brain tissues. Briefly, cerebral cortices were lysed in RIPA buffer; after centrifugation, insoluble material was extracted in formic acid, as described elsewhere[30]. RIPA- and formic-acid-extracted samples were diluted, and Aβ levels were measured by sandwich ELISA, as above.

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Caenorhabditis elegans early embryogenesis and vulval morphogenesis require chondroitin biosynthesis

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Defects in glycosaminoglycan biosynthesis disrupt animal development and can cause human disease[1–4]. So far much of the focus on glycosaminoglycans has been on heparan sulphate. Mutations in eight *squashed vulva* (*sqv*) genes in *Caenorhabditis elegans* cause defects in cytokinesis during embryogenesis and in vulval morphogenesis during postembryonic development[5,6]. Seven of the eight *sqv* genes have been shown to control the biosynthesis of the glycosaminoglycans chondroitin and heparan sulphate[6–11]. Here we present the molecular identification and characterization of the eighth gene, *sqv-5*. This gene encodes a bifunctional glycosyltransferase that is probably localized to the Golgi apparatus and is responsible for the biosynthesis of chondroitin but not heparan sulphate. Our findings show that chondroitin is crucial for both cytokinesis and morphogenesis during *C. elegans* development.

Glycosaminoglycans (GAGs) or mucopolysaccharides have been of great interest to biologists for decades[2–4,12–15]. Analyses of mutations that cause developmental defects in *Drosophila melanogaster* have shown that GAG biosynthesis, in particular the synthesis of heparan sulphate (HS), is important for intercellular signalling mediated by the Wingless, Hedgehog and fibroblast growth factor pathways[2]. In addition, mutations in GAG biosynthesis have been implicated in human diseases, including a progeroid variant of the connective tissue disorder Ehlers–Danlos syndrome[1] and hereditary multiple exostoses[3], which is characterized by inappropriate chondrocyte proliferation and bone growth. Much of the focus on GAGs has been on HS, in part because of the many ligands that it binds, its action in growth factor signalling, and its role in *Drosophila* development[2,14]. Studies of chondroitin sulphate (CS), another chief class of GAGs in vertebrates, have focused on the development of cartilage, tendon and bone[4].

The *C. elegans* genes *sqv-1* to *sqv-8* are important for both embryonic development and postembryonic vulval morphogenesis[5]. The progeny of mutants homozygous for strong loss-of-

function *sqv* mutations die during embryogenesis, with most arresting at the one-cell stage. This arrest is caused by a defect in the initiation of cytokinesis. This defect may be caused by a failure to form a fluid-filled extracellular space between the plasma membrane and the eggshell[6]. During the L4 larval stage, *sqv-1* to *sqv-8* mutants fail to expand the extracellular space of the vulva, which is the opening through which sperm and eggs pass in adult hermaphrodites. These mutants form a partially functional vulva but are normal in vulval cell proliferation, migration and fusion. We previously proposed that during both embryogenesis and vulval morphogenesis in *C. elegans*, GAGs that are attached to extracellular matrices drive the formation of fluid-filled extracellular spaces[6]. In sea urchin fertilization, the secretion of GAGs is thought to cause the swelling of a fluid-filled space between the vitelline envelope and the plasma membrane[16].

The molecular identities of seven of the *sqv* genes indicate a defect in the biosynthesis of two types of GAG present in *C. elegans*, HS and chondroitin[6–11]. SQV-4 (UDP-glucose dehydrogenase) synthesizes UDP-glucuronic acid (UDP-GlcA) in the cytoplasm[10], which is translocated into the lumen of the Golgi apparatus by the SQV-7 nucleotide-sugar transporter[9]. SQV-7 also translocates UDP-galactose and UDP-*N*-acetylgalactosamine[9]. SQV-1 catalyses the decarboxylation of UDP-GlcA[6], forming the first nucleotide-sugar donor required for GAG biosynthesis, UDP-xylose. In the lumen of the Golgi apparatus, UDP-xylose, UDP-galactose and UDP-GlcA are used as substrates of the SQV-6 xylosyltransferase[11], the SQV-3 galactosyltransferase I (refs 7,8), the SQV-2 galactosyltransferase II (ref. 11) and the SQV-8 glucuronosyltransferase I (refs 7,8) to build onto the protein core the GAG linkage tetrasaccharide (GlcAβ1,3Galβ1,3Galβ1,4Xylβ-*O*-serine) on which GAG backbones polymerize. Evidence that four such glycosylation reactions are essential for mammalian GAG polymerization has been obtained from studies of mutant hamster cell lines[14]. Mutation in the human homologue of the *sqv-3* galactosyltransferase I gene has been implicated as the cause of a progeroid variant of the connective-tissue disorder Ehlers–Danlos syndrome[17,18], which is characterized by loose skin and hypermobile joints. It seems likely that homologues of other *sqv* genes are involved in similar disorders.

