Hyaluronan Synthases*

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Minireview

In 1934, Meyer and Palmer isolated a novel, high Mₐ, glycosaminoglycan from the vitreous of the eye (1). They showed that this substance contained a hexuronic acid, an amino sugar, and no sulfobetes and proposed the name hyaluronic acid (hyaluronan, HA), from the Greek hyaloid (vitreous) and uronic acid. It took 20 years before Weissmann and Meyer (2) finally established the precise structure of the repeating disaccharide unit of hyaluronic acid (GlcAβ(1→3)GlcNAcβ(1→4)). The number of repeating disaccharides in an HA molecule can exceed 30,000, a Mₐ >10⁶. MedLine surveys for reports describing the structure, synthesis, degradation, and biology of HA reveal a steadily increasing interest in this biopolymer during the four decades following the determination of its structure: 790 papers published from 1966 to 1975; 2200 from 1976 to 1985; over 3300 from 1986 to 1996. During this time, HA has been identified in virtually every tissue in vertebrates and has achieved widespread use in various clinical applications, most notably and appropriately as an intra-articular matrix supplement (3) and in eye surgery. This period has also seen a transition from the original perception that HA is primarily a passive structural component in the matrix of a few connective tissues and in the capsule of certain strains of bacteria to a recognition that this ubiquitous macromolecule is dynamically involved in many biological processes: from modulating cell migration and differentiation during embryogenesis (4) to regulation of extracellular matrix organization and metabolism (5) to important roles in the complex processes of metastasis, wound healing, and inflammation (6, 7). Further, it is becoming clear that HA is highly metabolically active and that cells focus much attention on the processes of its synthesis and catabolism. For example, the half-life of HA in tissues ranges from 1 to 3 weeks in cartilage (8) to <1 day in epidermis (9). In this report, we describe recent advances that provide exciting new insights into the biosynthetic side of these metabolic processes.

HA Biosynthesis

It is now clear that a single protein utilizes both sugar substrates to synthesize HA (10). The abbreviation HAS, for the HA synthase, has gained widespread support for designating this class of enzymes and should now be accepted as standard nomenclature. Markovitz et al. (11) successfully character-ized the HAS activity from Streptococcus pyogenes and discovered the enzyme’s membrane localization and its requirements for sugar nucleotide precursors and Mg²⁺. Prehm (12) found that elongating HA, made by B6 cells, was digested by hyaluronidase added to the medium and proposed that HAS resides at the plasma membrane. Philipson and Schwartz (13) also showed that HAS activity cofractionated with plasma membrane markers in mouse oligodendroglial cells. HAS assembles high Mₐ HA that is simultaneously extruded through the membrane into the extracellular space (or to make the cell capsule in the case of bacteria) as glycosaminoglycan synthesis proceeds. This mode of biosynthesis is unique among macromolecules since nucleic acids, proteins, and lipids are synthesized in the nucleus, endoplasmic reticulum/Golgi, cytoplasm, or mitochondria. The extrusion of the growing chain into the extracellular space would also allow unconstrained polymer growth, thereby achieving the exceptionally large size of HA, whereas confinement of synthesis within a Golgi or post-Golgi compartment could limit the overall amount or length of the polymers formed. High concentrations of HA within a confined lumen could also create a high viscosity environment that might be deleterious for other organelle functions.

In 1983, Prehm (14) proposed a novel mechanism for HA synthesis that was distinctly different from that for other glycosaminoglycans, such as chondroitin sulfate and heparan sulfate. These latter glycosaminoglycans are elongated on core proteins by transfer of an appropriate sugar from a sugar nucleotide onto the nonreducing terminus of a growing chain (15). However, Prehm (14) proposed that HA synthesis occurs at the reducing terminus of a growing HA chain by a two-site mechanism (Fig. 1). In this mechanism, the reducing end sugar of the growing HA chain (either in the GlcNac or GlcA site) would remain covalently bound to a terminal UDP, and the next sugar to be added from the second site would be transferred as the UDP-sugar onto the reducing end sugar with displacement of its terminal UDP. The HA chain would then be in the second site. This unusual mode of synthesis does not occur with the eukaryotic heparan sulfate synthase (16) or the bacterial, K5 (17), or K4 (18) polysaccharide synthases, each of which utilizes the same nucleotide sugar substrates, and it remains to be verified using purified recombinant HAS.

