Mammalian heparanase, strongly implicated in the regulation of cell growth, migration, and differentiation, plays a crucial role in inflammation, angiogenesis, and metastasis. There is thus a clear need for understanding how heparanase activity is regulated. Cells can generate an active form of the enzyme from a larger inactive precursor protein by a process of secretion-recapture, internalization, and proteolytic processing in late endosomes/lysosomes. Cell surface heparan sulfate proteoglycans are the sole known components with a role in this trafficking of the heparanase precursor. Here, we provide evidence that heparan sulfate proteoglycans are not strictly required for this process. More importantly, by heparanase transfection, binding, and uptake experiments and by using a combination of specific inhibitors and receptor-defective cells, we have identified low density lipoprotein receptor-related proteins and mannose 6-phosphate receptors as key elements of the receptor system that mediates the capture of secreted heparanase precursor and its trafficking to the intracellular site of processing/activation.

Heparan sulfate proteoglycans (HSPGs) play a key role in the self-assembly of extracellular matrices and sequester a variety of growth factors, cytokines, chemokines, and coagulation factors in these structures (1). Degradation of heparan sulfate (HS) thus not only alters the composition and stability of the extracellular matrix but also mobilizes molecules involved in morphogenesis, development, tissue repair, inflammation, autoimmunity, and angiogenesis. Although there is evidence that several HS-degrading endoglycosidases (heparanases) may exist (2), at this time only one transcript encoding a functional heparanase (heparanase-1) has been identified (3–6). Under normal conditions, heparanase-1 expression is restricted to placental trophoblasts, keratinocytes, and blood-borne cells, probably reflecting the requirement of heparanase activity for cell diapedesis and extravasation associated with inflammatory processes, wound healing, and pregnancy (7, 8). Up-regulation of heparanase-1 occurs in nephrosis, cirrhosis, diabetes, and a number of human cancers (3, 4, 7) and markedly promotes tumor angiogenesis and metastasis (9). Understanding how heparanase-1 activity is generated and controlled is thus of major medical interest.

Heparanase-1 is synthesized as an inactive ~65-kDa precursor that subsequently undergoes proteolytic cleavage, yielding ~8- and ~50-kDa protein subunits that heterodimerize to form the active enzyme (10–13). Heparanase-1 is thought to be mostly an intracellular enzyme, because cells rapidly bind and internalize secreted pro-heparanase-1 (14–16), transferring the internalized precursor to late endosomes/lysosomes in which it is processed into the mature active form of the enzyme and stays localized (15). Recently, it has been suggested that the uptake or reuptake of secreted heparanase-1 precursor is mediated by cell membrane HSPGs, in particular the syndecans (16). The experiments reported here confirm a role for HSPGs in heparanase-1 uptake but indicate that these are not the sole elements involved and are not strictly required. Rather, we found that low density lipoprotein receptor-related protein (LRP) and possibly other receptor-associated protein (RAP)-sensitive receptor(s), as well as mannose 6-phosphate (Man-6-P) receptors are key to this process.

**EXPERIMENTAL PROCEDURES**

*Plasmid Construction*—Heparanase-1 cDNA was prepared from CaCo2 cell mRNA by reverse transcription-PCR and cloned into pcDNA 3.1/zeo (+) (Invitrogen). QuikChange site-directed mutagenesis (Stratagene) was used to mutate all six N-glycosylation Asn residues into Gln residues (ΔS1–6). All constructs were verified by sequencing.

*Antibodies*—The synthetic peptide YGPDVGQPRRKTAKM, corresponding to the sequence in the ~50-kDa subunit of mature heparanase-1, was coupled to keyhole limpet hemocyanin for immunization. Rabbit anti-heparanase-1 antibodies were affinity-purified on the same peptide coupled to EAH-Sepharose. Mouse monoclonal anti-actin antibody was obtained from Sigma-Aldrich. Mouse monoclonal antibodies 3G10 (recognizing desaturated glucuronate created by enzymatic cleavage of heparan sulfate with bacterial heparitinase) (17) and 2E9 (recognizing an epitope in the cytoplasmic domain of syndecan-1) (18) were prepared as described previously.