By physically mapping chromosomal deletions, we localized *sqv-5* to a region of roughly 200 kilobases (kb) between *fog-3* and the left endpoint of *qDf10* (Methods and Supplementary Fig. S1). A *Bam*HI–*Pst*I fragment of 18,446 nucleotides of cosmid K09A8 containing a single complete predicted gene (*T24D1.1*) rescued the *sqv-5* mutant phenotype. Introducing a nonsense or frameshift mutation in *T24D1.1* eliminated this rescuing activity. The *sqv-5 n3039* allele is a nonsense mutation in the *T24D1.1* open reading frame (ORF). We isolated a mutation (*n3611*) that deleted most of the *T24D1.1* ORF and caused the same *sqv* mutant phenotype as that of *sqv-5(n3039)* worms. We conclude that *sqv-5* corresponds to *T24D1.1*.

We found three discrepancies between our DNA sequencing results and those of the *C. elegans* Sequencing Consortium, one of which caused us to modify the predicted gene structure of *T24D1.1* to that shown in Supplementary Fig. S1. From the sequences of *sqv-5* complementary DNA and products from the 5′ rapid amplification of cDNA ends (RACE) products, we identified two alternatively spliced forms of *sqv-5* cDNAs, which encode predicted proteins of 734 and 736 amino acids. We detected a transcript of 3.6 kb on a northern blot (Supplementary Fig. S2), consistent with the size predicted by our cDNA and 5′ RACE results.

Of the 734 amino acids in the short form of the SQV-5 protein, 270 (37%) are identical to a human CS synthase (ref. 19, Fig. 1 and Supplementary Table S1). We also identified and determined the

