TLP-1 is an asymmetric cell fate determinant that responds to Wnt signals and controls male tail tip morphogenesis in *C. elegans*

Xiaojun Zhao[1],*, Ying Yang[2],*, David H. A. Fitch[2] and Michael A. Herman[1],†

[1]Program in Molecular, Cellular and Developmental Biology, Division of Biology, Kansas State University, Manhattan, KS 66506, USA
[2]Department of Biology, New York University, New York, NY 10003, USA
*These authors contributed equally
†Author for correspondence (e-mail: mherman@ksu.edu)

SUMMARY

We have isolated mutations defining a new gene, *tlp-1*, that affect asymmetric cell fates and morphogenesis during the development of the *C. elegans* tail. *tlp-1* mutations cause defects in the specification of asymmetric cell fates in the descendants of the T blast cell, whose polarity is controlled by Wnt signaling and cause abnormal male tail development leading to the formation of a posterior protrusion reminiscent of 'leptoderan', or pointy tailed, nematode species. In wild-type *C. elegans* males, which have a 'peloderan' or rounded tail, retraction of the tail tip hypodermis involves a temporally ordered set of cell fusions and changes in cell shape that appear to be heterochronically delayed in *tlp-1* males, suggesting that subtle changes in these events can bring about evolutionary changes in morphology. *tlp-1* encodes a C2H2 zinc-finger protein that is a member of the Sp family of transcription factors. A TLP-1::GFP fusion protein is expressed in the nuclei of many cells during early embryogenesis and then becomes restricted primarily to posterior cells. At hatching, it is expressed in several head neurons, the posterior intestine cells, tail hypodermal cells, the T cells and specific T-cell descendents in a pattern that suggests TLP-1 may be asymmetrically expressed during the divisions of the T cell lineage. Furthermore, the asymmetry of TLP-1 expression and function appears to be controlled by Wnt signals that control T cell polarity. These results suggest that *tlp-1* encodes a transcription factor required for cellular asymmetry that functions downstream of Wnt signals that control cell polarity, as well as in cell fusion and patterning in the *C. elegans* tail.

Key words: *C. elegans*, Cell polarity, Asymmetric cell division, Cell fusion, Morphogenesis male tail, Evolution

INTRODUCTION

Two fundamental questions in developmental biology are understanding how morphogenesis is controlled and how changes in morphogenesis during evolution lead to the elaboration of different forms. For morphogenesis to occur properly during development, many cellular processes must be correctly controlled; among these are the establishment of different cell fates through asymmetric cell divisions, cell polarity and cell fusion. Changes in any of these processes can lead to changes in morphology that over time could lead to the genesis of different forms.

The polarities of specific cells in the *C. elegans* tail are controlled by Wnt signaling. The conserved Wnt signaling pathway is one of the major signaling pathways controlling animal development (reviewed by Cadigan and Nusse, 1997; Wodarz and Nusse, 1998). Through genetic and molecular studies, the identification of the components that function in a canonical signaling pathway, as well as the probable order of their action, has been determined. In short, Wnt signals are short-range secreted signals that act through Frizzled (Fz) receptors at the cell surface. Through the action of Dishevelled inside the cell, β-catenin is stabilized, causing it to accumulate in the cytoplasm and the nucleus, where it interacts with Tcf factors to activate target genes.

Mutations in the Wnt gene *lin-44* cause the polarities of certain cells in the *C. elegans* tail – the B, TL and TR cells – to be reversed (Herman and Horvitz, 1994; Herman et al., 1995). LIN-44 is expressed by the epidermal cells at the tip of the developing tail, which are posterior to the cells whose polarities are affected by *lin-44* mutations. LIN-44 function is required within the tail tip cells, suggesting that LIN-44 is secreted by these cells and affects the polarity of more anterior asymmetric cell divisions (Herman et al., 1995). Mutations in the Frizzled-related gene *lin-17* cause a loss of polarity in the same cells in which *lin-44* mutations cause a reversal of polarity (Sternberg and Horvitz, 1988; Sawa et al., 1996), suggesting that LIN-17 serves as the receptor for LIN-44. The Wnt pathway that controls the polarity of the TL and TR cells shares some components with the canonical Wnt pathway, such as the requirement for the Tcf homolog, POP-1, but may not require other components such as β-catenin (Herman, 2001),

suggesting that it is a novel Wnt pathway. The defects in cell polarity observed in *lin-44* and *lin-17* mutants lead to defects in morphogenesis as evidenced by the formation of abnormal male tail structures in both mutants (Sternberg and Horvitz, 1988; Herman and Horvitz, 1994).

Another cellular function that often affects morphogenesis is cell fusion. In vertebrates, the formation of multinucleated cells, or syncytia, is involved in the formation of skeletal muscles, bones and, in mammals, the placenta. In *C. elegans*, one third of all the somatic nuclei in the adult animal are part of one of a few syncytia that are formed in reproducible spatial and temporal patterns (Shemer and Podbilewicz, 2000). For example, a detailed analysis of male tail development has revealed that a specific temporal order of cell fusions and cell shape changes lead to the formation of the male tail. *C. elegans* male tail tip morphogenesis begins after the bursal ray cell divisions are complete (Nguyen et al., 1999). The tail tip cells then lose adhesion with the cuticle and recede anteriorly. This tail tip retraction is accompanied by changes in cell shape, position and cell contacts. The retraction of the tail tip causes the adult male tail to be blunt ended, or 'peloderan', where the bursal fan extends posteriorly and envelopes the tail tip. By contrast, these changes in cell shape, position and cell contacts do not occur in hermaphrodites, and the pointed larval shape is retained in the adult. In other nematode species, such as *Oscheius myriophila*, the male tail tip cells also fail to retract during morphogenesis of the tail, causing the adult male tail to be pointed, or 'leptoderan', and protrude beyond the posterior edge of the adult fan (Nguyen et al., 1999).

To further understand the roles cell polarity and cell fusions play in morphogenesis, we have isolated mutations identifying a new gene, *tlp-1* (*T* cell *l*ineage defective and *lep*toderan tail). We show that *tlp-1* is required for the specification of asymmetric cell fates in the descendants of the T cell, as well as for cell fusions and cell shape changes of the tail tip hypodermal cells that lead to drastic changes in male tail morphology. *tlp-1* encodes a nuclear-localized C2H2 zinc-finger protein and is asymmetrically expressed during the divisions of the T cell lineage. Expression studies and cell lineage analysis in double mutants suggest that *tlp-1* is expressed in response to *lin-44* and *lin-17* genes in the control of T cell polarity. Our results suggest that *tlp-1* encodes a transcription factor required for morphogenesis of the *C. elegans* tail by regulating asymmetric cell fates in response to Wnt signaling.

MATERIALS AND METHODS

Nematodes were cultured by standard techniques (Sulston and Hodgkin, 1988). The following mutations were used: LGI, *lin-44(n1792), lin-17(n671), unc-29(e1072)*; LGIV, *bli-6(sc16), unc-24(e138), dpy-20(e1282), unc-26(e205), tlp-1(bx85, mh17, ny14), egl-23(n601), tra-3(e1107), dpy-4 (e1166)*; and LGV, *him-5(e1490)*. Rearrangements used are LGIV, V, *nT1(IV, V), nDf27, sDf21, sDf23*. Male defects were studied using strains containing the *him-5(e1490)* mutation, which increases the frequency of spontaneous male progeny (Hodgkin et al., 1979).

Isolation of *tlp-1* alleles and genetic mapping
tlp-1 alleles were isolated in separate screens for phasmid dye-filling defective (Pdy) mutants (*mh17*), male abnormal (Mab) mutants (*bx85*)

and males with leptoderan-like (Lep) tails (*ny14*) using ethyl methanesulfonate (EMS), diepoxybutane (DEB) and trimethypsoralen followed by u.v. irradiation as a mutagens, respectively. All three alleles failed to complement each other for the Pdy and Lep defects, indicating that all were alleles of *tlp-1*. *tlp-1* was mapped to *LGIV* (not shown, Fig. 4). Three-factor crosses further defined the map position of *tlp-1* to the region between *unc-26* and *egl-23* (see Fig. 4A). Complementation tests revealed that *tlp-1* is not uncovered by *sDf21* and *sDf23*, but may be partially uncovered by *nDf27* (Table 1).

Cell lineage analysis and microscopy
Living animals were observed using Nomarski optics; cell nomenclature and cell lineage analysis were as previously described (Sulston and Horvitz, 1977). Fates of the T cell descendants were determined by nuclear morphologies according to Herman and Horvitz (Herman and Horvitz, 1994). Phasmid dye-filling as an indicator of normal T cell polarity was scored as previously described (Herman and Horvitz, 1994). *jam-1::gfp*, a marker of adherens junctions, was used to visualize cell boundaries (Mohler et al., 1998) and to monitor cell fusions (accompanied by adherens junction degradation).

