Components of the transcriptional Mediator complex are required for asymmetric cell division in *C. elegans*

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Summary

Asymmetric cell division is a fundamental process that produces cellular diversity during development. In *C. elegans*, the Wnt signaling pathway regulates the asymmetric divisions of a number of cells including the T blast cell. We found that the *let-19* and *dpy-22* mutants have defects in their T-cell lineage, and lineage analyses showed that the defects were caused by disruption in the asymmetry of the T-cell division. We found that *let-19* and *dpy-22* encode homologs of the human proteins MED13/TRAP240 and MED12/TRAP230, respectively, which are components of the Mediator complex. Mediator is a multi-component complex that can regulate transcription by transducing the signals between activators and RNA polymerase in vitro. We also showed that LET-19 and DPY-22 form a complex in vivo with other components of Mediator, SUR-2/MED23 and LET-425/MED6. In the *let-19* and *dpy-22* mutants, *tlp-1*, which is normally expressed asymmetrically between the T-cell daughters through the function of the Wnt pathway, was expressed symmetrically in both daughter cells. Furthermore, we found that the *let-19* and *dpy-22* mutants were defective in the fusion of the Pn.p cell, a process that is regulated by *bar-1*/β-catenin. Ectopic cell fusion in *bar-1* mutants was suppressed by the *let-19* or *dpy-22* mutations, while defective cell fusion in *let-19* mutants was suppressed by *lin-39*/Hox mutations, suggesting that *let-19* and *dpy-22* repress the transcription of *lin-39*. These results suggest that LET-19 and DPY-22 in the Mediator complex repress the transcription of Wnt target genes.

Key words: Mediator, Wnt, Asymmetric cell division, *C. elegans*

Introduction

In every organism, asymmetric cell divisions are crucial to the generation of cell diversity (Hawkins and Garriga, 1998; Horvitz and Herskowitz, 1992). In *Drosophila*, asymmetric divisions of neuroblasts cause the Prospero protein to be segregated into only one daughter cell, the one that becomes a ganglion mother cell (GMC). Prospero is a transcription factor that is required for the GMCs to adopt their fates correctly (Betschinger and Knoblich, 2004; Roegiers and Jan, 2004). In budding yeast, asymmetric cell division contributes to the mating-type switch, which involves the rearrangement of specific DNA segments at the MAT locus. This process is catalyzed by the HO endonuclease, which is expressed in mother but not daughter cells. Thus, mating-type switching occurs only in mother cells (Amon, 1996; Nasmyth, 1993). Transcription of the HO gene is dependent on the SWI/SNF chromatin remodeling complex and the Mediator complex. During telophase, SWI/SNF binds to the HO promoter in the nucleus of mother cells, and recruits the Mediator complex and RNA polymerase to facilitate the transcription of HO (Cosma, 2002; Cosma et al., 1999).

The Mediator complex was first identified in yeast as a complex associated with RNA polymerase that can support activated transcription in vitro (reviewed by Myers and Kornberg, 2000). A number of mammalian complexes related to yeast Mediator have since been identified, the TRAP, DRIP, ARC and SMCC complexes, which have nearly identical subunit compositions (Malik and Roeder, 2000). These complexes can mediate the activities of various transcription factors, such as Sp1, thyroid hormone receptor and p53, to activate or repress transcription. The largest Mediator complexes contain about 20 subunits, but they seem to be divided into functional and physical submodules. It has been suggested that yeast Mediator can be divided into four modules: Srb4, Gal11/Sin4, Med9/Med10 and Srb8-Srb11. For example, yeast mutants of the Gal11/Sin4 module components (Gal11, Rgr1, Sin4, Med2 and Pgd1) exhibit similar phenotypes (Jiang et al., 1995; Jiang and Stillman, 1995), and the presence of Gal11, Sin4 and Pgd1 in the complex depends

on Rgr1 (Li et al., 1995). Under highly stringent conditions, the Srb8-Srb11 module is isolated as a separate entity from the other components of Mediator (Borggrefe et al., 2002), and this module has repressive functions in yeast (Carlson, 1997; Chang et al., 2001; Holstege et al., 1998). CDK8 and cyclin C, the human homologs of Srb10 and Srb11, respectively, also repress activator-dependent transcription in vitro (Akoulitchev et al., 2000). In addition, the human ARC-L complex, a large Mediator complex, is transcriptionally inactive and contains CDK8 and Cyclin C, as well as MED12 and MED13 (homologs of Srb8 and Srb9, respectively) (Taatjes et al., 2002). Therefore, Srb8/MED12, Srb9/MED13, Srb10/CDK8, and Srb11/Cyclin C associate with each other physically and functionally in yeast and human cells. In *Drosophila*, the MED12 and MED13 homologs are involved in the development of the eye and wing (Janody et al., 2003; Treisman, 2001). However, little is known about how these complexes are regulated or contribute to animal development.

In *C. elegans*, the asymmetric division of certain blast cells, including the T blast cell, is regulated by *lin-17/frizzled* and *lin-44/wnt* (Herman et al., 1995; Sawa et al., 1996). In *lin-17* mutants, the asymmetry of the division is disrupted, resulting in symmetric division (Sternberg and Horvitz, 1988). In *lin-44* mutants, the polarity of the division is reversed (Herman and Horvitz, 1994). It has been proposed that the LIN-44 signal, which acts through the LIN-17 receptor, provides polarity to cells that undergo asymmetric division (Sawa et al., 1996). The Wnt pathway, which controls the polarity of the T cell, shares some components with the canonical Wnt pathway, such as a Tcf homolog POP-1 (Herman, 2001). We have previously shown that PSA-1 and PSA-4, components of the SWI/SNF complex, are required for the asymmetric division of the T cell during mitosis, suggesting that distinct cell fates are determined by alteration of the chromatin structure (Sawa et al., 2000). Recently, it was reported that a putative transcription factor, TLP-1, is expressed asymmetrically in the T-cell daughters, and this asymmetric expression is regulated by the Wnt signaling pathway, suggesting that *tpl-1* is one of the target genes of the Wnt signal (Zhao et al., 2002). However, it is not clear how the Wnt signal regulates the transcription of its target genes.

