Identification of a Drosophila Gene Encoding Xylosylprotein β4-Galactosyltransferase That Is Essential for the Synthesis of Glycosaminoglycans and for Morphogenesis*

Received for publication, April 22, 2002, and in revised form, September 4, 2002
Published, JBC Papers in Press, September 4, 2002 DOI 10.1074/jbc.M203873200

Yoko Nakamura‡§, Nicola Haines‡§, Jihua Chen, Tetsuya Okajima‡, Keiko Furukawa‡, Takeshi Urano‡, Pamela Stanley†, Kenneth D. Irvine‡, and Koichi Furukawa**

From the ‡Department of Biochemistry II, Nagoya University School of Medicine, 65 Tsurumai, Showa-ku, Nagoya 466-0065, Japan, the †Howard Hughes Medical Institute, Waksman Institute and Department of Molecular Biology and Biochemistry, Rutgers, The State University, Piscataway, New Jersey 08854, and the ‡Department of Cell Biology, Albert Einstein College of Medicine, New York, New York 10461

In mammals, the xylosylprotein β4-galactosyltransferase termed β4GalT7 (XgaT-1, EC 2.4.1.133) participates in proteoglycan biosynthesis through the transfer of galactose to the xylose that initiates each glycosaminoglycan chain. A Drosophila cDNA homologous to mammalian β4-galactosyltransferases was identified using a human β4GalT7 cDNA as a probe in a BLAST analysis of expressed sequence tags. The Drosophila cDNA encodes a type II membrane protein with 322 amino acids and shows 49% identity to human β4GalT7. Extracts from L cells transfected with the cDNA exhibited marked glycosyltransferase activity specific for a xylopyranoside acceptor. Moreover, transfection with the cloned cDNA restored glycosaminoglycan synthesis in β4GalT7-deficient Chinese hamster ovary cells. In transfected lysates the properties of Drosophila and human β4GalT7 resembled each other, except that Drosophila β4GalT7 showed a less restricted specificity and was active at a wider range of temperatures. Drosophila β4GalT7 is expressed throughout development, with higher expression levels in adults. Reduction of Drosophila β4GalT7 levels using expressed RNA interference (RNAi) in imaginal discs resulted in an abnormal wing and leg morphology similar to that of flies with defective Hedgehog and Decapentaplegic signaling, which are known to depend on intact proteoglycan biosynthesis. Immunohistochemical analysis of tissues confirmed that both heparan sulfate and chondroitin sulfate biosynthesis were impaired. Our results demonstrate that Drosophila β4GalT7 has the in vitro and in vivo properties predicted for an ortholog of human β4GalT7 and is essential for normal animal development through its role in proteoglycan biosynthesis.

Proteoglycans are polyanionic molecules consisting of different core proteins covalently attached to a variety of glycosaminoglycan (GAG) chains. They are ubiquitously expressed not only on the cell surface but also in extracellular matrices (1) and are generated in a cell type-specific manner resulting in characteristic sulfation of GAG chains. The strictly regulated expression patterns of proteoglycans suggest that they play regulatory roles in development, cell proliferation, and differentiation (2) and in organogenesis (3). Mutational analyses in the mouse and in Drosophila have identified specific cell types and signaling pathways that are defective if proteoglycan synthesis is disrupted (4, 5).

The biosynthesis of sulfated GAG chains on proteoglycans is initiated by the addition of Xyl to Ser or Thr residues in the core protein, followed by the addition of two Gal residues and a GlcUA residue to form the common core sequence (1). Addition of a GlcNAc or a GalNAc residue to the terminal GlcUA of the core leads to the formation of heparin/heparan sulfate or chondroitin/dermatan sulfate GAG chains, respectively. Glycosyltransferases involved in the sequential transfer of individual sugars have recently been cloned, including glucuronyltransferase I (6, 7), galactosyltransferase I (xylosylprotein 4-β-galactosyltransferase, β4GalALT, XgalT1) (8, 9), galactosyltransferase II (10), and xylosyltransferase (11).