Cloning of *tlp-1*
Cosmids, plasmids and gel-purified PCR fragments derived from cosmid T23G4 or F52D4 were injected (10-60 ng/μl) using either pRF4 [a plasmid containing the semidominant *rol-6(su1006)* allele], pTG96 (a *sur-5::gfp* fusion construct kindly provided by Dr Min Han, University of Colorado) or pEL166 (a *str-1::gfp* fusion construct kindly provided by Dr Erik Lundquist, University of Kansas) (Troemel et al., 1997) (40-100 ng/μl) as markers into the mitotic germline of *tlp-1* hermaphrodites (Mello and Fire, 1995). Yeast genomic DNA isolated from strains containing the desired YAC DNAs were isolated (Qiagen[R]) and used for injection (40-100 ng/μl). A 10,110 bp PCR product amplified from F52D4 was digested with *Sca*I and cloned into pBluescript to obtain pXZS5, which contained a 7519 bp fragment that rescued the T cell defect of *tlp-1* mutants. We scored each line for either phasmid dye-filling or Lep defects and sometimes both.

The sequences of mutant alleles *mh17* and *bx85* were determined from PCR-amplified genomic DNA fragments using primers that amplified candidate genes in the *tlp-1* region. DNA lesions in T23G4.1 were identified from at least two independent PCR-amplified *mh17* and *bx85* genomic DNA fragments. *ny14* is a deletion allele for which both approximate end points were identified by PCR (see Fig. 4A). Briefly, PCR products from *ny14* were obtained from regions covered by YAC segment Y45F10D and cosmid H08M01 on the right, but not from any of the tested regions covered by cosmid C47A4. Additionally, segments including regions of ORFs T23G4.1, T23G4.2 and T23G4.3 could not be amplified from *ny14*.

The nucleotide sequence of genomic DNA through the *tlp-1* locus was determined by the *C. elegans* Genome Consortium and primers used were based on this sequence. The 1065 bp *tlp-1* coding region was amplified by PCR from cDNAs synthesized using Promega™ Reverse Transcription System. The trans-spliced leader 1 (SL1) primer was used to obtain a 292 bp 5′ fragment. An oligo(dT) primer was used to obtain 273 bp and 527 bp fragments that included the full 3′-UTR. All PCR fragments were cloned into pTOPOXL (Invitrogen™) and the sequence of both strands of the cDNA clones were determined (accession AY046083).

Expression experiments
A *tlp-1::gfp* fusion that placed five additional residues, the GFP-coding sequence and the *unc-54* 3′ UTR at the end of the TLP-1 coding sequence was constructed by PCR. Briefly, a 7973 bp PCR fragment amplified from F52D4 was cloned into pTOPOXL to obtain pXZS4, which was digested with *Kpn*I and *Eco*RV and ligated into pPD95.75 (from A. Fire, Carnegie Institute, Baltimore, MD) digested

with *Kpn*I and *Sma*I to obtain pZXE7. The *tlp-1::gfp* construct begins 5767 bp upstream of exon 1 and places the *gfp* tag at the end of the *tlp-1*-coding region. The cosmid C45D10, containing *unc-29(+)*, was used as a co-injectable marker (Lackner et al., 1994). *mhEx50* is a transgenic array generated in the *unc-29* background containing the *tlp-1::gfp* fusion and C45D10 that rescues the phasmid dye-filling defect and T cell defects. Similar results were obtained with two other independently generated arrays. JAM-1::GFP expression was observed using a strain of genotype *unc-30(e191) tlp-1(bx85) jcIs1[unc-29(+); jam-1::gfp rol-6(su1006)]; him5(e1490)*.

Phylogenetic analyses

To identify sequences related to TLP-1, available data banks were searched using BLASTP and PSI-BLAST using fragments of TLP-1 and *Drosophila* NocA that included the SPLALLA or C2H2 zinc-finger motifs. Additionally, Entrez and WormPD were queried with keywords 'Sp1' and 'C2H2' to identify additional candidate proteins. Preliminary neighbor-joining analyses were used to identify sequences most similar to TLP-1. We limited our in-depth phylogenetic analysis to 45 of these predicted protein sequences (listed in Fig. 4D), all of which have one or more predicted C2H2 zinc-finger motifs and 15 of which have the highly conserved SPLALLA motif. Although we assumed that the database sequences were correct, the human Sp3 sequence is clearly truncated at the N terminus, and *C. elegans* F45H11.1 appears to be truncated at the C terminus in the middle of the second zinc finger.

Multiple alignments of predicted protein sequences were performed using ClustalX 1.81 (Thompson et al., 1997) and MacClade 4.03 (Maddison and Maddison, 2001). Initially, the SPLALLA motif (alignment positions 154-173), Btd box (Supp et al., 1996; positions 979-985) and putative C2H2 zinc-finger motifs (positions 1020-1154) were aligned by hand in MacClade, and the sequences flanking these motifs were aligned as separate regions in ClustalX (accession ALIGN_000176 – can be retrieved from the EMBLALIGN database at http://srs.ebi).

PAUP* (Swofford, 2001) was used to perform weighted-parsimony, in which amino acid changes were weighted by the minimum number of nonsynonymous nucleotide changes required, and neighbor-joining distance analyses to determine the possible relationships among the 45 predicted C2H2 proteins, with particular emphasis on the proteins most closely related to TLP-1. Phylogenetic analyses were restricted to the 123 least ambiguously aligned characters (positions) of 1409 total; alignment between divergent sequences was mostly arbitrary for the remaining characters. Of these 123 characters, 113 were informative for the cladistic analyses. Phylogenetic analyses on alternative alignments or additional characters were consistent with those using only the 123 characters. Trees collected from exhaustive and heuristic searches were compared statistically and strict consensus trees were constructed from trees that were not rejected in these tests. Uncollapsed branches in these consensus trees were deemed to be unambiguously supported. Additional tests of branch stability included bootstrap and jackknife tests and decay indices. Details of these analyses are available from D. F., as are the NEXUS data files.

RESULTS

tlp-1 mutants display T cell lineage defects

In wild-type hermaphrodites, the bilaterally symmetric TL and TR cells (collectively referred to as T cells) divide asymmetrically during the L1 stage: T.a generates four hypodermal cells and one neuron whereas T.p generates five neural cells (Fig. 1). In *lin-44* mutants, the polarity of the division of the T cell lineage is reversed: T.a generates five neural cells and T.p generates four hypodermal cells and one neuron (Herman and Horvitz, 1994). In *lin-17* mutants, the
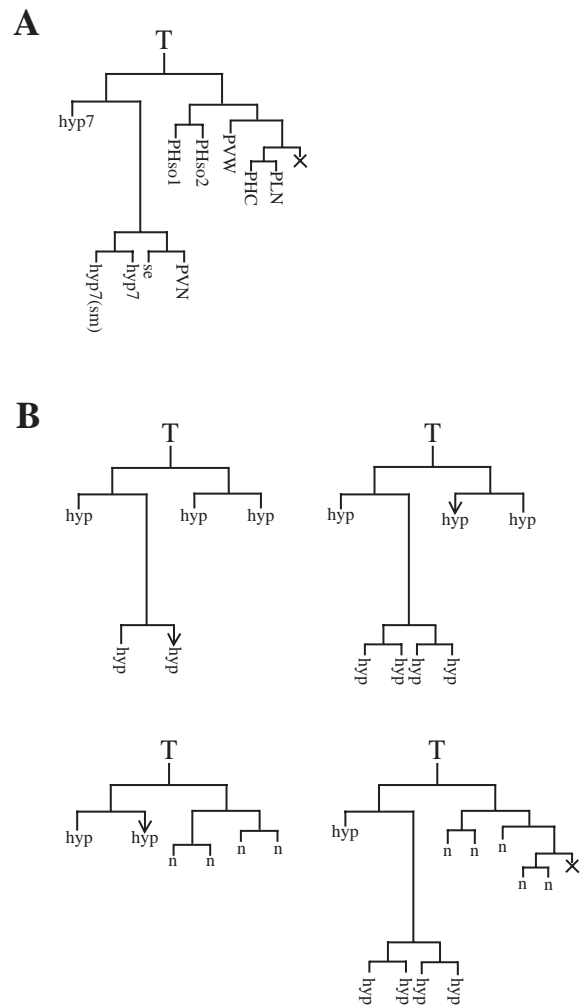

Fig. 1. Cell lineages of the T cell in wild-type and *tlp-1* hermaphrodites. (A) Wild-type hermaphrodite T cell lineage. The fates of many different cells in this lineage can be distinguished by nuclear morphology using DIC microscopy (Sulston and Horvitz, 1977; Herman and Horvitz, 1994). The hyp7 cells T.aa, T.apaa and T.apap join the hypodermal syncytium, but T.apaa has a smaller nucleus and is designated hyp7(sm). PHso1, PHso2, PVW, PHC and PLN have similar nuclear morphologies. × indicates apoptosis. The seam cell (se) is a specialized hypodermal cell. (B) We analyzed T cell lineages on both sides of three *ny14*, two *bx85* and two *mh17* mutants (14 total T cell lineages). Arrows indicate that a particular cell was sometimes observed to divide in a subset of the lineages. Six *ny14* lineages and one *bx85* lineage had the T cell division pattern that is shown in the upper left; however, in two *ny14* lineages and one *bx85* lineage, the T.app cell divided once to generate two hypodermal cells. Two *mh17* lineages had T cell division pattern shown in the upper right; however, in one, the T.pa cell divided to generate two hypodermal cells. Two *bx85* lineages had the T cell division pattern shown in the lower left; however, in one, the T.ap cell divided to generate two hypodermal cells. Two *mh17* and one *bx85* had the T cell division pattern shown in the lower right corner; however, in one of the *mh17* lineages, the T.pa cell did not divide and generated a hyp cell.

