elegans* at 49 positions with one 13 nucleotide gap, the fourth-to-last nucleotide corresponded to the nucleotide mutated in *pal-1(e2091)* (Fig. 2C). We hypothesize that this mutated sequence corresponds to the binding site for a transcription factor necessary for expression of *pal-1* in V6.

Suppressor mutations in sop-1 *restore* pal-1 *activity to V6*

To identify genes acting to regulate the *pal-1*-initiated transcription factor cascade leading to rays, we isolated suppressor mutations that reverted the V6 ray loss phenotype of *pal-1(e2091)* (see Materials and Methods). The X-linked gene *sop-1* was identified by four recessive suppressor mutations (Fig. 1D,E; Table 1, lines 3–6). These suppressor mutations were specific to the function of *pal-1* in generation of rays, and did not suppress the embryonic lethal phenotypes of a *pal-1(0)* mutation (data not shown). When the *pal-1(e2091)* mutation was crossed out, none of the *sop-1* alleles had any obvious mutant phenotype.

Table 1. *V6 rays in various genetic backgrounds*

	Genotype	Wild-type V6 rays (%)	Total sides
1	Wild-type male	100	>1000
2	*pal-1(e2091)*	4	214
3	*pal-1(e2091); sop-1(bx92)*	94	2470
4	*pal-1(e2091); sop-1(bx93)*	96	262
5	*pal-1(e2091); sop-1(bx103)*	62	412
6	*pal-1(e2091); sop-1(bx107)*	87	426
7	*pal-1(e2091); sop-1* (RNAi)	97	226
8	*pal-1(ct224); sDp3*	96.4	792
9	*pal-1(ct224); sop-1(bx92); sDp3*	95.7	956
10	*pal-1(e2091) mab-5(e1239); sop-1(bx92)*	0[a]	456
11	*pal-1(e2091); sop-1(bx92) bar-1(ga80)*	48	434
12	*bar-1(ga80)*	100	50
13	*sop-1(bx92) bar-1(ga80)*	100	50
14	*pal-1(e2091); egl-20(n585); sop-1(bx92)*	91	444

Percent of sides with a full set of normal V6 rays (rays 2–6). Strains with *sDp3* were grown at 25°C.
[a]2.8% of male sides (13/456) have 1–2 V6 rays.

sop-1 mutations appeared to restore rays by reactivating *pal-1* activity in the V6 lineage. We gained evidence in support of this conclusion by showing that *sop-1* mutations could not suppress ray loss due to *pal-1(ct224)*, a null mutation consisting of a 4.2-kb deletion extending from within exon 1 to the end of *pal-1*. Because *sop-1* mutations do not suppress the embryonic lethal phenotype of *pal-1(ct224)*, it was necessary to carry out this experiment in genetic mosaic animals. To generate mosaics in which *pal-1* activity was absent from the V6 lineage but present elsewhere, we employed the free duplication *sDp3*, a chromosomal fragment carrying a wild-type *pal-1* gene copy. In ~4% of *pal-1(ct224); sDp3* animals, *sDp3* is lost in the V6 lineage, resulting in a Pal phenotype (Table 1, line 8) (Hunter et al. 1999). We found that the frequency of such mosaic Pal males was unaffected by introduction of *sop-1(bx92)* (Table 1, line 9). This indicates that ray loss in V6 lineages lacking *pal-1* activity is not suppressed by *sop-1(bx92)*. We conclude that suppression requires *pal-1* activity, and does not occur via a *pal-1*-independent bypass pathway.

One function of *pal-1* is to activate the *Hox* gene *mab-5*, which is not expressed in V6 in *pal-1(e2091)* (Salser and Kenyon 1996; Hunter et al. 1999). If the effect of *sop-1* suppressor mutations was to reactivate the normal pathway of ray development initiated by *pal-1*, then generation of rays in *pal-1(e2091); sop-1* should require *mab-5* gene function. We found that this was the case (Table 1, line 10).

sop-1 mutations might result in *pal-1*-dependent activation of *mab-5* in a *pal-1(e2091)* background by restoring or raising the level of *pal-1* activity in V6, or by making *mab-5* sensitive to a small amount of *pal-1* gene function still supplied by the *pal-1(e2091)* allele. The following observation argues in favor of the former, that the effect of *sop-1* mutation is to elevate the level of *pal-1* activity in V6. Salser and Kenyon (1996) found that although *pal-1* functioned to turn on *mab-5* expression in V6, *mab-5* activity alone in the absence of a wild-type *pal-1* allele was insufficient to generate a wild-type V6 lineage and normal V6 rays. This was shown by experiments in which MAB-5 was supplied from a heat-shock transgene. In a *mab-5(–) pal-1(e2091)* double mutant, MAB-5 supplied from a heat-shock transgene was not effective in restoring rays, but was effective in a *mab-5(–) pal-1(+)* background (Salser and Kenyon 1996). Therefore, *pal-1* has a function in addition to activation of *mab-5*. Mutation in *sop-1*, which fully restores rays in *pal-1(e2091)*, must result in the provision of this additional activity, as well as in activation of *mab-5*. The simplest hypothesis is that both effects are brought about by elevation of *pal-1* expression in V6.

To gain further evidence for reactivation of *pal-1(e2091)* in V6 by *sop-1* mutations, we studied MAB-5 and PAL-1 expression in V6 by means of antibody staining. As shown previously, in wild type, MAB-5 could be detected in three lateral epidermal cells in late embryogenesis, identified as P9/10, P11/12, and V6 (Fig. 3A) (Cowing and Kenyon 1992; Hunter et al. 1999). In *pal-1(e2091)*, only two cells were stained, consistent with the loss of PAL-1 expression in V6 (Fig. 3B) (Hunter et al. 1999). In *pal-1(e2091); sop-1(bx92)*, three MAB-5-positive cells were visible ($n = 4/4$) (Fig. 3C). PAL-1 staining was weak and difficult to localize to individual cells, but was consistent with expression in the cell identified as V6 (Fig. 3C). Therefore, results of antibody staining supported the conclusion that *mab-5* is reactivated in V6, and were consistent with elevation of PAL-1 level in this cell.