We have identified mutations in the *let-19* and *dpy-22* genes that affect the asymmetric division of the T cell. The *let-19* and *dpy-22* mutations cause symmetrical expression of *tlp-1* in the T-cell daughters. We cloned these genes and found that they encode homologs of MED13 and MED12, components of the transcriptional Mediator complex. LET-19 and DPY-22 also function in the fusion of the Pn.p cells, a process that is also regulated by the Wnt signaling pathway. These results indicate that LET-19 and DPY-22 encode components of the Mediator complex and regulate asymmetric cell division, as the complex does in yeast.

Materials and methods

Genetics

The methods for the culture and genetic manipulation of *C. elegans* were as described (Brenner, 1974). Transgenic animals were generated as described (Mello et al., 1991). The *let-19* and *let-425* mutants are sterile and were maintained as heterozygotes over GFP-balancers, *mIn1[mIs14]* and *nT1[qIs51]*, respectively. The *dpy-22(os38)* mutants are fertile but semi-sterile. They were in most cases maintained as rescued strains with an extrachromosomal array (*osEx89*) carrying a genomic subclone of the *dpy-22* gene (pPS6.10) and *col-10*::GFP. Homozygous or non-array-bearing mutants were identified as non-green animals under the fluorescence dissecting scope. Animals were grown at 22.5°C unless otherwise noted. The Psa phenotype was determined as described (Sawa et al., 2000). Expression of *tlp-1*::GFP in the T-cell daughters was analyzed after the V6 cell division. *qIs74* was used for GFP::POP-1 (Siegfried et al., 2004).

Cloning

pAY104 (a rescuing plasmid for *let-19*) contained both a 9.1 kb *Pst*I fragment of F07H5 (with a 0.4 kb sequence from the Lorist6 cosmid vector) and a 4.1 kb *Pst*I fragment of F07H5 subcloned into the pBSK vector. The *let-19*::GFP construct (pAY105) was made by inserting a 0.1 kb PCR fragment (from the *Bst*EII site to the C terminus of the *let-19* gene) and a GFP fragment from pPD95.79 (a gift from A. Fire) into the *Bst*EII site of the *let-19* rescuing plasmid (pAY104). To identify mutations, we sequenced the PCR products amplified from *let-19* and *dpy-22* mutants using internal primers. The mutations were confirmed by sequencing different PCR fragments. The *sur-2*::HA construct (pAY106) consisted of a 10.4 kb *Sac*I-*Ban*I fragment of F39B2, a 0.15 kb PCR fragment just upstream of the stop codon and a HA fragment subcloned into the pT7Blue vector. The expression of GFP::POP-1, *tlp-1*::GFP, *let-19*::GFP and *dpy-22*::GFP was analyzed by confocal microscopy (Zeiss LSM510), while that of *mab-5*::GFP was analyzed by epifluorescence microscopy.

Preparation of nuclear extracts and co-immunoprecipitation analysis

HS490 [harboring SUR-2::HA in a *sur-2(ku9)* mutant background] and HS518 [harboring SUR-2::HA and LET-19::GFP in a *sur-2(ku9)* mutant background] strains were grown in liquid culture as described previously (Stiernagle, 1999). To prepare nuclear extracts, the animals were harvested and homogenized essentially as described previously (Mains and McGhee, 1999), except that the nuclear pellets were obtained from sonicated homogenates of mixed-stage animals, including embryos, larvae and adults, and the nuclear pellets were extracted with NEB350 [nuclear extraction buffer: 20 mM HEPES (pH 7.6), 350 mM KCl, 2 mM EDTA, 25% glycerol, 0.5 mM DTT, 1 mM PMSF, 10 μM E-64, and 0.1% Nonidet P-40]. The nuclear extracts were then co-immunoprecipitated with an anti-Flag antibody (M2, Sigma) or anti-GFP antibody (3E6, Quantum biotechnologies) conjugated to protein A-Sepharose beads (Amersham Pharmacia Biotech) overnight at 4°C. The immunoprecipitates were washed four times with NEB270 (same as NEB350 except containing 270 mM KCl), and eluted with Laemmli sample buffer. For detection of LET-19::GFP and SUR-2::HA, samples were separated by SDS-PAGE (5%), and transferred onto PDVF membranes (Immobilon P, Millipore) by electroblotting for 180 minutes in 10 mM CAPS [3-(cyclohexylamino)-1-propanesulfonic acid; pH 11.0] transfer buffer containing 7.5% methanol. The membranes were immunoblotted with anti-GFP (JL-8, CLONTECH) and anti-HA (12CA5, Boehringer Mannheim), and bound antibodies were visualized with HRP-conjugated antibodies against mouse IgGs (BioRad) using a chemiluminescence reagent (Western Lightning, Perkin Elmer Life Sciences). To detect MED-6, an immunoblot analysis was performed with anti-MED-6, as described previously (Kwon et al., 1999).

