Researchers move closer to producing heparin in the lab

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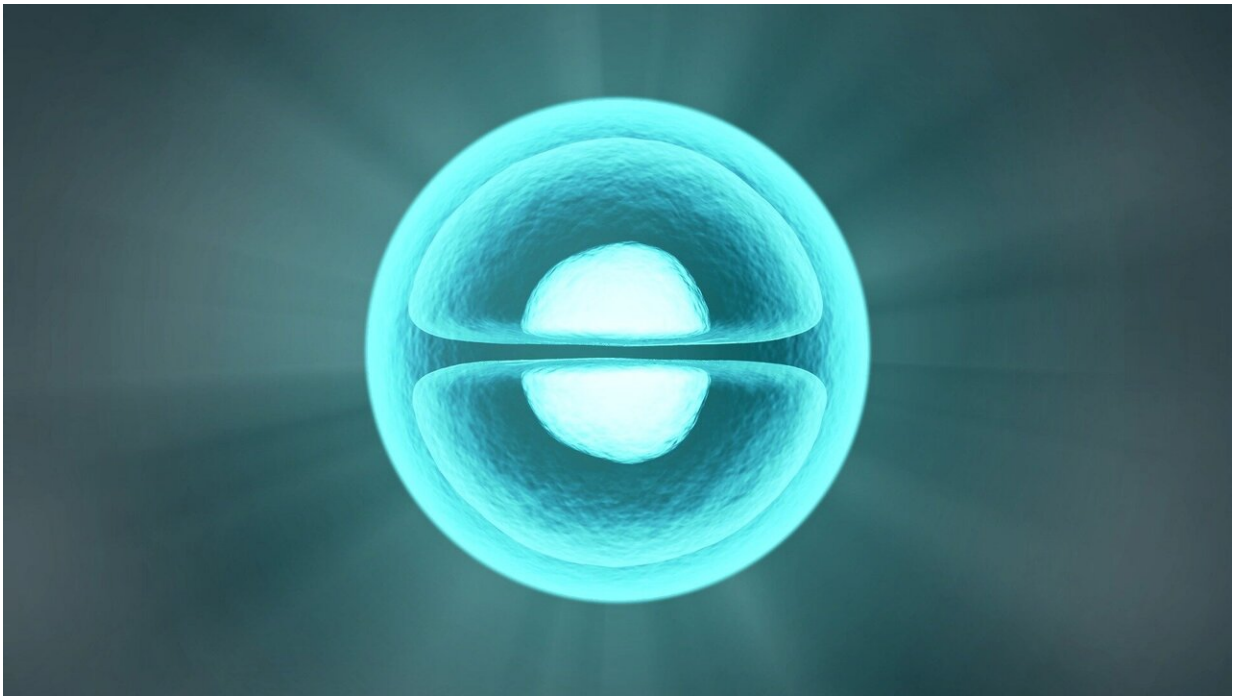

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In a recent study published in the *Proceedings of the National Academy of Sciences (PNAS)*, University of California San Diego researchers moved one step closer to the ability to make heparin in cultured cells. Heparin is a potent anti-coagulant and the most prescribed drug in hospitals, yet cell-culture-based production of heparin is currently not possible.

In particular, the researchers found a critical gene in heparin biosynthesis: ZNF263 (zinc-finger protein 263). The researchers believe this gene regulator is a key discovery on the way to industrial heparin production. The idea would be to control this regulator in industrial cell lines using genetic engineering, paving the way for safe industrial production of heparin in well-controlled cell culture.

Heparin is currently produced by extracting the drug from pig intestines, which is a concern for safety, sustainability, and security reasons. Millions of pigs are needed each year to meet our needs, and most manufacturing is done outside the USA. Furthermore, ten years ago, contaminants from the pig preparations led to dozens of deaths. Thus, there is a need to develop sustainable recombinant production. The work in *PNAS* provides new insights on exactly how cells control synthesis of heparin.

Heparin regulation

Heparin is a special subtype of a more general class of carbohydrates, called heparan sulfates, that are produced by a wide range of cells, both in the human body, as well as in cell culture. Yet, heparin is exclusively produced in a special type of blood cells called mast cells. To this day, heparin cannot be successfully produced in cell culture.

Researchers at UC San Diego reasoned that heparin synthesis must be under the control of certain gene regulators (called transcription factors), whose tissue-specific occurrence might give mast cells the unique ability to produce heparin.

Since regulators for heparin were not known, a research team led by UC San Diego professors Jeffrey Esko and Nathan Lewis used bioinformatic software to scan the genes encoding enzymes involved in heparin production and specifically look for sequence elements that could

represent binding sites for transcription factors. The existence of such a binding site could indicate that the respective gene is regulated by a corresponding gene regulator protein, i.e. a transcription factor.

"One DNA sequence that stood out the most is preferred by a transcription factor called ZNF263 (zinc-finger protein 263)," explains UC San Diego professor Nathan E. Lewis, who holds appointments in the UC San Diego School of Medicine's Department of Pediatrics and in the UC San Diego Jacobs School of Engineering's Department of Bioengineering.

"While some research has been done on this gene regulator, this is the first major regulator involved in heparin synthesis," said Lewis. He is also Co-Director of the CHO Systems Biology Center at the UC San Diego Jacobs School of Engineering.

Using the gene-editing technology, CRISPR/Cas9, the UC San Diego researchers mutated ZNF263 in a human cell line that normally does not produce heparin. They found that the heparan sulfate that this cell line would normally produce was now chemically altered and showed a reactivity that was closer to heparin.

Experiments further showed that ZNF263 represses key genes involved in heparin production. Interestingly, analysis of gene expression data from human white blood cells showed suppression of ZNF263 in mast cells (which produce heparin in vivo) and basophils, which are related to mast cells. The researchers report that ZNF263 appears to be an active repressor of heparin biosynthesis throughout most cell types, and mast cells are enabled to produce heparin because ZNF263 is suppressed in these cells.

This finding could have important relevance in biotechnology. Cell lines used in industry (such as CHO cells that normally are unable to produce

heparin) could be genetically modified to inactivate ZNF263 which could enable them to produce heparin, like mast cells do.

Philipp Spahn, a project scientist in Nathan Lewis' lab in the Departments of Pediatrics and Bioengineering at UC San Diego, described further directions the team is pursuing: "Our bioinformatic analysis revealed several additional potential gene regulators which can also contribute to heparin production and are now exciting objects of further study."

More information: ZNF263 is a transcriptional regulator of heparin and heparan sulfate biosynthesis, *Proceedings of the National Academy of Sciences* (2020). DOI: 10.1073/pnas.1920880117

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