*Recombinant RAP*—His6-RAP (plasmid provided by Dr. T. Willnow) was expressed in BL21(DE3)pLysS bacteria (Invitrogen) and purified using a nickel-nitrioltriacetic acid resin column (Qiagen) according to the manufacturer’s instructions. His6-RAP was checked for purity by SDS-PAGE and stored at ~80 °C in Tris-buffered saline.
Heparanase-1 Uptake

Cell Cultures and Transfection—All of the cells used were immortalized cell lines. Wild-type mouse embryonic fibroblasts (MEFs) were derived from wild-type mice. Wild-type HEK 293-T cells, wild-type CHO K1 cells, and MEFs that are genetically deficient in LRP (PEA13) (19) were obtained from ATCC. HS-deficient CHO cells (CHO 677) (20) were provided by Dr. J. D. Esko (University of California, San Diego). CHO cells deficient in LRP (CHO 13-5-1) (21) were provided by Dr. D. FitzGerald (National Institutes of Health, Bethesda, MD). MEF deficient in both the cation-independent (~300-kDa) and cation-dependent (~46-Da) Man-6-P receptor (22) were provided by Dr. K. von Figura (University of Göttingen, Germany). Cells were routinely grown in Dulbecco’s modified Eagle’s medium/nutrient mixture F-12 (Invitrogen) supplemented with 10% fetal calf serum (HyClone). MEFs were incubated with ~[35S]HS was prepared from metabolically labeled cultured human lung fibroblasts (17). Lysates from heparanase-1- or mock-transfected cells were incubated with ~[35S]HS in 30 mM phosphate buffer (pH 5.5) overnight at 37 °C. Digested material was fractionated by SDS-PAGE (4−15% gradient gel) and detected by autoradiography.

Endoglycosidase Digestions— Methanol/chloroform-precipitated cell extracts were suspended in 0.5% SDS and 0.1 M β-mercaptoethanol and boiled for 5 min. For PNGase F digestion (Roche Applied Science) the solution was adjusted to 3% Nonidet P-40, 50 mM Tris/HCl (pH 8.6), and 0.5 units of PNGase F. For Endo H digestion (Roche Applied Science) the solution was adjusted to 75 mM sodium acetate (pH 5.5), 0.05% phenylmethylsulfonyl fluoride, and 5 milliunits of Endo H. Both digestions were incubated overnight at 37 °C followed by Western blotting, as described above.

RESULTS

HSPGs Are Not Strictly Required for Heparanase-1 Uptake—HEK 293-T cells that were transiently transfected with heparanase-1 accumulated both the inactive precursor (~65 kDa) and the mature active form (~50 kDa) of the enzyme in roughly similar amounts (Fig. 1A, lane 2). The culture medium of these cells contained large amounts of the pro-enzyme but no mature enzyme (Fig. 1A, lane 4). Yet, many other heparanase-1-transfected cells released hardly any pro-enzyme in their culture medium (see below for examples). Monensin inhibited the accumulation of processed enzyme (Fig. 1B) and heparanase activity (Fig. 1C) in HEK 293-T cells, consistent with the late endosome/lysosome being the primary heparanase-1-processing organelle (15). The capacity of cells to internalize secreted heparanase-1 precursor and to convert the internalized protein into an active form of the enzyme was evident from adding spent culture medium, conditioned by heparanase-1-transfected HEK 293-T cells (Fig. 1A), to cultured mammalian cell lines, e.g. wild-type CHO K1 cells (Fig. 1, D and E) and wild-type MEFs (Fig. 1, F and G). Both cell types quickly bound the ~65-kDa heparanase-1 precursor and progressively converted the precursor into mature ~50-kDa protein (Fig. 1, D and F). In MEFs, this resulted in markedly enhanced heparanase activity (Fig. 1G). Treating the cells with trypsin removed none of the mature form but, depending on the length of the incubation with precursor, removed most or part of the precursor form of heparanase-1 (Fig. 1E), suggesting that all processing occurs inside the cells. Below, we describe similar transfection and uptake experiments using various cell lines with known receptor deficiencies.

