Enzymological Characterization of Recombinant Xenopus DG42, A Vertebrate Hyaluronan Synthase*

(Received for publication, January 28, 1997, and in revised form, December 5, 1997)

Philip E. Pummill, Ann Mary Achyuthan, and Paul L. DeAngelis‡

From the Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73104

We have characterized the hyaluronan (HA) synthase activity of the Xenopus DG42 gene product in vitro. The recombinant enzyme produced in yeast does not possess a nascent HA chain and, therefore, is an ideal model system for kinetic studies of the synthase’s glycosyltransferase activity. The enzymatic rate was optimal from pH 7.6 to 8.1. Only the authentic sugar nucleotide precursors, UDP-glucuronic acid (UDP-GlcA) and UDP-N-acetylglucosamine (UDP-GlcNAc), were utilized to produce a large molecular weight polymer. UDP-glucose or the galactose epimers of the normal substrates did not substitute. The Michaelis constant, K_m, of recombinant DG42 in membranes was 60 ± 20 and 235 ± 40 μM for UDP-GlcA and UDP-GlcNAc, respectively, which is comparable to values obtained previously from membranes derived from vertebrate cells. The apparent energy of activation for HA elongation is about 15 kilocalories/mol. DG42 polymerizes HA at average rates of about 80 to 110 monosaccharides/s into a nascent HA chain and, therefore, is an ideal model for the initial HA synthase to be identified was Xenopus laevis (African clawed frog) protein, DG42 (for differentially expressed in gastrulation), with a previously unknown function (6) was found to be quite similar at the amino acid sequence level to the bacterial HasA enzyme (3, 4) as well as fungal chitin synthases (7). These observations led to the hypothesis that this vertebrate protein was also a HA synthase (4, 7). DG42 contains predicted transmembrane segments clustered at both the amino and carboxyl termini; this positioning is similar to that of the membrane-associated regions found in HAS (4). DG42 was subsequently shown to be involved in HA biosynthesis by overexpression studies. Infection of mammalian cells with a recombinant vaccinia virus construct containing the DG42 cDNA directed these cells to produce more HA than the uninfected host cells alone or vector-infected cells (8). Definitive proof that DG42 was a bona fide HA synthase was obtained through overexpression studies in Saccharomyces cerevisiae, an eukaryotic host that does not normally make the HA polysaccharide. Yeast with the cloned DG42 cDNA on an expression plasmid produced a functional HA synthase (9). The recombinant enzyme transferred both GlcA and GlcNAc residues from UDP-sugar nucleotide donors to form a high molecular weight polymer. This material was degraded by the specific HA lyase from Streptomyces (9), an enzyme that does not digest any other GAG (10). The resulting fragments from the yeast-derived polymer were identical to those generated from authentic vertebrate HA as deemed by high performance liquid chromatography analysis (9). The HA synthase activity of the recombinant yeast was localized to the membrane fraction in agreement with both the predictions derived from the DG42 primary sequence and the previous characterizations of the HA synthase from mammalian sources.

In 1996, at least four reports were made of mammalian homologs possessing ~50% identity to the DG42 protein (11–14). Two of these reports utilized polymerase chain reaction and degenerate primers based on the hasA and DG42 sequences to obtain their clones (12, 14). The cDNAs corresponding to these homologs, when overexpressed on recombinant plasmids, substantially increased HA production of transfected mammalian cells in comparison to the host cells’ basal levels. It appears that at least three putative hyaluronan synthases encoded by three separate but related genes, named HAS1, HAS2, and HAS3, exist in human and mouse. The Xenopus DG42 gene is most closely related to mammalian HAS1 based upon conservation of exon/intron boundaries. In this report,

