Seminars in Cell & Developmental Biology 21 (2010) 638–645

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

Role of glycosylation of Notch in development

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ARTICLE INFO

Article history:
Available online 10 March 2010

Keywords:
Protein glycosylation
Notch
Development
Pofut1/Ofut1
Fringe
Rumi

ABSTRACT

The Notch pathway is one of the major signaling pathways required for proper development in metazoans. Notch activity is regulated at numerous levels, and increasing evidence reveals the importance of "protein glycosylation" (modification of Notch receptors with sugars) for its regulation. In this review we summarize the significance of the Notch pathway in development and the players responsible for its glycosylation, and then discuss the molecular mechanisms by which protein glycosylation may regulate Notch function.

Contents

1. Introduction

Notch signaling is essential for proper development in metazoans, and defects in this pathway result in a number of human diseases [1,2]. Notch is regulated at numerous overlapping levels, including endocytosis, ubiquitination, intracellular trafficking, degradation, and glycosylation [2–6]. Many genes impinge on this pathway, and the number of these genes continues to increase with the improved techniques for genome-wide analysis [7]. This review focuses on regulation of the Notch pathway by glycosylation.

1.1. Role of Notch in development and disease

The Notch phenotype was originally described in *Drosophila* nearly 100 years ago as an X-linked, dominant mutation which showed irregular "notches" at the tips of the wings [8]. Subsequent

Abbreviations: Cax, compact axial skeleton; CHO, Chinese hamster ovary; CMP, cytidine monophosphate; Dll, Delta-like ligand; DSL, Delta/Serrate/LAG-2; DOS, Delta and OSM-11-like proteins; ECD, extracellular domain; EGF, epidermal growth factor-like; ER, endoplasmic reticulum; Fuc, fucose; Gal, galactose; GDP, guanosine diphosphate; Glc, glucose; GlcA, glucuronic acid; GlcNAc, *N*-acetylglucosamine; GMD, GDP-mannose 4,6-dehydratase; NICD, Notch intracellular domain; NRR, negative regulatory region; Pofut1, Protein *O*-fucosyltransferase-1; Poglut, Protein *O*-glucosyltransferase; RBP-Jκ, recombination signal sequence-binding protein-Jκ; site 2, S2; site 3, S3; T-ALL, T cell acute lymphoblastic leukemia; T/ICD, transmembrane/intracellular domain; UDP, uridine diphosphate.

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doi:10.1016/j.semcdb.2010.03.003

work demonstrated that Notch plays key roles in development of many tissues in flies, including formation of neurons and glial cells, leg segments, eyes, heart, muscles, and blood lineages [2,9,10]. *Drosophila* has a single Notch receptor, while mammals have four [1]. Targeted disruption of the four mouse *Notch* genes demonstrated that these genes play important roles in development of many tissues. Loss of mouse *Notch1* results in an embryonic lethal phenotype with severe defects in somitogenesis [11,12]. Subsequent studies showed that Notch1 is also involved in neurogenesis and vasculogenesis [13,14]. Deletion of mouse *Notch2* also results in an embryonic lethal phenotype with apoptotic cell death in a wide variety of tissues, especially neural tissues, from embryonic day 9.5 [15]. *Notch3*$^{-/-}$ mice are viable and fertile, but have defects in arterial differentiation and maturation of vascular smooth muscle [16]. Although *Notch4*$^{-/-}$ mice are viable and fertile [14], loss of *Notch4* exacerbates the vascular remodeling defects observed in *Notch1*$^{-/-}$ embryo [14], suggesting partially overlapping function of *Notch1* and *4* during embryogenesis. Aberrant Notch signaling leads to multiple human disorders [1,17]. Mutations of *Notch* and the components of this pathway are implicated in human developmental disorders such as Alagille Syndrome and Spondylocostal Dysostosis, adult onset diseases such as CADASIL and Multiple Sclerosis, and cancers such as T cell acute lymphoblastic leukemia (T-ALL) and colon cancer.

