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SUP-17, a *Caenorhabditis elegans* ADAM protein related to *Drosophila* KUZBANIAN, and its role in LIN-12/NOTCH signalling

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SUMMARY

LIN-12/NOTCH proteins mediate cell-cell interactions that specify cell fates. Previous work suggested that *sup-17* facilitates *lin-12* signalling in *Caenorhabditis elegans*. Here, we show that *sup-17* encodes a member of the ADAM family of metalloproteases. SUP-17 is highly similar to *Drosophila* KUZBANIAN, which functions in *Drosophila* neurogenesis, and the vertebrate ADAM10 protein. Furthermore, we show by genetic analysis that the extracellular domain of LIN-12 appears to be necessary for *sup-17* to facilitate *lin-12* signalling and that *sup-17* does not act downstream of *lin-12*. Finally, we show by cell ablation experiments that *sup-17* can act cell autonomously to facilitate *lin-12* activity. We discuss the implications of our observations for LIN-12/NOTCH signalling and how our results complement and extend results obtained from genetic analysis of *kuz* in *Drosophila*.

Key words: *lin-12*, *Notch*, cell fate, metalloprotease, *C. elegans*, signalling

INTRODUCTION

Many cell-cell interactions that specify cell fate during animal development are mediated by receptors of the LIN-12/NOTCH family and ligands of the Delta/Serrate/LAG-2 (DSL) family (reviewed in Artavanis-Tsakonas et al., 1995). Much of the work on *lin-12* has focused on its role in two cell-fate decisions during *Caenorhabditis elegans* development. One of these decisions, the anchor cell (AC)/ventral uterine precursor cell (VU) decision, occurs during development of the hermaphrodite gonad; the other, vulval precursor cell (VPC) specification, occurs during development of the vulva.

During hermaphrodite gonadogenesis, *lin-12*-mediated cell-cell interactions occur between two cells, named Z1.ppp and Z4.aaa. These two cells are initially equivalent in their developmental potential, in that each has an equal chance of becoming the anchor cell (AC), a terminally differentiated cell type that is necessary for vulval development, or a ventral uterine precursor cell (VU), which contributes descendants to the ventral uterus. However, in any given hermaphrodite, only one of these cells will become the AC, while the other becomes a VU (Kimble and Hirsh, 1979).

The process of lateral specification of Z1.ppp and Z4.aaa depends on interactions between them (Kimble, 1981; Seydoux and Greenwald, 1989). It also depends on *lin-12* activity, since elevating *lin-12* activity causes both Z1.ppp and Z4.aaa to become VUs, while reducing *lin-12* activity causes both Z1.ppp and Z4.aaa to become ACs (Greenwald et al., 1983). Genetic mosaic analysis and reporter gene studies have revealed that initially both Z1.ppp and Z4.aaa express *lin-12* and *lag-2*, but a stochastic small variation in ligand and/or receptor activity is subsequently amplified by a feedback mechanism that influences *lin-12* and *lag-2* transcription (Seydoux and Greenwald, 1989; Wilkinson et al., 1994). This circuitry ensures that only one of the two cells chosen at random will become an AC and the other will become a VU.

The hermaphrodite vulva is normally formed from descendants of three of the VPCs (P5.p, P6.p and P7.p) (Sulston and Horvitz, 1977). However, there are six VPCs, P3.p-P8.p, each of which has the potential to adopt one of three fates: 1°, 2° or 3° (Sulston and White, 1980; Sternberg and Horvitz, 1986). The 1° fate and 2° fates are termed 'vulval' fates, because they lead to the production of vulval cells. The 3° fate is termed the 'non-vulval' or 'hypodermal' fate, because it leads to the production of cells that join the hyp7 hypodermal syncytium.

In wild-type hermaphrodites, P3.p-P8.p always adopt the same pattern of fates: 3°-3°-2°-1°-2°-3°. This pattern appears to be the outcome of three different signalling events (reviewed in Greenwald, 1997). One of these signalling events, lateral signalling (Sternberg, 1988), is thought to involve a signal emanating from P6.p that promotes the 2° fate in the neighboring cells P5.p and P7.p. LIN-12 is thought to be the receptor for the lateral signal, since elevating *lin-12* activity causes all VPCs to adopt the 2° fate, while reducing *lin-12* activity prevents any VPC from adopting the 2° fate (Greenwald et al., 1983).

Many components of the LIN-12/NOTCH pathway, or factors influencing the activity of the LIN-12/NOTCH pathway, have been evolutionarily conserved. An example of a

conserved downstream effector is the transcription factor known as Su(H) in *Drosophila*, LAG-1 in *C. elegans* and CBF1 in mammals (see Christensen et al., 1996, and references therein). An example of a conserved factor that may influence LIN-12/NOTCH signalling more indirectly is a protein known as SEL-12 in *C. elegans* and Presenilin 1 in mammals (Levitan and Greenwald, 1995; Shen et al., 1997; Wong et al., 1997). It is therefore likely that as new genes are identified in one system, they will be found to play conserved roles in other systems.

Genetic screens based on suppression or enhancement of *lin-12* mutations have identified a number of genes that influence *lin-12* activity (Sundaram and Greenwald, 1993; Levitan and Greenwald, 1995; Tax et al., 1997). Defects in the AC/VU decision and VPC specification result in distinctive phenotypes that facilitate such screens. For example, constitutive *lin-12* activity resulting from *lin-12(d)* mutations causes an egg-laying defective (Egl) phenotype and a Multivulva (Muv) phenotype; *lin-12(d)* mutants are Egl because they lack an AC, which plays several critical roles in the development of a functional vulva, and Muv because the descendants of each VPC self-organize into a pseudovulva that protrudes from the ventral side of the hermaphrodite.

The *sup-17* gene was defined by mutations that suppress the Egl phenotype of *lin-12(d)* mutations (Ferguson and Horvitz, 1985; Tax et al., 1997). Tax et al. (1997) analyzed *sup-17* genetically, and established that *sup-17* loss-of-function alleles suppressed all *lin-12(d)* alleles tested and all aspects of the *lin-12(d)* phenotype examined. They also showed that putative null alleles of *sup-17* cause embryonic lethality that can be maternally rescued. Finally, they showed that in *sup-17* putative null mutants, the AC/VU decision is made normally, but that there are defects in VPC specification consistent with reduced lateral signalling.

Here, we report sequence analysis and additional genetic characterization of *sup-17*. We find that *sup-17* encodes a member of the ADAM family of metalloproteases (Wolfsberg et al., 1995; Wolfsberg and White, 1996). In particular, SUP-17 is highly similar to *Drosophila* KUZBANIAN, which functions in *Drosophila* neurogenesis (Rooke et al., 1996; Pan and Rubin, 1997), and ADAM10, which is a known protease (Howard and Glynn, 1995; Lunn et al., 1997). Our data suggest that *sup-17* acts cell autonomously to facilitate *lin-12* signalling and that *sup-17* acts on or in concert with the extracellular domain of LIN-12. We propose a model for LIN-12/NOTCH activation that accounts for the genetic interactions between *sup-17* and *lin-12* and the presumed biochemical function of SUP-17 as an extracytosolic protease.

