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SPOTLIGHT

# What sugar does to your pores

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**FG-repeat nucleoporins at the center of the nuclear pore complex (NPC) are highly modified with *O*-GlcNAc. In this issue, Yoo and Mitchison (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202010141) use optogenetic probes to show that *O*-GlcNAc enhances permeability of the NPC, accelerating transport in both directions.**

*O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) is an abundant post-translational modification found on thousands of nuclear, cytoplasmic, and mitochondrial proteins in multicellular organisms (1). This single sugar modification of serine or threonine residues is reversible: it is added by *O*-GlcNAc transferase (OGT) and removed by *O*-GlcNAcase (OGA). Intracellular *O*-GlcNAc levels respond acutely to nutritional cues and often increase in response to stressful stimuli. *O*-GlcNAc was discovered nearly 40 yr ago, and nucleoporins (NUPs) were among the first proteins reported to be *O*-GlcNAcylated (2). Glycoproteomics analysis has since identified *O*-GlcNAc sites on the majority of NUPs, with some carrying 10 or more *O*-GlcNAc sites (3).

Although NUPs are among the most highly *O*-GlcNAcylated proteins, the function of *O*-GlcNAc in the nuclear pore complex (NPC) has remained enigmatic. Much of the *O*-GlcNAc in the NPC is found on FG NUPs, proteins named for their extensive, intrinsically disordered phenylalanine-glycine (FG) repeat regions. The *O*-GlcNAcylated FG domains project into the central channel of the NPC, forming a selective permeability barrier that regulates traffic in and out of the nucleus (4). Small proteins transit the pore through passive diffusion while larger proteins (cargo) are escorted through by karyopherins. Facilitated transport through the NPC depends on direct, yet transient, interactions between karyopherins and FG domains of NUPs.

Testing the function of *O*-GlcNAc in the NPC has proved challenging. The large number of *O*-GlcNAc sites in the NPC make analysis by mutagenesis impractical. Simple knockout of OGT is unworkable because it is required for mammalian cell proliferation. In vitro studies conducted by Görlich and co-workers examined the role of *O*-GlcNAc using a model system in which purified FG regions of NUPs assemble into elastic hydrogels that recapitulate transport properties of intact NPCs (5). They showed that *O*-GlcNAc modification of NUP98 altered both the physical properties and the selectivity of the resulting hydrogel. Additional clues have come from cellular assays where *O*-GlcNAc was shown to regulate the cellular stability of NUPs, with reductions in *O*-GlcNAc levels leading to increased NUP degradation and compromising the integrity of the selectivity filter (6, 7).

What has been missing is a method to accurately measure the rate of protein transport through the NPC in intact cells. In this issue, Yoo and Mitchison solve this challenge by taking advantage of two optogenetic probe molecules originally designed for a different purpose (8). Each probe molecule contains a nuclear export signal (NES), a nuclear localization sequence (NLS), and the light-oxygen-voltage-sensing 2 (LOV2) domain (9). The import probe is designed such that the NLS packs against the LOV domain in the dark state and remains concealed from nuclear import machinery. Under these conditions, the NES dominates, and the probe is localized primarily to the cytoplasm. Upon application of 447 nm light, the LOV2 domain undergoes a conformation change that reveals the NLS, causing the probe to be translocated to the nucleus. The export probe has the opposite design, localizing to the nucleus in the dark state and translocated to the cytoplasm upon illumination. Both probe molecules also include the mCherry fluorescent protein, allowing their locations to be monitored in real time by live-cell imaging. Yoo and Mitchison recognized that these probes could be repurposed to measure nuclear import and export rates, and to determine how these rates change under different conditions.

The researchers first validated that the probes could indeed be used to measure nuclear transport rates in live cells. Next, they perturbed global *O*-GlcNAc levels using siRNA or small molecule inhibitors to modulate OGT or OGA activity and measured the impact on nuclear import and export rates. Reduced *O*-GlcNAc levels decreased both import and export rates, while elevated *O*-GlcNAc levels increased both rates (Fig. 1). Remarkably, transport rates were linearly correlated with *O*-GlcNAc levels over the entire range of *O*-GlcNAc levels the researchers could achieve. An important caveat is that the *O*-GlcNAc perturbations applied were global and affect *O*-GlcNAcylation of thousands of proteins, making it impossible to ascribe the observed changes in transport rate solely to

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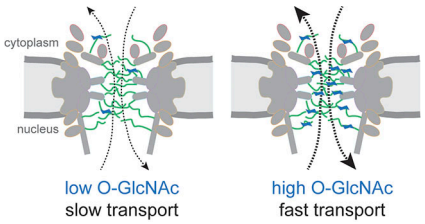


Figure 1. ***O*-GlcNAc increases transport rates through the nuclear pore.** Intrinsically disordered FG NUPs (green) form a selectivity filter in the central channel of NPCs (gray). Increased *O*-GlcNAc (blue) modification of FG NUPs results in accelerated transport in and out of the nucleus.

changes in NPC glycosylation. To address this, the researchers created heterokaryons in which cells with low *O*-GlcNAc levels were fused with cells with high *O*-GlcNAc levels. The resulting multinucleated cells contain some nuclei with low *O*-GlcNAc levels and others with high *O*-GlcNAc levels. Analysis of these cells revealed that the nuclear import rate was determined by the *O*-GlcNAc level of the nucleus with only a minor contribution from the cytoplasm. Additional experiments excluded the possibility that the Ran transport pathway was modulated by *O*-GlcNAc levels. Yoo and Mitchison concluded that *O*-GlcNAcylation of the NPC is what drives the observed alterations in transport rates (8).

How might *O*-GlcNAcylation of the NPC affect transport rates? Multiple models have been proposed to explain exactly how karyopherin–cargo complexes transit the selectivity filter at the center of the NPC (10). The molecular details of the interactions that occur and the physical properties of the FG domains remain active areas of investigation. The finding that *O*-GlcNAc accelerates facilitated transport can be incorporated into any of the disparate models for translocation across the NPC. As a bulky and hydrophilic modification, *O*-GlcNAc has the potential to change the chemical and physical properties of FG regions, potentially sterically interfering with FG domain interactions or modulating FG region dynamics. Notably, Yoo and Mitchison found that increased *O*-GlcNAc levels also led to an increased rate of passive diffusion through the NPC, suggesting that at least some of *O*-GlcNAc's effects must be on properties of the NPC that are independent of interactions with karyopherins.

*O*-GlcNAc levels change rapidly in response to nutrition cues and stressful stimuli, but cells also employ mechanisms to rapidly regulate OGT and OGA levels to restore *O*-GlcNAc homeostasis (11, 12). Nonetheless, altered *O*-GlcNAc levels are observed in a number of chronic diseases including cancer, diabetes, and neurodegenerative diseases (13). The *O*-GlcNAc–dependent modulation of nuclear transport rates suggests an unexplored mechanism by which altered *O*-GlcNAc levels might contribute to these disease states. The optogenetic method to measure nuclear import and export rates will be a powerful tool to further investigate exactly how nuclear transport is altered under conditions of dysregulated *O*-GlcNAcylation.

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