Glycosaminoglycans (GAG), linear polysaccharides based on a repeating disaccharide that usually consists of an amino sugar and a negatively charged sugar, are essential constituents of higher animals. Hyaluronan (HA), heparin, and chondroitin, dermatan, and keratan sulfates are members of this class of carbohydrates. HA is a prominent GAG that plays roles as a structural element and a recognition molecule in vertebrates (1). The initial HA synthase to be identified was HasA from Streptomyces thermosaccharolyticus (2). The protein that catalyzes the production of HA, the HA synthases, was the first glycosyltransferases capable of forming the di-saccharide repeat of a GAG to be cloned and described at the molecular level. The initial HA synthase to be identified was HasA of Strep. pyogenes which is the enzyme responsible for the formation of an extracellular capsule of HA in this human bacterial pathogen (2, 3). The HasA protein is strongly associated with the phospholipid membrane and is predicted to possess 4 or 5 membrane-spanning segments (3, 4). The enzyme utilizes UDP-GlcA and UDP-GlcNAc precursors found in the cytosol and extrudes the growing HA chain out of the cell during polymerization. HasA, a single protein, transfers both GlcA and GlcNAc residues to HA based on genetic and biochemical evidence (3, 5). A Xenopus laevis (African clawed frog) protein, DG42 (for differentially expressed in gastrulation), with a previously unknown function (6) was found to be quite similar at the amino acid sequence level to the bacterial HasA enzyme (3, 4) as well as fungal chitin synthases (7). These observations led to the hypothesis that this vertebrate protein was also a HA synthase (4, 7). DG42 contains predicted transmembrane segments clustered at both the amino and carboxyl termini; this positioning is similar to that of the membrane-associated regions found in HAS (4). DG42 was subsequently shown to be involved in HA biosynthesis by overexpression studies. Infection of mammalian cells with a recombinant vaccinia virus construct containing the DG42 cDNA directed these cells to produce more HA than the uninfected host cells alone or vector-infected cells (8). Definitive proof that DG42 was a bona fide HA synthase was obtained through overexpression studies in Saccharomyces cerevisiae, an eukaryotic host that does not normally make the HA polysaccharide. Yeast with the cloned DG42 cDNA on an expression plasmid produced a functional HA synthase (9). The recombinant enzyme transferred both GlcA and GlcNAc residues from UDP-sugar nucleotide donors to form a high molecular weight polymer. This material was degraded by the specific HA lyase from Streptomyces (9), an enzyme that does not digest any other GAG (10). The resulting fragments from the yeast-derived polymer were identical to those generated from authentic vertebrate HA as deemed by high performance liquid chromatography analysis (9). The HA synthase activity of the recombinant yeast was localized to the membrane fraction in agreement with both the predictions derived from the DG42 primary sequence and the previous characterizations of the HA synthase from mammalian sources.
we have characterized the requirements and kinetics of recombinant DG42 produced in yeast.

**EXPERIMENTAL PROCEDURES**

**Production of Recombinant DG42 and HasA Enzyme—**All reagents were from Sigma unless noted otherwise. The construction and the use of the DG42 expression plasmid for studies in yeast were described by DeAngelis and Achyuthan (9). Briefly, the DG42 cDNA, encoding a polypeptide of 588 residues, was cloned into the pYES2 vector (Invitrogen) under control of the GAL1 promoter to form pYES/DG+. Upon induction with galactose, active DG42 accumulated in the plasma membrane fraction. Membranes were prepared by the same glass-bead disruption protocol except for three alterations: (i) the more soluble and stable protease inhibitor aminoethylbenzenesulfonyl fluoride was substituted for phenylmethanesulfonyl fluoride; (ii) the repeated freeze-thawing cycles were omitted; and (iii) some preparations were lysed utilizing a MiniBeadbeater-8 (Biospec). Preparations with about 10-fold higher specific activity than our previous report were obtained when all of these modifications were utilized. Protein was quantitated by the Coomassie dye-binding assay (15) with a bovine serum albumin standard (Pierce).

A DNA fragment encoding the open reading frame of 419 residues corresponding to streptococcal HasA (original Val codon switched to Met; Ref. 3) was also subcloned by standard methods into the pYES2 yeast expression vector to produce pYES/HasA. Membranes from cells with this construct were prepared in the same fashion as pYES/DG+. The samples derived from pYES/HasA constructs contained substantial HA synthase activity and a unique 42-kDa protein could be detected on Western blots with antibodies against HasA; membranes from cells with vector alone possessed neither activity nor the immunoreactive band (not shown).

**Polysaccharide Synthase Assays—**The incorporation of sugars into high molecular weight HA polysaccharide was monitored using UDP-[14C]GlcA (291 mCi/mmol; ICN) and/or UDP-[3H]GlcNAc (27.3 Ci/mmol; NEN Life Science Products Inc.) precursors as described previously (9). For determining optimal reaction conditions, the membrane preparations were incubated at 30 °C for 1 h, unless noted otherwise, in a buffer typically containing: 50 mM buffer, 0–300 mM divalent metal ion, 1 mM diithothreitol (DTT), 0–150 μM UDP-GlcA, and 0–300 μM UDP-GlcNAc. Reactions were terminated by the addition of SDS to 2% (w/v). Descending paper chromatography (65:35, ethanol, 1 M ammonium acetate, pH 5.5) was utilized to separate products from substrates; the radioactive polymer at the origin of the paper chromatogram were detected by liquid scintillation counting. As for characterization of the kinetic optima of DG42 were set so that <5% of the radiolabeled substrate was consumed and the enzyme concentration was in the linear range of the column.

