sqv-3, *-7*, and *-8*, a set of genes affecting morphogenesis in *Caenorhabditis elegans*, encode enzymes required for glycosaminoglycan biosynthesis

Dorota A. Bulik*†, Ge Wei*‡, Hidenao Toyoda*§, Akiko Kinoshita-Toyoda*§, W. Ross Waldrip§, Jeffrey D. Esko‡, Phillips W. Robbins†, and Scott B. Selleck§¶

†Department of Molecular and Cell Biology, Boston University, Boston, MA 02118; ‡Department of Cellular and Molecular Medicine, Glycobiology Research and Training Center, University of California at San Diego, La Jolla, CA 92093; and §Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721

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***sqv* (*squashed vulva*) genes comprise a set of eight independent loci in *Caenorhabditis elegans* required zygotically for the invagination of vulval epithelial cells and maternally for normal oocyte formation and embryogenesis. Sequencing of *sqv-3*, *sqv-7*, and *sqv-8* suggested a role for the encoded proteins in glycolipid or glycoprotein biosynthesis. Using a combination of *in vitro* analysis of SQV enzymatic activities, *sqv*$^+$-mediated rescue of vertebrate cell lines, and biochemical characterization of *sqv* mutants, we show that *sqv-3*, *-7*, and *-8* all affect the biosynthesis of glycosaminoglycans and therefore compromise the function of one specific class of glycoconjugates, proteoglycans. These findings establish the importance of proteoglycans and their associated glycosaminoglycans in epithelial morphogenesis and patterning during *C. elegans* development.**

The cell surface and surrounding extracellular matrix is composed of a large repertoire of glycans attached to both proteins and lipids, a feature long appreciated by cell biologists and described as the glycocalyx. Although the structural variety of these glycoconjugates has been well characterized, their functions during development are only beginning to be appreciated. The nematode worm *Caenorhabditis elegans* provides a genetically tractable organism ideally suited for the analysis of glycan function during tissue assembly and morphogenesis. Toward this end, a number of groups have begun to explore the pathways of glycosylation represented in *C. elegans* (1–3).

Vulval morphogenesis in *C. elegans* has served as a powerful model for studying a variety of morphogenetic and signaling processes. One event critical for vulval development is the invagination of the vulval epithelium. This process is disrupted in *sqv* (*squashed vulva*) mutants, which define a set of eight loci required for epithelial morphogenesis (4). In addition to zygotic loss of *sqv* function affecting vulval assembly, *sqv* genes are required maternally for oocyte formation and embryogenesis. Maternal contributions of *sqv* function are essential for viability and the normal development of *sqv*/+ progeny, indicating that *sqv* genes likely affect a broad spectrum of patterning events during development. Molecular cloning and sequencing of three *sqv* genes revealed homology to a number of vertebrate enzymes involved in glycosylation (1) and all bear homology to at least one enzyme known to affect glycosaminoglycan biosynthesis.

Heparan sulfate and chondroitin sulfate are the major glycosaminoglycans found on vertebrate and invertebrate proteoglycans. These chains are covalently attached to the protein cores of proteoglycans via a tetrasaccharide linkage fragment (GlcA-Gal-Gal-Xyl-serine) (5), which is assembled on specific serine residues of the protein core. *sqv-3* is similar to a mammalian β1,4 galactosyl transferase I, an enzyme that adds the first galactose (Gal) residue to xylose of the linkage tetrasaccharide (6, 7). Mutations in this gene are associated with Elhers–Danlos syndrome, a human genetic disorder characterized by many developmental abnormalities (8). *sqv-7* is related to a nucleotide-sugar transporter required for the transport of GDP-mannose across Golgi membranes in *Leishmania donovani* (1). Glycosylation requires nucleotide sugar donors and SQV-7 might transport these substrates from the cytoplasm into the Golgi. Finally, *sqv-8* is related to three glucuronyl transferases, GlcAT-I, GlcAT-P, and GlcAT-D (9–12). GlcAT-P and GlcAT-D are involved in synthesis of the HNK-1 epitope (3-*O*-sulfated GlcA-Gal-GlcNAc-) found at high levels on glycoproteins and glycolipids in the developing nervous system (13). GlcAT-I has a different function, adding the terminal glucuronic acid (GlcA) of the glycosaminoglycan linkage tetrasaccharide (9, 10). These sequence relationships suggest the possibility that *sqv* genes may encode enzymes required for glycosaminoglycan biosynthesis.