Results

let-19 and *dpy-22* are required for asymmetric division regulated by the Wnt signaling pathway

In *lin-17* and *lin-44* mutants, the disruption of asymmetric T-cell division results in the absence of phasmid socket cells (Psa

Table 1. The Mediator complex is required for the asymmetry of the T-cell division

Genotype	% Psa	
Wild type	0	*n*=460*
dpy-22(os38)	43	*n*=252
dpy-22(os26)	38	*n*=50
dpy-22(e652)	2	*n*=94
dpy-22(bx93)†	0	*n*=70
dpy-22(bx103)†	0	*n*=50
dpy-22(os38sib)	39	*n*=200
let-19(os33+M)	69	*n*=138
let-19(os36+M)	78	*n*=134
let-19(mn19+M)	73	*n*=120
let-19 (os33+M); dpy-22(os38sib)	88	*n*=232
sur-2(ku9)	4	*n*=170
let-425(s385+M)	0	*n*=72
let-425(RNAi)	9	*n*=56

The +M designation in a genotype indicates that the animals had a wild-type maternal contribution of the gene function. Genotypes described as 'sib' represent the non-array-bearing siblings from the same brood as the array-bearing animals. *n*, number of phasmids scored.

*Data from Sawa et al. (Sawa et al., 2000).

†Contains *pal-1(e2091); him-5(e1490)*.

phenotype), which are generated by the T.p cell. We identified mutants of the *let-19* (Herman, 1978) and *dpy-22* (Hodgkin and Brenner, 1977) genes in a screen for the Psa phenotype (Sawa et al., 2000) (Table 1). In addition to the Psa phenotype, as shown in Table 1, the *let-19* mutants had Dpy (dumpy), Muv (multivulva) and Sterile phenotypes, and the *dpy-22* mutants had Dpy, Muv and Egl (egg-laying defective) phenotypes. We determined the T-cell lineage in the *let-19* and *dpy-22* mutants (Fig. 1). In both mutants, symmetric division was observed, which led to the production of four hypodermal cells, as seen in the *lin-17* mutants (type I lineage), indicating that these genes are required for the T cell to divide asymmetrically. To understand how these genes regulate this division, we analyzed the expression of two genes that are asymmetrically expressed between T.a and T.p in wild-type animals.

In the embryo, the Wnt pathway functions through a β-catenin homolog, WRM-1, to downregulate the levels of POP-1/Tcf in the posterior daughter of the EMS blastomere. The level of POP-1 is also lower in the posterior daughters of many cells that divide along anteroposterior axis, including that of the T cell (Herman, 2001; Lin et al., 1998). To determine the localization of POP-1 in the T cell, we used a GFP::POP-1 fusion protein (Siegfried et al., 2004). In wild-type animals, the level of GFP::POP-1 was lower in the posterior daughter of the T cell (*n*=15, Fig. 2A). As reported previously (Herman, 2001), the level of GFP::POP-1 was symmetric in the *lin-17* mutants (*n*=2, Fig. 2B). In *let-19* (*n*=6) and *dpy-22* (*n*=6) animals, the levels of POP-1 were higher in the anterior T-cell daughter, just as in wild-type animals (Fig. 2C,D). These results indicate that *let-19* and *dpy-22* do not regulate the POP-1 level, and suggest that *let-19* and *dpy-22* function downstream of *pop-1*.

Zhao et al. showed that *tlp-1* encodes a transcription factor that is required for the asymmetric T-cell division that functions downstream of Wnt signaling (Zhao et al., 2002). In wild-type animals, the *tlp-1*::GFP fusion gene was expressed in the T.p cell, but not in the T.a cell (Fig. 3A). In *lin-17* animals, TLP-1 expression was diminished (Fig. 3B). We then investigated the expression of *tlp-1*::GFP in *let-19* and *dpy-22*

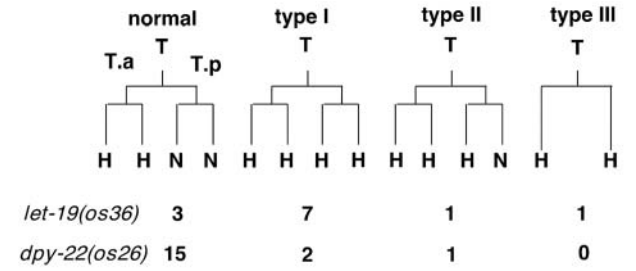

Fig. 1. Abnormal T-cell lineages in *let-19* and *dpy-22* mutants at the L1 stage. The fates of cells (H, hypodermal; N, neural) were determined by nuclear morphology. The number of animals that showed a given lineage is indicated below the diagrams.

mutants to determine whether the *let-19* and *dpy-22* genes might regulate TLP-1 expression (Fig. 3C,D; Table 2). We observed that GFP was expressed symmetrically in both the T.a and T.p cells in *let-19* animals (9/11) and *dpy-22* animals (8/13). These results suggest that the asymmetric *tlp-1* expression is regulated by *let-19* and *dpy-22*. Furthermore, we observed the symmetrical expression of *tlp-1*::GFP in a double-mutant between *lin-17* and *let-19*, a pattern similar to that seen in the *let-19* mutant (Fig. 3E; Table 2). These results indicate that *let-19* is epistatic to *lin-17* and support the idea that *let-19* acts downstream of the Wnt pathway.