We previously isolated cDNAs of human galactosyltransferase I on the basis of BLAST searches using the sequence of Caenorhabditis elegans sqv-3 (8) and mapped the gene to the human β4GalALT locus (12). sqv-3 is one of three genes isolated by Herman et al. (13, 14) from C. elegans mutants defective in vulval epithelial invagination. They predicted that these proteins encode components of a conserved glycosylation pathway that assemble a C. elegans carbohydrate moiety. In fact, it was demonstrated that sqv-8 encodes GlcUA transferase I (15), and sqv-7 encodes a nucleoside-diphosphate sugar transporter (16). As for sqv-3, after we reported the cloning and characterization of human β4GalALT (hβ4GalALT) (8), sqv-3 was shown to encode the C. elegans homolog of galactosyltransferase I (15).

In the present study, we isolated cDNA clones of a Drosophila gene encoding a xylosylprotein β4-galactosyltransferase. A BLAST search of expressed sequence tags (ESTs) for Drosophila was performed using the rat β4GalALT (Drosophila β4GalALT) EST as a query sequence. The cloned cDNA inserts showed 69% identity at the nucleotide level to the sequences found in the rat β4GalALT EST database.
Drosophila βGalT7 in Proteoglycan Synthesis and Morphogenesis

FluoroCytochemistry Analysis—CHO pβG-B761 cells were transfected with 10 μg of plasmid DNA using LipofectAMINE® (Invitrogen) according to the manufacturer's instructions. Three days later, cells were trypsinized and prepared for flow cytometry using mAb 10E4 at a dilution of 1:25 (40 μg/ml) and FITC-conjugated goat anti-mouse IgM (μ chain specific) (Zymed Laboratories Inc.) on a FACSCalibur with CellQuest® version 3.1f software (Becton Dickinson).

B5 Using a Drosophila Expression Panel—A Drosophila RapidScan® gene expression panel was purchased from OriGene Technologies, Inc. (Rockville, MD). The product contains first-strand cDNAs prepared from different Drosophila tissues and developmental stages. Forward primer 331 F (5′-ACTGCTGTCGCTTGTTTCCGAG-3′) and the reverse primer 730 R (5′-TCTCTTAAATCCCCACCCACCCA-3′) were selected to avoid a fragment of 100 bp. PCR conditions were: denaturation of the template at 95°C for 1 min, followed by 95°C for 30 s, 60°C for 30 s, 68°C for 30 s, and 68°C for 1 min for either 25 or 35 cycles.

RNA Interference Constructs—Based on the predicted genomic structure of D4fGalT7 (CG11780), the second exon and second intron of the βGalT gene were cloned from Drosophila genomic DNA by PCR using Taq and Pvu DNA polymerases (Expand® Long Template PCR System, Roche Molecular Biochemicals) and primers (5′-CAAGAATTCACCTGCGTTGGCGGGCTGTC-3′ and 5′-CCCAGATCTCTGGAGGTGGAATTGACATAA-3′) with EcoRI and BgII restriction sites. This fragment was cloned into the pUAST vector (21) using the EcoRI and BgII sites in the pUAST/LacZ vector (Stratagene) to prevent recombination between the inverted repeats. Two independent transgenic lines for pUAST-D4fGalT7, UAS-iβGalT7 [A], and UAS-iβGalT7 [B], both on the second chromosome, were generated by F-element-mediated transformation.