Consistent with a possible role for HSPG in heparanase-1 uptake or reuptake, HS-deficient CHO 677 cells (20) that were transiently transfected with heparanase-1 released large amounts of the ~65-kDa heparanase-1 precursor into their culture medium, conditioned by heparanase-1-transfected HEK 293-T cells (Fig. 2A, lane 4). Heparanase-1-transfected wild-type CHO K1 cells, in contrast, released none (Fig. 2A, lane 3). Compared with wild-type CHO K1 cells, lysates of CHO 677 cells still contained considerable (almost similar) amounts of the ~50-kDa active enzyme (Fig. 2A, lane 2 versus lane 1). Yet, in comparison with wild-type CHO K1 cells, HS-deficient CHO 677 cells showed a moderate delay in binding and uptake/processing of exogenous, added pro-heparanase-1 (Fig. 2B). Consistently, treatment of wild-type CHO K1 cells with bacterial heparitinase slightly decreased heparanase-1 binding and delayed uptake/processing (Fig. 2C, left panel). The heparitinase digestion was effective, as shown by the appearance of the 3G10 epitope (17), decorating discrete bands corresponding to the molecular masses of core proteins of different HSPGs (e.g. ~65-kDa syndecan-1, ~60-kDa glypican, ~35-kDa syndecan-4) (Fig. 2C, right panel). Because CHO 677 cells are deficient in HS but overexpress chondroitin sulfate (20), we then investigated whether chondroitin sulfate proteoglycans might be responsible for the remaining heparanase-1 binding and uptake, by pretreating CHO 677 cells with chondroitinase ABC. Although the treatment was effective (appearance of a discrete ~65-kDa band corresponding to the core protein of syndecan-1, a hybrid HS/chondroitin sulfate proteoglycan (18)) (Fig. 2D, right panel), we could not detect any effect on heparanase-1 binding and internalization (Fig. 2D, left panel). It had been suggested that added
heparin competes with cell surface HSPGs for heparanase-1 binding and thus inhibits uptake (16). Our data confirmed that the binding and uptake of heparanase-1 can be totally inhibited by the addition of increasing concentrations of heparin (1–50 μg/ml). Yet, inhibition not only occurs in wild-type CHO K1 cells (Fig. 2E, left) but also in HS-deficient CHO 677 cells (Fig. 2E, right), implying that heparin also inhibits heparanase-1 binding to other receptor(s). From all of these data, we conclude that HSPGs are not strictly required for the capture or recapture and internalization of the heparanase-1 precursor and are not the key players in this process. Rather, they may have a role in regulating its efficiency and/or kinetics.

