TGF-β signaling[*]

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Abstract

TGF-β superfamily ligands play fundamental roles in the development and physiology of diverse animal species. Genetic and genomic analyses in the model organism *Caenorhabditis elegans* have contributed to the understanding of TGF-β-related signal transduction mechanisms. In this chapter, I describe the currently characterized TGF-β-related signals and signal transduction cassettes in *C. elegans*. Homology searches of the genome identify five TGF-β-related genes, for which functions have been identified for three. Two of the TGF-β-related genes, *daf-7* and *dbl-1*, function through conventional signaling pathways. These signaling pathways are comprised of ser/thr kinase receptors, Smads, and transcription co-factors. A third TGF-β-related gene, *unc-129*, functions in axonal guidance using novel signaling mechanisms. Thus, TGF-β-related signaling in *C. elegans* proceeds via both conserved and novel paradigms that can inform studies in other animal systems.

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1. Overview

TGF-β is the prototype of a large family of secreted peptide growth factors in metazoans that includes activin and the Bone Morphogenetic Proteins (BMPs) in vertebrates. The canonical TGF-β signal transduction pathway (ten Dijke and Hill, 2004) is comprised of two transmembrane ser/thr kinase receptors (type I and type II) and two or three intracellular Smad signal transducers. Upon ligand binding, the type I and type II receptors are recruited into a heteromeric complex and the type II receptor phosphorylates the type I receptor on the conserved GS domain. The type I receptor kinase is thereby activated, and phosphorylates Smads of the R-Smad (receptor-regulated) subfamily. R-Smads form heteromeric complexes with Co-Smads and accumulate in the nucleus to regulate gene transcription. A third group of Smads, I-Smads, act as inhibitors of pathway signaling by interfering with productive molecular interactions. Smads contain two regions of homology, the N-terminal MH1 domain that binds DNA and the C-terminal MH2 domain that participates in intermolecular interactions and activates transcription.

In *C. elegans*, five TGF-β-related genes can be identified by sequence homology: *daf-7*, *dbl-1*, *unc-129*, *tig-2*, and Y46E12BL.1 (Table 1). No biological roles have yet been described for *tig-2* or Y46E12BL.1. The *unc-129* signaling pathway is still being elucidated, but it does not seem to depend on known TGF-β-related receptors or Smads. Conventional signaling pathways have been identified that act downstream of *daf-7* and *dbl-1*, and are referred to as the Dauer pathway (Figure 1) and the Sma/Mab pathway (Figure 2), respectively. Most of the components of these two pathways were identified by forward genetic approaches, and often the signal transduction components were identified before the relevant ligands. More recent work using reverse genetics, yeast two-hybrid, and DNA microarray analysis continues to identify components, modifiers, and targets of these pathways and to uncover new biological roles for existing pathways and components. A summary of the TGF-β signaling components in *C. elegans* and their likely *Drosophila* and mammalian orthologs is given in Table 1. In addition to the genes described below, there is one potential I-Smad gene in the genome, *tag-68* (Newfeld et al., 1999), whose function has not yet been determined.

Table 1. TGF-β signaling components in *C. elegans*

Component	Gene name	*Drosophila* ortholog	Mammalian ortholog
Ligand	*daf-7*	*Alp23B*	GDF11
	dbl-1	*dpp*	BMP5
	unc-129	–	–
	tig-2	*gbb*	BMP8
	Y46E12BL.1	–	–
Type I Receptor	*daf-1*	*babo*	TGF-βRI
	sma-6	*tkv*	BMPRIB
Type II Receptor	*daf-4*	*put*	ACTRIIB
R-Smad	*daf-8*	*Smox*	–
	daf-14	Similar to *Smox*	Smad2
	sma-2	*Mad*	Smad1
	sma-3	Similar to *Mad*	Smad5
Co-Smad	*daf-3*	–	–
	sma-4	*Medea*	Smad4
I-Smad	*tag-68*	Similar to *Dad*	Similar to Smad6
Transcription factors	*daf-5*	–	Similar to Sno/Ski
	daf-12	Hr96 (nuclear hormone receptor)	–
	sma-9	*shn* (Zn finger protein)	Similar to HIVEP1
	lin-31	*fd95Cb*	FKH-5

