Homologs of the Hh signalling network in *C. elegans*[*]

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Abstract

In *Drosophila* and vertebrates, Hedgehog (Hh) signalling is mediated by a cascade of genes, which play essential roles in cell proliferation and survival, and in patterning of the embryo, limb buds and organs. In *C. elegans,* this pathway has undergone considerable evolutionary divergence; genes encoding homologues of key pathway members, including Hh, Smoothened, Cos2, Fused and Suppressor of Fused, are absent. Surprisingly, over sixty proteins (i.e. WRT, GRD, GRL, and QUA), encoded by a set of genes collectively referred to as the Hh-related genes, and two co-orthologs (PTC-1,-3) of fly Patched, a Hh receptor, are present in *C. elegans*. Several of the Hh-related proteins are bipartite and all can potentially generate peptides with signalling activity, although none of these peptides shares obvious sequence similarity with Hh.

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In addition, the *ptc*-related (*ptr*) genes, which are present in a single copy in *Drosophila* and vertebrates and encode proteins closely related to Patched, have undergone an expansion in number in nematodes. A number of functions, including roles in molting, have been attributed to the *C. elegans* Hh-related, PTC and PTR proteins; most of these functions involve processes that are associated with the trafficking of proteins, sterols or sterol-modified proteins. Genes encoding other components of the Hh signalling pathway are also found in *C. elegans,* but their functions remain to be elucidated.

1. Introduction

The *Drosophila hedgehog (hh)* mutant was identified by Nüsslein-Volhard and Wieschaus in their classic genetic screen that led to the identification of key embryonic segment polarity genes (Nüsslein-Volhard and Wieschaus, 1980). Subsequently, the single *Drosophila* and the multiple vertebrate Hh proteins, Sonic Hedgehog (Shh), Indian Hedgehog (Ihh) and Desert Hedgehog (Dhh), have collectively proven to be key effectors in patterning the embryo, limb buds and neural tube, organ formation, cell proliferation, axon guidance and cell survival (for reviews, see Ingham and McMahon, 2001; Lum and Beachy, 2004). Mutations in genes constituting the Hh signalling pathway are also implicated in various human syndromes and cancers (Bale, 2002; Beachy et al., 2004; Pasca di Magliano and Hebrok, 2003; Taipale and Beachy, 2001). Details of the pathway are summarised in Figure 1 (Ingham and McMahon, 2001; Lum and Beachy, 2004). The various proteins involved in the Hh and Ptc signalling pathway, including those present in *C. elegans*, are listed in Table 1.
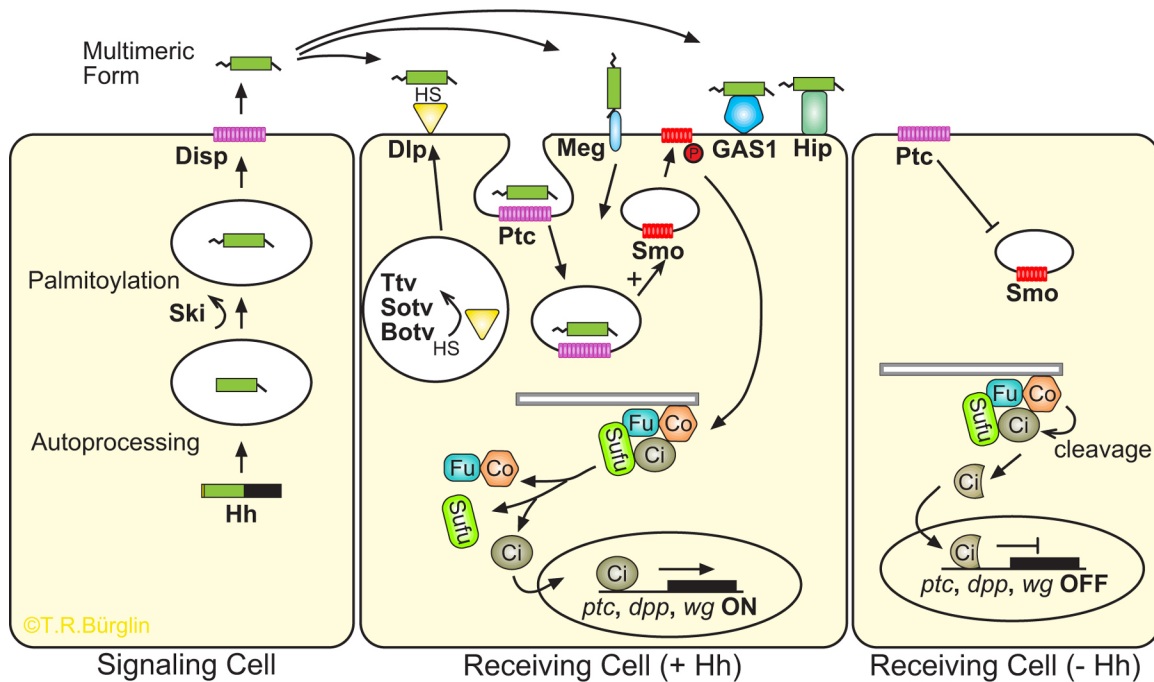