LRP Involvement in Heparanase-1 Precursor Uptake—Because the roles of LRP in the uptake of proteases, protease inhibitor complexes, lipoproteins, and lipases are well established (23, 24), we then tested whether this receptor might be involved in the uptake or reuptake of secreted heparanase-1 precursor. MEFs deficient in LRP (PEA13 cells) (19) that were transiently transfected with heparanase-1 released large amounts of the ~65-kDa precursor in their culture medium (Fig. 3A, left panel, lane 4). Transfected wild-type MEFs (which express LRP abundantly), in contrast, released none (Fig. 3A, left panel, lane 3). Comparing LRP-deficient CHO 13-5-1 cells (21) with wild-type CHO K1 cells yielded similar results (Fig. 3A, right panel). As these data were suggestive of a role for LRP, this role was further assessed by examining the effect of RAP, a receptor-associated protein that inhibits ligand binding to several members of the low density lipoprotein-receptor family. The addition of increasing concentrations of RAP to heparanase-1-transfected MEFs (Fig. 3B) or CHO K1 cells (data not shown) resulted in progressively increasing amounts of the ~65-kDa heparanase-1 precursor in the culture medium. The addition of RAP to transiently transfected PEA13 cells (Fig. 3C) or CHO 13-5-1 cells (data not shown) also increased the amount of heparanase-1 precursor that these cells released in the culture medium, suggesting that RAP-sensitive receptors other than LRP (e.g. sortilin, very low density lipoprotein receptor, megalin, etc.) might also be involved in heparanase-1 precursor uptake. Yet, RAP is a known ligand for heparin and may bind to HSPGs in some cell types (25), while not binding to HSPGs in cells that produce other, less or differently sulfated forms of HS (26). The addition of RAP to heparanase-1-transfected CHO 677 cells markedly enhanced the amount of heparanase-1 precursor that these cells released in the culture medium and reduced the amount of mature enzyme in the lysate of the cells (Fig. 3D), confirming a role for LRP (and possibly other RAP-sensitive receptors) in heparanase-1 binding and uptake and suggesting that this role might be less apparent in the presence of HSPGs. Consistent with a role for LRP, uptake experiments detected a moderate but clear difference between MEFs and PEA13 cells in the rate of heparanase-1 conversion (Fig. 3E, lane 1 versus lane 3, showing inverted ratios of precursor over mature protein after 120 min of incubation with heparanase-1 precursor at 37 °C). Consistently, the processing of the heparanase-1 precursor by MEFs decreased when RAP was included during the uptake experiment, reducing it to the level of processing by PEA13 cells (Fig. 3E, lanes 2 and 3). At the same time, the precursor associated with MEFs remained more accessible to trypsin in the presence of RAP (Fig. 3F, lanes 3 and 4) than in the absence of RAP (Fig. 3F, lanes 1 and 2), suggesting that the net effect of RAP is reduced uptake and, consequently, conversion. RAP also had a small effect on uptake/processing by PEA13 cells (Fig. 3E, lane 4), confirming that RAP-sensitive receptors other than LRP might also mediate heparanase-1 precursor uptake. The inclusion of RAP moderately reduced the levels of precursor and severely reduced the levels of mature enzyme associated with CHO 677 cells (Fig. 3G), confirming a role for LRP (and possibly other RAP-sensitive receptors) in heparanase-1 binding and uptake and suggesting that this role might be less apparent in the presence of HSPGs. Consistently, increasing concentrations of RAP also progressively reduced the amount of heparanase-1 precursor that became associated with MEFs when these cells were incubated with heparanase-1 at 4 °C (preventing internalization) for 60 min (time sufficient to reach maximal binding), implying that LRP and/or other RAP-sensitive receptors are involved in the binding of the heparanase-1 precursor (Fig. 3H).
Man-6-P Receptor Involvement in Heparanase-1 Precursor Uptake

As most of the soluble lysosomal hydrolases are transported via Man-6-P receptors (27), we then investigated whether this receptor system might also play a role in the capture or recapture of heparanase-1. If so, then heparanase-1 glycosylation should affect the trafficking and activation of the precursor protein. Treatment of heparanase-1-transfected HEK 293-T cells with tunicamycin A (TM) (an inhibitor of N-glycosylation) or replacement of all six Asn residues in the protein that compose sites for N-glycosylation (28) by Gln residues resulted in heparanase-1 migrating as only one single band of 50 kDa, corresponding to the predicted molecular mass of the nonglycosylated form of proheparanase-1 (Fig. 4, A and B, left panels), and no gain in heparanase activity (Fig. 4, A and B, right panels), suggesting that the maturation of the pro-enzyme is inhibited. These results are in clear contrast to an earlier claim that the N-glycosylation of heparanase-1 is not required for its activation and activity (28). Although we have no formal explanation for this discrepancy, we suggest that the metabolic stability of the (pre-formed) active enzyme (half-life of ~30 h) (16) may hamper detecting effects of TM on heparanase activity in stably transfected cells (28). Cell extracts of transiently heparanase-1-transfected HEK 293-T cells were then digested in vitro with either PNGase F (cleaving all Asn-linked oligosaccharides) or Endo H (removing only the high mannose and hybrid types of Asn-linked oligosaccharides). Western blots of these digestions (Fig. 4 C) revealed that the 65-kDa precursor and the 50-kDa mature enzyme are equally sensitive to PNGase F and Endo H, both digestions leaving none of the proteins intact and leading to multiple bands (in the case of Endo H, including also major bands that correspond to the molecular masses of the nonglycosylated forms (~50 kDa for the precursor and ~43 kDa for the mature enzyme)). A similar result was obtained for the secreted heparanase-1 precursor (data not shown). To ensure that these results were not due to the levels of expression or dependent on the cell type, we repeated these experiments with stably heparanase-1-transfected Madin-Darby canine kidney type II cells, obtaining similar results (Fig. 4 D). Inclusion of protease inhibitors during the digestions had no effect on the banding pattern (shown for Endo H in Fig. 4 E). Moreover, neither enzyme degraded a heparanase-1-mutant in which all six N-glycosylation Asn residues had been replaced by Gln residues (Fig. 4 F). Both enzymes also failed to degrade the nonglycosylated form of proheparanase-1.