2. Dauer pathway

The dauer larva is an alternative third larval stage specialized for survival under harsh environmental conditions (high population density, low food availability, high temperature; Cassada and Russell, 1975; Golden and Riddle, 1984a; Golden and Riddle, 1984b). The decision to enter the dauer stage is made in the L1 and L2 stages. When dauer larvae encounter more favorable environments, they exit dauer and resume development, entering the L4 stage. Large-scale genetic screens were conducted for mutations that affect the decision to enter dauer (Riddle et al., 1981; Golden and Riddle 1984a; Malone and Thomas, 1994). The mutants isolated are either dauer-constitutive (Daf-c: entering dauer inappropriately under favorable environmental conditions) or dauer-defective (Daf-d: failing to enter dauer under adverse conditions). Genetic epistasis and detailed phenotypic analysis allowed these genes to be grouped into three classes (Thomas et al., 1993). Subsequent molecular analysis established that the genes in each class encode components of three different signaling pathways: a TGF-β-related pathway, an insulin-related pathway, and a cyclic nucleotide pathway. In this section, I will refer to the TGF-β-related pathway that functions in dauer development as the Dauer TGF-β pathway. See appropriate chapters for more information on dauer development and the insulin and cyclic nucleotide signaling pathways.

2.1. Identification of Dauer TGF-β pathway components

DAF-7 is the TGF-β-related ligand for the Dauer pathway (Figure 1; Ren et al., 1996). *daf-7* mutants are Daf-c, indicating that the gene has a role in promoting continuous, non-Dauer development. DAF-7 is expressed in the pair of ASI sensory neurons under favorable environmental conditions (Ren et al., 1996; Schackwitz et al., 1996). Its expression is repressed under dauer-inducing conditions, leading to a loss of Dauer pathway signaling and entry into the dauer stage. Four other Daf-c genes are characterized by alleles that confer phenotypes, and have genetic interactions similar to those of mutations in *daf-7*, suggesting that they function with *daf-7*. These are *daf-1*, *daf-4*, *daf-8*, and *daf-14*. Genetic epistasis analysis has predicted that three Daf-d genes act downstream of this pathway: *daf-3*, *daf-5*, and *daf-12* (Thomas et al., 1993). Mutations in any of these three Daf-d genes suppress the effects of mutations in *daf-7*, *daf-1*, *daf-4*, *daf-8* or *daf-14*. *daf-3* and *daf-5* seem to function specifically in this pathway since they do not strongly suppress mutations in other Daf-c genes. In contrast, *daf-12* can suppress mutations both in *daf-7* class genes and in other Daf-c genes, suggesting that it integrates signals from multiple pathways. In addition to the Daf-c phenotype, DAF-7 pathway genes also have a less well-characterized role in egg laying. The egg-laying defect is suppressed by mutations in *daf-3* and *daf-5* (Trent et al., 1983). Mutations in two other genes required for egg laying, *egl-4* and *egl-32*, are also suppressed by *daf-3* and *daf-5* mutations, indicating a possible interaction with this pathway. *egl-4* has additional phenotypes, suggesting roles in body size regulation (see below) and chemosensation, and encodes multiple cGMP-dependent protein kinase isoforms (Fujiwara et al., 2002; L′Etoile et al., 2002; Hirose et al., 2003).

