RESEARCH HIGHLIGHT

A sticky solution to protein-selective sugar installation

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O-GlcNAc is a widespread form of intracellular glycosylation that is added to thousands of substrates by one enzyme, O-GlcNAc transferase (OGT), and removed by one other enzyme, O-GlcNAcase (OGA); the generality of this biochemistry has made it extremely challenging to alter the levels of O-GlcNAc on selected proteins to investigate the consequences of specific modifications. In a recent *Cell* paper, Zhu et al. develop an RNA aptamer-based strategy to localize endogenous OGT to endogenous proteins of interest, resulting in increased O-GlcNAc modification.

O-GlcNAc is the addition of an *N*-acetylglucosamine sugar to serine/threonine residues and typically refers to this type of glycosylation in multicellular organisms (Fig. 1a).[1] In metazoans, O-GlcNAc is added to proteins throughout the cytosol, nucleus, and mitochondria by the enzyme O-GlcNAc transferase (OGT). Another enzyme, O-GlcNAcase (OGA), can remove the modification, rendering O-GlcNAc similar to other dynamic posttranslational modifications (PTMs) like phosphorylation. However, phosphorylation of specific proteins can be achieved through the substrate selectivities of hundreds of kinases and phosphatases. This has allowed researchers to investigate the consequences of certain phosphorylation events through the application of selective small-molecule kinase and phosphatase inhibitors. OGT and OGA inhibitors have also been developed, but their application to living systems results in the global down- or up-regulation of the entire repertoire of O-GlcNAc modifications added and removed by these sole enzymes. Additionally, O-GlcNAc cannot be easily mimicked by amino acid substitutions like aspartate/glutamate can for serine/threonine phosphorylation, and genetic codon expansion cannot to date install O-GlcNAc. Given these limitations, most studies of specific O-GlcNAc sites in cells or organisms have relied on mutating the serine and/or threonine sites to alanine, thus preventing the modification. Unfortunately, these mutations can have their own effects apart from O-GlcNAc and can block other PTMs; therefore, this type of experiment should be combined with inhibitors or other controls to confirm the importance of O-GlcNAc in the results. This limitation is one of the reasons why thousands of O-GlcNAc-modified proteins have been identified but the biochemical effects of the vast majority of these modifications are completely unknown.

Several groups have made progress in bridging this gap using a range of chemical and biological approaches.[2] For example, my own lab uses synthetic protein chemistry to prepare O-GlcNAc-modified proteins in a site-specific fashion for subsequent biological studies.[3] While this yields chemically-defined proteins with site-specific and bona-fide O-GlcNAc, it requires specialized skills and is limited in its applications. Recently, van Aalten and colleagues took advantage of the fact that OGT will modify some cysteine residues with GlcNAc and that this "S-GlcNAc" is stable against removal by OGA. Specifically, they mutated an O-GlcNAc-modified serine in OGA to cysteine and showed that it increased the modification stoichiometry to ~70%.[4] However, the broad generalizability of this approach still needs to be explored. Finally, the Woo lab has designed a series of OGT or OGA constructs that can be targeted to proteins of interest with nanobodies to increase or decrease O-GlcNAc stoichiometry, respectively.[5,6] This technique is selective at the protein level, but it requires expression of engineered proteins and/or the generation of novel nanobodies to target endogenous proteins.

