
TGF- β signaling in *C. elegans*^{*}

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Abstract

Transforming Growth Factor- β (TGF- β) superfamily ligands regulate many aspects of cell identity, function, and survival in multicellular animals. Genes encoding five TGF- β family members are present in the genome of *C. elegans*. Two of the ligands, **DBL-1 and **DAF-7**, signal through a canonical receptor-Smad signaling pathway; while a third ligand, **UNC-129**, interacts with a noncanonical signaling pathway. No function has yet been associated with the remaining two ligands. Here we summarize these signaling pathways and their biological functions.**

1. Overview

The Transforming Growth Factor- β (TGF- β) superfamily of intercellular signaling molecules constitutes a significant, ancestral means of cell-to-cell communication in eukaryotic animals (De Robertis, 2008). TGF- β signaling is required during development and homeostasis for several vital processes, including patterning the embryonic body plan and later specifying and maintaining cell identities (Wu and Hill, 2009). Alteration of TGF- β signaling causes birth defects, disorders, and diseases in humans (Padua and Massagué, 2009; Wu and Hill, 2009).

Seminal contributions based on research in the *C. elegans* system have proven instrumental in understanding the TGF- β signaling pathway, its transcriptional targets, and its regulators, as we discuss here. In comparison to the 30+ human TGF- β members, *C. elegans* has only five TGF- β ligands with non-redundant, non-lethal functions (Patterson and Padgett, 2000). TGF- β signaling pathways are highly conserved at the molecular and functional level, as TGF- β pathway components and regulators identified in *C. elegans* have been shown to be used by higher organisms in a related fashion (Table 1).

Table 1. TGF- β superfamily signaling is conserved in eukaryotes.

Component	<i>C. elegans</i> gene name	<i>Drosophila</i> gene name	Human gene name	Molecule or family
Ligand	<i>dbl-1</i>	<i>dpp</i>	BMP5	Transforming Growth Factor- β
	<i>daf-7</i>	<i>dawdle</i>	GDF11	
	<i>unc-129</i>	-	-	
	<i>tig-2</i>	<i>gbb</i>	BMP8	
	<i>tig-3</i>	dActivin	BMP2	
Type I receptor	<i>sma-6</i>	<i>tkv</i>	BMPRII	ser/thr kinase receptor
	<i>daf-1</i>	<i>babo</i>	TGF- β RI	
Type II receptor	<i>daf-4</i>	<i>put</i>	ACTRII	
R-Smad	<i>sma-2</i>	<i>Mad</i>	Smad1	Smad
	<i>sma-3</i>	<i>Mad</i>	Smad5	
	<i>daf-8</i>	<i>Smox</i>	Smad8	
	<i>daf-14</i>	<i>Smox</i>	Smad2	
Co-Smad	<i>sma-4</i>	<i>Medea</i>	Smad4	
	<i>daf-3</i>	<i>Medea</i>	Smad4	
I-Smad	<i>tag-68</i>	<i>Dad</i>	Smad6	

Transcription Factors	<i>sma-9</i>	<i>schnurri</i>	Shn1/Shn2/Shn3	Zn finger protein
	<i>lin-31</i>	<i>fd96C</i>	FoxB1	HNF3/forkhead family
	<i>mab-31</i>	-	-	
	<i>daf-5</i>	-	Sno/Ski	Proline-rich protein
	<i>daf-12</i>	Hr96	Vitamin D receptor	nuclear hormone receptor
Extracellular Regulators	<i>lon-1</i>	-	-	CRISP PR-protein
	<i>lon-2</i>	Dally, Dally-like	GPC1-GPC6	glypican
	<i>sma-10</i>	kekkon	LRIG	leucine rich and immunoglobulin-like domains protein
	<i>crm-1</i>	CRIM	CRIM/CHRD/chordin	cysteine-rich
	<i>drag-1</i>	-	RGMA, RGMB	repulsive guidance molecule (RGM)
	<i>adt-2</i>	-	-	ADAMTS

This large family of secreted peptide growth factors in metazoans comprises a number of subfamilies, including TGF- β (the founding member of the family), activin, Nodal, Growth and Differentiation Factors (GDFs), and the Bone Morphogenetic Proteins (BMPs) (Herpin et al., 2004). The canonical TGF- β signal transduction pathway is composed of two pairs of transmembrane serine/threonine kinase receptors (type I and type II) and two or three intracellular Smad signal transducers (ten Dijke and Hill, 2004). Upon TGF- β ligand binding, the type I and type II receptors are recruited into a heterotetrameric complex and the type II receptors phosphorylate the type I receptors' conserved GS (glycine-serine) 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 (common mediator Smads) and accumulate in the nucleus to regulate gene transcription (Massagué et al., 2005). A third group of Smads, I-Smads (inhibitory Smads), usually inhibits pathway signaling by interfering with productive molecular interactions of the other Smads (Itoh et al., 2000). Smads contain two regions of homology, the N-terminal MAD homology 1 (MH1) domain that binds DNA and the C-terminal MH2 domain that participates in intermolecular interactions and regulates transcription (Wrana and Attisano, 1996).

In *C. elegans*, five TGF- β -related genes have been identified by sequence homology: *dbl-1* (p.k.a. *cet-1*), *daf-7*, *unc-129*, *tig-2*, and *tig-3* (Table 1). No biological roles have yet been described for *tig-2* or *tig-3* (see previous version of this chapter, TGF- β signaling; Suzuki et al., 1999). The *unc-129* signaling pathway acts through a netrin receptor, UNC-5, rather than known TGF- β -related receptors or Smads (MacNeil et al., 2009). Conventional signaling pathways have been identified that act downstream of *dbl-1* (Figures 1A-C, 2) and *daf-7* (Figures 1D, 3). Many of the components of these two pathways were initially identified in forward genetic screens. Subsequent work using RNA interference (RNAi), reverse genetics, yeast two-hybrid assays, and DNA microarray analyses have continued to identify components, modifiers, and targets of these pathways and to uncover new biological roles for existing pathways and components. In addition to the genes described below, there is one potential I-Smad gene in the genome, *tag-68*, whose function has not yet been determined (Newfeld et al., 1999).

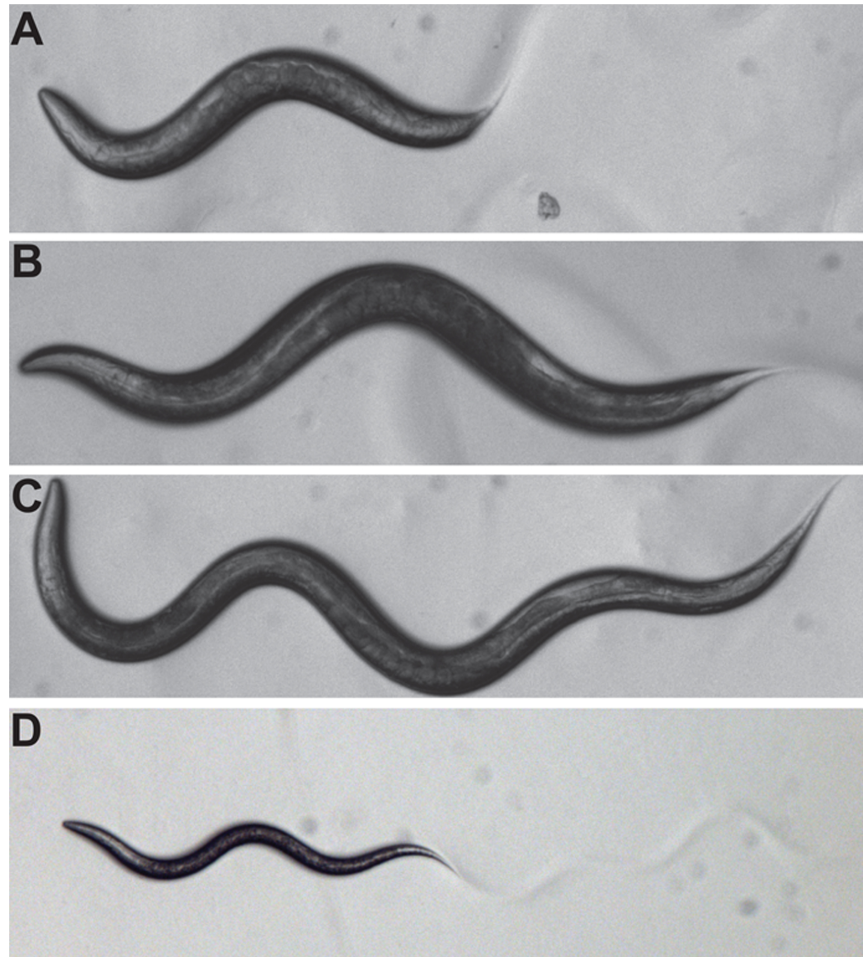


Figure 1. Phenotypes of *DBL-1* and *DAF-7* TGF- β -related pathways. A) An animal lacking functional *DBL-1* is smaller than wild type. B) Wild-type animal. C) An animal overexpressing functional *DBL-1* is longer than wild type. D) Animals lacking functional *DAF-7* enter dauer at 25 °C. All animals were staged and imaged at a similar age at 25 °C (when animals in A-C were 24-hour adults). The anterior is left; dorsal is up. Representative individuals are shown. All images were taken at the same magnification and treated identically.

2. *DBL-1* pathway

The *DBL-1* pathway was first associated with two mutant phenotypes, small body size (Sma) and male tail abnormal morphology (Mab), and is also known as the Sma/Mab pathway (Padgett et al., 1998; Savage et al., 1996). Subsequent studies have shown that the *DBL-1* pathway also regulates innate immunity, reproductive aging, mesodermal patterning, and chemosensation (Almedom et al., 2009; Foehr et al., 2006; Luo et al., 2009; Mallo et al., 2002; Vashlishan et al., 2008). In spite of these numerous functions, *DBL-1* pathway activity is not essential for viability, a feature that has greatly facilitated genetic manipulations (Morita et al., 1999; Suzuki et al., 1999).

The core components of the pathway are the ligand *DBL-1* (Dpp and BMP-like-1) (Morita et al., 1999; Suzuki et al., 1999), type II receptor *DAF-4* (Estevez et al., 1993), type I receptor *SMA-6* (Krishna et al., 1999), R-Smads *SMA-2* and *SMA-3*, and Co-Smad *SMA-4* (Figure 2) (Savage et al., 1996). Overall, these components show the greatest degree of homology to BMP subfamily ligands and signal transducers (Table 1) (Patterson and Padgett, 2000). In addition to the core components, modulators of the pathway regulate signal strength, some of them in a tissue-specific manner. Extracellular or transmembrane regulators include *LON-1* (Maduzia et al., 2002; Morita et al., 2002), *LON-2* (Gumienny et al., 2007), *SMA-10* (Gumienny et al., 2010), *CRM-1* (Fung et al., 2007), *DRAG-1* (Tian et al., 2010), and *ADT-2* (see section 2.7) (Fernando et al., 2011). Transcription factors that regulate signaling downstream of the receptors include *SMA-9* (Liang et al., 2003), *LIN-31*/forkhead (Baird and Ellazar, 1999), and *MAB-31* (see section 2.8) (Wong et al., 2010). *dbl-1* expression is itself regulated in motor neurons by transcription factor *UNC-3* (Kratsios et al., 2011).