2.2. Receptors

Molecular cloning of the *daf-7* class genes has revealed that they comprise a TGF-β signaling pathway. *daf-1* and *daf-4* encode type I and type II TGF-β receptors, respectively (Georgi et al., 1990; Estevez et al., 1993). The molecular cloning of *daf-4* was pivotal in the recognition of this pathway as a TGF-β signaling pathway, since DAF-4 showed homology to the newly identified TGF-β type II receptors. (The cloning of *daf-1* preceded this time, so its homology was not recognized until later). Importantly, DAF-4 was shown to bind human BMP ligands in cell culture, illustrating the conservation of these pathway components from nematodes to humans (Estevez et al., 1993). *daf-4* is the sole type II receptor gene in *C. elegans*; it functions both in the Dauer pathway and in the Sma/Mab pathway by interacting with different pathway-specific components. *daf-4* is expressed widely during all stages of *C. elegans* development (Patterson et al., 1997; Gunther et al., 2000). Alternative polyadenylation of the *daf-4* locus produces a truncated protein containing only the extracellular domain that acts as a negative regulator of DAF-7 function (Gunther and Riddle, 2004). *daf-1*, the type I receptor, is also widely expressed, primarily in the nervous system (Gunther et al., 2000). Genetic evidence suggests that DAF-1 is capable of signaling, albeit weakly, in the absence of phosphorylation by DAF-4. Thus, in a *daf-4* mutant background, depletion of *daf-1* causes a more severe mutant phenotype and overexpression of *daf-1* partially rescues the mutant phenotype. The ability of DAF-1 to be constitutively active is supported by the phylogeny of TGF-β receptors that indicates that DAF-1 is somewhat related to both type I and type II receptors (Newfeld et al., 1999). BRA-1 is a DAF-1 binding protein identified by homology to human BMP receptor-associated molecule BRAM1, which negatively regulates DAF-7 signaling (Morita et al., 2001).
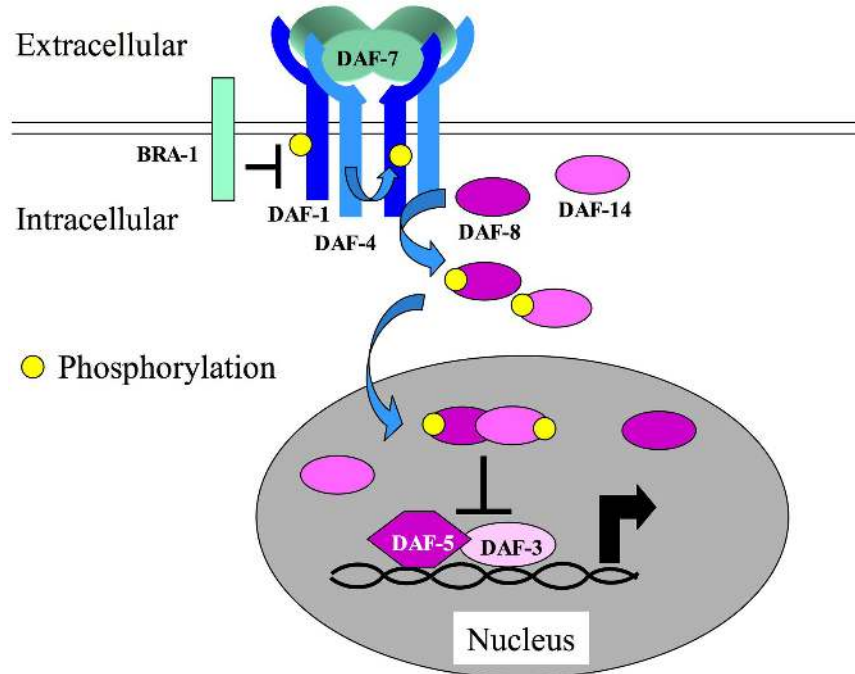
Figure 1. The Dauer TGF-β-related pathway . DAF-7 promotes continuous, nonDauer development. The DAF-7 signal is transduced by DAF-1 type I receptor, DAF-4 type II receptor, and DAF-8 and DAF-14 Smads. These components, when activated, inhibit the functions of DAF-3 Smad and DAF-5 Sno/Ski, which promote Dauer development. BRA-1 is a negative regulator of DAF-1.

2.3. Transcription factors

The Smads participating in the Dauer pathway are encoded by *daf-8*, *daf-14*, and *daf-3*. DAF-8 and DAF-14 are related to R-Smads, except that DAF-14 lacks strong homology to the MH1 domain (Estevez 1997; Inoue and Thomas, 2000). These R-Smads have been shown to have partially redundant functions (Inoue and Thomas, 2000). *daf-3* encodes a Smad distantly related to Co-Smads (Patterson et al., 1997). All of these Smads are expressed broadly. While DAF-8 and DAF-14 function to transduce the *daf-7* signal, DAF-3 activity is negatively regulated by the pathway. In the absence of DAF-3, such as in a *daf-3* mutant, animals enter the non-Dauer L3 stage regardless of the presence or absence of DAF-7 signaling (Patterson et al.1997). Thus, the output of Dauer TGF-β pathway signaling culminates in the inhibition of *daf-3* activity and is dispensable in the absence of *daf-3*. This type of antagonistic interaction differs from the mechanism of inhibition by I-Smads in other systems. *daf-5* has the same type of genetic interactions with the Dauer TGFβ pathway as *daf-3*. Cloning of *daf-5* revealed homology to the Sno/Ski family of oncogenes that negatively regulate TGF-β signaling in vertebrates (da Graca et al., 2004; Tewari et al., 2004). DAF-5 physically interacts with DAF-3 to promote dauer development. Finally, like *daf-3* and *daf-5* mutations, *daf-12* mutations suppress the Daf-c phenotype of Dauer pathway mutants. Unlike *daf-3* and *daf-5*, however, *daf-12* also shows strong suppression of mutations in the insulin-related and cyclic nucleotide pathways. *daf-12* encodes a nuclear hormone receptor that is required for regulating multiple stage-specific aspects of nematode development (Antebi et al., 2000; Snow and Larsen, 2000). The *daf-12* locus is complex, encoding multiple alternatively spliced isoforms, and is capable of mutating to Daf-d, Daf-c, and heterochronic phenotypes independently.

