Metabolic Engineering of Hyaluronic Acid Production in Streptococci

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Hyaluronic acid (HA) is a ubiquitous polysaccharide in vertebrates, where it serves a wide variety of biological functions in fertilization, morphogenesis, tissue remodeling and homeostasis, cell-cell signaling, lubrication and water retention. The wide utility of this polymer is the basis for a USD 2 billion plus pharmaceutical and cosmetic market. Reticence to the use of animal derived HA has revitalized the market for HA produced by fermentation of group C streptococci.

Hyaluronic acid synthase (HAS) is similar to other membrane-associated glycosyl transferases producing abundant beta-linked polysaccharides such cellulose, chitin and beta-glucan. While the mechanisms of elongation are well understood, the mechanism of termination and hence molecular weight control is not. Intrinsic features of HAS such as conserved cysteine residues are believed to dictate the maximum chain length that can be retained against the torque exerted by the expanding polymer. The molecular weight realized in microbial culture, however, is much less than this maximum and – significantly – much less than the molecular weight that can be realized through extraction from rooster comb.

The molecular weight realized in streptococcal cultures is greatly affected by culture parameters such as sugar source and oxygen availability indicating that resource availability (energy and carbon) is a major factor. Over the past decade, we have explored with limited success various process and strain engineering strategies to release more resources for HA production. Through systematic overexpression of each gene in the HA pathway and comprehensive omics analysis of the resultant strains, we have now successfully broken the 5 MDa barrier, up from 1-2 MDa for wildtype strains.

The study highlights the power of systems biotechnology to tackle quality traits such as molecular weight. The key to the success in this case was to achieve a proper balance between the two activated HA precursors, UDP-N-acetyl glucosamine and UDP-glucuronic acid. Presumably, HAS terminates polymerization if a precursor enters the enzyme out of turn. Through the understanding gained through the systems approach, it was possible to identify a number of strain and bioprocess engineering strategies that led to high Mw.