sop-1 *encodes a homolog of a component of the human transcription Mediator complex*

We identified genomic DNA encoding the *sop-1* locus by complementation rescue (Fig. 4A). Rescuing activity was localized to a fragment containing a single predicted gene, F47A4.2, comprising 19 exons encoding a protein of 3498 amino acids (Fig. 4B,C). We confirmed most of the predicted intron/exon boundaries by sequencing cDNAs (see Materials and Methods). To gain further evidence that the large predicted gene was a single functional locus, we introduced frameshift mutations into the amino-terminal region and also constructed amino-terminal and carboxy-terminal deletions. Each of these alterations abolished or severely reduced rescuing activity (Fig. 4B). Finally, reporter genes constructed by in-frame insertion of green fluorescent protein (GFP) respectively into the fourth and seventeenth exons had similar expression patterns, consistent with the assignment of these exons to a single transcriptional unit (Fig. 5).

sop-1 encodes a homolog of human TRAP230, a component of the transcriptional mediator complex (Fig. 4D) (Ito et al. 1999). SOP-1 and TRAP230 are ~40% similar and 22% identical in five regions spanning the coding sequence. Both proteins have a glutamine-rich region at

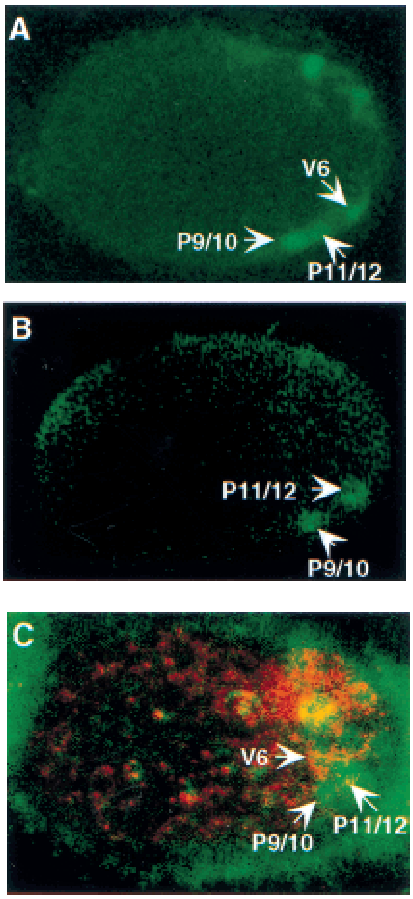

Figure 3. Expression of MAB-5::LACZ and PAL-1 in embryos (confocal fluorescence photomicrograph; anti-LACZ, green; anti-PAL-1, red). (*A*) MAB-5::LACZ in wild-type embryo. (*B*) MAB-5::LACZ in *pal-1(e2091)*. In *A* and *B* PAL-1 staining was weak and is not shown. (*C*) MAB-5::LACZ and PAL-1 in *pal-1(e2091); sop-1(bx92)*. Weak overlap (yellow) is detectable in the cell identified by position as V6. The strongly expressing cell above the arrowhead pointing to V6 is in a different plane of focus.

the carboxy-terminal end (798 amino acids long and 31% glutamine in SOP-1, 127 amino acids long and 67% glutamine in TRAP230). SOP-1 also contains two ligand-dependent nuclear hormone receptor recognition motifs (LXXLL), as does TRAP230. Apart from the glutamine-rich region and the LXXLL motifs, SOP-1 and TRAP230 are novel proteins containing no other recognizable sequence motifs. SOP-1 is the only predicted TRAP230 homolog in the *C. elegans* genome.

The similarity of SOP-1 to a known component of RNA polymerase II holoenzyme argues strongly that relief of the *pal-1(e2091)* mutational block by *sop-1* mutation occurs at the level of *pal-1* transcriptional initiation, and not at a later step involving RNA processing, transport, or expression. Suppression at the level of transcriptional initiation is consistent with the conclusion that the point mutation in the fifth intron in *pal-1(e2091)* prevents *pal-1* transcription and not with models that postulate this mutation affects RNA processing or other posttranscriptional event.

We localized the four *sop-1* mutations by transformation rescue experiments employing combinations of gene subregions derived from wild-type and mutant DNA and sequenced subregions that could not support rescue (see Materials and Methods). In *bx103*, the weakest allele, an invariant residue necessary for splice donor activity at the start of the seventh intron is mutated from G to A (Fig. 4B). In *bx92*, *bx93*, and *bx107*, CAG glutamine codons are mutated to TAG amber stop codons (Fig. 4B). In *bx93*, the strongest allele, the terminal one-third of the protein, including the entire glutamine-rich domain, is predicted to be truncated (950 amino acids). *bx92* and *bx107* lie downstream of *bx93* within the glutamine-rich region and are predicted to truncate respectively 333 and 299 carboxy-terminal amino acids. Among the three stop codon mutations, the degree of suppression increases with increasing protein truncation (Table 1, lines 3,4,6). However, on microinjection into *pal-1(e2091); sop-1(bx92)*, DNA from even the strongest allele, *bx93*, gave up to 20% rescue (see Materials and Methods). Therefore all of these mutants may retain some gene activity.

To determine the expression pattern of *sop-1*, we constructed three GFP reporters (Fig. 5A). EM#290 contains 2.2 kb of upstream genomic sequence plus the first four SOP-1 exons joined in-frame to GFP. EM#291 and EM#292 are functional reporters that have GFP inserted in-frame into an amino-terminal and a carboxy-terminal exon, respectively. During embryogenesis, all three reporters are expressed in all cells starting after the 8-cell stage of embryogenesis (Fig. 5B). Fluorescence is nuclear, and for EM#291 and EM#292 but not EM#290, beginning in late embryogenesis, it is punctate (Fig. 5C). In postembryonic development and in adult animals, EM#291 and EM#292 are expressed mainly in neurons and fluorescence is punctate (Fig. 5D), whereas EM#290 is expressed widely (Fig. 5E,F). Expression of these reporters in V6 during embryogenesis was observed and is consistent with cell-autonomous function of *sop-1* in regulation of *pal-1* expression. Nuclear localization is consistent with a function in transcription.