MATERIALS AND METHODS

General methods and strains

General methods are described by Brenner (1974). The wild-type parent for all strains was *C. elegans* var. Bristol strain N2. Strains were grown at 20° unless otherwise noted. Mutations used were:

LGI: *sup-17(n1258)*, *sup-17(n1316)*, and *sup-17(n1319)* (Tax et al., 1997). The integration site of *arIs12 [lin-12(intra)]* (Struhl et al., 1993) is between *dpy-5* and *unc-13* on LGI (C.W., unpublished observations).

LGIII: *lin-12(n137)* and *lin-12(n302)* (Greenwald et al., 1983); *lin-12(ar170)* (Hubbard et al., 1996).

YAC engineering

Y40F10 contains approximately 180 kb of *C. elegans* genomic DNA and is able to rescue the maternal effect lethal phenotype of *sup-17(n1258)*; none of the cosmid clones in the region rescue *sup-17(n1258)*, implying that *sup-17* lies in the gap between two cosmids K11A11 and F36A2 (see Fig. 1). Truncated derivatives of Y40F10 were engineered by homologous recombination in yeast, and the rescue experiments using these truncated YACs confirmed that *sup-17* was located within a gap of approximately 60 kb.

DY1 and DY2 were made using the method described in Lewis et al. (1992). Two 1 kb fragments, A and B, corresponding to the right end of K11A11 and the left end of F36A2, respectively (see Fig. 1), were amplified by PCR from K11A11 and F36A2 DNA, respectively. The A and B fragments were cloned into a YAC fragmentation vector, pBCL (Lewis et al., 1992) to make the plasmids pC1 and pC3, respectively. pC1 was linearized and transformed into a yeast strain containing Y40F10. After selecting for $Trp^+Lys^+Ura^-$ recombinants, colonies that contained the correct truncated YAC derivative, DY1, were isolated and verified. DY2 was generated by the same method, using pC3.

DY3 was made by deleting a large (approximately 50 kb) segment of DY2. Three DNA fragments (Y, URA3 and A, see Fig. 1) were cloned into Bluescript SK+(Stratagene) in the order Y-URA3-A to make the plasmid pC7. Y is a 1 kb fragment amplified by PCR from the left arm of the YAC vector, and lies just before the left end of the insert but after the ARS/CEN sequence. The yeast URA3 fragment was a gift from R. Rothstein. The Y-URA3-A fragment was cut out from pC7 and transformed into a yeast strain containing DY2. $Trp^+Ura^+Lys^+$ colonies were selected and the resulting YAC derivative called DY3. DY3 was confirmed to cover only the region between K11A11 and F36A2. DY3 has an insert of about 60 kb and rescues *sup-17*. DY3 was sent to the Sanger Centre to be sequenced.

For each step, the truncated YAC DNA was separated from yeast chromosomal DNA by pulsed field gel electrophoresis (BioRad). The identity of the truncated YAC was confirmed by three criteria: (1) genetic linkage of yeast markers, (2) the size of the YAC DNA and (3) and Southern blotting to detect the changes of restriction sites around the recombination area.

Cloning *sup-17* by germline tranformation rescue

Each DNA construct was tested for its ability to rescue the maternal effect lethality of *sup-17(n1258ts)*. DNA (10 μg/ml) was coinjected with the cloned *rol-6* dominant marker plasmid pRF4 (100 μg/ml) as described by Mello et al. (1991). Stable transformed lines were established at 20°C, the permissive temperature. L4 Roller hermaphrodites from each line were shifted to 25°C; the presence of viable progeny indicated rescue. As described above, we generated truncated YACs to narrow down the rescue region to a 60 kb YAC DY3. Partial sequence of DY3 was obtained from the Sanger Centre. A 7 kb fragment that was predicted to encode a KUZBANIAN homolog was amplified by PCR. Two independent PCR products were cloned into Bluescript SK+ to generate pK3 (#1 and #2). The plasmids were injected together, and later separately into *sup-17(n1258)*. pK3 is able to fully rescue the maternal effect lethality of *sup-17(n1258)*. It also reverses the non-Multivulva phenotype of *sup-17(n1258); lin-12(n137)* to a Multivulva phenotype.

Molecular analysis

Standard methods were used for the manipulation of recombinant DNA (Sambrook et al., 1989). Sequence comparisons and alignments were obtained using Blast (Altschul et al., 1990) through the NCBI web site and GCG (version 8, Devereux et al., 1984) programs.

One cDNA clone, yk23h2, contains the full-length cDNA of *sup-17*. yk23h2was generously provided by Dr Yuji Kohara. We used the manufacturer's protocol (Stratagene) to excise a plasmid clone from the λZAP II cDNA vector. The complete sequence of yk23h2 insert was obtained by sequencing one strand, and the sequence was

confirmed by comparison with the genomic sequence of DY3 from the Sanger Centre. The 3′ end of *sup-17* was assigned based on the finding of a poly(A) sequence in this cDNA 17 nucleotides 3′ to a polyadenylation signal (see Fig. 2).

The 5′ end of *sup-17* was determined using a modified protocol for 5′ RACE (Stratagene). cDNA was synthesized from total RNA using the manufacturer's protocol. After two rounds of PCR using nested gene-specific primers and SL1 (Krause and Hirsh, 1987) or SL2 (Huang and Hirsh, 1989), we detected a product only when SL1 was used. Sequence of the PCR product confirmed the 5′ end of *sup-17* as shown in Fig. 2. The yk23h2 clone contains the entire *sup-17* coding sequence, and is missing only the *trans*-spliced leader sequence.

The lesions associated with *sup-17(n1319)* and *sup-17(n1316)* mutations were found by direct sequencing of single-stranded PCR products (Allard et al., 1991; Kaltenboeck et al., 1992). Because *sup-17* null mutants produce only inviable embryos, we amplified the *sup-17* gene from individual adult hermaphrodites.

Laser microsurgery

sup-17(n1258) hermaphrodites were grown at 20°C and shifted to 25°C after laser microsurgery, along with unoperated control animals. The operated animals were later checked to confirm the operation was successful. The nucleus of Z4 was ablated in newly hatched L1 larvae, and the isolation of Z1.ppp was accomplished by a series of ablations: first, Z2, Z3 and Z4; then Z1.a; then Z1.pa; and finally Z1.ppa. The presence of an AC was scored during the late L3 stage. VPCs were ablated in early L2 larvae, several hours before VPC induction and *lin-12* specification of the 2° fate (Greenwald et al., 1983; Sternberg and Horvitz, 1986). The VPC cell fates were followed through the L3 to the L4 stage.