2.4. Protein interactions in the Dauer TGF-β pathway

In large-scale interactome mapping analysis of this pathway using the yeast two-hybrid system, physical interactions among 59 proteins, including DAF-1 type I receptor, DAF-4 type II receptor, DAF-3 Smad, DAF-5 Sno/Ski, and several novel interactors were identified (Tewari et al., 2004). In these screens, a physical interaction between DAF-3 and DAF-5 was shown. Among the novel interactors was DAF-21/HSP90, which was previously shown to function in the cyclic nucleotide pathway and now found to interact physically with DAF-1 and DAF-4. TTX-1, a homeodomain protein required for thermosensation, is linked with both DAF-3 Smad and DAF-4 type II receptor indirectly via secondary interactions. DAF-3 Smad also shows direct or indirect associations with SWI3 and SNF5 homologs (Y11G7B.23 and R07E5.3, respectively).

3. Sma/Mab pathway

The Sma/Mab pathway regulates body size and the development of male-specific sensory rays and copulatory spicules (Savage et al., 1996). Thus, mutants of the pathway are small in size (Small) and mutant males have patterning defects in the male tail (Male abnormal). The normal male tail contains nine bilaterally symmetrical pairs of sensory organs, known as rays, embedded in a cuticular fan (Sulston et al., 1980). In Sma/Mab pathway mutants, defects in the specification of ray identities lead to the fusion of pairs of rays (4-5, 6-7, and 8-9) with varying penetrance. The defects in ray identity specification are accompanied by changes in neurotransmitter expression by ray neurons (Lints and Emmons, 1999). Furthermore, in mutant males, the copulatory spicules are crumpled. The Mab defects prevent homozygous mutant males from mating successfully. For more information on male tail development and function, see chapters on Male development and Male mating behavior.

3.1. DBL-1: the Sma/Mab pathway ligand

DBL-1 is the TGF-β-related ligand for the Sma/Mab pathway (Figure 2; Morita et al., 1999; Suzuki et al., 1999). *dbl-1* mutations were generated using two strategies: (1) reverse genetics, by deletion of *dbl-1* (DPP/BMP-like) sequences (Morita et al., 1999; Suzuki et al., 1999); and (2) forward genetics, by isolation of Sma mutants and subsequent mapping and complementation tests (Suzuki et al., 1999); Savage-Dunn et al., 2003). DBL-1 acts as a dose-dependent regulator of body size (Morita et al., 1999; Suzuki et al., 1999). Loss of *dbl-1* activity results in smaller animals, while *dbl-1* overexpression results in longer animals. In *dbl-1* mutant males, the spicules are crumpled and there are defects in the specification of rays 5, 7, and 9. Overexpression of *dbl-1* in males leads to fusions of rays 3 and 4 (Suzuki et al., 1999). *dbl-1* is expressed primarily in neurons in both hermaphrodites and males (Morita et al., 1999; Suzuki et al., 1999). Although overexpression of *dbl-1* leads to gain-of-function phenotypes, overexpression of the downstream signaling components does not, suggesting that the concentration of ligand is limiting.
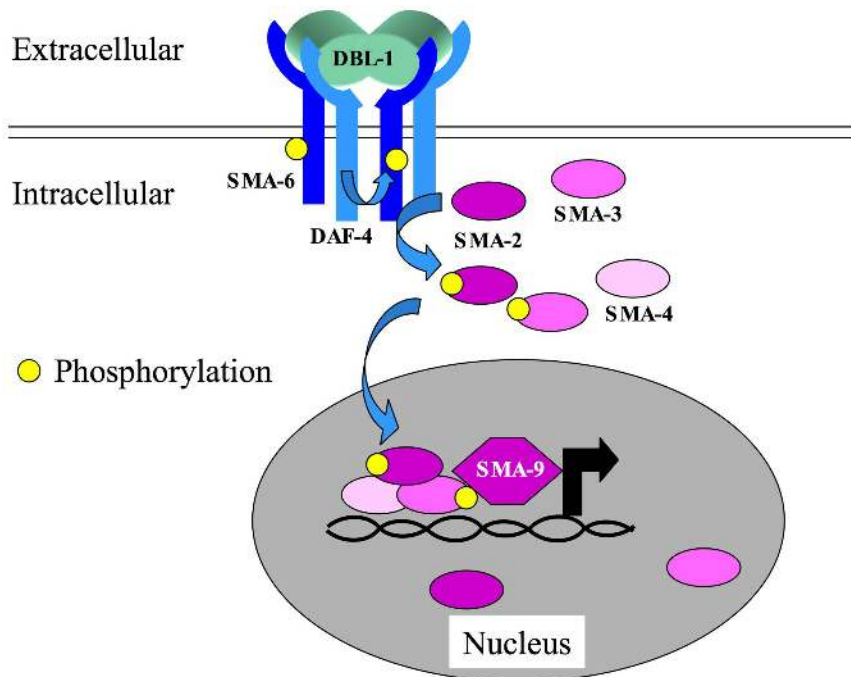