Despite the defects in the T lineage, the *tlp-1* expression in other cells appeared to be normal in the *let-19* and *dpy-22* mutants. Specifically, *tlp-1*::GFP was not expressed in seam cells other than the T cells and was expressed in the posterior but not the anterior gut cells in the *let-19* (*n*=8) and *dpy-22* (*n*=10) mutants, as well as in wild-type animals (*n*=8). Therefore, these genes regulate the *tlp-1* expression specifically in the T-cell lineage.

let-19 and *dpy-22* are required for cell fusion regulated by the Wnt signaling pathway

To further investigate the roles of these genes in Wnt signaling, we analyzed the phenotypes of the *let-19* and *dpy-22* mutants in other developmental events regulated by the Wnt signaling

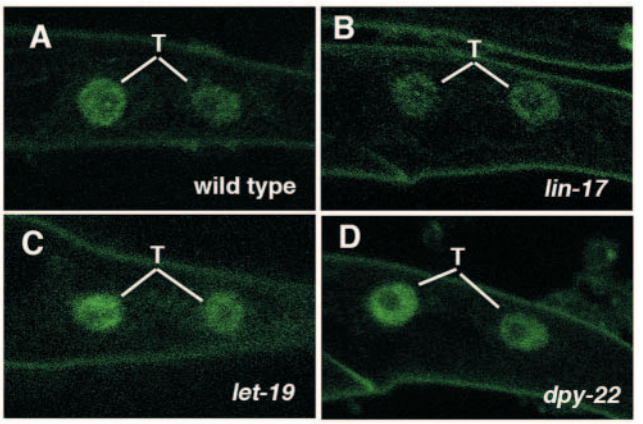

Fig. 2. Asymmetric expression of POP-1 in the T-cell division is not affected by *let-19* and *dpy-22* mutations. Expression of GFP::POP-1 in L1 larvae of wild-type (A), *lin-17(n3091)* (B), *let-19(mn19)* (C) and *dpy-22(os38)* (D). Anterior is towards the left, ventral towards the bottom. The daughters of the T cells are indicated.

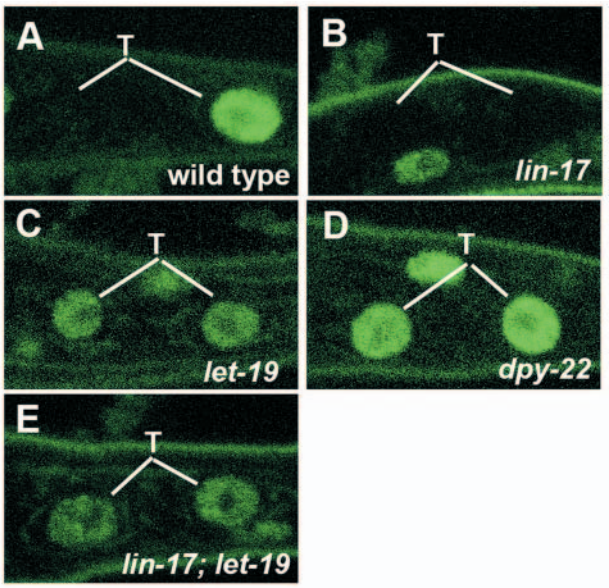

Fig. 3. Symmetric expression of TLP-1 in *let-19* and *dpy-22* mutants after the T-cell division. Expression of *tlp-1*::GFP in L1 larvae of wild-type (A), *lin-17(n3091)* (B), *let-19(mn19)* (C), *dpy-22(os38)* (D) or *lin-17(n3091); let-19(mn19)* (E). Anterior is towards the left, ventral towards the bottom. The daughters of the T cells are indicated.

pathway. Wnt signaling is known to regulate cell fusion (Eisenmann et al., 1998). The ventral hypodermal cells, called Pn.p cells (P1.p through P11.p), can assume alternative fates. In wild-type animals, the two anterior and three posterior Pn.p cells fuse with the hypodermal syncytium (F fate), while the six central cells (P3.p through P8.p) do not fuse and become precursor cells for the vulva (VPCs). (The P3.p cell adopts the F fate in about 50% of animals.) In mutants of the *bar-1* gene, which encodes β-catenin, cell fusion occurs ectopically, producing fewer VPCs than in wild-type animals (Eisenmann et al., 1998). BAR-1 maintains the expression of LIN-39/Hox, which inhibits cell fusion. In *lin-39* mutants, all the Pn.p cells fuse (Clark et al., 1993; Wang, 1993). We quantified the unfused ventral hypodermal cells in the *let-19* and *dpy-22* mutants, using an adherens junction marker, *ajm-1*::GFP (Koppen et al., 2001). We first found that the Pn.p cells in these mutant animals sometimes underwent an extra division in the late L1 stage, producing extra hypodermal cells. A similar phenotype was reported for *lin-25* mutants (Tuck and Greenwald, 1995). Despite the presence of these extra

Table 2. Expression of *tlp-1*::GFP

Genotype	No expression	T.a > T.p	T.a < T.p	T.a=T.p	*n*
Wild type	0	0	20	0	20
let-19(mn19+M)	1	0	1	9	11
dpy-22(os38)	1	0	4	8	13
lin-17(n3091)	16	5	5	3	29
lin-17(n3091); let-19(mn19+M)	4	0	2	12	18

The +M designation in a genotype indicates that the animals had a wild-type maternal contribution of the gene function.

Table 3. *let-19* can suppress the *bar-1* cell fusion phenotype

	Average number of unfused ventral hypodermal cells	
Wild type	5.5	*n*=28 (0)*
bar-1(ga80)	3.2	*n*=15 (0)*
let-19(mn19+M)	7.9	*n*=16 (5)*
let-19(mn19+M); bar-1 (ga80)	9.5	*n*=15 (8)*
dpy-22(os38)	7.5	*n*=26 (5)*
bar-1(ga80) dpy-22(os38)	6.5	*n*=20 (3)*
lin-39(n1760)	0.0	*n*=14 (0)*
let-19(mn19+M); lin-39(n1760)	0.0	*n*=15 (0)*

*Numbers of animals that had unfused P2.p or P9.p.
All the strains contained *jcIs1* (*ajm-1*::GFP). The +M designation in a genotype indicates that the animals had a wild-type maternal contribution of the gene function.