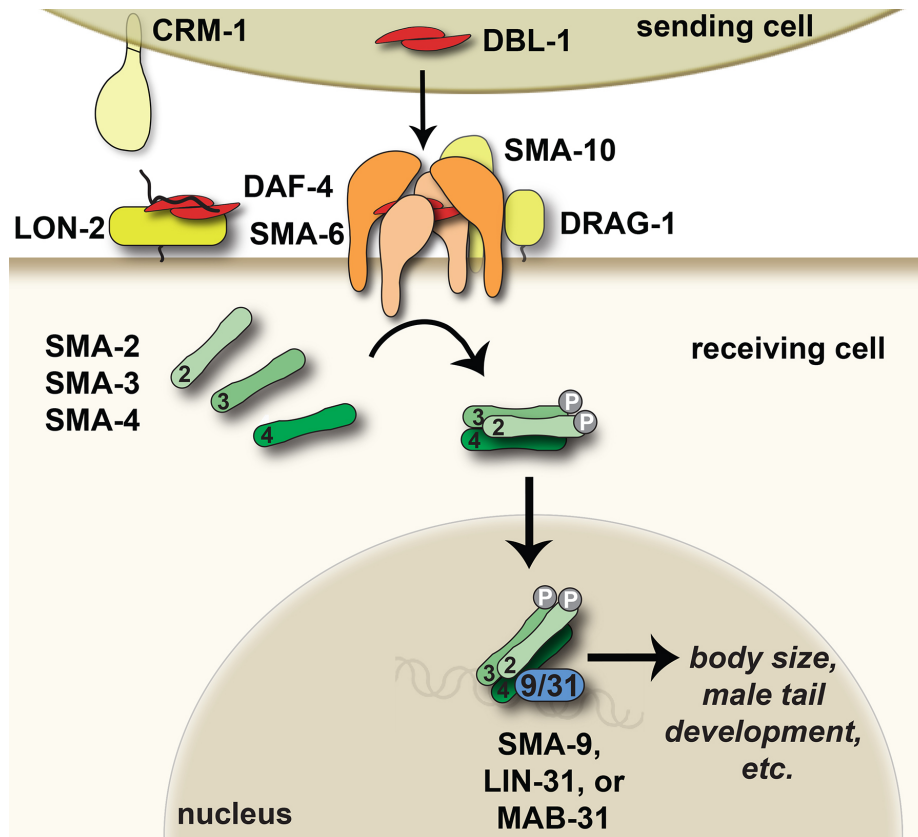


Figure 2. The Sma/Mab TGF- β -related pathway. DBL-1 regulates body size, male tail morphogenesis, cell lineage decisions, and innate immune responses. The DBL-1 signal (red) is received by a heterotetrameric receptor composed of two SMA-6 type I receptor and two DAF-4 type II receptor subunits (light and dark orange, respectively). Extracellular regulators (yellows) of DBL-1 signaling include CRM-1, which acts at the sending cell membrane or in the extracellular space to promote DBL-1 signaling, SMA-10 and DRAG-1, which promote DBL-1 signaling at the membrane of the receiving cell, and LON-2/glypican, which inhibits DBL-1 signaling at the receiving cell. The DBL-1 signal is transduced by SMA-2, SMA-3, and SMA-4 Smads (greens). Transcription factors (blue) that act with Smads to carry out DBL-1-mediated responses include SMA-9/Schnurri, LIN-31/forkhead, and MAB-31. Cartoons are based on *C. elegans* or other published homologous protein structures and functions. See text for more details.

2.1. Body size regulation

Sma mutant alleles were originally identified in Sydney Brenner's classic screen by their relatively proportional reduction in size (Brenner, 1974). Later, a directed genetic screen for mutant animals with small body size expanded the collection of Sma alleles (Savage-Dunn et al., 2003). Both of these screens identified mainly components of the DBL-1 pathway, establishing this pathway as the predominant regulator of body size in *C. elegans*. On the other hand, conserved signaling pathways that affect body or organ size in other organisms, including insulin signaling, TOR kinase, and Hippo-Warts pathways, appear to play a more minor role in size regulation in *C. elegans* and act in parallel with the DBL-1 pathway (Cai et al., 2009; Halder and Johnson, 2010; Jones et al., 2009; Oldham et al., 2000; Schmelzle and Hall, 2000). MAPK signaling (through SMA-5/MAPK) also affects body size in *C. elegans* in a manner independent from DBL-1 signaling (Watanabe et al., 2007; Watanabe et al., 2005). These studies suggest that DBL-1 acts synergistically with, instead of through, these other signaling pathways to regulate body size.

DBL-1 is a dose-dependent regulator of growth, since its functional loss (by mutation or RNAi) causes small body size, its overexpression results in long body size, and *dbl-1*(+/-) heterozygotes have an intermediate phenotype (Figure 1A-C) (Morita et al., 1999; Suzuki et al., 1999). Sma/Mab pathway mutant animals hatch as first larval stage (L1) larvae indistinguishable in size from the wild type (Savage-Dunn et al., 2000; Suzuki et al., 1999). Postembryonic growth is reduced in Sma/Mab mutant animals, resulting in relatively smaller body lengths and volumes (Hirose et al., 2003; Savage-Dunn et al., 2000). 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 mutant animals, organ size measurements have shown that different organs are reduced in size to different degrees. The degree of reduction in the size of hypodermal (epidermal) seam cells and

the large hypodermal syncytium hyp7 is most proportional to the degree of reduction in body size (Nagamatsu and Ohshima, 2004; Nyström et al., 2002; Wang et al., 2002). In contrast, neuronal soma size is not modulated by the DBL-1 pathway (Goldsmith et al., 2010). Studies directing expression of the receptors and Smads to particular tissues have demonstrated that the hypodermis, but not the intestine, plays a major role in regulation of body size (Inoue and Thomas, 2000a; Schulenburg et al., 2004; Wang et al., 2002; Yoshida et al., 2001). The gonad regulates body size, too, but through a mechanism independent of DBL-1 signaling (Patel et al., 2002).

What are the molecular mechanisms of cell size control targeted by DBL-1 pathway Smads? A correlation between body size and hypodermal ploidy has been described for some mutant strains (Flemming et al., 2000; Lozano et al., 2006; Morita et al., 2002; Nyström et al., 2002), but is not sufficient to account for all of the differences in size seen in mutant animals (Nagamatsu and Ohshima, 2004; Nyström et al., 2002). Expression profile analyses suggest that the DBL-1 pathway regulates body size in a complex way, in part through regulation of cuticle collagen and metabolism genes, as well as through signaling molecules such as insulin-like ligands and Hedgehog-related warthog ligands (see section 6.2) (Liang et al., 2007; Roberts et al., 2010). A cuticle collagen gene, *lon-3*, and an extracellular matrix protease gene, *adt-2*, may act downstream of or in parallel with the DBL-1 pathway to regulate body size (Suzuki et al., 2002).

2.2. Male tail development

The normal adult male tail ends in a mating structure containing nine bilaterally symmetrical pairs of sensory rays embedded in a cuticular fan (Sulston et al., 1980). In Sma/Mab pathway mutant males, defects in the specification of ray identities lead to the fusion of rays (4-5, 6-7, and 8-9) with varying penetrance (Baird and Ellazar, 1999; Krishna et al., 1999; Savage et al., 1996; Suzuki et al., 1999). Ray fusion in *sma-6(wk7)* males is preceded by ectopic formation of adherens junctions between cells of different rays (Hahn and Emmons, 2003). Ray identity specification is also abnormal, with the neurons of rays 5, 7, and 9 losing their dopaminergic fate (Lints and Emmons, 1999). Conversely, ubiquitous expression of *dbl-1* driven by a heat shock promoter can induce ectopic expression of dopaminergic fate in neurons of rays 3, 4, 6, and 8 (Lints et al., 2004). To specify ray identities, DBL-1 pathway signaling regulates the expression of two transcription factors. DBL-1 pathway activity establishes dopaminergic fates in the A neurons of rays 5, 7, and 9 by blocking the activity of DMD-3/ DM (Doublesex/MAB-3) domain transcription factor (Siehr et al., 2011). DBL-1 pathway signaling is required for the expression of *ceh-13*/ labial Hox family transcription factor in the B γ rectal blast cell lineage and ray complexes 5, 7, and 9, though males lacking *ceh-13* still have functional copulatory structures (Stoyanov et al., 2003). This positive regulation of a labial homolog by a TGF- β superfamily member is conserved in *Drosophila* (Immergluck et al., 1990).

In addition to the sensory ray defects, the copulatory spicules are crumpled in mutant adult males (Baird and Ellazar, 1999; Krishna et al., 1999; Suzuki et al., 1999). These Mab defects prevent homozygous mutant males from mating successfully. A weak mutation in *sma-6* or *sma-4* that affects body size does not result in male ray fusion or spicule crumpling, indicating that body size is more sensitive to DBL-1 dosage than the Mab phenotypes (Krishna et al., 1999).

In a screen for factors involved in the morphogenesis of the male tail tip, an additional role for Sma/Mab components was uncovered (Nelson et al., 2011). RNAi knockdown of *daf-4*, *sma-2* or *sma-3* resulted in a pointed (leptoderan) rather than a rounded tail tip. Male tail morphogenesis was associated with nuclear localization of tagged SMA-3, where SMA-3 could direct target gene activity, but the hermaphrodite tail at the same developmental stage maintained tagged SMA-3 in the cytoplasm, consistent with the model that active DBL-1 pathway signaling is required to remodel the male tail. Whether this role also depends on apical junction remodeling, as the male tail ray studies suggest, remains to be discovered. For more information on male tail development and function, refer to [Male development](#).

