Yeast-derived Recombinant DG42 Protein of Xenopus Can Synthesize Hyaluronan in Vitro*

Paul L. DeAngelis and Ann Mary Achyuthan
From the Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190

We demonstrate in this report that the Xenopus DG42 gene product made in the yeast Saccharomyces cerevisiae can synthesize authentic high molecular weight hyaluronan (hyaluronic acid; HA) in vitro. Saccharomyces are eukaryotes that do not naturally produce HA or any other molecules known to contain glucuronic acid. Therefore bakers' yeast is a good model system to determine the enzymatic activity of the DG42 protein, which is the topic of recent debate. Membrane extracts prepared from cells expressing DG42 encoded on a plasmid incorporated [14C]glucuronic acid and [3H]acetylglucosamine from exogenously supplied UDP-sugar nucleotides into a high molecular mass (10^6 to 10^7 Da) polymer in the presence of magnesium ions. Both sugar precursors were simultaneously required for elongation. Control extracts prepared from cells with the vector plasmid alone or the DG42 cDNA in the antisense orientation did not display this activity. The double-labeled polysaccharide product synthesized in vitro was deemed to be HA by enzymatic analyses; specific HA lyase could degrade the polymer, but it was unaffected by protease or chitinase treatments. The fragments generated by HA lyase were identical to fragments derived from authentic vertebrate HA as compared by high performance liquid chromatography. We conclude that DG42 is a membrane-associated HA synthase capable of transferring both glucuronic acid and N-acetylg glucosamine groups.

Glycoconjugates of the extracellular matrix are essential constituents of multicellular organisms throughout embryogenesis and adult life. One such molecule, HA, is a linear polysaccharide composed of alternating GlcA and GlcNAc residues (reviewed in Ref. 1). A recent controversy in carbohydrate biosynthesis is focused on the identity of the eukaryotic HA synthases (2). The function of DG42 was unknown at the time of its discovery as a major transcript accumulated during Xenopus gastrulation (3). The sequence similarity to some enzymes with a GlcNAc transferase activity, at first noted with Rhizobium NodC and fungal chitin synthases (4, 5) followed by streptococcal HA synthase (6, 7), yielded a valuable clue to the possible function of DG42. At least two groups have overexpressed members of an apparent class of candidate HA synthases, Xenopus DG42 (8) or a putative mouse analog HAS (76.7% identity to DG42; Ref. 9), in mammalian cells and observed increases in HA biosynthesis above basal levels. On the other hand, another group has reported that a DG42-fusion protein derived from in vitro translation reactions can produce chitin-like chains of 2 to 6 GlcNAc monomers, but not HA polysaccharide (10). The same group subsequently detected an activity from detergent extracts of zebra fish embryos that could make chitin oligosaccharides (11). The peak of this activity on gel filtration chromatography profiles contained a putative DG42 analog that was detected by cross-reaction with an antibody against Xenopus DG42. The chitin oligosaccharide synthase activity was discrete from an HA-synthesizing activity that eluted earlier on the gel filtration profiles (11).

To avoid the difficulties in interpreting relative increases in HA biosynthesis in mammalian expression systems, we have performed studies employing bakers' yeast as the heterologous host. There are no reports of HA or UDP-GlcA production, capsule formation, or exopolysaccharide synthesis by any Saccharomyces in the Medline data base since 1986. Since the entire genome of Saccharomyces cerevisiae has been sequenced, we searched the Saccharomyces Genome Database (Stanford) for proteins similar to known UDPglucose 6-dehydrogenases (EC 1.1.1.22), the enzymes that catalyze UDP-GlcA precursor production. We used the primary sequences of the enzymes from bovine liver (A54926; Ref. 12) or from Group A Streptococcus (HasA, A46089; Ref. 13) as queries in computer homology analyses (TBLASTN version 1.4.9; Ref. 14). We could not identify a potential yeast analog (smallest sum probability = 0.992–0.82). On the other hand, parallel searches using HasA, the streptococcal HA synthase (L20853; Ref. 6), as the query detected its sequence similarity (smallest sum probability = 10^{-4}–10^{-5}) to all three of the yeast chitin synthases, CHS1, CHS2, and CHS3. Thus far we report that the Xenopus DG42 gene product produced in yeast can synthesize high molecular weight HA in vitro.