Figure 1. Hh encodes a secreted morphogen that activates a signal transduction pathway. Figure is based on studies from *Drosophila* and mammals and is adapted from Cohen (2003). A description of genes and abbreviations is provided (Table 1; for reviews, see Lee et al., 1992; Mohler and Vani, 1992; Tabata et al., 1992; Tashiro et al., 1993). Left panel: Hh is produced, autoprocessed and modified by Ski. N-Hh is released by Disp. Middle panel: The N-Hh signal is propagated by the HSPG Dlp, which is modified by transferases such as Ttv, Sotv, Botv that add heparan sulphate (HS). Other molecules that can bind Hh signal are megalin, Hip and GAS1. N-Hh is an antagonistic ligand that represses the Patched (Ptc) receptor, before being internalised, and releases the latent activity of the Smoothened (Smo) seven-pass membrane protein, which is related to the Frizzled family of receptors (Alcedo et al., 1996; Chen and Struhl, 1998; Nusse, 2003; van den Heuvel and Ingham, 1996). In turn, Cos2 is released from microtubules and the Cos2/Fu/Su(fu)/Ci complex releases Ci, which can then enter the nucleus to activate transcription (Hooper, 2003; Jia et al., 2003; Lum et al., 2003; Ogden et al., 2003; Ruel et al., 2003). Several molecules involved in modifying the Cos2//Fu/Su(fu)/Ci complex are not shown. Right panel: In the absence of N-Hh, Ptc inhibits Smo, the Cos2/Fu/Su(fu)/Ci complex remains associated with microtubules, Ci is phosphorylated and cleaved by Cos2. The Ci fragment now acts as a transcriptional repressor. Details of the pathway relevant for *C. elegans* are provided in the text. Grey bar represents microtubules.

2. Hh pathway components in *C. elegans*

What role does the Hh/Ptc signalling pathway play in *C. elegans* development? To address this question, it is first reasonable to ask whether the major proteins of the pathway are encoded by the *C. elegans* genome, a task that is simplified by the availability of the complete genome sequence (Table 1; *C. elegans* Sequencing Consortium, 1998). Surprisingly, genes encoding recognisable Hh and Smo proteins are absent. In addition, homologs for Cos2, Fu and Su(fu), which transduce the Hh signal from Smo to Ci, are absent. The Ci homolog itself, TRA-1, is not essential for embryonic pattern formation, but continues to act at the transcriptional level to control sexual cell fate decisions and male gonadal development (Hodgkin, 1983; Mathies et al., 2004), although there is no evidence that the nuclear localisation or activity of TRA-1 is controlled in the same manner as Ci (Kuwabara et al., 2000; Zarkower and Hodgkin, 1992). It has been speculated that the *C. elegans* sex determination pathway could be evolutionarily related to the Hh/Ptc pathway because additional similarities have also been noted between the two pathways (Kuwabara et al., 1992).

Table 1. Components of the Hh signaling network. Note: the function of many of the *C. elegans* components is not clear and may be different from that in *Drosophila* and/or mammals. The abbreviations used for the hh pathway in Figure 1 are highlighted in yellow in this table. Slashes separate synonymous gene names, parentheses contain the abbreviations of the full gene names, comas separate paralogs.

Role	*C. elegans*	*Drosophila*	Mammals
Signal			
Hint and associated domains	*qua-1, wrt-1 - wrt-10, grd-1 - grd-17, grl-1 - grl-32, hog-1*	*hedgehog (hh)*	*Sonic hedgehog (Shh), Indian hedgehog (Ihh), Desert hedgehog (Dhh)*
Palmitoylation, membrane bound O-acyl transferase, MBOAT	*Y57G11C.17, ZC101.3*	*rasp/skinny hedgehog (ski)/sightless*	*Ski1/Skn/HHAT*
12-Pass TM, SSD	*che-14, ptd-2*	*dispatched (disp)*	*DISP*
Signal modulators			
Hip module (homologs in plants, bacteria)	–	–	*Hedgehog interacting protein (Hip1/Hip)*
GPI-anchored external plasma membrane glyco-protein (growth arrest when overexpressed)	*phg-1*	–	*Growth arrest specific 1 (Gas1)*
Low density lipoprotein receptor family	*lrp-1*		*Megalin/gp330/LRP2*
Glycosyltransferases involved in Heparan sulfate proteoglycan (HSPG) synthesis, exostosin domain	*rib-1* (lacks C-term. putative catalytic region with DXD motif)	*tout-velu (ttv)*	*EXT1*
	rib-2	*brother of tout-velu (botv)*	*EXTL3*
	–	*sister of tout-velu (sotv)*	*EXT2*
Glypican: heparan sulfate proteoglycan anchored to the cell membrane by a GPI-anchor	*gpn-1*	*dally-like (dlp/dly)*	*Glypican-6*
Receptor			
12-Pass TM, SSD	*ptc-1, ptc-3*	*patched (ptc)*	*PTCH1, PTCH2*
Signal transduction			
7TM serpentine receptors	–	*smoothened (smo)*	*SMO*