Decrease of sop-1 *gene function results in suppression of* pal-1(e2091)

To gain information regarding the effect of *pal-1(e2091)* suppressor mutations on *sop-1* gene activity, we used the RNAi (RNA interference) technique to reduce or eliminate gene function (Fire et al. 1998). We asked whether reduction of *sop-1* activity resulted in suppression of *pal-1(e2091)*. Double-stranded RNA covering the ninth exon of *sop-1* was microinjected into the syncytial gonad of adult *pal-1(e2091)* hermaphrodites. Progeny embryos were scored for viability and adult males were scored for suppression of the Pal ray-loss phenotype. No loss of viability was observed, and the Pal phenotype of males was suppressed (Table 1, line 7; Materials and Methods). This result indicates that suppression of *pal-1(e2091)* re-

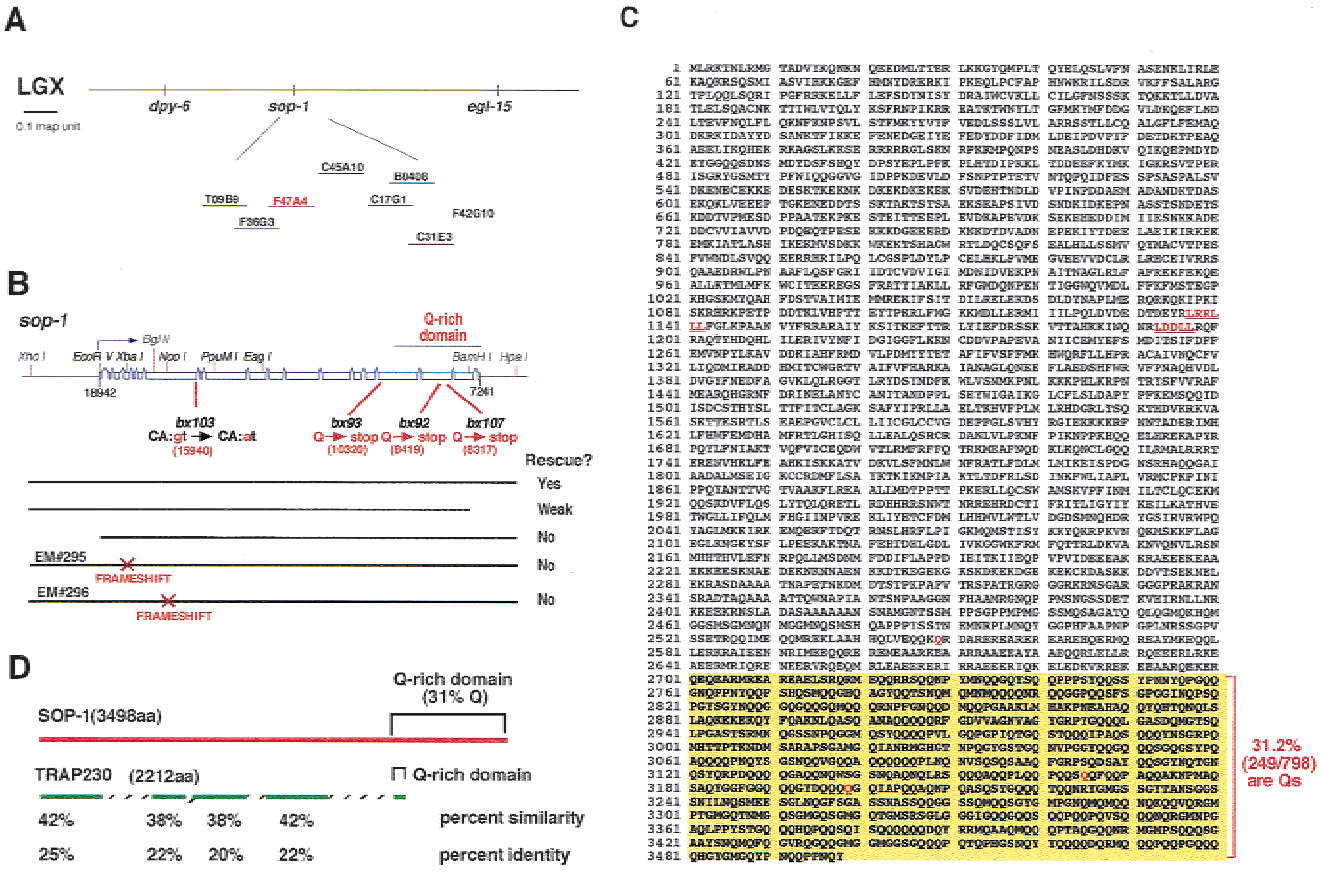

Figure 4. *sop-1* encodes a TRAP-230 homolog. (*A*) Map position of *sop-1* in the genetic interval defined by the cloned genes *dpy-6* and *egl-15*. The cosmids shown were tested for rescue. Cosmid F47A4 restored the Pal phenotype in *pal-1(e2091); sop-1(bx92)* mutants. (*B*) The structure of *sop-1* (*top*). The exons and introns predicted by GeneFinder are shown. The structure from exon 9 to end was confirmed by sequencing cDNAs (see Materials and Methods). The numbers in parenthesis are the positions of the mutations in F47A4. *bx103* changes an invariant intron donor CA:gt to CA:at. (*Bottom*) Rescuing activity of various genomic regions (see Materials and Methods). (*C*) The predicted amino acid sequence of SOP-1. The carboxy-terminal Q-rich domain is highlighted. The two LxxLL motifs are highlighted in red and underlined. The three marked Q residues correspond to the positions of the mutations in *bx93*, *bx92*, and *bx107*. (*D*) Schematic of similarity between SOP-1 and TRAP-230.

sults from decrease of *sop-1* gene function, rather than from some special property of the four suppressor mutations. This is consistent with the recessive nature of these mutations. The experiment does not indicate whether or not *sop-1* is an essential gene, because we do not know whether gene function was eliminated completely in every cell by the RNAi technique.