EXPERIMENTAL PROCEDURES

Plasmid Construction— Molecular biology reagents were from Promega, and, unless noted, all other reagents were from Sigma. The DG42 gene was obtained from the cDNA plasmid pC2902 (3; generously provided by I. David). The coding region of 588 residues was amplified by 15 cycles of the polymerase chain reaction with Tag DNA polymerase and oligonucleotide primers (Ransom Hill Bioscience) designed to add flanking EcoRI restriction sites (15). The cassette was subcloned by standard methods into pYES2 (Invitrogen), an episomal shuttle plasmid possessing the yeast GAL1 promoter for foreign gene expression, using Escherichia coli as the intermediary host (15). The plasmids were characterized with respect to insert size and orientation by restriction mapping; pYES/DG+ or pYES/DG− contain the gene in the protein

* This work was supported by a medical research scholar grant from the University of Oklahoma Medical Alumni Association and National Research Initiative Grant for Sustaining Animal Health and Well-Being 94-37294-0929 from the U. S. Department of Agriculture (to P. L. D.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, 940 Stanton L. Young Blvd., Oklahoma City, OK 73190. Tel.: 405-271-2227; Fax: 405-271-3092; E-mail: Paul-DeAngelis@UOKHSC.edu.

‡ This work was supported by a medical research scholar grant from the University of Oklahoma Medical Alumni Association and National Research Initiative Grant for Sustaining Animal Health and Well-Being 94-37294-0929 from the U. S. Department of Agriculture (to P. L. D.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The abbreviations used are: HA, hyaluronan, hyaluronate, or hyaluronic acid; GlcA, glucuronic acid; GlcNAc, N-acetylg glucosamine.
Yeast-derived DG42 Can Synthesize HA

**Expression and Membrane Preparation**—For expression experiments, the various constructs were grown to midlogarithmic phase (0.2–0.8 A_{600}^\text{nm}) in SM-U with 1.5% raffinose and 5% glycerol instead of glucose. Galactose, the inducer of the GAL1 promoter, was added to 2%, and the cultures were grown for a further 5–6 h. All the following steps were performed at 4 °C or on ice. Cells were collected and washed twice with 50 mM sodium phosphate buffer, pH 7.5, 20 mM MgCl_2, 1 mM dithiothreitol, 0–180 mM ethylenediaminetetraacetic acid (EDTA). The cell pellet was resuspended in 1 ml of a lysis buffer, SB, composed of the above wash buffer, pH 7.5, 20 mM MgCl_2, 1 mM dithiothreitol, 0–180 mM EDTA, and washed by an ultracentrifugation (100,000 g, 30 min). The resulting membrane pellet was resuspended by gentle ultrasonication in SB and washed by an ultracentrifugation (100,000 g, 1 h). The supernatant fraction was resuspended in SB and stored frozen in aliquots at –80 °C until needed. Lysis was performed by three cycles of freeze/thawing followed by vigorous mixing (1 min vortex/1 min ice, 6 cycles) with glass beads (50% total volume; 400–600 μm). The lysate was removed after the beads had settled and was subjected to ultracentrifugation (20 s, setting 3, Heat Systems W-380 with microtip probe). The debris and unbroken cells were removed from the lysate by low speed centrifugation (2,000 g, 1 min). The supernatant fraction was centrifuged at 100,000 g for 5 min. The resulting membrane pellet was resuspended by gentle ultracentrifugation in SB and washed by another round of ultracentrifugation. The final membrane fraction was resuspended in SB, stored frozen in aliquots at –80 °C. Protein was quantitated by the Coomassie binding assay using a bovine serum albumin standard (Pierce; Ref. 16).