Heparanase-1 Uptake

FIGURE 2. HSPGs are not strictly required for the binding and internalization of heparanase-1 precursor. A, heparanase-1 accumulation in transfected cells; Western blot, using anti-heparanase-1, of cell extract and medium samples derived from wild-type CHO K1 and HS-deficient CHO 677 cells for the indicated lengths of time either without any pretreatment or supplement (B) after treatment for 4 h at 37 °C with bacterial heparitinase (C) or chondroitinase ABC (D) or with the addition of the indicated concentrations of heparin (E). Total cell lysates were subjected to Western blotting using anti-heparanase-1 and anti-actin (B, C, left panel; D, left panel; and E) with 3G10 monoclonal antibody to detect the desaturated uronates that are generated by heparitinase (C, right panel) or with 2E9 monoclonal antibody to detect the core protein of syndecan-1 (D, right panel). Open arrowheads point at the ~65-kDa heparanase-1 precursor, filled arrowheads point at the ~50-kDa mature active heparanase-1, and arrows point at actin.
cosylated heparanase-1 precursor accumulating in TM-treated cells (not shown), further ensuring that the degradation of the glycosylated precursor was not due to contaminating protease activities. These data imply that most of the Asn-linked oligosaccharides on the precursor and mature form of heparanase-1 are of the non-complex type. Possibly, these contain Man-6-P residues, preventing their conversion to complex carbohydrates.

The sole addition of Man-6-P (10 mM) had little or no effect on the uptake and conversion of heparanase-1 precursor by wild-type MEFs (Fig. 5A, lanes 1 and 3). However, in the presence of RAP (2 μM) Man-6-P further inhibited the conversion of heparanase-1 precursor into the mature form (Fig. 5A, compare lanes 1 and 2 and lanes 2 and 4). A similar combination of Man-6-P and RAP completely prevented the accumulation of mature heparanase-1 in heparitinase-treated MEFs (Fig. 5A, lanes 5–8), and all of the precursor protein associating with these cells was accessible to trypsin (not shown). In contrast, in MEFs that lacked LRP (Fig. 5B) the sole addition of Man-6-P (10 mM) markedly reduced conversion of heparanase-1 precursor; the further addition of RAP had little additional inhibitory effect on this conversion. Similarly, in wild-type CHO K1 cells (Fig. 5C) and in LRP-deficient CHO 13-5-1 cells (Fig. 5D) the combination of Man-6-P and RAP was more effective than either Man-6-P or RAP alone in reducing the con-
version of precursor, and in HS-deficient CHO 677 cells (Fig. 5E) it completely inhibited precursor uptake and conversion. Neither glucose-6-phosphate (10 mM) nor mannose (10 mM) had significant effects on uptake and conversion (Fig. 5, C–E). Finally, we also supplied pro-heparanase-1 to MEFs that lacked Man-6-P receptors (22) (Fig. 5F). All of the heparanase-1 precursors associating with these cells remained in precursor form, which was perhaps to be expected, because such MEFs are likely to be deficient in the lysosomal enzymes required for the processing of internalized heparanase-1 precursor. In the absence of RAP, most of this heparanase-1 resisted treatment of the cells with trypsin, confirming that substantial internalization can occur in the absence of Man-6-P receptors. Adding RAP markedly reduced the accumulation of trypsin-resistant (internalized) heparanase-1 precursor in these cells. Significantly, the internalization of heparanase-1 precursor was completely blocked by RAP when these MEFs were pretreated with heparitinase (Fig. 5F, lane 10). As expected, adding Man-6-P had no effect on heparanase-1 uptake by these cells. Together, these data clearly demonstrate that HSPGs, RAP-sensitive receptors, and Man-6-P receptors cooperate in the binding and internalization of heparanase-1 precursor.