For determining the temperature dependence of DG42 activity, 360 μM UDP-GlcA and 1 mM UDP-GlcNAc were employed in 30-min assays to measure maximal velocity measurements. For the sugar nucleotide specificity studies, one of the authentic HA precursors was substituted with a closely related structural analog. Km values for the substrates were obtained by holding one radiolabeled UDP-sugar at a constant and saturating concentration while titrating the other UDP-sugar. The data were analyzed by graphing on Hanes-Woolf plots.

**Analysis of HA Polymerization Rate—**Membranes (385 μg of protein) were incubated with 400 μM UDP-[14C]GlcA (1 μCi) and 900 μCi unlabelled UDP-GlcNAc in 50 mM Tris, pH 7.6, 20 mM MgCl2, and 1 mM DTT (550 μl reaction volume) at 30 °C and samples (100 μl) of the reaction mixture were withdrawn at various times. The synthesis was inactivated by the addition of SDS to 0.5% and the samples were deproteinized by Pronase® treatment (0.5 mg/ml final, overnight at 37 °C; Boehringer Mannheim). A parallel study with membranes containing yeast-derived recombinant HasA was performed as above except that the buffer was pH 7.0. The unincorporated precursors and small molecules were removed by ultrafiltration (3 buffer changes with a Microcon® 3 unit; Amicon). After clarification by centrifugation (16,000 × g, 5 min), one-third of the sample was injected onto a Sephacryl S-500HR gel filtration column (Pharmacia; 1 × 51 cm, 40 ml) equilibrated in 50 mM Tris, pH 7.4, and 0.5 mM MgCl2. The column was eluted at 0.5 ml/min and radioactivity in the fractions (1 ml) was quantitated by liquid scintillation counting after adding EconoSafe mixture (4.5 ml, the manufacturer (using defined microsphere standards and extrapolation) to be ~2 × 1010 Da. Even if the extended size was actually 1.5 × 1012 Da, however, our polymerization rates would be only 10% lower because the HA peaks used for the rate determinations eluted at midrange or higher Kav values.

We found that the transport and the availability of sugar nucleotides across the phospholipid membranes were not the rate-limiting steps with our yeast preparations. Similar HA polymerization experiments using treatments that permeabilize lipid vesicles (preincubation with 0.05% (w/v) digitonin (final) or a pore-forming protein, perfringolysin O (1 μg/150 μg of membrane protein; generously provided by R. Tweten, University of Oklahoma)) did not alter the chromatography profiles from experiments using untreated membranes (data not shown).

**RESULTS**

**Buffer Optima for DG42 HA Synthase Activity—**The DG42 enzyme in membranes was assayed under various conditions to determine the optimal pH, metal ion concentration, and ionic strength for HA polymerization. The enzyme displayed a pH optimum around neutrality and the highest activities were observed in Tris-based buffers at pH 7.6 to 8.1 (Fig. 1). The synthase retained ~80% of maximal activity from pH 7.0 to 8.4. The enzyme activity was linear for at least 2 h at pH 7.6 (data not shown). The enzyme did not perform as well in phosphate buffer; in this case, the phosphate ion probably chelates a substantial proportion of the required Mg2+ ion (data not shown).

We explored the possibility that metal ions other than Mg2+ could substitute as a cofactor for HA polymerization using reactions buffered at pH 7.6. No other metal ion, including Mn2+, Co2+, Cu2+, or Ni2+, was as effective as Mg2+ (Fig. 2). No more than 13 or 23% of the incorporation observed for 20 mM Mg2+ was detected when Ni2+ or Mn2+, respectively, were substituted at the same concentration.