Figure 2. The Sma/Mab TGFβ-related pathway . DBL-1 regulates body size and male tail morphogenesis. The DBL-1 signal is transduced by SMA-6 type I receptor, DAF-4 type II receptor, SMA-2, SMA-3, and SMA-4 Smads, and SMA-9 Schnurri.

3.2. Sma/Mab pathway signal transducers

Candidate signaling components of the Sma/Mab pathway were identified by the study of mutants with similar Sma and Mab phenotypes. Some of these mutants had existed for many years in strain collections (*sma-2*, *sma-3*, *sma-4*, *sma-6*), while others were identified by reverse genetics and additional genetic screens (as with *dbl-1* itself and *sma-9*). The receptors for DBL-1 are encoded by *sma-6* (type I), which is pathway-specific (Krishna et al., 1999), and *daf-4* (type II), which also functions in the Dauer pathway (Estevez et al., 1993). The signal transducers

for this pathway are SMA-2, SMA-3, and SMA-4 (Savage et al., 1996), which, together with *Drosophila Mad*, were the founding members of the Smad family. SMA-2 and SMA-3 are R-Smad homologs, and SMA-4 is a Co-Smad homolog. Unlike the partial redundancy between DAF-8 and DAF-14, R-Smads, no function of either SMA-2 or SMA-3 is detectable in the absence of the other partner, or of SMA-4 Co-Smad (Savage-Dunn et al., 2000). This observation has led to the suggestion that the functional Smad complex for this pathway is a heterotrimer (SMA-2/SMA-3/SMA-4). While early models of TGF-β signaling suggested that R-Smad phosphorylation and heteromeric complex formation were necessary for nuclear translocation, it has more recently become evident that Smads engage in dynamic nucleocytoplasmic shuttling (Xiao et al., 2001; Inman et al., 2002; Xu et al., 2002; Nicolas et al., 2004). Similarly, a rescuing SMA-3::GFP fusion protein is found in both the cytoplasm and the nucleus (Wang et al., 2002). In wild-type animals, the protein accumulates to high levels in the nucleus, while in *sma-6* type I receptor mutants, the protein is more evenly partitioned. Nuclear accumulation is not influenced by R-Smad (*sma-2*) or Co-Smad (*sma-4*) depletion.

Expression patterns reported for the receptors and Smads are similar and include expression in the pharynx, intestine, and hypodermis. Genetic mosaic analyses and directed expression studies of *daf-4*, *sma-6*, and *sma-3* have indicated that the expression of signaling components in the hypodermis is necessary and sufficient for body size regulation (Inoue and Thomas, 2000; Yoshida et al., 2001; Wang et al., 2002). The expression in the pharynx and intestine may reflect a more recently uncovered role for the Sma/Mab pathway in innate immunity (Mallo et al., 2002; see Signaling in the immune response). Some of the transcriptional target genes of this pathway were identified by differential hybridization of a cDNA array (Mochii et al., 1999). One of these target genes is *sma-6*, indicating the existence of a positive feedback loop. Some of the other target genes were expressed in the intestine and had functional domains, such as C-type lectin domains, suggestive of a role in immune recognition of pathogens. In addition, the Hox gene *ceh-13*, a *labial* homolog, has been identified as a direct or indirect target of Sma/Mab signaling in the male tail (Stoyanov et al., 2003). *ceh-13* expression in the B cell lineage and in rays 5, 7, and 9 is dependent on the function of the Sma/Mab pathway.