**DISCUSSION**

In this study we show that receptors involved in the binding and internalization of secreted heparanase-1 precursor include HSPGs, LRP, possibly other RAP-sensitive receptor(s), and Man-6-P receptors. Mutant cell lines, undoubtedly deficient in at least one of these receptor systems, indicate that each of these receptors is individually dispensable. Thus, different types of cells are likely to use different combinations of these receptors for regulating their heparanase activities.

Persistent uptake and activation of latent heparanase-1 in the absence of HS, i.e. by HS-deficient CHO 677 cells and by heparitinase-treated cells, indicate that HSPGs are not the sole elements involved and are not strictly required for uptake. Moreover, adding RAP to MEFs that lack Man-6-P receptors or adding the combination of RAP and Man-6-P to wild-type MEFs or CHO cells severely reduces internalization of heparanase-1, suggesting that HSPGs, by themselves, are not effective...
Heparanase-1 Uptake

In the absence of HS and functional Man-6-P receptors (e.g. CHO 667 cells that are exposed to Man-6-P, or Man-6-P receptor-deficient MEFs that are treated with heparitinase) binding and internalization of heparanase-1 precursor persists at a substantial rate. This residual uptake is totally abolished when RAP is also included, unambiguously identifying LRP and possibly other RAP-sensitive receptors as bona fide and major pro-heparanase-1 internalization receptors. The effects of Man-6-P on precursor internalization, evident in LRP-deficient cells or in the presence of RAP, and indirectly the extreme sensitivity of Man-6-P receptor-deficient MEFs to the combination of heparitinase and RAP indicate that Man-6-P receptors work along with LRP and/or other RAP-sensitive receptors in the internalization of the heparanase-1 precursor. Thus, the various mutants are equally (if not primarily) helpful in demonstrating the participation of the "alternative" receptor systems as they are in showing the contribution of the mutant receptor under question. Multiple different internalization receptors would seem to provide robustness to this uptake mechanism, possibly reflecting a strong need for cells to keep extracellular concentrations of heparanase-1 precursor at low levels.

It is not clear to what extent the secretion-recapture route represents a physiological path for cells to direct the heparanase-1 precursor from the endoplasmic reticulum (the site of biosynthesis) to lysosomes, in which it is processed into the mature active form of the enzyme. In most cells with easily detectable endogenous heparanase activity, e.g. non-transfected HEK 293-T cells, heparanase-1 is hardly if at all detectable at the protein level. Possibly, mechanisms for direct sorting from early secretory pathway compartments to lysosomes may be overwhelmed in instances of excessive heparanase-1 production as in transfected cells. In that case it remains to be established whether such direct sorting of heparanase-1 is mediated by the same receptors that were implicated here in the uptake or reuptake of the secreted heparanase-1 precursor, but LRP and in particular Man-6-P receptors are logical candidates.