**Oligosaccharide Synthase Assays**—The incorporation of sugars into high molecular weight HA polysaccharide was monitored by using UDP-[14C]GlcA (291 mCi/mmol; ICN) and/or UDP-[3H]GlcNAc (27.3 Ci/mmol; DuPont NEN) precursors. Membrane preparations were incubated at 30 °C in a volume of 100 μl in a buffer typically containing: 50 mM Tris, pH 7.5, 20 mM MgCl_2, 1 mM dithiothreitol, 0–180 μM UDP-GlcA, 0–300 μM UDP-GlcNAc. Reactions were terminated by the addition of SDS to 2% (w/v). Either paper chromatography (17) or gel filtration chromatography on Sephadex G-25 was done at 23°C. The digests were then subjected to paper chromatography or gel filtration chromatography on Sephadex G-25 (Pharmacia Inc.) to separate products from substrates; the radioactive polymers at the origin of the paper chromatogram or in the column fractions were detected by liquid scintillation counting.

The identity of the polysaccharide products were assessed by treatment with: (i) HA-specific hyaluronate lyase of Streptomyces hyalurolyticus (EC 4.2.2.1; Ref. 18) in ammonium acetate, pH 5.5, at 250 units/ml, (ii) Serratia marcescens chitinase (EC 3.2.1.14) in 50 mM sodium phosphate, pH 6.5, at 10 units/ml, and (iii) Streptomyces cyaneus protease (Pronase, Calbiochem) in 50 mM Tris, pH 7.5 at 96 proteolytic units/ml. Incubations were performed for 3.5 h at 37 °C except for (ii) which was done at 23°C. The digests were then subjected to paper chromatography or gel filtration chromatography on Sephadex G-25 (Pharmacia).

**Oligosaccharide Mapping**—Carbohydrate digests were analyzed by liquid chromatography utilizing the method of Gherezhgher et al. (19). Polysaccharide samples were exchanged into 50 mM ammonium acetate buffer, pH 5.5, and digested with HA lyase at 400 units/ml at 37 °C for 6.5 h. The oligosaccharides for standards were prepared by digestion of authentic HA from rooster comb. The digests were lyophilized twice to remove the volatile salts before resuspension and injection onto a Vario-Disk AX-5 and unhydrolized (4 cm × 50 cm) column with 10% methanol. The column was eluted isocratically with 4% methanol, 0.3 M ammonium formate, pH 5.5, at 1 ml/min. The standard oligosaccharides were monitored by UV absorbance; the action of the enzyme creates a 4,5-double bond that was detected at 232 nm (18). The radiolabeled sugars were measured by liquid scintillation counting of the fractions (0.5 min).

**RESULTS**

**Sugar Incorporation by DG42 in Vitro**—Membrane preparations derived from cells with the plasmid pYES/DG+ encoding DG42 in the correct orientation behind the GAL promoter incorporated the sugars from UDP-GlcNAc and UDP-GlcA into high molecular weight product (Table I). Both precursors were simultaneously required for polymerization. Mg^{2+} ion is also required for catalysis; membranes incubated with EDTA instead of metal ion did not incorporate radioactivity from the precursors into polymer. Specific activities of ~5 pmol of GlcA transferred (μg of protein)−1 h−1 were typically obtained. Preparations derived from cells with either pYES2 vector or the antisense plasmid, pYES/DG−, did not display any similar activity.

**Characterization of the DG42 Protein**—Membranes derived from cells with pYES/DG+ were compared before and after the high speed washing step described under “Experimental Procedures.” The second membrane pellet contained on average ~86% of the activity observed in the first pellet even though the total protein concentration was reduced by ~40%. No activity was found in the washes. This result suggests that DG42 is associated with the membranes. Total yeast cell lysates possessed only about 10% of the activity observed with the membranes, but this decrease is most likely due to the detrimental effects of cytosolic enzymes on the sugar precursors. By Western blot analysis using antisera to a DG42-fusion protein supplied by I. Dawid (3), only membranes derived from yeast with pYES/DG+ possessed a unique immunoreactive band migrating at ~67 kDa by SDS-polyacrylamide gel electrophoresis (data not shown). This estimate is very similar to the predicted size from the deduced sequence (3) and the experimental values of radiolabeled embryo extracts (3) or lysates from cells infected with recombinant DG42-vaccinia virus (8).

