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/LTRAP240 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; Rögers 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
Materials and methods

Genetics

The methods for the culture and genetic manipulation of \textit{C. elegans} as described (Brenner, 1974). Transgenic animals were generated as described (Mello et al., 1991). The \textit{let-19} and \textit{dpy-22} mutants are sterile and were maintained as heterozygotes over GFP-balancers, \textit{mhh[mtx14]} and \textit{nT1[qls51]}, respectively. The \textit{dpy-22} (os38) mutants are fertile but semi-sterile. They were in most cases maintained as rescued strains with an extrachromosomal array (\textit{osEx89} carrying a genomic subclone of the \textit{dpy-22} gene (pAP61.10) and \textit{col-10::GFP}. Heterozygous or non-array-bearing mutants were identified as non-green animals under the fluorescence dissecting scope. Animals were grown at 22.5\degree C unless otherwise noted. The \textit{Psa} phenotype was determined as described (Sawa et al., 2000). Expression of \textit{tlp-1::GFP} in the T-cell daughters was analyzed after the V6 cell division. \textit{qls74} was used for \textit{GFP::POP-1} (Siegelfried et al., 2004).

Cloning

\textit{pAP104} (a rescuing plasmid for \textit{let-19}) contained both a 9.1 kb \textit{Pst} fragment of \textit{F07H5} (with a 0.4 kb sequence from the \textit{Lorist6} cosmid vector) and a 4.1 kb \textit{Pst} fragment of \textit{F07H5} subcloned into the pBSK vector. The \textit{let-19::GFP} construct (\textit{pAP105}) was made by inserting a 0.1 kb PCR fragment (from the \textit{BstEII} site to the C terminus of the \textit{let-19} gene) and a GFP fragment from \textit{p9D95.79} (a gift from A. Fire) into the \textit{BstEII} site of the \textit{let-19} rescuing plasmid (\textit{pAP104}). To identify mutations, we sequenced the PCR products amplified from \textit{let-19} and \textit{dpy-22} mutants using internal primers. The mutations were confirmed by sequencing different PCR fragments. The \textit{sur-2::HA} construct contained of a 1.04 kb \textit{Sac-BamI} fragment of \textit{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 \textit{GFP::POP-1}, \textit{tlp-1::GFP}, \textit{let-19::GFP} and \textit{dpy-22::GFP} was analyzed by confocal microscopy (Zeiss LSM510), while that of \textit{mab-5::GFP} was analyzed by epifluorescence microscopy.

Preparation of nuclear extracts and co-immunoprecipitation analysis

\textit{HS490} [harboring \textit{SUR-2::HA} in a \textit{sur-2(ku9)} mutant background] and \textit{HS518} [harboring \textit{SUR-2::HA} and \textit{LET-19::GFP} in a \textit{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, larval and adults, and the nuclear pellets were extracted with \textit{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\degree C. The immunoprecipitates were washed four times with \textit{NEB270} (same as \textit{NEB350 except containing 270 mM KCl}) and eluted with Laemmli sample buffer. For detection of \textit{LET-19::GFP} and \textit{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 \textit{MED-6}, an immunoblot analysis was performed with anti-MED-6, as described previously (Kwon et al., 1999).

Results

\textit{let-19} and \textit{dpy-22} are required for asymmetric division regulated by the Wnt signaling pathway.

In \textit{lin-17} and \textit{lin-44} mutants, the disruption of asymmetric T-cell division results in the absence of phasmid socket cells (\textit{Psa}...
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 in 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 (Fig. 1). In the T.p cell. We identified genes that are generated by the T.p cell. We identified genes that 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 mutants to determine whether the let-19 and dpy-22 genes might regulate TLP-1 expression (Fig. 3C,D). 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). 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 path.

