For Wnt Signaling, Fucosylation of LRP6 Is a Bitter Pill

Adnan Shami Shah,[1] Hongyan Sun,[1] and Jeremy M. Baskin[1,*]
[1]Department of Chemistry and Chemical Biology and Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY 14853, USA
*Correspondence: jeremy.baskin@cornell.edu
https://doi.org/10.1016/j.chembiol.2020.08.003

In this issue of *Cell Chemical Biology*, Hong et al. (2020) use *in situ* chemoenzymatic labeling to discover that fucosylation of the Wnt co-receptor LRP6 induces its endocytosis and downregulates Wnt/β-catenin signaling. Their findings reveal a glycosylation-based mechanism for regulating Wnt signaling that could be targeted in cancer.

The promise of molecular medicine relies in large part on our ability to understand and exploit the intricate details of key cellular and physiological signaling pathways that go awry in disease. Among many evolutionarily conserved pathways, Wnt signaling stands out as a major regulator of important cell-fate decisions critical to organogenesis during development, including proliferation, polarization, and migration, as well as tissue homeostasis in adults (MacDonald et al., 2009). Dysregulation of this pathway that pushes its levels outside of the physiological, homeostatic range leads to a variety of devastating diseases, including several cancers (Nusse and Clevers, 2017).

The canonical, β-catenin-dependent Wnt signaling pathway initiates at the plasma membrane, where secreted Wnt proteins engage a Frizzled (FZD) receptor along with a key co-receptor, low-density lipoprotein receptor-related protein 5 or 6 (LRP5/6), to form a Wnt–FZD–LRP5/6 complex on the surface of the Wnt-receiving cell. This binding event induces recruitment of the cytosolic protein Dishevelled (DVL) to the complex on the cytosolic face of plasma membrane, which initiates a cascade that blocks and disassembles a β-catenin destruction complex, resulting in the stabilization and nuclear translocation of β-catenin. Once in nucleus, β-catenin forms an active complex with TCF/LEF transcription factors, leading to expression of several Wnt target genes.

Though the core components of canonical Wnt signaling have been long established, elucidation of mechanisms controlling its modulation in specific environments represents a vital and active area of research. Not only is this work critical to understand context-dependent regulation of physiological Wnt signaling in development and tissue homeostasis, but also successful therapeutic modulation of Wnt signaling is likely to take a targeted rather than all-or-none approach, given the physiological importance of Wnt signaling. In this issue of *Cell Chemical Biology*, Hong et al. (2020) use a chemoenzymatic approach to reveal a mechanism for regulating Wnt signaling that occurs via glycosylation of the co-receptor LRP6.

Glycosylation is among the most structurally and functionally complex of the myriad protein posttranslational modifications. Addition of specific glycans to protein backbones is a way to tune protein stability, interactions, activities, and localization. Several core Wnt signaling proteins, including the Wnts themselves, Frizzled receptors, and LRP5/6, are glycoproteins. Because glycosylation is not directly genetically encoded, classic reverse genetic tools have been supplemented by many powerful chemical methods that have proven instrumental to uncovering functions of glycans *in vivo* (Wang and Davis, 2013).

In situ glycan engineering is one such approach that enables the precise, enzyme-mediated generation of specific glycan structures on cell-surface glycoproteins. The Wu lab at Scripps has pioneered a technique termed *in situ* fucosylation (ISF) that allows precise engineering of fucose monosaccharides onto glycoproteins of interest (Zheng et al., 2011). Fucose is a compelling sugar to study, as the simple presence or absence of this one (often terminal) monosaccharide can completely change the function of the glycoprotein to which it is attached (Murrey et al., 2006; Schneider et al., 2017).

ISF is a chemoenzymatic method involving delivery to the extracellular milieu of both a recombinant, soluble glycosyltransferase and its nucleotide sugar donor, resulting in glycosylation of native cell-surface glycoproteins bearing the appropriate acceptor groups (Figure 1). In ISF, the glycosyltransferase is a fucosyltransferase (FucT) from *H. pylori*, which permits attachment of an

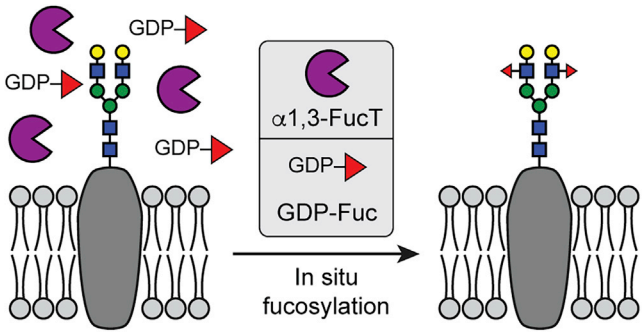

Figure 1. Schematic Representation of *In Situ* Fucosylation (ISF)
Treatment of intact cells with *H. pylori* α1,3-fucosyltransferase (FucT) and GDP-fucose (GDP-Fuc) results in α1,3-linked fucosylation of N-acetyllactosamine (LacNAc) residues within cell-surface glycans.