The dependence of HA polymerization on ionic strength was measured by addition of NaCl to reactions at pH 7.6 containing 20 mM Mg2+ (data not shown). The Tris buffer and Mg2+ alone contribute an ionic strength of 0.11 molal. The activity remained fairly constant up to ~0.4 molal. At higher ionic strengths, however, we observed a decrease, which was most pronounced with the addition of NaCl to the reaction mixture. The highest ionic strengths tested were 1 M NaCl (data not shown).
Characterization of Recombinant DG42, a HA Synthase

Before the advent of the recombinant enzymes, several groups had studied the native HA synthase(s) derived from various vertebrate cell lines. The enzymes' requirements and Km values for the UDP-sugar substrates were measured (17–21). There were potential complications with these studies because (i) multiple HA synthase isozymes exist, and (ii) the enzymes isolated from mammalian cells possess partially elongated nas-
cent chains and/or completed HA chains. We have utilized the yeast expression system to circumvent these pitfalls. First, since yeast do not normally make HA, the activity of a cloned synthase can be analyzed without the contributions of other endogenous HA synthases that are found in the mammalian systems. Second, yeast is a host which does not form the re-endothelial HA synthases that are found in the mammalian systems. Second, yeast is a host which does not form the re-

curating UDP-GlcA precursor of HA.

In contrast, the current model advocated by Prehm (22, 23) is that HA is polymerized by transfer of sugars to the non-reducing end of the nascent chain. One line of evidence that led to this hypothesis was that the nascent HA polymers synthesized by mammalian cell membranes apparently contain a covalently attached UDP moiety (23).

In the current study, it was found that the HA synthase activity was dependent on the availability of UDP-GlcA, whereas the activity of the isolated enzyme (23) was much lower. This suggests that the HA synthase activity in vivo may be limiting factor in the synthesis of HA.

Table 1: Sugar nucleotide specificity of recombinant DHG42 hyaluronan synthase

| Second sugar nucleotide present | [14C]GlcA dpm | [3H]GlcNAc dpm | % |
|---------------------------------|--------------|---------------|---|
| None                            | 90 (0.3)     | 350 (2.2)     |   |
| UDP-GlcA                        | ND           | 16,100 (100)  |   |
| UDP-GlcNAc                      | 30,300 (100) | ND            |   |
| UDP-Glc                        | 80 (0.3)     | 240 (1.6)     |   |
| UDP-GalA                       | ND           | 320 (2.0)     |   |
| UDP-GalNAc                     | 70 (0.2)     | ND            |   |

The small amount of radioactive material at the origin in this reaction was chitin polysaccharide produced by the endogenous chitin synthase activity; the material was susceptible to degradation by chitinase 63 (provided by P. Robbins), and it was also formed by control membranes derived from cells with vector or antisense plasmids under similar reaction conditions. DG42 incorporated only the authentic HA precursors into the polysaccharide.

Preparation. This feature facilitates analysis of HA biosynthesis since all polymerization occurs de novo. In particular, the controversy surrounding the direction of HA polymer growth may be answered in the near future utilizing the yeast system. Most other known carbohydrates are synthesized by the addition of the new saccharide residue from an activated sugar nucleotide to the nonreducing end of the nascent chain. One line of evidence that led to this hypothesis was that the nascent HA polymers synthesized by mammalian cell membranes apparently contain a covalently attached UDP moiety (23).

All of the HA synthases described to date, from Gram-positive Streptococcus and Gram-negative Pasteurella bacteria and
from vertebrates, including *Xenopus*, utilize UDP-sugar nucleotide precursors at neutral pH (9, 17–21, 24, 25). These HA synthases require a divalent metal ion to function, but the enzymes display different preferences in vitro. Mg$^{2+}$ supports the highest efficiency polymerization of HA for all enzymes tested to date, except for *Pasteurella* enzyme for which 1 mM Mn$^{2+}$ serves 2-fold better than 10 mM Mg$^{2+}$ (25).

The temperature dependence of HA synthase activity of DG42 suggests that the apparent $E_a$, the energy of activation for elongating HA, is $\sim 15$ kcal/mol. This value is similar to that observed for a wide spectrum of other biosynthetic enzymes. If the transfer of one of the sugar groups, either GlcA or GlcNAc, to the HA chain had a greater energy barrier, then our calculated apparent $E_a$ value would be a reflection of the reaction with the higher activation energy. However, the GlcA and GlcNAc groups are both transferred by a UDP donor and both of the resulting glycosidic bonds are $\beta$-linked. Therefore, the actual $E_a$ values may be very similar for both reactions catalyzed by HAS. To make the proper assumptions concerning the similarities in reactive encounters or transition states during catalysis, it would be useful to know if the HASs possess (i) a common binding site that interacts with both UDP-sugars or (ii) two distinct binding sites for UDP-GlcA and UDP-GlcNAc.