A variety of mutant cell lines defective in proteoglycan synthesis have been identified and biochemically characterized. Interestingly, these mutants divide normally in culture, suggesting that glycosaminoglycan chains are dispensable in isolated cells (14). In contrast, glycosaminoglycans and their associated proteoglycans have been shown to affect a wide range of essential biological processes *in vivo* (15). Recent genetic studies have demonstrated that glycosaminoglycans and proteoglycans are critical for signaling mediated by a number of growth factors during development, including members of the Wnt, transforming growth factor β, fibroblast growth factor (FGF), and Hedgehog families (16–24). To date, however, there has been no functional analysis of glycosaminoglycans in *C. elegans*. Recent studies have demonstrated that glycosaminoglycans of the chondroitin and heparan sulfate types are present in *C. elegans* and bear structural features common to both *Drosophila* and vertebrate polysaccharides (25). We set out to determine whether three members of the *sqv* class of genes recently described (1) affect glycosaminoglycan biosynthesis and therefore provide an inroad into understanding the function of this abundant class of molecules during development.

Materials and Methods

***C. elegans* Stocks and Genetics.** Routine culture and genetics of *C. elegans* were performed according to published methods (26).

Abbreviations: GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; Gal, galactose; FGF, fibroblast growth factor; CHO, Chinese hamster ovary; NM, naphthalenemethanol; UA, uronic acid.

*D.A.B., G.W., H.T., and A.K.-T. contributed equally to this work.

¶To whom reprint requests should be addressed. E-mail: selleck@u.arizona.edu.

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All strains were grown at 20°C. Mutant strains used included MT7554: *sqv-3(n2842) unc-69(e587)/qC1 dpy-19(e1259) glp-1(q339)*, SP174: *sqv-8(mn63) unc-4(e120)/mnC1 dpy-10(e128) unc-52(e444)*, and MT7562: *sqv-7(n2839)* (1, 4).

SQV-3 Mediated Rescue of Galactosyl Transferase I-Defective Chinese Hamster Ovary (CHO) Cells. CHO cells were cultivated in Ham's F-12 medium supplemented with 10% FBS as described (27). Mutant pgsB-618 was previously shown to lack heparan sulfate and chondroitin sulfate because of a deficiency of galactosyltransfersase I (28). Analysis of heparan sulfate production by radiolabeling and staining cells with biotinylated FGF-2 was performed as described (27, 29).

Expression of the SQV3 and SQV8 Proteins. Reverse transcription–PCR generated cDNA fragments containing the full-length ORF of *sqv-3* or *sqv-8* were directly cloned into pcDNA3.1/V/His-TOPO according to manufacturer's specifications (Invitrogen). The proteins were expressed as a C-terminal fusion with the V5 epitope, permitting ready detection of the recombinant proteins with monoclonal-α-V5 antibody. The *sqv-3* and *sqv-8* bearing constructs were transiently transfected into COS-7 cells grown to 60–80% confluence by using Lipofectamine (Life Technologies, Grand Island, NY). Transfected cells were scraped from the culture dishes, and membrane fractions were isolated by differential centrifugation.

***In Vitro* Galactosyl Transferase (SQV3) and Glucuronyl Transferase I (SQV-8) Assays.** Galactosyl transferase activity was determined as described with some modifications (30). Briefly, the assay mixture in a final volume of 25 μl contained: 100 mM Mes (pH 6.0), 50 mM KCl, 1% Triton, 15 mM $MgCl_2$, 0.4 mM (150,000 dpm) UDP-[^{14}C]Gal, 0.001–10 mM acceptor, and 5 μg of a membrane fraction as the enzyme source. The reaction mixture was incubated at 17°C for 1 h, a reaction time within the linear range of the enzyme activity (data not shown). The standard assay mixture for glucuronyl transferase contained: 100 mM Hepes (pH 6.5), 10 mM $MnCl_2$, 2.5 mM ATP, 0.2 mM (150,000 dpm) UDP-[^{14}C]GlcA, 1% Triton X-100, 5–10 mM acceptor, and 5 μl of a membrane fraction. The reaction mixture was incubated for 3 h, a reaction time determined to be within the linear range of enzyme activity at 17°C. Reaction mixtures from both sets of assays were applied to 1 ml OASIS C_{18} cartridge (Waters), washed with 5 column volumes of water followed by the elution of reaction products in 1 ml methanol. This material was vacuum dried, and radioactivity was determined. Mock-transfected COS-7 cells or cells transfected with plasmids bearing the *sqv-3* or *sqv-8* coding region in the reverse orientation served as controls for determining background activity.

Worm Extract Preparation. Mixed-stage N2 worms were grown in liquid culture medium in the presence of *Escherichia coli* (OP 50 strain), collected by centrifugation and purified from bacteria by sucrose floatation. A packed, frozen *C. elegans* pellet (0.5 ml) was homogenized by using a Dounce homogenizer in 20 mM ice-cold sodium phosphate buffer (pH 7.2) followed by centrifugation (4 min, at 2,000 $\times$ *g*). A supernatant solution was collected and used immediately for *in vitro* enzyme assay as described above.