hypodermal cells in the *let-19* and *dpy-22* animals, cell fusion occurred less frequently than in wild-type animals (Table 3). Specifically, in five out of 16 *let-19(mn19)* animals and in two of 26 *dpy-22(os38)* animals, neither P2.p nor P2.pp fused. In addition, in four out of 26 *dpy-22(os38)* animals, P9.p (and in one animal, P9.pa, P9.pp and P10.p) did not adopt the F fate. Furthermore, we found that *let-19* and *dpy-22* mutations efficiently suppressed the *bar-1* mutant phenotype (Table 3). Unfused P2.p or P9.p cells were still observed in the *let-19; bar-1* or *bar-1 dpy-22* double mutants. By contrast, the *let-19* mutations did not suppress the *lin-39* mutant phenotype. These results suggest that *let-19* and *dpy-22* function to repress the *lin-39*/Hox expression that is regulated by *bar-1*/β-catenin.

egl-20/Wnt and *bar-1*/β-catenin regulate the posterior migration of the QL neuroblast (Maloof et al., 1999; Rocheleau et al., 1997; Thorpe et al., 1997). Neither *let-19* (*n*=22) nor *dpy-22* (*n*=20) mutant was defective in QL-cell migration and neither significantly suppressed the QL-cell migration defects that occur in *bar-1* mutants (defective in 22/22 in *let-19; bar-1* and 25/27 in *bar-1 dpy-22*). Although Wnt signaling and the LIT-1 MAP kinase regulate endoderm induction in embryos (Maloof et al., 1999; Rocheleau et al., 1997; Rocheleau et al., 1999; Thorpe et al., 1997), the RNAi of *let-19* did not cause the gutless phenotype (*n*>50), nor did it suppress the gutless phenotype in *lit-1*/NLK mutants (*n*=94). In addition, although mutants of *ln-17/frizzled* and *pop-1*/TCF often lack gonad arms because of the absence of distal tip cells (Siegfried and Kimble, 2002; Sternberg and Horvitz, 1988), *let-19* (*n*=34) and *dpy-22* (*n*=23) mutants had the normal number of gonad arms. These results suggest that *let-19* and *dpy-22* are specifically involved in the Wnt signaling pathway in the T and Pn.p cells.

let-19 and *dpy-22* encode components of the transcriptional mediator complex

let-19 was mapped to the right of *rol-6* on chromosome II (Sigurdson et al., 1984). *let-19* mutants were rescued by cosmid F07H5 and a subclone of F07H5 that contains the predicted gene K08F8.6 (Fig. 4A). The RNAi of this gene was embryonically lethal, but escapers mimicked the Psa and extra Pn.p phenotypes of *let-19* (data not shown). We sequenced this gene in the *let-19* mutants and found mutations in all the alleles, confirming that K08F8.6 was the *let-19* gene. All the alleles had nonsense mutations, indicating that they were strong loss-of-function mutants. Consistent with this, all the alleles were fully recessive, and the Muv phenotype of *mn19* homozygotes was similar to that of *mn19/mnDf46*, a deficiency

