Probing Beneath the Surface: Discovering O-GlcNAc on Intracellular Proteins

The Subcellular Distribution of Terminal N-Acetylglucosamine Moieties. Localization of a Novel Protein-Saccharide Linkage, O-Linked GlcNAc

In the early 1980s, when first-year graduate student Carmen-Rosa Torres brought Gerald Hart the first evidence of O-linked N-acetylglucosamine, or O-GlcNAc, he was sure the data were wrong. Only after making Torres repeat the experiments for a year and jumping into the lab himself was Hart finally convinced. Torres and Hart’s discovery of O-GlcNAc was published in 1984 in the Journal of Biological Chemistry (JBC).

O-GlcNAc is an oligosaccharide bound to a hydroxyl moiety on a serine or threonine residue of a protein. It is formed when the enzyme O-GlcNAc transferase (OGT) transfers a GlcNAc moiety from UDP-GlcNAc to a specific amino acid residue, a process known as O-GlcNAcylation. In the 1980s, scientists were confident that all glycosylated proteins were either transported outside of the cell or confined to bound, intracellular compartments after processing. The 1986 JBC paper that came out of Hart’s laboratory at Johns Hopkins University School of Medicine laid out the first proof of O-GlcNAc in the cytosol and nucleus. The discovery described in the paper, now recognized as a JBC Classic, violated the reigning dogma of its time. Even within the glycobiology community, the idea that glycoproteins would be functioning inside the cell was hard to accept. “Frankly, it was controversial enough that when we presented the findings at conferences, I had people basically saying to my face that they didn’t believe the results,” says Gordon Holt, who was a graduate student in Hart’s lab and a coauthor on the 1986 paper. (Torres was working on another project at that time.)

O-GlcNAc is found on many different proteins throughout the cell. Its function varies based on to which protein it is attached. Much like phosphorylation, one known purpose of O-GlcNAc is to control signaling in response to nutrients. Holt, who is now the chief science officer at NorthShore Bio, describes O-GlcNAc as an orthogonal method of regulating the same proteins: “It’s like a rivet—if there’s a carbohydrate at a phosphorylation site, then that phosphorylation site is no longer regulated by classical phosphorylation pathways. Instead, it becomes regulated by the O-GlcNAc regulatory pathways.”

Methods for studying phosphorylation have advanced rapidly in recent years, thanks in part to the existence of site-specific antibodies, whereas methods for studying O-GlcNAc continue to lag behind. Researchers have only recently begun to recognize the importance of O-GlcNAc to chronic diseases. Prolonged elevation of O-GlcNAc, as in hyperglycemia, can cause transcriptional processes to go haywire. “It’s something that we have to understand mechanistically very carefully, because it is essential for understanding lots of the intracellular signaling pathways, and the way the cell can function when glucose goes up or down,” says Vincent Hascall at the Cleveland Clinic.
Hart says a lot of deleterious effects of diabetes are caused by elevated O-GlcNAc, adding, “O-GlcNAc has also been elevated in every cancer type that’s been examined to date.” Low brain O-GlcNAcylation has also been linked to Alzheimer disease. Several drug companies now are developing treatments that inhibit O-GlcNAc hydrolase (OGA), the enzyme governing O-GlcNAc removal.

In their 1986 experiment, Hart and Holt used highly purified fractions of cells to pinpoint where the bulk of O-GlcNAc modification was located. Hart remembers sitting Holt down to break the news that preparation for the experiment would take him close to a year. Holt listened and nodded, then grabbed an ice bucket and went around to different labs at Johns Hopkins, collecting all the organelle samples needed—what Holt calls his “cup of sugar in the form of organelles”—from some of the leading experts in organelle purification. The preparation was completed in just a few days. “It was pretty amazing,” says Hart. “He had a graduate student network. They know what’s going on in the place way better than the faculty do.”

Faculty and students teased Holt for taking a shortcut. “It occurred to me in retrospect that the ice-bucket strategy was the path of least resistance, but it was also critically important, because it got us unquestionably pure organelles,” says Holt. “Our results needed to be taken seriously as a result.” After obtaining the samples, they attached radioactive sugar residues to galactosyltransferase, an enzyme purified from cow’s milk. They used radioactive tags to identify to which proteins the enzyme attached in each organelle. “The prevailing opinion was that intracellular cytosolic proteins were not glycoproteins,” says Hascall. “They showed in this paper, which opened up a whole new field, that that was not true—there is, in fact, a very specific glycosylation on a number of intracellular proteins.”

“It’s not very often in your career that you run across something completely novel and totally unexpected,” says Hart. “When you do, you should probably follow your data.”

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Vince Hascall at the Cleveland Clinic nominated the paper as a Classic. Alexandra Taylor (alexandraataylor@gmail.com) is a master’s candidate in science and medical writing at Johns Hopkins University.