Results

Molecular Cloning of the D4fGalT7 Gene—The gene mutated in the sqv-3 mutant of C. elegans is similar in amino acid sequence to members of the βGalT gene family of humans and other vertebrates (15) and is required for vulval invagination and oocyte development in C. elegans (13). The human gene corresponding to sqv-3 is now termed βGalT7 (12). To find a Drosophila ortholog, the NCBI Data Bank and Berkeley Dro-
Drosophila Genome Project of EST cDNA clones were probed with the deduced amino acid sequence of h/4GalT7 cDNA. One cDNA was obtained from the databases (CK02622 contig, accession number AA142310). This cDNA is encoded by a gene predicted by the genomic sequence of Drosophila, CG11780, located at 96B16 on chromosome 3. Based on the biochemical and functional studies outlined below, we have named this Drosophila gene /4GalT7 (symbol /4G7). The cDNA contains a complete open reading frame encoding a protein of 322 amino acids with a molecular mass of 36,468 Da (Fig. 1A). It has two potential N-linked glycosylation sites. The position of the AUG start codon was determined according to the Kozak consensus sequence (26). Hydropathy analysis (27) indicates one prominent hydrophobic segment of 26 residues in length in the amino-terminal region, predicting that the protein has type II transmembrane topology characteristic of many other glycosyltransferases cloned to date (Fig. 1B). Comparison of the primary structure of the identified cDNA and h/4GalT7 (GenBank™ accession number AB026800 (6)) revealed that 144 out of the 322 amino acids (49%) are identical (Fig. 2). Approximately 30% identity was found between the newly cloned gene and other human /4GalTs (now termed /4GalT-1 through /4GalT-6). In addition, /4GalT7 contains the three short amino acid sequence motifs conserved among all members of the /4GalT family (28–33), i.e. FNRA, DVD, and WGREDDE as indicated in Fig. 2 (34, 35).

**Restoration of GAG Synthesis by a Cloned D/4GalT7 cDNA**

To investigate whether D/4GalT7 is involved in the biosynthesis of GAGs in vivo, CHO mutant pgsB-761 (17) cells that lack /4GalT7 activity were transiently transfected with pMIKneo-D/4GalT7. pgsB-761 cells are mutant for /4GalT7 and, consequently, are defective in GAG biosynthesis. The ability of CHO cells to synthesize GAGs can be monitored with an antibody that recognizes heparan sulfate, mAb10E4. As shown in Fig. 3, about 50% of the cells transfected with /4GalT7 cDNA reacted with mAb10E4, as did a similar number of cells transfected with a h/4GalT7 cDNA, whereas mock-transfected cells were negative. These results show that the D/4GalT7 corrected the inability of pgsB-761 mutant cells to synthesize proteoglycans, indicating that the cloned cDNA encodes a Drosophila ortholog of /4GalT7.

**Galactosyltransferase Activity of D/4GalT7**

To analyze the galactosyltransferase activity of D/4GalT7, the expression vectors pMIKneo-D/4GalT7 or pMIKneo-h/4GalT7 were transfected into L cells, and 3 days later cell extracts were prepared for galactosyltransferase assay to compare the activities of h/4GalT7 and D/4GalT7 under various conditions. Back-
To analyze the expression of Drosophila β4GalT7, RT-PCR was performed using a Drosophila RapidScan® gene expression panel (Fig. 6). Although the Dβ4GalT7 gene was ubiquitously expressed, relatively higher expression levels were observed in RNA from adults compared with embryos at early developmental stages.

**Spatio-temporal Expression Pattern of the Dβ4GalT7 Gene**—To analyze the expression of Dβ4GalT7, RT-PCR was performed using a Drosophila RapidScan® gene expression panel (Fig. 6). Although the Dβ4GalT7 gene was ubiquitously expressed, relatively higher expression levels were observed in RNA from adults compared with embryos at early developmental stages.

