Temperature-sensitive Glycosaminoglycan Biosynthesis in a Chinese Hamster Ovary Cell Mutant Containing a Point Mutation in Glucuronyltransferase I*

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In previous studies, we reported the isolation and characterization of a Chinese hamster ovary cell mutant (pgsG) defective in glucuronyltransferase I (GlcATI). This enzyme adds the terminal GlcA residue in the core protein-linkage tetrasaccharide (GlcAβ1,3Galβ1,3Galβ1, 4Xylβ- O-) on which glycosaminoglycan assembly occurs (Bai, X. M., Wei, G., Sinha, A., and Esko, J. D. (1999) J. Biol. Chem. 274, 13017–13024; Wei, G., Bai, X. M., Sarkar, A. K., and Esko, J. D. (1999) J. Biol. Chem. 274, 7857–7864). Here we show that incorporation of 35SO4 into glycosaminoglycans in the mutant is temperature-sensitive, with greater synthesis occurring at 33 °C compared with 37 °C. Wild-type cells show the opposite thermal dependence. Rabbit antisera to hamster GlcATI failed to detect cross-reactive material in pgsG cells by immunofluorescence and Western blotting. Furthermore, expression of chimeric proteins composed of mutant GlcATI fused to IgG binding domain of protein A or to green fluorescent protein did not yield the proteins at the expected mass. The green fluorescent protein-tagged version appeared as a truncated protein, and immunofluorescence showed large perinuclear bodies at 30 °C. At 37 °C, the fusion protein was not readily detectable. Sequencing cDNAs from mutant and wild-type cells revealed a single base transition (G331A) in the open reading frame in pgsG cells, which resulted in a Val-111 → Met substitution. These data suggest that pgsG cells contain a labile form of GlcATI that causes conditional expression of glycosaminoglycans dependent on temperature.

Proteoglycans play important roles as regulators in many biologic processes, such as cell adhesion, proliferation, differentiation, and cytokine and growth factor action (1, 2). Most of these activities depend on interactions of proteins with sulfated oligosaccharide sequences present in the glycosaminoglycan (GAG) chains that distinguish proteoglycans from other glycopolypeptide conjugates. GAG assembly initiates by the formation of a tetrasaccharide, GlcAβ1,3Galβ1,3Galβ1,4Xylβ-O-attached to the side chain hydroxyl group of specific serine residues (3). This oligosaccharide serves as a primer for chain elongation, forming either heparan sulfate or chondroitin sulfate depending upon the addition of a GlcNAc or GalNAc residue, respectively (3, 4). The chains then polymerize by the alternating addition of GlcA and GlcNAc or GalNAc residues and undergo modifications by multiple sulfotransferases and epimerases, thus generating binding sites for various ligands (5, 6). Nearly all of the enzymes involved in GAG assembly have now been identified, molecularly cloned, and in many cases mutated in cells or model organisms (6).

Glucuronyltransferase I (GlcATI) catalyzes the final biosynthetic step in the formation of the linkage region tetrasaccharide. This enzyme has been purified and cloned from several sources (7–12). It belongs to a family of three glucuronyltransferases that catalyze the formation of GlcAβ1,3Gal-linkages in different glycoconjugates (13–16). Negishi and co-workers (17, 18) have crystallized GlcATI with bound trisaccharide acceptor (Galβ1,3Galβ1,4Xyl) and UDP-GlcA donor. The enzyme appears as a dimer in the crystal structure (17, 18). In vivo, it is located in the Golgi along with other enzymes involved in GAG assembly (19).

We described previously a set of Chinese hamster ovary cell mutants designated pgsG that lack GlcATI enzyme activity and fail to make both heparan sulfate and chondroitin sulfate (20). In the course of studying the Caenorhabditis elegans ortholog of GlcATI (21), we discovered that GAG assembly in the pgsG mutants depended on the temperature at which the cells were grown. Here we show that the pgsG mutants contain a point mutation in the GlcATI gene, which renders GAG synthesis temperature-sensitive.