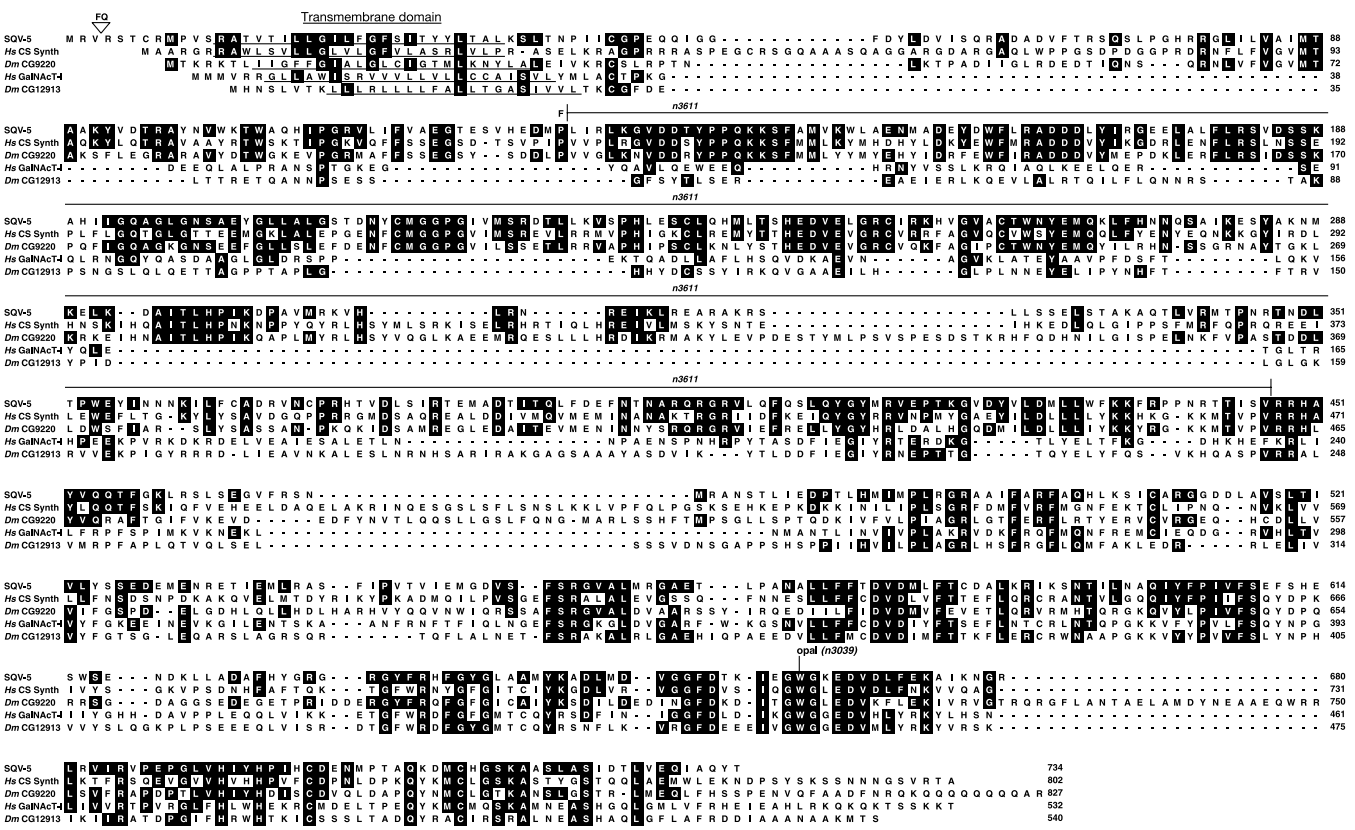

Figure 1 Sequence alignment of SQV-5 and homologues. Human CS synthase (*Hs* CS Synth), *Drosophila melanogaster* CS synthase homologue (*Dm* CG9220), human CS GalNAcT-I (*Hs* GalNAcT-I) and *Drosophila* CS GalNAcT-I homologue (*Dm* CG12913) are compared with SQV-5. The extent of the *sqv-5(n3611)* deletion and the location of the *sqv-5(n3039)* nonsense allele are indicated. Two of the six cloned 5′ RACE products contained six extra nucleotides at the 5′ end of the second exon, reflecting an alternatively spliced mRNA; the addition of two amino acids (FQ) in the longer alternatively spliced form of SQV-5 is indicated.

sequences of a cDNA from a *Drosophila melanogaster* gene that is predicted to encode a protein of 832 amino acids, of which 270 are identical to SQV-5 (37%). SQV-5 is less similar to the human CS *N*-acetylgalactosaminyltransferase I (GalNAcT-I)[20], with which it shares 109 amino acids (20% identity). However, because SQV-5 is the only protein in the *C. elegans* genome with extensive similarity to the human CS GalNAcT-I, we considered SQV-5 also to be a candidate CS GalNAcT-I. By contrast, the *Drosophila* genome contains a second gene that probably encodes an orthologue of the human CS GalNAcT-I (37% identity) (Fig. 1 and Supplementary Table S1). All five proteins contain a single predicted transmembrane domain near the amino terminus, consistent with a type II transmembrane topology. Such N-terminal transmembrane domains are typical of glycosyltransferases, which have active sites that face the lumen of the Golgi apparatus.