Role	*C. elegans*	*Drosophila*	Mammals
Serine/threonine kinase (homologs in plants)	–	*fused (fu)*	*FU*
Sufu domain (homologs in bacteria)	–	*Suppressor of fused (Su(fu))*	*SUFU*
Kinesin-like	–	*costal 2/costa (cos)*	*KIF27, KIF7*
Zinc finger transcription factor	*tra-1*	*cubitus interruptus (ci)*	*Gli1, Gli2, Gli3*

The *C. elegans* genome encodes an abundance of what will be collectively referred to as Hh-related (Hh-r) proteins, three Patched proteins and 24 Patched-related proteins (PTR). Four Frizzled receptor homologues (see Wnt signaling), as well as a series of molecules participating in the early stages of Hh signalling are also present. Given our current understanding of the Hh/Ptc signal transduction pathway in other organisms, the presence of multiple *C. elegans* Hh-r, PTC and PTR proteins and the absence of Hh, Smo and other components is unexpected and raises a number of questions about the evolution and the function of these genes in *C. elegans*.

3. Hh-r genes, the structure

The Hh proteins are composed of an N-terminal signalling domain, Hh-N, and a C-terminal autoprocessing domain, Hh-C. Hh-C has autoproteolytic activity, which cleaves Hh and subsequently attaches a cholesterol moiety to the Hh-N C-terminus (see scheme Figure 2A; Lee et al., 1994; Porter et al., 1996). Hh-C shares sequence similarity with the self-splicing inteins; hence, this region was named Hint (Dalgaard et al., 1997; Hall et al., 1997; Koonin, 1995). The 60 terminal residues of Hh-C are named the sterol recognition region (SRR) because they are required for adding cholesterol to Hh-N (Figure 2A; Beachy et al., 1997). Here, we refer to the combined Hint-SRR domain as the Hog domain.

Surprisingly, early database searches uncovered eight distinct *C. elegans* ORFs sharing similarity to Hh (Bürglin, 1996; Porter et al., 1996). Further investigation revealed that the sequence similarity shared by Hh and these ORFs was confined only to the Hog domain. Analyses of the N-terminal regions revealed several new motifs (Bürglin, 1996; Porter et al., 1996), which were also found in other ORFs that lacked Hog domains. In mimicry of the bipartite "Hedge-hog" structure, the three new gene families were named, *warthog (wrt)*, *groundhog (grd)* and *ground-like (grl)* and the protein domains found at their respective N-terminal regions were called Wart, Ground and Ground-like (Aspöck et al., 1999; Bürglin, 1996). The final additions to the nomenclature are two previously unnamed single copy genes, *qua-1* for *quahog* (T05C12.10/M110, Hao et al., 2006b), and an ORF consisting only of the Hog domain, *hog-1* (W06B11.4). The structural organization of Hh and the Hh-r genes is shown in Figure 2B.

Over 60 Hh-r genes have been identified in *C. elegans*, most of which are expressed, although a few appear to be pseudogenes (e.g., *grd-17*; Figure 2B). Many of these genes share a one to one orthology to *C. briggsae* genes, although several have arisen through recent duplications within the *C. elegans* lineage. Preliminary searches of the ongoing *Brugia malayi* genome project reveal that representatives of the different *C. elegans* families are also present (Figure 2B). This clearly demonstrates that these Hh-r gene families were already present in the last common ancestor of *C. elegans* and *B. malayi* more than 300 million years ago.