EXPERIMENTAL PROCEDURES

Cell Culture—Chinese hamster ovary cells (CHO-K1, ATCC CCL-61) were obtained from the American Type Culture Collection (Manassas, VA). The isolation of mutants pgsG-110, -114, and -224 was described previously (20). The cells were grown under an atmosphere of 5% CO2 in air and 100% relative humidity and maintained in Ham’s F-12 growth medium (Hyclone Laboratories) supplemented with 7.5% (v/v) fetal bovine serum (Hyclone Laboratories), 100 µg/ml streptomycin sulfate, and 100 units/ml penicillin G. Sulfate-free medium was prepared from individual components (22), substituting chloride salts for sulfate, and fetal bovine serum that had been dialyzed against phosphate-buffered saline (23).

Purification of Glycosaminoglycan Chains—Cells were labeled for 6–24 h with 10 µCi/ml H35SO4 (1600 Ci/mmol, PerkinElmer Life Sciences) in sulfate-free medium as indicated in the figure legends.

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1 The abbreviations used are: GAG, glycosaminoglycan; CHO, Chinese hamster ovary; GlcA, β-glucuronic acid; GlcATI, UDP-GlcA: Galβ1,3Gal-R β1,3glucuronyltransferase; PBS, phosphate-buffered saline; GFP, green fluorescent protein; MES, 2-(N-morpholino)ethanesulfonic acid; GST, glutathione S-transferase; FTIC, fluorescein isothiocyanate.
Radionabeled GAGs were isolated from the growth medium and the cells by anion-exchange chromatography as described (24) and quantified by liquid scintillation chromatography, and the counts were expressed relative to the amount of cell protein that was analyzed. In some experiments, the radionabeled GAGs were analyzed by anion-exchange high pressure liquid chromatography (20). The elution position of chondroitin sulfates and heparan sulfates was determined by enzymatic treatment of the chains (24).

**In Vitro Assay of GlcATI Activity**—GlcATI activity was assayed under optimized conditions as described (10, 20). The standard reaction (25 μl) contained 10–30 μg of total cell homogenates, 0.05% Triton X-100, 50 mM MES, pH 6.5, 10 mM MnCl2, 0.2–0.4 μCi of UDP-[1,3-3H]GlcA, 100 μM UDP-GlcA, and 10 mM synthetic Galβ3Galβ-O-naphthalenemethanol as acceptor (25). The reaction was diluted with 1 ml of 0.5 mM NaCl and applied to a Sep-Pak C18 cartridge (100 mg, Waters). After washing the cartridge with 2 ml of water, the product was eluted with 50% methanol, dried, and counted by liquid scintillation. The reaction was proportional to protein and time at 37 °C for at least 4 h.

**DNA Sequencing**—mRNA from mutant and wild-type CHO cells was purified, and first-strand cDNA was produced using random primers (Superscript™ Preampification System for First Strand cDNA synthesis, Invitrogen). The 5′- and 3′-primers were 5′-CTTAAAGCGGTC-CGCCGGACCCC-3′ and 5′-TGGAGGGGCTGCTCTGTCTGTTCTGTA-3′. PCR was carried out with Pfu polymerase (Clontech; 25 cycles at 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min, followed by a final incubation at 72 °C for 7 min) in a 2400 Thermocycler (PerkinElmer Life Sciences). The PCR products were cloned into pCR-Script Amp SK+ (Stratagene). At least two clones from each PCR were sequenced on both strands by the dideoxy chain termination method using Taq polymerase (dye terminator cycle sequencing, PerkinElmer Life Sciences) with a DNA automatic sequencer (ABI PRISM genetic analyzer).

**Production of Rabbit Polyclonal Antiserum against GlcATI-1**—A polyclonal antiserum was prepared against recombinant GlcATI fused to glutathione S-transferase. A cDNA fragment consisting of GlcATI without its transmembrane and cytosolic domains (residues 29–335) was amplified by PCR using Pfu polymerase (Clontech) and cloned into the EcoRI and XhoI sites of pQE-X-KG expression vector (Amersham Biosciences). The resulting cDNA encoded a fusion protein composed of GST, a thrombin cleavage site, and the catalytic domain of hamster GlcATI. The GST fusion protein was produced after isopropyl-1-thio-β-D-galactopyranoside induction of a 1-liter culture of *Escherichia coli* DH5α containing the plasmid. The cells were recovered by centrifugation and resuspended in 20 ml of cold PBS containing 1 mM EDTA, 0.1% β-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride, and 5 mM benzamidine. The sample was sonicated twice for 30 s on ice, adjusted to 1% (v/v) Triton X-100, and centrifuged at 4 °C at 10,000 × g for 10 min. Glutathione-agarose beads (Sigma) were equilibrated with PBS, and 1 ml of a 50% slurry was added to the supernatant. The suspension was mixed gently at 4 °C for 30 min using a rotary mixer and then briefly centrifuged to recover the beads. After washing the beads 4 times with cold PBS, the fusion protein was eluted by incubating the beads twice with 1 ml of fresh, reduced glutathione (10 mM) followed by centrifugation. The eluant was diluted 10-fold with cold PBS and repurified on fresh beads. GST-GlcATI was injected into New Zealand White rabbits (0.5 mg/injection, Robert Sargeant, Ramona, CA). The antiserum was obtained after 5–6 injections, spaced apart by 6–12 weeks.