HPLC and Western Blot Analysis of Chondroitin-Modified Proteoglycans. *C. elegans* cultures were synchronized as described, and mutant *sqv-3(n2842)* and *sqv-8(mn63)* L4 larva were picked by scoring for the associated *unc* phenotype. *sqv-7(n2839)* mutant L4 larvae were picked from a synchronized culture. Five hundred worms were lyophilized and washed with acetone. Proteoglycans were extracted in 400 μl of 1% CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), 0.15 M NaCl, 10 mM disodium EDTA, 10 mM *N*-ethylmaleimide, 1 mM PMSF, 2% 2-propanol, 50 mM sodium phosphate buffer (pH 6.0) for 16 h at 0°C with constant stirring. The resulting crude proteoglycan mixture was applied to a column of DEAE-Sephacel, and the proteoglycans that eluted with 2.0 M NaCl in 6 M urea, 50 mM sodium phosphate buffer (pH 6.0) were collected and resuspended in 20 μl of water. A 5-μl aliquot of this material was used for Western blot analysis by using an anti-ΔDi-0S monoclonal antibody (1-B-5, Seikagaku) and ECL Western blotting detection system (Amersham Pharmacia) as described (31). Unsaturated disaccharides were generated from chondroitin by chondroitinase ABC and ACII digestion of 2-μl samples and quantitated by postcolumn fluorometric derivitization after HPLC (25).

HPLC Analysis of Heparan Sulfate. Mixed-stage cultures of N2 and *sqv-7(n2839)* were grown on enriched peptone plates. Heparan sulfate was extracted from 10 mg of lyophilized worms and prepared for disaccharide compositional analysis. After exhaustive enzymatic digestion of partially purified glycosaminoglycans with a mixture of heparin lyase I, II, and III, unsaturated disaccharides were identified and measured by comparison to standards by using fluorometric postcolumn HPLC, according to published procedures (25).

Results

***sqv-3* Rescues Chondroitin and Heparan Sulfate Synthesis in a CHO Cell Mutant Defective in Galactosyl Transferase I.** Glycosaminoglycan biosynthesis has been elucidated by a variety of strategies, including the isolation and assay of relevant biosynthetic enzymes and the identification of somatic cell mutants defective in specific biosynthetic steps. pgsB-618 is a CHO cell line deficient in galactosyl transferase I, an enzyme required for assembly of the core protein linkage tetrasaccharide (28) and one of the vertebrate proteins related to SQV-3. As shown below, this mutant cell line does not synthesize any glycosaminoglycans and as a result is defective in the binding of FGF-2, a heparan sulfate-binding growth factor (Fig. 1). To determine whether *sqv-3* might encode a relevant galactosyl transferase, pgsB-618 cells were transfected with a *sqv-3*$^+$-bearing vector and assayed for FGF-2 binding by cell sorting. As shown in Fig. 1, *sqv-3*$^+$ expression restored FGF binding to near wild-type levels (Fig. 1). To confirm that SQV-3 encoded the galactosyl transferase, *sqv-3*-transfected cells were labeled with $^{35}SO_4$ and the glycosaminoglycans were analyzed by HPLC. Expression of SQV-3 restored the synthesis of both chondroitin and heparan sulfate in the galactosyl transferase I-defective CHO cells to nearly wild-type levels (Fig. 2).

SQV-3 Expressed in COS-7 Cells Generates Galactosyl Transferase Activity. The rescue of the glycosaminoglycan defect of pgsB-618 cells by *sqv-3*$^+$ provides evidence that this gene encodes a protein with galactosyl transferase I activity. To determine directly whether SQV-3 can catalyze the transfer of Gal to appropriate substrates *in vitro*, we expressed SQV-3 in COS-7 cells and measured enzymatic activity in a membrane fraction derived from these transfected cells (Table 1). SQV-3-expressing cells showed significantly greater activity than that of control preparations from cells transfected with vector alone or vector with *sqv-3*$^+$ in the reverse orientation. The activity obtained from *sqv-3*$^+$-transfected cells showed the appropriate specificity for β-linked xylosides vs. α-linked analogs, in contrast to the behavior of the vertebrate enzyme (32). The membrane preparation also transferred Gal to *N*-acetylglucosamine (GlcNAc)-containing acceptors, but the K_m of the SQV-3-encoded enzyme is lower for the β-xyloside substrate ($^{app}K_m$ 47 μM) compared with α-GlcNAc ($^{app}K_m$ 0.68 mM) and β-GlcNAc substrates ($^{app}K_m$ 0.44 mM). SQV-3 activity with xylose α-*O*-*p*-nitrophenyl,