2.3. Innate immunity

The DBL-1 pathway was implicated in innate immunity when it was shown that some genes regulated by DBL-1 signaling were also induced upon *S. marcescens* infection (Mallo et al., 2002; Mochii et al., 1999). DBL-1 highly regulates the expression of genes associated with mounting an effective innate immune response, including lectins, digestive enzymes like lysozymes and lipases, the PGP (P-glycoprotein) subclass of ATP-binding cassette (ABC) transporter family, and a caenacin (*cnc*) antimicrobial peptide gene cluster (Alper et al., 2007; Liang et al., 2007; Mallo et al., 2002; Mochii et al., 1999; Roberts et al., 2010). Functional loss of many DBL-1 core pathway genes increases sensitivity to a number of pathogens that infect broad ranges of hosts, from nematodes to humans,

and include bacteria *E. coli*, *E. faecalis*, *P. aeruginosa* strain PA14, *S. enterica*, *S. typhimurium* strain SL1344, and *Se. marcescens*, and the nematophagous fungus *D. coniospora* (Mallo et al., 2002; Tenor and Aballay, 2008; Zugasti and Ewbank, 2009; So et al., 2011; Portal-Celhay et al., 2012). *sma-3* was isolated in a screen to identify genes that when mutated lead to an enhanced susceptibility to pathogens (Esp), specifically *P. aeruginosa* strain PA14 (Kurz and Tan, 2004). All three Smads are required for a response to *P. aeruginosa* strain PA14, but for a response to *D. coniospora*, only *sma-3* is needed, not *sma-2* or *sma-4* (Zugasti and Ewbank, 2009). This work suggests that different Smad pathways are used to regulate distinct sets of immune effectors to provide resistance to specific pathogens (Zhang and Zhang, 2009). DBL-1 pathway mutant animals may also have increased sensitivity to infection because they fail to effectively kill and digest pathogenic bacteria (So et al., 2011). DBL-1 receptors and Smads act in the pharynx and intestine, where they may contribute to innate immune responses (Krishna et al., 1999; Mallo et al., 2002; Patterson et al., 1997; Reece-Hoyes et al., 2007; Wang et al., 2002; Yoshida et al., 2001). This role for TGF- β signaling in immune responses appears to be conserved, as the *Drosophila* homolog of DBL-1, Decapentaplegic (Dpp), is upregulated upon infection by *Micrococcus luteus* (Irving et al., 2001), and mammalian TGF- β s also play roles in immune responses (Yoshimura et al., 2010). For more information, see [Signaling in the immune response](#).

2.4. Aging and longevity

The DBL-1 pathway plays a minor role in determining lifespan. Under standard culture conditions, *dbl-1* mutant populations have a somewhat reduced average lifespan relative to controls (Mallo et al., 2002; So et al., 2011). However, there is evidence that this reduced lifespan is due to increased susceptibility of DBL-1 pathway mutant animals to the slight pathogenic effects of the standard bacterial food strain, OP50, to which animals lacking DBL-1 are more susceptible than the wild type. Consistent with this hypothesis, *dbl-1* mutant animals grown on OP50 accumulate a greater number of live bacterial cells in their intestines than wild-type animals grown under the same conditions (So et al., 2011). Furthermore, growth of *dbl-1* mutant animals on heat-killed OP50 increases their survival (Mallo et al., 2002; So et al., 2011). When 5-fluoro-2'-deoxyuridine (FUdR) is added to plates to prevent DNA synthesis, thus also preventing progeny production and bacterial replication, animals lacking DBL-1 no longer have reduced survival but instead populations show moderate increases in lifespan (Luo et al., 2009; Mallo et al., 2002). These results suggest that the pathogenic effect of OP50 is obscuring a moderate lifespan-extending effect of *dbl-1* inactivation. Consistent with this hypothesis, *dbl-1* overexpression under these conditions reduces lifespan (Luo et al., 2009). Another proposed mechanism by which DBL-1 pathway mutations may extend lifespan is through the established lifespan extending factor dietary restriction (So et al., 2011), since expression of some digestive enzymes is positively correlated with DBL-1 pathway activity (Liang et al., 2007; Roberts et al., 2010).

In contrast to its minor effects on lifespan, the DBL-1 pathway plays a major role in the regulation of reproductive aging (Luo et al., 2010; Luo et al., 2009). As in humans, cessation of reproduction in *C. elegans* normally occurs in mid-adulthood. In wild-type hermaphrodites, the mean reproductive span is approximately 3.5 days, while that for *dbl-1* mutant animals is more than 7 days (Luo et al., 2009). Other DBL-1 pathway alleles also permit significantly longer reproductive spans than controls (Luo et al., 2009). This result indicates that the DBL-1 pathway normally restricts reproductive span and promotes timely cessation of reproduction. Mechanistically, the DBL-1 pathway regulates reproductive aging by modulating germline and oocyte quality (Luo et al., 2010). Not only is the population of proliferating germ cells maintained later in life in *sma-2* mutant animals, these cells show significantly reduced indicators of decline, including cavities, graininess, and cellularization. These healthier germ cells produce fertilizable oocytes later in life than controls. Oocytes from older *sma-2* mutant hermaphrodites have visibly improved oocyte morphology, a lower frequency of chromosomal nondisjunction, and greater success in developing into viable embryos than oocytes from similarly aged wild-type animals. Using tissue-specific expression of *sma-3*, Luo et al. demonstrated that the DBL-1 pathway acts non-autonomously in somatic tissues to regulate reproductive aging (Luo et al., 2010). Finally, this regulation of reproductive aging is independent of the FoxO transcription factors *daf-16* and *pha-4* (Luo et al., 2009), which mediate DAF-7/insulin signaling and dietary restriction regulation of longevity, respectively (see [section 6.2](#)). Thus, the DBL-1 pathway uncouples the regulation of lifespan from that of reproductive aging.

2.5. Mesodermal patterning

In the postembryonic mesodermal lineage, the M blast cell divides to produce descendants that contribute to the sex muscles (SM fate) and to the coelomocytes (CC fate), pseudocoelomic scavenger cells (Sulston and Horvitz, 1977). In genetic screens for mutant animals with alterations in the M lineage, two *sma-9*/Schnurri alleles were identified (Foehr et al., 2006). These alleles cause a duplication of SMs at the expense of CCs. Surprisingly, alleles affecting core DBL-1 signaling components produce no M lineage defect. Instead, these mutant alleles can function as suppressors of the *sma-9* M lineage phenotype (SOSMLP) in double mutant combinations. While SMA-9/Schnurri promotes body size signaling, this phenomenon suggests that *sma-9* antagonizes DBL-1 pathway signaling to promote dorsal M lineage fates (Foehr and Liu, 2008). The SOSMLP phenotype was used to screen for novel regulators of the DBL-1 pathway. *drag-1*, which encodes a membrane-associated repulsive guidance molecule (RGM) protein, was isolated from this screen and identified as a positive regulator of DBL-1 activity (Tian et al., 2010). This was the first evidence that an RGM molecule regulates TGF- β signaling *in vivo*, though *in vitro* evidence in mammalian systems has shown that RGM proteins can act as co-receptors for the TGF- β member BMP6 (Andriopoulos et al., 2009; Babitt et al., 2007; Tian et al., 2010; Xia et al., 2008).

2.6. Chemosensation and neuronal function

DBL-1 is expressed in and secreted from cholinergic neurons, which excite neighboring body muscles (Duerr et al., 2008; Morita et al., 1999; Suzuki et al., 1999). The DBL-1 pathway plays a clear role in how nematodes respond to excitatory acetylcholine inputs, as mutations in *dbl-1*, *sma-6*, *sma-3*, or *sma-4* confer hypersensitivity to cholinergic agonists levamisole and nicotine, and/or the acetylcholinesterase inhibitor aldicarb (Almedom et al., 2009; Vashlishan et al., 2008). Increased DBL-1 pathway signaling results in animals that are resistant to levamisole (Gumienny laboratory, unpublished observations). Hypersensitivity of muscle cells to these drugs is caused by mutations that increase acetylcholine secretion or decrease inhibitory GABAergic synaptic signaling (Loria et al., 2004; Gracheva et al., 2006; McEwen et al., 2006). Loss of DBL-1 results in both pre- and post-synaptic defects at GABAergic synapses, suggesting that this sensitivity occurs through a defect in GABAergic signaling (Vashlishan et al., 2008). However, the molecular basis of how the DBL-1 pathway may normally promote GABA transmission is unknown.

The DBL-1 receptors SMA-6 and DAF-4 and SMA-4/Smad also appear to play a role in maintaining normal serotonin levels by acting downstream of a serotonin pathway calcium channel, UNC-2, and repressing expression of *tph-1*, a tryptophan hydroxylase gene required for serotonin synthesis (Estevez et al., 2004). These transmission defects may underlie the slow movement observed in *dbl-1*(-) animals (Maniere et al., 2011).

Another neuronal function attributed to DBL-1 is its effect on fluorescently tagged, misfolded superoxide dismutase SOD1(G85R) expressed in neurons (Wang et al., 2009). *C. elegans* overexpressing a human SOD1 variant that causes amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease) in humans develop aggregates of this protein within the expressing neurons and develop motor defects over time. Loss of *dbl-1* activity exacerbated protein aggregation and led to earlier onset of movement defects, suggesting that DBL-1 normally either prevents aggregation or promotes degradation of this misfolded protein.

2.7. Ligand, receptors, and their modulators

DBL-1 was identified both by forward genetics using the small body size phenotype and by reverse genetics due to its homology to *Drosophila* Dpp and mammalian BMPs (Morita et al., 1999; Suzuki et al., 1999). DBL-1 is secreted from neurons in the head and nerve cords. Heterologous expression of *dbl-1* demonstrates that the tissue source of DBL-1 is less important than the level of expression for DBL-1 signaling. This suggests that DBL-1 expression levels may normally depend on cues processed by the neurons that express DBL-1 (Savage-Dunn et al., 2011). A major target of DBL-1 for body size regulation appears to be the hypodermis, which cradles the nerve cords along their entire length. The hypodermis expresses the receptors for DBL-1, 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).

Between the TGF- β secreting cells and the receiving cells, the TGF- β does not simply diffuse to its receptors on the receiving cell, but is tightly regulated. In *C. elegans*, four transmembrane proteins have been identified that have tissue-specific roles in extracellular regulation of DBL-1 activity: the negative regulator LON-2/glypican and the positive regulators SMA-10/LRIG, CRM-1/CRIM, and DRAG-1/Dragon. LON-2, one of the two glypicans in *C.*

elegans, is a heparan sulfate proteoglycan that negatively regulates DBL-1 signaling (Gumienny et al., 2007). Like *Drosophila* glypican Dally, LON-2 binds BMP members and thereby is proposed to regulate signaling (Gumienny et al., 2007; Kirkpatrick et al., 2006). Loss of functional heparan sulfate modifying enzymes results in small animals that may be a result of LON-2 over-repressing DBL-1, since LON-2 is required for the small phenotype of the heparan sulfate enzyme mutant animals (Gumienny et al., 2007).

SMA-10 is a positive regulator of DBL-1 signaling. It is member of the leucine-rich repeats and immunoglobulin-like domains (LRIG or LRRIG) family (Gumienny et al., 2010). This conserved transmembrane protein binds to both SMA-6/type I and DAF-4/type II receptors in cell culture assays, as does one of its mammalian homologs, LRIG1.

