The *Caenorhabditis elegans* SOMI-1 zinc finger protein and SWI/SNF promote regulation of development by the *mir-84* microRNA

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Hundreds of microRNAs (miRNAs) have been discovered in metazoans and plants, and understanding of their biogenesis has advanced dramatically; however, relatively little is known about the cofactors necessary for miRNA regulation of target gene expression. In *Caenorhabditis elegans*, the conserved miRNA *let-7* and its paralogs, including *mir-84*, control the timing of stage-specific developmental events. To identify factors required for the activity of *mir-84* and possibly other miRNAs, we screened for mutations that suppress the developmental defects caused by overexpression of *mir-84*. Mutations in the *somi-1* gene prevent these defects without affecting the expression level of *mir-84*. Loss of *somi-1* also causes phenotypes similar to deletion of *mir-84*, showing that *somi-1* is necessary for the normal function of this miRNA. *somi-1* encodes a zinc finger protein that localizes to nuclear foci and binds the promoters of *let-60/RAS*, *lin-14*, and *lin-28*, genes that may be targeted by *mir-84* and similar miRNAs. Genetic evidence shows that *somi-1* inhibits *lin-14* and induction of the vulval precursors by the *let-60/RAS* pathway. Proteomic and genetic screens identified conserved chromatin-remodeling and homeodomain transcription factor complexes that work with *somi-1* to regulate differentiation. Our results suggest that *somi-1* coordinates a nuclear response that complements the activity of *mir-84*.

[*Keywords*: microRNAs; *mir-84*; *let-7*; SWI/SNF; chromatin; differentiation]

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microRNAs (miRNAs) are ~21-nucleotide (nt) RNAs found in plants and throughout metazoan phylogeny. The hundreds of known miRNAs are involved in many processes, including timing of developmental events, apoptosis, organ development and function, and many others (for review, see Alvarez-Garcia and Miska 2005).

Animal miRNAs negatively regulate target mRNAs by binding sites in the 3′ untranslated region (UTR), which typically have perfect or near-perfect complementarity to nucleotides 2–8 (the "seed" region) of the miRNA, but contain mismatches to the rest of the miRNA (Wightman et al. 1993; Doench and Sharp 2004). In some cases, miRNA-bound mRNAs are directed to cytoplasmic processing bodies to be degraded by nucleases or held intact but their translation inhibited (Bagga et al. 2005; Ding et al. 2005). How the fate of particular mRNAs that are targeted by miRNAs is determined is unknown.

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The first two miRNAs discovered, *lin-4* and *let-7*, act in the heterochronic pathway to control the timing of stage-specific developmental events in *Caenorhabditis elegans* (Ambros and Horvitz 1984; Lee et al. 1993; Reinhart et al. 2000). *lin-4* down-regulates its target genes, *lin-14* and *lin-28*, in early larval development, allowing stage-specific progression in patterns of division of epidermal and vulval precursor cells (VPCs) (Lee et al. 1993; Wightman et al. 1993; Moss et al. 1997). *let-7* acts later in larval development to down-regulate target genes such as *lin-41* and *hbl-1* to allow accumulation of the LIN-29 transcription factor, which directs terminal differentiation of epidermal cells and exit from the molting cycle at the adult stage (Rougvie and Ambros 1995; Reinhart et al. 2000; Slack et al. 2000; Abrahante et al. 2003; Lin et al. 2003).

let-7 is conserved across bilaterian phylogeny (Pasquinelli et al. 2000), and some targets of this miRNA are also conserved. *let-7* regulates orthologs of *lin-41* in vertebrate development (Lancman et al. 2005) and the *RAS* oncogene in worms and humans (Johnson et al. 2005). Likewise, orthologs of *lin-4* and *let-7* regulate vertebrate orthologs of *lin-28*. By inhibiting maturation of *let-7*, Lin28 promotes

pluripotency of stem cells (for review, see Nimmo and Slack 2009). Conservation of heterochronic miRNAs and their target mRNAs suggests that cofactors for the function of these miRNAs may also be broadly conserved.

Three *C. elegans* paralogs of *let-7*—*mir-84*, *mir-48*, and *mir-241*—act with *let-7* to control certain developmental events. These miRNAs are identical at the 5′ ends, but diverge at the 3′ ends (Lau et al. 2001). *mir-84* is most similar (18 of 22 nt identity) to *miR-98* of humans and other vertebrates, shown using ClustalW to align *let-7* with 71 of its paralogs in 10 animal species (Supplemental Fig. S1). Human *miR-98* targets the *HMGA2* oncogene (Hebert et al. 2007). In *C. elegans*, cessation of molting in adults is directed by *let-7* redundantly with its paralogs (Abbott et al. 2005; Hayes et al. 2006). Similarly, all three *let-7* paralogs redundantly down-regulate *hbl-1* during larval development, directing timing of divisions of the VPCs and hypodermal seam cells (Abbott et al. 2005).

While *mir-84* acts with its paralogs in these pathways, high gene dosage of *mir-84* is sufficient to induce precocious division of the VPCs to cause a penetrant defect in vulval morphogenesis so that animals never lay eggs, which then hatch within the parent (Hayes and Ruvkun 2005; Johnson et al. 2005). To identify factors required for function of *mir-84* and potentially other miRNAs, we performed a genetic screen for suppressors of this defect, referred to as Somi (suppressor of overexpressed miRNA) mutants. Here, we describe the *somi-1* gene, which encodes a new class of zinc finger protein. In addition to suppressing the phenotypes induced by high *mir-84* gene dosage, loss of *somi-1* enhances heterochronic phenotypes caused by mutations in *let-7* and its paralogs, showing that *somi-1* itself regulates the timing of developmental events. SOMI-1 binds the promoters of particular genes that may be targets of *mir-84* and *let-7* and copurifies with a SWI/SNF-like chromatin-remodeling complex that promotes differentiation. Our results suggest that *somi-1* coordinates a nuclear response that complements regulation by these miRNAs.

Results

To explore the function of *let-7* and paralogous miRNAs, we generated transgenic strains that overexpress the *mir-84* miRNA (Hayes et al. 2006). These animals show precocious development of vulval and epidermal cells similar to *lin-14* and other precocious heterochronic mutants (Ambros and Horvitz 1984; Hayes and Ruvkun 2005; Johnson et al. 2005). The VPCs divide precociously in animals bearing the *mgIs45[mir-84++]* transgene (Supplemental Fig. S2), causing abnormal eversion of the vulva (the Evl phenotype) (Fig. 1A) that prevents all *mgIs45*-bearing animals from laying eggs (Table 1).

To identify genes required for function of *mir-84* (and perhaps other miRNAs), we mutagenized *mir-84*-overexpressing animals and recovered the very rare eggs laid by the F_2 mutant descendants, putative Somi mutants. We focused on the *somi-1(mg415)* mutant, which strongly suppressed the Evl phenotype. *somi-1* mutants appear

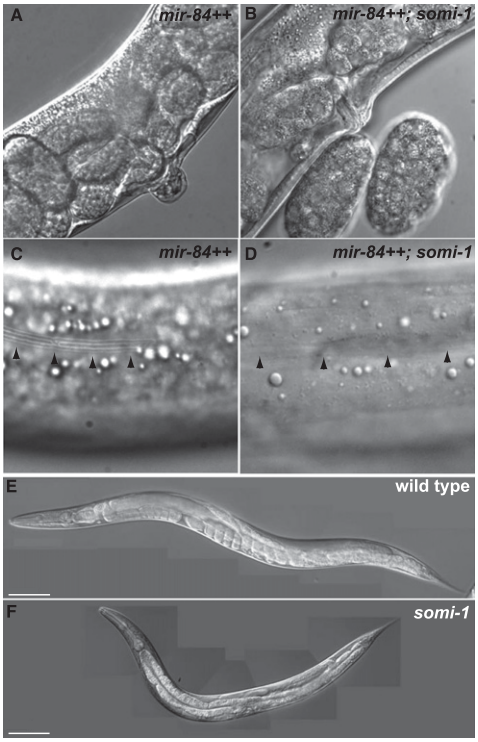

Figure 1. Loss of *somi-1* suppresses precocious development caused by overexpression of *mir-84*. (*A*) *mir-84*-overexpressing adult with a vulval protrusion. (*B*) *mir-84*-overexpressing *somi-1(mg415)* mutant adult with a functional vulva next to two embryos. (*C*) Precocious alae (arrowheads) on the cuticle of a *mir-84*-overexpressing L4 larva. (*D*) A *mir-84*-overexpressing *somi-1(mg415)* mutant L4 larva lacking alae (arrowheads). Wild-type (*E*) and *somi-1(mg415)* (*F*) young adults 74 h after egg lay at 20°C. Bars, 100 μm.

superficially wild type, except that adults have slightly reduced body length, ~12% shorter in young adults (Fig. 1E,F). Genetic mapping (see the Supplemental Material) placed *mg415* within a 1.9-map unit region of chromosome five. RNAi against one gene of the 412 tested in this interval, M04G12.4, enabled *mgIs45*-bearing animals to lay eggs. Sequencing of M04G12.4 in the *mg415* mutant revealed a single cytosine-to-thymine transition (Fig. 2A) predicted to cause a glutamine to an ochre nonsense codon substitution (Fig. 2B). Sequencing of other Somi mutants revealed that two of them, *mg431* and *mg432*, bear distinct nonsense mutations in *somi-1* (Fig. 2).