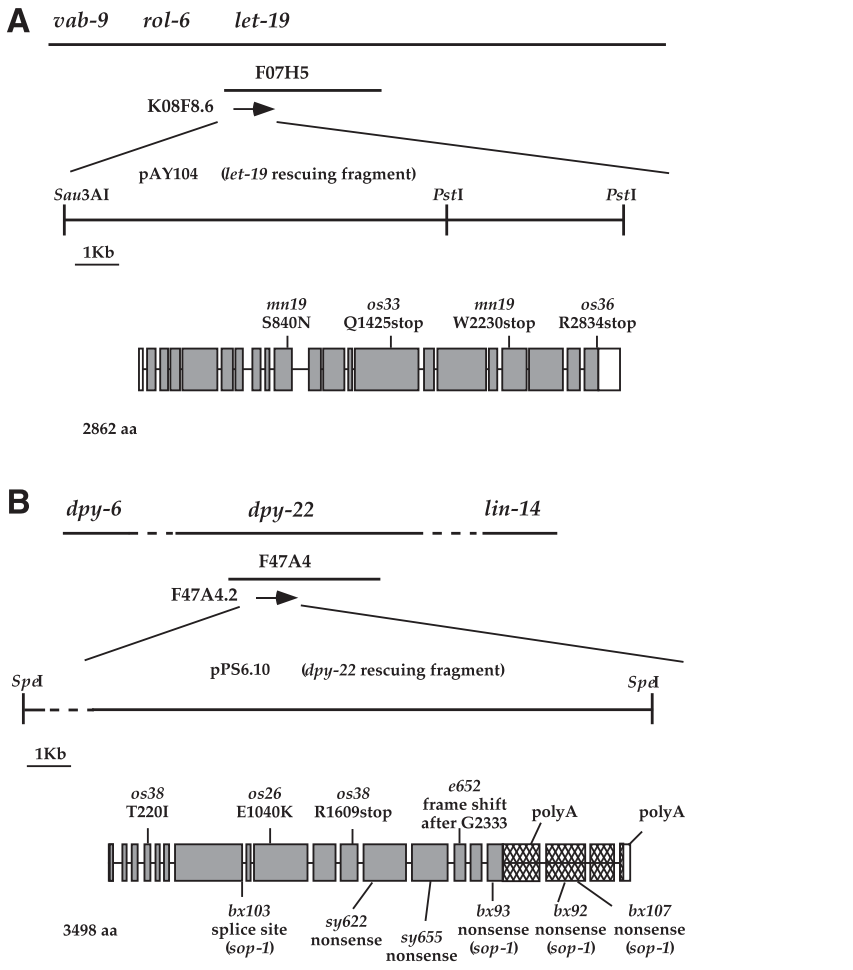

Fig. 4. Molecular cloning of *let-19* and *dpy-22*. Genetic maps of the *let-19* (A) and *dpy-22* (B) loci with rescuing cosmids. Structures of the genes and rescuing constructs are shown with the coding regions in gray and the Q-rich domain in *dpy-22* hatched. The molecular lesions of the mutations are indicated. The *sop-1*-class mutations of *dpy-22* are from Zhang and Emmons (Zhang and Emmons, 2000). The *sy622* and *sy655* mutations are from Moghal and Sternberg (Moghal and Sternberg, 2003). The total lengths of the protein products are indicated on the left. (C) Protein sequence comparisons of the C-terminal regions of MED13 homologs from *C. elegans* (Ce), human (Hs), mouse (Mm), rat (Rn), *D. melanogaster* (Dm), *D. discoideum* (Dd), *S. pombe* (Sp) and *S. cerevisiae* (Sc). The consensus sequence (Cons) is indicated in the top row. The numbers indicate positions in the complete peptide sequences. Black and gray backgrounds indicate identical or similar amino acids, respectively, in at least four aligned sequences. Amino acids considered similar are R/K/H, S/T, I/L/V/M, E/D, Q/N and F/Y/W. Stop signals are indicated by asterisks. The mutation site (R2834stop) of *let-19(os36)* is indicated in italics.

in which the *let-19* locus is deleted (data not shown). *let-19* encodes a protein of 2862 amino acids that has been reported to be homologous to mammalian MED13, a component of the Mediator complex (Ito et al., 1999).

One of the *let-19* mutants, *os36,* which has a similar phenotype to the other mutant alleles, contained a nonsense mutation that was predicted to truncate the last 29 amino acids of the protein, indicating that the C-terminal region of the LET-19 protein is essential to its function. The MED13 homologs include *C. elegans* LET-19, mammalian MED13, *D. melanogaster* Skuld (Skd), *D. discoideum* AMIB and yeast Srb9. All these homologs have conserved domains in their C-terminal regions (Fig. 4C) (Boube et al., 2002; Wang et al., 2004). These data imply that the C-terminal region of the MED13 family proteins is important to their function.

We searched for homologs of other components of Mediator in the *C. elegans* genome and found that a MED12 homolog mapped to the same region of chromosome X as *dpy-22*. We found that *dpy-22* was rescued by cosmid F47A4 and a subclone of F47A4 that contains the MED12 homolog, F47A4.2 (Fig. 4B). The RNAi of this gene mimicked the Dpy Psa and the fertile phenotype of *dpy-22* (data not shown). This gene was previously identified as the *sop-1* gene (Zhang and Emmons, 2000). *sop-1* was identified from mutations that suppress the *pal-1* mutant. In contrast to the *dpy-22* mutants, which have a variety of phenotypes, as described above, *sop-1* mutants did not exhibit any phenotypes by themselves. Most of the *sop-1* mutants had nonsense mutations near the C terminus that truncated the glutamine-rich domain of the protein, except for *bx103,* which contained a splice-site mutation (Zhang and Emmons, 2000). We identified the mutations in three *dpy-22* mutants (Fig. 4B). Among them, *os38*, which had the strongest phenotype, contained a nonsense mutation in the middle of the coding sequence, in addition to a missense mutation near the N terminus, suggesting that it was a strong loss-of-function mutant. *sop-1* mutants are likely to be weak loss-of-function mutants of the *dpy-22* gene. *dpy-22/sop-1* was shown to be expressed ubiquitously during development (Zhang and Emmons, 2000).

sop-1 mutations can suppress *pal-1* mutants for the production of rays from the V6 cells in males. However, *dpy-22(os38)* and *let-19(mn19)* males without the *pal-1* mutation were missing most of the rays [0 rays/sides of animals in *let-19(mn19) n*=10 and 1.9 rays in average in *dpy-22(os38) n*=16]. (Both T-derived and V6-derived rays appeared to be similarly affected in the *os38* animals.) We then analyzed the expression of the *mab-5* gene, which acts downstream of *pal-1* for ray production. *mab-5*::GFP was often not expressed in the V6 cells in the *dpy-22(os38)*, *let-19(mn19)* or *pal-1* mutants (Table

Table 4. Expression of *mab-5*::GFP in V6 at the early L1 stage

Genotype		% Expression	
N2	Hermaphrodites	91	*n*=53
N2	Males	98	*n*=50
dpy-22(os38)	Hermaphrodites	21	*n*=29
dpy-22(os38)	Males	18	*n*=11
let-19(mn19+M)	Hermaphrodites	48	*n*=82
let-19(mn19+M)	Males	38	*n*=31
pal-1(e2091)	Hermaphrodites	8	*n*=25
pal-1(e2091)	Males	12	*n*=17

All the animals contain *muIs16* (*mab-5*::GFP) and *him-5(e1490)*.

4). Therefore, strong loss-of-function mutants of *dpy-22* have the opposite effects of weak loss-of-function mutants (*sop-1* class) on *mab-5* expression in the V6 cells. If both classes of mutations affect the transcription of *pal-1*, *let-19* and *dpy-22* are likely to be involved in *pal-1* transcription through its intronic enhancer element, which controls *pal-1* expression (Zhang and Emmons, 2000). By contrast, Zhang and Emmons suggested that the *sop-1*-class of *dpy-22* mutations activates *pal-1* transcription through another element, only when the intronic element is defective. Therefore, Mediator may regulate *pal-1* expression through two distinct promoter elements. It is also plausible that *let-19* and *dpy-22* mutations directly disrupt the transcription of *mab-5*, while *sop-1*-class mutations affect that of *pal-1*.

