10 *ABSTRACT*

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Regulating Signal Transduction with Glycosylation

profile

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Dr. Robert S. Haltiwanger did his doctoral work in the laboratory of Dr. Robert L. Hill at Duke University. In Dr. Hill's laboratory, Dr. Haltiwanger studied the specificity of several mammalian lectins and performed the initial purification and characterization of the macrophage mannose receptor. Dr. Haltiwanger then moved to the laboratory of Dr. Gerald W. Hart at Johns Hopkins for postdoctoral work. In Dr. Hart's laboratory, he identified, purified and characterized the enzyme responsible for addition of *O*-GlcNAc to proteins, the *O*-GlcNAc transferase. He also worked on identification of a number of proteins bearing the *O*-GlcNAc modification and mapping the sites of *O*-GlcNAc modification on those proteins. Dr. Haltiwanger began his independent career in 1992 when he accepted an appointment as an assistant professor in the Department of Biochemistry and Cell Biology at the State University of New York at Stony Brook, where he was promoted to associate professor in 1998. Since at Stony Brook, Dr. Haltiwanger has continued to work on unusual *O*-linked carbohydrate modifications, including *O*-fucose and *O*-glucose. His work in this area has led him into the role of these modifications in the regulation of signal transduction events.

Key words Signal transduction, Notch, Fringe, Cripto, Nodal, EGF repeat, *O*-fucose

The complexity of carbohydrate structures on cell surfaces led researchers over 30 years ago to propose a role for complex carbohydrates in the transfer of information across the plasma membrane. Although the structural complexity of carbohydrates has posed a significant barrier to determining their biological functions, recent work in a number of laboratories has revealed examples of signal transduction events regulated by alterations in protein glycosylation. For example, carbohydrate modifications of epidermal growth factor-like (EGF) repeats are known to modulate several signaling pathways, including those involving uPA, Notch and Nodal/Cripto. EGF repeats are small (approximately 40 amino acids) protein modules containing six conserved cysteines forming three conserved disulfide bonds. Two forms of *O*-linked carbohydrate modifications, *O*-fucose and *O*-glucose, occur at putative consensus sites within EGF repeats. *O*-glucose modifications occur between the first and second conserved cysteines at the sequence $C^1XS/TXPC^2$, and *O*-fucose modifications between the second and third cysteine at the sequence C^2XXGGS/TC^3 (1). Recent studies have demonstrated that although the C^2XXGGS/TC^3 can be used to accurately predict whether proteins will be modified with *O*-fucose, fucose is found on more sites than predicted using this sequence (2). A new broader *O*-fucose consensus sequence has been proposed: $C^2X_{3-5}S/TC^3$.

The first demonstration that *O*-fucose modifications play a role in signal transduction came from studies on the interaction of urinary-type plasminogen activator (uPA) with its receptor (3). The uPA receptor is a GPI-anchored, cell-surface receptor capable of activating several kinase cascades and inducing cell division. The EGF repeat from uPA is necessary and sufficient for activation of the uPA receptor, and removal of *O*-fucose from the uPA EGF repeat abrogates its ability to activate. Interestingly, the deglycosylated EGF repeat binds to the uPA receptor with the same affinity as the glycosylated EGF repeat, suggesting that the *O*-fucose plays a role in activation of the receptor.

More recently, *O*-fucose modifications have been demonstrated to play a key role in regulation of Notch signal transduction (Fig. 1A). Notch is a cell surface receptor that plays key roles in numerous stages of development, and several human diseases result from defects in Notch signaling. Notch becomes activated upon binding to its ligands (Delta, Serrate/Jagged families), which are also cell surface, transmembrane proteins. Thus, Notch is activated only when ligand is expressed on an adjacent cell. Both Notch and its ligands contain multiple EGF repeats, many of which bear *O*-fucose modifications (2, 4). The Fringe protein was identified as a modulator of Notch function, capable of increasing Notch activation from Delta, but inhibiting Notch activation from Serrate/Jagged (5). Fringe has recently been shown to be an *O*-fucose: β1,3-*N*-acetylglucosaminyltransferase, capable of modifying *O*-fucose on Notch (2, 6). The recent demonstration that Notch ligands are also substrates for Fringe (2) raises the interesting possibility that Fringe could function through altering *O*-fucose structures on Notch, its ligands, or both. Current studies are focusing on the mechanism through which changes in *O*-fucose structures alter Notch activation.

Very recently *O*-fucose modifications on the EGF-CFC protein, Cripto, have been demonstrated to play an essential role in Nodal signaling (Fig. 1B) (7, 8). Nodal is a member of the TGFβ superfamily, and defects in Nodal signaling cause severe phenotypes in establishment of polarity in vertebrate embryos. Nodal is believed to activate activin-like type I and type II cell surface receptors, and activation of the receptors is dependent on the presence of the EGF-CFC proteins (Cripto, Cryptic, FRL, OEP). Each EGF-CFC protein contains a truncated EGF repeat containing an *O*-fucose consensus site as well as a domain unique to these proteins called CFC. Both human and mouse Cripto are known to be modified with *O*-fucose (7, 8). Mutation of the modified serine to alanine eliminates fucosylation and abrogates Cripto-dependent Nodal signaling, demonstrating that the *O*-fucose is essential for proper Cripto function.