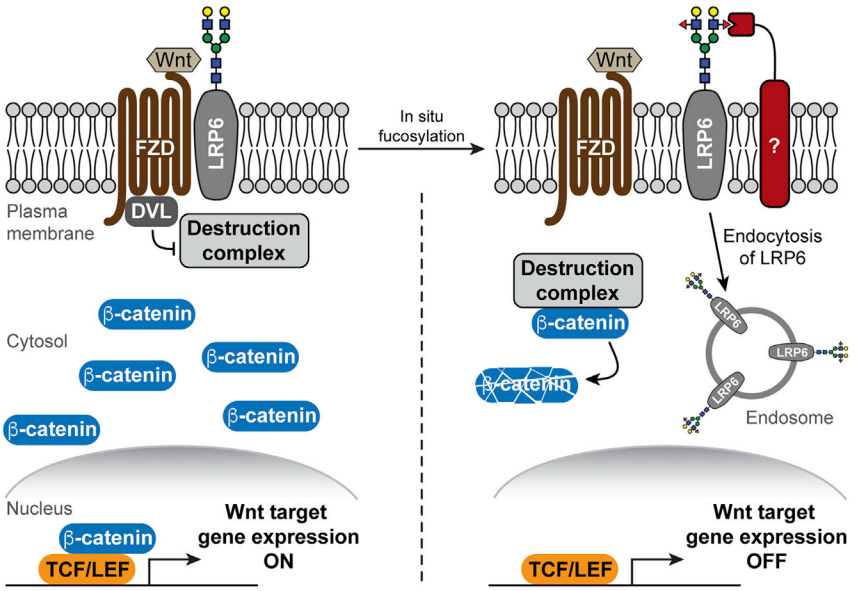

Figure 2. ISF Reveals an Inhibitory Role for Fucosylation of LRP6 on Wnt Signaling

(A) In the absence of ISF, Wnt stimulation leads to productive formation of a Wnt–FZD–LRP6 complex at the cell surface of Wnt-receiving cells, enabling Dishevelled (DVL) recruitment to this complex at the plasma membrane, preventing β-catenin from degradation by the destruction complex, and enabling β-catenin-dependent transcription of Wnt target genes by TCF/LEF transcription factors.

(B) In the presence of ISF, α1,3-fucosylation of LRP6 results in its endocytosis, relocalizing it to endosomal compartments. Under these conditions, the Wnt–FZD–LRP6 complex fails to form at the cell surface, and Wnt stimulation does not lead to β-catenin stabilization and transcription of Wnt target genes. Shown in maroon is an unknown fucose-binding endocytic adaptor that is proposed to be involved in the internalization of fucosylated LRP6. Hence, ISF reveals that fucosylation of LRP6 inhibits Wnt signaling.

α1,3-linked fucose group from the universal fucose donor GDP-fucose (GDP-Fuc) to N-acetyllactosamine (LacNAc) disaccharide groups present on a variety of glycans (Zheng et al., 2011). Because of the relaxed preferences of *H. pylori* FucT with respect to its donor substrate, ISF allows incorporation of unnatural, bioorthogonally tagged fucose analogs from the corresponding GDP-Fuc analogs, enabling subsequent visualization and enrichment of tagged glycoproteins.

Peng Wu and co-workers previously established that modulation of fucose levels alters Wnt signaling by using the overexpression of a GDP-Fuc transporter, SLC35C1 (Feng et al., 2014). Yet the precise mechanism underlying this phenomenon remained mysterious. Here, Hong et al. (2020) use ISF as the centerpiece of a study in which they establish that fucosylation of LRP6 is a means for cells to downregulate Wnt signaling (Hong et al., 2020). They find that α1,3-linked fucosylation of LRP6 causes its internalization by endocytosis, thus reducing the cell-surface LRP6 pool available to respond to extracellular Wnt ligands.

To reveal and support this mechanism, they first established a clever way to perform ISF *in vivo* on zebrafish embryos by microinjection of the FucT and GDP-Fuc into the chorion but outside the embryo and demonstrated the phenomenon of fucosylation as inhibitory to Wnt signaling *in vivo*. Subsequent experiments using a variety of mutant CHO cell lines show that the effects of ISF on Wnt signaling correlate with the availability of the acceptor glycan, N-linked LacNAc, on the cell surface. The authors then showed that fucosylation increases the endocytosis of LRP6, which prevented it from recognizing and propagating the Wnt signal (Figure 2). Remarkably, the addition of free fucose prevented both the internalization of fucosylated LRP6 and the inhibitory effect of ISF on Wnt signaling, suggesting that an endocytic adaptor for fucosylated LRP6 may exist. Identification of such a potential adaptor is an exciting future direction.