The Wart, Ground, Ground-like and Qua domain each have a characteristic pattern of conserved cysteine residues (Figure 2C). The Ground and Ground-like domains differ in their arrangement of these conserved cysteine residues (Aspöck et al., 1999). In some instances, it has been possible to predict likely disulfide bond arrangements because of pairwise cysteine substitutions (Figure 2C; Aspöck et al., 1999). The Wart domain is about twice the size of the Ground and Ground-like domain; however, the C-terminal half of the Wart domain has a cysteine arrangement similar to that of the Ground domain and both share a short conserved motif similarly positioned between cysteine residues (FDøI, FEøV, respectively, Figure 2C; Aspöck et al., 1999). Thus, it is likely that the C-terminal part of the Wart domain and the Ground domain was derived from the same ancestral domain. The short conserved FDøV "core" motif is also present in the Hh-N core, although its position relative to the cysteine residues is different (Figure 2C; Aspöck et al., 1999).
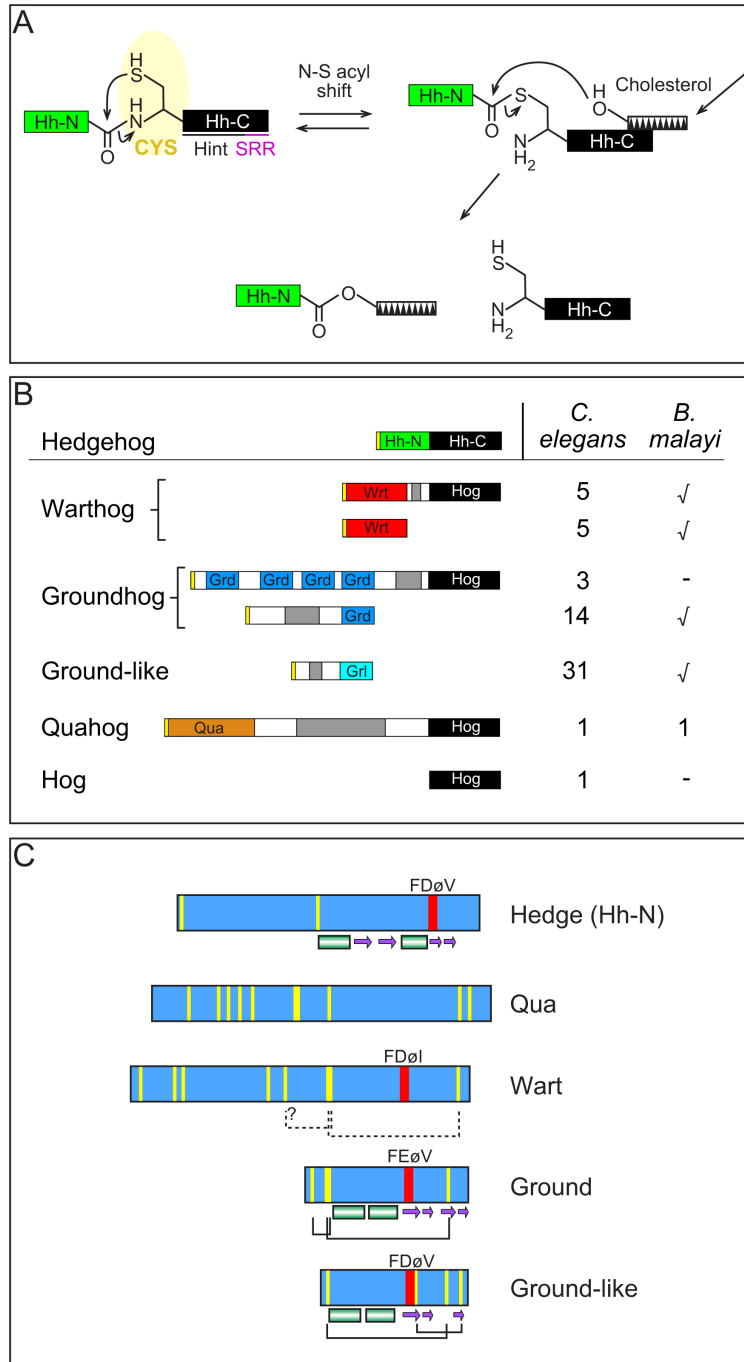
Figure 2. Structural features of the *C. elegans* Hh-r proteins . A) Intramolecular autoprocessing of Hh. The extent of the Hint domain and the sterol recognition region (SRR) is underlined beneath Hh-C; in *C. elegans* the SRR region within the Hog domain is called ARR, since it is unknown if it binds cholesterol (Beachy et al., 1997). The conserved first residue (cysteine) of the Hint domain is highlighted in yellow. The toothed box represents cholesterol. Acids and bases assisting in catalysis are not shown (adapted from Hall et al., 1997). B) Schematic representation of the conserved domains in Hh, WRT, GRD, GRL, QUA-1 and HOG-1 proteins. The yellow box at the N-terminus indicates the signal sequence for secretion and the black box represents the Hog domain. The green, red, blue, light blue and orange boxes indicate the Hh-N ("Hedge"), Wart, Ground, Ground-like, and Qua domains, respectively. The grey regions indicate regions with repetitive sequences of varying lengths. The first column on the right indicates the number of members of each family that are present in *C. elegans*. The second column shows a tick mark when members of these families have been detected in *B. malayi*, but the precise number has not yet been determined. C) Schematic representation of the Hh-N, Qua, Wart, Ground, and Ground-like domains. Yellow lines represent conserved cysteine residues. The red bar indicates a short conserved motif that often starts with a phenylalanine, followed by an acidic residue and two hydrophobic residues; ø stands for a small hydrophobic residue. Black solid lines beneath the domains mark predicted disulfide bonds, dashed lines are tentative predictions. The cylinders and arrows beneath Hh-N indicate alpha helices and beta-sheets, respectively, taken from the solved 3D structure (Hall et al., 1995). Cylinders and arrows beneath the Ground and Ground-like domain were produced by secondary structure prediction of the multiple aligned sequences (Aspöck et al., 1999).

4. Modification and function of Hh-r genes

All Hh-r genes in *C. elegans*, apart from *hog-1*, are predicted to encode secreted molecules. Porter et al. (1996) have shown that WRT-1, similar to Hh, undergoes autoprocessing; thus it is expected that other Hh-r proteins with a Hog domain will also have autocatalytic properties. However, it remains unclear whether the Hh-r proteins can bind cholesterol or other sterols, because the region corresponding to the SRR of Hh is slightly different in the Hh-r proteins (see Figure 1A in Aspöck et al., 1999).

An expression survey of *wrt* and *grd* genes revealed that they are expressed primarily in ectodermal tissues, particularly the hypodermis and *seam* cells, but can also be found in neurons, neuron-associated cells and gland cells (Aspöck et al., 1999). *qua-1* is also expressed in the hypodermis (Hao et al., 2006b). An internal deletion in *qua-1* results in severe molting defects (Hao et al., 2006b). Functional studies of other wrt genes are in progress and preliminary results indicate that they also play important roles in development. For example, *wrt-5* functions in the hypodermis during embryogenesis (Hao et al., 2006a). At present it is still unclear how Hh-r molecules function, and whether their activity could relate to the function of the PTC and PTR proteins.