3.3. Transcription co-factors

SMA-9 is a transcriptional co-factor for the Sma/Mab pathway (Liang et al., 2003). Identified in a genetic screen for small mutants (Savage-Dunn et al., 2003), *sma-9* encodes a large zinc finger transcription factor related to *Drosophila* Schnurri (Liang et al., 2003). Phenotypic analysis of *sma-9* indicates that it functions in a subset of Sma/Mab signaling outcomes: early but not late larval growth; ray 9 but not ray 5 and 7 morphogenesis; regulation of neurotransmitter expression in rays 5, 7, and 9; and spicule morphogenesis with reduced penetrance. This observation suggests that SMA-9 may be required for transcriptional regulation of a subset of Sma/Mab pathway target genes. SMA-9 is nuclear localized and detected in most, if not all, cells by immunocytochemistry. The *sma-9* locus shows extensive alternative splicing that could give rise to isoforms with different numbers of zinc finger motifs and *trans* splicing that could produce shorter N-terminal and C-terminal isoforms. Another transcriptional co-factor that may be involved in a tissue-specific output of this pathway is the forkhead transcription factor LIN-31. *lin-31* was originally identified by its role in vulval development (Miller et al., 1993), but *lin-31* mutant males also have a crumpled spicule defect like that of Sma/Mab pathway mutants (Baird and Ellazar, 1999). Since forkhead transcription factors in other systems function as Smad co-factors (Chen et al., 1996), LIN-31 may similarly interact with SMA-2/SMA-3/SMA-4 specifically to regulate spicule morphogenesis.

rnt-1 encodes a runt domain transcription factor that may function in parallel with the Sma/Mab pathway in regulation of body size and male tail morphogenesis (Ji et al., 2004). *rnt-1* was identified by its homology to mammalian RUNX transcription factors (Bae and Lee, 2000), which interact with Smads (Hanai et al., 1999; Zhang et al., 2000; Alliston et al., 2001). A *rnt-1* deletion mutant is small and double mutants with *rnt-1* and *sma-2*, *sma-3*, *sma-4*, or *sma-6* are smaller than any of the single mutants (Ji et al., 2004). *rnt-1* is expressed in the hypodermal seam cells and the intestine (Nam et al., 2002). *rnt-1* mutants also have defects in the male tail, but these are more severe and less specific than the Sma/Mab pathway defects, indicating that *rnt-1* has additional functions outside of this pathway (Ji et al., 2004). RNT-1 physically interacts with SMA-4 Smad, providing a possible mechanism for the synergistic interactions suggested by the mutant phenotypes.

3.4. Body size regulation

Body size and morphology are regulated by several different mechanisms in *C. elegans*. Dumpy (Dpy) mutants are often defective in cuticle collagen genes (Johnstone, 2000) or dosage compensation genes (Plenefisch et al., 1989). A smaller number of genes can mutate to result in long (Lon) animals. Sma mutants were originally identified by Sydney Brenner by their relatively proportional reduction in size (Brenner, 1974). Most of these Sma

mutants are defective in components of the TGF-β Sma/Mab pathway, indicating that this pathway is a major regulator of body size in *C. elegans*. In other animals, components of insulin signaling pathways and TOR kinase signaling regulate cell size (Schmelzle and Hall, 2000; Oldham and Hafen, 2003). In *C. elegans*, however, mutations in these components have not been shown to affect body size but rather play a role in dauer development (Kimura et al., 1997; Jia et al., 2004).