Still, cells that are not synthesizing elevated levels of heparanase-1 on their own, e.g. quiescent vascular endothelial cells, may capture and activate heparanase-1 precursor secreted by cells that overexpress heparanase-1, e.g. tumor cells, with potential "paracrine" effects on HS-dependent signaling pathways in these "recipient" cells. LRP levels and activity are known to be substantially decreased in many tumors (23). Based on frequent loss of heterozygosity and functional mutations in tumors from cancer patients, and from receptor expression studies in tumor cell lines, it is proposed that the ~300-kDa Man-6-P (Man-6-PIGF-II) receptor represents an important tumor suppressor gene, possibly by mediating the endocytosis and degradation of IGF-II, in this way opposing its growth-promoting effects (27). Our findings could extend such a proposal whereby the loss of LRP and the Man-6-PIGF-II receptor leads to the accumulation of (tumor) heparanase-1 in the extracellular space where it might be taken up and activated by (receptor-expressing) surrounding endothelial cells (promoting e.g. tumor angiogenesis). Moreover, it has been shown that the ~65-kDa heparanase-1 precursor can enhance Akt and in some cases mitogen-activated signaling. The importance of this is highlighted recently by an important study for the role of heparanase-1 in the growth and invasion of breast tumors.
Heparanase-1 Uptake

protein kinase signaling by an unidentified receptor, stimulating endothelial cell migration and invasion (29). The signaling role of the inactive precursor may be enhanced by the loss of LRP and Man-6-P receptors in tumor cells. A corollary of identifying LRP (and/or other RAP-sensitive receptors) as a binding receptor for the heparanase-1 precursor could be that it also functions as the receptor that mediates the signaling functions of the inactive precursor in endothelial cells.

In summary, our studies identify HSPGs, LRP, possibly other RAP-sensitive receptors, and Man-6-P receptors as responsible for the capture or recapture of the heparanase-1 precursor and its delivery to the intracellular compartments in which it is activated. This may have major implications for the development of novel strategies for inhibiting heparanase-1 activation, inflammatory processes, and cancer progression.

Acknowledgment—We thank all who have provided reagents and advice.

