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Metabolic control of hyaluronan synthases

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ABSTRACT

Hyaluronan (HA) is a glycosaminoglycan composed by repeating units of D-glucuronic acid (GlcUA) and N-acetylglucosamine (GlcNAc) that is ubiquitously present in the extracellular matrix (ECM) where it has a critical role in the physiology and pathology of several mammalian tissues. HA represents a perfect environment in which cells can migrate and proliferate. Moreover, several receptors can interact with HA at cellular level triggering multiple signal transduction responses. The control of the HA synthesis is therefore critical in ECM assembly and cell biology; in this review we address the metabolic regulation of HA synthesis. In contrast with other glycosaminoglycans, which are synthesized in the Golgi apparatus, HA is produced at the plasma membrane by HA synthases (HAS1-3), which use cytoplasmic UDP-glucuronic acid and UDP-N-acetylglucosamine as substrates. UDP-GlcUA and UDP-hexosamine availability is critical for the synthesis of GAGs, which is an energy consuming process. AMP activated protein kinase (AMPK), which is considered a sensor of the energy status of the cell and is activated by low ATP:AMP ratio, leads to the inhibition of HA secretion by HAS2 phosphorylation at threonine 110. However, the most general sensor of cellular nutritional status is the hexosamine biosynthetic pathway that brings to the formation of UDP-GlcNAc and intracellular protein glycosylation by *O*-linked attachment of the monosaccharide β-N-acetylglucosamine (O-GlcNAcylation) to specific aminoacid residues. Such highly dynamic and ubiquitous protein modification affects serine 221 residue of HAS2 that lead to a dramatic stabilization of the enzyme in the membranes.

1. Introduction

The complex molecular structure that surrounds cells in tissues is defined as extracellular matrix (ECM) which has been reported to have many critical functions in determining the cellular microenvironment. A large body of evidence supports the concept that ECM not only acts in the structural architecture of the cells and tissues, but also regulates cell behavior in both physiological and pathological conditions controlling nutrient and waste exchanges, cell–cell and cell–matrix interactions, and diffusion of signaling molecules. Nowadays, the active role of ECM in cell physiology and pathology determination, for instance, proliferation, movements and differentiation of the cells as well as the stem cell niche is becoming more evident. ECM is also critical in cell dysfunctions that characterize many pathologies as cancer or cardiovascular diseases (Vigetti et al., 2006a; Akita et al., 2008; Acloque et al., 2009).

Among the different components of ECM, polysaccharides (i.e.,glycosaminoglycans, GAGs) have the highest variability and represent the most dynamic structures in tissues. In fact, several chemical modifications as epimerization, acetylation, O-, and N-sulfation are critical to determine the properties of chondroitins, heparan sulfate, and keratan. A plethora of enzymes are known to tailor GAG molecules during pathophysiological processes (Afratis et al., 2012). A further degree of variability is represented by the proteoglycan (PG) core protein, to which the GAG chains are linked. In fact, PGs can have several isoforms and can be differently modified by proteases and other post-translational modifications. Hyaluronan (HA) is an atypical GAG being not covalently bond to any PG core proteins, not epimerizated and not sulfated, although tumor necrosis factor alpha-stimulated protein 6 and its covalent binding to HA can mediate the transfer of the heavy chain of inter alpha trypsin inhibitor to –COOH of HA leading to a complex known as Serum-derived Hyaluronan-associated Protein (SHAP), that has proinflammatory properties and is involved in many pathologies (Jiang et al., 2011).