Mutants in the Sma/Mab pathway hatch as L1 larvae indistinguishable in size from wild type (Suzuki et al., 1999; Savage-Dunn et al., 2000). Postembryonic growth is then reduced, resulting in a reduction in both body length and volume (Savage-Dunn et al., 2000; Hirose et al., 2003). Sma/Mab mutant adults contain the same number of nuclei as wild-type adults, indicating that cell size must be smaller (Nagamatsu and Ohshima, 2004). Despite the relatively proportional appearance of Sma/Mab mutants, organ size measurements have shown that different organs are reduced in size to different degrees. The degree of reduction in the size of hypodermal seam cells and hyp7 is most proportional to the degree of reduction in body size (Wang et al., 2002; Nagamatsu and Ohshima, 2004), consistent with the defined focus of action of Sma/Mab signaling components in the hypodermis. In contrast to these results, *sma-1* βH-spectrin mutants have a defect in embryonic elongation, but not postembryonic growth, and organ size measurements reveal a more severe reduction in pharynx length than in seam cell length (Wang et al., 2002). The molecular mechanisms of cell size control by these genes remain elusive. A correlation between body size and hypodermal ploidy has been described for some mutants (Flemming et al., 2000; Morita et al., 2002; Nystrom et al., 2002), but may not be sufficient to account for all of the differences in size seen in mutant animals (Nagamatsu and Ohshima, 2004; Nystrom et al., 2002).

Several genes can mutate to a Lon phenotype similar to *dbl-1* overexpression, resulting in an increase in body length but not in volume. *lon-1* is a putative target of the Sma/Mab pathway and encodes a member of the CRISP (cysteine-rich secretory protein) family (*Mad*uzia et al., 2002; Morita et al., 2002). Mutations in *lon-1* suppress the small body size phenotype of Sma/Mab pathway mutants, suggesting that *lon-1* acts downstream. *lon-3* is a cuticle collagen gene for which loss-of-function mutants are long but overexpressing animals are small (Nystrom et al., 2002; Suzuki et al., 2002). The Lon phenotype of *lon-3*, together with the Dpy phenotypes of other cuticle collagen genes, indicates a role for cuticle collagens in constraining the body size and shape of *C. elegans*. In addition to these long mutants, large body size mutants with increased body volume were sought in a genetic screen (Hirose et al., 2003). In this screen, multiple alleles of the gene *egl-4* were isolated. *egl-4*, which encodes cGMP-dependent protein kinases, has additional phenotypes indicating roles in egg laying (see above) and sensory processing (Daniels et al., 2000; Fujiwara et al., 2002; L′Etoile et al., 2002). In *egl-4* mutants, most of the major organs except the gonad are increased in volume without an increase in the number of nuclei (Hirose et al., 2003) and there is evidence of an increase in fluid retention (Nagamatsu and Ohshima, 2004). The gene is expressed in neurons, hypodermis, intestine, and body wall muscles (Fujiwara et al., 2002; L′Etoile et al., 2002; Hirose et al., 2003). Expression in neurons or in hypodermis can rescue the organ size defects nonautonomously (Nakano et al., 2004). Mutations in Sma/Mab pathway components can completely suppress the large body size phenotype of *egl-4*, suggesting that *egl-4* acts upstream of the TGF-β pathway (Hirose et al., 2003).

4. UNC-129

unc-129, a TGF-β-related gene (Colavita et al., 1998), was isolated in a genetic screen (Colavita and Culotti, 1998) designed to identify genes that interact with *unc-5*, which encodes a transmembrane receptor in the UNC-6/netrin pathway necessary for axon guidance along the dorsoventral axis. *unc-129* mutants, like *unc-5*, *unc-6*, and *unc-40* mutants, have defects in the trajectories of some dorsally migrating motoraxons (Colavita and Culotti, 1998). In addition, both *unc-129* and *dbl-1* mutations can enhance a defect in the migration of the gonadal distal tip cells in a weak *unc-5* mutant background (Merz et al., 2003). UNC-129 is expressed in several tissues including dorsal body wall muscle, in which its expression is sufficient to provide normal axon guidance cues (Colavita et al., 1998). UNC-129 is unlikely to interact with the identified TGF-β superfamily receptors DAF-4, DAF-1, or SMA-6, since mutations in the receptor genes do not cause defects in axon guidance. In addition to its axon guidance role, a redundant role for *unc-129* in male tail sensory ray development has been identified (Ikegami et al., 2004). In this pathway, *unc-129* interacts with the semaphorin gene, *mab-20* and the plexin gene, *plx-2*, and acts in parallel with semaphorin/*mab-20* and ephrin/*efn-4*, to prevent sensory ray fusions between adjacent rays. Interestingly, *unc-129* is expressed in alternating rays (1, 3, 5, and 7), possibly to keep their identities distinct from those of their neighbors.

5. Acknowledgements

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