RESULTS

Genomic localization of *sup-17*

sup-17 had been genetically mapped between *fer-1* and *unc-29* (Ferguson and Horvitz, 1985; Tax et al., 1997). Transgenes containing cosmids and yeast artificial chromosomes (YACs) from this region were assessed for *sup-17(+)* activity by looking for rescue of the maternal effect embryonic lethal phenotype of *sup-17(n1258ts)* (Tax et al., 1997) (Fig. 1 and data not shown). Y40F10, a YAC containing approximately 180 kb of *C. elegans* genomic DNA, contained rescuing activity. This YAC spans a gap in the cosmid physical map (Waterston et al., 1997). To delineate the region of Y40F10 containing *sup-17(+)* activity, we generated deletions using yeast genetic methods (see Fig. 1 and Materials and methods). DY3, a YAC containing 60 kb of *C. elegans* genomic DNA, spanned the gap and rescued the maternal effect lethal phenotype of *sup-17(n1258ts)*. DY3 was then provided to the *C. elegans* genome sequencing consortium for sequence analysis.

sup-17 encodes a conserved ADAM protein that is similar to Drosophila KUZBANIAN and human ADAM10

A BLAST search (Altschul et al., 1990) using the sequence of predicted proteins encoded by the DY3 sequence (GENEFINDER; see Edgley et al., 1997) revealed that one predicted protein was a member of the ADAM family (contains A Disintegrin And Metalloprotease domain). The predicted protein displayed a high degree of sequence similarity to the product of the *Drosophila kuzbanian* (*kuz*) gene, also a member of the ADAM family (Rooke et al., 1996), and the bovine and human ADAM 10 proteins (Howard and Glynn, 1995; Wolfsberg et al., 1995; Wolfsberg and White, 1996; Lunn et al., 1997). Because *kuz* has been implicated in neurogenesis (Rooke et al., 1996), a process that also involves *Notch*, we hypothesized that the *kuz*-related gene might correspond to *sup-17*.

To assay the *sup-17(+)* activity of the *kuz*-related gene, we constructed pK3, a plasmid containing the predicted gene (see Materials and methods), and assessed the ability of transgenes containing pK3 to rescue the maternal effect lethality of *sup-17(n1258)* as described above. We also assessed the ability of pK3 transgenes to reverse the suppression of *sup-17(n1258)*; *lin-12(n137)*, restoring the Muv phenotype of *lin-12(n137)*. By both of these assays, pK3 appeared to have *sup-17(+)* activity, suggesting that the *kuz*-related gene is indeed *sup-17*. Sequence analysis of mutations, described below, confirmed the identity of the *kuz*-related gene as *sup-17*.

Our analysis of the genome project database revealed three expressed sequence tags corresponding to three *sup-17* cDNAs that had been identified. One of these cDNAs, yk23H2, generously provided by Y. Kohara, proved to contain a full-length *sup-17* cDNA. This cDNA was completely sequenced on one strand, and was found to agree with the GENEFINDER prediction. The cDNA sequence shown in Fig. 2 reflects the combined information obtained from the genome sequence and cDNA sequence. Information about the predicted protein sequence is given in Figs 3 and 4.

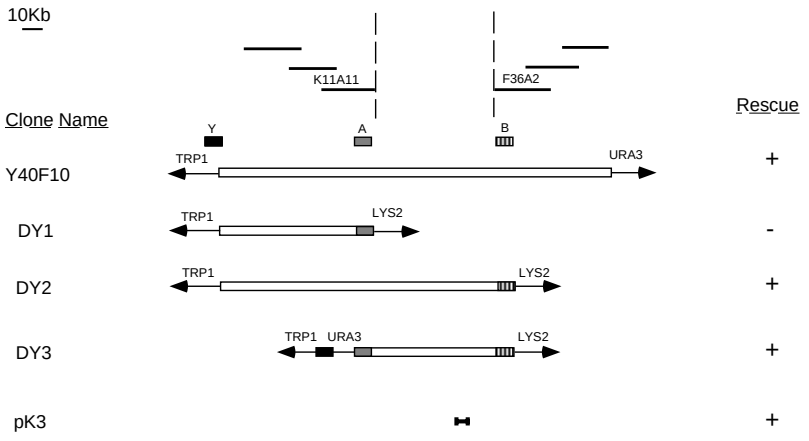

Fig. 1. Molecular cloning of *sup-17*. Cosmid clones such as K11A11 and F36A2 are represented by black lines, and YAC clones are indicated by open bars. pK3 is a plasmid containing the 7 kb *sup-17* coding region amplified by PCR of *C. elegans* genomic DNA. The ability of these clones to rescue the maternal effect lethality of *sup-17(n1258)* is indicated; + indicates a clone was able to rescue, – indicates a clone was unable to rescue. For details of the rescue experiments see Materials and methods. Also shown in the figure are the yeast markers present in the YAC constructs, and three regions of sequence (Y, A and B) that were used in yeast recombination experiments. See Materials and methods for details. The *C. elegans* genomic regions included in each clone are drawn to scale. Flanking regions from the YAC vectors and the Y, A and B regions are not drawn to scale.