Despite the defects in the V6 cell, the *mab-5*::GFP expression in other cells did not appear to be significantly affected in the *let-19* or *dpy-22* mutants. At the early L1 stage, *mab-5*::GFP was not expressed in other seam cells in *let-19* (17/17), *dpy-22* (17/17) or wild-type (11/11) animals, while it was expressed in the P9/10 and P11/12 cells in the *let-19* (16/17; no expression in P9/10 in one animal), *dpy-22* (17/17) and wild-type (11/11) animals. Therefore, *let-19* and *dpy-22* are involved in the *mab-5* expression specifically in the V6 cells.

let-19 and *dpy-22* are expressed symmetrically in the T-cell daughters

To analyze the expression patterns of *let-19* and *dpy-22*, we made constructs in which the *let-19* and *dpy-22* genes were fused in-frame to the GFP (green fluorescent protein) gene at the ends of their coding sequences. Each construct rescued the *let-19* or *dpy-22* phenotypes, respectively, indicating that the fusion proteins were functional. Using these constructs, we found both *let-19* and *dpy-22* to be expressed in most cells during embryogenesis and in many if not all cells in developing larvae (data not shown). As shown in Fig. 5, both genes were expressed in the T cell and the T-cell daughters. GFP fluorescence was observed in both of the daughter nuclei, indicating that there was no asymmetry in the expression patterns of *let-19* and *dpy-22* during T-cell division.

LET-19 interacts with SUR-2 and LET-425 in vivo

We examined the interaction between LET-19 and other putative components of Mediator, LET-425/MED6 and SUR-2/MED23 (Kwon and Lee, 2001; Singh and Han, 1995). To this end, GFP-tagged LET-19 and HA-tagged SUR-2 were co-expressed in *sur-2* mutants. The vulva-less phenotype of the

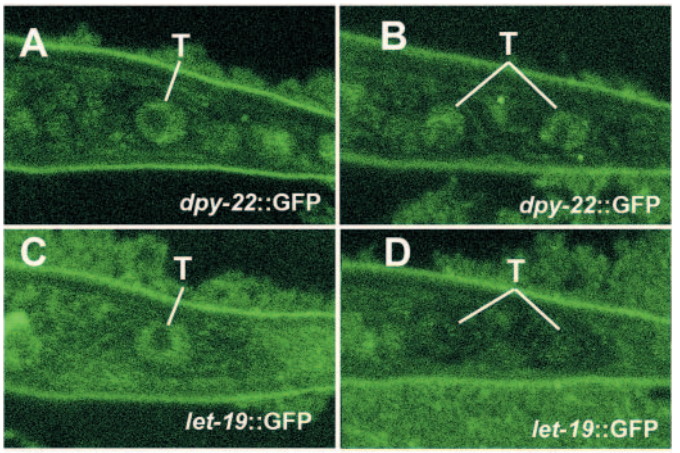

Fig. 5. Symmetric expression of LET-19 and DPY-22 in the T-cell division. Expression of *dpy-22*::GFP (A,B) and *let-19*::GFP (C,D) in the T cell. The T cell is in telophase in B and D. Anterior is towards the left, ventral towards the bottom.

sur-2 mutants was rescued by *sur-2*::HA, indicating that the SUR-2::HA fusion protein was functional. Nuclear extracts were prepared from mixed-stage animals and protein association was examined by immunoprecipitation (IP) with the anti-GFP antibody, followed by immunoblotting with anti-HA and anti-LET-425. As shown in Fig. 6, LET-425 and the functional SUR-2::HA fusion protein could be co-immunoprecipitated with LET-19::GFP, confirming that these proteins were present in the same complex in vivo.

LET-425/MED6 functions in the T-cell division

We also examined whether SUR-2/MED23 and LET-425/MED6 were required for the T-cell division to be asymmetric. We found that *sur-2* mutants showed weak defects in the T-cell division asymmetry (Table 1). *let-425* homozygous mutants obtained from heterozygotes did not show defects in the asymmetry of this division (Table 1) and had very minor developmental defects, probably owing to the maternal contribution, although these mutants are sterile (Kwon and Lee, 2001). However, although RNAi of *let-425* causes the embryonic lethal phenotype (Kwon et al., 1999), we found that the escapers of the lethality showed defects in the asymmetry of the T-cell division similar to those of the *let-19* and *dpy-22* mutants. Therefore, SUR-2/MED23 and LET-425/MED6 are also involved in the T-cell division regulated by the Wnt signaling pathway.

Discussion

Asymmetric cell division and the Mediator complex

When cells divide asymmetrically, the daughter cells are likely to acquire distinct cell fates by transcribing different sets of genes. Mediator complexes are key regulators of transcription (Myers and Kornberg, 2000). We identified *let-19* and *dpy-22* mutations that affected the asymmetric T-cell division. We showed that *let-19* and *dpy-22* encode proteins similar to MED13 and MED12, respectively. We showed that the LET-425/MED6 and SUR-2/MED23 proteins co-immunoprecipitated with LET-19. Because SUR-2 and LET-

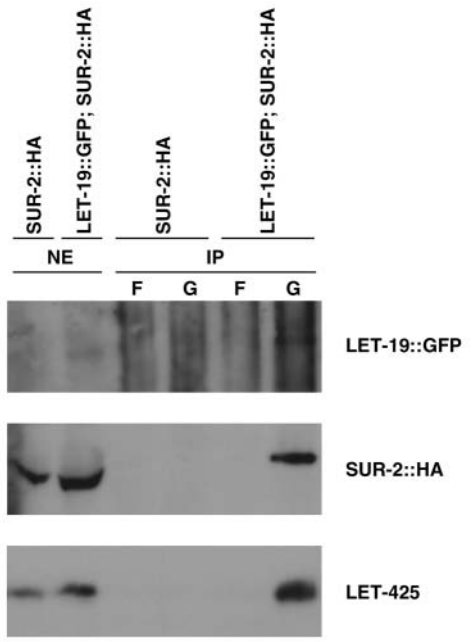

Fig. 6. Association of LET-19 with SUR-2 and LET-425 in vivo. Nuclear extract (NE) was prepared from *sur-2* mutant animals expressing only HA-tagged SUR-2 or both GFP-tagged LET-19 and HA-tagged SUR-2. Nuclear extracts and immunoprecipitation (IP) with anti-Flag (F) and anti-GFP (G) antibodies were analyzed by immunoblotting using antibodies against GFP, HA and LET-425.