RNAi Identifies a Role for Dβ4GalT7 in Morphogenesis—

Proteoglycans aid the transport and reception of a number of signaling molecules in Drosophila, including Hedgehog (HH), Wingless (WG), fibroblast growth factor, and Decapentaplegic (DPP) (4, 5). Disruption of these signaling events leads to mis-patterning and loss of tissues during development. If Dβ4GalT7 is involved in the generation of proteoglycans in vivo, a reduction in the function of this enzyme should result in phenotypes in the fly similar to those observed when proteoglycan-dependent signaling pathways are disrupted. As Dβ4GalT7 mutants are not available, we used RNA interference (RNAi) to reduce the levels of the β4GalT7 transcript (37, 38). A construct (β4GalT7i) containing two copies of exon 2 of the β4GalT7 gene in an inverted repeat, such that it could fold back on itself to generate a 622-bp double-stranded RNA (dsRNA) stem with an 89-bp loop, was expressed using the UAS-Gal4 technique (21). The roles of HH, DPP, and WG signaling pathways have been well studied in the developing imaginal discs of Drosophila (39), and, to examine requirements for β4GalT7, dsRNA was expressed under the control of Gal4 lines that drive expression in these tissues. The patched (ptc) promoter drives expression in cells that receive the HH signal. The distance between veins three and four was significantly reduced in the wings of ptc-Gal4 UAS-β4GalT7 flies (Fig. 7B). A weak reduction in the vein three to vein four distance was also observed when dsRNA for β4GalT7 was expressed throughout the wing using the scalloped-Gal4 driver (data not shown). The loss of tissue in this region of the wing is typical of wing
phenotypes of weak mutations in genes in the HH signaling pathway (39–41). The *apterous* (*ap*) and *engrailed* (*en*) promoters drive expression in the dorsal and posterior compartments of the wing, respectively. When dsRNA for *β4GalT7* was expressed under *ap-Gal4* or *en-Gal4* control, the corresponding regions of the wing were reduced in size (Fig. 7, C and D); this reduction can also be detected in the developing wing imaginal disc (Fig. 8B). In the case of *en-Gal4* UAS-*iβ4GalT7* flies, the loss of tissue appeared most pronounced between the posterior edge of the wing and the most posterior wing vein, vein five (Fig. 7C). In the case of *ap-Gal4* UAS-*iβ4GalT7* flies, tissue loss was also more pronounced toward the edges of the wing, manifest most obviously in the complete deletion of vein two from the dorsal surface of the wing (Fig. 7D). Because growth of the wing along the anterior-posterior axis is regulated primarily by DPP (39), these phenotypes can best be explained as resulting from impairment of DPP signaling. Notably, the bristles at the edge of the wing, the wing margin, were unaffected under all conditions examined. These structures are established through WG and Notch signaling, and we infer that these pathways were relatively unaffected. The *Distal-less* (*Dil*) promoter drives expression in the distal regions of all appendages. When dsRNA for *β4GalT7* was expressed under *Dil-Gal4* control, the most dramatic phenotypes were observed in the legs, which were truncated distally (Fig. 7F). Similar distal truncations were also occasionally (~15% of legs) observed when *iβ4GalT7* was expressed under the control of *da-Gal4*, which drives expression ubiquitously (data not shown). Distal-proximal outgrowth of the leg is dependent upon the combined action of the HH, DPP, and WG signaling pathways, and impairment of any of these pathways could cause truncations similar to those observed (39).

** Fig. 4. Donor and substrate specificities of *Dβ4GalT7* and *hβ4GalT7*. pGEM 3-Dβ4GalT7 was transfected into Kc cells (upper), pMIKneo-*hβ4GalT7* into L cells (lower). A, substrate specificity of *Dβ4GalT7* and *hβ4GalT7*. Various acceptors were incubated in the standard assay mixture using membrane extracts of L cells transfected with pMIKneo-Dβ4GalT7 (upper), or pMIKneo-*hβ4GalT7* (lower). Each substrate was used at the concentration of 2 mM. Both transfectants were harvested, and the membrane fractions were prepared as a source of the enzyme. Enzyme assays proceeded as described under “Experimental Procedures.” B, three kind of sugar donors were compared in the incorporation into *p-Nph-β-D-Xyl as an acceptor substrate. Both transfectants were harvested, and the membrane fractions were prepared as a source of the enzyme. Enzyme assays proceeded as described under “Experimental Procedures.”
GAGs can be impaired by RNAi-mediated repression of β4GalT7 expression.

**DISCUSSION**

The results presented here show that Dβ4GalT7 encodes the ortholog of hβ4GALT7. Based on predicted amino acid sequence, Dβ4GalT7 is more similar to mammalian β4GalT7 than to the six other mammalian β4GalTs (28–33). In vitro assays showed that it encodes a β4GalT with a preference for xylose as acceptor rather than GlcNAc or Glc. In addition, expression of Dβ4GalT7 restores GAG synthesis to the CHO mutant pgsB-761, which lacks β4GalT7 activity due to a mutation in the hamster β4GalT7 gene (17), whereas RNAi of Dβ4GalT7 impairs GAG synthesis in Drosophila tissues. Therefore, Dβ4GalT7 functions as hβ4GalT7 in vivo and in vitro indicating that it is required for the biosynthesis of GAG core sugars in the linkage region of proteoglycans in the fly.