All three alleles of *somi-1* strongly suppressed the Evl phenotype when they were outcrossed to wild type and introduced to a strain bearing an unmutagenized copy of *mgIs45* (Fig. 1A,B; Table 1). Similar results were obtained for a deletion mutation in M04G12.4 (*tm562*) that eliminates 530 base pairs (bp) encoding the final third of exon two and the following splice donor site. A transgene bearing the M04G12.4 ORF restored the Evl phenotype to *mgIs45; somi-1(mg415)* animals (90%, n = 49) compared with siblings lacking the transgene (6%, n = 17), showing that *somi-1* is encoded by M04G12.4 (Table 1).

Table 1. *Loss of* somi-1 *suppresses the precocious development caused by overexpression of* mir-84

Genotype	Evl [% (n)]	Alae formation at L4 stage (% [n])[c]	Alae formation at adult stage (% [n])[c]
Wild type	0% (20)	0% (10)	100% (23)
mgIs45[mir-84++]	100% (22)[a]	70% (30)[a]	nd
mgIs45/ +	81% (27)	nd	nd
somi-1(mg415)	0% (20)	0% (10)	100% (22)
mgIs45; somi-1(mg415)	10% (39)[a]	0% (21)	100% (11)
mgIs45; somi-1(mg431)[d]	20% (50)[a]	nd	nd
mgIs45; somi-1(mg432)[d]	24% (45)[a]	nd	nd
mgIs45; somi-1(tm562)	9% (45)[a]	18% (11) [b]	100% (12)
mgIs45; control RNAi	100% (103)	nd	nd
mgIs45; somi-1 RNAi	36% (74)[a]	nd	nd
mgIs45; somi-1(mg415)[e]	6% (17)	28% (18)	nd
mgIs45; somi-1(mg415); mgEx740[somi-1(+)][e]	90% (49)[a]	75% (8) [b]	nd
mgIs45; somi-1(mg415)[e]	6% (17)	17% (12)	100% (9)
*mgIs45; somi-1(mg415); mgEx739[*SOMI-1::GFP*]*[e]	70% (27)[a]	73% (11)[b]	100% (6)

(nd) Not determined.
[a]$P < 0.0001$ compared with control, Fisher's exact test, two-tailed.
[b]$P < 0.05$ compared with control, Fisher's exact test, two-tailed.
[c]One side of each animal was examined by Nomarski optics, and the percentage of animals bearing complete or partial alae is indicated.
[d]*somi-1* was marked with *wIs54[scm::gfp]* in these strains.
[e]Siblings that either carried or lacked the transgenes encoding *somi-1* (*mgEx740* or *mgEx739*) were scored.

somi-1 *encodes a zinc finger protein with predicted nuclear localization*

somi-1 encodes two differentially spliced C_2H_2-type zinc finger isoforms (Fig. 2A), with one lacking the N terminus encoded by the first exon. SOMI-1 is conserved in the nematodes *Caenorhabditis briggsae* and *Caenorhabditis remanei* (Fig. 2B), but database searches reveal no orthologs in organisms other than nematodes, although members of the zinc finger class of nucleic acid-binding proteins exist across phylogeny. The three nonsense mutations we identified in *somi-1* are predicted to cause severely truncated SOMI-1 proteins lacking the zinc finger domain and nuclear localization signal (NLS), and thus are likely null alleles.

As a zinc finger protein, SOMI-1 is predicted to bind nucleic acids (Laity et al. 2001). We found a single NLS in SOMI-1 (Fig. 2B), consistent with its nuclear localization (see below) and suggesting that it may regulate transcription or other nucleic acid processing mechanisms. In addition to the zinc finger domain, a similar sequence lacks the second histidine residue of a canonical zinc finger (Fig. 2B). Seven consecutive proline residues at the C terminus are perfectly conserved in *C. briggsae* and *C. remanei*.

somi-1 *facilitates* mir-84 *function in differentiation of the hypodermis*

Loss of *somi-1* suppressed not only precocious vulval development caused by *mir-84* overexpression, but also precocious appearance of alae, cuticular structures secreted by the hypodermal seam cells, which normally form in adults. Animals bearing *mgIs45* formed alae precociously, at the third larval molt (70%, $n = 30$), but this defect was fully suppressed by the *somi-1(mg415)* mutation ($n = 21$) (Fig. 1C,D; Table 1).

mir-84 and its paralog, *let-7*, together direct terminal differentiation of the seam cells and exit from the molting cycle (Hayes et al. 2006). Animals bearing the *let-7(mg279)* mutation, a weak allele that reduces abundance of *let-7* RNA (Bracht et al. 2004), can fail to exit the molting cycle at the adult stage and instead enter a supernumerary molt. Loss of *mir-84* enhances the penetrance of *let-7(mg279)* supernumerary molting (Hayes et al. 2006). We reasoned that if *somi-1* facilitates function of *mir-84* in the hypodermis, then loss of *somi-1* should also enhance *let-7(mg279)*. We assayed supernumerary expression of a green fluorescent protein (GFP) reporter, *mlt-10p::gfp-pest*, which peaks in expression before each of the larval molts and is not expressed in wild-type adults (Frand et al. 2005). When *somi-1(mg415); let-7(mg279)* double and *let-7(mg279)* single mutants were grown in synchrony, a much higher percentage of the double mutants expressed *mlt-10p::gfp-pest* at 72 h of growth (Fig. 3A,B). *let-7 mir-84* double mutant adults similarly express *mlt-10::gfp* sooner after the fourth molt than *let-7(mg279)* single mutants (Supplemental Fig. S3; Hayes et al. 2006). We obtained similar results when *somi-1* was inactivated by RNAi in a *let-7(mg279)* mutant, but did not see supernumerary expression of *mlt-10p::gfp-pest* in wild-type or *mir-84* single mutant animals in which *somi-1* was inactivated (Supplemental Fig. S3) or in *somi-1* single mutants (Fig. 3A). Consistent with these results, inactivation of *somi-1* enhanced the lethality of a *let-7(n2853)* point mutant (Table 2). These results suggest that *somi-1* and *mir-84*, together with *let-7*, direct terminal differentiation of the seam cells, exit from the molting cycle, and formation of the vulva. Furthermore, *somi-1* is not merely required for phenotypes induced by expression of *mir-84* from a transgene, but facilitates activity of the endogenous miRNA.

mir-48 and *mir-241* act with *mir-84* to control larval divisions of the seam cells and exit from the molting cycle in adults (Abbott et al. 2005). Supernumerary expression of *mlt-10p::gfp-pest* in *mir-48 mir-241* adults began earlier