Several studies attempted to solubilize, identify, and purify HAS from strains of Streptococci that make a capsular coat of HA as well as from eukaryotic cells (11–13, 19–21). Although the streptococcal (19, 20) and murine oligodendroglial enzymes (21) were successfully detergent-solubilized and studied, efforts to purify an active HAS for further study or molecular cloning remained unsuccessful for decades. Prehm and Mausolf (19) used periodate-oxidized UDP-GlcA or UDP-GlcNAc to affinity label a protein of ~52 kDa in streptococcal membranes that co-purified with HA. This led to a report (22) claiming that the Group C streptococcal HAS had been cloned, which was unfortunately erroneous. This study failed to demonstrate expression of an active synthase and may have actually cloned a peptide transporter. Triscott and van de Rijn (20) used digiotin to solubilize HAS from streptococcal membranes in an active form. van de Rijn and Drake (23) selectively radiolabeled three streptococcal membrane proteins of 42, 33, and 27 kDa with S-azido-UDP-GlcA and suggested that the 33-kDa protein was HAS. As shown later (24, 25), however, HAS actually
a multigene family encoding distinct isozymes. Two genes
were discovered in 1996 by four laboratories revealing that HAS is
initially thought to be the same mouse and human enzyme.

However, through an extraordinary circumstance, each of the
four laboratories had discovered a separate HAS isozyme in
two species. Using a similar functional cloning approach to
that of Itano and Kimata (29), Shyjan et al. (30) identified the
human homolog of HAS1. A messenteric lymph node cDNA
library was used to transfect murine mucosal T lymphocytes
that were then screened for their ability to adhere in a rosette
assay. Adhesion of one transfected was inhibited by antisera
to CD44, a known cell surface HA-binding protein, and was
abrogated directly by pretreatment with hyalurondase. Thus,
resetting by this transf ectant required synthesis of HA. Cloning
and sequencing of the responsible cDNA identified hs
HAS1. Itano and Kimata (31) also reported a human HAS1
cDNA isolated from a fetal brain library. The hsHAS1 cDNAs
reported by the two groups, however, differ in length; they
encode a 578 (30) or a 545 (31) amino acid protein. HAS activity
has only been demonstrated for the longer form.

Based on the molecular identification of spHAS as an au-
thentic HA synthase and regions of near identity among DG42,
spHAS, and NodC (a β-GlcNAc transferase nodulation factor in
Rhizobium), Spicer et al. (32) used a degenerate RT-PCR ap-
proach to clone a mouse embryo cDNA encoding a second dist-
inct enzyme, which is designated mmHAS2. Transfection of
mmHAS2 cDNA into COS cells directed de novo production of
an HA cell coat detected by a particle exclusion assay, thereby
providing strong evidence that the HAS2 protein can synthe-
size HA. Using a similar approach, Watanabe and Yamaguchi
(33) screened a human fetal brain cDNA library to identify
hsHAS2, Fulop et al. (34) independently used a similar strategy
to identify mmHAS2 in RNA isolated from ovari an cumulus
cells actively synthesizing HA, a critical process for normal
cumulus oophorus expansion in the pre-ovulatory follicle. Cu-
mulus cell-oocyte complexes were isolated from mice immedi-
ately after initiating an ovulatory cycle, before HA synthesis
begins, and at later times when HA synthesis is just beginning
(3 h) or already apparent (4 h). RT-PCR showed that HAS2
mRNA was absent initially but expressed at high levels 3–4 h
later suggesting that transcription of HAS2 regulates HA syn-
thesis in this process. Both hsHAS2 and mmHAS2 are 552

**The HAS Family**

The elusive HA synthase gene was finally cloned by a trans-
spoon mutagenesis approach (24), in which an acapsular mu-
tant Group A strain was created containing a transspoon in-
terruption of the HA synthase operon. Known sequences of the
transspoon allowed the region of the junction with streptococ-
cal DNA to be identified and then cloned from wild-type cells.
The encoded spHAS (25) was 5–10% identical to a family of
other proteins. HasB is a UDP-glucose dehydrogenase, which is
required to convert UDP-glucose to UDP-GlcA, one of the sub-
strates for HA synthesis (26). HasC is a UDP-glucose pyrophos-
phorylase, which is required to convert glucose 1-phosphate
and UTP to UDP-glucose (27). Co-transfection of both hasA and
hasB genes into either acapsular Streptococcus strains or Ent-
eroccus faecalis conferred them with the ability to synthesize
HA and form a capsule (24, 25). This provided the first strong
evidence that HasA is an HA synthase.