These examples demonstrate that the presence or absence of a simple *O*-fucose monosaccharide on an EGF repeat can regulate a signal transduction pathway. Elongation of the *O*-fucose by proteins such as Fringe offers an extra level of modulation. The gene for the enzyme responsible for addition of *O*-fucose to EGF repeats, the protein *O*-fucosyltransferase (*O*-FucT-1), has recently been identified (9). Studies are currently underway in several laboratories to determine whether this enzyme is developmentally regulated, and if so, how that regulation affects the signaling pathways modulated by *O*-fucose. In addition, work is just beginning on the *O*-glucose modification of EGF repeats. Many of the predicted *O*-glucose sites on proteins such as Notch are evolutionarily conserved, suggesting they will play a similarly important role in regulating signaling. These and other results suggest that we are just beginning to uncover the varied and important biological roles played by complex carbohydrates in regulating signal transduction pathways.

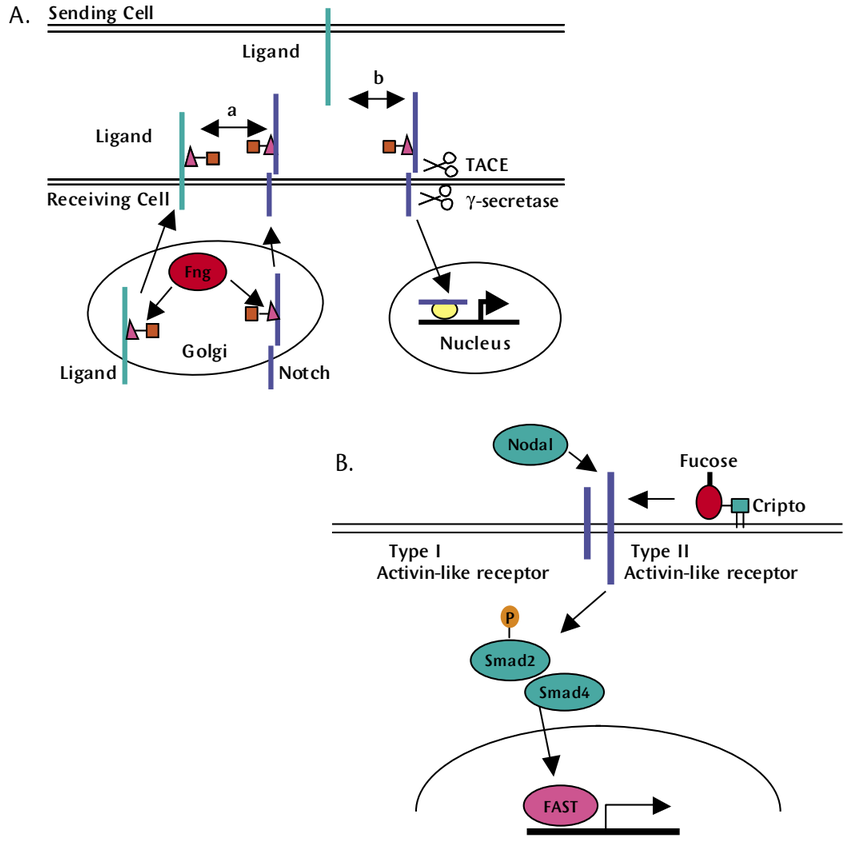

Figure 1. Notch and Nodal/Cripto signaling pathways are modulated by O-fucose modifications.
A. Ligand expressed in the Sending Cell binds to and activates Notch in the Receiving Cell. Activation of Notch is mediated by two sequential proteolytic events, catalyzed by TACE and γ-secretase, resulting in the release of the cytoplasmic domain of Notch from the membrane. The cytoplasmic domain then translocates to the nucleus where it binds to members of the CSL family of transcriptional regulators and activates several downstream gene products. Ligand expressed in the same cell as Notch can inhibit Notch activation (cell-autonomous inhibition). Fringe (Fng) modifies both Notch and ligand as they move through the Golgi apparatus by addition of GlcNAc (squares) to O-fucose residues (triangles). The GlcNAc is subsequently capped by galactose and sialic acid (not shown), although recent work suggests only the galactose is required for alteration of Notch function (10). The change in sugar structure modulates Notch activation. Two potential models for how the sugars modulate Notch activation are shown. (a) Modulation of cell autonomous inhibition by ligand. (b) Direct modulation of ligand binding.

B. Nodal activation of type I/type II activin-like receptors requires the presence of Cripto. Cripto is attached to the membrane through a GPI anchor, and the O-fucose on Cripto is essential for it to serve as a co-activator. Activation of the type I/type II activin-like receptors by the Nodal/Cripto complex results in the phosphorylation-induced accumulation of the Smad proteins in the nucleus where they interact with members of the FAST family of transcriptional activators and activate transcription of several downstream gene products.

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