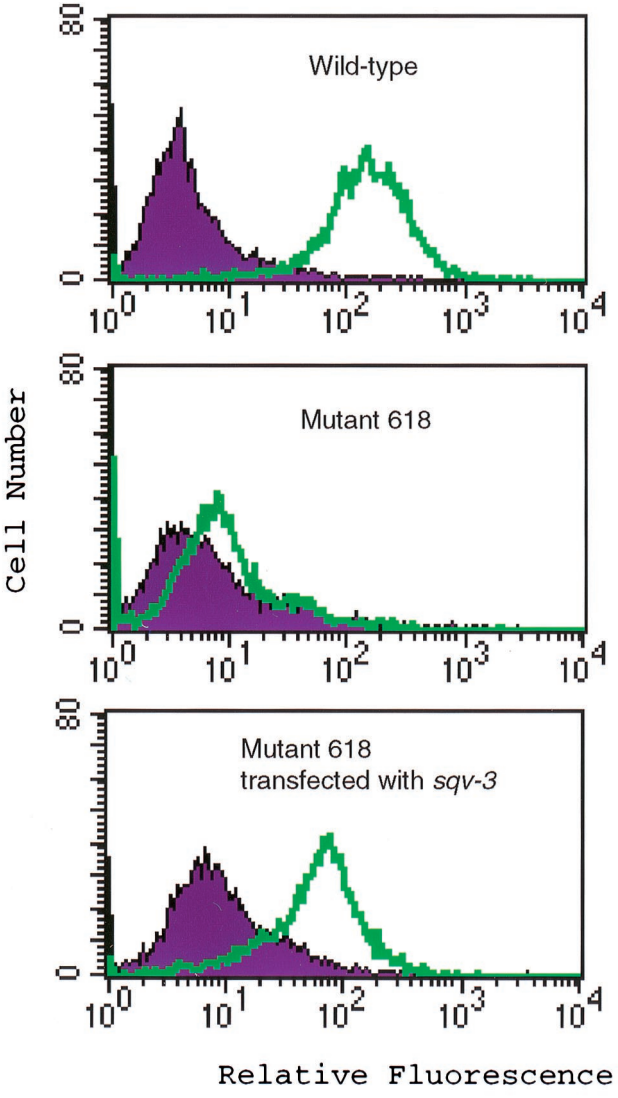

Fig. 1. *sqv-3*$^+$ restores FGF-2 binding in mutant pgsB-618 cells. A stable cell line was derived from Gal transferase I-deficient mutant (pgsB-618) by stable transfection with sqv-3$^+$ cDNA. Wild-type, mutant, and transfected cells were incubated at 37°C and treated with biotinylated FGF-2 and FITC-labeled streptavidin before cell sorting (29). (*Top*) Wild-type CHO cells. (*Middle*) Mutant pgsB-618 cells. (*Bottom*) Mutant pgsB-618 transfected with *sqv-3*$^+$. The shaded curve represents sorts done on cells without added biotin-FGF.

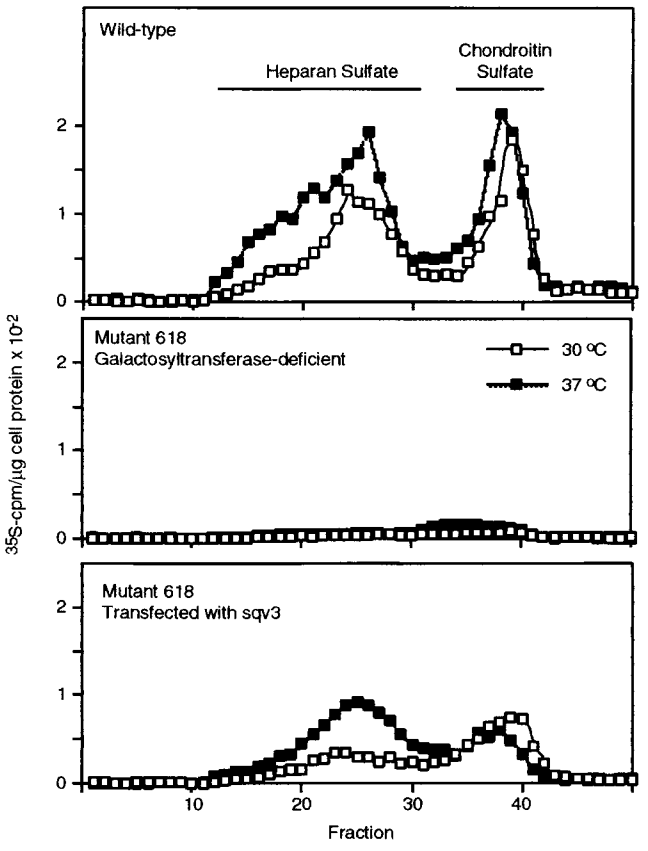

Fig. 2. Correction of CHO pgsB-618 by *sqv-3*$^+$. A stable cell line was derived from a Gal transferase I-deficient mutant (pgsB-618) by transfection with *sqv-3*$^+$ cDNA using Lipofectamine and drug selection. Multiple drug-resistant clones were picked and radiolabeled with $^{35}SO_4$ for 3 days. The radioactive glycosaminoglycans were isolated, and samples were analyzed by anion exchange HPLC as described (27). ■, Cells were grown at 37°C. □, Cells were grown at 30°C. The identification of heparan sulfate and chondroitin sulfate was established by chondroitinase ABC and nitrous acid degradation.