**Analysis of the Oligosaccharide Product**—Membranes derived from the pYES/DG plasmids were incubated with both UDP-[14C]GlcA and UDP-[3H]GlcNAc, and the reaction mixtures were analyzed by gel filtration chromatography on Sephacryl S-500HR (Fig. 1). A double-labeled polymer produced by membranes of cells with pYES+eluted with an apparent molecular mass ranging from 10^6 to 10^7 Da; this value is based on the elution position of the blue dextran standard (average 2 × 10^6 Da; Pharmacia) and the resin exclusion limit (≥ 2 × 10^7 Da). No such polymer was detected in parallel runs with the antisense plasmid control (data not shown).

The polysaccharide product synthesized by membranes from pYES/DG+ cells and purified on the S-500HR column was deemed to be HA by its complete sensitivity to the specific streptomyces HA lyase. Untreated material remained at the origin of the paper chromatogram, but at least 99% of the ^3H and ^14C label migrated away from the origin after digestion. These labeled fragments also eluted in the totally included...
Yeast-derived DG42 Can Synthesize HA

Yeast membranes were treated with degradative enzymes to assess the nature of the remainder of the material in fractions 14–24 was pooled and aliquots were treated with both radiolabeled precursors diluted to roughly equivalent specific activity. After 30 min, additional unlabeled precursors were added to 180 μM each, and the incubation was continued for 90 min. SDS was added to 2% (w/v), and the reaction was heated at 95 °C for 1 min. The mixture was clarified by centrifugation and injected onto a Sephadex S-500HR column (1 × 50 cm) equilibrated in 0.2 M NaCl, 5 mM Tris, pH 8. The radioactivity in a portion of each fraction (0.2 ml of 1 ml) was measured. After 30 min, additional unlabeled precursors were added to the reaction, and the incubation was continued for 90 min. SDS was added to 2% (w/v), and the reaction was heated at 95 °C for 1 min. The mixture was clarified by centrifugation and injected onto a Sephadex S-500HR column (1 × 50 cm) equilibrated in 0.2 M NaCl, 5 mM Tris, pH 8. The radioactivity in a portion of each fraction (0.2 ml of 1 ml) was measured.

The fragments derived from HA lyase digestion of the radiolabeled polysaccharide were further characterized by high performance liquid chromatography on a Micropak AX-5 amino acid column (fractions 14–24; depicted in Fig. 1) and treated with HA lyase. The radiolabeled fragments (1/4 of pool) were analyzed by chromatography on a Micropak AX-5 amino column as described in the text. The arrowhead marks the elution time for unretained molecules. The column was standardized with an HA lyase digest of authentic HA (5 μg). Two UV-absorbing peaks were observed in the standard that eluted at 5.9 min and 8.5 min (not shown); these species correspond to the unsaturated tetrasaccharide and hexasaccharide, respectively, and are the final products formed by the Streptomyces enzyme (18, 19). The radiolabeled peaks (14C, squares; 3H, circles) eluted with the same retention times as the oligosaccharides derived from pure authentic HA. Yeast-derived recombinant DG42 can make HA.

FIG. 1. Size exclusion chromatography of polymer product. Membranes from cells with pYES/DG* (100 μg of protein) were incubated with both radiolabeled precursors diluted to roughly equivalent specific activity (80 μM, 0.2 μCi each) under typical buffer conditions with Mg2+. After 30 min, additional unlabeled precursors were added to 180 μM each, and the incubation was continued for 90 min. SDS was added to 2% (w/v), and the reaction was heated at 95 °C for 1 min. The mixture was clarified by centrifugation and injected onto a Sephadex S-500HR column (1 × 50 cm) equilibrated in 0.2 M NaCl, 5 mM Tris, pH 8. The radioactivity in a portion of each fraction (0.2 ml of 1 ml) was measured.