REFERENCES

1. Bernfield, M., Gotte, M., Park, P. W., Reizes, O., Fitzgerald, M. L., Lincecum, J., and Zako, M. (1999) Annu. Rev. Biochem. 68, 729–777
2. Bame, K. J. (2001) Glycobiology 11, 918–988
3. Vladavsky, I., Friedmann, Y., Elkin, M., Aingorn, H., Atzmon, R., Ishai-Michaeli, R., Bitan, M., Pappo, O., Peretz, T., Michal, I., Spector, L., and Pecker, I. (1999) Nat. Med. 5, 793–802
4. Hulett, M. D., Freeman, C., Hamdorf, B. J., Baker, R. T., Harris, M. J., and Parish, C. R. (1999) Nat. Med. 5, 803–809
5. Kussie, P. H., Hulmes, J. D., Ludwig, D. L., Patel, S., Navarro, E. C., Seddon, A. P., Giorgio, N. A., and Bohlen, P. (1999) Biochem. Biophys. Res. Commun. 261, 183–187
6. Toyoshima, M., and Nakajima, M. (1999) J. Biol. Chem. 274, 24153–24160
7. Vladavsky, I., and Friedmann, Y. (2001) J. Clin. Invest. 108, 341–347
8. Parish, C. R., Freeman, C., and Hulett, M. D. (2001) Biochim. Biophys. Acta 1471, M99–M108
9. Goldshmidt, O., Zcharia, E., Abramovitch, R., Metzger, S., Aingorn, H., Friedmann, Y., Schirrmacher, V., Mitrani, E., and Vladavsky, I. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 10031–10036
10. Fairbanks, M. B., Mildner, A. M., Leone, J. W., Cavey, G. S., Mathews, W. R., Drong, R. F., Slightom, J. L., Bienkowski, M. J., Smith, C. W., Bannow, C. A., and Heinrikson, R. L. (1999) J. Biol. Chem. 274, 29587–29590
11. Nardella, C., Lahm, A., Pallao, M., Brunetti, M., Vannini, A., and Steinkuhler, C. (2004) Biochemistry 43, 1862–1873
12. Levy-Adam, F., Miao, H. Q., Heinrikson, R. L., Vladavsky, I., and Ilan, N. (2003) Biochem. Biophys. Res. Commun. 308, 885–891
13. McEnery, E., Young, K., Hirokawa, N., Bennett, J., Bhaman, M., Felix, R., Turner, P., Stamps, A., McMillan, D., Saville, G., Ng, S., Mason, S., Snell, D., Schofield, D., Gong, H., Townsend, R., Gallagher, J., Page, M., Parekh, R., and Stubberfield, C. (2003) Biochem. J. 373, 423–435
14. Nadav, L., Eldor, A., Yacoby-Zeevi, O., Zamir, E., Pecker, I., Ilan, N., Geiger, B., Vladavsky, I., and Katz, B. Z. (2002) J. Cell Sci. 115, 2179–2187
15. Zetser, A., Levy-Adam, F., Kaplan, V., Gingis-Velitski, S., Bashenko, Y., Schubert, S., Flugelman, M. Y., Vladavsky, I., and Ilan, N. (2004) J. Cell Sci. 117, 2249–2258
16. Gingis-Velitski, S., Zetser, A., Kaplan, V., Ben-Zaken, O., Cohen, E., Levy-Adam, F., Bashenko, Y., Flugelman, M. Y., Vladavsky, I., and Ilan, N. (2004) J. Biol. Chem. 279, 44084–44092
17. David, G., Bai, X. M., Van der Schueren, B., Cassiman, J. J., and Van den Berghe, H. (1992) J. Cell Biol. 119, 961–975
18. Lories, V., Cassiman, J. J., Van der Berghe, H., and David, G. (1989) J. Biol. Chem. 264, 7009–7016
19. Willnow, T. E., and Herz, J. (1994) J. Cell Sci. 107, 719–726
20. Liddiolt, K., Weiske, J. L., Kiser, C. S., Lugemwa, F. N., Bame, K. J., Cheifetz, S., Massague, J., Lindahl, U., and Esko, J. D. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 2267–2271
21. FitzGerald, D. J., Fyryling, C. M., Zdanovskov, A., Saelinger, C. B., Kounnas, M., Winkles, J. A., Strickland, D., and Leppla, S. (1995) J. Cell Biol. 129, 1533–1541
22. Pohlmann, R., Boeker, M. W., and von Figura, K. (1995) J. Biol. Chem. 270, 27311–27318
23. Herz, J., and Strickland, D. K. (2001) J. Clin. Investig. 108, 779–784
24. Strickland, D. K., and Ranganathan, S. (2003) J. Thromb. Haemost. 1, 1663–1670
25. Ji, Z. S., and Mahley, R. W. (1994) Arterioscler. Thromb. 14, 2025–2031
26. Vassiliou, G., and Stanley, K. K. (1994) Carbohydr. Res. 254, 645–654
27. Goldshmidt, O., Zcharia, E., Abramovitch, R., Metzger, S., Aingorn, H., Friedmann, Y., Schirrmacher, V., Mitrani, E., and Vladavsky, I. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 10031–10036
28. Simizu, S., Ishida, K., Wierzba, M. K., and Osada, H. (2004) J. Biol. Chem. 279, 2697–2703
29. Gingis-Velitski, S., Zetser, A., Flugelman, M. Y., Vladavsky, I., and Ilan, N. (2004) J. Biol. Chem. 279, 23536–23541
Cellular Uptake of Mammalian Heparanase Precursor Involves Low Density Lipoprotein Receptor-related Proteins, Mannose 6-Phosphate Receptors, and Heparan Sulfate Proteoglycans

Veronique Vreys, Nathalie Delande, Zhe Zhang, Christien Coomans, Anton Roebroek, Joachim Dürr and Guido David

J. Biol. Chem. 2005, 280:33141-33148.
doi: 10.1074/jbc.M503007200 originally published online July 26, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M503007200

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 29 references, 16 of which can be accessed free at http://www.jbc.org/content/280/39/33141.full.html#ref-list-1