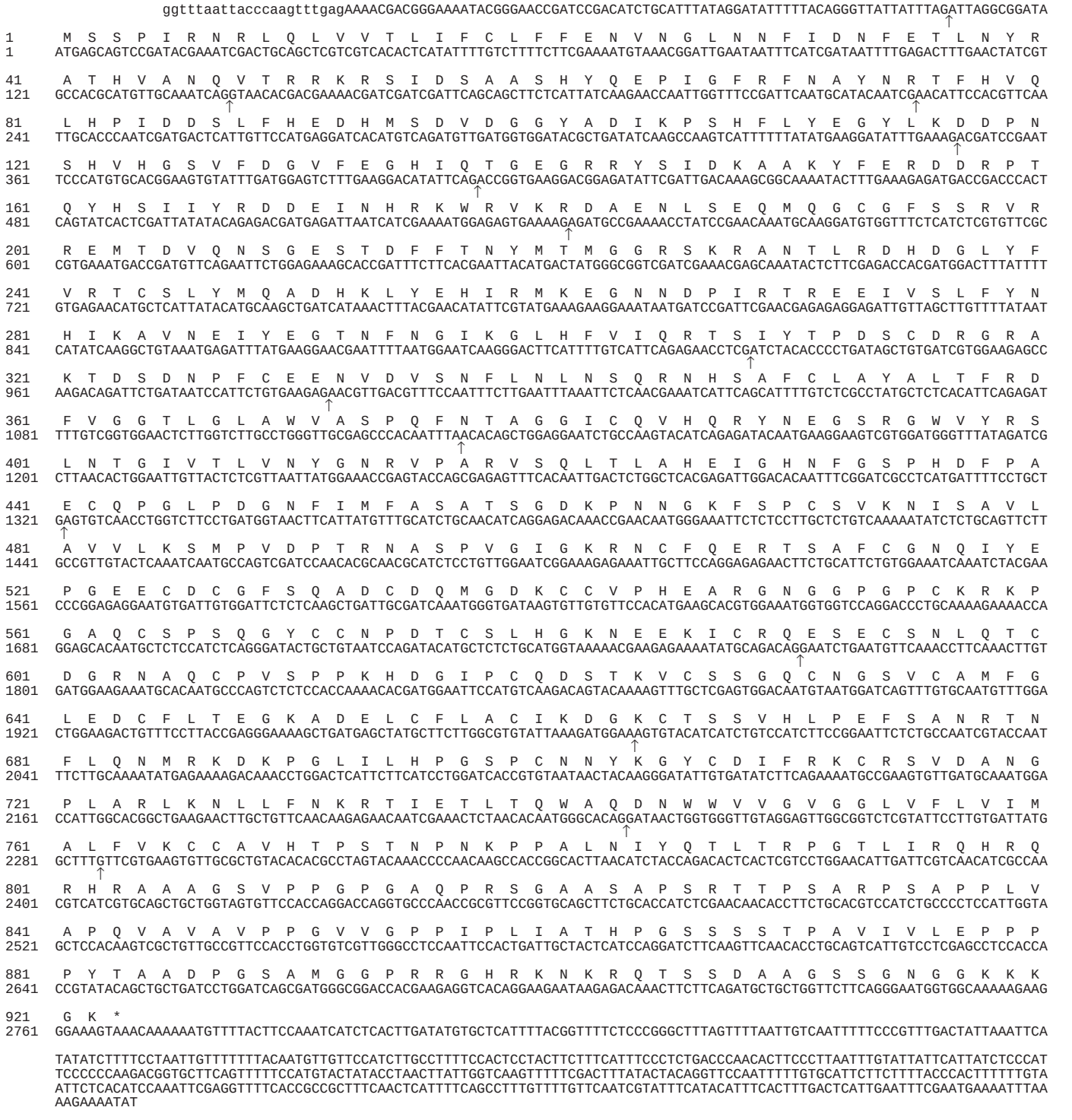

Fig. 2. *sup-17* cDNA sequence and predicted protein product. Nucleotides are numbered beginning with the first base of the predicted start codon. The SL1 sequence (Krause and Hirsh, 1987) is shown at the 5′ end in lower-case letters; a predicted *trans*-splice acceptor sequence (CTTCCAG) is present in the genomic sequence before the *trans*-splice junction. Arrows indicate the position of *cis*-splice junctions. The termination codon is marked by an asterisk. Three independent cDNAs, available in the genome project database, terminate 17 nucleotides downstream of a consensus polyadenylation signal sequence (AATGAA). We have sequenced the 3′ end of one of them (yk23h2), and a poly(A) sequence is present after the cDNA, as shown. For details, see Materials and methods. This sequence has been submitted to GenBank (accession number AFO24614).

Sequence analysis of *sup-17* mutations

To confirm the rescue data and to identify potential molecular null alleles, we determined the sequence alterations in *sup-17* mutants by direct sequencing of amplified genomic DNA

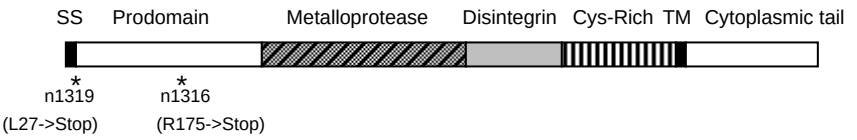

Fig. 3. Schematic depiction of SUP-17. The hallmark motifs of the ADAM family and sequence alterations associated with two null mutations are shown. Note that Tax et al. (1997) have shown that *n1319* is suppressible by an amber suppressor tRNA, and we have found that the lesion in *n1319* is an amber codon.

products (see Materials and methods). Several alleles of *sup-17* appear to be null alleles by genetic criteria (Tax et al., 1997), and we have identified sequence alterations associated with two of these alleles (*n1316* and *n1319*; see Fig. 3). This result confirms that the rescuing construct corresponds to the *sup-17* gene. The sequence changes associated with null mutations also support the genetic evidence suggesting that they eliminate *sup-17* activity, since both alleles are nonsense mutations that would truncate the SUP-17 protein soon after the predicted signal sequence.

We examined the phenotype of *sup-17(n1319)*, a probable molecular null allele, to see if they displayed defects in the AC/VU decision. All hermaphrodites (25/25) had a single AC, as had been previously described for *sup-17(n1258ts)* by Tax et al. (1997). The fact that completely eliminating *sup-17* activity does not affect the AC/VU decision may be due to redundant activity provided by another gene(s) (see Discussion). Nevertheless, we were able to exploit the genetic interactions between *sup-17* and mutations in *lin-12* to explore issues pertaining to *sup-17* function.

Reducing *sup-17* activity does not suppress the constitutive activity of *lin-12(intra)*

We examined the effect of reducing *sup-17* activity on the activity of the intracellular domain of LIN-12. Expression of *lin-12(intra)* causes phenotypes associated with LIN-12 activation (Struhl et al., 1993). The phenotype is similar to that caused by *lin-12(d)* mutations, which are missense mutations in the extracellular domain of LIN-12 that cause apparent ligand-independent activation (Greenwald and Seydoux, 1990). However, we found that a mutation in *sup-17* does not suppress *arIs12[lin-12(intra)]*, in contrast to the robust suppression of *lin-12(n137)*, a *lin-12(d)* allele that displays greater constitutive activity than *arIs12* (Table 1). Thus, the presence of the extracellular domain of LIN-12 appears to be necessary for *sup-17* to facilitate *lin-12* signalling.

Cell autonomy of *sup-17* function

To examine the cell autonomy of *sup-17* suppression of *lin-12(d)* mutations, we performed laser ablation experiments to remove the signalling cells during the AC/VU decision and VPC specification. This experiment enabled isolated cells of different genotypes to be compared with respect to their intrinsic level of constitutive *lin-12* activity. If *sup-17* acts within the same cell to facilitate *lin-12* activity, then we would expect the cell-intrinsic level of constitutive *lin-12* activity to be lower in a *sup-17(–)* than in a *sup-17(+)* background. Conversely, if *sup-17* acts nonautonomously, the cell-intrinsic level of *lin-12* activity would be the same in a *sup-17(–)* as in a *sup-17(+)* background.

If all gonadal cells except for Z1.ppp or Z4.aaa are ablated in wild-type hermaphrodites, the isolated cell becomes an AC (Kimble, 1981), presumably because LIN-12 is not activated. However, if all gonadal cells except for Z1.ppp or Z4.aaa are ablated in *lin-12(n302)* hermaphrodites, the isolated cell becomes a VU (Greenwald and Seydoux, 1990), suggesting that the level of constitutive *lin-12* activity in this mutant is relatively high. We ablated all gonadal cells except for Z1.ppp in *sup-17(n1258); lin-12(n302)* hermaphrodites, and found that the isolated Z1.ppp became an AC (Table 2). In Table 2, we also show additional data for experiments in which we ablated Z4, the precursor to Z4.aaa, and found that Z1.ppp becomes an AC in *sup-17(n1258); lin-12(n302)* hermaphrodites, while it becomes a VU in *lin-12(n302)* hermaphrodites (Table 2). These ablation experiments suggest that *sup-17* can facilitate *lin-12* signalling cell autonomously for the AC/VU decision. Although these experiments do not address the possibility of an additional nonautonomous role for *sup-17* in the signalling cell, the extent of suppression of *lin-12(n302)* can be completely accounted for by the *sup-17* cell-autonomous function.

We performed similar ablation experiments to test if *sup-17* mutations reduce *lin-12(d)* activity autonomously in the VPCs (Table 2). If a single VPC is isolated by ablating all other VPCs in wild type, it usually adopts the 1° fate (if near the AC) or the 3° fate (if it is far from the AC or the AC is absent) (Sulston and White, 1980; Sternberg and Horvitz, 1986); it generally does not adopt the 2° fate because LIN-12 has not been activated by lateral signalling. However, if all VPCs but P4.p are ablated with a laser microbeam in *lin-12(n137)* hermaphrodites, P4.p adopts a vulval (2°) fate, indicating that the level of constitutive *lin-12* activity in this mutant is high (Table 2). When we ablated all VPCs but P4.p in *sup-17(n1258); lin-*

Table 1. Genetic interactions between *sup-17* and *lin-12*

Reducing *sup-17* activity does not suppress the constitutive activity of *lin-12(intra)*

Genotype	#hermaphrodites with ectopic pseudovulvae/total (%)
arIs12[lin-12(intra)]	67/70 (96%)
arIs12[lin-12(intra)] sup-17	98/104 (95%)
lin-12(n137)	>200 (100%)
sup-17; lin-12(n137)	9/70 (13%)

***sup-17* reduces *lin-12* activity in the absence of a *lin-12(d)* mutation**

Genotype	#hermaphrodites with 2 AC/total
lin-12(ar170)	9/40 (23%)
sup-17; lin-12(ar170)	22/25 (88%)

The *sup-17* allele used was *sup-17(n1258ts)*. Hermaphrodites were grown at 15° C in (A) and at 20° C in (B).