HA is simply composed by the disaccharide unit D-glucuronic acid (GlcUA) and D-N-acetyl-glucosamine (GlcNAc) linked by alternating β-1,4 and β-1,3 glycosidic bonds. HA is considered a molecule rich in biological information being the polymer length critical to determine HA effects (Stern et al., 2006). In fact, the number of HA disaccharide repetitions can greatly vary in determining the polymer molecular mass that ranges from 5 to 10,000 kDa. Further, high molecular weight HA can be fragmented in short oligosaccharides by different mechanisms including degrading enzymes (i.e., hyaluronidases), and free radicals (Stern et al., 2006). Moreover, low molecular mass HA can be directly synthesized by dysregulation of HA synthases (HASes) as well as due to an alter precursor availability. Therefore, the rheostatic effects of HA largely depend on the size of the polymeric chain. Further, several proteins with receptor functions have been described to bind HA directly affecting cell behavior (Jiang et al., 2011; Vigetti et al., 2011b). In

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the light of these premises, HA cannot be considered a merely space filling molecule but a ubiquitous microenvironment component able to trigger different cell signaling cascades and to modulate many physiological and pathological processes. This review focuses on the fine mechanism that links cellular metabolism and HA synthesis also describing the known post-translational modifications of HASes in mammals.

1.1. HA synthases

In mammals HA is synthesized by three different isoenzymes named HAS 1, 2, and 3. HASes are plasma membrane proteins that use cytoplasmic UDP-GlcNAc and UDP-GlcUA as precursors to polymerize the HA chain and to extrude it out of the cell without the necessity of a primer or an anchor protein or lipid. However purification experiments revealed that bacterial HAS (which shares several structural similarities with mammalian enzymes (Weigel and DeAngelis, 2007)) is strictly associated with cardiolipin and the catalytic activity is dependent on such lipid (Weigel et al., 1997). Although the crystallographic data are not yet available, HASes would have a complex architecture. In fact, a single protein codes for many functions as binding of two distinct UDP-sugars and binding of two distinct HA acceptor or donor species. Moreover HASes transfer two different sugars in two different linkages, catalyze repetitive sugar polymerization, and transfer HA across the membrane (Weigel and DeAngelis, 2007; Hubbard et al., 2012).

Little is known about HASes structure, topological *in silico* analyses revealed that Class 1 mammalian and Streptococcal HASes have 2 N-terminal transmembrane domains and several of C-terminal transmembrane domains or membrane associated domains (Jiang et al., 2011). In Fig. 1 are reported the predicted structural organization of mammals (Fig. 1A) and Streptococcal HASes (Fig. 1B). These domains are responsible for the catalytic activities of these proteins (Heldermon et al., 2001). On the other hand, the only member of Class 2 HAS, expressed by *Pasteurella multocida*, has a shorter C-terminal domain with only 4 transmembrane regions and 2 membrane associated domains. Interestingly, only a limited number of pathogenic bacteria show the ability to produce HA, which is used by these bacteria to shield themselves from the host immune system (DeAngelis, 1999). The cytosolic domain shares similarities with chitin synthases highlighting the importance of this portion of the protein for the catalysis. The overexpression of HAS2 and 3 lead to the formation of hetero and homodimers in Chinese hamster ovary (CHO) cells (Karousou et al., 2010) and this observation is consistent with the evidence that HAS activity resides in a large protein complex in plasma membranes (Asplund et al., 1998).

HASes possess different biochemical properties (Itano and Kimata, 2002). HAS1 is less catalytically active than HAS2, which, in turn, is intrinsically less active than HAS3. Moreover, HAS1 and HAS2 synthesize a polymer of high molecular weight whereas HAS3 produces shorter chains (~2×10^6 Da *vs* ~2×10^5 Da, respectively) (Itano et al., 1999). Interestingly, the lower intrinsic activity of HAS1 comes from the fact that it cannot produce HA when the cellular level of UDP-sugars is low (Rilla et al., 2013).