polarity of division of the T cell lineage is lost: both T.a and T.p generate hypodermal cells (Sternberg and Horvitz, 1988). The defects in T cell polarity observed in both *lin-17* and *lin-*

Table 1. Penetrance of *tlp-1* defects

Genotype	Percentage of phasmid dye-filling (n)	Percentage presence of phasmid socket cells (n)	Percentage of male Lep (n)
Wild type	98 (472)	99 (110)	0 (>100)
tlp-1(bx85)	28 (952)	57 (412)	100 (>200)
tlp-1(mh17)	19 (1000)	46 (206)	100 (>100)
tlp-1(ny14)	2 (328)	1 (156)	100 (>100)
tlp-1(ny14)/tlp-1(bx85)	36 (836)	ND	ND
tlp-1(ny14)/tlp-1(mh17)	27 (586)	ND	ND
tlp-1(ny14)/+	100 (226)	ND	ND
tlp-1(bx85)/sDf21	ND	ND	0 (29)
tlp-1(bx85)/sDf23	ND	ND	0 (53)
tlp-1(bx85)/nDf27	ND	ND	74 (23)
tlp-1(mh17)/sDf21	100 (232)	ND	ND
tlp-1(mh17)/nDf27	77 (278)	ND	ND
tlp-1(mh17)/sDf23	99 (306)	ND	ND
unc-29(e1072);tlp-1(mh17); mhEx50	90 (150)	85 (98)	100 (35)
unc-29(e1072);tlp-1(ny14); mhEx50	0 (92)	71 (244)	100 (32)
tlp-1(mh17)/+	99 (200)	ND	0 (74)
lin-44	1 (550)	ND	15 (59)*
lin-44; tlp-1(mh17)/+	ND	ND	37 (52)*

Phasmid dye-filling was used as an indicator of normal T-cell polarity (Herman and Horvitz, 1994). There is one phasmid on each side of the animal. *n*, number of phasmids scored; ND, not determined.

Presence of phasmid socket cells was scored during the L2 stage. *n*, number of phasmids scored.

Male Lep tails were scored in strains containing *him-5(e1490)*. *n*, number of animals scored.

*Values were determined to be significantly different (*P*<0.01) using Fisher's exact test.

Fig. 2. *tlp-1* males have leptoderan-like tails. (A) Adult *wild-type* males have a round shaped, or peloderan, male tail morphology. (B) *tlp-1* males have a pointed, leptoderan, male tail morphology. Scale bar: 10 μm.

44 mutants cause the two neurons of the phasmid, a sensory structure in the tail, to fail to fill with fluorescent dyes (Herman et al., 1995; Sawa et al., 1996).

Screens for additional mutations that resulted in phasmid dye-filling (Pdy) defects resulted in the identification of the *mh17* allele of *tlp-1*. In addition, separate screens for mutations that caused male tail defects yielded two additional *tlp-1* alleles, *bx85* and *ny14*, which also display phasmid dye-filling defects (Table 1). Both the Pdy and Lep defects are recessive. The penetrances of the Pdy defect of *mh17* and *bx85* mutations are similar but *ny14* is more penetrant, although this may not be due to the removal of *tlp-1* function alone.

Analysis of the T cell lineages of each *tlp-1* mutant showed that cell fates of the posterior T cell daughter, T.p, and the posterior daughter of the T.ap cell, T.app, were variably defective (Fig. 1). We observed four basic patterns of defective cell lineages from which we conclude that *tlp-1* mutations cause a loss of asymmetry in the divisions of the T.p and T.ap cells, most often resulting in the loss of cell divisions and neural cell fates (Fig. 1B).

tlp-1 mutant males have an abnormal tail morphology reminiscent of male tails of leptoderan nematode species

Tail tip morphogenesis in *C. elegans* males involves changes in cell adhesion of the tail tip cells to the cuticle followed by an anterior movement of the tip cells which causes the adult male tail to be blunt ended, or 'peloderan'.
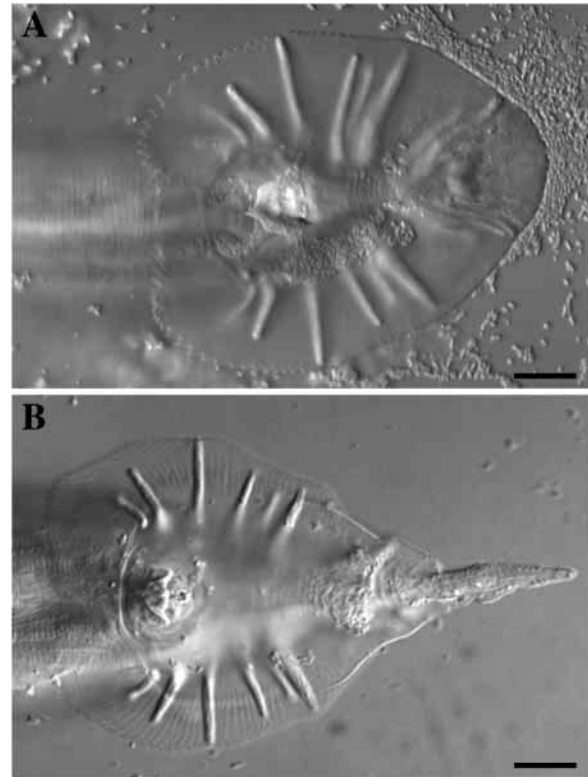
To further understand male tail morphogenesis and tail tip cell retraction, we have isolated mutations that result in a leptoderan (Lep) male tail. The leptoderan tails of *bx85* males, as well as those of *ny14* and *mh17*, are prominent and variable in length (Fig. 2). The Lep defect is completely penetrant in all three *tlp-1* mutant alleles (Table 1). Interestingly, the tail tips in these mutants seem to differentiate fan cuticle, as excess cuticle surrounds the pointy tail tip, unlike *lep-1* mutants (Nguyen et al., 1999). Regions of the fan are often narrow and misshapen. In addition, rays 8 and 9 (60% of sides, *n*=58) or 7-9 (17% of sides) were often missing, consistent with the T cell lineage defects we observed. In a few animals we observed fusions of rays 3 and 4 or 4 and 5 and an ectopic T cell-derived ray. Gross morphologies of the tail tips of *tlp-1* hermaphrodites are unaffected, although there are often defects at the cellular level (see below).

Lep tail defects in *tlp-1* males are correlated with defects in cell fusions

As cell fusion is a prominent event in male tail tip morphogenesis, we wanted to know if the Lep defect of *tlp-1* mutants might be due to failure in cell fusions. The distribution of adherens junctions was observed in live worms using JAM-1::GFP (Fig. 3), a fusion of the green fluorescent protein (*gfp*) (Chalfie et al., 1994) to the *j*unction *a*ssociated *m*olecule, *jam-1*, that localizes to cell boundaries (Mohler et al., 1998). In wild-type males, the tail tip cells fuse before the Rn.p cells fuse to form the 'set' ('tail seam') syncytium. These fusions

Fig. 3. Tail hypodermal cell defects in *tlp-1* animals. Adherens junctions are labeled by JAM-1::GFP and shown in green. (A,C,E) Wild type. (A) Late L4 male, left-ventral view at a stage when the ray cells begin to cluster and the tail tip cells fuse to form the tail tip syncytium. Cell fusions indicated by punctate adherens staining. (C,E) Adult hermaphrodites, left-ventral and left views, respectively. Note that hyp11 is located dorsally above hyp8. (B,D,F) *tlp-1(bx85)*. (B) Male, ventral view, same stage as that in A, but fusion of tail tip cells has not yet occurred. In addition, only two T-ray primordia are visible on the left side of this animal and all appear to be missing on the right side. (D,F) Hermaphrodites, left view. (D) hyp8 and hyp11 do not appear to be bounded by adherens junctions and could have fused with hyp7; adherens 'outlines' of phasmids are not formed or are formed aberrantly. (F) Although the adherens junctions of the phasmid look normal, the tail tip cells are not organized as in wild type, in that at least one extra cell appears in the group of tail tip cells. Scale bar: 20 μm.
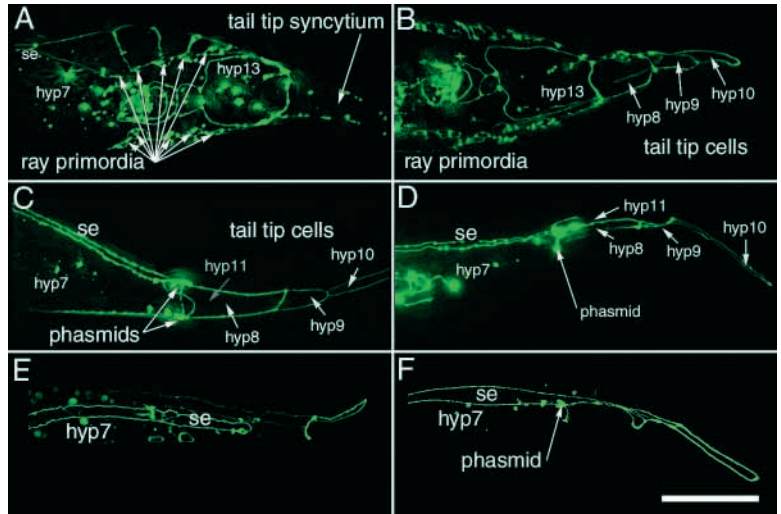
correspond to a punctate 'broken' pattern of JAM-1::GFP, owing to the degradation of the junction (Fig. 3A). In *tlp-1(bx85)* mutants a punctate JAM-1::GFP pattern usually appears long after the 'set' has formed (Fig. 3B). That is, compared with wild type, fusion of the male tail tip hypodermal cells is heterochronically delayed in *tlp-1* mutants.