Galα-β-*O*-2-naphthol, and *N*-acetylgalactosamine (GalNAc)α-β-*O*-*p*-nitrophenyl was below the limit of detection (<0.1 pmol). Interestingly, the temperature optimum for SQV-3 was 17°C, well below that of the vertebrate enzyme and in the range expected for an enzyme from an invertebrate organism. Correction of pgsB cells did not show marked thermal dependence in the temperature range of 30–37°C (Fig. 2).

SQV-8 Expressed in COS-7 Cells Produces Glucuronyl Transferase Activity with the Appropriate Substrate Specificity *in Vitro*. *sqv-8* encodes a protein with homology to three distinct glucuronyl transferases (GlcAT-I, GlcAT-P, and GlcAT-D) that play a role in the synthesis of different glycoconjugates. GlcAT-I is required for synthesis of the glycosaminoglycan linker, catalyzing the addition of GlcA to Gal. To determine whether SQV-8 has GlcAT-I activity, COS-7 cells were transfected with *sqv-8*$^+$, and a membrane fraction was used to measure GlcAT transferase activity *in vitro* (Table 1). Membrane fractions from *sqv-8*$^+$-transfected cells showed a significant increase in GlcAT-I activity over that of mock-transfected cells, with the appropriate preference for β-Gal. These preparations had the highest activity with Galβ1,3Galβ-*O*-NM, the *in vitro* substrate most closely resembling the natural substrate for GlcAT-I (9). SQV-8 activity with Galα-*O*-1-naphthol, GalNAcα-*O*-*p*-nitrophenyl, and Gal-

Table I. Galactosyl transferase I (SQV-3) and glucuronyl transferase I (SQV-8) protein activity expressed in COS-7 cells

Acceptor	GalT activity, pmol/h/assay*	GlcAT-I activity, pmol/h/assay*
Xylose-β-nitrophenyl	82 ± 12	
GlcNAc-β-nitrophenyl	57 ± 9	
GlcNAc-α-nitrophenyl	37 ± 10	
Galβ1,3-Galβ-*O*-NM		137.4 ± 19.8
Galβ-*O*-2-naphthol		8.6 ± 0.5
Gal1,3β-GalNAc-α-1-*O*-benzyl		11.8 ± 1
Galβ1,3-GlcNAcβ-*O*-NM		12.1 ± 0.8
Galβ1,4-GlcNAcβ-*O*-NM		11 ± 1.2

*Values are means of two independent experiments run in duplicate. NM, naphthalenemethanol; assays were run for 1–3 h at the optimum temperature (17°C).

NAcβ-*O*-*p*-nitrophenyl was below the limit of detection (<0.1 pmol). Like SQV-3, SQV-8 has a temperature optimum at 17°C.

SQV-8 also transfers GlcA to Galβ-*O*-naphthol, Galβ1,3GalNAcα-*O*-benzyl, a core I disaccharide characteristic of *O*-linked mucins, Galβ1,4GlcNAcα-*O*-NM, a substrate for HNK-1 biosynthesis, and Galβ1,3-GlcNAcα-*O*-NM. However, the activity with these substrates was at least 10-fold lower than that observed with the linkage region disaccharide analog Galβ1,3Galβ-*O*-NM, suggesting that the enzyme's physiological function is in glycosaminoglycan biosynthesis.

To confirm that glucuronyl transferase I activity exists in *C. elegans*, a whole worm extract was used as an enzyme source. Similar to the membrane fractions derived from SQV-8-expressing COS cells, the glucuronyl transferase detected in worm extracts preferentially used Galβ1,3-Galβ-*O*-NM as a substrate (10.5 ± 3.8 pmol/h), whereas activity with other acceptors was below the limits of detection.