FIG. 2. Mapping of HA lyase digests. High molecular weight, double-labeled material from recombinant pDG42+ yeast was purified on the S-500HR column (fractions 14–24; depicted in Fig. 1) and treated with HA lyase. The radiolabeled fragments (1/4 of pool) were analyzed by chromatography on a Micropak AX-5 amino column as described in the text. The arrowhead marks the elution time for unretained molecules. The column was standardized with an HA lyase digest of authentic HA (5 μg). Two UV-absorbing peaks were observed in the standard that eluted at 5.9 min and 8.5 min (not shown); these species correspond to the unsaturated tetrasaccharide and hexasaccharide, respectively, and are the final products formed by the Streptomyces enzyme (18, 19). The radiolabeled peaks (14C, squares; 3H, circles) eluted with the same retention times as the oligosaccharides derived from pure authentic HA. Yeast-derived recombinant DG42 can make HA.

DISCUSSION

As noted in the introduction and a commentary elsewhere (2), a definitive answer on the nature of the enzymatic activity of the DG42 polypeptide was not previously available. Due to the innate ability of mammalian cells to produce HA, the earlier experiments in the other model cell systems could not rule out the possibility that components, such as catalytic or regulatory species, distinct from the DG42 polypeptide were also required for HA biosynthesis. It is quite plausible that the HAS protein of Itano and Kimata (9) actually complemented the defect of their particular cell line, and perhaps the HAS molecule alone was not sufficient for HA production. In the case of Meyer and Kreil (8), overexpression of DG42 could have potentially elevated the concentration of a limiting biosynthetic component in a cell line with a low endogenous HA production capability, rather than the DG42 polypeptide be an actual HA synthase. These scenarios seem quite possible in light of information reported by Semino et al. (11) that some irrelevant molecules, such as an integrin subunit and p34, could increase HA production when overexpressed in mammalian cells.

Bakers’ yeast does not possess any of the required components specific to HA elongation and, therefore, in some respects is a better model system to dissect HA biosynthesis than the vertebrate cell systems with the endogenous ability to produce HA. The data in this report clearly show that the recombinant DG42 gene product copolymerizes GlcA and GlcNac groups to form the repeating, linear HA polysaccharide. The size of the polymer produced by recombinant yeast membranes was comparable to high quality, purified vertebrate HA polysaccharide (1). Therefore, Xenopus DG42, and, very probably, mouse HAS (9) are genuine HA synthases which require no other catalytic subunits for HA elongation.

The attempt by Semino and Robbins (10) to detect HA polymerization with recombinant DG42 in an in vitro system did not succeed. We believe that their recombinant DG42 molecule could not make HA for perhaps several reasons: (i) the protein was produced with a potentially disruptive T7-peptide tag at the amino terminus, (ii) no lipids or membranes were available during translation in the in vitro system, or (iii) other protein-folding or post-translational modification machinery is required. Any one or all of these reasons could cause misfolding, and the DG42 polypeptide would not be able to elongate HA. For example, we have found evidence that the streptococcal HasA amino terminus is important for activity; deletion of the first 24 residues results in an inactive product.2 Furthermore, we could detect neither significant protein production nor HA synthase activity of recombinant DG42 produced by several foreign gene expression systems employing E. coli as the host (data not shown). The DG42 protein prepared in the yeast system, however, was active with respect to HA elongation in our very first experiment.

HA is produced during the entire lifetime of higher animals. One of the most obvious previous arguments against DG42 being an eukaryotic HA synthase was the apparent temporal

2. P. L. DeAngelis and P. H. Weigel, unpublished data.
restriction of DG42 expression to the period of gastrulation (3). Our observation that DG42 can indeed produce HA suggests that several genes may exist in the vertebrate genome that encode different developmental or tissue-specific isozymes of HA synthase. It is quite likely that a family of eukaryotic HA synthases will be found upon further examination of sequenced genomes as well as the result of directed explorations.

DG42 alone can transfer both GlcA and GlcNAc groups to form HA. In almost all known cases of carbohydrate biosynthesis, one glycosyltransferase transfers only one type of sugar subunit to form a certain, specific linkage. There is precedent, however, for one glycosyltransferase having the ability to transfer two distinct sugars. Based on genetic and biochemical evidence, the streptococcal HA synthase, HasA, has been demonstrated to transfer both GlcA and GlcNAc to the growing HA chain. Only one gene encoding one protein on a recombinant plasmid is required for HA synthesis in both Gram-positive and Gram-negative bacteria, as long as the UDP-GlcA precursor is present or supplied (6, 20). Immunoaffinity-purified HasA can also synthesize HA chains (17).