To make affinity columns, purified GST and GST-GlcATI were cross-linked to CNBr-activated Sepharose 4B (Amersham Biosciences). Rabbit antiserum was diluted 1:3 with PBS and mixed with GST-GlcATI-Sepharose (1 ml) at 4 °C overnight. The resin was collected by low speed centrifugation and washed with PBS until the absorbance at 280 nm of the supernatant was ≤0.01. The antibodies were eluted twice with 4 ml of a buffer containing 0.05 M glycine and 0.15 M NaCl, pH 2.3, neutralized immediately with 1 M Tris base, pH 9.5, and dialyzed against PBS at 4 °C. Antibodies to GST were removed by passing the antiserum through a glutathione-agarose column.
through a column containing GST, and the material was then concentrated by membrane centrifugation (Centricon 10) to 0.5 mg/ml. The antiserum appeared to be specific based on its strong reaction with purified GST-GlcATI fusion protein by Western blotting and the reaction of a single band in extracts from wild-type CHO cells.

Expression of Wild-type and Mutant GlcATI Fusion Proteins—cDNA encoding amino acids 30–335 of GlcATI (stem region and catalytic domain) was prepared and fused in-frame to the C terminus of the IgG-binding domain of protein A in pRK5-F10-PROTA (26). The fusion proteins were transiently expressed in COS-7 cells, isolated by IgG affinity chromatography from the medium, and analyzed by SDS-PAGE.

The full-length coding sequences of mutant and wild-type GlcATI were prepared by PCR and fused in-frame to the N terminus of green fluorescent protein (GFP) in vector pEGFP-N1 (Clontech). These constructs were transiently transfected into CHO-K1 cells using LipofectAMINE 2000 (Invitrogen). For Western blots, the cells were harvested 48 h after transfection. For labeling studies, [35S]methionine was added 24 h after transfection, and 1 day later the cells were harvested and analyzed by SDS-PAGE, autoradiography, and Western blotting.

SDS-PAGE Analysis—Samples were boiled in reducing SDS sample buffer (Invitrogen) and analyzed by SDS-PAGE on a 4–20% Tris-gly-
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Fig. 5. A protein A-pgsG GlcATI fusion protein is not stable. Mutant and wild-type forms of GlcATI lacking their transmembrane and cytoplasmic domains were fused to the IgG binding domain of protein A and expressed in COS-7 cells (“Experimental Procedures”). The fusion proteins were affinity-purified from the conditioned medium using IgG-agarose beads and analyzed by SDS-gel electrophoresis. The gels were silver-stained or analyzed by Western blotting using rabbit polyclonal anti-GlcATI antibody, and the bands were captured using a DKC-5000 digital camera (Sony) and densitometrically analyzed. The cells were stained with Hoechst dye, and photomicrographs were taken in epi-fluorescence under a Zeiss microscope filled with green barrier filter for FITC emission. The digitized images were captured using a DRC-5000 digital camera (Sony) and rendered in Adobe Photoshop.

Wild-type and mutant GlcATI-GFP were transiently transfected into wild-type and mutant CHO cells on coverslips using LipofectAMINE™ (Invitrogen). Three days later, cells were made permeable with 0.1% Triton X-100 in PBS and then fixed with 1% paraformaldehyde in PBS. The cells were washed three times with 50 mM Tris-HCl, pH 7.5, containing 0.15 M NaCl. Lysis buffer (1 ml) was added (50 mM Tris-HCl, pH 7.5, 0.15 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, 1 mM phenylmethylsulfonyl fluoride, and 5 mM EDTA). After incubation for 20 min at room temperature, the cell lysates were washed with lysis buffer and collected for SDS-PAGE.