tlp-1 mutants display additional tail tip cell defects

In wild-type hermaphrodites and larval males, lateral adherens junctions surrounding the posterior epidermal cells are contiguous from anterior to posterior: adherens junctions border the lateral seam ('se'), surround the phasmids at the posterior end of the seam, continue posteriorly between hyp8 and hyp9 (on the ventral side) and hyp11 (on the dorsal side), finally extending along the pointy hyp10 cell (Fig. 3C,E). In *tlp-1* mutants, the adherens border between hyp8 and hyp11 is often missing, such that a 'blank space' appears between the 'se' and the tail tip cells (Fig. 3D). Because of abnormal arrangements of nuclei in these tails, we could not discern whether hyp11 or hyp8 were present, but hyp9 and hyp10 nuclei were always present. The lack of adherens junctions could indicate that specific cells (e.g. hyp8 and hyp11) have ectopically fused with the hyp7 body syncytium. If so, such fusions must have occurred very early in development, as these defects can already be seen in L1 and L2 animals.

In other cases, extra adherens junctions are formed in the tail tip (Fig. 3F), indicating either the presence of an extra epidermal cell or extra adherens junctions built by one of the tail tip cells. These presumed extra cells might be generated by the abnormal T cell lineages observed in *tlp-1* mutants. These cells are not phasmid socket cells transformed from their normal fate, as characteristic phasmid structures can be seen in some of these animals (Fig. 3F). All of these variations suggest that *tlp-1* mutations cause changes in the presence or fates of particular cells in the tails of both sexes, thereby affecting tail tip cell fusions.

Why are *tlp-1* male tails Lep?

The fusion and retraction of the male tail tip cells might be triggered by cell signals emanating from an anterior source or by a developmental program intrinsic to the tail tip cells or

both. It is unlikely that a fusion signal emanates from the T cell lineage as other mutations that affect the T cell lineage, such as *lin-17*, do not cause Lep tails, although *lin-44* males have a low penetrance Lep tail defect. Furthermore, we killed the T cells with a laser microbeam (Sulston and White, 1980) in five wild-type males, but did not observe a Lep defect. It is also possible that the abnormal T cell fates physically interfere with such a signal emanating from other cells. This was tested by killing both TL and TR cells in five *tlp-1* males; all had Lep tails, suggesting that the abnormal T cell lineages in *tlp-1* males do not block an anterior signal for fusion and retraction.

Molecular cloning of *tlp-1*

Microinjection of either cosmid F52D4 or T23G4 rescued both the T cell and Lep defects of *tlp-1(mh17)* and *tlp-1(bx85)* mutants. Additionally, a 7519 bp fragment derived from F52D4 rescued the phasmid dye-filling defects of *tlp-1(mh17)* and *tlp-1(bx85)* mutants, but not the phasmid dye-filling defect of *tlp-1(ny14)* mutants (Fig. 4A). Interestingly, a rescuing *tlp-1::gfp* transgene did not rescue the phasmid dye-filling defect of *ny14* mutants either, but did restore the presence of phasmid socket cells, suggesting the presence of another gene in the region deleted by *ny14* that may be involved in phasmid function but not in asymmetric cell fate determination (Table 1). Furthermore, neither the 7519 bp fragment nor the *tlp-1::gfp* construct rescued the Lep defect of *bx85* males but a 10,110 bp fragment partially rescued the Lep defect of *mh17* and *bx85* males (Fig. 4A). The *tlp-1::gfp* construct contained all the sequences 5′ to the coding region present on the 10,110 bp genomic fragment and extends 3421 bp further 5′ than T23G4, suggesting that other sequences, which must lie 3′ to the coding region, are necessary for the expression of *tlp-1* required for proper retraction and fusion of the tail tip cells. Alternatively, the GFP moiety may interfere with tail tip-specific activity of TLP-1.

We found DNA lesions in each *tlp-1* mutation that affected the coding region of the predicted gene T23G4.1 (Fig. 4B), confirming that we have identified the *tlp-1* locus. *ny14* is a deletion that extends beyond cosmid C47A4 on the right and predicted gene T23G4.4 on the left (not shown), thus removing all of T23G4.1, the *tlp-1*-coding region. The *mh17* mutation is
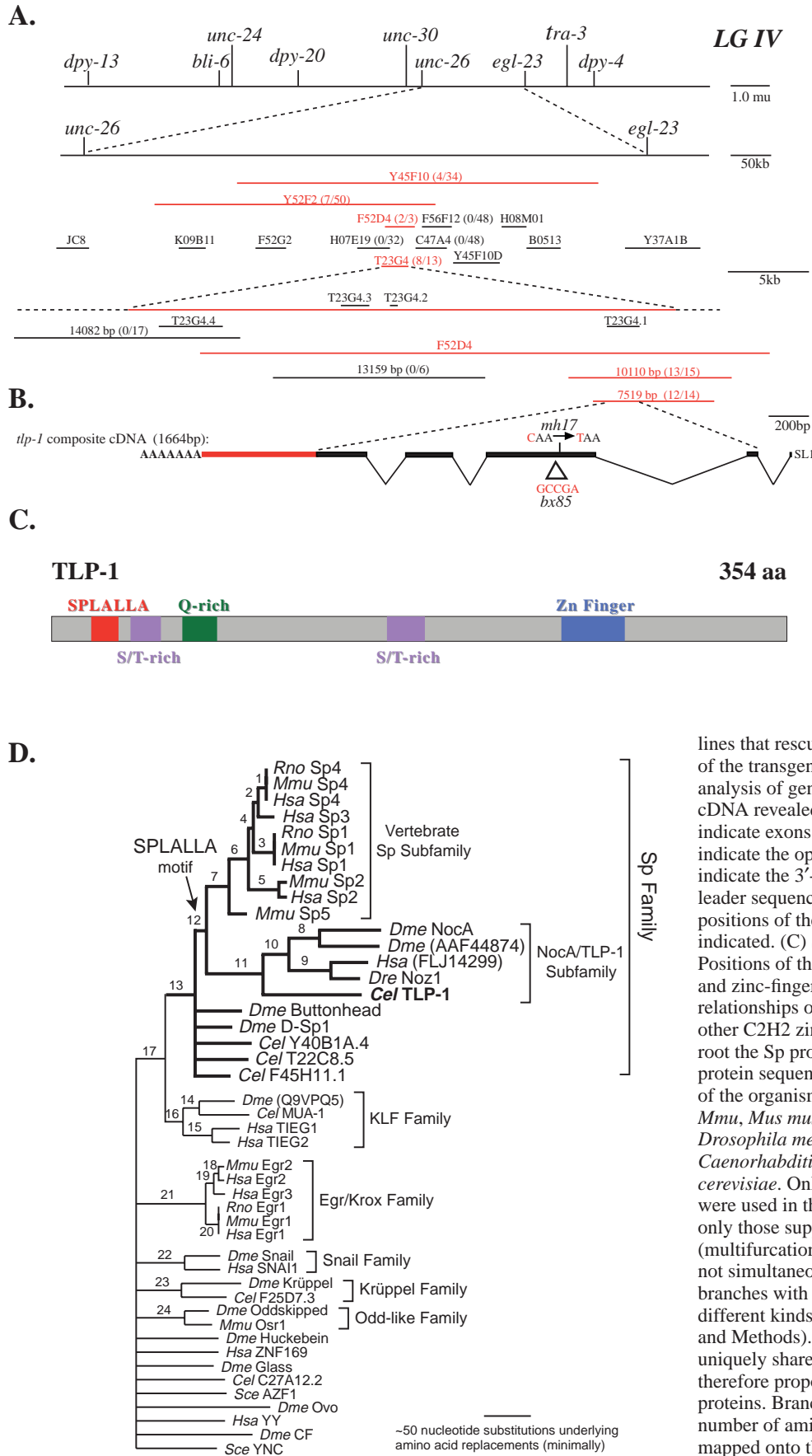
A.

B.

C.

D.