Zhu et al. describes a novel approach to this problem by taking advantage of RNA aptamers (Fig. 1b).[7] First, the authors used Systematic Evolution of Ligand by Exponential Enrichment (SELEX) that enables the selection of nucleotide binders (DNA/RNA oligonucleotides) of targets of interest including proteins. Using SELEX, an RNA aptamer that tightly binds OGT without inhibiting its activity was discovered. This was then linked to a known aptamer that binds to GFP. After some optimization, transfection of cells with this aptamer relocalized endogenous OGT to GFP-tagged proteins and resulted in notable increases of O-GlcNAc modification on these proteins. This proof-of-principle experiment demonstrated nicely that increasing the "stickiness" between OGT and its substrates can yield a selective increase in modification of that protein, allowing the effects of those O-GlcNAc modifications to be potentially studied in isolation. However, this aptamer still required the engineering of target proteins to be GFP tagged. To overcome this limitation, the authors focused on the key O-GlcNAc-modified protein β-catenin. β-Catenin has multiple roles in cells, including transcriptional regulation upon Wnt signaling, a critical pathway in development and many cancers. Previous work had demonstrated that β-catenin can be O-GlcNAc modified in a way that inhibits phosphorylation and thus blocks protein degradation. Accordingly, the authors used SELEX to find yet another aptamer that can bind to endogenous β-catenin. Upon linking this to the OGT aptamer, the authors created a reagent that was able to increase the O-GlcNAc modification of *endogenous* β-catenin by *endogenous* OGT. This is a significant achievement, as it requires no protein engineering and only the simple transfection of cells with DNA or RNA. Using this aptamer, the authors found that O-GlcNAc increases the interaction of β-catenin with another transcriptional regulator EZH2 upon induction of Wnt signaling, altering the transcriptional output.

These results are exciting because of the potentially widespread impact of these data. If the method turns out to be generalizable, interested researchers could simply fuse an aptamer sequence for

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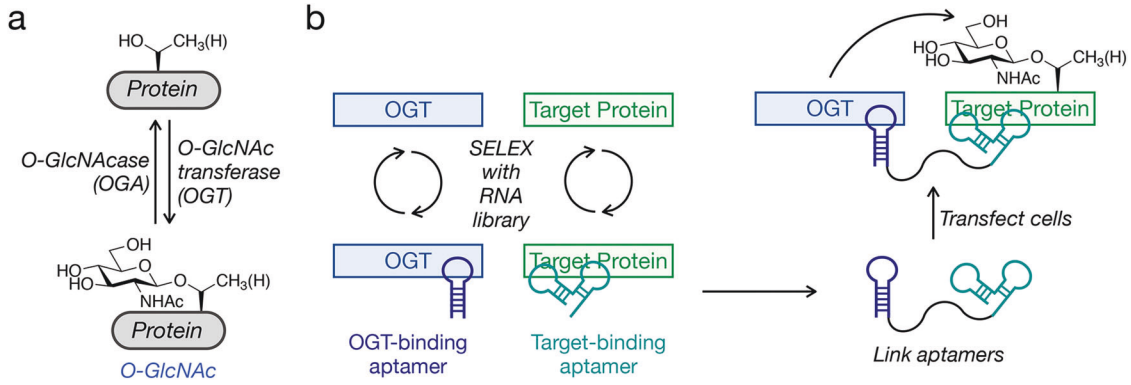

Fig. 1 O-GlcNAc and aptamer targeting. a O-GlcNAc is an intracellular posttranslational modification controlled by two enzymes, OGT and OGA. **b** The aptamer approach taken in Zhu et al. SELEX can be used to create RNA aptamers that bind OGT or a protein target of interest. The two aptamers can then be linked and transfected into cells to recruit OGT and induce selective O-GlcNAc modification.

their protein of interest to the OGT aptamer to create a tool that can be readily produced and applied by anyone with standard molecular and cellular biology skills. In fact, aptamer development services are commercially available, potentially circumventing this rate-limiting step. Like any new technology, more optimization and characterization is, of course, needed. Beyond the question of generality, the current aptamers only increase O-GlcNAc by ~4–5 fold, which may not be enough to uncover biological roles. Additionally, as the authors point out, the sites of O-GlcNAc modification induced by the aptamer might not necessarily be the same as those installed by the native OGT/substrate interaction. Despite these unknowns, I believe that the field should be very excited about the potential of this new resource.

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ADDITIONAL INFORMATION

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