A more extensive study of genetic interactions between *sup-17* and *lin-12(d)* mutations is described in Tax et al. (1997).

Table 2. Cell autonomy of *sup-17* function

AC/VU decision*

Genotype	Cell(s) remaining after ablation	0 AC	1 AC
lin-12(n302)	Z1 descendants	11	0
sup-17; lin-12(n302)	Z1 descendants	1	15
lin-12(n302)	Z1.ppp	4†	0†
sup-17; lin-12(n302)	Z1.ppp	0	3

VPC specification‡

Genotype	VPC remaining after ablation	Vulval fate	Non-vulval fate
lin-12(n137)	P4.p	16	0
sup-17; lin-12(n137)	P4.p	7‡	8‡

The *sup-17* allele used was *sup-17(n1258ts).* Hermaphrodites were grown and operated at 20°C, then shifted to 25°C.

*All unoperated *lin-12(n302)* hermaphrodites have the 0 AC defect (Greenwald et al., 1983). We examined 20 unoperated *sup-17; lin-12(n302)* hermaphrodites, and found that all contained a single AC.

†Data of Greenwald and Seydoux (1990). Note that a typographical error in Table 1 of that paper lists *n302* as '*n349*'.

‡All hermaphrodites included in this table lacked an AC. In unoperated *sup-17; lin-12(n137)* hermaphrodites, P4.p always adopts the non-vulval fate. However, P5.p or P6.p may adopt a vulval fate; when all VPCs but P4.p are ablated, P4.p shifts its position, and hence it may behave like P5.p or P6.p in some cases. The critical observation here is that an isolated P4.p sometimes adopts a non-vulval fate in *sup-17; lin-12(n137)* hermaphrodites but not in *lin-12(n137)* hermaphrodites.

12(n137) hermaphrodites that lacked an AC, we found that the isolated P4.p in some cases adopted the nonvulval (3°) fate (Table 2). These results suggest that *sup-17* acts to facilitate *lin-12*-mediated signalling cell autonomously during VPC specification.

sup-17 reduces *lin-12* activity in the absence of a *lin-12(d)* mutation

To investigate whether *sup-17* mutations require that LIN-12 contain a *lin-12(d)* mutation to affect *lin-12* activity, we examined the phenotype of *sup-17(n1258); lin-12(ar170)* hermaphrodites. *lin-12(ar170)* is a hypomorphic allele of *lin-12* that was generated in a *lin-12(+)* background (Hubbard et al., 1996, 1997). A mutation in *sup-17* enhances the 2 AC defect of *lin-12(ar170)* (Table 1). This result indicates that loss of *sup-17* activity can lower the activity of *lin-12* in the absence of a *lin-12(d)* mutation.

DISCUSSION

Genetic analysis has established that *sup-17* facilitates *lin-12* signalling (Ferguson and Horvitz, 1985; Tax et al., 1997; this work). Here, we have shown that *sup-17* encodes an ADAM protein that is very similar to *Drosophila* KUZBANIAN (KUZ), which functions in *Drosophila* and vertebrate neurogenesis (Rooke et al., 1996; Pan and Rubin, 1997) (Fig. 4). Furthermore, we provide genetic data showing that *sup-17* acts cell autonomously and does not act downstream of *lin-12*. We discuss our findings and the related *Drosophila* data, and consider how SUP-17/KUZ facilitates LIN-12/NOTCH signalling.

ADAM proteases

The ADAM family includes certain snake venom proteins, proteins that have been implicated in cell fusion events during fertilization and myogenesis, and proteins involved in matrix disruption during metastasis (reviewed in Wolfsberg et al., 1995; Wolfsberg and White, 1996). Recently, ADAM proteins have been implicated in the processing of a peptide growth factor from a precursor protein (Lunn et al., 1997). The ADAM family is distinguished by an ordered set of domains (reviewed in Wolfsberg and White, 1996). In the extracellular region there is a prodomain, a Zn^{2+}-activated metalloprotease-like domain, a distintegrin-like domain and a cysteine-rich domain; after a transmembrane domain, there is a cytoplasmic tail. The prodomain is thought to regulate the binding of Zn^{2+} to the metalloprotease domain, and the disintegrin domain is thought to mediate interactions with the extracellular matrix or cell surface receptors. The cysteine-rich domain of some ADAM proteins contains a hydrophobic region similar to viral fusion proteins, suggesting that this region may mediate membrane fusion (Huovila et al., 1996). The large number and widespread distribution of ADAM proteins suggest that they may play numerous different roles during development (e.g. Wolfsberg et al., 1995; Alfandari et al., 1997). The predicted topology of the ADAM proteins suggests that their substrates are extracytosolic or extracellular proteins.

Wolfsberg et al. (1995) and Wolfsberg and White (1996) have used strict phylogeny criteria to group the ADAMs into cross-species orthologs. ADAM10, SUP-17 and KUZ have a metalloprotease domain that is highly conserved among themselves, but is not well conserved with other ADAMs, suggesting that they form a subfamily. Since bovine ADAM10 has been shown biochemically to cleave myelin basic protein (Howard and Glynn, 1995) and proTNFα (Lunn et al., 1997), it is likely that ADAM10 subfamily members share the enzyme activity to cleave membrane-anchored proteins.

SUP-17 is the second ADAM protein described from *C. elegans*. The first, ADM-1, was identified by a search of the genome project database for *C. elegans* ADAM proteins (Podbilewicz, 1996). The mRNA and protein expression patterns suggest that *adm-1* may be involved in cell fusion events during hypodermal development and fertilization (Podbilewicz, 1996). More recent database searches have revealed the existence of several additional ADAM proteins in *C. elegans* with the consensus protease active site; of these *C. elegans* ADAM proteins, the one that is most closely related to SUP-17 is similar to the TNFα converting enzyme (Moss et al., 1997). Perhaps functional redundancy with one or more of these ADAM proteins explains why *sup-17* null mutants do not display a profound *lin-12(0)* phenotype (Tax et al., 1997 and C.W., unpublished observations).