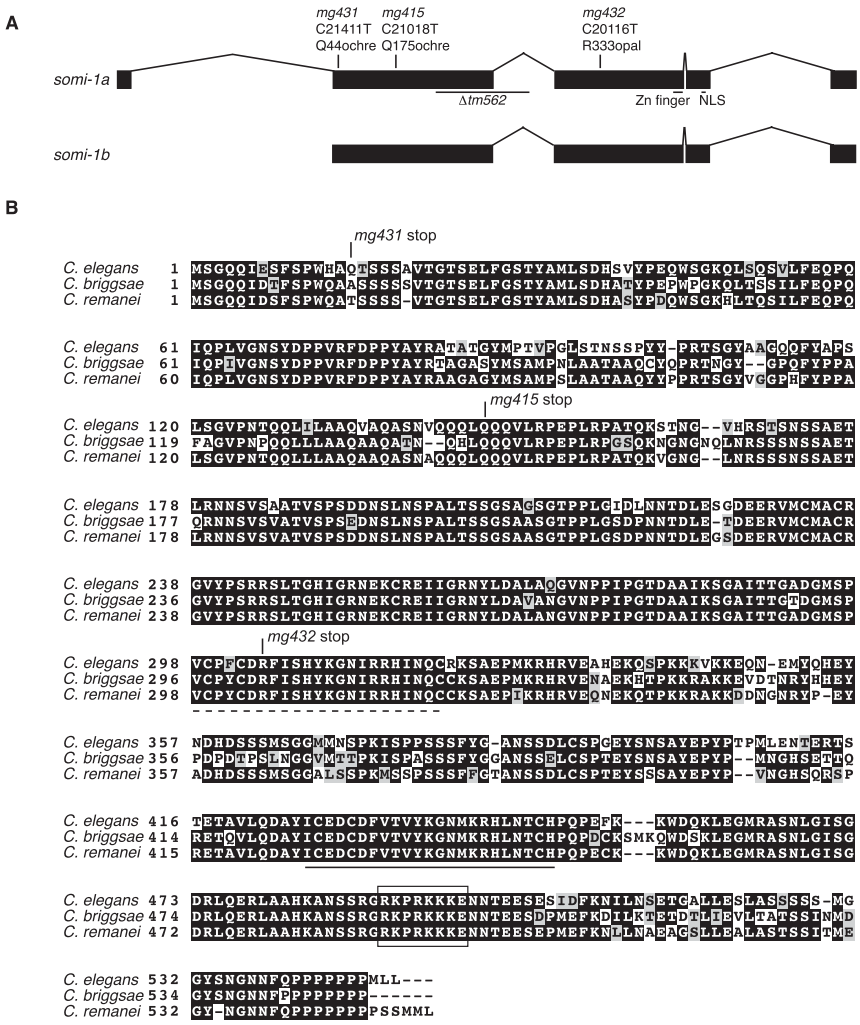

Figure 2. *somi-1* encodes two C_2H_2 zinc finger proteins that are conserved in nematodes. (*A*) Gene structures for *somi-1a* and *somi-1b* isoforms, which are supported by expressed sequence tag data (not shown). Boxes denote exons and lines denote introns. Positions of mutations in *somi-1*, the zinc finger domain, and predicted NLS are shown. Nucleotide numbering refers to cosmid M04G12.4 (accession no.: emb|Z88103) and amino acid numbering refers to M04G12.4A (accession no.: ref|NP_506320.3). (*B*) Alignment of SOMI-1B sequences from *C. elegans* and two related nematodes. Black shading denotes amino acid identity and gray shading denotes similarity. The zinc finger domain is underlined, and a similar sequence that lacks the second histidine residue is marked with a dashed line. The NLS is boxed, and positions where the protein is truncated in *somi-1* nonsense mutants are marked by vertical lines. The alignment was produced with ClustalW 1.83 and shading was produced with Boxshade 3.21. Accession numbers are as follows: *C. elegans*: ref|NP_872161.2|; *C. briggsae*: ref|XP_002647630.1; and *C. remanei*: ref|XP_003114157.1.

when *somi-1* was inactivated by RNAi compared with animals exposed to dsRNA homologous to no worm gene (hereafter, control RNAi) (Fig. 3C). Animals lacking all three of these *let-7* paralogs often burst at the last larval molt, similar to *let-7*-null mutants (Abbott et al. 2005). *somi-1(RNAi)* enhanced this lethality (43%, $n = 273$) in *mir-48 mir-241* mutants compared with control RNAi (27%, $n = 268$). Thus, *somi-1* acts with *mir-48* and *mir-241* to promote stability of the vulva and exit from the molting cycle, potentially by facilitating function of *mir-84*.

To examine the role of *somi-1* in miRNA-regulated temporal specification of the hypodermis, we observed expression of *col-19::gfp* (Abrahante et al. 1998). Synthesis of the adult-specific collagen COL-19 is directed by the LIN-29 transcription factor (Rougvie and Ambros 1995), and *let-7* acts with its paralogs to promote full expression of *col-19::gfp* in the hypodermal syncytium and seam cells (Abbott et al. 2005; Hayes et al. 2006). Gravid *let-7(mg279)* mutants in which *somi-1* was inactivated by RNAi expressed *col-19::gfp* in the seam cells but not the hypodermis (Fig. 3D), a phenotype similar to *let-7(mg279) mir-84(tm1304)* double mutants (Hayes et al. 2006). In contrast, the majority of *let-7* mutants exposed to control RNAi expressed GFP in both the seam cells and hypodermis. Wild-type animals nearly all had full expression of *col-19::gfp* even upon RNAi against *somi-1*. These results confirm that *somi-1* acts in the heterochronic pathway to specify developmental timing of the hypodermis.

Finally, we asked whether *somi-1* is required for precocious development caused by overexpression of other *let-7* family miRNAs. We expected loss of *somi-1* to suppress the vulval defect caused by overexpression of *mir-48* (Li et al. 2005), which is similar to that caused by overexpression of *mir-84*; however, worms bearing the *veIs48 [mir-48(ve33)]* transgene had a completely penetrant Evl phenotype even when *somi-1* was inactivated by mutation or RNAi (Supplemental Table S1). In contrast, only 7% ($n = 83$) of animals bearing a transgene that drives precocious expression of *let-7* under the *lin-4* promoter (Hayes and Ruvkun 2006) expressed *col-19::gfp* at the L4 stage when fed *somi-1(RNAi)* compared with 48% ($n = 177$) of animals fed control RNAi (Supplemental Table S1). This suggests that function of *mir-48* is not mediated by *somi-1* and that *somi-1* may promote inhibition of one or more targets that are specific to *mir-84* and *let-7* but not *mir-48*, at least when each miRNA is overexpressed.

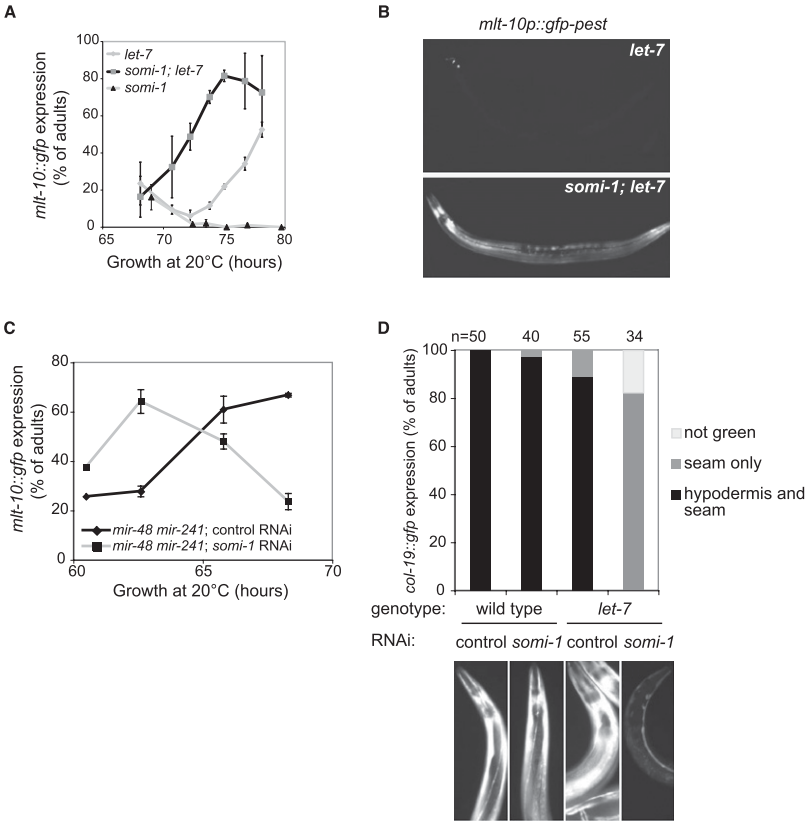

Figure 3. Loss of *somi-1* enhances defects in exit from the molting cycle and differentiation of the hypodermis in adults with mutations in *let-7* or its paralogs. (*A*) Supernumerary expression of *mlt-10p::gfp-pest*, a reporter for molting, in populations of GR1436, GR1605, and GR1707 mutant adults plated as synchronized L1 larvae. The combined results of two experiments are shown. A total of at least 90 animals were scored at each time point. (*A*,*C*) Error bars indicate SEM. (*B*) Typical *mlt-10p::gfp-pest* expression in *let-7(mg279)* single and *somi-1(mg415); let-7(mg279)* double mutants at 74.5 h of growth. (*C*) *mlt-10p::gfp-pest* expression in *mir-48 mir-241(nDf51)* mutant adults plated as synchronized L1 larvae to *somi-1* or control RNAi. The combined results of three experiments are shown. A total of at least 40 animals were scored at each time point. (*D*) Loss of *somi-1* enhances the failure of *let-7(mg279)* mutants to express a reporter for the adult-specific collagen COL-19 in the hypodermal syncytium. Animals were fed *somi-1* or control RNAi, and their progeny were scored as gravid adults for expression of *col-19::gfp* using a Zeiss SV-6 microscope. The combined results of two independent experiments are presented. (*B*,*D*) Images of typical animals were captured using the same exposure settings and were processed identically.