Immunostaining GlcATI in Wild-type and Mutant CHO Cells—Both wild-type and pgsG mutant cells were grown on glass coverslips at low density. The cells were first made permeable by treatment with PBS containing 0.1% Triton and 0.1% bovine serum albumin (10 min) and then blocked with 1% bovine serum albumin in PBS for 1 h. The cells were then stained with antibodies to GlcATI or α-mannosidase II (kindly provided by Dr. Marilyn Farquhar, University of California, San Diego) and FITC-conjugated goat anti-rabbit IgG secondary antibodies. Cells were viewed by epifluorescence under a Zeiss microscope filled with a green barrier filter for FITC emission. The digitized images were captured using a DRC-5000 digital camera (Sony) and rendered in Adobe Photoshop.

Wild-type and mutant GlcATI-GFP were transiently transfected into wild-type and mutant CHO cells on coverslips using LipofectAMINE™ (Invitrogen). Three days later, cells were made permeable with 0.1% Triton X-100 in PBS and then fixed with 1% paraformaldehyde in PBS. The cells were stained with Hoechst dye, and photomicrographs were taken and processed as described (19).

RESULTS AND DISCUSSION

Glycosaminoglycan Synthesis Is Conditionally Defective in pgsG Cells—In the course of studying the GlcATI ortholog of C. elegans (SQV-8), a cDNA clone for the C. elegans gene was introduced into pgsG mutant cells to test if it corrected the GAG deficiency (21). In these studies, the cells were grown at 30 °C because some enzymes from nematodes are more active at lower temperatures. pgsG cells made GAGs after transfection with SQV-8 GlcATI, but the same results were obtained when the cells were transfected using a control vector lacking any insert. Further analysis showed that the mutant produced GAGs to the same extent as wild-type cells at all temperatures below 30 °C, whereas it produced significantly less GAG at higher temperatures (35–37 °C) (Fig. 1A). Some residual synthesis occurred at elevated temperatures presumably due to a small amount of residual enzyme activity (as shown below). Analysis of the composition of the [35S]GAGs generated at 30 °C showed that pgsG cells made the normal amount of heparan sulfate and chondroitin sulfate as compared with wild-type cells grown under identical conditions (Fig. 2). At 37 °C, the mutant made a small amount of [35S]labeled material that migrated like GAG chains. Furthermore, the heparan sulfate chains from both mutant and wild-type cells grown at 30 °C bound FGF-2 to the same extent (data not shown and see Ref. 27).

To determine how rapidly the growth temperature affected GAG synthesis, cells were grown under permissive conditions (30 °C) in the presence of [35S]SO4 2− and then shifted to non-permissive conditions (37 °C) (Fig. 1B). When maintained at 30 °C, both mutant and wild-type cells generated GAGs at the same rate, which paralleled the growth of the cells (doubling time ~30 h). When shifted to 37 °C, wild-type cells increased the rate of synthesis of GAGs dramatically, balancing the extent of GAG synthesis with the increase in growth rate (doubling time ~14 h). In contrast, when the mutant was shifted to 37 °C, [35S]GAGs no longer accumulated in the cultures. The doubling time of the mutant was unaffected, indicating that the decrease in GAG synthesis was independent of growth. The decrease in the amount of [35S]GAGs recovered from the cultures 1–2 days after temperature shift presumably reflects partial turnover of membrane proteoglycans through endocytosis and lysosomal degradation (28, 29).