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% Psa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>0 n=460*</td>
</tr>
<tr>
<td>dpy-22(os38)</td>
<td>43 n=252</td>
</tr>
<tr>
<td>dpy-22(os26)</td>
<td>38 n=50</td>
</tr>
<tr>
<td>dpy-22(e652)</td>
<td>2 n=94</td>
</tr>
<tr>
<td>let-19(os33+M)</td>
<td>69 n=138</td>
</tr>
<tr>
<td>let-19(os36+M)</td>
<td>78 n=134</td>
</tr>
<tr>
<td>let-19(mn19+M)</td>
<td>73 n=120</td>
</tr>
<tr>
<td>let-19(os33+M)</td>
<td>88 n=232</td>
</tr>
<tr>
<td>dpy-22(os38)</td>
<td>4 n=170</td>
</tr>
<tr>
<td>let-425(s385+M)</td>
<td>0 n=72</td>
</tr>
<tr>
<td>let-425(RNAi)</td>
<td>9 n=58</td>
</tr>
</tbody>
</table>

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).

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**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.

**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.
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., 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; Thorpe et al., 1997), the RNAi of let-19 did not cause the gutless phenotype (n=94). In addition, although mutants of lin-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.

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

<table>
<thead>
<tr>
<th>Average number of unfused ventral hypodermal cells</th>
<th>n=28 (0)*</th>
<th>n=15 (0)*</th>
<th>n=16 (5)*</th>
<th>n=15 (8)*</th>
<th>n=26 (5)*</th>
<th>n=20 (3)*</th>
<th>n=14 (0)*</th>
<th>n=15 (0)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>5.5</td>
<td>4.0</td>
<td>4.0</td>
<td>3.2</td>
<td>7.9</td>
<td>6.5</td>
<td>0.0</td>
<td>7.9</td>
</tr>
<tr>
<td><em>bar-1</em>(ga80)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>let-19</em>(mn19+M)</td>
<td>3.2</td>
<td>2.0</td>
<td>2.0</td>
<td>3.0</td>
<td>4.0</td>
<td>4.0</td>
<td>0.0</td>
<td>4.0</td>
</tr>
<tr>
<td><em>dpy-22</em>(os38)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>bar-1</em>(ga80) <em>dpy-22</em>(os38)</td>
<td>9.5</td>
<td>8.0</td>
<td>8.0</td>
<td>9.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td><em>lin-39</em>(n1760)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>let-19</em>(mn19+M); <em>lin-39</em>(n1760)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*Numbers of animals that had unfused P2.p or P9.p.
All the strains contained *jcs1* (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.

eegl-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=94). In addition, although mutants of *lin-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.

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No expression</th>
<th>T.a &gt; T.p</th>
<th>T.a &lt; T.p</th>
<th>T.a=T.p</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>Wild type</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td><em>let-19</em>(mn19+M)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td><em>dpy-22</em>(os38)</td>
<td>1</td>
<td>0</td>
<td>4</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td><em>lin-17</em>(n3091)</td>
<td>16</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td><em>lin-17</em>(n3091); <em>let-19</em>(mn19+M)</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>12</td>
<td>18</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>% Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2 Hermaphrodites</td>
<td>91 n=53</td>
</tr>
<tr>
<td>N2 Males</td>
<td>98 n=50</td>
</tr>
<tr>
<td>dpy-22(os38) Hermaphrodites</td>
<td>21 n=29</td>
</tr>
<tr>
<td>dpy-22(os38) Males</td>
<td>18 n=11</td>
</tr>
<tr>
<td>let-19(mn19+M) Hermaphrodites</td>
<td>48 n=82</td>
</tr>
<tr>
<td>let-19(mn19+M) Males</td>
<td>38 n=31</td>
</tr>
<tr>
<td>pal-1(e2091) Hermaphrodites</td>
<td>8 n=25</td>
</tr>
<tr>
<td>pal-1(e2091) Males</td>
<td>12 n=17</td>
</tr>
</tbody>
</table>

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

### sur-2 mutants were 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-
425 are also involved in the asymmetric T-cell division. 

**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.

**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 *dpv-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 *dpv-22* mutants, rather than the hypodermal fate of the T.a cell being altered. This puzzling contradiction can be explained if *let-19* and *dpv-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 *dpv-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-I(e2091)* mutation was suppressed not only by *dpv-22*/soa-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.
**References**


Mediator controls asymmetric cell division