Many growth factors, cytokines, and other signaling molecules control the transcription of HAS genes and are recently reviewed elsewhere (Tammi et al., 2011). Once transcribed, to reach the plasma membrane, HAS proteins have to be synthesized in the endoplasmic reticulum and to follow the secretory pathway. Interestingly, microsomal membrane preparations, without plasma membrane contaminations, showed the capacity to synthesize HA in vitro. These properties are evident after several cell treatments that modulate ER stress, protein N-glycosylation and phosphorylation (Vigetti et al., 2009a). These latter evidences suggest that HASes enzymes, once translated, are subjected to a fine regulation that is largely unknown, nevertheless can be reasonable to hypothesize that modifications of

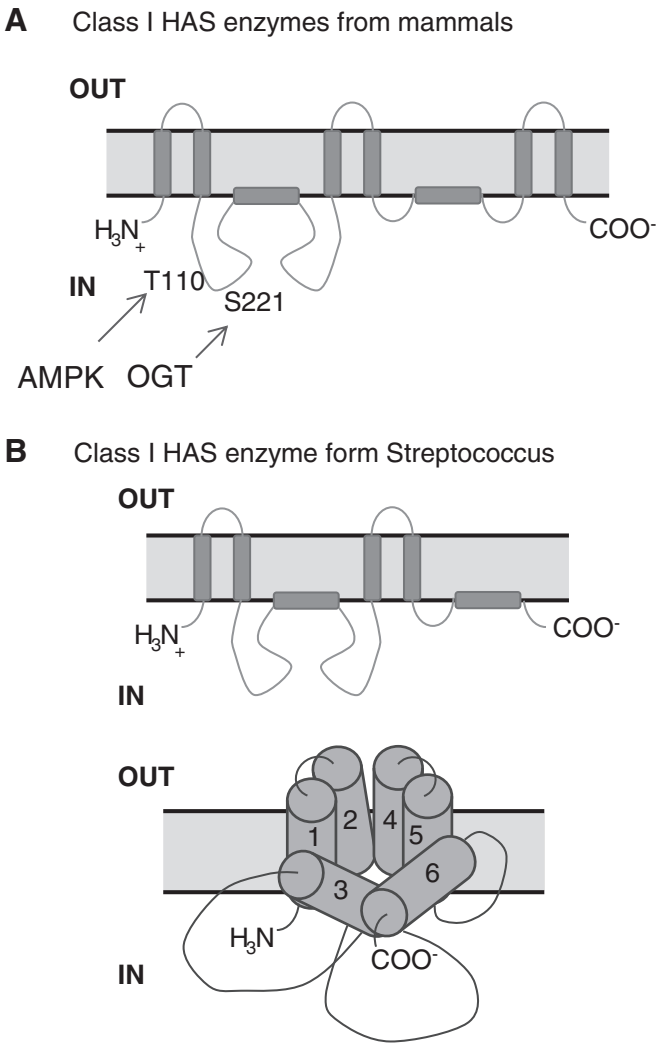

Fig. 1. A. Schematic representation of Class 1 mammal HAS with 6 transmembrane domains and 2 membrane associated domains. B. Schematic representation of Streptococcal Class 1 HAS with 4 transmembrane domains and 2 membrane associated domains (B, upper panel). In the lower panel there is a representation of Class 1 HAS in which the two cytoplasmic loops and the central pore for HA chain extrusion in the extracellular space are highlighted.

HASes can have a critical role in HASes activity. Moreover, evidences *in vitro* on purified bacterial (*P. multocida*) HAS showed that a monodispersed HA chain can be obtained by finely tuning the reaction stoichiometry. In fact the molar ratio of precursors and acceptor molecules has an important role in enzyme kinetics (Jing and DeAngelis, 2004; DeAngelis, 2008). This point highlights the crucial role of UDP-sugar precursors and, therefore, of the entire cell metabolism that produces HAS substrates.

2. Role of UDP-GlcUA and cellular energy homeostasis

UDP-GlcUA has many functions in mammalian cells in addition to being a component of the GAGs with the exception of keratan sulfate. In fact, UDP-GlcUA is the donor substrate for glucuronidation reactions that take place in liver to detoxify the organism and in other cell types (Vigetti et al., 2009b). Moreover, UDP-GlcUA can be decarboxylated to UDP-xylose which is critical for the linking of GAGs to proteoglycan core proteins.

To investigate the role of UDP-GlcUA in GAG synthesis, our group overexpressed UDP-glucose dehydrogenase, the enzymes involved in the oxidation of UDP-glucose to form UDP-GlcUA (Fig. 2), in human

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