CRM-1, a member of the cysteine-rich motor neuron protein (CRIM) family, also acts in the intercellular milieu to facilitate DBL-1 signaling (Fung et al., 2007). Although CRM-1 is expressed in the same ventral cord neurons as DBL-1, this transmembrane protein functions in a cell non-autonomous way to promote DBL-1 signaling in the hypodermis. Post-translational processing or alternative splicing to make soluble CRM-1 isoforms may account for this activity. In vertebrate systems, CRIM has also been shown to bind BMPs, but reduces BMP processing and secretion, thereby antagonizing BMP activity (Wilkinson et al., 2003).

The third extracellular positive regulator of DBL-1 signaling is DRAG-1, the sole member of the repulsive guidance molecule (RGM) family in *C. elegans*. DRAG-1 promotes normal body size and mesoderm development through the DBL-1 pathway (Tian et al., 2010). Like its conserved family members in other species, DRAG-1 is GPI-linked to the extracellular membrane surface. Other RGM members have been shown to physically bind specifically to some BMPs and both BMP receptor types (Corradini et al., 2009).

In addition to these transmembrane regulators of DBL-1 signaling, ligand activity may also be regulated through interactions with the extracellular matrix. For example, DBL-1 genetically acts with the extracellular matrix constituent UNC-52/perlecan in directing migrating distal tip cells (Merz et al., 2003). Perlecan in other systems interacts with the TGF- β regulator fibrillin-1 (Tiedemann et al., 2005). DBL-1 signaling is also positively regulated in part by ADT-2 (p.k.a. SMA-21), a member of the ADAMTS (a disintegrin and metalloprotease with thrombospondin motifs) family of secreted metalloproteases (Fernando et al., 2011). Animals lacking functional ADT-2 are smaller than normal and, in this background, expression of a DBL-1-responsive transcriptional reporter is decreased. However, ADT-2 is also required for normal fibril formation of cuticle collagens, which may affect body size in a DBL-1-independent manner (Fernando et al., 2011). ADAMTS metalloproteases assemble and degrade extracellular matrix (Colige et al., 1995; Collins-Racie et al., 2004; Fernandes et al., 2001; Kuno et al., 2000; Somerville et al., 2003; Tortorella et al., 2000; Tortorella et al., 2005; Wang et al., 2003). Mouse cells lacking ADAMTS5 have increased responsiveness to TGF β 1 treatment (Velasco et al., 2011), while geleophysic dysplasia patients with altered ADAMTSL2 show increased TGF- β activity (Le Goff et al., 2008). ADAMTSL2 binds the TGF- β inhibitor latent TGF- β -binding protein 1 (LTBP1), which does not have an identified homolog in *C. elegans* (Le Goff et al., 2008).

2.8. Intracellular signaling components and regulation of gene expression

The main intracellular 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 of transcription factors (Sekelsky et al., 1995). SMA-2 and SMA-3 are receptor-regulated Smads (R-Smads), and SMA-4 is a Co-Smad. Smads engage in dynamic nucleocytoplasmic shuttling, with retention in the nucleus stimulated by pathway activation (Inman et al., 2002; Nicolás et al., 2004; Xiao et al., 2001; Xu et al., 2002). In *C. elegans*, a rescuing SMA-3:GFP fusion protein normally accumulates to high levels in the nucleus, while in *sma-6* type I receptor mutant animals, the protein is more evenly partitioned between nucleus and cytoplasm (Wang et al., 2002). Nuclear accumulation is not influenced by R-Smad (SMA-2) or Co-Smad (SMA-4) depletion. SMA-2 and SMA-3 R-Smads are not redundant for body size and male tail patterning (Savage-Dunn et al., 2000), suggesting that the functional Smad complex is a heterotrimer containing two different R-Smads (SMA-2/SMA-3/SMA-4). In contrast, mounting an innate immune response against the fungus *D. coniospora* has provided evidence of a noncanonical DBL-1 signaling mechanism that relies on SMA-3 but not SMA-2 or SMA-4 (see section 2.3) (Zugasti and Ewbank, 2009). This result suggests that during an innate immune response, the functional Smad complex is an R-Smad homo-multimer that does not incorporate a Co-Smad. Expression of the Smads and their receptors in the hypodermis, pharynx, and intestine are involved in immune responses, while the body size regulatory aspect of Smads is localized only in the hypodermis and pharynx (Krishna et al., 1999; Schulenburg et al., 2004; Wang et al., 2002; Yoshida et al., 2001).

Another intracellular modulator of TGF- β signaling in *C. elegans* is KIN-29 (p.k.a. SMA-11 and SNS-8), a serine/threonine kinase that acts downstream of DBL-1 to regulate body size (Lanjuin and Sengupta, 2002; Maduzia et al., 2005). *kin-29* is expressed in a number of sensory neurons, including some that express *dbl-1*, where it appears to affect levels of some olfactory receptors (Lanjuin and Sengupta, 2002). KIN-29 is also expressed in the hypodermis, where DBL-1 receptors and downstream players and targets reside, though its targets in the hypoderm are unknown (Maduzia et al., 2005). Body size is partially restored in *kin-29* mutant animals if wild-type *kin-29* is expressed in either neurons or hypodermis, but the molecular mechanism of this rescue is currently unknown.

SMA-9 is a transcription factor in the Sma/Mab pathway (Liang et al., 2003). Identified in genetic screens for small (Savage-Dunn et al., 2003) or mesodermal patterning mutant animals (Foehr et al., 2006), *sma-9* encodes a large zinc finger transcription factor related to *Drosophila* Schnurri (Liang et al., 2003). SMA-9 is nuclear localized and is detected in most, if not all, somatic cells by immunohistochemistry. Phenotypic analyses 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. In mesodermal patterning, SMA-9 acts antagonistically with the DBL-1 pathway (see section 2.5) (Foehr et al., 2006). SMA-9 is not required for DBL-1 transcriptional regulation of antimicrobial peptide gene expression following fungal infection (Zugasti and Ewbank, 2009). These complexities in function may depend on alternative splicing to encode isoforms of SMA-9 that have varying zinc finger motif numbers. This model has been demonstrated in the case of SMA-9 in male tail development, where alternative transcript processing results in a truncated SMA-9 required for male tail patterning but not for body size regulation (Yin et al., 2010).

Additional transcriptional partners have been identified that play tissue-specific roles in TGF- β signal transduction. For example, the Forkhead/Fox transcription factor LIN-31, originally identified by its role in vulval development (Miller et al., 1993), is involved in spicule morphogenesis with the Sma/Mab pathway (Baird and Ellazar, 1999). Yeast two-hybrid experiments demonstrate that LIN-31 can physically interact with SMA-3 (Wang et al., 2005). In vertebrates, FoxO and FoxH transcription factors are Smad transcriptional partners (Massagué et al., 2005), suggesting that this Smad-Fox transcriptional partnership is evolutionarily conserved. Another tissue-specific transcription partner is MAB-31. *mab-31* was identified in an RNAi screen for genes required for normal male tail development (Wong et al., 2010). Loss of *mab-31* function, either by RNAi or in a deletion mutant, results in ray fusions indistinguishable from the core Sma/Mab pathway mutant phenotype. MAB-31:GFP localizes to the nucleus, and its localization does not depend on SMA-6 receptor or SMA-4 Co-Smad, suggesting constitutive nuclear localization rather than nucleocytoplasmic shuttling. MAB-31 is conserved among nematodes, but shows no apparent homology to proteins in other organisms. These three transcription factors, SMA-9, LIN-31 and MAB-31, can be considered integral components of the DBL-1 pathway based on the close similarity of their mutant phenotypes to those of other DBL-1 pathway mutant animals. Other transcription factors that are required for DBL-1-regulated responses are more pleiotropic and are described below.

Body size regulation by the DBL-1 pathway may, like other TGF- β pathways, require accessory transcription factors. Smads, by themselves, interact poorly with DNA and complex with other transcription factors to mediate TGF- β responses (reviewed by Massagué et al., 2005; Schmierer and Hill, 2007). In *C. elegans*, RNT-1 (RUNX family transcription factor) has been identified as a transcription factor that physically interacts with SMA-4 Smad *in vitro* and is required for normal body size, as loss of RNT-1 function results in small animals (Ji et al., 2004). In mammals, RUNX transcription factors also interact with Smads (Hanai et al., 1999; Selvamurugan et al., 2004; Zhang et al., 2000). *rnt-1* is expressed in the seam cells and the intestine, but not apparently in the hypodermis, so how RNT-1 affects hypodermally-expressed SMA-4 remains unclear (Nam et al., 2002). Double mutant combinations with *rnt-1* and *sma-2*, *sma-3*, *sma-4*, or *sma-6* result in animals smaller than animals harboring a single mutant allele, suggesting that *rnt-1* acts at least partly in parallel to DBL-1 signaling for body size, possibly through different transcriptional targets or tissues (Ji et al., 2004). This SMA-4—RNT-1 interaction appears to be inhibited by another transcription factor, BRO-1 (brother (*Drosophila* transcription factor partner) homolog), which competes with DBL-1 signaling by interacting with RNT-1 in a complex with DNA (Shim and Lee, 2008).

In the male tail rays, DBL-1 promotes a dopaminergic fate in neurons of rays 5, 7, and 9 (Lints and Emmons, 1999). Regulation of this fate requires genetic interaction with the Hox gene *egl-5* and the DM domain transcription factor gene *mab-23* (Lints and Emmons, 1999, 2002). EGL-5 is required for rays 3-6 to be competent to express the dopaminergic fate in response to DBL-1 signaling (Lints and Emmons, 1999). On the other hand, DBL-1 induction of dopaminergic fate in ray 5 requires suppression of *mab-23* activity (Lints and Emmons, 2002). MAB-23 normally functions to repress dopaminergic fate, since in *mab-23* mutant adult males, rays 1, 3, and 6 ectopically express dopaminergic fate (Lints and Emmons, 2002).

Another gene that acts downstream of the **DBL-1** pathway in specification of ray identity is **mab-21**, which encodes a novel, conserved protein (Chow et al., 1995; Morita et al., 1999). **mab-21** is required in the structural cell of ray 6 to specify ray 6 identity, and its Mab phenotype is epistatic to that of the **DBL-1** pathway (Ho et al., 2001; Morita et al., 1999). In *C. elegans*, SIN-3, a histone deacetylase complex subunit, associates with **MAB-21** (Choy et al., 2007). Interestingly, vertebrate Mab21 and Sin3 both physically interact with Smads and modulate BMP signaling (Baldessari et al., 2004). These results support a model whereby a complex of Smads, **MAB-21**, and SIN-3 repress transcription of TGF- β target genes by recruiting a histone deacetylation complex to repress transcription of targeted genes (Inoue and Imamura, 2008).