**Fig. 1. Proposed mechanism of HA synthesis.** The repeating
disaccharide (shown in brackets) is synthesized by extension of the
polymer at the reducing end via a two-site mechanism (14), as described
in the text. Definitive evidence for this unusual mode of saccharide
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amino acids in length and are 98% identical. mmHAS1 is 583 amino acids long and 95% identical to hsHAS1, which is 578 amino acids long. Most recently Spicer et al. (35) used a PCR approach to identify a third HAS gene in mammals. The mmHAS3 protein is 554 amino acids long (Fig. 2) and 57, 71, 56, and 28% identical, respectively, to mmHAS1, mmHAS2, DG42, and spHAS. Spicer et al. (42) have also localized the three human and mouse genes to three different chromosomes (HAS1 to hsChr 19/mmChr 17; HAS2 to hsChr 8/mmChr 15; HAS3 to hsChr 16/mmChr 8). Localization of the three HAS genes on different chromosomes and the appearance of HA throughout the vertebrate class suggest that this gene family is ancient and that isozymes appeared by duplication early in the evolution of vertebrates. The high identity (≥30%) between the bacterial and eukaryotic HASs also indicates a common ancestral gene. Perhaps primitive bacteria usurped the HAS gene from an early vertebrate ancestor before the eukaryotic gene products became larger and more complex. Alternatively, the bacteria could have obtained a larger vertebrate HAS gene and deleted regulatory sequences nonessential for enzyme activity.

The discovery of *X. laevis* DG42 by Dawid and co-workers (28) played a significant role in these recent developments, even though this protein was not known to be an HA synthase. Nonetheless, that DG42 and spHAS were 30% identical was critical for designing oligonucleotides that allowed identification of mammalian HAS2 (32, 34). Ironically, definitive evidence that DG42 is a *bona fide* HA synthase was reported only after the discoveries of the mammalian isozymes, when DeAngelis and Achyuthan (43) expressed the recombinant protein in yeast (an organism that cannot synthesize HA) and showed that it synthesizes HA when isolated membranes are provided with the two substrates. Meyer and Kreil (44) also showed that lysates from cells transfected with cDNA for DG42 synthesize elevated levels of HA. Now that its function is known, DG42 can, therefore, be designated *xlHAS* (Fig. 2).

### Structural Features of the HAS Proteins

Fig. 3 depicts the common predicted structural features shared by all the HAS proteins, including a large central domain, Ala-145-Pro-317, in spHAS is particularly conserved, with few gaps, in the family. hs, *Homo sapiens*; mm, *Mus musculus*; sp, *S. pyogenes*; xl, *X. laevis*.
HAS genes encoding different synthases strongly support the emerging consensus that HA is an important regulator of cell behavior and not simply a structural component in tissues. Thus, in less than 6 months, the field moved from one known, cloned HAS (spHAS) to recognition of a multigene family that promises rapid, numerous, and exciting future advances in our understanding of the synthesis and biology of HA.