The enzymatic activity of the β4GalT that adds Gal to Xyl was originally studied by Roden’s group (43, 44), and was further characterized using CHO mutants defective in that activity (17) and in the study of clinical cases (45) with a genetic defect in the biosynthesis of dermatan sulfate proteoglycan. The identification of human β4GalT7 as the gene responsible for Xyl β4GalT activity (8) revealed that this enzyme almost exclusively utilizes xylose as an acceptor substrate. In the present study, we show that Dβ4GalT7 has a slightly broader specificity in acceptor utilization. Intriguingly, the acceptor specificity of the C. elegans homolog, sqv-3 (16), is even broader, because transfer of Gal to p-Nph-β-D-GlcNAc or p-Nph-a-D-GlcNAc was 69.5% and 45.1% compared with p-Nph-β-D-Xyl, respectively (16). Thus, the acceptor specificity of β4GalT7 enzymes from different species varies, with that of humans being most restricted. Although a few studies have characterized the chemical structures of Drosophila proteoglycans (46, 47), and glycolipids (48), we need to accurately and comprehensively investigate the existing carbohydrate structures in flies with biochemical approaches to clarify the potential significance of differences in the acceptor specificity of β4GalT7 among species.

We also investigated optimal conditions for in vitro activity of β4GalT7 from Drosophila and human, and found that, with the exception of temperature, they prefer similar assay conditions. Thus, the biochemical properties of Dβ4GalT7 are well

**Fig. 5.** Influence of temperature, pH, divalent cations, and detergents on the activity of hβ4GalT7 and Dβ4GalT7. A, pGEM 3-Dβ4GalT7 (closed circles) or pGEM 3 (opened circles) alone was transfected into Kc cells by CELLFECTIN reagent (upper). L cells were transiently transfected with pMIKneo-hβ4GalT7 by DEAE-dextran method (lower). Membrane fractions were the source of enzyme and assays were performed as described under “Experimental Procedures.” B, influence of pH on the activity of Dβ4GalT7 and hβ4GalT7. The enzyme sources in B–D were prepared as described above. The buffers used were 100 mM MES-NaOH, pH 5.5–6.5 (opened squares or circles) or 100 mM HEPES-NaOH, pH 6.5–7.5 (closed squares or circles). C, influence of divalent cations. The effects of divalent cations on the Dβ4GalT7 activity were determined under standard assay conditions with different divalent cations or EDTA at a final concentration of 10 mM. D, influence of detergents on the activity of Dβ4GalT7 and hβ4GalT7. The effects of different detergents on the Dβ4GalT7 activity were determined under standard assay conditions with different detergents at final concentrations of 1%.

**Fig. 6.** Dβ4GalT7 gene expression levels in different developmental stages and tissues. Four different cDNA concentrations were used, i.e., the reverse transcript (1×) and 1:100 dilution (0.01×). The amount of cDNA in each well at the highest concentration is ~1 ng. PCR was performed as indicated 25 cycles (upper) and 35 cycles (lower). Both transfectants were harvested, and the membrane fractions were prepared as a source of the enzyme. Enzyme assays proceeded as described under “Experimental Procedures.”
The difference in temperature optimum correlates with the difference in typical body temperature: because flies are cold-blooded, their body temperature is variable and is typically 10–20 °C lower than that of humans.

Ever since the activating and inhibitory effects of proteoglycans on cell proliferation were elucidated (49, 50), important biological roles for GAG chains on proteoglycans have been increasingly recognized. Their importance for human health was emphasized by the discovery that two different mutations in the human $β_{4}$GalT7 gene were identified in a case of Ehlers-Danlos syndrome (a progeroid variant), revealing that the patient was a compound heterozygote of $β_{4}$GalT7, a transcriptional target of HH signaling in vivo.