Many protein-coding genes in the heterochronic pathway bear potential target sites for *let-7* family miRNAs (Reinhart et al. 2000), and inactivation of some of these genes causes precocious developmental phenotypes like those caused by overexpression of *mir-84* (Ambros and Horvitz 1984). The *lin-14* 3′ UTR bears several potential targets for *mir-84* (Hayes and Ruvkun 2005), and expression of *mir-84* begins in the early L1 stage (Esquela-Kerscher et al. 2005; Hayes et al. 2006), suggesting that *mir-84* may act with *lin-4* during early larval development to regulate *lin-14*. Indeed, elevated expression of *mir-84* inhibits *lin-14* in a 3′ UTR-dependent way (Hayes and Ruvkun 2005). The *lin-14(n360)* mutation, which prevents synthesis of LIN-14B but leaves LIN-14A unaffected, causes precocious formation of alae. This defect is suppressed when LIN-14A levels are elevated by loss of the *lin-4* miRNA (Reinhart and Ruvkun 2001), suggesting loss of *somi-1* could similarly raise expression of particular heterochronic genes. Indeed, only half of *lin-14(n360)* mutants formed alae at the L4 stage when *somi-1* was inactivated, in contrast to all *lin-14(n360)* mutants fed control RNAi (Supplemental Table S1). Similarly, the Evl phenotype was less penetrant upon inactivation of *lin-14* in *somi-1* mutants than in wild type (Supplemental Table S1). Thus, loss of *somi-1* may suppress the effects of overexpression of *mir-84* at least in part by increasing expression of *lin-14*. In contrast, loss of *somi-1* did not suppress the Evl phenotype of a temperature-sensitive allele of *lin-14*, and RNAi against other heterochronic genes caused similarly penetrant precocious phenotypes in wild-type and *somi-1* mutant animals.

somi-1 *is not required for expression of* mir-84

Loss of *somi-1* could suppress *mir-84* overexpression phenotypes and phenocopy a *mir-84*-null mutant by reducing expression of *mir-84*. All animals with two copies of the *mgIs45[mir-84++]* transgene ($n = 22$) but only 81% ($n = 27$) of animals with one copy of this transgene formed a vulval protrusion that prevented egg laying (Table 1), suggesting that loss of half of the expression of *mir-84* from *mgIs45* can only weakly suppress precocious vulval development. Northern blotting showed that loss of *somi-1* did not alter the level of endogenous *mir-84* in two independent samples (Supplemental Fig. S4A), and expression of *mir-84* from *mgIs45* in a *somi-1* mutant was similar to wild type in two cases and decreased by 44% in one case (Supplemental Fig. S4B). Thus, change in *mir-84* abundance

Table 2. *Loss of* somi-1 *enhances the lethality of a* let-7 *point mutant*

Genotype	RNAi	Lethality (% [n])
Wild type	Control	0% (95)
Wild type	*somi-1*	0.6% (172)
let-7(n2853)	Control	4% (566)
let-7(n2853)	*somi-1*	81% (505)[a]

Wild-type or GR1434 L4 animals were placed on bacteria expressing dsRNA homologous to the indicated gene or no worm gene, and their progeny were scored for bursting at the L4-to-adult transition.

[a] $P < 0.0001$ compared with control, Fisher's exact test, two-tailed.

in total RNA was too small to account for the nearly complete suppression by loss of *somi-1* of the vulva formation defect caused by *mir-84* overexpression. Variation in expression of *mir-84* from the transgene could be due to spontaneous partial silencing of the transgene, a process we do not believe *somi-1* is involved in. Northern analysis cannot exclude the possibility that *somi-1* is required for *mir-84* expression in particular tissues. However, we found no change in expression of *mir-84::gfp* (Hayes et al. 2006) in a *somi-1* mutant (data not shown). These results suggest that *somi-1* does not regulate expression of *mir-84*, but acts genetically downstream from *mir-84*.

SOMI-1 is localized to nuclear foci and coexpressed with mir-84

To determine in which tissues *somi-1* acts, we generated transgenes that fuse sequence 5′ of the transcriptional start of *somi-1a* to GFP. The *somi-1p::gfp* transcriptional fusion gene was expressed in the hypodermal seam cells (Fig. 4A) and the somatic gonad and VPCs (Fig. 4B). This expression pattern is similar to that of reporters for *mir-84* (Esquela-Kerscher et al. 2005; Johnson et al. 2005; Hayes et al. 2006) and shows that *somi-1* is expressed in tissues that develop precociously when *mir-84* is overexpressed. Seam cell expression of *somi-1p::gfp* was most common in L4 larvae and adults, suggesting that *somi-1* is up-regulated upon differentiation of this tissue (data not shown). The earliest *somi-1p::gfp* expression was in comma stage embryos (Fig. 4C), whereas *mir-84::gfp* expression was first detected in larvae (Hayes et al. 2006). *somi-1p::gfp* was also expressed in body wall muscle (Fig. 4A) and certain neurons in the head (Fig. 4D) and tail (data not shown), which do not express *mir-84::gfp*. The partial overlap in spatial and temporal expression patterns of GFP reporters for *somi-1* and *mir-84* is consistent with *somi-1* not only facilitating function of *mir-84*, but also acting independently of *mir-84*.

Immunofluorescence using antisera raised against a full-length His-SOMI-1A fusion protein confirmed the expression pattern revealed by *somi-1p::gfp*, except that hypodermal expression of SOMI-1 was more evident by immunostaining. SOMI-1 was detected in the nuclei of embryos beginning at the comma stage and in larvae of all stages and adults in hypodermal, body wall muscle, and most other cells in wild type (Fig. 4E) but not in a *somi-1(mg415)* mutant (Fig. 4F). SOMI-1 was also detected in the somatic gonad, including the distal tip (Fig. 4G) and sheath cells (data not shown).

Fusion of the *somi-1* promoter and genomic protein-coding sequence to the N terminus of GFP yielded a rescuing SOMI-1::GFP reporter (Table 1) that was typically localized to the nucleus but excluded from the nucleolus. SOMI-1::GFP was often concentrated in nuclear foci, which were most apparent in embryos (Fig. 5A), but could also be detected in larvae (Fig. 5B). SOMI-1 immunofluorescence also concentrated in nuclear foci and colocalized extensively with DNA, as visualized by staining with diamino-2-phenylindole (DAPI) (Fig. 5C). SOMI-1::GFP was expressed in the same tissues as the transcriptional *somi-1p::gfp* fusion gene as well as the hypodermal syncytium and gut (data not shown). This expression pattern matches SOMI-1 immunostaining and suggests that enhancers embedded downstream from the start codon that are absent from *somi-1p::gfp* drive expression of *somi-1* in some tissues.

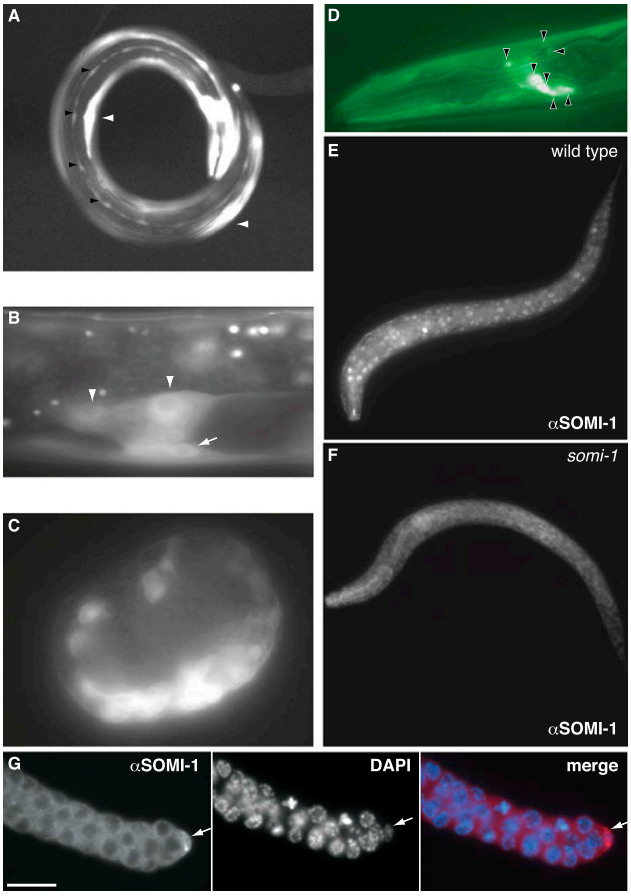

Figure 4. *somi-1* is expressed in tissues affected by loss of paralogs of *let-7* and in other tissues. (*A–D*) *somi-1p::gfp::somi-1 3′ UTR* expression. (*A*) Adult expressing *somi-1p::gfp* in the hypodermal seam cells (black arrowheads), body wall muscle (white arrowheads), and neurons. (*B*) *somi-1p::gfp* in the somatic gonad (arrowheads) and a VPC (arrow) of an L3 larva. (*C*) Onset of *somi-1p::gfp* expression in a comma stage embryo. (*D*) *somi-1p::gfp* in head neurons (arrowheads) of an L3 larva. GFP image overlaid on Nomarski image. (*E*) SOMI-1 immunostaining in the nuclei of hypodermal, muscle, and other cells of a wild-type L1 larva. (*F*) Immunostaining of a *somi-1(mg415)* mutant L1 larva showing only background staining. (*G*) SOMI-1 immunostaining in the distal tip cell (arrow) of the somatic gonad. Faint cytoplasmic staining of the germ cells was sometimes also seen in *somi-1* mutants and may be nonspecific. Bar, 10 μm.