In vertebrates, CS synthase catalyses the alternating, stepwise addition of GlcA and *N*-acetylgalactosamine (GalNAc) to the nascent chain, resulting in the polymerization of the CS backbone. Protein extracts prepared from whole worms that were homozygous for the *sqv-5(n3611)* null allele, heterozygous for *sqv-5(n3611)* or wild-type were assayed for glucuronosyltransferase (GlcAT-II) and *N*-acetylgalactosaminyltransferase (GalNAcT-II) activities of CS synthase. Significant enzymatic activities were observed in the extracts of the wild-type worms in both assays using chemically desulphated CS as the acceptor (Fig. 2a, b). Worms heterozygous for *sqv-5(n3611)* contained slightly more than a half of the enzymatic activity observed in wild-type worms, and no significant enzymatic activity above the negative control was observed in the extracts of the homozygous mutant worms. About half the enzymatic activity was observed in assays consisting of half wild-type and half *sqv-5* null extracts (wild type, 1,466 ± 144; *sqv-5*, 105 ± 35; wild type + *sqv-5*, 809 ± 57, control, 68 ± 24 c.p.m.; mean ± s.d.), indicating that the mutant worms do not contain an inhibitor of the enzyme. These findings establish that *sqv-5* controls chondroitin synthase activity in *C. elegans* and, in combination with our sequence data, show that the SQV-5 protein has synthase activity. By contrast, we observed no difference in *N*-acetylglucosaminyltransferase II (GlcNAcT-II) activity, which is required for HS synthesis, in extracts of *sqv-5(n3611)* mutants (Fig. 2c), suggesting that chondroitin but not HS biosynthesis is disrupted in *sqv-5* mutants.

The addition of the first GalNAc residue to the linkage tetrasaccharide is thought to be catalysed by a different enzyme to that involved in CS backbone polymerization[19,20]. This reaction can be assayed using glucuronic acid β1,3galactose-*O*-naphthalenemethanol as the acceptor instead of desulphated CS. Using this assay, we observed significant enzymatic activity in extracts of wild-type worms (Fig. 2d). About one-half of the enzymatic activity above that of the negative control was present in extracts of worms heterozygous for *sqv-5(n3611)*, whereas homozygous null worms lacked activity. These findings indicate that SQV-5 functions in both the initiation and the elongation of chondroitin chains. It is notable that SQV-5, which has a similar degree of amino acid sequence identity to the human GalNAcT-I as to the human CS synthase (Supplementary Table S1), has this additional GalNAcT-I activity, as the human CS synthase apparently lacks this activity[19].

To assay changes in chondroitin and HS content in *sqv-5* mutants, we isolated GAGs from wild-type and *sqv-5(n3611)* adult hermaphrodites. The amount of chondroitin was reduced from 182 ± 52 fmol per worm (mean ± s.d.) in wild type to 24 ± 20 fmol per worm in *sqv-5* mutants. The amount of HS was below the limits of detection; the relative amount of HS is 150- to 250-fold less than chondroitin in *C. elegans*[21,22]. We also suppressed *sqv-5* function by RNA-mediated interference (RNAi) achieved by feeding the worms dsRNA[23]. *sqv-5* RNAi-treated L4 larvae had reduced vulval extracellular spaces reminiscent of mutants homozygous for a weak loss-of-function mutation in other *sqv* genes (Supplementary Fig. S3). The *sqv-5* RNAi-treated adults treated were reduced in brood size (29 ± 23 (mean ± s.d.), $n = 47$) as compared with vector RNA-treated adults (264 ± 33, $n = 31$). These observations suggest that *sqv-5* function is incompletely suppressed in these worms, because worms homozygous for either *sqv-5* mutant allele have average brood sizes of zero. Intact proteoglycans were isolated from L4 larvae that had been treated with *sqv-5* or vector RNAi and digested with either chondroitinase ABC or heparin lyase II. These enzyme treatments of chondroitin and HS resulted in 'stub' oligosaccharides, consisting of the linkage tetrasaccharide and one disaccharide repeat containing a terminal 4,5-unsaturated uronic acid, which were recognized by monoclonal antibodies against either chondroitin or HS, respectively. Western blot analysis using the anti-chondroitin antibody detected a major proteoglycan band with a relative molecular mass of about 120,000 ($M_r \approx 120$K) and several minor bands. The main band of 120K was reduced 5–8-fold in *sqv-5* RNAi-treated worms as compared with vector RNA-treated worms (Fig. 2e, f). Western blot analysis using the anti-HS antibody detected three proteoglycans of 24K, 30K and 48K, which did not vary in amount between vector and *sqv-5* RNAi-treated worms. Thus, loss of *sqv-5* function selectively reduces chondroitin quantities.