Downstream transcriptional targets of the **DBL-1** pathway have been determined using microarray analyses. Liang et al. compared expression profiles of **dbl-1** and **sma-9** mutant populations to the wild type at the L2 stage (Liang et al., 2007); Roberts et al. compared **sma-6** mutant populations with **dbl-1(++)** overexpressing animals at the L4 stage (Roberts et al., 2010); Luo et al. compared **sma-2** and **sma-4** mutant populations to wild type at the L4 stage (Luo et al., 2010). All three groups identified similar target gene categories, including cuticle collagens, hedgehog-related genes, metabolism genes, and innate immunity genes. These results are similar to those obtained in a smaller scale arrayed cDNA hybridization experiment (Mochii et al., 1999). Differences in the specific genes identified in these experiments may be due to stage-specific regulation of target genes. In addition to the somatic targets of the pathway, germline genes showed significant decreases in expression levels (Liang et al., 2007; Luo et al., 2010; Roberts et al., 2010). By performing expression profiles on isolated unfertilized oocytes, Luo et al. found germline-specific **sma-2** target genes that have no overlap with the target genes identified in the L4 stage (Luo et al., 2010). One of the functions of these target genes is to mediate the regulation of reproductive aging (see section 2.4).

3. **DAF-7** pathway

The **DAF-7** pathway was first elucidated by its regulation of the dauer/continuous development switch. The core components of this pathway are: the ligand **DAF-7** (Ren et al., 1996), type II receptor **DAF-4** (Estevez et al., 1993), type I receptor **DAF-1** (Georgi et al., 1990), R-Smads **DAF-8** (Park et al., 2010) and **DAF-14** (Inoue and Thomas, 2000a), and antagonistic Co-Smad **DAF-3** (Patterson et al., 1997) (Figure 3). The Sno/Ski homolog **DAF-5** acts downstream of the pathway and binds **DAF-3/Co-Smad**, an interaction that is conserved in other systems (da Graca et al., 2004; Inoue and Imamura, 2008; Tewari et al., 2004). **DAF-7** is expressed only in the pair of ASI sensory neurons (Ren et al., 1996; Schackwitz et al., 1996), while other pathway components have widespread expression. **DAF-7** pathway components are in general quite highly diverged from TGF- β signaling components in other species, but have more sequence features in common with TGF- β /Activin/Nodal/GDF pathway components than with BMP components (see previous version of this chapter, TGF- β signaling).

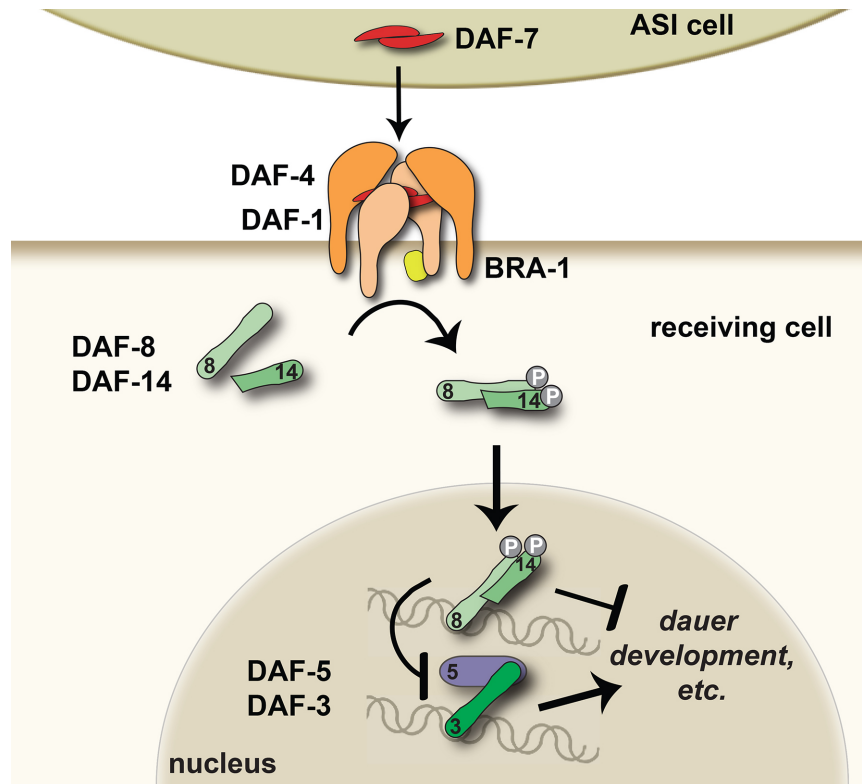


Figure 3. The dauer TGF- β -related pathway. DAF-7 promotes continuous, non-dauer development. The DAF-7 signal (red) is received by a heterotetrameric receptor composed of two DAF-1 type I receptor and two DAF-4 type II receptor subunits (light and dark orange, respectively). BRA-1/BMP receptor associated protein (BRAM) (yellow) is a negative intracellular regulator of DAF-1. The DAF-7 signal is transduced by DAF-8 and DAF-14 Smads (light greens). These components, when activated, inhibit the functions of DAF-3/Co-Smad (dark green) and DAF-5/Sno/Ski (indigo), which promote dauer development. The Smad/Ski model shown is not definitive. Cartoons are based on *C. elegans* or other published homologous protein structures and functions. See text for more details.

3.1. Regulation of dauer development

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 predauer stages (Swanson and Riddle, 1981). When dauer larvae sense more favorable environments, they exit dauer and resume development. Large-scale genetic screens were conducted for mutations that affect the decision to enter dauer (Golden and Riddle, 1984b; Malone and Thomas, 1994; Riddle et al., 1981). The mutant strains isolated were either dauer-constitutive (Daf-c: constitutively entering dauer inappropriately under favorable environmental conditions) or dauer-defective (Daf-d: defective in entering dauer under adverse conditions). Genetic epistasis and detailed phenotypic analyses revealed three dauer gene classes (Thomas et al., 1993). Subsequent molecular analyses 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 (see Dauer). Animals defective in the core components of the *daf-7*/TGF- β dauer pathway are Daf-c. Mutations in the downstream genes *daf-3*/Co-Smad and *daf-5*/Sno/Ski cause Daf-d phenotypes that suppress the Daf-c phenotypes of the upstream components (Figure 3) (Thomas et al., 1993).

DAF-7 pathway mutant phenotypes are temperature sensitive, with increased penetrance of dauer at 25°C than at 15°C (Golden and Riddle, 1984b). Temperature modulates the animal's response to other dauer-inducing cues, pheromone and food shortage. *daf-7*/TGF- β expression is responsive to changes in temperature, pheromone, and food (Ren et al., 1996; Schackwitz et al., 1996). However, *daf-3* and *daf-5* mutant alleles suppress the *daf-7* pathway mutant Daf-c phenotype at 25°C but enhance it at 27°C, and *daf-3* or *daf-5* mutant animals retain pheromone responsiveness, suggesting that DAF-7 pathway signaling, pheromone levels, and temperature are three different cues the organism must integrate in its decision to enter dauer (Ailion and Thomas, 2000; Ren et al., 1996; Thomas et al., 1993). For more information, refer to the WormBook chapter Dauer.

Both dauer entry and exit decisions appear to require **DAF-7**. *daf-7* expression decreases with dauering and increases during dauer exit induced by presence of food or a shift to a permissive temperature (Ren et al., 1996; Schackwitz et al., 1996). Shifting dauers with a wild-type background to a permissive temperature or from high to low pheromone concentration in the presence of food recovers dauers, but dauer recovery is greatly reduced in *daf-7* mutant animals (Ren et al., 1996). Dauer recovery of *daf-7* mutants is further delayed or prevented when the nuclear hormone receptor NHR-69 or Notch LIN-12d is lacking, and is enhanced at 25 °C by muscarinic agonist neurotransmitters or if the Notch pathway (GLP-1 or LAG-2) or insulin INS-18 is not functional (Tissenbaum et al., 2000; Ouellet et al., 2008; Park et al., 2012b). These results suggest that **DAF-7** signaling is an important part of recovering from dauer as well as bypassing dauer in reproductively favorable environments.

3.2. Fat storage

In adult nematodes, the **DAF-7** pathway responds to environmental signals by modulating energy consumption. In particular, *daf-7*, *daf-4*, and *daf-1* mutant animals grown under favorable environmental conditions accumulate more intestinal fat than wild type, in spite of reduced feeding (Greer et al., 2008). These metabolic changes are similar to those seen in dauers or in other nematodes grown under unfavorable conditions. Like the *Daf-c* phenotype, these metabolic phenotypes are dependent on *daf-3*/Smad (Greer et al., 2008). *egl-4*/cGMP-dependent protein kinase may act upstream of *daf-7* to promote fat deposition in the intestine (Raizen et al., 2006). To identify specific effectors of the *daf-7*(-) fat accumulation phenotype, a suppressor screen was performed. It identified a gain-of-function allele of *egl-30*, which encodes an α subunit of a heterotrimeric G protein that promotes acetylcholine secretion (Lackner et al., 1999; Miller et al., 1999; Greer et al., 2008). How this fat accumulation suppressor interacts with the **DAF-7** pathway is undetermined. In addition, loss of two metabotropic glutamate receptors, **MGL-1** and **MGL-3**, specifically eliminated the increased fat phenotype of *daf-7* mutant animals. These results are consistent with a model where **DAF-7** signaling during reproductively favorable conditions inhibits **DAF-3** activity and permits appropriate fat deposition. In an unfavorable environment, **DAF-7** is inactivated, thereby activating **DAF-3** and increasing fat storage levels through **MGL-1** and **MGL-3** metabotropic receptors (Greer et al., 2008). For more information, refer to the WormBook chapter *Obesity and the regulation of fat metabolism*.

3.3. Behavioral functions

In addition to the *Daf-c* and fat storage phenotypes, animals lacking **DAF-7** display behavioral defects in egg laying, roaming, quiescence, and feeding. The egg-laying defect is a partial retention of embryos in the uterus, reminiscent of the egg retention that occurs under low food conditions. Like other phenotypes of **DAF-7** pathway mutant strains, the egg-laying defect is suppressed by mutations in *daf-3*/Co-Smad and *daf-5*/Sno/Ski (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*/cGMP-dependent protein kinase has additional phenotypes that overlap with those of both *dbl-1* and *daf-7*, including body size regulation (see section 6.2), fat accumulation (see section 3.2), and chemosensation (see section 6.2) (Fujiwara et al., 2002; Hirose et al., 2003; L'Etoile et al., 2002). *egl-32*, which is uncloned, is also required for normal egg laying and chemosensation, though the molecular basis of these phenotypes and whether they are **DAF-7** dependent is unknown (Daniels et al., 2000; McGovern et al., 2007).