DG42 displays a greater affinity for the UDP-GlcA precursor than UDP-GlcNAc, a characteristic of all other known HA synthases. We found that the $K_m$ values of recombinant DG42 in yeast for the precursors were higher but quite similar in magnitude to values (UDP-GlcA, 3–50 $\mu$M; UDP-GlcNAc, 21–100 $\mu$M) reported by others for the membrane-associated enzyme derived from adult human, murine, or chicken cells (17–

**FIG. 6.** Gel filtration analysis of HA biosynthesis by DG42 and HasA synthases. $[^{14}C]$GlcA-labeled reactions were analyzed over a time course as described under “Experimental Procedures.” One-third of each time point was loaded onto the Sephacryl S-500HR column. Panel A, DG42 (2.5 min, ●; 5 min, □; 15 min, ▲); Panel B, HasA (2.5 min, ○; 5 min, □; 15 min, ▲); Panel C, reactions after a 45-min elongation period, normalized by setting the fraction with the highest level of radioactivity in each profile to 100% (DG42, ● maximal incorporation 910 dpm; HasA, ○ maximal incorporation 3330 dpm). The void volume and the totally included volumes were 17 and 38 ml respectively. The fractions used for product size estimations are marked with brackets. The peak of blue dextran 2000 (average $\sim 2 \times 10^6$ Da) eluted at 26–29 ml. DG42 makes high molecular weight HA quickly, but the product appears somewhat polydisperse in comparison to HA made by HasA.
Characterization of Recombinant DG42, a HA Synthase

21. The variance in $K_m$ values may be due to the intrinsic characteristics among the different HA synthase isozymes, but further analysis will be required to resolve if this disparity is just a matter of different source species, purification protocols, or assay methods.

Yeast-derived DG42 exhibited exquisite specificity for the authentic sugar nucleotide precursors of the HA polysaccharide. The galactose epimers and UDP-Glc could not substitute for UDP-GlcA and UDP-GlcNAc. Similarly, streptococcal HasA only incorporated the authentic precursors into polymer (5). This selectivity suggests that the enzyme needs to make several critical contacts with the substrates, including the substituents at C-2 and C-4 of GlcNAc, and the substituents at C-4 and C-6 of GlcA.

We estimated the average rates of HA polymerization by measuring the length of the HA chains produced in vitro by gel filtration and then dividing the average size by the reaction time. Our method of calculating the product size is limited by filtration and then dividing the average size by the reaction measuring the length of the HA chains produced in vitro and C-6 of GlcA.

eral critical contacts with the substrates, including the sub-

This selectivity suggests that the enzyme needs to make several critical contacts with the substrates, including the substituents at C-2 and C-4 of GlcNAc, and the substituents at C-4 and C-6 of GlcA.

We estimated the average rates of HA polymerization by measuring the length of the HA chains produced in vitro by gel filtration and then dividing the average size by the reaction time. Our method of calculating the product size is limited by several factors: (i) the experimental difficulty in separating polymers in the $10^6$-$10^8$ Da range with existing aqueous-based chromatography media; (ii) the relative lack of defined high molecular weight standards; and (iii) the intrinsic polydispersity nature of polysaccharides. To combat the imprecision of the gel filtration estimates of $M_r$, we used data from sequential reaction times and calculated the size across the median fractions of the HA peak. We presented conservative estimates of the polymerization rate based on the average size of the product molecules, but some of the HA chains are longer than the average value. Therefore, the enzyme may elongate HA at even higher rates. Our parallel studies indicate that both yeast-derived recombinant DG42 and HasA polymerize HA rapidly.

Interestingly, comparison of the gel filtration profiles reveals that the ultimate size of the HA products from the two enzymes are different. DG42 produces HA polysaccharide with a smaller average size ($6-12 \times 10^6$ Da) than that formed by HasA ($2 \times 10^7$ Da). The HA size distribution produced by DG42, nonetheless, is comparable to high quality HA isolated from vertebrate tissues (1).

Overall, the findings in this report substantiate yeast as a useful expression system for studies of HA synthases. Apparently no post-translational modifications unique to vertebrates, and absent in Saccharomyces, are required for correct enzymatic function of DG42, a putative HAS1-type vertebrate synthase. Furthermore, since yeast-derived HasA also functioned very well as a HA synthase, no other bacterial-specific components for HA synthesis are required; this is further evidence that one glycosyltransferase can indeed utilize two distinct substrates.