***sqv-3, -7,* and *-8* Mutations Affect the Levels of Chondroitin and Chondroitin-Modified Proteoglycans *in Vivo*.** The rescue of galactosyl transferase defective cells with *sqv-3*$^+$, the *in vitro* activity of SQV-3 and SQV-8, and the homology of SQV-7 to a putative nucleotide-sugar transporter, provides evidence that these three *sqv* genes all are required for synthesis of glycosaminoglycans *in vivo*. Recently, methods for structural analyses of glycosaminoglycans in *C. elegans* have been developed that provide direct measures of chondroitin and heparan sulfate (25). Adult *C. elegans* contain high levels of chondroitin and heparan sulfate composed of disaccharide units also found in *Drosophila* and vertebrates. Using these methods, we determined that *sqv-3*, *sqv-7*, and *sqv-8* L4 stage animals derived from *sqv*/+ hermaphrodites all showed significantly reduced levels of chondroitin-derived disaccharides (wild-type N2, 170 pg/body; *sqv7*, 68 pg/body; *sqv-3*, 50 pg/body; *sqv-8*, 8 pg/body). Animals bearing a viable *sqv-7* allele showed the smallest decrease in chondroitin levels. None of the *sqv* alleles tested, including those that are required maternally for viability, completely abolish chondroitin biosynthesis. Given that both the *sqv-3* and *sqv-8* alleles contain stop codons within the normal ORF and are therefore likely null alleles, zygotic defects in these genes are likely not sufficient to completely block glycosaminoglycan biosynthesis during development. This finding suggests that a strong maternal effect exists, as recently shown in *Drosophila* mutants altered in glycosaminoglycan biosynthesis (16–18, 20, 23, 33).

We also have examined the effect of *sqv* mutations on chondroitin-modified proteoglycans by using a monoclonal antibody (anti-ΔDi-0S) that recognizes an unsaturated uronic acid derivative in disaccharides generated by chondroitinase ABC digestion. Treatment of partially purified proteoglycans from *C. elegans* with chondroitinase ABC after electrophoretic transfer to the membrane revealed a set of large, broad, molecular weight bands detected by anti-ΔDi-0S staining (Fig. 3*A*). Most of the immunoreactive material migrates above the 215-kDa marker, in the size range typical of chondroitin-modified proteoglycans. If the material is subjected to chondroitinase ABC digestion before SDS/PAGE, anti-ΔDi-0S staining detects a collection of bands ranging in molecular mass from around 70 kDa to more than 215 kDa, showing that there are numerous chondroitin-modified proteoglycans in wild-type *C. elegans*. Detection of chondroitin-modified material from *C. elegans* required digestion with chondroitinase ABC, as documented for chondroitin-modified proteoglycans from vertebrates (Fig. 3*A*, lane 3).

Homozygous L4 worms derived from heterozygous hermaphrodites bearing mutations in *sqv-3* or *sqv-8* show dramatic reductions in chondroitin-modified proteoglycans; *sqv-3* shows a markedly reduced level of the higher molecular weight species, and there was no detectable material from an equivalent number of *sqv-8* animals (Fig. 3*B*). The viable *sqv-7* allele shows a different pattern of anti-ΔDi-0S staining. The immunoreactive proteoglycans are of a smaller average molecular weight, suggesting that chondroitin chain length is reduced in this mutant relative to wild-type or *sqv-3* mutants. Although *sqv-3(n2842)* and *sqv-7(n2839)* possessed similar levels of chondroitin-derived disaccharide measured by HPLC (50 and 68 pg/animal for *sqv-3*, and *sqv-7*, respectively) *sqv-7(n2839)*-derived proteoglycans showed greater anti-ΔDi-0S staining, consistent with the presence of a greater number of chondroitin-modified proteoglycans bearing shorter chondroitin chains.

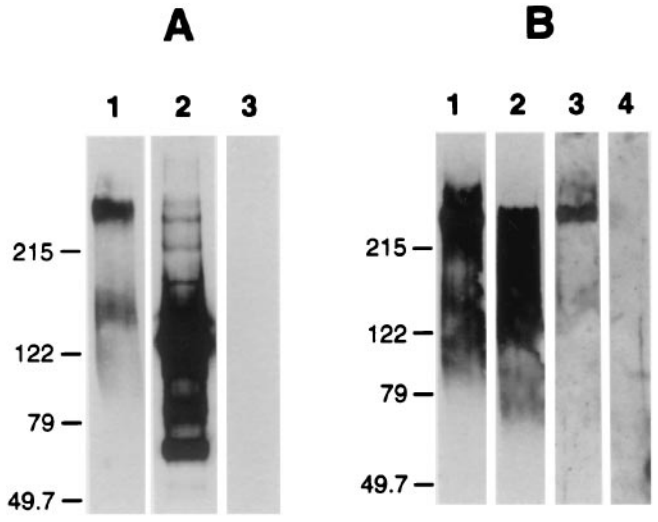

Fig. 3. Western blot analysis of chondroitin-modified proteoglycans in wild-type (N2), *sqv-3*, *sqv-7*, and *sqv-8* homozygous L4 animals, detected with anti-ΔDi-0S antibody. (*A*) Analysis of wild-type proteoglycans, digestion with chondroitinase ABC after (lane 1) or before (lane 2) electrophoresis and blotting. Proteoglycans in lane 3 were not treated with chondroitinase, showing that enzymatic digestion is required for antibody detection of these molecules. (*B*) Comparison of chondroitin-modified proteoglycans from wild-type (lane 1), *sqv-7* (lane 2), *sqv-3* (lane 3), *sqv-8* (lane 4) mutants, where chondroitinase digestion was performed after electrophoresis and blotting.