To study the expression and subcellular localization of the SQV-5 protein, we generated affinity-purified rabbit polyclonal antibodies against a SQV-5 and glutathione *S*-transferase (GST) fusion protein. Anti-SQV-5 antibodies stained several punctate foci in the cytoplasm of the vulva, the uterus and oocytes (Fig. 3a–c). This punctate staining was not seen in worms homozygous for the *sqv-5(n3611)* null allele (data not shown).

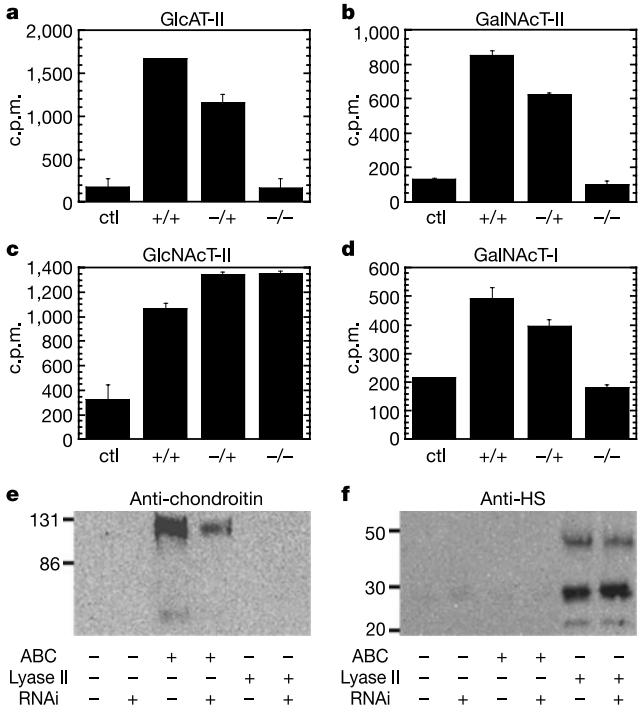

Figure 2 Glycosyltransferase activities were assayed using cell-free extracts prepared from wild type (+/+) and *sqv-5(n3611)* heterozygotes (−/+) and homozygotes (−/−). Activities measured without an acceptor (ctl) are also shown. Results are the mean ± s.e.m. from representative experiments. **a**, GlcAT-II assay. **b**, GalNAcT-II assay. **c**, GlcNAcT-II assay. **d**, GalNAcT-I assay. **e**, **f**, Intact proteoglycans were purified from vector (−) or *sqv-5* (+) RNAi-treated worms and treated with chondroitinase ABC and/or heparan lyase II or buffer. Western blot analysis was done with monoclonal antibodies specific for chondroitin (**e**) or HS (**f**), which were visualized using secondary antibodies conjugated to horseradish peroxidase.

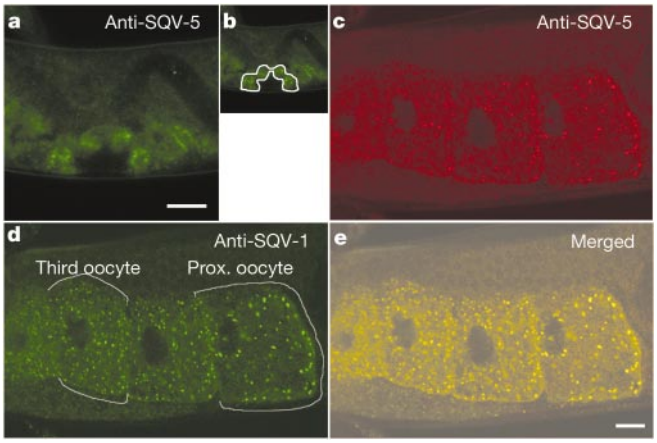

Figure 3 Wild-type worms were stained with antibodies specific for SQV-5 and/or SQV-1 (ref. 6). **a**, L4 vulva stained with rabbit antibodies against SQV-5. **b**, Diagram of vulva shown in **a**. **c**, Adult oocytes stained with rabbit antibodies against SQV-5. **d**, Adult oocytes stained with rat antibodies against SQV-1. Staining was visualized using secondary antibodies conjugated to fluorescein isothiocyanate and/or Texas red. **e**, Merged image of **c** and **d** showing colocalization of the SQV-1 and SQV-5 proteins in oocytes. Scale bar, 10 μm.