It will be interesting to examine other vertebrate HA synthases to determine if the enzymological characteristics of a particular synthase isozyme customize its function in different tissues of the body and/or at various times during development. A comparison of the enzymology of the other GAG glycosyltransferases, yet to be molecularly cloned, to the HA synthases should also prove illuminating in a mechanistic as well as evolutionary sense.

Acknowledgments—We thank Drs. Paul H. Weigel and Pierre Neuenschwander for helpful discussions. We also thank Dr. Andrew P. Spicer for sharing preliminary information concerning the multiple vertebrate HAS genes.

REFERENCES

1. Laurent, T. C., and Fraser, J. R. E. (1992) FASEB J. 6, 2397–2404
2. DeAngelis, P. L., Papaconstantinou, J., and Weigel, P. H. (1993) J. Biol. Chem. 268, 14568–14571
3. DeAngelis, P. L., Papaconstantinou, J., and Weigel, P. H. (1993) J. Biol. Chem. 268, 19181–19184
4. DeAngelis, P. L., Yang, N., and Weigel, P. H. (1994) Biochem. Biophys. Res. Commun. 199, 1–10
5. DeAngelis, P. L., and Weigel, P. H. (1994) Biochemistry 33, 9033–9039
6. Rosa, F., Sargent, T. D., Rebert, M. L. Michaels, G. S., Jamrich, M., Grunz, H., Jonas, E., Winkles, J. A., and Dawid, I. B. (1988) Dev. Biol. 129, 114–123
7. Atkinson, E. M., and Long, S. R. (1992) Mol. Plant-Microbe Interact. 5, 439–442
8. Meyer, M. F., and Krell, G. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 4543–4547
9. DeAngelis, P. L., and Achyuthan, A. M. (1996) J. Biol. Chem. 271, 23657–23660
10. Ohy, T., and Kaneko, Y. (1970) Biochem. Biophys. Acta 196, 607–609
11. Iwao, N., and Kimata, K. (1996) J. Biol. Chem. 271, 9875–9878
12. Spicer, A. P., Augustine, M. L., and McDonald, J. A. (1996) J. Biol. Chem. 271, 23400–23406
13. Shyjan, A. M., Heldin, P., Butcher, E. C., Yoshino, T., and Briskin, M. J. (1996) J. Biol. Chem. 271, 23395–23399
14. Watanabe, K., and Yamaguchi, Y. (1996) J. Biol. Chem. 271, 22945–22948
15. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
16. Determan, H. (1968) Gel Chromatography, Springer-Verlag Inc., New York
17. Ishimoto, N., Tomin, H. M., and Stromberg, J. L. (1966) J. Biol. Chem. 241, 2052–2057
18. Appel, A., Horwitz, A. L., and Dorfman, A. (1979) J. Biol. Chem. 254, 12199–12203
19. Philipson, L. H., and Schwartz, N. B. (1980) J. Biol. Chem. 255, 5017–5023
20. Ng, K. F., and Schwartz, N. B. (1989) J. Biol. Chem. 264, 11776–11783
21. Malinowski, N. M., Cysewski, R. L., and August, E. M. (1995) Biochem. Mol. Biol. Int. 35, 1123–1132
22. Prehm, P. (1983) Biochem. J. 211, 181–189
23. Prehm, P. (1983) Biochem. J. 211, 191–198
24. Markowitz, A., Cifonelli, J. A., and Dorfman, A. (1959) J. Biol. Chem. 234, 2343–2350
25. DeAngelis, P. L. (1996) Biochem. 35, 9768–9771
Enzymological Characterization of Recombinant *Xenopus* DG42, A Vertebrate Hyaluronan Synthase

Philip E. Pummill, Ann Mary Achyuthan and Paul L. DeAngelis

*J. Biol. Chem.* 1998, 273:4976-4981.
doi: 10.1074/jbc.273.9.4976

Access the most updated version of this article at [http://www.jbc.org/content/273/9/4976](http://www.jbc.org/content/273/9/4976)

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

[Click here](http://www.jbc.org/content/273/9/4976.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 24 references, 15 of which can be accessed free at [http://www.jbc.org/content/273/9/4976.full.html#ref-list-1](http://www.jbc.org/content/273/9/4976.full.html#ref-list-1)