Wild-type sop-1 *blocks a bypass pathway stimulated by* β*-catenin*

Previous studies had suggested that *pal-1(e2091)* could be suppressed by activation of the Wnt signal transduction pathway (Hunter et al. 1999). We therefore investigated whether the Wnt pathway was responsible for suppression of *pal-1(e2091)* in a *sop-1* mutant background. The known components of the Wnt pathway active in the male tail are shown in Figure 6. Evidence for involvement of this pathway in ray development came from studies of *pry-1*. In a *pry-1* mutant, the ray developmental program is activated in anterior seam cells, which as a result generate anterior rays (*pry* stands for polyray) (Maloof et al. 1999). Activation of the ray pathway results from the ectopic expression of *mab-5* and requires the function of *bar-1*/β-catenin but not of the EGL-20 ligand or the LIN-17 receptor. It was therefore proposed that *pry-1* acts downstream of the receptor but upstream of *bar-1*/β-catenin to block inappropriate activation of the Wnt signal transduction pathway.

Mutations in *pry-1* suppress *pal-1(e2091)* (Hunter et al. 1999). By inference it was concluded that *pal-1(e2091)* could be suppressed by activation of the Wnt signal transduction pathway in V6. We therefore tested the effect of a *bar-1* mutation on ray development in *pal-1(e2091); sop-1*. The number of rays in *pal-1(e2091); sop-1(bx92)* was significantly reduced by introduction of *bar-1(ga80)* (Table 1, line 11). *bar-1(ga80)* had an effect only in the presence of both the *pal-1(e2091)* mutation and a *sop-1* mutation. *bar-1(ga80)* did not reduce the number of rays in *pal-1*(+); *sop-1*(+) (Hunter et al. 1999; Table 1,

line 12). Therefore *bar-1* is normally not required for expression of *pal-1* or any other gene in the ray developmental pathway. Nor did *bar-1(ga80)* reduce the number of rays in *pal-1(+); sop-1(bx92)* (Table 1, line 13). Therefore, a *sop-1* mutation does not make *pal-1* or any other gene sensitive to *bar-1* function.

The requirement for *bar-1* only in a *pal-1(e2091); sop-1* mutant provides evidence that *sop-1* mutations allow *pal-1* activation via a bypass pathway, rather than via reactivation of the normal pathway requiring the fifth

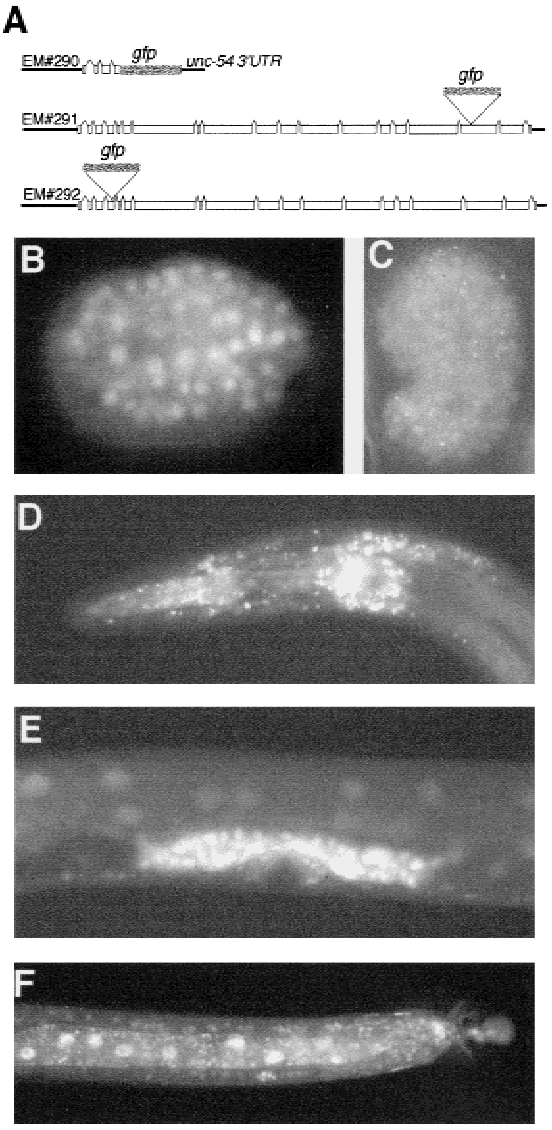

Figure 5. SOP-1 is nuclear and expressed ubiquitously. (*A*) The structure of *sop-1::gfp* reporter genes. EM#291 and EM#292 have in-frame insertions of *gfp* and are functional. EM#290 includes an SV40 nuclear localization signal. (*B–D*) Expression of EM#292 (EM#291 was similar). (*B*) Ubiquitous expressed in the early embryo. (*C*) Expression during late embryogenesis showing some nuclei with punctate localization. (*D*) Punctate expression in neurons in the head of an L4 larva. (*E,F*) Expression of EM#290. (*E*) Expression in developing vulval cells. (*F*) Expression in gut nuclei and neurons in the male posterior region.

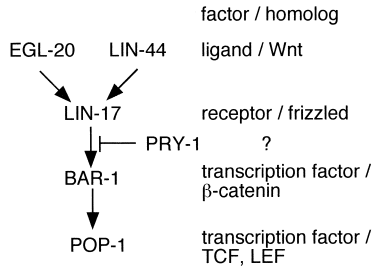

Figure 6. The known components of the Wnt pathway in the male seam.

pal-1 intron. It is unlikely that *sop-1* mutations restore binding of the putative intronic factor, because the activation pathway involving this factor does not require *bar-1*. Rather, *sop-1* mutations activate a different pathway with a different genetic requirement. In wild type, by blocking the pathway stimulated by *bar-1*, *sop-1(+)* enforces the requirement for the intronic *cis*-regulatory element.