Like other animals, *C. elegans* divide their time between alternative behavioral states of inactive dwelling and active roaming, with this allocation responsive to food availability (see *Chemosensation in C. elegans*). During dwelling, which occurs 80% of the time under normal laboratory conditions, animals move slowly, alternating between forward and backward movement, with little net displacement. In roaming, animals move in a more continuous forward direction. When food resources are low or poor, the time allocated to roaming will increase (Fujiwara et al., 2002). *daf-7* mutants, however, are defective in triggering roaming behavior in response to unfavorable food conditions (Ben Arous et al., 2009). Another distinct behavioral response to food is quiescence, defined as the complete cessation of movement and feeding for periods of at least 10 seconds (You et al., 2008). Quiescence is not normally seen with standard laboratory food (OP50 bacteria), but can be triggered by feeding HB101 bacteria, which is considered a higher quality food (You et al., 2008). This quiescence may be analogous to satiety in mammals. The induction of quiescence is defective in *daf-7*, *daf-1*, and *daf-4* mutant animals, as well as in insulin signaling defective animals (Ren et al., 1996; You et al., 2008). Furthermore, genetic interactions suggest that both **DAF-7** and insulin pathways function via the cGMP-dependent protein kinase **EGL-4** to induce quiescence.

Another behavioral alteration described in animals defective in the **DAF-7** pathway is the clumpy phenotype (Thomas et al., 1993). These mutant animals tend to aggregate on a bacterial lawn, in contrast to the dispersed localization of the standard laboratory strain N2. In natural isolates of *C. elegans*, these two alternate behaviors are observed and have been attributed to polymorphisms in the *npr-1* neuropeptide receptor gene (de Bono and Bargmann, 1998). This aggregation behavior is proposed to be a way to avoid hyperoxia, as local oxygen levels are decreased within aggregations (Gray et al., 2004). Like *npr-1*-defective animals, animals lacking **DAF-7** also display a robust avoidance of hyperoxia and aggregate in the presence of food, unlike N2 wild-type animals, which aggregate to avoid hyperoxia only in the absence of food (Chang et al., 2006). Since *npr-1* and *daf-7* act in different cell types, however, they are believed to act in parallel in a distributed neuronal network regulating these behaviors.

Animals lacking a functional **DAF-7** pathway have reduced feeding rates as measured by pharyngeal pumping (Greer et al., 2008). These reductions are similar to those seen in wild-type nematodes treated exogenously with the neurotransmitters tyramine or octopamine (Horvitz et al., 1982). By blocking the tyramine or octopamine biosynthetic pathways or signal transduction pathways in *daf-7* mutant animals, Greer et al. showed that the reduced pumping in these animals depends on these neurotransmitters (Greer et al., 2008). The dauer, egg-laying and fat retention phenotypes, on the other hand, were unaltered by elimination of tyramine or octopamine signaling and therefore act through a different mechanism than **DAF-7**-regulated pharyngeal pumping. These results suggest that **DAF-7** is an environmental sensor to determine not only the dauer decision, but also to modulate the animal's behaviors to maintain energy balance at other stages. For more information on the regulation of these behaviors, refer to the WormBook chapters [Egg-laying](#), [Neuropeptides](#), [Biogenic amine neurotransmitters](#), [Acetylcholine](#), and [GABA](#).

3.4. Aging and longevity

The output of **DAF-7** pathway signaling is integrated with insulin signaling in the regulation of dauer entry and exit. Early reports suggested that the functions of these two pathways diverged in adulthood, with insulin signaling but not TGF- β signaling regulating longevity (Kenyon et al., 1993; Larsen et al., 1995). It has subsequently been shown, however, that matricide masked a long-lived phenotype of **DAF-7** pathway mutant strains (Shaw et al., 2007). This death of adult hermaphrodites is a consequence of embryos hatching internally and is another effect of the *daf-7* mutant egg-laying defect. When lifespan assays were conducted on FudR to prevent embryonic development, a significant increase in lifespan was observed in *daf-7*, *daf-1*, *daf-4*, *daf-8*, and *daf-14* mutant strains relative to wild type (Shaw et al., 2007).

The lifespan extension of *daf-7* mutant populations was dependent upon *daf-16*/FoxO, the downstream transcription factor in the insulin pathway, suggesting that the TGF- β pathway regulates longevity upstream of and via the insulin pathway (Shaw et al., 2007). Consistent with this hypothesis, the expression profiles of **DAF-7** and insulin mutants are highly similar to each other. Furthermore, the insulin-like ligands *ins-7* and *ins-18* are transcriptionally regulated by the **DAF-7** pathway, providing a mechanism for regulation of the insulin pathway (Liu et al., 2004; Shaw et al., 2007; Narasimhan et al., 2011).

3.5. Germline proliferative zone

A negative regulatory influence of the **DAF-7** pathway on the germline proliferative zone was suggested by Park et al. (Park et al., 2010). Based on their studies, they concluded that **DAF-8**/R-Smad expressed in the distal tip cell negatively regulates the expression of *lag-2*, which encodes the DTC-expressed ligand for **GLP-1**/Notch, and thereby negatively regulates the proliferative pool of germ cells. This role for **DAF-8** appeared to be independent of **DAF-3** and **DAF-5** (Park et al., 2010).

However, in screens to identify genes involved in germline proliferation and differentiation, *daf-7*, *daf-1*, and *daf-4* were identified as positive regulators of the proliferative zone (Dalfó et al., 2012). **DAF-7** expressed from the ASI environment-sensing cells promotes the larval accumulation of germline progenitor cells in response to reproductively favorable environmental conditions. To coordinate this response, **DAF-7** requires the activity of its receptor **DAF-1** in the distal tip cells, which constitute the niche for germline stem cells. **DAF-3**, **DAF-5**, **DAF-8**, and likely **DAF-14** are also expressed in the distal tip cells (Patterson et al., 1997; Inoue and Thomas, 2000a; Park et al., 2010; da Graca et al., 2004). **DAF-3** and **DAF-5** limit **DAF-7**-mediated expansion of the proliferative zone, while **DAF-8** and **DAF-14**, like **DAF-7** and its receptors, promote expansion (Dalfó et al., 2012). Additional results support the idea that this pathway acts to influence the proliferation versus differentiation decision of larval germ cells (as opposed to influencing the cell cycle rate), but in a **GLP-1**/Notch-independent manner. Though currently

not reconciled, the opposite germline phenotypes observed by these two groups may be independent and may be controlled in different ways by DAF-7 pathway components. DAF-7's positive regulation of larval proliferative zone expansion is also independent of DAF-12/nuclear hormone receptor, DAF-18/PTEN, or DAF-16/FOXO, which are required for the DAF-7 response to dauerling cues (DAF-12) and life-span regulation (DAF-18 and DAF-16) (see above) (Dalfó et al., 2012). These results suggest that DAF-7 influences larval germline progenitors through an unidentified downstream pathway.

3.6. Ligand, receptors and transmembrane regulators of signaling

DAF-7/TGF- β -related ligand is expressed in the pair of ASI sensory neurons under favorable environmental conditions and is repressed under adverse conditions, such as increased population density and low food availability, leading to appropriate responses to these conditions (Ren et al., 1996; Schackwitz et al., 1996). Animals lacking functional DAF-7 are Daf-c, indicating that this TGF- β superfamily member has a role in promoting continuous, non-dauer development. Genetic analysis of dauer formation in the closely related free-living nematode *Caenorhabditis briggsae* revealed similar pathways involved in dauer regulation (Inoue et al., 2007). Specifically, the *C. briggsae* *daf-4* phenotype is Daf-c, while the *C. briggsae* *daf-3* phenotype is Daf-d.

DAF-7 orthologs have been identified in several species of parasitic nematodes, but their roles in responding to environmental cues appear to be diverged (Brand et al., 2005; Crook et al., 2005; Freitas and Arasu, 2005; Gomez-Escobar et al., 2000; McSorley et al., 2009). Not only does *Parastrongyloides trichosuri* *daf-7* fail to rescue *C. elegans* *daf-7* mutant phenotypes, its expression is different than *C. elegans* *daf-7* (Crook et al., 2010). In contrast with *C. elegans* *daf-7* (Ren et al., 1996), which is expressed in early larval stages but is downregulated upon entry into dauer, expression of parasitic nematode *daf-7* homologs increases during the infectious diapause state (Viney et al., 2005).

The type I and type II receptors for DAF-7 are encoded by *daf-1* and *daf-4*, respectively (Estevez et al., 1993; Georgi et al., 1990). 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 (Georgi et al., 1990; Kingsley, 1994)). 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 DAF-7 pathway and in the DBL-1 pathway by interacting with different pathway-specific components (Estevez et al., 1993).

daf-4 is expressed widely during all stages of *C. elegans* development (Gunther et al., 2000; Patterson et al., 1997). 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). Expression of *daf-1*/type I receptor in interneurons is sufficient to rescue the dauer, egg-laying, feeding rate, and fat storage defects of a *daf-1* mutant (Greer et al., 2008). Genetic evidence suggests that DAF-1/type I receptor is capable of signaling, albeit weakly, in the absence of phosphorylation by DAF-4/type II receptor. In a *daf-4* mutant background, depletion of *daf-1* causes a more severe mutant phenotype and overexpression of *daf-1* partially rescues the *daf-4* mutant phenotype (Gunther et al., 2000). Canonically, type I receptors differ from the constitutively active type II receptors in requiring phosphorylation for activation. 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).

At the receiving cell, DAF-7 signaling is negatively regulated by BRA-1, which binds DAF-1 (Morita et al., 2001). BRA-1 was identified by homology to human BMP receptor-associated molecule BRAM1, which negatively regulates BMP signaling by binding BMP receptor-1A (Kurozumi et al., 1998). DAF-7 pathway receptor activity also may be modulated intracellularly by OBR-3 (originally published as BIP), an oxysterol-binding domain protein that binds BRA-1 and BRA-2/BMP receptor associated protein (BRAM) (Sugawara et al., 2001). However, knockdown of OBR-3 function results in small animals with no phenotypes associated with the DAF-7 pathway, suggesting a role for OBR-3 in DBL-1 signaling instead.