Beyond the important fundamental advance that establishes LRP6 fucosylation as a new layer of regulation to Wnt/β-catenin signaling, this study also has therapeutic implications. Aberrant Wnt signaling occurs in many types of cancers, and as a result, many efforts have been made to target this pathway therapeutically (Nusse and Clevers, 2017). Yet, while many pharmacological agents targeting various components of Wnt signaling are in clinical trials, the pathway has proven challenging to selectively target in cancer because of its importance in tissue homeostasis, motivating the search for Wnt-related therapeutic targets that are modulators rather than core components of the pathway.

Changes in cell-surface glycosylation are a hallmark of cancer. Recent efforts to chemoenzymatically remodel the glycocalyx, notably by using sialidases to remove sialic acid residues, have proven highly powerful and exhibit great translational potential (Xiao et al., 2016). The current study suggests a complementary approach, that glycan editing using a glycosyltransferase and its nucleotide sugar donor, rather than a glycosidase, may be therapeutically beneficial to downregulate pathogenic Wnt signaling (Jiang et al., 2018). Significant hurdles remain, including selective and concurrent delivery of both the macromolecular enzyme and its small-molecule substrate to the tissue of interest, as well as methods to ensure minimal effects on non-diseased tissues. In principle, such a strategy could involve local microinjection or selective delivery based on targeting groups, taking advantage of recent advances in liposomal carriers and antibody-drug conjugates.

Collectively, this study establishes fucosylation of the Wnt co-receptor LRP6 as a new layer of regulation of the canonical Wnt/β-catenin signaling pathway. This work is a powerful illustration of how precision chemical glycobiology tools such as *in situ* chemoenzymatic labeling can lay the foundation for a mechanistic study that reveals and fleshes out the details of new roles for glycans in the regulation of cell signaling. It is also a vivid reminder that, despite the (justified) inclusion of the core Wnt/β-catenin pathway in textbooks and undergraduate biology curricula, our understanding of the mechanisms that tune, or regulate, Wnt signaling pathways remains incomplete. A desire to reveal and elucidate these mechanisms will inspire advances in fundamental cell biology and physiology

for years to come and motivate the development of new therapeutic strategies to treat pathological Wnt signaling in cancer.

ACKNOWLEDGMENTS

Work in J.M.B.'s lab is supported by the NIH (R01GM131101), the NSF (CAREER CHE-1749919), the Arnold and Mabel Beckman Foundation (Beckman Young Investigator), and the Alfred P. Sloan Foundation (Sloan Research Fellowship).

REFERENCES

Feng, L., Jiang, H., Wu, P., and Marlow, F.L. (2014). Negative feedback regulation of Wnt signaling via N-linked fucosylation in zebrafish. Dev. Biol. *395*, 268–286.

Hong, S., Feng, L., Yang, Y., Jiang, H., Hou, X., Guo, P., Marlow, F.L., Stanley, P., and Wu, P. (2020). In situ fucosylation of the Wnt co-receptor LRP6 increases its endocytosis and reduces Wnt/β-catenin signaling. Cell Chem. Biol., this issue, 1140–1150.

Jiang, H., López-Aguilar, A., Meng, L., Gao, Z., Liu, Y., Tian, X., Yu, G., Ovryn, B., Moremen, K.W., and Wu, P. (2018). Modulating Cell-Surface Receptor Signaling and Ion Channel Functions by In Situ Glycan Editing. Angew. Chem. Int. Ed. Engl. *57*, 967–971.

MacDonald, B.T., Tamai, K., and He, X. (2009). Wnt/β-catenin signaling: components, mechanisms, and diseases. Dev. Cell *17*, 9–26.

Murrey, H.E., Gama, C.I., Kalovidouris, S.A., Luo, W.I., Driggers, E.M., Porton, B., and Hsieh-Wilson, L.C. (2006). Protein fucosylation regulates synapsin Ia/Ib expression and neuronal morphology in primary hippocampal neurons. Proc. Natl. Acad. Sci. USA *103*, 21–26.

Nusse, R., and Clevers, H. (2017). Wnt/β-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. Cell *169*, 985–999.

Schneider, M., Al-Shareffi, E., and Haltiwanger, R.S. (2017). Biological functions of fucose in mammals. Glycobiology *27*, 601–618.

Wang, L.-X., and Davis, B.G. (2013). Realizing the Promise of Chemical Glycobiology. Chem. Sci. (Camb.) *4*, 3381–3394.

Xiao, H., Woods, E.C., Vukojicic, P., and Bertozzi, C.R. (2016). Precision glycocalyx editing as a strategy for cancer immunotherapy. Proc. Natl. Acad. Sci. USA *113*, 10304–10309.

Zheng, T., Jiang, H., Gros, M., del Amo, D.S., Sundaram, S., Lauvau, G., Marlow, F., Liu, Y., Stanley, P., and Wu, P. (2011). Tracking N-acetyllactosamine on cell-surface glycans in vivo. Angew. Chem. Int. Ed. Engl. *50*, 4113–4118.