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REFERENCES

1. Meyer, K., and Palmer, J. W. (1934) J. Biol. Chem. 107, 629–634
2. Weissmann, B., and Meyer, K. (1945) J. Am. Chem. Soc. 27, 1753–1757
3. Balaza, E. A. (1993) J. Rheumatol. 39, 3–9
4. Toole, B. P. (1990) Curr. Opin. Cell Biol. 2, 839–844
5. Laurent, T. C., and Fraser, J. R. E. (1992) FASEB J. 6, 2397–2404
6. Hall, C. L., and Turley, E. A. (1995) J. Neuro-Oncol. 26, 221–229
7. Lesley, J., and Kinecke, H. R. (1995) Adv. Immunol. 54, 271–335
8. Morales, T. I., and Hasseal, C. V. (1988) J. Biol. Chem. 263, 3632–3638
9. Tammi, R., Saamanen, A.-M., Maibach, H. I., and Tammi, M. (1991) J. Invest. Dermatol. 97, 126–130
10. DeAngelis, P. L., and Weigel, P. H. (1994) Biochemistry 33, 9033–9039
11. Markovitz, A., Cifoniello, A., and Dorman, A. (1959) J. Biol. Chem. 234, 2343–2350
12. Prehm, P. (1984) Biochem. J. 220, 597–600
13. Philipson, L. I., and Schwartz, N. B. (1984) J. Biol. Chem. 259, 5017–5023
14. Prehm, P. (1985) Biochem. J. 211, 191–198
15. Hasseal, V. C., Heinegard, D., K., and Wight, T. N. (1991) in Cell Biology of Extracellular Matrix (Hay, E. D., ed) 2nd Ed., pp. 149–175, Plenum Publishing Corp., New York
16. Bane, K. J., Chechet, S. M., and Weigel, P. H. (1992) Proc. Natl. Acad. Sci. U.S.A. 8 9, 2267–2271
17. Liholt, K., Fjelstad, M., Jann, K., and Lindahl, U. (1994) Carbohydr. Res. 255, 47–101
18. Liholt, K., and Fjelstad, M. (1997) J. Biol. Chem. 272, 2682–2687
19. Prehm, P., and Mausolf, A. (1986) Biochem. J. 235, 887–889
20. Trucott, M. X., and van de Rijn, I. (1986) J. Biol. Chem. 261, 6004–6009
21. Ng, K. F., and Schwartz, N. B. (1989) J. Biol. Chem. 264, 11776–11783
22. Lansing, M., Leillig, S., Mausolf, A., Martini, I., Crescenzi, F., Oregon, M., and Prehm, P. (1993) Biochim. Biophys. Acta 1179, 179–184
23. van de Rijn, I., and Drake, R. K. (1993) J. Biol. Chem. 268, 24302–24306
24. DeAngelis, P. L., Papacostaumious, J., and Weigel, P. H. (1993) J. Biol. Chem. 268, 14568–14571
25. DeAngelis, P. L., Papacostamious, J., and Weigel, P. H. (1993) J. Biol. Chem. 268, 19181–19184
26. Dougherty, B. A., and van de Rijn, I. (1993) J. Biol. Chem. 268, 7118–7124
27. Crater, D. L., Dougherty, B. A., and van de Rijn, I. (1995) J. Biol. Chem. 270, 28676–28680
28. Rosa, F., Sargent, T. D., Rebert, M. L., Michaels, G. S., Rebbert, M. L., and Grunz, H., Jonas, E., Winkles, J. A., and Dawid, I. B. (1988) Dev. Biol. 129, 114–123
29. Itano, N., and Kimata, K. (1996) J. Biol. Chem. 271, 9875–9878
30. Shyjan, A. M., Heldin, P., Butcher, E. C., Yoshino, T., and Briskin, M. J. (1996) J. Biol. Chem. 271, 23385–23399
31. Itano, N., and Kimata, K. (1996) Biochem. Biophys. Res. Commun. 222, 816–820
32. Spicer, A. P., Augustine, M. L., and McDonald, J. A. (1996) J. Biol. Chem. 271, 23400–23406
33. Watanabe, K., and Yamaguchi, Y. (1996) J. Biol. Chem. 271, 22945–22948
34. Fulop, C., Salustri, A., and Hasseal, C. V. (1997) Arch. Biochem. Biophys. 337, 261–266
35. Spicer, A. P., Olson, J. S., and McDonald, J. A. (1997) J. Biol. Chem. 272, 8957–8961
36. Rittig, M., Lutijen-Dreccol, E., and Prehm, P. (1992) Exp. Eye Res. 54, 455–460
37. Kleeves, L., Turley, E. A., and Prehm, P. (1990) Biochem. J. 268, 791–795
38. Rittig, M., Fliegel, C., Prehm, P., and Lutijen-Dreccol, E. (1990) Graefes Arch. Clin. Exp. Ophthalmol. 231, 313–317
39. Kleeves, L., and Prehm, P. (1994) J. Cell. Physiol. 106, 539–544
40. Pintuvvillies, A. A., Archer, W. C., Prehm, P., Bayliss, M. T., and Edwards, J. C. (1995) J. Histochem. Cytochem. 13, 263–273
41. Mitchell, B. S., Whitehouse, A., Prehm, P., Delpech, B., and Schumacher, U. (1996) Clin. Exp. Metastasis 14, 107–114
42. Spicer, A. P., Seldin, M. F., Olson, S. A., Brown, N., Wells, D. E., Doggett, N. A., Itano, N., Kimata, K., Inazawa, J., and McDonald, J. A. (1997) Genomics, in press
43. DeAngelis, P. L., and Achyuthan, A. M. (1996) J. Biol. Chem. 271, 23657–23660
44. Meyer, M. F., and Kreil, P. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 4543–4547
45. Heidelberg, C., Tiapak Simonie, V., DeAngelis, P., and Weigel, P. (1996) Mol. Biol. Cell, 5 (abstr.)
46. Yang, B., Yang, B., Savani, R., and Turley, E. A. (1994) EMBO J. 13, 286–296
47. Mian, N. (1986) Arch. Biochem. Biophys., in press
48. Nagahashi, S., Sudo, M., Ono, N., Sawada, R., Yamaguchi, E., Uchida, Y., Mio, T., Takagi, M., Ariyama, M., and Yamada-Okabe, H. (1995) J. Biol. Chem. 270, 13961–13967

FIG. 3. Proposed membrane topology for the HAS family. Very similar hydropathy plots and primary structure (28–71% identity) among all the HAS isoymes suggest that they are similarly organized within the membrane. The scheme depicts the N and C termini and the large central domain, between MD2 and MD3, inside the cell. The larger eukaryotic HASs (thick line) have additional amino acids in all regions (see Fig. 2) compared with the bacterial HASs (thin line), except for the highly conserved carboxyl 178 residues of the central domain and MD1–MD5. In particular, the carboxyl –25% of the eukaryotic HASs has two additional predicted membrane domains (MD6 and MD7), missing in the bacterial proteins. The conserved Cys is indicated by the circled C. MD5 can be modeled as an amphipathic helix, which would orient the C terminus of all HAS members inside.