In *Drosophila*, Hh is further modified by Skinny hedgehog (Ski), which palmitoylates the N-terminus of Hh-N, an essential process for Hh function (Chamoun et al., 2001). *C. elegans* has two co-orthologs, Y57G11C.17 and ZC101.3 (T.R. Bürglin, unpublished), but large-scale RNAi experiments have not uncovered any associated mutant phenotype. Modified Hh is secreted with the help of Dispatched; *C. elegans* has two Disp homologs, CHE-14 and PTD-2 (see below).

Secreted Hh forms a multimeric complex that moves through the extracellular matrix space. Heparan sulphate proteoglycans (HSPG) play an important role in Hh signal transduction. Three *Drosophila* genes, *tout-velu (ttv)*, *sister of tout-velu (sotv)*, and *brother of tout-velu (botv)* encode glycosyltransferases of the EXT family that are required for the biosynthesis of heparan sulfate and are essential for the movement of N-Hh (Han et al., 2004a; Takei et al., 2004). One target for these enzymes is Dally-like (Dlp), a glypican of the HSPG family that is also essential for Hh signalling (Desbordes and Sanson, 2003; Han et al., 2004b; Lum et al., 2003). *C. elegans* has two EXT family members, *rib-1* and *rib-2* (for review, see Berninsone and Hirschberg, 2002). *rib-1* is orthologous to ttv and EXT1, and *rib-2* is ortholgous to *botv* and EXTL3; however, orthologs of other EXT members are absent (Figure 3). The EXT proteins have two domains: the exostosin domain and a 300 amino acid C-terminal region that is highly conserved between members and probably corresponds to the catalytic transferase domain, which is missing in *rib-1*. Homozygous *rib-2* animals show delayed larval growth and reduced life span, while their progeny display more than 90% early embryonic lethality (Morio et al., 2003). The *C. elegans* Dally-like gene ortholog, *gpn-1*, shows no phenotype in large-scale RNAi screens.

Two other molecules that bind and restrict the availability and range of N-Hh are Hip and Growth arrest specific 1 (GAS1; for review, see Cohen, 2003). Hip is absent in both *Drosophila* and *C. elegans*. GAS1 is a GPI-linked protein identified by its ability to arrest cell cycle progression when overexpressed. The *C. elegans* ortholog *phg-1/phas-1* is expressed in the pharynx and may play a similar role in mitotic cell cycle regulation (Agostoni et al., 2002). Megalin, a member of the family of low-density lipoprotein receptors, is a third protein that binds to N-Hh (see Cohen, 2003). The *C. elegans* homolog *lrp-1* is expressed in the hypodermis; mutations in *lrp-1* produce molting defects (Yochem et al., 1999).
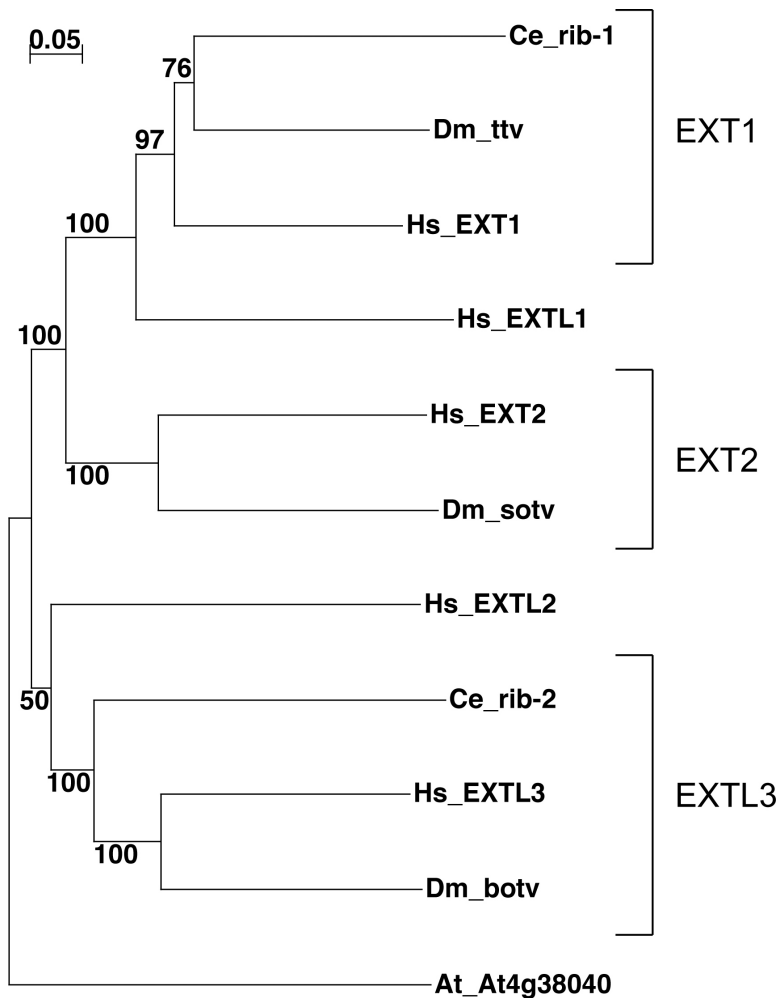
Figure 3. Phylogenetic tree of exostosin domain proteins from *C. elegans* (*Ce*), *Drosophila* (*Dm*) and human (*Hs*). A multiple sequence alignment was produced using Clustal X (Thompson et al., 1997) and the built-in neighbor joining was used to generate a tree using the default values (not corrected for multiple substitutions, gaps not excluded). 1000 bootstrap trials were run, and trial values are shown in the figure as percentages. An *Arabidopsis thaliana* (*At*) exostosin domain containing protein was included as an outgroup. The three families conserved between flies and human are indicated.