Functional relationship between SUP-17/KUZ and LIN-12/NOTCH

Much of our work on *sup-17* and *lin-12* has focused on their roles in the AC/VU decision and VPC specification. The AC/VU decision is a simple case of lateral specification involving two cells, Z1.ppp and Z4.aaa. As described more extensively in the Introduction, Z1.ppp and Z4.aaa interact with each other, so that a small stochastic difference in *lin-12* activity becomes amplified by a feedback mechanism that influences receptor (*lin-12*) and ligand (*lag-2*) transcription

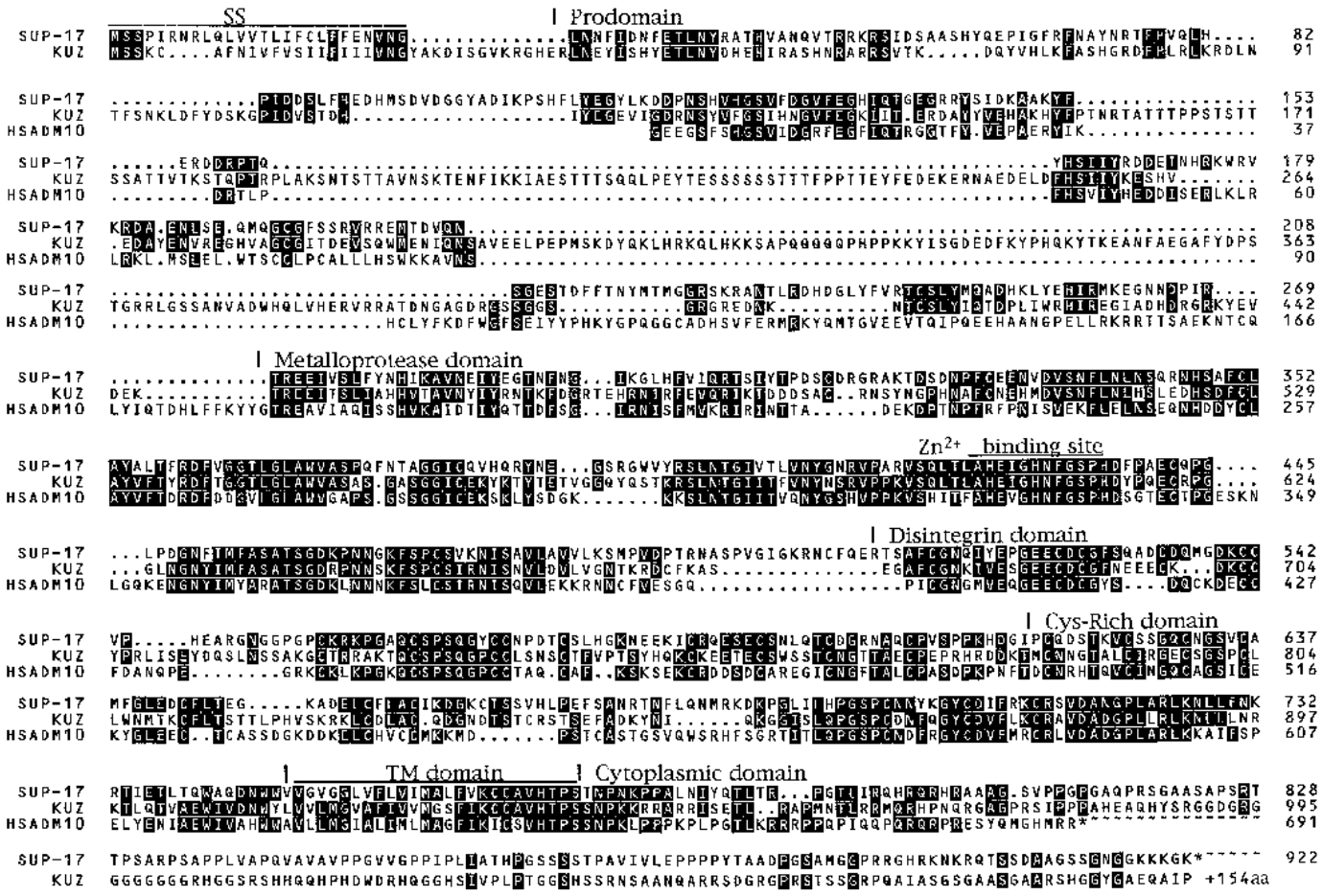

Fig. 4. SUP-17 aligned with *Drosophila* KUZ and human ADAM10. Residues that are identical in at least two of the three proteins are shown in reverse contrast letters. The *Drosophila* KUZ sequence is from Rooke et al. (1996) and the HSADM10 sequence is from GenBank (Accession number Z48579). The sequence amino-terminal to the prodomain of ADAM10 is not present in the database. A BLAST search using SUP-17 as the query sequence identified many members of the ADAM family. The most similar ones are the *Drosophila* and mouse KUZ proteins, and the bovine and human ADAM10 proteins (the bovine ADAM10 is also known as bovine metalloprotease, BMP). The next most similar group of proteins include TACE (TNFα convertase) and another *C. elegans* ADAM protein (GenBank accession number U70844). Numerous other ADAM family members were also identified. We note that the *C. elegans* expressed sequence tags identified as an ADAM10 ortholog by Wolfsberg et al. (1996) correspond to *sup-17* cDNAs. SS, signal sequence; TM, transmembrane.

(Seydoux and Greenwald, 1989; Wilkinson et al., 1994). Similarly, neighboring VPCs interact with each other, so that a lateral signal from one VPC causes its neighbor to adopt the 2° fate (see Sternberg, 1988; Greenwald, 1997).

Constitutive or elevated *lin-12* activity causes both Z1.ppp and Z4.aaa to become VUs, and all VPCs to adopt the 2° fate. One class of mutations causing constitutive or elevated activity include *lin-12(d)* mutations, an allelic series of hypermorphs that result from point mutations in the extracellular domain (Greenwald et al., 1983; Greenwald and Seydoux, 1990). Reduction or elimination of *sup-17* activity suppresses the effects of *lin-12(d)* mutations (Ferguson and Horvitz, 1985; Tax et al., 1997). The interaction between *sup-17* and *lin-12* is sensitive to both *sup-17* and *lin-12* dosage (Tax et al., 1997). *sup-17* is haploinsufficient: a deficiency of the locus is a semidominant suppressor. Furthermore, the efficacy of *sup-17* suppression depends on the level of *lin-12* activity: *sup-17* is a better suppressor of *lin-12(d)* alleles that modestly increase *lin-12* activity than of *lin-12(d)* alleles that greatly increase *lin-12* activity, although some degree of suppression has been observed for even the strongest *lin-12(d)* alleles (Tax et al., 1997).

In contrast, reducing *sup-17* activity cannot suppress mutant phenotypes associated with *lin-12(intra)*. The *lin-12(intra)* transgene expresses the intact intracellular domain under the control of *lin-12* regulatory sequences and behaves like a constitutively active receptor (Struhl et al., 1993). It is incompletely penetrant for its effects on the AC/VU decision and VPC specification, and hence does not elevate *lin-12* activity as much as the stronger *lin-12(d)* alleles. Furthermore, we have found that *sup-17* can enhance the AC/VU decision defect of a partial loss-of-function *lin-12* allele, implying that it does not require a *lin-12(d)* lesion to influence *lin-12* activity. Since *sup-17* can suppress *lin-12(d)* mutations but cannot suppress *lin-12(intra)*, we can infer that *sup-17* does not act downstream of the activated receptor.

We have found that the interaction between *sup-17* and *lin-12* occurs within the same cell. By using a laser microbeam to

isolate Z1.ppp or a vulval precursor cell from its normal source of signal, we showed that *sup-17* mutations reduce the cell-intrinsic level of constitutive *lin-12* activity in *lin-12(d)* mutants. This result suggests that *sup-17* acts cell autonomously and that its action is not dependent on ligand binding.