Development of rays in *pal-1(e2091); sop-1* was not affected by introduction of a mutation in *egl-20* (Table 1, line 14). We were unable to test the effect of the ligand gene *lin-44* or the receptor gene *lin-17* because mutations in these genes are pleiotropic and have severe effects on tail development. *lin-44* might act redundantly with *egl-20* to activate the Wnt pathway in the tail, explaining why there was no effect of an *egl-20* mutation. Or *sop-1* mutants could activate the Wnt signal transduction pathway in a ligand-independent manner similar to the action of *pry-1* mutants.

sop-1 *prevents activation of the* Hox *gene* egl-5 *by* bar-1

We asked whether *sop-1* acted at additional steps of the ray transcription factor cascade to prevent gene activation by the Wnt signal transduction pathway. One of the later steps of the cascade is activation of the *Hox* gene *egl-5* in V6.ppp (Ferreira et al. 1999). Normally, activation of *egl-5* is dependent on the function of *mab-5*. However, we found that in *sop-1* mutants, *egl-5* was activated weakly in the absence of *mab-5* gene function. Whereas in *mab-5(e1239)*, a null allele, there are no V6 rays (rays 2–6) or V5 rays (ray 1), and alae extend through the region where the V-rays normally form (Kenyon 1986), in *mab-5(e1239); sop-1(bx92)* mutants, although there were no normal sets of V6 rays, in 13% of male sides alae stopped short of the ray domain and in these cases an average of 1.5 rays were generated (n = 205 sides).This effect of *sop-1* required *egl-5* activity, because in a *mab-5(e1239) egl-5(n486); sop-1(bx92)* triple mutant, alae always extended into the ray domain and there were no V rays (n = 132 sides).

To determine whether activation of *egl-5* was stimulated by the Wnt signal transduction pathway, we introduced *bar-1(ga80)*. In this background in all male sides alae extended into the tail region and no rays were gen-

erated (n = 205). Thus as it does for *pal-1*, *sop-1* enforces regulatory stringency on *egl-5*. By preventing activation of *egl-5* by *bar-1*, *sop-1* makes *egl-5* expression completely dependent on *mab-5*.

sop-1 *mutations do not elevate gene expression generally*

The foregoing results showing activation of *pal-1* and *egl-5* by *sop-1* mutations might be explained if *sop-1* mutations had a global effect on transcription, possibly resulting in a general elevation of gene expression. To determine whether *sop-1* mutations nonselectively elevated the expression of all genes, we tested the effect of *sop-1* mutations on a known expression-sensitive locus unrelated to ray development. Loss-of-function mutations of *lin-15* cause a hermaphrodite multivulva (Muv) phenotype. The percentage of Muv animals in the hypomorphic background *lin-15(n765ts)* at 20°C is sensitive to changes in the level of global gene expression (Villeneuve and Meyer 1987). We found that the percent of *lin-15(n765ts)* Muv animals (>99%) was not affected by introduction of *sop-1(bx92)* [n = 424 for *lin-15(n765ts) sop-1(+)*; n = 576 for *lin-15(n765ts) sop-1(bx92)*]. Therefore *sop-1* mutations do not appear to elevate levels of gene expression generally, arguing that they act selectively on the regulation of only certain genes.

This result also shows that *sop-1* differs in its action from another putative Mediator component, *sur-2*. Loss-of-function mutations in *sur-2* suppress the effects of an activated Ras/MAP kinase pathway (Singh and Han 1995). In particular, they suppress the Muv phenotype of *lin-15(n765ts)*. Thus Mediator contains components with selective effects in promoting the actions of different transcriptional regulatory signals.

Discussion

Model for pal-1 *regulation*

Our results identify a component of the Mediator complex that is required for normal regulation of genes in a postembryonic transcriptional cascade. Expression of the *caudal* homolog *pal-1* in V6 in late embryogenesis initiates a transcription factor cascade that results in generation of rays in the late L4 male. We have shown that *pal-1* can be activated in V6 either by a pathway requiring an intronic *cis*-regulatory element, or by a pathway involving the action of BAR-1/β-catenin. The two pathways appear to initiate the cascade equally well , producing a normal set of rays. SOP-1, a homolog of a component of the human Mediator complex, suppresses the BAR-1-stimulated pathway normally, thus making *pal-1* expression dependent on the pathway that utilizes the *cis*-regulatory element. SOP-1 similarly makes expression of a later gene of the cascade, *egl-5*, entirely dependent on the transcription factor MAB-5 by suppressing the action of BAR-1/β-catenin on this gene. Thus the mode of regulation of *pal-1* and *egl-5* are influenced by a component of the Mediator complex.

The data presented here suggest the model for *pal-1* activation shown in Figure 7. This model postulates that a transcription factor (labeled I in the figure) binds an intronic enhancer to activate *pal-1* transcription (Fig. 7, top panel). The identity of this factor is unknown, as is its role in the transcription complex assembly pathway. It might act early, binding to chromatin-bound DNA to initiate the assembly pathway. Alternatively, it could act at later steps to recruit chromatin-remodeling factors or holoenzyme, or to release the preinitiation complex for transcription. We suggest that because the requirement for this factor can be overcome by mutation in a component of the Mediator, Factor I is more likely to act at one of the later steps of the pathway. We postulate that opening of the chromatin structure and recruitment of holoenzyme is accomplished by one or more other factors (Factor II in Fig. 7) as explained below.

In a *pal-1(e2091)* mutant background, the model postulates that the intronic factor cannot effectively bind to the mutated *cis*-regulatory element (Fig. 7, bottom panel). Under these circumstances, decrease of *sop-1* gene function allows *pal-1* transcription to be activated by an alternate pathway that is stimulated by BAR-1. Because BAR-1 together with the TCF/LEF family transcription factor POP-1 is likely to act on many genes to convey the anteroposterior position of a cell (Lin et al. 1998), we suggest that BAR-1 acts at a step in the assem-