5. SSD Proteins: PTC and PTC-related proteins

Three *ptc* genes, *ptc-1*,-2,-3 have been identified in *C. elegans*, although *ptc-2* is absent in *C. briggsae* and is likely to be a pseudogene arising from a recent duplication of *ptc-1*. In addition, 24 genes have been identified that encode proteins sharing both sequence and topological similarities to PTC; these proteins have been named PTR (for Patched-related; Figure 4).

Drosophila, human and mouse are each predicted to encode a single PTR protein; thus the PTR family appears to have undergone an expansion in *C. elegans* (Kuwabara et al., 2000). Moreover, all 24 *ptr* genes are present in *C. briggsae* and share a one to one orthology with *C. elegans*.
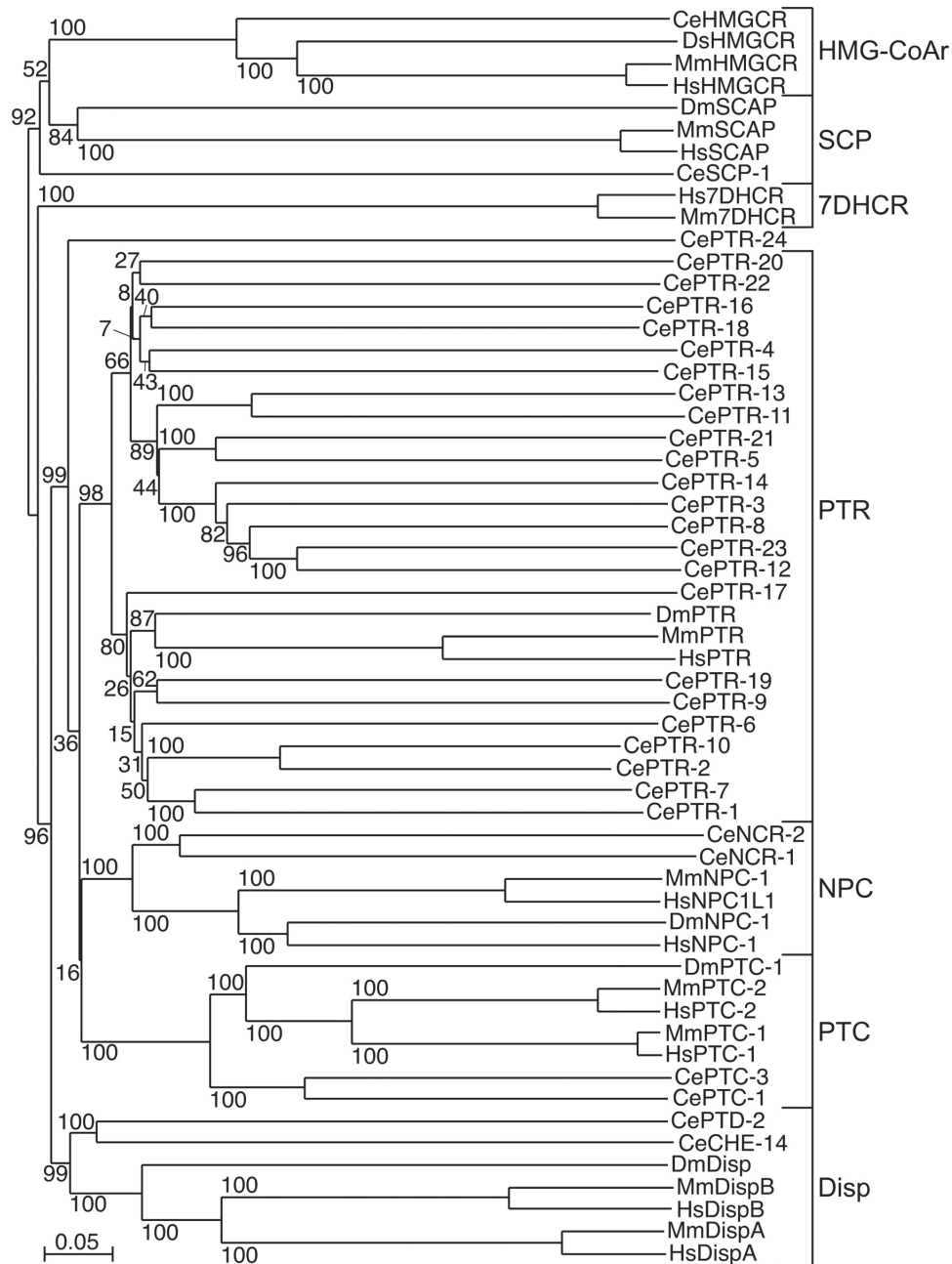
Figure 4. Phylogenetic tree of SSD proteins. A multiple sequence alignment of *C. elegans* (*Ce*), *Drosophila* (*Dm*) and human (*Hs*) SSD proteins was produced in Clustal X and the built-in neighbor joining was used to generate a tree using the default values (not corrected for multiple substitutions, gaps not excluded). 1000 bootstrap trials were run, and trial values are shown in the figure as percentages. Seven families of SSD proteins can be distinguished. Based on the bootstrap values, the PTC, NPC, Disp and PTR are more similar to each other. It is clear that the PTR family has expanded substantially in nematodes. PTR-24 does not fall within the clade of the PTR family, but we still believe it to be a divergent member of the PTR family. The PTR family itself seems to be composed of two subfamilies that each diverged and expanded to an equal extent. MSA data provided by O. Zugasti.