SOMI-1 binds the promoters of particular heterochronic genes and let-60/RAS

Given the localization of SOMI-1 to nuclear foci, we expected that *somi-1* might bind the promoters of particular heterochronic genes or others involved in development of the vulva and hypodermis. To test this, we performed

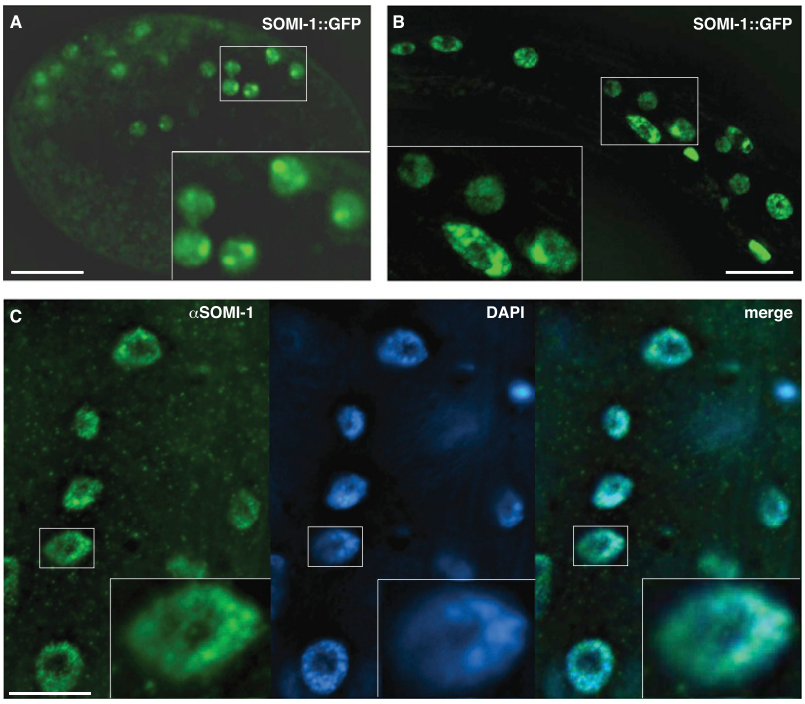

Figure 5. SOMI-1 localizes to nuclear foci. (*A*,*B*) *mgIs57[SOMI-1::GFP]* expression. (*A*) SOMI-1::GFP in the nuclei of a comma stage embryo. The image corresponds to one deconvolved optical section. (*B*) SOMI-1::GFP in hypodermal and neuronal nuclei of an L2 larva. Five deconvolved optical sections were flattened into one image. (*C*) SOMI-1 immunostaining (green) in hypodermal nuclei near the tail of a wild-type adult with DNA visualized by DAPI. Images correspond to one deconvolved optical section. (*A–C*) Bars, 10 μm. Boxed areas are magnified in *insets*.

chromatin immunoprecipitation (ChIP) on animals bearing SOMI-1::GFP. The promoter of *let-60/RAS* was enriched up to 4.6-fold over an intergenic control and that of *lin-28* was enriched up to 3.2-fold in two independent experiments on L2 larvae (Fig. 6A; Supplemental Fig. S5B). The *lin-14a* and *lin-14b* promoters were enriched variably in L2 animals (1.5-fold to 3.1-fold), but *lin-14a*, *lin-28*, and *let-60* promoters were enriched more than fourfold in ChIP performed on a mixture of gravid adults and embryos (Fig. 6A; Supplemental Fig. S5). We observed no or inconsistent enrichment of the promoters of *let-7*, *mir-84*, or other heterochronic or *EGF/RAS* pathway genes (*lin-45/RAF* and *let-23/EGF-R*), or those of selected transcription factors involved in vulval development, such as *egl-5* and *egl-13* (Fig. 6A; Supplemental Fig. S5).

somi-1 *facilitates regulation of* let-60/RAS *by* mir-84

mir-84 regulates the *C. elegans* gene *let-60*, and *let-7* regulates its human homolog, the oncogene *RAS* (Johnson et al. 2005). Activating mutations in *let-60* cause elevated induction of the VPCs, resulting in the appearance of ventral protrusions, the multivulva (Muv) phenotype (Beitel et al. 1990). *let-60(n1046)* mutants in which *somi-1* was inactivated by RNAi formed an average of 2.3 ± 1.0 (n = 214) ventral protrusions compared with 1.9 ± 1.0 (n = 221) for animals on control RNAi ($P < 0.0001$, Student's t-test), consistent with *somi-1* facilitating down-regulation of *let-60* (Fig. 6B). *somi-1(RNAi)* reduced 3.1-fold the percentage of adults with no ventral protrusions and increased 3.4-fold the percentage of adults with four protrusions compared with *let-60(n1046)* mutants treated with control RNAi ($P <$ 0.01, Fisher's exact test). In contrast, *somi-1(RNAi)* in a *mir-84*-null mutant background had no effect on the number of ventral protrusions in *let-60(n1046)*. Finally, the mean number of ventral protrusions in *n1046* mutants that overexpressed *mir-84* increased from 1.5 ± 1.0 (n = 146) to 2.1 ± 1.0 (n = 139) when *somi-1* was inactivated. The percentage of *mir-84++; let-60(gf)* animals with no protrusions was reduced 2.5-fold and the percentage with three protrusions was increased 2.6-fold by inactivation of *somi-1* ($P < 0.01$, Fisher's exact test). These data are consistent with *mir-84* negatively regulating *let-60/RAS* in a *somi-1*-dependent way. Together with ChIP data showing binding of SOMI-1 to the *let-60* promoter, they suggest that *somi-1* inhibits expression of *let-60* in VPCs that do not normally adopt vulval fates. The fact that deletion of *mir-84* abolishes enhancement of *let-60(gf)* by loss of *somi-1* suggests that *somi-1* mediates a nuclear response to the activity of *mir-84*. Given that SOMI-1::GFP was not enriched at the promoter of *mir-84* and loss of *somi-1* did not appear to change expression of reporters for *mir-84* in the VPCs (data not shown), *somi-1* is more likely to mediate response to, rather than expression of, *mir-84*.