In conclusion, the Xenopus DG42 protein is an HA synthase, a transferase that can form HA chains by alternating addition of both GlcA and GlcNAc groups; no other specific catalytic subunits appear to be required. It is extremely likely that most of the newly discovered vertebrate DG42 homologs (9) are also bona fide HA synthases. The elucidation of the details of the enzymatic mechanism should be an active field of research now that a prototype eukaryotic HA synthase has been identified and experimental model systems are available. Basic information on HA biosynthesis will surely aid our understanding of numerous phenomena including development, intercellular adhesion and recognition, cell motility, and cancer.

Acknowledgments—We thank Dr. Igor B. Dawid for providing the DG42 plasmid and antibody, Dr. Richard D. Cummings and Terry R. Smith for use of and aid with the HPLC chromatograph, Coy Heldermon and Drs. Paul H. Weigel and Valerie Tlapak-Simmons, for helpful discussions. We also thank Drs. Andrew P. Spicer and John A. McDonald for sharing preliminary data on the existence of multiple HAS analogs in vertebrates.

REFERENCES
1. Laurent, T. C., and Fraser, J. R. E. (1992) FASEB J. 6, 2397–2404
2. Varki, A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4523–4525
3. Rosa, F., Sargent, T. D., Rebbert, M. L. Michaels, G. S., Janz, M., Grunz, H., Jonas, E., Winkles, J. A., and Dawid, I. B. (1988) Dev. Biol. 129, 114–123
4. Bulawa, C. E. (1992) Mol. Cell. Biol. 12, 1764–1776
5. Atkinson, E. M., and Long, S. R. (1992) Mol. Plant-Microbe Interact. 5, 439–442
6. DeAngelis, P. L., Papaconstantinou, J., and Weigel, P. H. (1993) J. Biol. Chem. 268, 19181–19184
7. DeAngelis, P. L., Yang, N., and Weigel, P. H. (1994) Biochem. Biophys. Res. Commun. 199, 1–10
8. Meyer, M. F., and Kreil, G. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4543–4547
9. Itano, N., and Kimata, K. (1996) J. Biol. Chem. 271, 9875–9878
10. Semino, C. E., and Robbins, P. W. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3498–3501
11. Semino, C. E., Specht, C. A., Raimondi, A., and Robbins, P. W. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4548–4553
12. Hempel, J., Perozich, J., Romavacek, H., Hinrich, A., Kuo, I., and Feingold, D. S. (1994) Protein Sci. 3, 1074–1080
13. Dougherty, B. A., and van de Rijn, I. (1993) J. Biol. Chem. 268, 7118–7124
14. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
15. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J., and Struhl, K. (1995) Short Protocols in Molecular Biology, 3rd Ed., John Wiley & Sons, New York
16. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
17. DeAngelis, P. L., and Weigel, P. H. (1994) Biochemistry 33, 9033–9039
18. Ohyu, T., and Kaneko, Y. (1970) Biochim. Biophys. Acta 198, 667–669
19. Gherezghiher, T., Kekwick, M. C., Nordquist, R. E., and Wilkinson, C. P. (1987) J. Chromatogr. 413, 9–15
20. DeAngelis, P. L., Papaconstantinou, J., and Weigel, P. H. (1993) J. Biol. Chem. 268, 14568–14571
21. Lind, T., Lindahl, U., and Lidholt, K. (1993) J. Biol. Chem. 268, 20705–20708
Yeast-derived Recombinant DG42 Protein of *Xenopus* Can Synthesize Hyaluronan *in Vitro*

Paul L. DeAngelis and Ann Mary Achyuthan

*J. Biol. Chem.* 1996, 271:23657-23660.
doi: 10.1074/jbc.271.39.23657

Access the most updated version of this article at [http://www.jbc.org/content/271/39/23657](http://www.jbc.org/content/271/39/23657)

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 20 references, 10 of which can be accessed free at [http://www.jbc.org/content/271/39/23657.full.html#ref-list-1](http://www.jbc.org/content/271/39/23657.full.html#ref-list-1)