Fig. 4. *tlp-1* cloning and the *tlp-1* locus molecular analysis. (A) *tlp-1* was mapped to *LG IV* between *unc-26* and *egl-23*. Tested genomic clones are shown in relative positions below the genetic map. Red lines represent genomic clones that rescued the *tlp-1* T cell defect, in which the number of transgenic lines that rescued the T cell defect is indicated (>70% of the transgenic animals were rescued). (B) Sequence analysis of genomic DNA and 1664 bp composite cDNA revealed the intron/exon structure shown. Boxes indicate exons: specifically, black closed regions indicate the open reading frame; red closed portions indicate the 3′-UTR. Position of the SL1 (trans-spliced leader sequence) and the poly(A) tail are shown. The positions of the base changes in the *mh17*, *bx85* are indicated. (C) Schematic of the TLP-1 protein. Positions of the conserved SPLALLA, S/T-rich, Q-rich and zinc-finger domains are indicated. (D) Phylogenetic relationships of Sp-type proteins (larger font) and some other C2H2 zinc finger proteins (smaller font) used to root the Sp protein phylogeny. The names of the 45 protein sequences begin with a three-letter abbreviation of the organism binomen: *Rno*, *Rattus norvegicus*; *Mmu*, *Mus musculus*; *Hsa*, *Homo sapiens*; *Dme*, *Drosophila melanogaster*; *Dre*, *Danio rerio*; *Cel*, *Caenorhabditis elegans*; *Sce*, *Saccharomyces cerevisiae*. Only the least ambiguously aligned residues were used in the analyses. Relationships shown are only those supported with some degree of confidence (multifurcations represent ambiguous branching order, not simultaneous divergence). Numbers (1-24) identify branches with such support in at least one of several different kinds of phylogenetic analyses (see Materials and Methods). All proteins deriving from branch 12 uniquely share the SPLALLA motif, which we therefore propose is a shared-derived feature of these proteins. Branch lengths represent the minimum number of amino acid-altering nucleotide substitutions mapped onto the phylogeny by parsimony.

a point mutation that changed glutamine residue 77 to an ochre stop codon. The *bx85* mutation is a 5 bp deletion that causes a frameshift at residue 89 and a termination codon 162 residues later.

Overlapping cDNA clones specific to *tlp-1* were identified by RT-PCR from cDNA prepared from mixed-stage wild-type animals and sequenced. The positions of the four exons in the gene structure are the same as those the *C. elegans* genome project predicted for T23G4.1, indicating that this predicted gene corresponds to *tlp-1*. The composite cDNA contains the SL1 transpliced leader sequence (Krause and Hirsh, 1987), a 1062 nucleotide open reading frame, a 25 nucleotide 5′ untranslated region, a 459 nucleotide 3′ untranslated region and a poly(A) tail. Developmental northern analysis using the RT-PCR product containing the complete coding region as a probe detected a single 1.8 kb transcript present at all stages (not shown), consistent with the size of the 1664 bp composite cDNA. Together, these data suggest that the composite cDNA represents the full-length *tlp-1* message.

TLP-1 contains a C2H2-type zinc-finger domain

Translation of the *tlp-1* composite cDNA yielded a predicted protein of 354 amino acid residues containing two serine/threonine-rich regions, a glutamine-rich region and a zinc finger domain of the C2H2 type (Fig. 4C). Another highly conserved, previously undescribed motif of unknown function (SPLALLA) occurs near the N terminus (Fig. 4C). The C2H2-type zinc-finger domains are characterized by sequences that approximate the form (Tyr/Phe)-X-Cys-X_{2-4}-Cys-X_3-Phe-X_3-Leu-X_2-His-X_{3-5}-His, where X represents more variable amino acids (Berg and Shi, 1996). TLP-1 bears all the most conserved sequences of the C2H2 motif. However, instead of two to four amino acids between the two Cys residues, the variable part contains eight amino acids. The variable regions are speculated to mediate different interactions with other macromolecules (Berg and Shi, 1996). All C2H2 zinc-finger proteins studied so far are DNA-binding proteins (Iuchi, 2001), suggesting that TLP-1 functions as a transcription factor. Both *mh17* and *bx85* mutations truncate the protein before the zinc finger, suggesting its function is important.

TLP-1 is a member of the Sp1-like gene family

Phylogenetic analyses show that *tlp-1* is a member of a monophyletic Sp1-like family of C2H2-type zinc-finger transcription factor genes (thick lineages in Fig. 4D), and is most closely related to the *D. melanogaster nocA* (*no ocelli*) gene (Cheah et al., 1994), the *noz1* (*nocA*-related zinc finger 1) gene from zebrafish (Andreazzoli et al., 2001), and two uncharacterized genes predicted from the *D. melanogaster* and human genome projects. Because the relationships of these five sequences reasonably reflect the relationships of the organisms that harbor them, this NocA/TLP-1 subfamily is an orthology group.

Predicted proteins of this subfamily uniquely share the SPLALLA motif with the proteins of the monophyletic Sp subfamily (Sp1-Sp5) of vertebrates, constituting the primary evidence of common descent by gene duplication from an ancestral sequence with a SPLALLA motif (lineage 12, Fig. 4D). However, five other Sp-like sequences (Buttonhead, D-Sp1, Y40B1A.4, T22C8.5, and F45H11.1) do not share this SPLALLA motif. Because of poor resolution of relationships

among these latter sequences and between these sequences and the vertebrate Sp and NocA/TLP-1 subfamilies, several alternative evolutionary scenarios are possible besides that depicted in Fig. 4D. Specifically, one or all of the sequences without the SPLALLA motif could be most closely related to the vertebrate Sp subfamily. For example, D-Sp1 could be the *Drosophila* ortholog of the vertebrate Sp subfamily. Although possible, these alternative hypotheses are less parsimonious than that proposed in Fig. 4D.

Proteins in the NocA/TLP-1 subfamily have only one convincing C2H2 zinc finger, whereas the other Sp family members have three (as noted previously for NocA) (Cheah et al., 1994). Based on the supported phylogenetic hypotheses, the NocA/TLP-1 subfamily members lost the first and third zinc fingers. Having three zinc fingers is likely to be ancestral, because the most closely related C2H2 family, the KLF family, also has three zinc fingers (Knight and Shimeld, 2001).

All of the proteins in the Sp family have retained a sequence adjacent to the zinc-finger region called the 'Btd (Buttonhead) box' (Wimmer et al., 1996), although the consensus is reduced from that originally described: R-X(0-4)-C-X-C(/D/N)-P-N/Y-C. However, TLP-1 has an A instead of N or Y as the penultimate residue, and the other NocA/TLP-1 subfamily members do not have the R. Thus, there has been considerable evolution in the structure of the NocA/TLP-1 proteins after they diverged from other members of the Sp family, as indicated by the longer branch lengths of their lineages in the phylogeny (Fig. 4D). Consistent with this increased accumulation of differences, phenetic (distance-based) phylogenetic analyses artefactually place the NocA/TLP-1 subfamily outside of either the Sp or KLF families. In this case, the 5 non-SPLALLA Sp sequences and the vertebrate Sp subfamily are grouped on the basis of plesiomorphic (primitive) similarity alone. We do not know what adaptive significance there may be, if any, to this increased divergence.

TLP-1 expression pattern

A transgene in which *gfp* was fused in frame with *tlp-1* and whose expression was designed to be driven by the *tlp-1* promoter rescued the T cell defect but not the Lep defects of *tlp-1* mutants (Table 1). *tlp-1::gfp* expression begins to be barely detectable at the beginning of gastrulation at about 100 minutes of embryonic development (Fig. 5A). Shortly after the gastrulation begins, the level of expression increases and is detectable in the nuclei of most embryonic cells (Fig. 5B). This pattern appears to persist through gastrulation. However, at about 260 minutes, expression in the anterior of the embryo fades and expression in the posterior of the embryo persists and gets stronger (Fig. 5C). At the 1.5-fold stage, at about 400 minutes, we primarily observe expression in posterior nuclei (Fig. 5D) and this pattern continues throughout the rest of development. We have not characterized this expression pattern in detail, as we do not observe any embryonic defects in *tlp-1* mutants. However, we have observed expression in hyp9 and hyp10 in threefold embryos but not in the T cells (not shown).