We previously observed a similar punctate staining pattern using antibodies specific for the SQV-7 nucleotide-sugar transporter and the SQV-1 UDP-GlcA decarboxylase[6]. We used rat antibodies against SQV-1 and rabbit antibodies against SQV-5 to show that SQV-1 and SQV-5 proteins colocalize and thus seem to be present in the same cytoplasmic compartment (Fig. 3c–e). We suggest that nucleotide-sugar biosynthesis by SQV-1, nucleotide-sugar transport by SQV-7 (ref. 6) and elongation of the disaccharide region of chondroitin by SQV-5 are all catalysed in the same subcellular compartment, presumably the Golgi apparatus. Most glycosyltransferases involved in GAG biosynthesis have been shown to localize to the Golgi apparatus in vertebrates[15].

Our molecular identification of *sqv-5* defines the last step in the *C. elegans* biosynthetic pathway for chondroitin and suggests that defects in the biosynthesis of chondroitin account for the embryonic and vulval defects caused by mutations in all *sqv* genes (Fig. 4). By contrast,

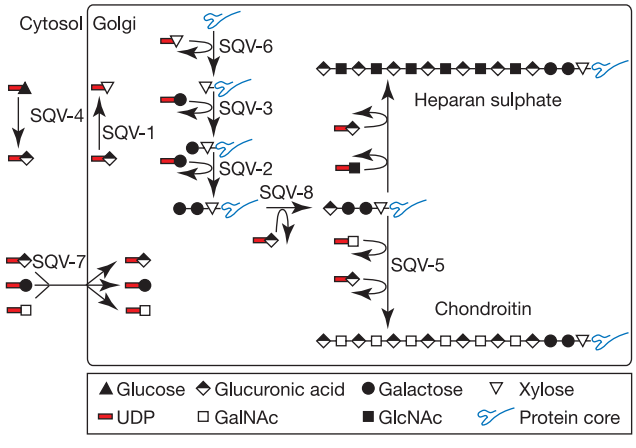

Figure 4 Model of the function of SQV proteins in the biosynthesis of heparan and chondroitin backbones. SQV-4 converts UDP-glucose to UDP-glucuronic acid[10]. SQV-7 transports UDP-glucuronic acid, UDP-galactose and UDP-*N*-acetylgalactosamine (UDP-GalNAc) from the cytoplasm to lumen of the Golgi apparatus[9]. SQV-1 converts UDP-glucuronic acid to UDP-xylose in the lumen of the Golgi apparatus[6]. SQV-6 is the GAG xylosyltransferase[11], SQV-3 is the GAG galactosyltransferase I (ref. 8), SQV-2 is the GAG galactosyltransferase II (ref. 11) and SQV-8 is the GAG glucuronosyltransferase I (ref. 8). SQV-5 is required for the synthesis of the repeating disaccharide region of chondroitin.

defects in the biosynthesis of HS leading to abnormalities in cell signalling pathways, such as the Wingless and Hedgehog pathways, are implicated as the cause of many developmental defects in *Drosophila*, including those defects arising from mutations that are predicted to disrupt both CS and HS biosynthesis[2]. Unlike HS, which is known to bind several ligands involved in morphogenesis, wound healing, host defence and energy metabolism[14], non-sulphated chondroitin is not known to bind to specific ligands.

In vertebrates, large amounts of CS are secreted into the extracellular matrix, where CS has a structural role and binds to ligands such as type I collagen[13]. The ability of chondroitin to interact with water, to cause swelling and to generate osmotic pressure on its surroundings could be responsible for its biological effects[12], including the expansion of the extracellular spaces of the *C. elegans* embryo and vulva. Studies of sea urchin gastrulation have led to the proposal that the secretion of CS proteoglycan can result in the hydration of the extracellular matrix and cause epithelial invagination[24]. Our studies provide support for such a mechanism. We cannot exclude other possibilities, however, such as mechanisms involving adhesion, cytoskeletal rearrangement or intercellular signalling[5,6]. Whatever the mechanism of chondroitin action, our findings show the importance of chondroitin in cytokinesis, early embryogenesis and epithelial morphogenesis in *C. elegans* and support the hypothesis that CS, like HS, has a broad and important role in development and disease. □