GlcATI Activity in pgsG Cells—The labeling studies suggested that GlcATI activity should be present in pgsG cells cultured at 30 °C and that it might be thermostable. To test this possibility, cell-free extracts were prepared from mutant and wild-type cells cultured at 30 °C and assayed at 30 and 37 °C using conditions optimized for the wild-type enzyme (10, 20). Wild-type cells expressed robust activity at both temperatures (Fig. 3, solid bars). In contrast, the mutant had low activity at both temperatures compared with the wild type, but the values were significantly above background (i.e. the signal obtained in the absence of added acceptor; open bars). Somewhat higher activity was observed at 30 °C compared with 37 °C in several experiments, but this difference was not statistically significant due to variation in the assay. Attempts to stabilize the residual activity in the mutant by altering the concentration of substrates and cofactors were not successful, making further analysis of the enzyme difficult. Although enzyme activity was low in the mutant at permissive conditions, it could account for GAG biosynthesis. CHO cells produce about ~1 μg of GAG/mg of cell protein, which is ~50 pmol of GAG chains/mg of cell protein assuming on average 100 disaccharides/chain. Residual enzyme activity in the mutant was reported to be 0.2 pmol/min/mg cell protein (10, 20). Given a generation time of ~14 h, the mutant could generate about 0.2 pmol/min/mg × (60 min/h × 14 h) = ~170 pmol of chains/mg cell protein, more than enough to account for the level of residual synthesis. In the wild type, GlcATI is expressed in large catalytic excess.

Mutant GlcATI Protein Is Missing in pgsG Cells—To determine the level of GlcATI protein in the mutant, cells were made permeable, reacted with polyclonal anti-GlcATI antibodies, and stained with FITC-conjugated goat anti-rabbit IgG. In wild-type cells, GlcATI localized to a perinuclear region (Fig. 4B), much like α-mannosidase II (Fig. 4A), a medial Golgi marker (19). Surprisingly, GlcATI was not detected in pgsG cells (Fig.
although α-mannosidase was detected (Fig. 4C). Similarly, we were not able to detect GlcATI in the mutant by Western blotting extracts prepared from the cells (data not shown).

To increase the level of expression of the mutant enzyme, chimeras were prepared containing the signal peptide from transin, the IgG-binding domain of protein A, and residues 30–335 of wild-type or mutant GlcATI (stem region and catalytic domain). Recombinant protein secreted into the medium was affinity-purified, separated by SDS-PAGE, and analyzed by Western blotting. Expression of a chimera containing wild-type GlcATI yielded a band of the expected mass (68 kDa) and was detected both by silver staining and by Western blotting with anti-GlcATI polyclonal antibodies (Fig. 5). In contrast, the chimera containing the mutant form of GlcATI was not detectable by chemical or antibody staining. This experiment was repeated with chimeras consisting of the full-length forms of GlcATI fused on their C termini to GFP. The cells were radiolabeled with [35S]methionine, and samples were immunoprecipitated with antiserum to GFP. SDS-PAGE and autoradiograms showed that the mutant chimera was not detectable by Western blotting with anti-GFP or anti-GlcATI antiserum (“Experimental Procedures”). The arrows mark the migration of GFP and GlcATI-GFP fusion proteins, and the arrowheads indicate the position of an unusual cleavage product generated from the mutant GlcATI-GFP fusion.
rathy showed multiple bands brought down by the antisera, with a particularly strongly labeled band in cells transfected with GFP fused to wild-type GlcATI (Fig. 6). This band was greatly reduced in cells transfected with mutant GlcATI-GFP. Instead, a protein migrating with a lower mass was observed (arrowhead, Fig. 6, left panel), suggesting that the fusion protein underwent proteolytic cleavage. Western blotting with anti-GFP or anti-GlcATI polyclonal sera confirmed that the cleavage product contained both GFP and a portion of GlcATI. These findings suggested that the mutation rendered GlcATI more susceptible to proteolysis, presumably resulting in its inactivation. When GFP-tagged forms of wild-type GlcATI were expressed in wild-type CHO cells grown at 30 and 37 °C, the wild-type chimera exhibited a typical Golgi localization pattern at both 30 and 37 °C (Fig. 7, C and D). In contrast, the mutant form of the enzyme showed unusually large perinuclear fluorescent loci, which may reflect aggregation of partially degraded protein (Fig. 7E, multipanel). At 37 °C, only one example of a cell was found with weak diffuse staining (Fig. 7F), similar to the pattern of staining seen in cells transfected with GFP (Fig. 7, A and B). Similar results were obtained when the GFP-tagged forms of the enzymes were transfected into pgsG cells (data not shown).