During larval development, we observed *tlp-1::gfp* expression in the posterior intestinal cells, several neuronal nuclei in the head (not shown), the tail tip cells in hermaphrodites and males and in descendants of the T cell lineage. The tail tip cell expression we observed was weak, but consistent with a role for *tlp-1* in the differentiation of these
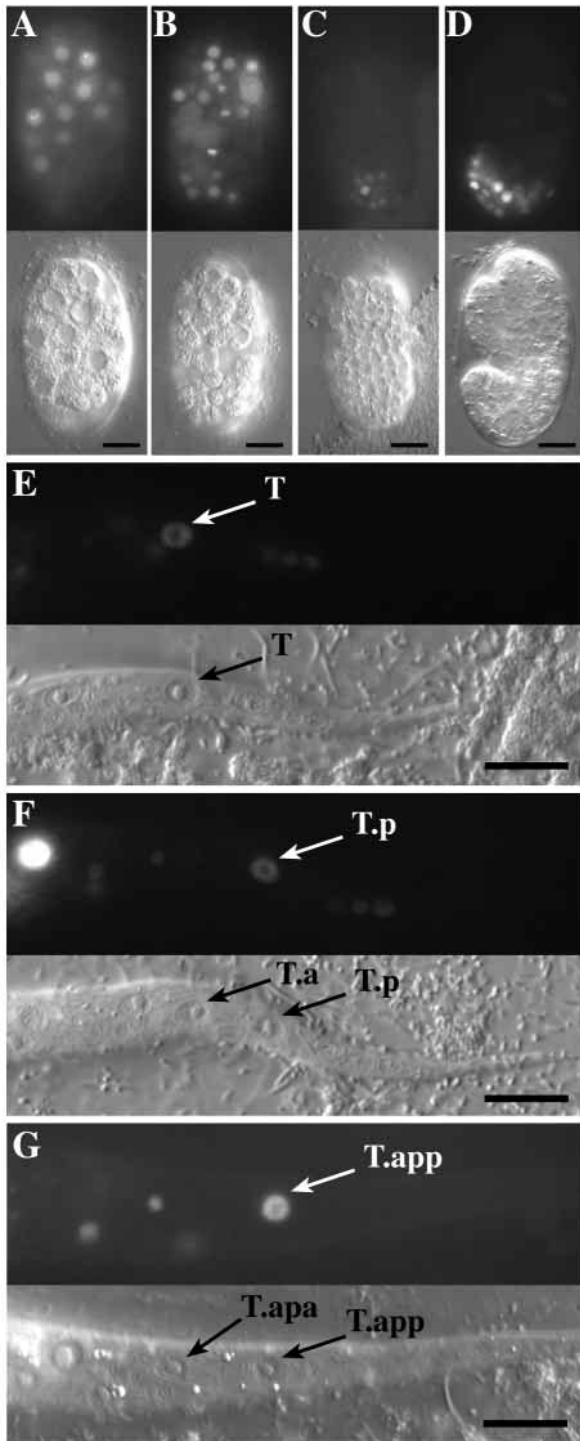
Fig. 5. *tlp-1* expression. A functional *tlp-1* reporter construct is strongly expressed in the nuclei of the posterior intestine cells, weakly expressed in the nuclei of T and its descendent cells, as well as the tail hypodermal cells. (A-G) Fluorescence (upper) and DIC optics (lower). Scale bars: 10 μm. (A) GFP expression in most nuclei of an embryo at approximately 100 minutes when four E cell descendants were present, (B) most nuclei of a gastrulating embryo, (C) the nuclei of posterior cells in an embryo at approximately 270 minutes and (D) the nuclei of posterior cells in a 1.5-fold embryo. (E) GFP expression in the T cell. We also observed that few animals had GFP expression in the hyp9, hyp10 and hyp11 cells. (F) GFP expression in the T.p cell and (G) the T.app cell.

cells that, when reduced or absent, could lead to the Lep tail defect we observed in *tlp-1* males.

We have examined *tlp-1::gfp* expression in the T cell descendants in some detail. We observed weak GFP expression in the T cell in 6% (*n*=124) of animals (Fig. 5E). After the division of the T cell, we observed stronger GFP expression in the posterior T cell daughter, T.p. but not in T.a (Fig. 5F). More specifically, of the animals that showed GFP expression in the T cell lineage (57/150), 88% of the animals examined showed GFP expression in T.p alone and 12% showed expression in T.a and T.p. This expression pattern correlates well with the cell lineage defects we observed in the T.p sublineage. We also observed GFP expression in the posterior T.ap daughter cell, T.app but not in T.ap or its anterior daughter cell T.apa (Fig. 5G). Specifically, all of the animals that showed expression in the T.ap lineage (11/56) showed GFP expression in T.app alone. We did not observe expression in T.apa in any animals. Although we did not observe expression in T.ap, the expression pattern correlates with the cell lineage defects we observed in the T.app division. In summary, these results suggest that TLP-1 is asymmetrically segregated or asymmetrically expressed in the T cell descendants.

TLP-1 responds to *lin-44* signaling

Mutations in *lin-44* cause the polarity of the first division of the T cell to be reversed (Herman and Horvitz, 1994). LIN-44 encodes a Wnt protein that is expressed from the tail tip hypodermis and signals polarity information to the more anterior T cells. We examined the expression of *tlp-1::gfp* in a *lin-44* mutant to determine whether TLP-1 localization might respond to LIN-44 polarity signals. We observed weak GFP expression in the T cell in 8% (*n*=108) of *lin-44* animals (not shown). Interestingly, we found that *tlp-1::gfp* expression is often reversed in *lin-44* mutants, reflecting the reversal in T cell polarity (Fig. 6A). Specifically, of the animals in which we observed GFP expression in the T cell lineage (20/86), 60% showed expression in T.a only, 20% showed expression in T.p only, and 20% showed expression in T.a and T.p. These results correlate with the effect of *lin-44* mutations on the T cell division: roughly 70% of the T cell divisions are reversed, 20% are symmetric and 10% are normal (Herman and Horvitz, 1994). This suggests that the asymmetry of *tlp-1* expression is controlled by the LIN-44 signaling pathway.

To further explore how *lin-44* might regulate *tlp-1*, we analyzed T cell lineages in a *lin-44; tlp-1* double mutant (Fig. 6B). In each lineage it appeared that the polarity of the T cell division was reversed, as it is in *lin-44* single mutants. However, the subsequent cell division patterns were reminiscent of *tlp-1* single mutants, in that we observed the loss of both neural cell fates and cell divisions in four lineages and only the loss of cell divisions in another four lineages. Thus it appears that in *lin-44; tlp-1* double mutants we observed the effects of both mutations, indicating that *tlp-1* mutants retain some T cell polarity information that can be affected by mutations in *lin-44*. In addition, *lin-44* and *tlp-1* appear to interact in the formation of the male tail. *lin-44* males display a weak Lep tail defect that is significantly enhanced by one mutant copy of *tlp-1* (Table 1). This suggests that *tlp-1* might also function with, and perhaps downstream of, *lin-44* in the development of the male tail.

DISCUSSION

tlp-1 is required for proper morphogenesis of the *C. elegans* tail. Mutations in *tlp-1* cause defects in tail morphogenesis by affecting two fundamental processes: specification of asymmetric cell fates and cell fusion. The T cell lineage defects observed in *tlp-1* mutants suggest that *tlp-1* is involved in the generation or execution of T cell fate asymmetry. Mutations in *tlp-1* also cause the normally rounded, peloderan male tail to be pointed, or leptoderan, apparently owing to a heterochronic delay in the highly ordered set of cell fusions and retractions of the tail tip hypodermis.

Molecular analysis revealed that *tlp-1* encodes a C2H2-type zinc-finger protein that is a member of the Sp family of transcription factors. Although the zinc-finger motifs of TLP-1 and its orthologs (Fig. 4D) are quite divergent from other Sp family members, they are probably most closely related to the Sp subfamily of vertebrate transcriptional activators. NocA and Noz1, as well as members of the other Sp subfamilies (e.g. mouse Sp4 and *D. melanogaster* Buttonhead and D-Sp1), are also transcription factors with roles in developing brain, CNS and peripheral sensory organs (Cheah et al., 1994; Supp et al., 1996; Wimmer et al., 1996; Schöck et al., 1999; Andreazzoli et al., 2001). TLP-1 is expressed in nuclei and has a functional role in the postembryonic development of the phasmid sensory organs in the *C. elegans* tail. Thus, the most parsimonious prediction for TLP-1 function would be as a transcription factor involved in patterning neuronal organs.

By the same logic, one might also predict an embryonic role for *tlp-1* in the developing brain. Although *tlp-1* has a dynamic and highly regulated embryonic expression pattern, we do not observe any embryonic defects in any of our *tlp-1* mutants, including *ny14*, which completely deletes the locus. That there are no major phenotypic consequences in homozygous null embryos suggests that there may be another gene acting

We also examined the localization of *tlp-1::gfp* expression in *lin-17* mutants. We observed weak *tlp-1::gfp* expression in 4.0% of T cells examined (*n*=202) in *lin-17* animals. Of the animals that showed GFP expression in the T cell lineage after the division of the T cell (11/140), 1.0% showed GFP expression in T.a alone, 5.0% showed expression in T.p alone and 2% showed expression in T.a and T.p (not shown). We also analyzed the T cell lineages in a *lin-17; tlp-1* double mutant. In each lineage, there was a loss of cell polarity, resulting in the generation of only hypodermal cell fates. In fact, both the pattern of cell divisions and cell fates generated was indistinguishable from that observed in *lin-17* single mutants, suggesting that *lin-17* is epistatic to *tlp-1* and that the neural cell fates we sometimes observed in *tlp-1* single mutants were dependant upon *lin-17* function (Fig. 6C); consistent with the observation that *lin-17* mutations cause a loss of *tlp-1::gfp* expression.

redundantly with *tlp-1* in its embryonic role. As a precedent for such redundancy, *buttonhead* and *Sp1* are redundant for postembryonic sensory organ development in *Drosophila*, although *buttonhead* alone is required for embryonic head segment formation (Schöck et al., 1999). There are three other as yet uncharacterized genes in *C. elegans* that belong to the Sp family (Y40B1A.4, T22C8.5, and F45H11.1) that could be good candidates for having overlapping function with *tlp-1*. However, if TLP-1 does not function in embryonic development, this would be a novel trait that arose subsequent to the divergence of TLP-1 from its orthologs; the embryonic expression pattern might then be a nonfunctional vestige of the ancestral embryonic function.