Because *sqv-7* encodes a protein with homology to a nucleotide-sugar transporter that could provide substrates required for glycosaminoglycan synthesis, it is not surprising that animals bearing mutations in this gene have a shorter average glycosaminoglycan chain length. *sqv-3 and sqv-8*, on the other hand, encode enzymes that probably affect only the biosynthesis of the linkage tetrasaccharide. If a linkage fragment is made in these animals, it stands to reason that it should provide a substrate for the generation of a full-length glycosaminoglycan chain.

***sqv-7* Affects Heparan Sulfate Biosynthesis.** To determine whether any of the *sqv* mutations affect heparan sulfate biosynthesis, we partially purified glycosaminoglycans from animals bearing a viable allele of *sqv-7* and determined the disaccharide composition by exhaustive heparin lyase digestion and HPLC. Animals homozygous for *sqv-7* showed an approximately 40% reduction in all disaccharide species derived from heparan sulfate (total heparan sulfate in wild-type N2 52.5 ng/mg and in *sqv-7* 30.8 ng/mg dried tissue). The composition of disaccharides from *sqv-7* homozygous animals was unchanged with respect to wild-type (ΔUA-GlcNAc, 55.2%; ΔUA-GlcNS, 9.6%; ΔUA2S-GlcNS, 20.6%; ΔUA2S-GlcNS6S, 14.6%) (Fig. 4). These findings show that *sqv-7* affects both chondroitin and heparan sulfate biosynthesis.

Discussion

We have demonstrated that the *C. elegans sqv-3* and *sqv-8* genes encode enzymes with galactosyl transferase and glucuronyl

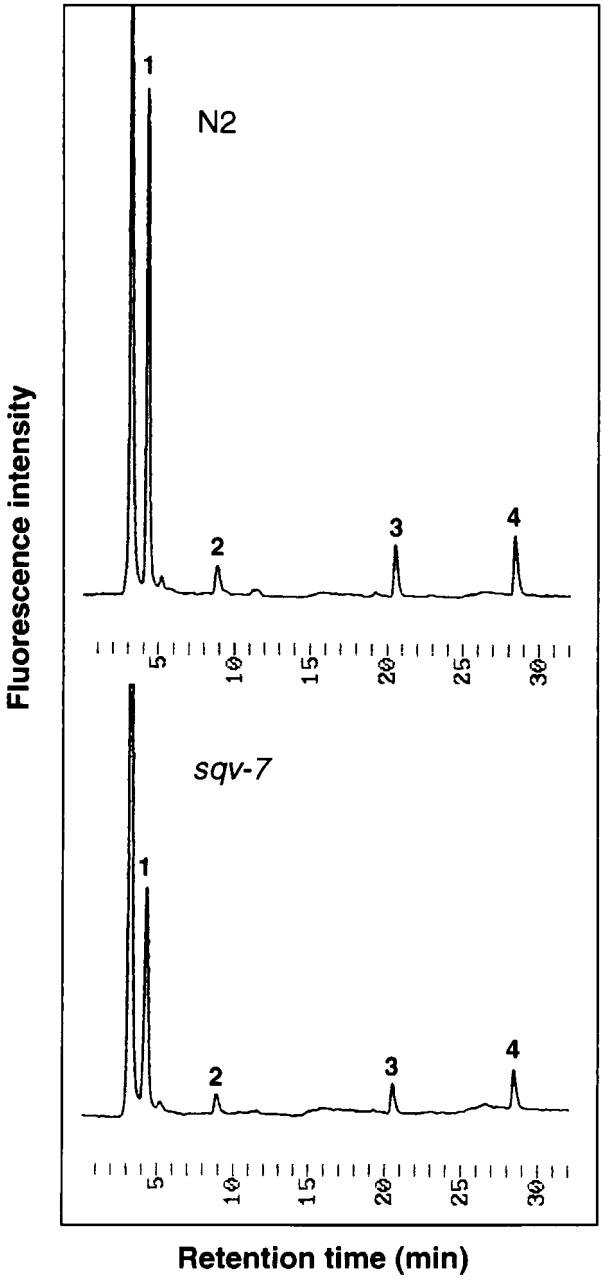

Fig. 4. HPLC profile of heparan sulfate-derived disaccharides from wild-type and *sqv-7* mutants. Peak 1, ΔUA-GlcNAc; peak2, ΔUA-GlcNS; peak 3, ΔUA2S-GlcNS; peak 4, ΔUA2S-GlcNS6S. Note the reductions in peak height for all disaccharides, including both unsulfated, mono-, di-, and trisulfated species. Levels for each disaccharide are given in the text.