The *C. elegans* PTC and PTR proteins share characteristics common to all known Ptc proteins. The PTC and PTR proteins are predicted to have 12-membrane spanning domains with cytoplasmic N- and C-termini. The membrane domains can be further sub-divided into two cassettes of 1+5, which are separated by a large intracellular loop. Each cassette contains a large extracellular loop predicted to facilitate Hh binding (Figure 5). Carried within the first set of TM domains is a sterol-sensing domain (SSD), which was first identified in SCAP, a protein involved in cholesterol homeostasis (Radhakrishnan et al., 2004).
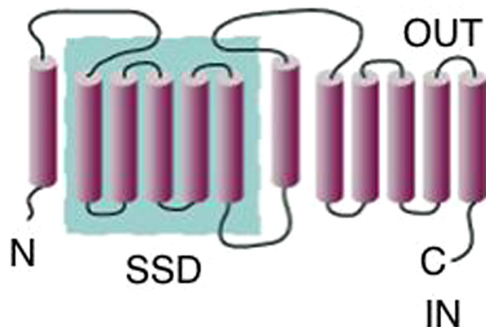
Figure 5. Predicted topologies of PTC and PTR proteins. PTC and PTR proteins are predicted to encode proteins with 12-membrane spanning domains and share similarity with RND (Resistance, Nodulation, cell Division) bacterial multidrug transporter proteins (Hooper and Scott, 1989; Ingham et al., 1991; Kuwabara and Labouesse, 2002; Nakano et al., 1989). Blue inset highlights the five consecutive membrane-spanning domains that form the sterol-sensing domain (SSD).

6. Role of PTC proteins in *C. elegans*

ptc-1 is the first member of the *ptc* and *ptr* family to be analysed in detail (Kuwabara et al., 2000). Surprisingly, it was found that *ptc-1* plays a crucial role in germline cytokinesis. Elimination of *ptc-1* activity either by RNAi or mutation produces sterile adults that carry multinucleate oocytes and sperm. In other respects, development of a *ptc-1* mutant is essentially wild type. *In situ* hybridization reveals that *ptc-1* mRNA is maternally provided, and becomes enriched in P4, the germ line precursor. Immunocytochemistry performed using a polyclonal antibody capable of detecting PTC-1 protein in wild type adult hermaphrodites reveals that PTC-1 protein is enriched in the plasma membrane of mitotic germ cells undergoing proliferation, or in membranes undergoing rapid expansions, such as oocytes. PTC-1 protein is particularly enriched in vesicles at the apex of the membrane furrows separating individual germ nuclei.

An analysis of *ptc-1* mutants further revealed that the absence of cytokinesis generated membrane furrows leads to a loss of cell autonomous development in the germline syncytium, as evidenced by the presence of clusters of nuclei in M-phase (Kuwabara et al., 2000). The involvement of *ptc-1* in cytokinesis has led to the hypothesis that PTC-1 could play a role in the transport of lipids, proteins or lipoproteins to the cleavage furrow. Importantly, a proteomic survey of proteins involved in mammalian cytokinesis reveals that Ptc-1 is a component of the mid-body (Skop et al., 2004). Hence, the involvement of PTC-1 in cytokinesis is likely to highlight an ancestral function for Ptc proteins, which is apparently independent of Smoothened.

A mutation has also been obtained for *ptc-3*. Preliminary phenotypic characterisation indicates that *ptc-3* is an essential gene; mutants arrest in late embryogenesis (P.E.K., unpublished; Zugasti et al., 2005). A survey of the other *ptr* genes by RNAi reveals that many have roles in molting, but also affect growth and morphogenesis (Zugasti et al., 2005). It has also been demonstrated that *C. elegans daf-6* encodes a Patched-related protein required for lumen formation (Perens and Shaham, 2005).