Mutant cells exhibiting temperature-sensitive GAG synthesis should prove useful for several types of studies. The mutant can be used for biosynthetic studies to synchronize the expression of GAGs and proteoglycans on the cell surface. Turnover studies can be performed in the mutants in the absence of biosynthesis, thus simplifying the analysis of proteoglycan turnover kinetics and distribution. The dependence of binding or activation of proteoglycan receptors by which GAGs play a role (e.g., receptor activation) can be titrated by using cells at different times after shift to permissive conditions or by growing cells at intermediate temperatures. Temperature-sensitive mutants also provide a tool for studying enzyme folding and oligomerization. Although conditional alleles in other genes involved in GAG assembly are not yet available, it should be possible to phenocopy the mutant described here by expressing transgenes that can be activated pharmacologically. This latter approach could be used in other CHO mutants non-conditionally defective in GAG biosynthesis (24, 27, 30–33), thus expanding the utility of existing mutants.

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REFERENCES
31. Tone, Y., Kitagawa, H., Imiya, K., Oka, S., Kawasaki, T., and Sugahara, K. (1999) FEBS Lett. 459, 415–420
32. Kitagawa, H., Tsuji, M., Hata, K., Sugita, M., and Kawasaki, T. (1999) Biochem. J. 358, 539–546
33. Oka, S., Terayama, K., Kawasaki, C., and Kawasaki, T. (1992) J. Biol. Chem. 267, 22711–22714
34. Terayama, K., Oka, S., Seiki, T., Ito, S., Nakamura, A., Kato, S., Kawasaki, T. (1997) J. Biol. Chem. 272, 6093–6098
35. Terayama, K., Seiki, T., Nakamura, A., Matsuzaki, K., Oka, S., Sugita, M., and Kawasaki, T. (1998) J. Biol. Chem. 273, 30295–30300
36. Seiki, T., Oka, S., Terayama, K., Imiya, K., and Kawasaki, T. (1999) Biochem. Biophys. Res. Commun. 260, 192–197
37. Pedersen, L. C., Tsuji, K., Kitagawa, H., Sugahara, K., Darden, T. A., and Negishi, M. (2000) J. Biol. Chem. 275, 34580–34585
38. Pedersen, L. C., Darden, T. A., and Negishi, M. (2002) J. Biol. Chem. 277, 21869–21873
39. Pinhal, M. A. S., Smith, B., Olson, S., Aikawa, J., Kimata, K., and Esko, J. D. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 12864–12869
40. Bai, X. M., Wei, G., Sinha, A., and Esko, J. D. (1999) J. Biol. Chem. 274, 13017–13024
41. Bulk, D. A., Wei, G., Toyoji, H., Kinosita-Toyoji, A., Waldrip, W. R., Esko, J. D., Robbins, P. W., and Selaee, S. B. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 10838–10843
42. Ham, R. G. (1965) Proc. Natl. Acad. Sci. U. S. A. 53, 288–293
43. Dalbec, R., and Vogt, M. (1954) J. Exp. Med. 99, 167–169
44. Bame, K. J., and Esko, J. D. (1989) J. Biol. Chem. 264, 8059–8065
45. Sarkar, A. K., and Esko, J. D. (1995) Carbohydr. Res. 279, 161–171
46. Wei, Z., Swezler, S. J., Ishihara, M., Orellana, A., and Hirschberg, C. B. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 3885–3888
47. Bai, X. M., and Esko, J. D. (1996) J. Biol. Chem. 271, 17171–17177
48. Bame, K. J., Zhang, L., David, G., and Esko, J. D. (1984) Biochem. J. 226, 1–8
49. Bai, X. M., Bame, K. J., Habuchi, H., Kimata, K., and Esko, J. D. (1997) J. Biol. Chem. 272, 23172–23179
50. Esko, J. D., Elgavish, A., Pezeshki, T., Taylor, W. H., and Weineke, J. L. (1986) J. Biol. Chem. 261, 15725–15733
51. Esko, J. D., Stewart, T. E., and Taylor, W. H. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 3197–3201
52. Esko, J. D., Weineke, J. L., Taylor, W. H., Ekborg, G., Rodén, L., Anantharamaiah, G., and Gawash, A. (1987) J. Biol. Chem. 262, 12189–12195
53. Lefkowitz, K., Weineke, J. L., Kaiser, G. S., Lagunoff, M., Bame, K. J., Cheifetz, S., Massague, J., Lindahl, U., and Esko, J. D. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 2267–2271