transferase I activity. Both galactosyl transferase I and glucuronyl transferase I are required for the synthesis of the linkage tetrasaccharide common to all glycosaminoglycans, including chondroitin and heparan sulfate (9, 29). Consistent with the demonstrated enzymatic activities, animals bearing *sqv-3* and *sqv-8* mutations have reduced levels of chondroitin and abnormal profiles of chondroitin-modified proteoglycans. Our analysis of *sqv-7* demonstrated that mutations in this gene compromise both chondroitin and heparan sulfate synthesis *in vivo*. These findings collectively show that glycosaminoglycan and proteoglycan biosynthesis are defective in *sqv* mutants.

The sequence homologies of *sqv-3*, *sqv-7*, and *sqv*-8 to vertebrate glycosyl transferases and a nucleotide-sugar transporter provided the basis for a model in which these three genes worked coordinately in the synthesis of a common glycoconjugate (1). Our analysis has proven this hypothesis correct and has further demonstrated that these *sqv* genes affect glycosaminoglycan and hence proteoglycan biosynthesis. Given the specificity of the vertebrate glycosaminoglycan biosynthetic enzymes, it is unlikely that *sqv-3* or *sqv-8* directly compromise the synthesis of other glycoproteins or glycolipids. *sqv-3*, *sqv-7*, and *sqv-8* are three of eight genes in the *sqv* class, identified on the basis of a common vulval invagination defect. Our findings suggest that other *sqv* loci might well affect glycosaminoglycan or proteoglycan synthesis. Glycosaminoglycan biosynthesis involves at least 16 different enzymes and the existing *sqv* genes could encode other enzymes in this pathway.

The biochemical analysis of *sqv* mutants by HPLC provides a sensitive, direct measurement of glycosaminoglycans *in vivo*. Using this method, all *sqv* mutants derived from heterozygous hermaphrodites examined had detectable levels of glycosaminoglycans. Our analysis included alleles of *sqv-3* and *sqv-8* that contain stop codons in the ORF (1). These findings suggest that maternal contributions of *sqv* mRNA or protein provide significant biosynthetic capacity. Indeed, both maternal and zygotic loss of *sqv* gene function is lethal, disrupting oocyte formation and embryogenesis. The importance of maternal contributions of *sqv* function is further revealed by the observation that a percentage of *sqv*/+ progeny derived from *sqv* homozygous hermaphrodites show morphological defects (4). Loss of only zygotic *sqv* function, however, produces predominantly a phenotype found late in development, where vulval epithelial cells fail to invaginate. These observations parallel studies of genes encoding glycosaminoglycan biosynthetic enzymes in *Drosophila*. *sugarless*, *sulfateless*, and *tout-velu* all affect glycosaminoglycan synthesis (25, 33) and show a strong maternal effect. Embryonic phenotypes are observed only in mutant embryos derived from females bearing germ-line homozygous mutant clones. A detailed analysis of *sqv* embryos derived from *sqv* hermaphrodites will be required to determine exactly what developmental processes are disrupted by the complete lack of glycosaminoglycan synthesis.

How might these molecules be involved in epithelial invagination? Proteoglycans could well affect the physical properties of cells that influence epithelial invagination, such as cell–cell adhesion and the interaction of cells with the extracellular matrix (34). In addition, recent work has demonstrated critical roles for cell surface proteoglycans in signaling. *division abnormally delayed*, a *Drosophila* member of the glypican family, modulates cellular responses to both Wingless and Decapentaplegic, members of the Wnt and transforming growth factor β families of growth factors, respectively (19, 21, 22). Defects in genes encoding glycosaminoglycan biosynthetic enzymes in *Drosophila* compromise signaling mediated by Wingless, Decapentaplegic, Hedgehog, and the FGF receptors, *branchless* and *breathless* (16–18, 20, 23, 25). It is therefore quite possible that the abnormalities in vulval invagination could stem from signaling defects. Whatever the molecular mechanisms, it is clear that vulval invagination is sensitive to loss of glycosaminoglycan biosynthesis.

The genetic and morphological analysis of the eight *sqv* loci identified to date suggests they are essential for a variety of patterning events during development. *C. elegans* offers several distinct advantages as a model organism for studying in detail the functions of glycosaminoglycans and proteoglycans *in vivo*. The availability of the complete genome sequence together with the technology for reverse genetics and sensitive analytical procedures for glycosaminoglycans provide the necessary tools to

understand in detail the biosynthesis and function of glycosaminoglycans during development of this genetically tractable organism.

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