3.7. Transcription factors and regulation of target gene expression

The Smads participating in this pathway are encoded by *daf-8*, *daf-14*, and *daf-3*. DAF-8 and DAF-14 are related to TGF- β R-Smads, except that DAF-14 lacks strong homology to the MH1 domain (Inoue and Thomas, 2000a; Park et al., 2010). These R-Smads have been shown to have partially redundant functions (Inoue and Thomas, 2000a; Park et al., 2010). Both of these Smads are expressed broadly. The Ser/Thr phosphatase PDP-1 has been identified as a negative regulator of DAF-8 and DAF-14 Smad activity (Narasimhan et al., 2011). In *Drosophila* and in mammals, PDP has similarly been identified as a Mad/Smad1 C-terminal phosphatase (Chen et al., 2006). *C. elegans* PDP-1 also modulates phenotypes of insulin signaling and nuclear localization of DAF-16/FoxO, providing evidence of crosstalk between the insulin and TGF- β pathways (see section 5.2) (Narasimhan et al., 2011).

daf-3 encodes a Smad distantly related to Co-Smads (Patterson et al., 1997). While DAF-8 and DAF-14 R-Smads function to transduce the DAF-7 signal, DAF-3 Co-Smad activity is negatively regulated by the pathway. In the absence of DAF-3, animals enter the non-dauer L3 stage regardless of the presence or absence of DAF-7 signaling (Patterson et al., 1997). Thus, while DAF-7 pathway signaling normally inhibits *daf-3* activity it is dispensable in the absence of *daf-3*. Furthermore, DAF-3 drives a negative feedback loop by repressing the expression of *daf-7* and *daf-8* (Park et al., 2010). These antagonistic interactions differ from the mechanisms of inhibition by I-Smads in other systems.

daf-5 has the same type of genetic interactions with the DAF-7 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). Ski has been shown in vertebrates to be a component of the histone deacetylase complex (HDAC) and binds Smads (Akiyoshi et al., 1999; Luo et al., 1999; Sun et al., 1999; Wang et al., 2000; Xu et al., 2000). DAF-5 physically interacts with DAF-3 to promote dauer development, and may act in a conserved fashion as other Sno/Ski members (Tewari et al., 2004). Ski may repress TGF- β superfamily signaling in two ways. First, Ski recruits the HDAC to maintain the chromatin structure around TGF- β target genes in a transcriptionally silent form (Akiyoshi et al., 1999; Luo et al., 1999). Second, Ski prevents target gene transcription by stabilizing inactive Smad complexes on Smad-binding elements in the regulatory regions of target genes (Akiyoshi et al., 1999; Luo et al., 1999; Sun et al., 1999; Suzuki et al., 2004; Xu et al., 2000).

Microarray analyses have been performed to identify transcriptional target genes of the DAF-7 pathway. Liu et al. compared expression levels in *daf-7*, *daf-8*, and *daf-14* mutant to wild-type populations during larval stages when the dauer decision was made and executed (Liu et al., 2004). Functional categories of identified target genes include insulin pathway genes, hedgehog- and patched-related genes, hormone biosynthetic genes, and cell cycle genes. Shaw et al. compared expression in adulthood in *daf-7* and *daf-1* mutant populations with expression in *daf-3* and *daf-5* Daf-d strains and in *daf-7*; *daf-3* double mutant strains (Shaw et al., 2007). The adult targets of the pathway showed relatively low correlation with the dauer targets, and instead were enriched in functional categories that regulate longevity such as regulators of metabolism. Both studies identified a large degree of overlap with transcriptional targets of the insulin pathway at the same developmental stages. Consistent with this outcome, the upstream sequences of many of the target genes contained binding sites for DAF-16/FoxO, the transcription factor that mediates insulin signaling.

The DAF-7 pathway also regulates chemoreceptor gene expression in sensory neurons. Mutations in this pathway were identified in a screen for altered expression of the chemoreceptor gene *sra-6*/serpentine receptor, class A (Nolan et al., 2002). Several additional chemoreceptor genes normally expressed in the ASH and ASI sensory neurons or in the AWC sensory neurons also depend on DAF-7 pathway function for expression (Lesch and Bargmann, 2010; Nolan et al., 2002). Chemoreceptor gene regulation is likely mediated directly by DAF-3, since DAF-3 binding sites are prevalent upstream of chemoreceptor genes (McCarroll et al., 2005).

3.8. Systems biology of the DAF-7 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). This work showed a physical interaction between DAF-3 and DAF-5. Among the novel interactors was DAF-21/HSP90, which interacts physically with DAF-1 and DAF-4 receptors. DAF-21 was previously shown to function in the DAF-11 cyclic nucleotide pathway, which processes dauer cues in parallel to the DAF-7 pathway. Thus, DAF-21 may act in

multiple dauer-regulating pathways, possibly to ensure proper protein folding at the high temperatures that trigger dauer entry. **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** also shows direct or indirect associations with **SWI3** and **SNF5** homologs (**Y11G7B.23** and **R07E5.3**, respectively), suggesting a function in chromatin regulation.

4. Comparison of **DBL-1** and **DAF-7** pathways

In this section, we summarize the similarities between the **DBL-1** and **DAF-7** pathways as well as the differences that may reflect functional specializations. Both **DBL-1** and **DAF-7** ligands function alone, with no evidence of functional heterodimers. Both ligands interact with the sole type II receptor in *C. elegans*, **DAF-4**, with specificity determined by the type I receptor recruited (**SMA-6** or **DAF-1**, respectively). In both pathways, two different R-Smads are required for full pathway activity, consistent with the functional Smad complex being a heterotrimer composed of three different subunits. However, while R-Smads **SMA-2** and **SMA-3** cannot replace one another, R-Smads **DAF-8** and **DAF-14** show some functional redundancy. In addition, each pathway has some biological roles in which a single R-Smad appears to function alone: *e.g.* **SMA-3** in pathogen resistance and **DAF-8** in germline mitosis. Unlike in vertebrates, *C. elegans* Co-Smads are pathway-specific. The Co-Smad **SMA-4** is similar to mammalian Smad4 in both sequence and function. On the other hand, **DAF-3** has sequence and functional features of both Co-Smads and I-Smads. In spite of the diversity of biological roles of these two pathways, the microarray analyses identified many of the same families of downstream target genes, with different individual genes represented in each case. These common families of target genes notably include those of the Hedgehog-related pathway and insulin-related ligand genes. Finally, both **DBL-1** and **DAF-7** pathways are controlled by tissue- and pathway-specific regulators (*i.e.*, **CRM-1**, **SMA-10**, **DRAG-1**, **LON-2** for **DBL-1**; **BRA-1** and **DAF-5** for **DAF-7**). As investigators continue to probe the functions of these pathways, it is likely that the number of modulators identified will continue to increase.

5. **UNC-129** pathway

Unlike the other *C. elegans* TGF- β -related proteins, **UNC-129** is a more ancestral protein with no obvious subfamily affiliation based on sequence or *in vivo* function (Colavita et al., 1998). Also unique to **UNC-129** is that it has no recognized type I and type II receptors. Rather, as confirmed in tissue culture, **UNC-129** interacts with the **UNC-5** netrin receptor, a transmembrane protein with two immunoglobulin-like domains and two type I thrombospondin motifs in the extracellular region that shares no obvious similarity with other TGF- β receptors (MacNeil et al., 2009).

5.1. Cell migration guidance

UNC-129 is expressed in several tissues, including dorsal body wall muscle, where it facilitates the long-range migrations of motor neurons and distal tip cells (DTCs) (Colavita et al., 1998; Gabel et al., 2008; MacNeil et al., 2009). Too little or too much **UNC-129** causes misguided **PVPR** and **PVQL**, which pioneer the left axon tract, and motor axons (**DA**, **DB**, **VD**, **DD**, **NSM**, and **SDQ**), producing the namesake uncoordinated (**Unc**) phenotype (Axäng et al., 2008; Colavita and Culotti, 1998; Colavita et al., 1998; Hedgecock et al., 1990; Qin and Powell-Coffman, 2004). **UNC-129** also plays a minor role in the migration of the pharyngeal **M2** motor neuron (Mörck et al., 2003). This role is facilitated by **MNM-2** (M neuron morphology abnormal-2), a Krüppel-like zinc finger protein that affects the **M2** sister cell **M3**, which helps guide **M2** (Rauthan et al., 2007). Although **UNC-129** is required for neuronal guidance during development, it does not appear to be required for axon guidance during regeneration (Gabel et al., 2008).

The two DTCs also use **UNC-129** in their U-shaped migrations that pattern the hermaphrodite gonad (Merz et al., 2003). **UNC-129** in the dorsal body wall muscle attracts the DTCs, promoting their lateral turn from the ventral muscle band and migration toward the dorsal muscle band (distal tip cell migration phase 2) (Merz et al., 2003). Although loss of **UNC-129** does not by itself affect DTC migrations, the need for **UNC-129** is revealed when levels of its receptor, **UNC-5**, are also reduced (MacNeil et al., 2009; Merz et al., 2003). Some gonads of animals with hypomorphic *unc-5* and loss of *unc-129* expression have both proximal and distal gonad ends positioned on the dorsal side, instead of having only the proximal end dorsally positioned and the distal end ventrally positioned. Ectopic expression of **UNC-129** in ventral body wall muscle instead of its normal dorsal body wall muscle site also causes DTCs to mis-migrate (Colavita et al., 1998; Kinnunen et al., 2005; Nash et al., 2000). An *unc-129* null mutation partially suppresses the enhancement by class I *unc-52*/perlecan alleles of *unc-5(e152)* DTC migration defects, suggesting that **UNC-52**, an extracellular matrix heparan sulfate proteoglycan, acts in part through

UNC-129 to direct DTC migration (Merz et al., 2003). This observation is supported by findings in other systems that perlecan modulates TGF- β bio-availability and activity (Lamoureux et al., 2007).

5.2. Male tail development

In addition to its axon guidance role, a role for *unc-129* in male tail sensory ray development has been identified (Ikegami et al., 2004). UNC-129 is expressed in specific ray neurons (rays 1, 3, 5, and 7) and their precursor Rn cells (Ikegami et al., 2004; Nash et al., 2000). Alteration of UNC-129 levels alone has no effect on rays or the formation of ray axon commissures, axon processes that run from one side of the body to the other circumferentially (Ikegami et al., 2004; Jia and Emmons, 2006; Roy et al., 2000). However, a role for UNC-129 is revealed in animals lacking gene products required for cell separation and neuron migrations. *unc-129* interacts genetically with the semaphorin 2a gene *mab-20* and the semaphorin receptor gene *plx-2*/plexin, and acts in parallel with *efn-4*/eprhin, to prevent sensory ray fusions between adjacent rays.

5.3. Regulation of *unc-129* signaling

UNC-129 attracts cells by promoting the formation of UNC-5/UNC-40 receptor heterodimers, which promote long-range cell migration, at the expense of UNC-5 homodimers, which promote short-range migration (Colavita et al., 1998; Gabel et al., 2008; MacNeil et al., 2009). Expression of *unc-129* in ventral body muscles is normally inhibited by the forkhead transcription factor UNC-130 (Nash et al., 2000). Loss of UNC-130 leads to a phenotype that closely resembles that of animals ectopically overexpressing *unc-129* in both dorsal and ventral body wall muscles (Nash et al., 2000). *unc-129* expression may also require the T-box transcription factor MAB-9, as loss of *mab-9* reduces expression of a short *unc-129* promoter fusion with GFP that expresses in DA and SB motor neurons in transgenic animals (Colavita et al., 1998; Pocock et al., 2008). The extracellular matrix constituent TEN-1/teneurin, a single-pass transmembrane protein with EGF-like domains, also appears to normally restrict the expression of *unc-129* in the dorsal muscle, as an *unc-129* gene fusion with GFP is 20-fold upregulated in *ten-1* loss-of-function animals (Mörck et al., 2010).