The genetic analysis of *sup-17* in *C. elegans* complements and extends the results from genetic studies of the *Drosophila kuz* gene. In *Drosophila*, NOTCH-mediated lateral signalling specifies many neural/epidermal cell fate decisions. For example, in the notum, the absence of *Notch* activity leads to neural hypertrophy at the expense of epidermis (Heitzler and Simpson, 1991); expression of the intracellular domain of NOTCH, here referred to as NOTCH(intra), causes the absence of sensory bristles (Lieber et al., 1993; Struhl et al., 1993).

Rooke et al. (1996) identified the *Drosophila kuzbanian* (*kuz*) gene in a screen for essential genes involved in lateral specification in the developing nervous system, and described the phenotype of viable *kuz* mosaics. Clones lacking *kuz* activity display some of the characteristic defects of a failure of lateral specification; however, differences in the phenotypes of *kuz* clones as compared to that of *Notch* clones led Rooke et al. (1996) to propose that *kuz* might be involved in two opposing processes, an autonomous role in the reception of the lateral signal that specifies epidermis and a different, non-autonomous role in promoting the neural fate. Recently, Pan and Rubin (1997) examined the effect of removing *kuz* activity on the phenotype caused by NOTCH(intra). They report that the phenotype of *kuz* mutant clones expressing NOTCH(intra) is not appreciably different from the phenotype caused by expression of NOTCH(intra) in a kuz^+ background. Their observation is comparable to our observation that expression of LIN-12(intra) causes a similar phenotype in either a *sup-17* mutant or *sup-17(+)* background.

Pan and Rubin (1997) have noted that the observation that *Notch(intra)* is epistatic to *kuz* does not in principle rule out the possibility that KUZ and NOTCH act in parallel pathways. That is, in principle it is possible that activating the signal-transducing activity of NOTCH bypasses the need for a parallel pathway that normally involves *kuz*. However, the interactions between *sup-17* and *lin-12* argue against this bypass model, since mutations in *sup-17* are efficient suppressors of *lin-12(d)* mutations (Tax et al., 1997; also see Results), which encode constitutively active receptors (Greenwald and Seydoux, 1990). Thus, the fact that mutations in *sup-17* do not suppress *lin-12(intra)* can be unambiguously interpreted as indicating that *sup-17/kuz* does not act downstream of *lin-12/Notch*.

SUP-17/KUZ and LIN-12/NOTCH processing

There is genetic evidence in both *C. elegans* and *Drosophila* that *kuz/sup-17* influences *lin-12/Notch* activity and facilitates signalling only if the extracellular domain of LIN-12/NOTCH proteins is present (Rooke et al., 1996; Pan and Rubin, 1997; Tax et al., 1997; this work). There is evidence for a cleavage event in the extracellular domain of *C. elegans*, *Drosophila* and human NOTCH proteins, and for the physical association of the N- and C-terminal cleavage products (Crittenden et al., 1994; Blaumueller et al., 1997; Pan and Rubin, 1997). Furthermore, Pan and Rubin (1997) have shown that KUZ is required in vivo for the proteolytic processing of NOTCH into two fragments, consistent with a cleavage event in the extracellular domain. Together, the data are consistent with the proposal that the LIN-12/NOTCH extracellular domain is a substrate of the KUZ/SUP-17 protease. An alternative possibility is that K0Z/SUP-17 is part of a protease cascade that processes the LIN-12/NOTCH extracellular domain.

There are many unanswered questions about how proteolysis influences LIN-12/Notch activity. Blaumueller et al. (1997) have proposed that proteolysis is essential for trafficking to the cell surface based on the observation that the majority of the human NOTCH2 protein is cleaved in the *trans*-Golgi network, so that most of the NOTCH2 protein present on the surface of cells is the cleaved form. At this time, however, there is no direct evidence that proteolysis is obligatory for NOTCH trafficking, since it is not known whether NOTCH is retained within the cell when the cleavage site is mutated or in *kuz* mutants. Furthermore, while it is known that the cleaved form can bind ligand (Blaumueller et al., 1997), it is not known whether the cleaved form, the uncleaved form, or both, can activate signal transduction. It is possible that the cleaved and uncleaved forms display different affinities for ligand, or have qualitatively or quantitatively different outputs in signal transduction.

If SUP-17 cleaves the extracellular domain of LIN-12, then reducing *sup-17* activity would reduce the level of receptor at the cell surface (if the principal role of cleavage is to facilitate trafficking to the surface) or the level of receptor able to be activated (if the cleaved form is more responsive to ligand or more active in signal transduction). In this context, it is interesting to note that the effect of *sup-17* on *lin-12(d)* mutations is to restore ligand sensitivity as well as to reduce the intrinsic level of activity. LIN-12(d) proteins behave as if they are ligand-insensitive constitutively active receptors (Greenwald and Seydoux, 1990). Yet in *sup-17; lin-12(d)* double mutants, ligand sensitivity appears to be restored: *sup-17(n1258); lin-12(n302)* hermaphrodites always have a single AC, as in wild type. We have previously provided genetic evidence that *lin-12(d)* mutations promote self-association (for example, dimerization) of LIN-12, and proposed that ligand binding activates signalling by promoting self-association (Greenwald and Seydoux, 1990). If *sup-17*-mediated cleavage is important for LIN-12 trafficking, then in *sup-17* mutants, the reduced amount of LIN-12(d) protein on the cell surface may reduce the probability of self-association in the absence of ligand. Alternatively, if *sup-17*-mediated cleavage is necessary for maximal signalling, then in *sup-17* mutants, ligand-independent signalling might be sufficiently reduced so that the effects of ligand become significant. An important challenge for the future will be to elucidate the function of the processing of to LIN-12/NOTCH extracellular domain with respect to ligand-dependent activation and signal transduction.

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REFERENCES

Alfandari, D., Wolfsberg, T. G., White, J. M. and DeSimone, D. W. (1997). ADAM13: a novel ADAM expressed in somitic mesoderm and neural crest cells during *Xenopus laevis* development. *Dev. Biol.* **182**, 314-330.

Allard, M. W., Ellsworth, D. L. and Honeycutt, R. L. (1991). The production of single-stranded DNA suitable for sequencing using the polymerase chain reaction. *BioTechniques* **10**, 23-26.

Altschul, S. F., Gish, W., Miller, W., Meyers, E. W. and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* **215**, 403-410.

Artavanis-Tsakonas, S., Matsuno, K. and Fortini, M. (1995). Notch Signaling. *Science* **268**, 225-268.

Blaumueller, C. M., Qui, H., Zagouras, P. and Artavanis-Tsakonas, S. (1997). Intracellular cleavage of Notch leads to a heterodimeric receptor on the plasma membrane. *Cell* **90**, 281-291.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71-94.

Christensen, S., Kodoyianni, V., Bosenberg, M., Friedman, L. and Kimble, J. (1996). *lag-1*, a gene required for *lin-12* and *glp-1* signaling in *C. elegans*, is homologous to human CBF1 and *Drosophila* Su(H). *Development* **122**, 1373-1383.

Crittenden, S. L., Troemel, E. R., Evans, T. C. and Kimble, J. (1994). GLP-1 is localized to the mitotic region of the *C. elegans* germ line. *Development* **120,** 2901-2911.

Devereux, J., Haeberli, P. and Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucl. Acids Res.* **12**, 387-395.

Edgley, M. L., Turner, C. A. and Riddle, D. L. (1997). On-line *C. elegans* resources. In *C. elegans II* (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess), pp. 1059-1062. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Ferguson, E. L. and Horvitz, H. R. (1985). Identification and characterization of 22 genes that affect the vulval cell lineages of the nematode *Caenorhabditis elegans*. *Genetics* **110**, 17-72.