Suppression of a *lin-14(lf)* or enhancement of a *let-60(gf)* mutation by loss of *somi-1* and the binding of SOMI-1::GFP to the promoters of these genes suggest that they may be up-regulated in a *somi-1* mutant. However, quantitative real-time RT–PCR (qRT–PCR) analysis of total RNA from synchronously grown animals at various stages did not reveal up-regulation of *lin-14*, *let-60*, or other potential targets of *mir-84*. Indeed, these transcripts typically had slightly (approximately twofold) reduced abundance in a *somi-1* mutant relative to wild type (data not shown). A *let-60p::gfp::let-60 3′ UTR* reporter also did not show up-regulation in the VPCs of a *somi-1* mutant, although the intense, ubiquitous expression of *let-60::gfp* could mask such a change (data not shown). Staining with LIN-14

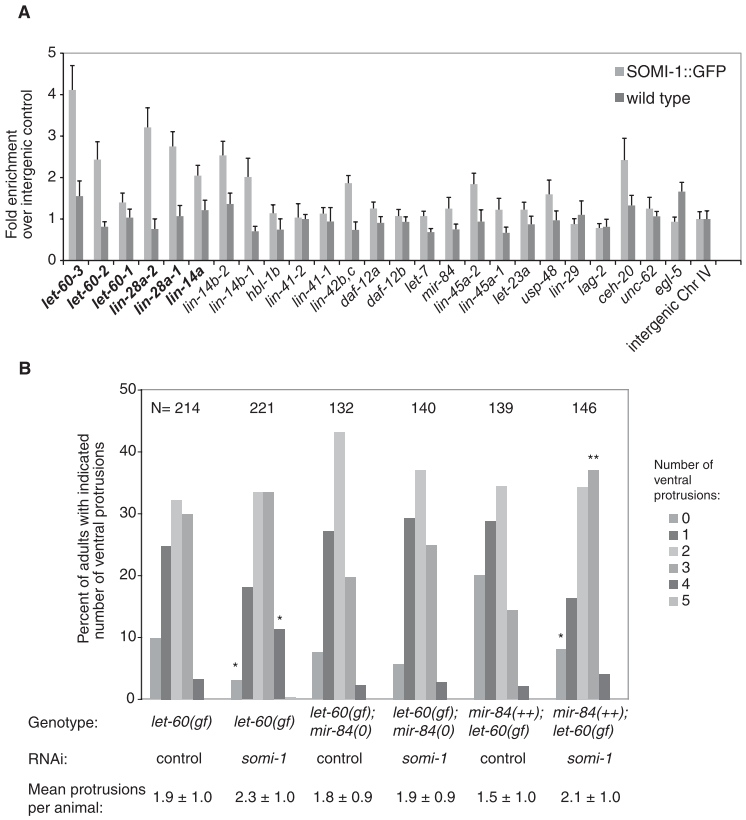

Figure 6. SOMI-1 is enriched at the promoters of *let-60/RAS*, *lin-14*, and *lin-28*. (*A*) Chromatin was isolated from α-GFP immunoprecipitates of L2 stage SOMI-1::GFP or wild-type worms and the purified DNA was assayed by qPCR with primers homologous to the promoters of candidate genes (see Supplemental Table S5). The abundance of each DNA relative to an intergenic control is plotted. Genes in bold were consistently enriched at least twofold in independent experiments. Error bars indicate SEM in qPCR replicates. (*B*) Inactivation of *somi-1* enhances a *let-60* gain-of-function mutant. MT2124, GR1689, or GR1690 animals were placed on *somi-1(RNAi)* or control bacteria, and the number of ventral protrusions, representing ectopic induction of the VPCs, was scored in each of their adult progeny using a dissecting microscope. The mean number of protrusions per animal ± SD and the percentage of animals with the indicated number of protrusions are indicated. Significant differences compared with control are indicated: (*) $P < 0.01$; (**) $P = 0.0001$, Fisher's exact test.

antibody was also not obviously changed in *somi-1* mutants (data not shown). Mutation of *somi-1* may cause changes in gene expression too subtle to detect with current methods that nonetheless cause phenotypic change. Alternatively, rather than affecting transcription, *somi-1* could promote targeting of transcripts for miRNA-mediated silencing upon export to the cytoplasm. Supporting this possibility, the promoter from which an mRNA is transcribed can affect its regulation by miRNAs (Kong et al. 2008).

SOMI-1 copurifies with SWI/SNF-like proteins that promote miRNA-mediated differentiation

To examine how *somi-1* acts with the *mir-84* miRNA, we looked for proteins that bind to SOMI-1. Functional SOMI-1::GFP was purified from mixed stage extracts by immunoprecipitation followed by identification of copurifying proteins (Supplemental Fig. S6A) by mass spectrometry (LC-MS/MS). This yielded several specific binding partners (Supplemental Table S2), although most were low in abundance, suggesting that they bind transiently, only in particular tissues, or at certain developmental stages.

We used RNAi tests to evaluate the functional importance of any specific binding partners. Because depletion of some interactors, such as SWSN-1 (Cui et al. 2004), causes an Evl phenotype in a wild-type background that could complicate interpretation of vulval phenotypes, we used hypodermal assays. We reasoned that reduced abundance of proteins that promote function of SOMI-1 should phenocopy *somi-1*, enhancing a *let-7(mg279)* mutant, whereas depletion of proteins that oppose function of SOMI-1 might suppress a *somi-1; let-7* double mutant. Inactivation of several candidates significantly increased the percentage of synchronously growing *let-7(mg279)* worms that showed supernumerary expression of *mlt-10::gfp* or lacked expression of *col-19::gfp* (Fig. 7B; Supplemental Table S2; Supplemental Fig. S7A). Most of the gene inactivations that enhanced *let-7* in both assays correspond to components of a chromatin-remodeling complex similar to yeast SWI/SNF or RSC and human BAF and PBAF, respectively (Fig. 7A; Supplemental Table S2). Functions of other categories of SOMI-1 interactors will be described separately. Depletion of the majority of SWI/SNF components for which RNAi clones were available enhanced *let-7(mg279)* in both assays (Fig. 7A). We did not observe supernumerary expression of *mlt-10::gfp* in wild-type animals treated with the RNAi clones we assayed (data not shown). Only *swsn-1* RNAi blocked expression of *col-19::gfp* in the majority of wild-type gravid adults (Supplemental Table S2; Supplemental Fig. S7B). This homolog of BAF155, a core component of BAF and PBAF, may be especially sensitive to depletion.

To further examine the genetic interaction between SWI/SNF and *let-7*, we observed *mlt-10::gfp* expression over time in mutants grown on the RNAi clones that caused the most penetrant defects in the *mlt-10::gfp* assay. *swsn-2.1* and *tag-298* encode orthologs of accessory components that promote the function of SWI/SNF complexes and their recruitment to particular loci (Kwon and Wagner 2007; Moshkin et al. 2007). Inactivation of *swsn-2.1* and *tag-298*, like *somi-1(RNAi)*, caused earlier onset of supernumerary

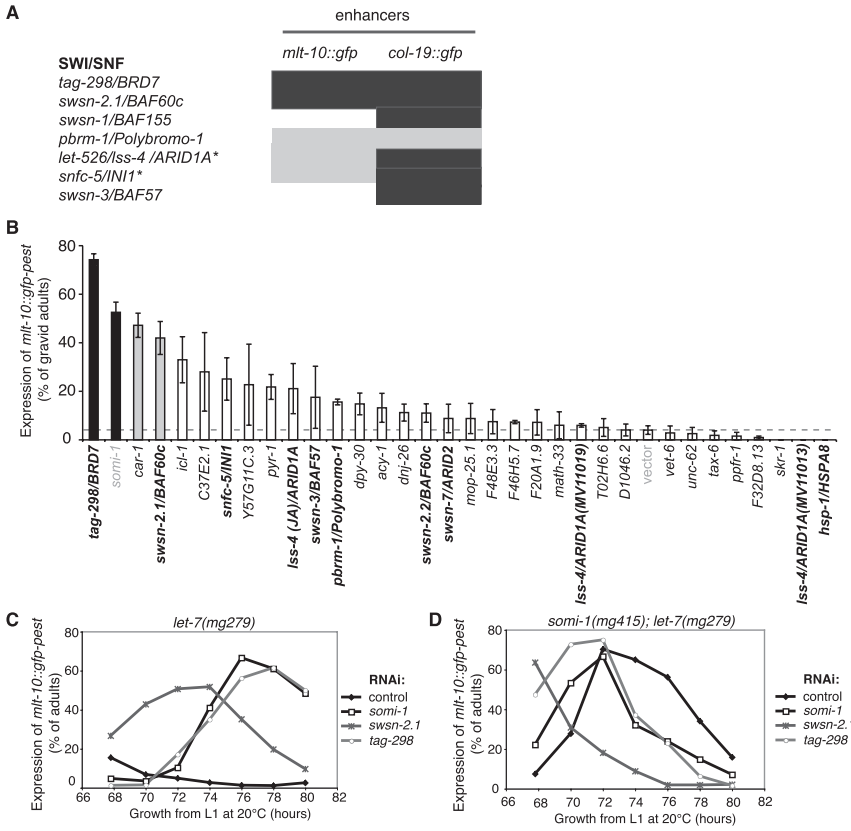

Figure 7. SWI/SNF-related proteins that copurify with SOMI-1 regulate differentiation of the hypodermis. (*A*) Many genes that enhance *let-7(mg279)* when inactivated encode homologs of components of the SWI/SNF chromatin-remodeling complex. Human orthologs are given. Black and gray boxes indicate strong and weak enhancement, respectively. *swsn-1* was not assayed for *mlt-10::gfp* expression because its inactivation slowed development. The asterisk (*) denotes genes whose products did not copurify with SOMI-1 but were assayed because they have a related function. (*B*) Chart of data from Supplemental Table S2. Synchronized *let-7(mg279)* L1 larvae were placed on the indicated RNAi clone and scored at ~76 h. The dashed line indicates percentage of gravid adults expressing *mlt-10p::gfp-pest* when treated with control bacteria. SWI/SNF-related gene names are bold, and *somi-1* and vector controls are gray. Error bars represent SEM for independent experiments. Bar colors show significant difference from control: (black) $P < 0.001$; (gray) $P < 0.05$, one-way ANOVA, Tukey's post hoc test. (*C*,*D*) Expression of *mlt-10p::gfp-pest* in the hypodermis of gravid GR1436 (*C*) or GR1605 (*D*) worms placed on the indicated RNAi bacteria as synchronized L1 larvae. At least 25 worms were scored at each time point.