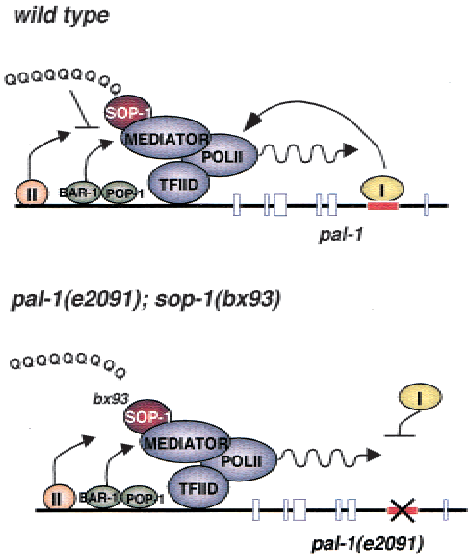

Figure 7. Model for *pal-1* regulation. In wild type, the intronic factor I allows a preinitiation complex containing SOP-1 to transcribe *pal-1*. The holoenzyme, consisting of the Mediator complex and PolII-core polymerase complex are brought to the promoter through the action of other factors, here represented by factor II. Such factors, together with the general A/P specifying factors BAR-1 and POP-1, are prevented by SOP-1 from activating transcription in the absence of factor I. In a *pal-1(e2091); sop-1(–)* background, the negative effect of SOP-1 is reduced, allowing *pal-1* transcription. The *bx92*, *bx93*, and *bx107* mutations reduce or eliminate the carboxy-terminal glutamaine-rich domain of SOP-1, implicating this domain in the SOP-1-mediated repression.

bly pathway after initiation of the pathway by a *pal-1*-specific factor. Initiation of the pathway is the function of the postulated Factor II (see Fig. 7). It seems unlikely that DNA binding by factors such as Factor II and BAR-1/POP-1 will be affected by mutation within the Mediator. A mutation in the Mediator would only be expected to affect recruitment of holoenzyme by DNA-bound factors or the function of holoenzyme after recruitment. Therefore, Factor II and BAR-1/POP-1 most likely also bind in wild type, as shown in Figure 7 (top panel). It is for this reason that we suggest that Factor I acts at a later step in the assembly pathway, after Factor II has initiated the assembly process. Although we show BAR-1/POP-1 as acting in the nucleus at the *pal-1* promoter, we have no direct evidence for nuclear action of BAR-1. One mode of BAR-1 action appears to be cytoplasmic, involving the LIT-1 kinase (Rocheleau et al. 1999; Shin et al. 1999), and this could be the pathway that is involved in preventing expression of *pal-1(e2091)*.

In addition, the model assumes that the effects of SOP-1 mutations occur at the *pal-1* promoter, but it is formally possible that they occur at the promoter of another gene that acts as an alternate *pal-1* activator.

C. elegans *Mediator complex*

Analysis of the *C. elegans* genome sequence reveals homologs of 11 components of the mammalian and yeast Mediator complexes (Boyer et al. 1999; Ito et al. 1999; Kwon et al. 1999; this work, data not shown). An additional seven components of the mammalian complex (TRAP220, TRAP150, TRAP100, TRAP95, TRAP80, CRSP34, CRSP70) are not represented in the *C. elegans* genome sequence (Ito et al. 1999; this work). A functional role in developmental gene regulation has been defined for one of the genes conserved between *C. elegans* and humans, *sur-2*. Loss-of-function mutations of *sur-2* were identified as suppressors of an activated Ras/MAP kinase pathway (Singh and Han 1995). Biochemical studies have shown that hSur-2 (human *sur-2*) is a binding target of the adenovirus E1A transcription factor, as well as of the Ras/MAP kinase-activated transcription factor Elk-1 (Boyer et al. 1999). However, hSur-2 did not appear to be involved in gene activation by Gal4-VP16, a transcription factor with a transcriptional activator domain of a different class. Therefore SUR-2/hSur-2 appears to be a Mediator target that is involved selectively in gene activation by the Ras/MAP kinase pathway. Two additional selective transcription factor targets have been identified in in vitro studies of human Mediator. TRAP220 is a binding and activation target of TR (thyroid hormone receptor) and p53, whereas TRAP80 is a binding and activation target of VP16 and p53 (Ito et al. 1999).

Our results suggest that SOP-1/TRAP230 may be a Mediator target of pathways regulating transcriptional response to the Wnt pathway. Because *sop-1* appears to block action of the Wnt signal transduction pathway, and because it is expressed widely, its effect may have to be relieved wherever the Wnt pathway acts. In this role, SOP-1 may act as an integrator of multiple signaling pathways impinging on the nucleus. One pathway known to act in concert with the Wnt signal transduction pathway in *C. elegans* is the Ras/MAP kinase pathway. Ras/MAP kinase and Wnt/*bar-1*/β-catenin act together to activate the Hox gene *lin-39* in vulval development (Eisenmann et al. 1998), and the Hox gene *egl-5* in the P12 neuroectoblast cell (Jiang and Sternberg 1998). The Ras/MAP kinase pathway does not appear to be involved in development of the rays, and as noted above, *sop-1*, unlike *sur-2*, does not suppress an activated ras pathway. Therefore, *sop-1* may integrate one or more non-Ras/MAP kinase pathway with the Wnt pathway.

The glutamine-rich domain of human TRAP230 functions as a transcriptional activation domain (Ito et al. 1999). This is in contrast to the results reported here in which the glutamine-rich domain appears to function to repress transcription. Mutation of human TRAP230 affecting the glutamine-rich region results in an elevated susceptibility to mental retardation in males (Philibert et al. 1998). Our results suggest that altered cell fate specification within the central nervous system, consequent on misexpression of such key cell-fate specification genes as *caudal* and Hox genes, could be the cause of this condition.

Activation of ray development by the Wnt pathway

The pathway that *sop-1* integrates with the Wnt pathway may be a signaling pathway regulated by cell contacts. Expression of the ray developmental program is regulated by the contacts that seam cells make with their neighbors. Normally, rays develop from only the three most posterior cells in two bilateral rows of nine seam cells, and not from seam cells in the anterior part of the body (Sulston and Horvitz 1977). If, however, anterior seam cells lose contact with their neighbors, they can undergo a cell fate transformation and generate rays (Sulston and White 1980; Austin and Kenyon 1994; Hunter et al. 1999). A similar effect of neighbor-cell contact is seen in *pal-1(e2091)*. In a *pal-1(e2091)* background, where rays are not generated by V6, the ray developmental program is activated in V6 if the posterior neighbor of V6 is ablated (Waring and Kenyon 1990). Activation of ray development following such neighbor-cell ablation, like activation by mutation of *sop-1*, is stimulated by the function of *bar-1* (Hunter et al. 1999). Thus, it is possible that the same pathway is activated both by loss of cell contact and by *sop-1* mutation. As we have shown, in a normal cellular context, *bar-1* is inhibited by *sop-1*. After cell ablation, SOP-1 action could be blocked by an inhibitory signal generated as a result of loss of cell contact.