7. Other SSD proteins provide clues to PTC and PTR function

Another clue to the function of PTC-1 in worms is provided by the presence of an SSD and its connections to sterols and protein transport. The SSD of SCAP, the first identified SSD protein, is capable of binding directly to cholesterol (Radhakrishnan et al., 2004; Figure 4). Other SSD protein families that are closely related to PTC are the Dispatched family, which includes CHE-14 and PTD-2, and the Niemann-Pick Type C family, which includes the NCR-1, -2 proteins in *C. elegans* (Kuwabara and Labouesse, 2002). *che-14* was first identified in a screen for animals that were defective in dye-filling (Perkins et al., 1986). Subsequent work demonstrated that *che-14* promotes apical sorting in epithelial cells. *che-14* mutants accumulate vesicles that are trapped near the apical surface, indicative of problems in protein secretion (Michaux et al., 2000). The phenotype of *che-14* mutants reveals that the problem is more likely to be associated with defects in exocytosis, rather than in endocytosis. The presence of an SSD in CHE-14 led the authors to speculate that CHE-14 might be involved in the secretion of proteins containing sterol or GPI adducts. Similarly, Disp promotes the exocytosis of Hh away from the cells from which it was synthesised (Burke et al., 1999). A protein transported by CHE-14 might be WRT-6, which is expressed in sensory socket cells (Aspöck et al., 1999).

Niemann-Pick disease type C1 (NPC1) is a neurodegenerative disease associated with defects in cholesterol storage/transport. In *C. elegans*, *ncr-1; ncr-2* double mutants are hypersensitive to cholesterol deprivation and inappropriately form dauers under favourable food conditions, a dauer-constitutive (Daf-c) phenotype (Sym et al., 2000). Recent studies further show that *ncr-1* is hypersensitive to progesterone, a known inhibitor of cholesterol trafficking (Li et al., 2004). Hence, in *C. elegans* there is a strong link between SSD proteins and the transport of sterols, sterol modified proteins or in the case of dauer formation, a sterol modified hormone.

C. elegans are cholesterol auxotrophs, requiring exogenous cholesterol for growth and survival (Chitwood, 1999; Hieb and Rothstein, 1968). Sterols are involved in at least two processes in the worm: the decision to enter diapause, which is mediated by a sterol-derived hormone, and molting, which is impaired by sterol depletion (Matyash et al., 2004). Animals depleted for cholesterol arrest in the second generation during early larval stages (Matyash et al., 2001; Merris et al., 2003). These L2 arrested larvae became dauer-like and fail to complete molting (Matyash et al., 2004).

Because worms require so relatively little cholesterol, it has been postulated that cholesterol is unlikely to be an essential architectural component of membranes and could play a more important role in signalling (Matyash et al., 2001). Mutations in *lrp-1*, *qua-1* and RNAi inhibition of most *ptr* genes, and post-embryonic RNAi of *ptc* gene activity all lead to defects in molting (Yochem et al., 1999; Zugasti et al., 2005; Hao et al., 2006b). Thus, taken together, it is speculated that the PTC and PTR proteins could promote the transport of sterols or sterol-modified proteins, possibly including sterol-modified Hh-r proteins.

8. Evolution

C. elegans lacks the typical Hh signal transduction cascade found in flies and vertebrates that is composed of Smo, Fu, Su(fu), and Cos2. Since homologs of Fu and Su(fu) are found in plants and bacteria (T.R.B., unpublished), it follows that this part of the pathway was lost in nematodes. Concomitantly, we have observed a large increase in the number of *C. elegans* Hh-r and *ptr* genes. Several lines of evidence point towards the Hh-r expansion being nematode specific: 1) Homologs of the Hh-r genes have so far not been identified outside of the nematodes. 2) Conversely, Hh is present in both arthropods and vertebrates and only missing in nematodes. 3) Phylogenetic analyses of the Hog domain and motif similarity shared by the Wrt and Grd domains indicate that the *wrt*, *grd* and *grl* gene families are derived from a single ancestral gene (Aspöck et al., 1999); *qua-1* may also be derived from the same ancestral gene, as its Hog domain is also more similar to those of the Wrt and Grd proteins than to that of Hh. 4) *C. briggsae* has fewer Hh-r genes than *C. elegans*, and *B. malayi* even fewer, which underscores the capacity of these genes to expand. The expansion of the *ptr* genes is likewise nematode specific, since *Drosophila* and humans have only a single copy.

There is a running debate whether or not nematodes should be classified as ecdysozoa. If nematodes are indeed ecdysozoa, or group at least within the protostome branch of ecdysozoa and lophotrochozoa (see chapter on evolution), then the loss, expansion and diversification of various Hh pathway components, which have been described here, are most parsimoniously explained by postulating that a *C. elegans* Hh signalling pathway did exist, but was disrupted at some unknown time in early nematode evolution. It also implies that the Hh-r genes were derived from Hh. On the other hand, if nematodes are an outgroup to coelomata, then one of several alternatives is that a complete Hh signalling pathway was never acquired.

9. Summary

It is clear that the Hh and Ptc homologs in *C. elegans* have undergone considerable evolutionary divergence when compared to their *Drosophila* and vertebrate counterparts. Studies in *C. elegans* are providing insights into the roles and mechanisms underlying the control of the Hh-r, PTC and PTC-related proteins and have established that these proteins can function independently of Smo. The involvement of Hh-r and *ptr* genes in molting suggests that at least some of the Hh-r and *ptr* genes can participate in similar pathways. Thus, it will be of particular interest to determine whether the Hh-r proteins and the PTC and PTR proteins have maintained a ligand/receptor relationship that could potentially mediate cell-cell signalling.

10. Acknowledgements

11. References

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