expression of *mlt-10::gfp* in the *let-7(mg279)* mutant (Fig. 7C; Supplemental Fig. S6B). To ask whether these SWI/SNF components require *somi-1* to inhibit the supernumerary molt, we inactivated *tag-298* and *swsn-2.1* in a *somi-1(mg415); let-7(mg279)* mutant background. We observed further enhancement (Fig. 7D; Supplemental Fig. S6C), indicating that SWI/SNF acts at least in part independently of SOMI-1 to inhibit the supernumerary molt. Consistent with this, an mCherry::TAG-298 fusion protein partly colocalized with SOMI-1::GFP foci in hypodermal nuclei but was also in parts of nuclei that lacked SOMI-1::GFP (Supplemental Fig. S8).

Finally, inactivation of *unc-62*, *ceh-20*, or *ceh-40*, which encode cofactors of homeobox (Hox) transcription factors (Van Auken et al. 2002), strongly restored expression of *col-19::gfp* in *let-7 mir-84* or *somi-1; let-7* adults (Supplemental Tables S2, S3; Supplemental Fig. S9). Inactivation of three of 46 additional homeodomain genes that we screened by RNAi—*ceh-6/POU3F3*, *ceh-12/MNX1*, and *ceh-13/HOXB1*—restored expression of *col-19::gfp* to the hypodermis of both *let-7 mir-84* and *somi-1; let-7* adults (Supplemental Table S3). Only a single peptide corresponding to UNC-62 suggested physical interaction of SOMI-1 with these factors. Thus, *somi-1* may oppose the activity of Hox complexes that promote larval fates in the hypodermis, but this regulation may be indirect. Consistent with this possibility, inactivation of *sop-2* or *sor-1*, which globally repress *Hox* gene expression, enhances the lethal bursting phenotype of *let-7(mg279)* (Zhang et al. 2006; Parry et al. 2007).

Discussion

Enrichment of SOMI-1 at the promoters of *lin-14*, *lin-28*, and *let-60/RAS*, transcripts of which may be targets of *let-7* and *mir-84*, and the requirement for *somi-1* for the defects caused by misexpression of *mir-84* and *let-7* suggest that *somi-1* mediates a nuclear response to the activity of these miRNAs. Genetic and ChIP evidence suggest that SOMI-1 inhibits *lin-14* and *let-60/RAS* in a way that complements their regulation by miRNAs (Supplemental Fig. S10). The identification of SOMI-1 and its interactors gives insight into how cytoplasmic inhibition of the target mRNAs of *let-7* and its paralogs is translated into changes in chromatin structure and gene expression that promote differentiation. *somi-1* provides competence to respond to *mir-84*: Like deletion of *mir-84*, *somi-1*-null mutations alone cause no dramatic defects in developmental timing, but *somi-1* is required for the defects caused by elevated expression of *mir-84*, and its loss phenocopies the developmental timing deficits in a *let-7* mutant background conferred by loss of *mir-84*.

let-7 promotes differentiation of the hypodermis in part by inhibiting *lin-41* to allow the function of LIN-29, which effects a transcriptional switch from larval to adult components of the cuticle (Rougvie and Ambros 1995; Slack et al. 2000). The fact that *somi-1* acts with *let-7* to promote full expression of the adult-specific collagen gene *col-19*, transcription of which is activated by LIN-29, means that *somi-1* acts at least in part in the canonical heterochronic

pathway. But the identification of targets of *let-7* in addition to *lin-41* (Abrahante et al. 2003; Lin et al. 2003; Grosshans et al. 2005) and the fact that overexpression of *mir-84* partly suppresses the supernumerary molting phenotype of a *lin-29*-null mutant (Hayes et al. 2006) indicate that factors in addition to LIN-29 promote differentiation of the hypodermis and exit from the molting cycle.

A small number of loci facilitate the function of *mir-84*. Our screen recovered three alleles of *somi-1*, about one-sixth of the mutants found in the screen of the F_2 progeny of the mutagenized worms. In addition, in a screen of the RNAi clones from chromosome four, about one-sixth of the genome, none robustly enabled egg laying in *mir-84*-overexpressing animals (Hayes and Ruvkun 2005). It is unlikely that this is because RNAi is ineffective in generating the Somi phenotype, because *somi-1* loss of function is phenocopied well by RNAi. Thus, identification of a small number of additional Somi mutants may facilitate a relatively complete picture of how *mir-84* regulates development.

Molecular function of SOMI-1

A molecular function of SOMI-1 is suggested by its identity as a zinc finger protein, its localization to nuclear foci, and its enrichment at particular loci. Many zinc finger proteins are transcription factors, although the zinc finger may also mediate interaction with RNA or proteins (Laity et al. 2001). Some proteins with a single zinc finger, such as SUPERMAN in *Arabidopsis* and the Trithorax-like GAGA factor in *Drosophila*, bind specific DNA sequences (Dathan et al. 2002), meaning that SOMI-1 could target other factors to particular loci. The nuclear foci formed by SOMI-1::GFP likely reflect endogenous SOMI-1 function, because SOMI-1::GFP rescues *somi-1* mutant defects. *C. elegans* HPL-2, a homolog of heterochromatin protein 1 (HP1) that inhibits vulval induction, is recruited to nuclear foci by the zinc finger transcription factor LIN-13 (Coustham et al. 2006). SOP-2 and SOR-1, which form a Polycomb-like complex that represses *Hox* genes, also localize to nuclear foci (Zhang et al. 2006). Although *somi-1* may oppose the function of some homeodomain genes, loss of *somi-1*, unlike *sop-2* or *sor-1*, does not globally derepress *Hox* gene expression (data not shown). However, localization of SOMI-1 to nuclear foci and its copurification with SWI/SNF-related proteins suggest that it may promote chromatin remodeling to inhibit expression of particular target genes of *mir-84*.

Chromatin-remodeling complexes and differentiation

It is increasingly clear that not only are new DNA sequence-specific transcription factors expressed during tissue differentiation, but the composition of general transcription factor complexes such as TFIID, Mediator, and SWI/SNF changes as well (D'Alessio et al. 2009). For example, the SWI/SNF subunits Baf45a and Baf53a act in neural progenitors of mice, but their replacement with Baf45b and Baf53b drives differentiation into post-mitotic neurons (Lessard et al. 2007). This switch is promoted by miRNAs *mir-9** and *mir-124* (Yoo et al. 2009).

Our results, together with prior identification of *lss-4*/*ARID1* as a target of *let-7* (Grosshans et al. 2005), suggest that *let-7* could promote a similar shift from LSS-4-containing BAF-like complexes to PBAF-like complexes containing PBRM-1, TAG-298/BRD7, and SWSN-2.1/BAF60c that act with SOMI-1 to promote differentiation of the hypodermis. Arid1a (the ortholog of LSS-4) and Arid2 are diminished compared with other SWI/SNF components upon differentiation of mouse embryonic stem cells (Kaeser et al. 2008), suggesting that down-regulation of LSS-4/Arid1a upon differentiation of particular tissues may be conserved. LSS-4 did not copurify with SOMI-1::GFP, in contrast to PBAF-specific subunits PBRM-1/Polybromo, TAG-298/BRD7, and SWSN-7/ARID2 (Kwon and Wagner 2007; Kaeser et al. 2008). Furthermore, three different *lss-4* RNAi clones caused the majority of *let-7(mg279)* mutants to burst at the vulva, an effect that we observed for no other SWI/SNF component nor *somi-1* (Supplemental Table S2). This may seem to contradict the identification of *lss-4* as a suppressor of *let-7* (Grosshans et al. 2005), but both the activity of *lss-4* during larval growth and its down-regulation in adults may be needed for normal development. Our RNAi conditions may have more strongly inactivated *lss-4* at the time of vulval specification. Unique association of the bursting phenotype with *lss-4(RNAi)* and absence of LSS-4 from the SOMI-1::GFP purification are consistent with SOMI-1 acting specifically with PBAF-like complexes to promote differentiation.