Unlike the alternate pathway in a *sop-1* background, the alternate pathway activated by loss of cell contact, both in the anterior seam in wild-type and in V6 in *pal-1(e2091)*, requires the Wnt ligand EGL-20 (Hunter et al. 1999). One possible explanation for the absence of EGL-20-dependence of the *sop-1* pathway is that EGL-20 and LIN-44, the two Wnt ligands known to be expressed in

the tail, normally act redundantly on V6. After T-cell ablation, access of LIN-44 to V6 might be blocked by cell debris or damage, thus giving rise to an artifactual dependence on EGL-20 in this experiment. Alternatively, activation of *bar-1* in *sop-1* mutants may be ligand independent. In this case, one effect of the normal context of cellular contacts may be to activate BAR-1/β-catenin.

Materials and methods

Nematodes

Strain maintenance, mutagenesis, and genetic analysis followed Brenner (1974); nematodes were grown at 20°C unless otherwise noted. Most strains carried the *him-5(e1490)* mutation, which gives a high frequency of males in selfing populations. The following additional mutations or strains were used (see Hodgkin 1997): LGII: *tra-2(q276)/unc-4(e120) dpy-10(e128)*; LGIII: *pal-1(e2091)*, *pal-1(ct224)*, *mab-5(e1239)*; LGIV: *egl-20(n585)*; LGX: *dpy-6(e14)*, *egl-15(n484)*, *bar-1(ga80).* Rearrangement: *sDP3*, a free duplication covering the left portion of LGIII including *pal-1*.

Identification of pal-1(e2091)

To localize the mutation in *pal-1(e2091)* either to the 5′ flanking promoter region or to the coding region, introns, and 3′ flanking region, we amplified the *pal-1* gene into two overlapping DNA fragments with the following primers (nucleotide numbers are from cosmid C38D4, GenBank accession no. Z46241): 5′ fragment—(31080–31055) 5′-ACCTGGTCGATACACTCAACGTGTGC-3′; (26622–26641) 5′-AATGAGACAGGTCGGCAAGG-3′; 3′ fragment—(28157–28131) 5′-GGAGCACCTGTTGATGCTGCAAAGGAG-3′; (21737–21762) 5′-GTCTCGAAGCGGCTCAAAATGTGGAG-3′ (Fig. 2A). Both fragments were amplified from either wild-type or *pal-1(e2091)* worms and digested with *Nco*I, which cut at position 28093. The 2.9-kb 5′ fragment (31080–28093) and 6.4-kb 3′ fragment (28093–21737) were gel-purified and ligated, and injected into *pal-1(e2091)* animals together with pRF4, which carries the dominant *rol-6 (su1006)* marker (Mello et al. 1991). F_1 and F_2 Rol males were scored for ray phenotype. The 3′ fragment from *pal-1(e2091)* failed to support rescue, showing that the mutation was downstream of most of the promoter region (Fig. 2A). Therefore, genomic DNA from *pal-1(e2091)* including all of the exons, introns, and ~1 kb of 3′ UTR was amplified and sequenced. The only differences from wild type were the two point mutations in intron 5 shown in Figure 2B.

Sequence of intron 5 from C. briggsae

The sequence of *C. briggsae pal-1* cDNA was kindly provided by C. Hunter (Harvard University, Cambridge, MA). Primers lying within regions corresponding to the fifth and sixth *C. elegans* exons (5′-TCGGCTTTCATAACTTCGGATCG-3′, 5′-GTGCTTCAGACATCTTAGAGTCG-3′) were used to amplify *C. briggsae* genomic DNA and the amplification product was sequenced.

Isolation and characterization of bx89, bx92, bx93, bx103, and bx107

sop-1 mutations were isolated as genetic suppressors of the V6 ray loss phenotype of *pal-1(e2091)*. F_2 or F_3 male progeny of EMS-treated *pal-1(e2091); him-5(e1490)* hermaphrodites were screened for the presence of V6 rays; mutations were recovered from sibling hermaphrodites. Approximately 4000 haploid genomes were screened, and 19 suppressor mutations in >10 genes were recovered, including the intragenic revertant *bx89*.

bx89 is an intragenic revertant of *pal-1(e2091)* by the following evidence. First, it is a fully penetrant dominant suppressor. Second, it mapped close to *pal-1.* Third, one of the two sequence differences between *pal-1(e2091)* and wild-type (T24264C) is reverted in *pal-1(e2091 bx89)* (Fig. 2B).

sop-1 mutants are rescued by maternal gene function: All male progeny from *tra-2/unc-4 dpy-10; pal-1(e2091); sop-1/+* hermaphrodites are Pal, whereas 25% are expected to be of genotype *tra-2; pal-1(e2091); sop-1* and hence suppressed.

bx92, *bx93*, *bx103*, and *bx107* were mapped by three-factor crosses to a single region on LG X between the cloned genes *dpy-6* and *egl-15*. Complementation tests were performed as follows. *tra-2; pal-1(e2091); sop-1(a)* males were mated to *dpy-10 unc-4; pal-1(e2091); him-5; sop-1(b)* hermaphrodites. Non-Dpy nonUnc hermaphrodite progeny were picked [genotype *tra-2/dpy-10 unc-4; pal-1; him-5/+; sop-1(a)/sop(b)*] and allowed to self. F_2 males (homozygous for *tra-2*) were scored. Because *sop-1* is rescued maternally, complementation should have resulted in nearly 100% Pal males, but <50% were observed. (For unknown reasons, the penetrance of *sop-1* suppression in *tra-2* mutant males is lower than in *him-5* males.)