When dsRNA for $β_{4}$GalT7 is expressed under ap-Gal4 or en-Gal4 control, wing tissue loss appears to be greatest at the anterior or posterior edges of the wing. Growth of the wing along the anterior-posterior axis is promoted by DPP, which is produced by cells in the middle of the wing, along the anterior-posterior compartment boundary, and then spreads from its site of synthesis. DPP is distributed in a concentration gradient, and cells at the edge of the wing receive the lowest amounts. Wing tissue loss may thus occur primarily at the edge of the wing in these flies, because these cells normally receive the lowest amounts of DPP and, hence, are particularly sensitive to HH signaling.

Because HH and DPP signaling requires proteoglycans (4, 5), the $β_{4}$GalT7 RNAi phenotype fits with its biochemical activity, and we infer that its requirement for HH and DPP signaling stems from its role in the synthesis of proteoglycans that in-
fluence transport or reception of these signaling molecules. However, the phenotypes of β4GalT7 RNAi flies are less severe than would be expected if HH or DPP signaling were completely eliminated, presumably because RNAi is only partially effective at reducing β4GalT7 levels. The lack of WG phenotypes in the adult wing, despite the known requirement for proteoglycans in WG signaling; likely also results from the incomplete impairment of GAG synthesis. Nonetheless, the relatively greater effect on DPP as opposed to WG signaling is unexpected.

In Drosophila, mutations have previously been isolated in four different genes involved in the elongation and modification of GAG chains: sugarless (sgl, UDP-glucose dehydrogenase), fringe connection (frc, UDP-sugar transporter), tout-velu (ttv, EXT, GlcUA/GlcNAc polymerase), and sulfatess (sft, N-deacetylase/N-sulfotransferase), and in one of the core proteins to which GAG chains are attached, daily (Glypican) (4, 5). Genetic analysis of these mutations has revealed the key role of proteoglycans in signaling pathways that are essential for growth and morphogenesis during development. However, WG signaling requires frc, sgl, sft, and daily, but not ttv; HH signaling requires frc, sft, ttt, and possibly sgl, but not daily; and DPP signaling requires daily and possibly sgl but has not been reported to require sft, ttt, or frc (4, 5). In part, the apparent lack of requirements for certain genes may stem from genetic redundancy, i.e., the Drosophila genome contains homologs of ttt and daily, and the full complement of UDP-sugar transporters is not known. However, the particular sensitivities of different signaling pathways to mutations at different steps of proteoglycan biosynthesis imply that different pathways have distinct requirements for specific proteoglycans.

Notably, β4GalT7 is the first gene to be examined phenotypically in Drosophila that participates in the synthesis of the core linker region to which GAG chains are attached. The genomic sequence predicts only a single β4GalT7 gene in Drosophila. The two other putative β4GalTs in Drosophila, encoded by CG8536 and CG14517, are more closely related to mammalian β4GalTs that utilize GlcNAc acceptors, and RNAi of these genes does not generate the wing phenotypes generated by expression of iβ4GalT7. Thus, β4GalT7 should in principle be required for all processes that require GAGs attached to core proteins through the GlcUA-Gal-Gal-Xyl linker, which would encompass all processes dependent upon heparan sulfate or chondroitin sulfate proteoglycans. Consistent with a broad role for β4GalT7 in wing morphogenesis, gene expression analysis by RT-PCR revealed that this gene is expressed throughout development, including the adult, and immunohistochemical staining has confirmed that it is required for the normal synthesis of both heparan and chondroitin sulfate.