Asymmetric expression and the control of cell fate in the T cell lineage

Loss of *tlp-1* function leads to the loss of specific T cell divisions and cell fates in the divisions of the posterior T cell daughter, T.p, and the T.ap cell. We observed the loss of neural cell fates and cell divisions in both affected cells (Fig. 1). What role does *tlp-1* play in controlling these divisions? One possibility is that *tlp-1* is involved only in the specification of neural cell fates. However, the loss of divisions in the T.p and T.ap sublineages, including the T.apa cell division which does not generate neural cell fates, suggests that *tlp-1* is not just involved in the specification of neural cell fates. For example, *tlp-1* may a play a role in the asymmetric expression of cell fate factors in response to cell polarity cues.

In fact, TLP-1 itself is asymmetrically expressed in the T cell lineage in response to cell polarity cues. We observed *tlp-1::gfp* expression in the T cell and the T.p cell. Later, *tlp-1::gfp* expression reappears in T.app, a descendant of the T.a and T.ap cells which never expressed it, suggesting that *tlp-1* is asymmetrically expressed rather than asymmetrically segregated in the T cell lineage (Fig. 7). However, it is also possible that *tlp-1* is expressed in T.ap, and perhaps T.apa, at levels below detection, which may explain why we observed defects in the division of T.apa. Alternatively, *tlp-1* may not be expressed in T.ap and T.apa fate may be determined by signals from T.app, which expresses *tlp-1*. Finally, *tlp-1* may be involved in a process required for proper T.apa division that gets set up in the T cell and is subsequently segregated to T.apa.

The precise mechanism by which *tlp-1* is asymmetrically expressed is not clear. However, the asymmetric expression of *tlp-1* is controlled by LIN-44/Wnt signals (Figs 6A, 7). This suggests that *tlp-1* may be involved in the specification of cell fates in response to LIN-44 signals that control cell polarity. Taken together with the effects of *lin-44* mutations on TLP-1 localization, it appears that a LIN-44 signal functions to polarize the T cell lineage but that TLP-1 is needed for correct cell fate specification within the polarized lineage. Thus, *tlp-1* appears to be a downstream target of a Wnt signal that functions to polarize cells.

In addition, we rarely observed *tlp-1::gfp* expression in *lin-17* animals, consistent with the idea that both T cell daughters take on a T.a-like cell fate, in which one would not expect to observe *tlp-1* expression (Fig. 7). Another transcription factor whose expression is asymmetric in the T cell lineage is POP-1/Tcf (Herman, 2001). POP-1 is also asymmetrically distributed at many divisions during embryonic and postembryonic development, with anterior daughter cells

Fig. 7. Model for TLP-1 function in the T cell lineage. *tlp-1* is asymmetrically expressed and functions in the T cell lineage. LIN-44/Wnt signal emanating from the tail hypodermal cells posterior to the T cell is indicated, as are LIN-17/Fz receptors, which are presumably present on the surface of the T cell. TLP-1 expression is indicated by 'TLP-1'. Hypodermal (hyp) T.a fate is represented by purple shading, neural (n) T.p fate is represented by green shading. Normal T.apa fate is represented by red shading and T.app fate by blue shading. TLP-1 is expressed in the T, T.p and T.app cells in wild-type animals, and affects the divisions of the T.p and T.ap cells. TLP-1 may not be expressed in T.ap, indicated by TLP-1? In *lin-44* animals, TLP-1 expression and T cell polarity are reversed. In *lin-17* animals, TLP-1 expression and cell polarity are lost.
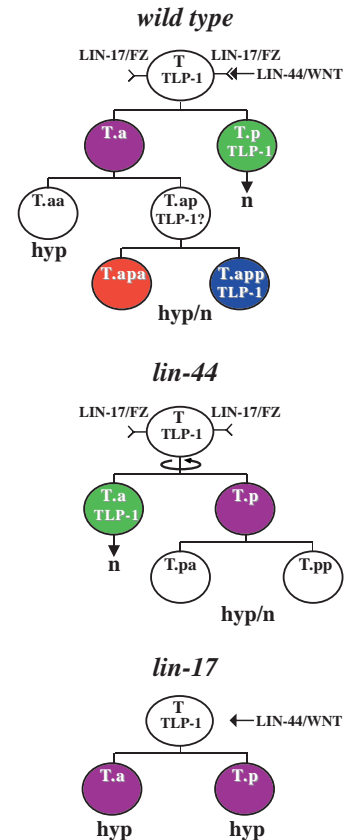
having higher levels than do posterior daughter cells (Lin et al., 1998; Herman, 2001). Interestingly, the expression of POP-1 is the opposite of TLP-1; POP-1 is expressed at high levels in T.a and at low levels in T.p. Furthermore, like TLP-1, POP-1 expression also responds to LIN-44/Wnt signals; in *lin-44* mutants, POP-1 expression is reversed, being expressed at high levels in T.p and at low levels in T.a. The mechanism by which asymmetric POP-1 expression is also not clear. In addition, for the T cell division, it does not appear that the different levels of POP-1 per se, specify cell polarity, as reducing *pop-1* function caused both T cell daughters to take on a hypodermal, or anterior, cell fate. This suggests that the high level of POP-1 observed in T.a may be nonfunctional and that a low level of a perhaps modified form of POP-1 is required for the posterior cell fate (Herman, 2001). Possibly this form of POP-1 is involved in regulating the expression of TLP-1, however this has not yet been tested. Alternatively, TLP-1 may be involved in regulating the distribution and modification of the active form of POP-1.

Why *are tlp-1* male tails Lep?

Male tail morphogenesis involves a specifically timed set of cell fusions and cell retractions (Nguyen et al., 1999). We observed that these specific cell fusions and retractions are heterochronically delayed in *tlp-1* males, leading to the failure of tail tip retractions and resulting in the leptoderan tail (Lep) defect. How are these cell fusions and retractions initiated and controlled and what role might TLP-1 play in the process? We considered two possibilities: either the fusion and retraction program is intrinsic to the tail tip cells; or an extrinsic cell

signal(s), from an unknown source(s) triggers the beginning of the fusions and retractions. TLP-1 is expressed in the tail tip cells hyp9 and hyp10 during embryogenesis. If the cell fusion and retraction program is intrinsic to the tail tip cells, *tlp-1* might function to control the fate of the tail tip cells or be required to initiate the cell fusion and retraction program. Interestingly, the tail tip cells are also posterior daughters of asymmetric divisions in the embryo, suggesting the possibility that tail tip cell fate may be part of a asymmetric cell fate governed by TLP-1. Our results did not rule out the existence of an extrinsic cell fusion signal. However, they did suggest that such a signal does not emanate from the T cell descendants and that the abnormal *tlp-1* T cell fates do not interfere with such a signal. As TLP-1 probably functions as a transcription factor, it could control the fate or the production of the signal within the signaling cell. Alternatively, it could control the fate of the tail tip cells or their competence to receive a signal. There is yet no evidence for such a signal, although *lin-44/Wnt* mutant males display a low level of Lep tails, suggesting that a role for Wnt signaling in the control of male tail tip morphogenesis. Furthermore, the frequency of Lep tails observed in *lin-44* males is significantly enhanced by *tlp-1* mutations, suggesting that *tlp-1* functions with *lin-44* in male tail morphogenesis as it does in the asymmetric divisions of the T cell.

Other *C. elegans* transcription factors have been shown to play a role in cell fusions. Three *C. elegans* Hox genes have been implicated in the control of cell fusions during both embryonic and postembryonic development (Clark et al., 1993; Wang et al., 1993; Brunschwig et al., 1999). During postembryonic development, *lin-39* and *mab-5* function to control the fusions of the ventral hypodermal cells, the Pn.p cells, to the hyp7 syncytium. In particular, the Hox gene *lin-39*, which encodes the *C. elegans* homolog of the *Drosophila Deformed* and *Sex combs reduced* genes (Clark et al., 1993; Wang et al., 1993), functions in hermaphrodites to control the fusion of the P(3-8).p cells to allow the formation of the vulval precursor cells. In this case, *lin-39* expression is required to prevent cell fusions (Clark et al., 1993; Wang et al., 1993). Another *C. elegans* Hox gene, *mab-5*, which encodes the *C. elegans* homolog of the *Drosophila Antennepedia* gene, functions to block P(9-11).p cell fusions in males (Kenyon, 1986). The expression of both *lin-39* and *mab-5* are controlled by *egl-27* (Ch'ng and Kenyon, 1999). *egl-27* encodes a product that contains an N-terminal region that has similarity to mammalian MTA (metastasis-associated) proteins. (Herman et al., 1999; Solari et al., 1999). MTA proteins have been biochemically linked to nuclear protein complexes that are involved in chromatin remodeling and histone deacetylation (Xue et al., 1998). Interestingly, mutations in *egl-27* also cause defects in the polarity of the T cell (Herman et al., 1999), suggesting a link between the control of cell fusion and cell polarity at the level of chromatin remodeling.