Antibodies and immunostaining

Simultaneous immunohistochemical staining of PAL-1 and MAB-5 followed the method of Hunter et al. (1999). MAB-5 was detected with mouse anti-β-GAL antibody (40-1a, J.R. Sanes, Johns Hopkins University, Baltimore, MD) in a strain (EM512) of genotype *pal-1(e2091); sop-1(bx92); muIs3. muIS3* is an integrated transgenic array consisting of *mab-5::lacZ* and the transformation marker pRF4 (Cowing and Kenyon 1992). Secondary antibody was labeled with FITC. Rabbit anti-PAL-1 antibodies were kindly provided by C. Hunter and were detected by means of secondary antibody labeled with Cy3.

Cloning of sop-1

sop-1 was cloned by complementation rescue. Cosmids from the genetic region of *sop-1* (A. Coulson, Sanger Center, Cambridge, UK) were injected into *pal-1(e2091); sop-1(bx92)* hermaphrodites at a concentration of 20–50 ng/μl (Fig. 4A). pRF4 was coinjected at a concentration of 100–200 ng/μl. F_2 Rol males were scored for presence of V6 rays. Cosmid F47A4 restored the ray loss phenotype of the unsuppressed *pal-1* background. Strong rescuing activity (86% of sides with Pal phenotype compared with 6% in uninjected) was localized to a 14-kb *Hpa*I–*Xho*I fragment (6081–20759) (Fig. 4B).

sop-1 gene structure from exon 9 to the end was confirmed by sequencing cDNA ESTs yk266a1, yk542c11, yk495a10, and yk492f6 kindly provided by Y. Kohara (MIG, Japan). These cDNA ESTs cover the region 14967–7040.

Because of its large size, *sop-1* was cloned in two fragments: the *Xho*I–*Eag*I DNA fragment of F47A4 (20759–13973) was cloned into pBluescript (EM#293); the *Hpa*I–*Bgl*II DNA fragment of F47A4 (6081–17057) was cloned into *Eco*RV–*Bam*HI sites of pBR322 (EM#294). To reconstruct the gene, the 8.4-kb *Eag*I–*Ppu*MI fragment of EM#293 (20759–15306) and the 10.3-kb *Bgl*I–*Ppu*MI fragment of EM#294 (6081–15306) were gel purified, ligated, and injected.

The 5′ upstream sequence and the first 35 amino acids were deleted by cleaving EM#293 with *Eco*RV and *Ppu*MI. The resulting 3.5-kb fragment (18837–15306) was gel purified, ligated to *Ppu*MI-cleaved EM#294, and tested for rescuing activity. Similarly, a carboxy-terminal deletion was constructed by

cleaving EM#294 with *Bam*HI and *Ppu*MI. The resulting 7.8-kb fragment, which lacked the 3′ UTR plus the 41 carboxy-terminal amino acids (7494–15306) was gel purified, ligated to *Ppu*MI-cleaved EM#293, and tested for rescue. Frameshift mutations were introduced into the amino terminal region by filling in with Klenow enzyme either the *Xba*I site (18089) (EM#295) or the *Nco*I site (16480) (EM#296) of EM#293 and religating the blunt ends. The resulting fragments were cleaved with *Ppu*MI, ligated to *Ppu*MI-cleaved EM#294, and tested for rescue.

Mapping the location of mutations in bx92, bx93, bx103, *and* bx107

To molecularly identify the *sop-1* mutations, they were first localized to regions of the *sop-1* gene, as follows. *sop-1* was amplified from mutants in two overlapping fragments. Fragment 1, ~6 kb, covered the amino-terminal portion of the gene [primers: 5′-GGCGTTTCAGATCAACGAGAACCG-3′ (20834–20811), 5′-AGCAATCTTGTCGTCAACTGCCTTC-3′ (14541–14565)], while fragment 2, ~10 kb, covered the carboxy-terminal portion [primers: 5′-TCTCAAGCATGGTGCAGTACATGG-3′ (16005–15982), 5′-GCTCAAGCGTTATCTTGATGACGC-3′ (6041–6064)]. Each fragment was digested with *Ppu*MI and ligated as appropriate with either the wild-type 10.4-kb *Bgl*I–*Ppu*MI fragment of EM#294 or the wild-type 8.4-kb *Eag*I–*Ppu*MI fragment of EM#293 and tested for rescuing activity. PCR amplification products that failed to support rescue were directly sequenced.

RNAi experiments

RNAi experiments were performed as described by Fire et al. (1998). The template DNA fragment, covering the ninth exon of *sop-1* (15724–14416), was amplified with the following primer pair: 5′-CACTAGAATTAACCCTCACTAAAGGGTCGTCG-GAAATATACATTCGCAGCAGG-3′, 5′-CACTAGTAATAC-GACTCACTATAGGGGATCATTCCGTGCGACTTACATC-GGC-3′. The amplification product was transcribed using MEGAscript T3 and T7 kit (Ambion). About 200 ng/μl dsRNA was injected into *pal-1(e2091); him-5* or *him-5* hermaphrodites. Eggs laid between 4 to 24 hr after injection were collected at 12 hr intervals.

sop-1 *reporter genes*

EM#290 contained 2.2 kb of *sop-1* 5′-upstream region and the first four exons. It was constructed by joining the *Xba*I fragment of F47A4 (21136–18089) in-frame into the *Xba*I site of pPD95.67, which contains an SV40 nuclear localization signal, *gfp* coding sequence, and *unc-54* 3′ UTR (for structure of *gfp* vectors, see Fire laboratory vector kit). EM#291 contained a *gfp* in-frame fusion at the carboxy-terminal *Not*I site (8918). It was constructed by inserting the 1-kb *Not*I fragment of pPD103.87 into the *Not*I site of EM#294. EM#292 contained a *gfp* in-frame insertion at the amino-terminal *Xba*I site (18089). It was constructed by inserting the 1-kb *Xba*I fragment of pPD102.33 into the *Xba*I site of EM#293. Before transformation, EM#291 and EM#292 were cleaved with *Ppu*MI and ligated to *Ppu*MI-cleaved EM#293 or EM#294, respectively.

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