Methods

sqv-5 mapping

We obtained Unc non-Vul and Vul non-Unc progeny from *unc-29(e1072) lin-11(n566)/sqv-5(n3039)* hermaphrodites. Five of ten Unc non-Vul progeny carried *sqv-5(n3039)*, and three of ten Vul non-Unc progeny carried *sqv-5(n3039)*, indicating that *sqv-5(n3039)* was located between *unc-29* and *lin-11* and to the left of *lin-11*. We examined the vulval phenotype of worms of the genotype *ces-1(n703) Df/sqv-5(n3039)*, using *qDf5*, *qDf7*, *qDf8*, *qDf9* and *qDf10*. All worms were Sqv, except for *ces-1(n703) qDf5/sqv-5(n3039)*. Because *qDf5*, *qDf7*, *qDf8*, *qDf9* and *qDf10* delete *fog-3*, but only *qDf5* and *qDf7* delete *lin-11* (ref. 25), *sqv-5* maps to the left of *fog-3*. Using single *qDf10* eggs, we amplified genomic DNA sequence corresponding to the cosmids K10C3 and C03C11. A polymerase chain reaction product of the expected length was amplified for K10C3 but not for C03C11 ($n = 10$), thus placing the left end point of *qDf10* between K10C3 and C03C11 (Supplementary Fig. S1).

sqv-5 cDNA

We determined the sequences of two cDNA clones, yk20d7 and yk21g9, corresponding to *T24D1.1*, and of six 5′ RACE products derived from mixed-stage RNA. The 5′ RACE products contained a 5′ SL1 *trans*-spliced leader, which is found at the 5′ end of many *C. elegans* transcripts. The *sqv-5* cDNA contained a 417-bp 5′ UTR, a 2,202-bp ORF and a 657-bp 3′ UTR sequence. We identified two alternatively spliced forms of the transcript by 5′ RACE. Two of six cloned 5′ RACE products represented a longer spliced form containing six additional base pairs at the 5′ end of the second exon (Supplementary Fig. S4).

Deletion allele of *sqv-5*

We isolated the deletion mutation *sqv-5(n3611)* from a library of worms mutagenized with ultraviolet illumination and trimethylpsoralen[26]. Mutant worms containing *sqv-5(n3611)* were backcrossed to the wild-type strain N2 six times. The deletion of 1,641 bp in *sqv-5(n3611)* removes nucleotides 1,661–3,301 of the *sqv-5* genomic DNA sequence file (GenBank accession code AY241925). *sqv-5(n3611)* is predicted to encode a truncated SQV-5 lacking 385 amino acids (amino acids 130–447) in the middle of SQV-5 and an alanine to phenylalanine substitution at amino acid 129.

Glycosyltransferase assays

We picked *sqv-5(n3611)*, *sqv-5(n3611)/hT2* and wild-type N2 hermaphrodites as L4 larvae by visual examination of the vulva using a dissecting microscope. The worms were allowed to grow for 23–27 h at 22 °C, and were then frozen in 50 mM Tris, pH 7.5 and stored at −70 °C. Samples were sonicated in 0.05% Triton X-100, 50 mM Tris and centrifuged at 15,000*g* for 10 min. The protein content of the cleared supernatant was assessed by the Bradford assay and portions of the extracts were used for the following assays. The chondroitin acceptor was prepared by desulphation of shark cartilage chondroitin-4-sulphate[27]. *N*-acetylheparosan was prepared from *Escherichia coli* K5 (ref. 28) and the disaccharide GlcAβ1,3Galβ-*O*-naphthalenemethanol was synthesized[28].