Although both heparan and chondroitin sulfate are affected in flies expressing iβ4GalT7, analysis of other genes suggests that its phenotypic effects can be largely accounted for by the influence on heparan sulfate. That is, biochemical analysis implies that sgl and ttt specifically influence heparan sulfate synthesis, and Dally is expected to be modified exclusively by heparan sulfate (like other glypicans). Because these genes influence HH or DPP signaling, there is in principle no need to invoke an influence on chondroitin sulfate in explaining the iβ4GalT7 phenotypes. The actual requirements for chondroitin sulfate during Drosophila development remain to be determined, because genes that exclusively affect this GAG have not yet been analyzed phenotypically. frc and sgl mutants are expected to impair both heparan and chondroitin sulfate synthesis, but aside from a requirement for frc in the Fringe-dependent modulation of Notch signaling (52, 53), the phenotypes of these mutations are similar to those of sgl. Nonetheless, it remains possible that the apparent differences in the sensitivities of different pathways to mutation or down-regulation of these genes reflect distinct requirements for different GAGs by signaling pathways. Importantly, the utility of reverse genetic approaches such as RNAi that require GAGs for signaling remains possible that the apparent differences in the sensitivity of different signaling pathways to mutations at different steps of proteoglycan biosynthesis imply that different pathways have distinct requirements for specific proteoglycans.
35. Gastinel, L. N., Cambillau, C., and Bourne, Y. (1999) EMBO J. 18, 3546–3557
36. Powell, J. T., and Brew, K. (1976) J. Biol. Chem. 251, 3645–3652
37. Kennerdell, J. R., and Carthew, R. W. (2000) Nat. Biotech. 18, 896–898
38. Kennerdell, J. R., and Carthew, R. W. (1998) Cell 95, 1017–1026
39. Brook, W. J., Diaz-Benjumea, F. J., and Cohen, S. M. (1996) Annu. Rev. Cell Dev. Biol. 12, 161–180
40. Strigini, M., and Cohen, S. M. (1997) Development 124, 4697–4705
41. Sanchez, H. E., Couso, J. P., Capdevila, J., and Guerrero, I. (1996) Mech. Dev. 55, 159–170
42. Vervoort, M., Crozatier, M., Valle, D., and Vincent, A. (1999) Curr. Biol. 9, 632–639
43. Schwartz, N. B., and Roden, L. (1975) J. Biol. Chem. 250, 5200–5207
44. Helting, T., and Roden, L. (1969) J. Biol. Chem. 244, 2791–2798
45. Quentin, E., Gladen, A., Roden, L., and Kresse, H. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 1342–1346
46. Toyoda, H., Kinoshita-Toyoda, A., and Selleck, S. B. (2000) J. Biol. Chem. 275, 2269–2275
47. Toyoda, H., Kinoshita-Toyoda, A., Fox, B., and Selleck, S. B. (2000) J. Biol. Chem. 275, 21856–21861
48. Seppe, A., Moreland, M., Schweingruber, H., and Tiemeyer, M. (2000) Eur. J. Biochem. 267, 3549–3558
49. Yayon, A., Klagsbrun, M., Esco, J. D., Leder, P., and Ornitz, D. M. (1991) Cell 64, 841–848
50. Raghaer, A. C., Kruvka, A., and Olwin, B. B. (1991) Science 252, 1705–1708
51. Okajima, T., Fukumoto, S., Furukawa, K., Urano, T., and Furukawa, K. (1999) J. Biol. Chem. 274, 28841–28844
52. Selva, E. M., Hong, K., Baeg, G. H., Beverley, S. M., Turco, S. J., Perrimon, N., Hacker, U. (2001) Nat. Cell Biol. 3, 809–815
53. Goto, S., Taniguchi, M., Muraoka, M., Toyoda, H., Sado, Y., Kawakita, M., Hayashi, S. (2003) Nat. Cell Biol. 3, 816–822
Identification of a *Drosophila* Gene Encoding Xylosylprotein β-4-Galactosyltransferase That Is Essential for the Synthesis of Glycosaminoglycans and for Morphogenesis

Yoko Nakamura, Nicola Haines, Jihua Chen, Tetsuya Okajima, Keiko Furukawa, Takeshi Urano, Pamela Stanley, Kenneth D. Irvine and Koichi Furukawa

*J. Biol. Chem.* 2002, 277:46280-46288.  
doi: 10.1074/jbc.M203873200 originally published online September 4, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M203873200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 52 references, 31 of which can be accessed free at http://www.jbc.org/content/277/48/46280.full.html#ref-list-1