6. Overlapping functions

6.1. Overlap between TGF- β pathways

In other systems, many TGF- β superfamily members act redundantly. In *C. elegans*, there is some overlap in function between DBL-1 and DAF-7. DBL-1 and DAF-7 pathways appear to both work to maintain a non-dauered animal. Expression of both is lost upon entering dauer conditions. Dauering by loss of DAF-7 pathway function reduces *dbl-1* expression (Liu et al., 2004), and *daf-7* is normally repressed in dauering conditions (Schackwitz et al., 1996). Not only does the DBL-1 pathway respond to dauering cues, but it appears to play a small role in preventing dauering. The *daf-1* Daf-c phenotype is enhanced in double mutant strains *sma-6(lf); daf-1(lf)* (Krishna et al., 1999; Maduzia et al., 2005) and *daf-7(lf); dbl-1(lf)* (Morita et al., 1999), indicating that *sma-6* and *dbl-1* normally function at a low level to repress dauer-promoting signaling.

DBL-1 and UNC-129 pathways both promote proper distal tip cell (DTC) migratory paths through UNC-5/netrin receptor (Merz et al., 2003; Nash et al., 2000). DBL-1, with receptor DAF-4, is proposed to act on the ventral side as a DTC repellant, while UNC-129 acts on the dorsal side to attract DTCs (Nash et al., 2000). This guidance role is not seen in animals where the function of either of these TGF- β s is lost. However, in a hypomorphic *unc-5* mutant background, mutation of either *dbl-1* or *unc-129* causes more DTC slipping than the *unc-5* hypomorphic background alone (Merz et al., 2003).

6.2. Interaction with other pathways

TGF- β pathways interact with other pathways to modulate signaling output, including insulins and Hedgehog-related warthogs (Guo and Wang, 2009). *C. elegans* provides a useful model system to study TGF- β signaling pathways relatively uncomplicated by pathways that can confound analyses in other systems. Many signaling pathways have fewer members in *C. elegans* than in other metazoans and provide clear, distinguishable phenotypes (Pires-daSilva and Sommer, 2003). For example, *C. elegans* contains highly diverged Hedgehog pathway members, which interact with TGF- β at the level of the receptors and/or Smads in other systems, but have no defined role in TGF- β signaling in the nematode (Hao et al., 2006). Largely non-overlapping Hedgehog-related pathways appear to be transcriptionally regulated by DBL-1 or DAF-7 signaling and may be players in transducing TGF- β superfamily signals (Liang et al., 2007; Liu et al., 2004; Roberts et al., 2010). The Hedgehog-related factors

upregulated by DBL-1 signaling are required for normal body size (Roberts et al., 2010; Zugasti et al., 2005). Other signaling pathways, including Wnt/Wg, EGF, and FGF, exist in the nematode but act in tissues distinct from those associated with TGF- β signaling (see [Wnt Signaling](#); [Genomic overview of protein kinases](#); [Vulval development](#)). In this section, we review some of the molecular pathway interactions that are conserved in other systems.

Insulin pathway interactions

In genetic studies of dauer development, three interacting signaling pathways were found to be involved: the DAF-7 pathway, an insulin signaling pathway, and a cyclic nucleotide pathway. In addition to their shared role in dauer development (see [section 3.1](#)), the insulin and DAF-7 pathways also possess common functions in the regulation of fat metabolism and longevity (see [sections 3.2](#) and [3.4](#)). In regulation of dauer development, inputs from the DAF-7 and insulin pathways must be integrated to determine the organism's response. Some of this integration is mediated by the nuclear hormone receptor DAF-12 (Antebi et al., 2000; Snow and Larsen, 2000). Like *daf-3* and *daf-5*, *daf-12* mutations suppress the DAF-7 pathway *Daf-c* phenotype. Unlike *daf-3* and *daf-5*, however, *daf-12* also shows strong suppression of mutations in the insulin-related and cyclic nucleotide pathways and 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. This structural complexity may contribute to its functional complexity. Crosstalk between the TGF- β and insulin dauer pathways may also be mediated in part by the regulation of insulin ligand expression by the DAF-7 pathway (Liu et al., 2004; Shaw et al., 2007). Finally, the DAF-7 pathway promotes secretion of the insulin-like ligand DAF-28 (Park et al., 2012a). This effect is mediated by an interaction between DAF-8/Smad and NHR-69/nuclear hormone receptor that represses expression of *exp-2*, a potassium channel gene, to promote DAF-28 secretion. Thus, DAF-7/TGF- β signaling can promote insulin pathway activity via multiple mechanisms.

Interaction of the insulin pathway with the DBL-1 pathway has been less well studied. The DBL-1 and insulin pathways both regulate reproductive aging, but they appear to do so in parallel (Luo et al., 2010). On the other hand, the DBL-1 pathway, like the DAF-7 pathway, regulates the expression of insulin-like ligands (Liang et al., 2007; Luo et al., 2010). Furthermore, the DBL-1 pathway regulates the expression of a subset of known DAF-16/FOXO transcriptional targets such as *fat-6* and *fat-7* (Liang et al., 2007). Finally, some *daf-2*/insulin receptor mutant animals have increased body size, suggesting an antagonistic interaction with the DBL-1 pathway in the regulation of growth (McCulloch and Gems, 2003). Additional studies will be necessary to determine whether these observations reflect a relevant regulatory interaction between the DBL-1 and insulin pathways.

Chemosensory pathways

Some signaling pathways and physiological processes affect body size by means that may partially merge with DBL-1 signaling. For example, several genes that function in chemosensation result in alterations in body size. These include *tax-6* and *cnb-1*, which encode the catalytic and regulatory subunits of calcineurin, respectively. Calcineurin is a protein phosphatase 2B (PP2B) that controls intracellular Ca²⁺ signaling and mediates transcriptional responses responsible for a variety of physiological processes in many systems. *tax-6* interacts with *kin-29* (a.k.a. *sma-11*), which encodes a serine/threonine kinase that is a downstream target of DBL-1 signaling (see [section 2.8](#)) (Maduzia et al., 2005; Singaravelu et al., 2007).

egl-4, a gene encoding cGMP-dependent protein kinase, acts downstream of chemosensory genes to regulate body size by repressing the DBL-1 pathway (Fujiwara et al., 2002; Hirose et al., 2003). *egl-4* is expressed in neurons, hypodermis, intestine, and body wall muscles (Fujiwara et al., 2002; Hirose et al., 2003; L'Etoile et al., 2002). Expression of *egl-4* in neurons or in hypodermis can rescue the organ size defects nonautonomously (Nakano et al., 2004). Interestingly, EGL-4 protein directly interacts with KIN-29 (Lanjuin and Sengupta, 2002; Maduzia et al., 2005; van der Linden et al., 2008). *egl-4* also acts downstream of chemosensory genes to regulate dauer entry and other metabolic responses (see [sections 3.2](#) and [3.3](#)).

Receptor tyrosine kinase signaling

scd-2 (suppressor of constitutive dauer-2) mutant alleles were identified in a genetic screen for suppressors of the TGF- β dauer-constitutive phenotype (Inoue and Thomas, 2000b) and as wild isolates from an oasis in a California desert (Reiner et al., 2008). SCD-2 is the *C. elegans* ortholog of anaplastic lymphoma kinase (ALK), a receptor tyrosine kinase. SCD-2, its ligand HEN-1, the adaptor SOC-1, and the downstream MAPK SMA-5 regulate dauer development upstream of DAF-3/Co-Smad but in parallel with DAF-7 (Reiner et al., 2008). The SCD-2

pathway is specific to the DAF-3-mediated dauer response, as it has no effect on the other phenotypes of the DAF-7 pathway.

In addition to MAPK pathways, receptor tyrosine kinases can also signal through the STAT pathway (Levy and Darnell, 2002). The *C. elegans* STAT homolog *STA-1* is expressed in chemosensory neurons in the head, and its expression is negatively regulated by *daf-7*, *daf-4*, and *daf-8* (Wang and Levy, 2006). Furthermore, loss of *sta-1* activity enhances the dauer-constitutive phenotypes of *daf-7*, *daf-4*, and *daf-14* mutant animals, but not of *daf-1* and *daf-8* mutants. These results indicate that *STA-1* negatively regulates dauer formation in a DAF-1- and DAF-8-dependent manner, and points to the existence of crosstalk between the STAT and TGF- β pathways.

7. Future directions

In humans, misregulation of TGF- β superfamily pathways leads to developmental defects and diseases, such as vascular defects, bone abnormalities including dwarfism, overgrowth syndromes including Marfan syndrome, midline defects, and cancers (Padua and Massagué, 2009; Serra and Chang, 2003; Wu and Hill, 2009). What challenges remain for the future that can be addressed using the *C. elegans* model system? We can anticipate that new genetic, molecular, and chemical screens will uncover additional protein, RNA, and lipid molecules that modulate TGF- β signaling, as well as new functions for known signaling components. New factors may function to stimulate or restrict TGF- β signaling in the sending cell, in the extracellular space, or in the cytoplasm or the nucleus of the receiving cell. Furthermore, studies of the transcriptional outputs of TGF- β signaling in *C. elegans* are still in their infancy, while studies of non-transcriptional outputs have, for the most part, not yet been carried out. These areas of research will shed light on the spectrum of possible cellular responses to TGF- β signaling, identifying both conserved and nematode-specific physiological roles.

An advantage of working in a genetic model system such as *C. elegans* is the ability to test perturbations within the context of the intact animal. For this reason, we expect that research in *C. elegans* will continue to provide insight into the difficult questions of how TGF- β signaling is integrated with other signaling pathways, with developmental and cell cycle timing, and with environmental cues. Furthermore, a critical area of study for the TGF- β field is to understand how TGF- β signaling achieves such remarkable context dependence. For example, many TGF- β superfamily ligands act as morphogens during development, specifying different outcomes at different concentrations (McDowell and Gurdon, 1999; Podos and Ferguson, 1999). In addition, TGF- β itself plays dual, opposing roles in cancer progression: as a tumor suppressor early in tumor progression and then as a pro-oncogenic factor later in carcinogenesis (Roberts and Wakefield, 2003). By studying how two simple TGF- β signaling pathways can achieve a wide variety of biological outcomes in *C. elegans*, mechanisms for context dependence will be identified that can then be tested in other systems. In summary, the simple, yet powerful, model system *C. elegans* promises to continue to provide insight into evolutionarily conserved molecules and mechanisms relevant to understanding not only dynamic cellular processes but also pathological states including developmental disorders and cancers.

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