Changes in cell polarity and cell fusion can lead to changes in morphogenesis that lead to evolution of different forms

The Lep tails observed in *tlp-1* males are morphologically distinct from those observed in leptoderan species of rhabditid nematodes related to *C. elegans*. Thus, *tlp-1* is not solely responsible for the observed variation, but is involved in processes responsible for such variation. Interestingly, mutations in *lep-1* also lead to the failure of male tail tip retraction and cause *C. elegans* males to have a tail morphology similar to that of leptoderan nematodes. The *lep-1* male tails resemble the tails of leptoderan nematodes more closely than do *tlp-1* male tails. Initial analysis of *lep-1* males revealed that the fusions of the tail tip cells are delayed. The tail hypodermal cells also fail to fuse with each other in closely related nematode species with naturally occurring leptoderan tails (Nguyen et al., 1999). Together these results suggest that cell fusions are integral to nematode male tail morphogenesis (Shemer and Podbilewicz, 2000), and that morphological diversity can thus result from evolutionary changes in cell fusions and cell shape. However, more work is needed to determine what the morphogenetic mechanism is and how the program is initiated. Finally, the Lep defects of both *lep-1* and *tlp-1* mutants suggest that changes in cell fusions can lead to changes in morphogenesis that may contribute to natural variation in form.

Note added in proof

Besides Noc, the other *D. melanogaster* ortholog of TLP-1 is ElbowB (accession no. AAF44874) (M. Wernet and C. Desplan, pers. comm.).

REFERENCES

Andreazzoli, M., Broccoli, V. and Dawid, I. B. (2001). Cloning and expression of *noz1*, a zebrafish zinc finger gene related to *Drosophila nocA*. *Mech. Dev.* **104**, 117-120.

Berg, J. M. and Shi, Y. (1996). The galvanization of biology: a growing appreciation for the roles of zinc. *Science* **271**, 1081-1085.

Brunschwig, K., Wittmann, C., Schnabel, R., Burglin, T. R., Tobler, H. and Muller, F. (1999). Anterior organization of the *Caenorhabditis elegans* embryo by the labial-like Hox gene *ceh-13*. *Development* **126**, 1537-1546.

Cadigan, K. M. and Nusse, R. (1997). *Wnt* signaling: a common theme in animal development. *Genes Dev.* **11**, 3286-3305.

Ch'ng, Q. and Kenyon, C. (1999). *egl-27* generates anteroposterior patterns of cell fusion in *C. elegans* by regulating Hox gene expression and Hox protein function. *Development* **126**, 3303-3312.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W. W. and Prasher, D. C. (1994). Green fluorescent protein as a marker for gene expression. *Science* **263**, 802-805.

Cheah, P. Y., Meng, Y. B., Yang, X., Kimbrell, D., Ashburner, M. and Chia, W. (1994). The *Drosophila l(2)35Ba/nocA* gene encodes a putative Zn finger protein involved in the development of the embryonic brain and the adult ocellar structures. *Mol. Cell. Biol.* **14**, 1487-1499.

Clark, S. G., Chisholm, A. D. and Horvitz, H. R. (1993). Control of cell fates in the central body region of C. elegans by the homeobox gene *lin-39*. *Cell* **74**, 43-55.

Herman, M. A. (2001). *C. elegans* POP-1/TCF functions in a canonical Wnt pathway that controls cell migration and in a noncanonical Wnt pathway that controls cell polarity. *Development* **128**, 581-590.

Herman, M. A. and Horvitz, H. R. (1994). The *Caenorhabditis elegans* gene *lin-44* controls the polarity of asymmetric cell divisions. *Development* **120**, 1035-1047.

Herman, M. A., Vassilieva, L. L., Horvitz, H. R., Shaw, J. E. and Herman, R. K. (1995). The C. elegans gene *lin-44*, which controls the polarity of

certain asymmetric cell divisions, encodes a Wnt protein and acts cell nonautonomously. *Cell* **83**, 101-110.

Herman, M. A., Ch'ng, Q., Hettenbach, S. M., Ratliff, T. M., Kenyon, C. and Herman, R. K. (1999). EGL-27 is similar to a metastasis-associated factor and controls cell polarity and cell migration in *C. elegans*. *Development* **126**, 1055-1064.

Hodgkin, J., Horvitz, H. R. and Brenner, S. (1979). Nondisjunction mutants of the nematode *Caenorhabditis elegans*. *Genetics* **91**, 67-94.

Iuchi, S. (2001). Three classes of C2H2 zinc finger proteins. *Cell Mol. Life Sci.* **58**, 625-635.

Kenyon, C. (1986). A gene involved in the development of the posterior body region of C. elegans. *Cell* **46**, 477-487.

Knight, R. D. and Shimeld, S. M. (2001). Identification of conserved C2H2 zinc-finger gene families in the Bilateria. *Genome Biol.* **2**.

Krause, M. and Hirsh, D. (1987). A trans-spliced leader sequence on actin mRNA in C. elegans. *Cell* **49**, 753-761.

Lackner, M. R., Kornfeld, K., Miller, L. M., Horvitz, H. R. and Kim, S. K. (1994). A MAP kinase homolog, *mpk-1*, is involved in *ras*-mediated induction of vulval cell fates in *Caenorhabditis elegans*. *Genes Dev.* **8**, 160-173.

Lin, R., Hill, R. J. and Priess, J. R. (1998). POP-1 and anterior-posterior fate decisions in C. elegans embryos. *Cell* **92**, 229-239.

Maddison, D. and Maddison, W. (2001). *MacClade: Analysis of Phylogeny and Character Evolution*. Sunderland, MA: Sinauer Associates.

Mello, C. C. and Fire, A. (1995). DNA transformation. In *Caenorhabditis elegans: Modern Biological Analysis of an Organism. Methods in Cell Biology* (ed. H. Epstein and D. Shakes), pp. 452-482. San Diego: Academic Press.

Mohler, W. A., Simske, J. S., Williams-Masson, E. M., Hardin, J. D. and White, J. G. (1998). Dynamics and ultrastructure of developmental cell fusions in the Caenorhabditis elegans hypodermis. *Curr. Biol.* **8**, 1087-1090.

Nguyen, C. Q., Hall, D. H., Yang, Y. and Fitch, D. H. A. (1999). Morphogenesis of the *Caenorhabditis elegans* male tail tip. *Dev. Biol.* **207**, 86-106.

Sawa, H., Lobel, L. and Horvitz, H. R. (1996). The *Caenorhabditis elegans* gene *lin-17*, which is required for certain asymmetric cell divisions, encodes a putative seven-transmembrane protein similar to the *Drosophila frizzled* protein. *Genes Dev.* **10**, 2189-2197.

Schöck, F., Purnell, B. A., Wimmer, E. A. and Jackle, H. (1999). Common and diverged functions of the *Drosophila* gene pair *D-Sp1* and *buttonhead*. *Mech. Dev.* **89**, 125-132.

Shemer, G. and Podbilewicz, B. (2000). Fusomorphogenesis: cell fusion in organ formation. *Dev. Dyn.* **218**, 30-51.

Solari, F., Bateman, A. and Ahringer, J. (1999). The *Caenorhabditis elegans* genes *egl-27* and *egr-1* are similar to MTA1, a member of a chromatin regulatory complex, and are redundantly required for embryonic patterning. *Development* **126**, 2483-2494.

Sternberg, P. W. and Horvitz, H. R. (1988). *lin-17* mutations of *Caenorhabditis elegans* disrupt certain asymmetric cell divisions. *Dev. Biol.* **130**, 67-73.

Sulston, J. and Hodgkin, J. (1988). Methods. In *The Nematode Caenorhabditis elegans*, vol. 1 (ed. W. B. Wood), pp. 587-606. COld Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sulston, J. E. and Horvitz, H. R. (1977). Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. *Dev. Biol.* **56**, 110-156.

Sulston, J. E. and White, J. G. (1980). Regulation and cell autonomy during postembryonic development of *Caenorhabditis elegans*. *Dev. Biol.* **78**, 577-597.

Supp, D. M., Witte, D. P., Branford, W. W., Smith, E. P. and Potter, S. S. (1996). Sp4, a member of the Sp1-family of zinc finger transcription factors, is required for normal murine growth, viability, and male fertility. *Dev. Biol.* **176**, 284-299.

Swofford, D. (2001). *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sunderland, MA: Sinauer Associates.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G. (1997). The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **24**, 4876-4882.

Troemel, E. R., Kimmel, B. E. and Bargmann, C. I. (1997). Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in *C. elegans*. *Cell* **91**, 161-169.

Wang, B. B., Muller-Immergluck, M. M., Austin, J., Robinson, N. T., Chisholm, A. and Kenyon, C. (1993). A homeotic gene cluster patterns the anteroposterior body axis of C. elegans. *Cell* **74**, 29-42.

Wimmer, E. A., Frommer, G., Purnell, B. A. and Jackle, H. (1996). *buttonhead* and *D-Sp1*: a novel *Drosophila* gene pair. *Mech. Dev.* **59**, 53-62.

Wodarz, A. and Nusse, R. (1998). Mechanisms of Wnt signaling in development. *Annu. Rev. Cell Dev. Biol.* **14**, 59-88.

Xue, Y., Wong, J., Moreno, G. T., Young, M. K., Cote, J. and Wang, W. (1998). NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. *Mol. Cell* **2**, 851-861.