Vertebrate orthologs of the PBAF subunits that act with *somi-1* and *let-7* to promote differentiation of the hypodermis have analogous roles. The human ortholog of TAG-298, BRD7, is a candidate tumor suppressor required for p53-mediated senescence and potentially other differentiation events (Burrows et al. 2010; Drost et al. 2010). BAF60c, the vertebrate ortholog of SWSN-2.1, mediates interactions between BAF complexes and transcription factors (Debril et al. 2004) and promotes skeletal muscle differentiation (Simone et al. 2004). Regulation of the vertebrate ortholog of LIN-41 by *let-7* is proposed to control muscle differentiation (Lancman et al. 2005), raising the possibility that *let-7* and BAF60c cooperate in this process.

SWI/SNF complexes in the balance appear, like *somi-1*, to inhibit VPC induction. Worms mutant for *swsn-1*, which encodes a core component of all SWI/SNF complexes, have an impenetrant Muv phenotype that is synergistically enhanced by mutation of *lin-15*, an inhibitor of *lin-3/EGF* expression (Cui et al. 2004). In contrast, mutations in several SWI/SNF accessory subunits impair vulval induction (Cui et al. 2006; Lehner et al. 2006). Mutations in accessory subunits may both positively and negatively affect transcription of various target genes, as observed for inactivation of the orthologs of *lss-4* and *tag-298* in mammalian cells (Kaeser et al. 2008).

In *C. elegans*, SWI/SNF complexes act with several signaling pathways to control the fate of hypodermal cells. For example, the larval asymmetric division of the T cell, the most posterior of the seam cells, which differentiate under the control of *let-7* at the adult stage, is regulated by SWI/SNF, together with Wnt signaling (Sawa et al. 2000), the Hox protein NOB-1/Abd, and cofactors

PSA-3/Meis and CEH-20/Pbx (Arata et al. 2006), and the nuclear hormone receptor encoded by *nhr-25* (Hajduskova et al. 2009), itself a target of *let-7* (Hayes et al. 2006). SOMI-1 may promote alterations in chromatin structure, mediated by a PBAF-like complex, that occur in response to expression of *mir-84* and *let-7* and oppose the activity of larval fate-promoting factors.

somi-1 *may regulate several aspects of vulval development*

We showed here that *somi-1* interacts genetically or physically with regulators of vulval development. One or more of these interactions may explain why loss of *somi-1* suppresses the precocious vulval development that results when *mir-84* is overexpressed. Activation of a *let-60/RAS*–MAP kinase cascade in the P6.p cell by expression of *lin-3/EGF* in the adjacent anchor cell during the L3 stage specifies 1° vulval fate. *RAS* signaling also inhibits expression of *lin-12/Notch* in P6.p, establishing lateral inhibition that specifies 2° vulval fate in the neighboring VPCs (Sternberg 2005). Given that *mir-84* targets *let-60*, elevated expression of *mir-84* might be expected to cause a *let-60(lf)* phenotype. However, *let-60* hypomorphs are vulvaless, due to lack of the inductive signal (Beitel et al. 1990). The precocious divisions of the VPCs and Evl phenotype of *mir-84*-overexpressing animals are more like those of *lin-14* and *lin-28* mutants. *lin-14* and *lin-28* control the timing of re-entry of the VPCs into the cell cycle, thus regulating when they can respond to signals that specify cell fate (Euling and Ambros 1996; Ambros 1999; Li and Greenwald 2010). SWI/SNF proteins also have a role in vulva formation. The simplest hypothesis is that *mir-84* overexpression causes precocious down-regulation of *lin-14* in the VPCs, which is suppressed by up-regulation of *lin-14* in a *somi-1* mutant. This is consistent with suppression of *lin-14(lf)* mutations by inactivation of *somi-1*. Given the binding of SOMI-1 to the promoters of *lin-14*, *lin-28*, and *let-60/RAS* and its physical interaction with SWI/SNF components, *somi-1* may influence vulval development at multiple levels.

Functions of paralogous miRNAs

Many miRNAs are members of paralogous families, which may redundantly regulate the same or related target mRNAs (Lau et al. 2001; Miska et al. 2007). In some cases, loss of single members of paralogous families has yielded phenotypes (Reinhart et al. 2000), but deletion of paralogs of *let-7*, for example, caused obvious defects only in animals lacking two or more of these miRNAs (Abbott et al. 2005). Overexpression of particular miRNAs can reveal the function of members of paralogous families: Overexpression of *lin-4* or *let-7* causes precocious heterochronic phenotypes the opposite of those caused by a loss-of-function mutation of each miRNA (Feinbaum and Ambros 1999; Reinhart et al. 2000). Similarly, apoptosis was inhibited by increased expression of the *bantam* miRNA in *Drosophila*, but was promoted by deletion of *bantam* (Brennecke et al. 2003). One concern is that off-target effects could result if an overexpressed miRNA binds to target sequences it would not regulate at physiological levels of expression. Yet the phenotypes caused by overexpression of *mir-84* (precocious development of hypodermal and vulval tissues) are consistent with loss-of-function phenotypes of genes in the heterochronic pathway, in which *let-7* and its paralogs act. Genetic screens for suppression of the phenotypes conferred by overexpression of miRNAs can also identify components of the pathways in which they act.

somi-1 is required for the precocious phenotypes that result when *mir-84* is overexpressed, but is dispensable for similar defects caused by elevated expression of *mir-48*. This suggests that these paralogous miRNAs, identical in their 5′ "seed" sequences and both expressed in the VPCs and seam cells during larval development (Li et al. 2005; Hayes et al. 2006), may recognize at least partly distinct sets of targets even when overexpressed. The miRNA 5′ seed sequence is proposed to be most important in target selection (Doench and Sharp 2004), but complementarity of the 3′ end of *let-7* to its target sites is important for regulation of *lin-41* (Vella et al. 2004), and additional determinants influence target site function (Didiano and Hobert 2006; Bartel 2009).

Materials and methods

Mutagenesis screen

C. elegans of genotype *mgIs45[mir-84++] I; wIs54[scm::gfp] V* were mutagenized with ethylmethane sulfonate. Rare eggs laid by the progeny of ~3700 F_1 animals were picked to new plates to establish putative Somi mutant strains. Male tail defects caused by overexpression of *mir-84* (data not shown) hampered assignment of the Somi isolates to complementation groups.

Rescue of somi-1

An 8-kb fragment containing 5 kb of M04G12.4 coding sequence and predicted 3′ UTR and 3 kb of sequence upstream was amplified from *C. elegans* genomic DNA with Expand High-Fidelity polymerase (Roche) and primers GH183 5′-caacccgggGCAGAA AATTAGGTCTGGAATCG-3′ (sequence homologous to *somi-1* is capitalized) and GH176 5′-TACTGGGCGGTCATATGTTC-3′. Three separate PCRs were pooled for injections at 0.5 ng/μL with plasmid specifying *ttx-3::dsRed* (courtesy of Ho Yi Mak) at 50 ng/μL, yielding transgene *mgEx740*, which was crossed into strain GR1576 to create strain GR1579 mg*Ex740[somi-1(+)]; mgIs45 [mir-84++] I; somi-1(mg415) V*.

Assay for egg laying defects

L4 larvae or adults that formed a clear vulval protrusion were scored as Evl. Otherwise, animals were moved singly to plates and typically scored the next day for ability to lay eggs. Those unable to lay eggs were scored as Evl. In RNAi experiments, animals grown for two generations on RNAi bacteria were moved as adults to plates seeded with strain OP50 for scoring the Evl phenotype. Infertile animals were not scored.

Microscopy

Images were captured on a Zeiss Axioplan microscope using a Hamamatsu ORCA-ER digital camera and Openlab (Improvision)

or Axiovision (Zeiss) software. Deconvolution was done with Axiovision.

RNAi

RNAi was performed essentially as described by Fraser et al. (2000) and modified by Hayes et al. (2006), where the *lin-28* RNAi clone is described. Typically, L4 larvae were placed on RNAi bacteria and their F_1 progeny were scored. Control bacteria expressed dsRNA homologous to no worm gene.

ChIP

In brief, worms were frozen and ground under liquid nitrogen. The resulting powder was cross-linked using 1% formaldehyde in PBS and sonicated. SOMI-1::GFP was immunoprecipitated with polyclonal rabbit-α-GFP antibody (Clontech) and Protein A Dynal beads (Invitrogen). Protein–DNA complexes were eluted and treated with RNase A and proteinase K. DNA was purified with a ChIP DNA purification kit (Zymo Research) and analyzed by qPCR.

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