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The dynamic metabolism of hyaluronan regulates the cytosolic concentration of UDP-GlcNAc

Vincent C. Hascall *[a], Aimin Wang [a], Markku Tammi [b], Sanna Oikari [b], Raija Tammi [b], Alberto Passi [c], Davide Vigetti [c], Richard W. Hanson [d], Gerald W. Hart [e]

[a] Biomedical Engineering, Cleveland Clinic, OH 44195, United States
[b] School of Medicine, Institute of Biomedicine/Anatomy, University of Eastern Finland, 70211 Kuopio, Finland
[c] Dipartimento di Scienze e Morfologiche, Universita degli Studi dell'Insubria, 21100 Varese, Italy
[d] Department of Biochemistry, Case Western Reserve University, Cleveland, OH 44106, United States
[e] Department of Biological Chemistry, The Johns Hopkins School of Medicine, Baltimore, MD 21205-2185, United States

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ABSTRACT

Hyaluronan, a macromolecular glycosaminoglycan, is normally synthesized by hyaluronan synthases at the plasma membrane using cytosolic UDP-GlcUA and UDP-GlcNAc substrates and extruding the elongating chain into the extracellular space. The cellular metabolism (synthesis and catabolism) of hyaluronan is dynamic. UDP-GlcNAc is also the substrate for O-GlcNAc transferase, which is central to the control of many cytosolic pathways. This Perspective outlines recent data for regulation of hyaluronan synthesis and catabolism that support a model that hyaluronan metabolism can be a rheostat for controlling an acceptable normal range of cytosolic UDP-GlcNAc concentrations in order to maintain normal cell functions.

1. Introduction

The review article in this issue by Vigetti et al. (2014) provides an insightful description of the mechanisms for regulating the stability and activity of hyaluronan synthase 2 (HAS2), the HAS that is required for development in vertebrates (McDonald and Hascall, 2002). Cytosolic UDP-GlcNAc is one of the substrates for hyaluronan synthesis and is also the substrate for the cytosolic enzyme, O-GlcNAc transferase (OGT). OGT transfers GlcNAc from UDP-GlcNAc (O-GlcNAcylation) to serines/threonines in many intracellular proteins, which is often essential for regulating their functions. For example, O-GlcNAc is often the alternate structure for sites of phosphorylation on kinases. The activity of OGT depends on cytosolic UDP-GlcNAc levels. For example, hyperglycemic glucose stress, with consequent increases in UDP-GlcNAc concentration, leads to aberrant O-GlcNAcylation on many more cytosolic proteins, which can compromise cellular functions (Ma and Hart, 2013). The following Perspective outlines how regulation of HAS2 activity could be an important regulator for maintaining cytosolic UDP-GlcNAc concentrations within an acceptable level for normal cell function.

2. Hyaluronan biosynthesis

Hyaluronan is a glycosaminoglycan composed of a disaccharide, (glucuronate-β1,3-N-acetylglucosamine-β1,4-), that can be repeated more than 10,000 times to form a macromolecule >10 MDa in size. Unlike the other glycosaminoglycans (chondroitin sulfate, heparin/heparan sulfate, keratan sulfate), biosynthesis of hyaluronan does not require a core protein and is not synthesized in the Golgi. Instead, it is normally produced at the plasma membrane by one or more of the 3 hyaluronan synthases (HAS1, 2, 3) in the mammalian genomes (Weigel et al., 1997; Tammi et al., 2011).

HASes are synthesized in the endoplasmic reticulum (ER) and carried by transport vesicles, likely through the Golgi, to the plasma membrane in an inactive form. Once embedded in the plasma membrane they can be activated, most likely through a phosphokinase C (PKC) phosphorylation pathway (Fig. 1A) (Wang and Hascall, 2004). The cytosolic domains of HASes then utilize cytosolic substrates (UDP-GlcUA and UDP-GlcNAc) and add them alternately onto the reducing end of the chain at the expense of removing the anchoring UDP, and the elongating chain is extruded through the plasma membrane into the extracellular space. During transport to the plasma membrane, the cytosolic domain is exposed to the cytosol. Therefore, it is important not to activate HASes before they are properly embedded in the plasma membrane.

Further, regulation of hyaluronan synthesis is relatively simple as it depends only on single enzymes. It is also relatively energy efficient as synthesis of one of the substrates, UDP-glucuronic acid (UDP-GlcUA), recovers the equivalent of two ATPs, 5 from double oxidation of NADPH, and minus 3 from the oxidation of UDP-glucose. It is also quite likely that the rate of hyaluronan synthesis depends on the cytosolic concentrations of the UDP-sugar substrates (Jokela et al., 2008; Rilla et al., 2013).

* Corresponding author at: Department of Biomedical Engineering, ND20, Cleveland Clinic, Cleveland, OH 44195, United States. Tel.: +1 216 445 5676; fax: +1 216 444 9198.
E-mail address: hascalv@ccf.org (V.C. Hascall).