425 are also involved in the asymmetric T-cell division, LET-19 and DPY-22 function in the Mediator complex to regulate the asymmetry of the T-cell division. In addition to the Mediator complex, we have previously shown that a chromatin-remodeling complex, SWI/SNF, is involved in this asymmetric division of the T cell (Cui et al., 2004; Sawa et al., 2000). In yeast, both SWI/SNF and the Mediator complex are required for the expression of the HO endonuclease that is transcribed only in mother cells upon asymmetric cell division (Cosma, 2002). Therefore, our results further indicate that the mechanism of asymmetric cell division is conserved between yeast and *C. elegans*.

Transcriptional repression of Wnt target genes by DPY-22 and LET-19

Two distinct Mediator complexes have been reported in mammals. The CRSP complex is active for Sp1-dependent transcription, while the larger complex, ARC-L, is transcriptionally inactive (Taatjes et al., 2002). Compared with CRSP, ARC-L has several additional components, including MED12 and MED13, which are homologs of DPY-22 and LET-19, respectively. In yeast, Srb8/MED12 and Srb9/MED13 form a sub-complex and do not always participate in the Mediator complex (Borggrefe et al., 2002; Myers and Kornberg, 2000). Similarly, in *C. elegans*, LET-19/MED13 and DPY-22/MED12 may be present only in the ARC-L-like but not in the CRSP-like complex. Because the *let-19* and *dpy-22* mutations induce symmetric cell division, similar to *lin-17* mutants, activation of the LIN-44/LIN-17 signaling pathway might convert the ARC-L-like complex to the CRSP-like complex, by causing the release of a sub-complex containing LET-19 and DPY-22. Our data suggest that LET-19 and DPY-22 are involved in preventing the expression of TLP-1 in the T.a cell, raising the possibility that the LET-19-DPY-22 subcomplex directly inhibits the expression of *tlp-1*, a candidate Wnt signal target in the T-cell division. In this case, the ARC-L-like complex may inhibit the expression of *tlp-1* in the T.a cell, while the CRSP-like complex may activate transcription of *tlp-1* in the T.p cell.

In addition to the *tlp-1* expression, in the fusion of the Pn.p cells, our results indicate that LET-19 and DPY-22 function in transcriptional repression of the *lin-39*/HOX gene. In this case, the Wnt signal mediated by *bar-1*/β-catenin may release LET-19 and DPY-22 from the Mediator complex, resulting in the induction of *lin-39* expression. By contrast, in the absence of the Wnt signal, LET-19 and DPY-22 may participate in the Mediator to inhibit the expression of *lin-39*, resulting in cell fusion.

Despite defects in *tlp-1* expression in the T.a cell, the neural fate of the T.p cell is abnormal in *let-19* and *dpy-22* mutants, rather than the hypodermal fate of the T.a cell being altered. This puzzling contradiction can be explained if *let-19* and *dpy-22* regulate the transcription of other genes required for neural fates in the T.p cell. Another possibility is that the expression of the *tlp-1* gene in the T.a cell may affect the fate of the T.p cell, although interactions between the T.a and T.p cells have not been reported.

Functions of MED13 and MED12 in the Mediator complex

In yeast, the Srb8-11 subgroup forms a specific module, which is present in holoenzyme preparations from cells growing exponentially in rich glucose medium, but is absent in stationary-phase cells (Holstege et al., 1998). Genetic analyses indicate that the Srb8-11 module is involved in the negative regulation of a small subset of genes (Carlson, 1997; Holstege et al., 1998). In *Drosophila*, loss of either the *skuld(skd)*/MED13 or *kohtalo(kto)*/MED12 gene has exactly the same effect. It was also reported that the Skd and Kto proteins interact with each other (Janody et al., 2003; Treisman, 2001). In *C. elegans*, we have shown here that mutations in either *let-19* or *dpy-22* cause similar defects in T-cell division and fusion of the Pn.p cells. They also share the Dpy and Muv phenotypes. A recent paper reported that the male tail phenotype caused by the *pal-1(e2091)* mutation was suppressed not only by *dpy-22/sop-1* mutations, but also by the reduced expression of *let-19* (Wang et al., 2004). These observations strongly suggest that MED13 and MED12 function as a unit, which is conserved evolutionally. A remaining question is, what are the roles of Cdk8 and Cyclin C, the other components of the Srb8-11 submodule? Do Cdk8 and Cyclin C also have a function similar to MED13 and MED12? Future studies of these molecules will contribute to our understanding of the roles of the Srb8-11 submodule in the Mediator complex.

In yeast, although disruption of Srb4/MED4 affects the transcription of most genes (93% of 5361 genes examined), that of Srb10/CDK8 affects only a small subset of them (3%) (Holstege et al., 1998). In *Drosophila*, Skd/MED13 and Kto/MED12 are specifically required for proper photoreceptor differentiation (Treisman, 2001), and Skd is involved in the regulation of segment identity (Boube et al., 2002). In *C.*

elegans, disruption of LET-19 at the embryonic stage affects the expression of a subset of developmentally regulated genes (Wang et al., 2004). We have shown that *let-19* and *dpy-22* mutants have defects in specific developmental events that are regulated by Wnt signaling. These mutations affect the expression of the *tlp-1* gene specifically in the T-cell lineage and that of *mab-5* in the V6 cell. These results indicate that the Srb8-11 submodule acts on specific genes in specific developmental contexts.

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