The GlcAT-II activity of chondroitin synthase was measured by mixing 1.3×10^5 c.p.m. of UDP-[1-^{3}H]glucuronic acid donor (20 Ci mmol^{-1}), 6 μg of β-glucuronidase-treated chondroitin acceptor and 3 μg of worm extract in a volume of 25 μl containing 0.05% Triton X-100, 10 mM $MnCl_2$, 100 μM ATP and 25 mM MES buffer, pH 6.5. The GalNAcT-II activity of chondroitin synthase was detected by mixing 3×10^5 c.p.m. of UDP-[1-^{3}H]GalNAc donor (38.5 Ci mmol^{-1}), 12 μg of chondroitin acceptor and 15 μg of

worm extract in 25 μl containing 0.05% Triton X-100, 10 mM $MnCl_2$ and 25 mM MES buffer, pH 6.5. The GlcNAcT-II activity of HS polymerase was assayed by mixing 5 μCi of UDP-[6-^{3}H]GlcNAc, 12 μg of *N*-acetylheparosan acceptor and 10 μg of worm extract in 25 μl containing 20 mM $MnCl_2$, 0.45% Triton X-100 and 25 mM MOPS buffer, pH 6.5. We incubated GlcAT-II, GlcNAcT-II and GalNAcT-II reactions for 2 h at 25 °C and separated the products from free nucleotide-sugars by DEAE-Sephacel (Pharmacia)[29].

GalNAcT-I activity was assayed as described for GalNAcT-II, except that 5×10^5 c.p.m. of UDP-[1-^{3}H]GalNAc donor and 12 mM GlcAβ1,3Galβ-*O*-naphthalenemethanol acceptor were used. Reactions were incubated 3 h at 25 °C, and products were separated from free nucleotide-sugars using a Sep-Pak C18 cartridge (Waters Corp.) as described[28]. Reactions were linear with time and amount of protein.

Chondroitin and HS characterization

Between 220 and 250 *sqv-5(n3611)* and wild-type worms were collected as described for glycosyltransferase assays, except that the worms were lyophilized and homogenized in acetone. Free GAGs were isolated by alkali extraction as described[30], except that they were extracted overnight in 0.5 M NaOH, 1 M $NaBH_4$ at 4 °C, and neutralized with 1 M HCl. Chondroitin was digested with 20 mU of chondroitinase ABC (Seikagaku) and analysed by high-performance liquid chromatography with post-column derivatization of the disaccharides[22].

RNAi was done essentially as described[23], except that L1-stage hermaphrodites were placed onto Petri plates containing bacteria and grown for 44–48 h at 20 °C before being collected as L4-stage hermaphrodites. We isolated intact proteoglycans by anion-exchange chromatography as described[30], except that we sonicated the worms in 0.5% Triton X-100 and protease inhibitor cocktail (Sigma). For western blots, intact proteoglycans were digested with chondroitinase ABC or heparin lyase II and incubated with monoclonal antibodies to chondroitin (1-B-5, Seikagaku) or HS (F69-3G10, Seikagaku).

Anti-SQV-5 antibodies

The *sqv-5* ORF was cloned into vectors pGEX-4T3 and pMAL-c2 to generate GST–SQV-5 and maltose-binding protein (MBP)–SQV-5 fusion proteins, respectively. The GST–SQV-5 and MBP–SQV-5 fusion proteins were purified by isolating insoluble proteins from inclusion bodies, followed by SDS–PAGE and electro-elution. We injected GST–SQV-5 into two rabbits (Covance). Anti-SQV-5 antibodies were affinity-purified by binding and elution from MBP–SQV-5 fusion protein, as described[10].

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Chondroitin proteoglycans are involved in cell division of *Caenorhabditis elegans*

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Glycosaminoglycans such as heparan sulphate and chondroitin sulphate are extracellular sugar chains involved in intercellular signalling. Disruptions of genes encoding enzymes that mediate glycosaminoglycan biosynthesis have severe consequences in *Drosophila* and mice[1–5]. Mutations in the *Drosophila* gene *sugarless*, which encodes a UDP-glucose dehydrogenase, impair developmental signalling through the Wnt family member Wingless, and signalling by the fibroblast growth factor and Hedgehog pathways. Heparan sulphate is involved in these pathways[6–8], but little is known about the involvement of chondroitin. Undersulphated and oversulphated chondroitin sulphate chains have been implicated in other biological processes, however, including adhesion of erythrocytes infected with malaria parasite to human placenta and regulation of neural development[9,10]. To investigate chondroitin functions, we cloned a chondroitin synthase hom-