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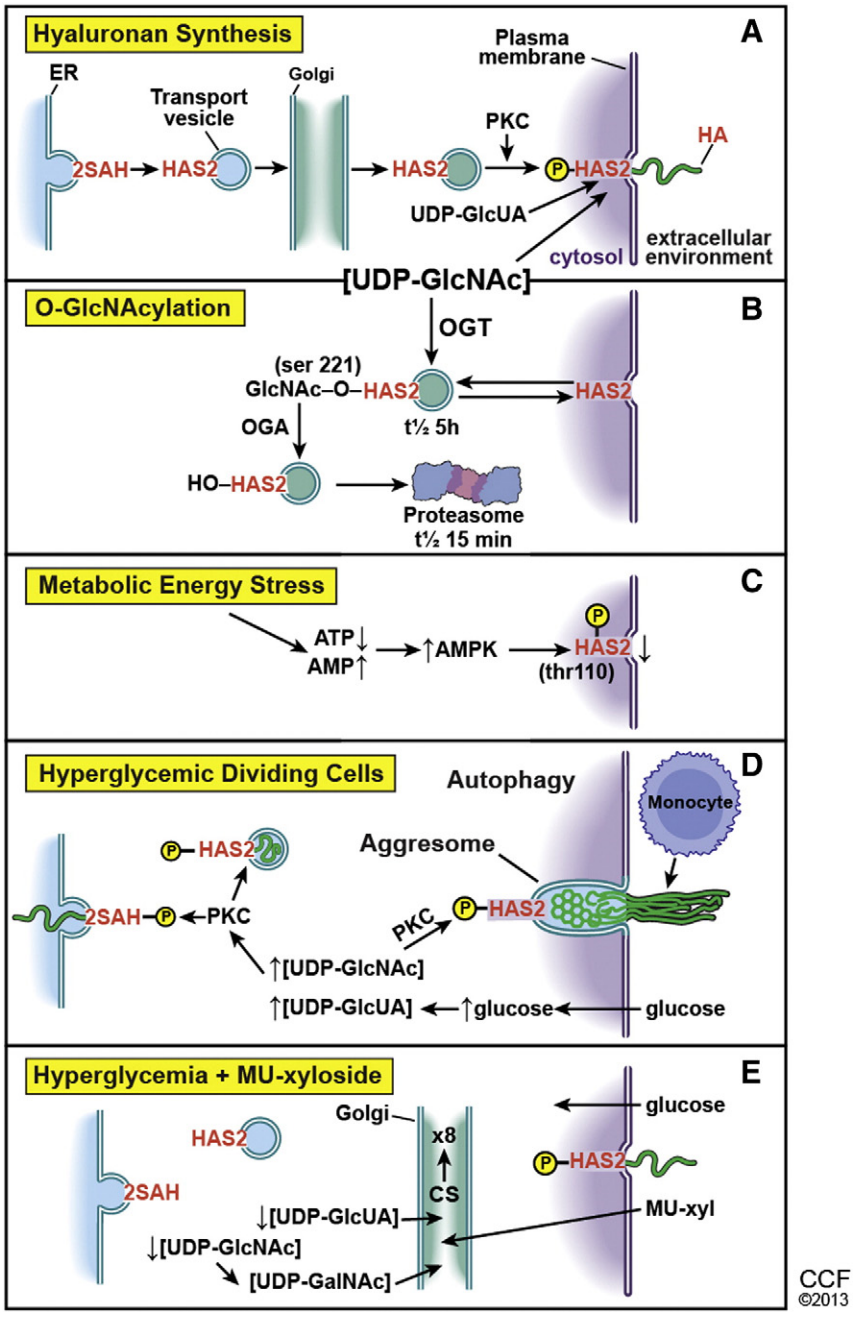

Fig. 1. Models for regulation of HAS2 synthesis and its relation with UDP-GlcNAc concentration and OGT activity.

3. Hyaluronan synthase 2 (HAS2) (see the Vigetti et al., 2014 review)

Hyaluronan synthase 2 is expressed by most, if not all, cells and is essential for life. While the HAS1 and 3 null mice are developmentally normal, the HAS2 null mouse dies at an early embryonic stage when the heart is formed. Normal heart development requires endothelial cells to undergo epithelial–mesenchymal transition (EMT), which occurs when HAS2 is upregulated. Organ cultures of the wild type heart tube endothelial cells undergo EMT at the initiation of hyaluronan synthesis by HAS2. In contrast, organ cultures of the HAS2 null heart endothelium do not undergo any EMT, but do so if hyaluronan is added to the culture medium (Camenisch et al., 2002; McDonald and Hascall, 2002).

HAS2 is dynamically regulated by cytosolic pathways that are involved in regulation of O-GlcNAcylation. The stability of cytosolic HAS2 is significantly increased when serine 221 is O-GlcNAcylated (t1/2 of ~5 h). In the absence of O-GlcNAc on this serine, HAS2 is rapidly degraded in proteasomes (t1/2 of ~15 min) (Fig. 1B) (Vigetti et al., 2012). It is possible that O-GlcNAcylation of this serine is a key for regulating whether or not HAS2 remains inactivated, which would allow the enzyme to migrate to the surface after its synthesis in the ER. Further, it would allow HAS2 to be internalized in an inactive form into cytosolic vesicles for storage or for eventual lysosomal or proteasomal degradation as outlined in Fig. 1B. It is also possible that removal of the GlcNAc and phosphorylation of this site or of another serine residue is critical for its normal activation in the plasma membrane. In any event, it is necessary to maintain HASes in non-active configurations when they are in any membrane exposed to cytosol except the plasma membrane, as shown by the pathological autophagic response of dividing hyperglycemic cells discussed below.

During energy stress, the ratio of AMP/ATP increases with consequent activation of AMP kinase. AMP kinase phosphorylates threonine