Greenwald, I., Sternberg, P. and Horvitz, H. R. (1983). The *lin-12* locus specifies cell fates in *Caenorhabditis elegans*. *Cell* **34**, 435-444.

Greenwald, I. and Seydoux, G. (1990). Analysis of gain-of-function mutations of the *lin-12* gene of *Caenorhabditis elegans*. *Nature* **346**, 197-199.

Greenwald, I. (1997). Development of the Vulva. In *C. elegans II* (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess), pp. 519-542. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Heitzler, P. and Simpson, P. (1991). The choice of cell fate in the epidermis of *Drosophila*. *Cell* **64**, 1083-1092.

Howard, L. and Glynn, P. (1995). Membrane-associated metalloproteinase recognized by characteristic cleavage of myelin basic protein: assay and isolation. *Meth. Enzym.* **248**, 388-395.

Huang, X.-Y. and Hirsh, D. (1989). A second trans-spliced RNA leader in the nematode *Caenorhabditis elegans*. *Proc. Nat. Acad. Sci. USA* **86**, 8640-8644.

Hubbard, E. J. A., Dong, Q. and Greenwald, I. (1996). Evidence for physical and function association between EMB-5 and LIN-12 in *Caenorhabditis elegans*. *Science* **273**, 112-115.

Hubbard, E. J. A., Wu, G., Kitajewski, J. and Greenwald, I. (1997). sel-10, a negative regulator of lin-12 activity in C. elegans, encodes a member of the CDC4 family of proteins. *Genes Dev.* (in press).

Huovila, A.-P. J., Almeida, E. A. C. and White, J. M. (1996). ADAMs and cell fusion. *Curr. Opin. Cell Biol.* **8**, 692-699.

Kaltenboeck, B., Spatafora, J. W., Zhang, X., Kousoulas, K. G., Blackwell, M. and Storz, J. (1992). Efficient production of single-stranded DNA as long as 2 kb for sequencing of PCR-amplified DNA. *BioTechniques* **12**, 164-171.

Kimble, J. and Hirsh, D. (1979). The post-embryonic cell lineages of the hermaphrodites and male gonads in *Caenorhabditis elegans*. *Dev. Biol.* **87**, 396-417.

Kimble, J. (1981). Alterations in cell lineage following laser ablation of cells in the somatic gonad of *Caenorhabditis elegans*. *Dev. Biol.* **87**, 286-300.

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

Levitan, D. and Greenwald, I. (1995). Facilitation of *lin-12*-mediated signalling by *sel-12*, a *Caenorhabditis elegans S182* Alzheimer's disease gene. *Nature* **377**, 351-354.

Lewis, B. C., Shah, N. P., Braun, B. S. and Denny, C. T. (1992). Creation of a yeast artificial chromosome fragmentation vector based on lysine-2. *GATA* **9**, 86-90.

Lieber, T., Kidd, S., Alcamo, E., Corbin, V. and Young, M. W. (1993). Antineurogenic phenotypes induced by truncated *Notch* proteins indicate a role in signal transduction and may point to a novel function for *Notch* in nuclei. *Genes Dev.* **7**, 1949-1965.

Lunn, C. A., Fan, X., Dalie, B., Miller, K., Zavodny, P. J., Narula, S. K. and Lundell, D. (1997). Purification of ADAM10 from bovine spleen as a TNFa convertase. *FEBS Lett.* **400**, 333-335.

Mello, C. C., Kramer, J. M., Stinchcomb, D. T. and Ambros, V. A. (1991). Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. *EMBO J.* **10**, 3959-3970.

Moss, M. L. et al. (1997). Cloning of a disintegrin metalloproteinase that processes precursor tumour-necrosis factor-a. *Nature* **385**, 733-736.

Pan, D. and Rubin, G. M. (1997). Kuzbanian controls proteolytic processing of Notch and mediates lateral inhibition during Drosophila and vertebrate neurogenesis. *Cell* **90**, 271-280.

Podbilewicz, B. (1996). ADM-1, a protein with metalloprotease- and disintegrin-like domains, is expressed in syncytial organs, sperm, and sheath cells of senosry organs in *Caenorhabditis elegans*. *Mol. Biol. Cell* **7**, 1877-1893.

Rooke, J., Pan, D., Xu, T. and Rubin, G. M. (1996). KUZ, a conserved metalloprotease-disintegrin protein with two roles in *Drosophila* neurogenesis. *Science* **273**, 1227-1231.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Seydoux, G. and Greenwald, I. (1989). Cell autonomy of *lin-12* function in a cell fate decision in *C. elegans*. *Cell* **57**, 1237-1245.

Shen, J., Bronson, R. T., Chen, D. F., Xia, W., Selkoe, D. J. and Tonegawa, S. (1997). Skeletal and CNS defects in *Presenilin-1* deficient mice. *Cell* **89**, 629-639.

Sternberg, P. W. and Horvitz, H. R. (1986). Pattern formation during vulval development in *C. elegans*. *Cell* **44**, 761-772.

Sternberg, P. W. (1988). Control of cell fates within equivalence groups in *C. elegans*. *Trends Neurosci*. **11**, 259-64.

Struhl, G., Fitzgerald, K. and Greenwald, I. (1993). Intrinsic activity of the Lin-12 and Notch intracellular domains in vivo. *Cell* **74**, 331-45.

Sulston, J. and Horvitz, H. R. (1977). Postembryonic 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.

Sundaram, M. and Greenwald, I. (1993). Suppressors of a *lin-12* hypomorph define genes that interact with both *lin-12* and *glp-1* in *Caenorhabditis elegans*. *Genetics* **135**, 765-783.

Tax, F. E., Thomas, J. H., Ferguson, E. L. and Horvitz, H. R. (1997). Identification and characterization of genes that interact with *lin-12* in *Caenorhabditis elegans*. *Genetics*, in press.

Waterston, R. H., Sulston, J. E. and Coulson, A. R. (1997). The genome. In *C. elegans II* (ed. D. L. Riddle, T. Blumenthal, B. J. Meyer and J. R. Priess), pp. 23-46. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Wilkinson, H., Fitzgerald, K. and Greenwald, I. (1994). Reciprocal changes in expression of the receptor *lin-12* and its ligand *lag-2* prior to committment in a *C. elegans* cell fate decision. *Cell* **79**, 1187-1198.

Wolfsberg, T., Straight, P. D., Gerena, R. L., Huovila, A.-P. J., Primakoff, P., Myles, D. G. and White, J. M. (1995). ADAM, a widely distributed and developmentally regulated gene family encoding membrane proteins with a disintegrin and metalloprotease domain. *Dev. Biol.* **169**, 378-383.

Wolfsberg, T. G. and White, J. M. (1996). ADAMs in fertilization and development. *Devel. Biol.* **180**, 389-401.

Wong, P. C., Zheng, H., Chen, H., Becher, M. W., Sirinathsinghji, D. J. S., Trumbauer, M. W., Chen, H. Y., Price, D. L., Van der Ploeg, L. H. T. and Sisodia, S. S. (1997). Presenilin 1 is required for *Notch1* and *Dll1* expression in the paraxial mesoderm. *Nature* **387**, 288-292.

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