1.2. Notch basics

Notch receptors are large type I transmembrane proteins [2]. Their basic molecular structure is evolutionarily conserved and consists of three domains: an extracellular domain (ECD) with 29–36 tandem epidermal growth factor-like (EGF) repeats and a unique negative regulatory region (NRR) which consists of three Lin-12/Notch repeats and a heterodimerization domain; a single transmembrane domain; and an intracellular domain with an RBP-Jκ (recombination signal sequence-binding protein-Jκ) association module domain, several nuclear localization sequences, seven ankyrin repeats, and a transactivation domain that harbors proline/glutamic acid/serine/threonine-rich motifs responsible for rapid degradation. The mature receptor is a heterodimer with the ECD tethered to the transmembrane/intracellular domain (T/ICD) through non-covalent, calcium dependent interactions. The heterodimer is formed by cleavage of the nascent polypeptide at site 1 by a furin-like protease in the Golgi [18,19].

Notch ligands are also type I transmembrane proteins with a similar overall architecture: an ECD containing an N-terminal DSL (Delta/Serrate/LAG-2) motif, specialized tandem EGF repeats termed the DOS (Delta and OSM-11-like proteins) domain, and several tandem EGF repeats; a single transmembrane domain; and a small intracellular domain [20]. *Drosophila* has two ligands, Delta and Serrate, while mammals have three Delta-like ligands (Dll1, 3, and 4) and two Serrate homologues (Jagged1 and 2).

Notch activation is initiated by ligand binding, and accomplished through a proteolytic mechanism [21]. The first cleavage occurs at site 2 (S2), just outside the membrane on the T/ICD, and is catalyzed by a metalloprotease of the ADAM family. In the absence of ligand, S2 appears to be covered by the NRR, sterically blocking access of the ADAM protease to the site. Ligand binding results in a conformational change in the NRR, exposing the site and allowing cleavage [22–24]. Subsequently, cleavage at site 3 (S3) in the Notch transmembrane domain by the γ-secretase complex results in the release of the Notch intracellular domain (NICD), and translocation of the NICD into the nucleus [25]. Interaction between NICD and DNA binding proteins such as RBP-Jκ, activate target gene transcription [26].

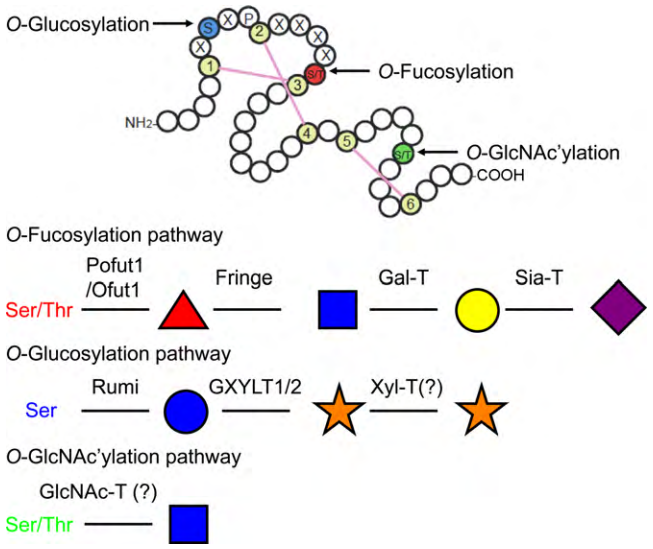

Fig. 1. *O*-Glycosylation of EGF repeats. Upper panel shows a single EGF repeat with the sites for addition of *O*-fucose, *O*-glucose, and *O*-GlcNAc. *O*-Fucose is attached to Ser/Thr in $C^2XXXX(\underline{S/T})C^3$ (red). *O*-Glucose is attached to Ser in $C^1X\underline{S}XPC^2$ (blue). *O*-GlcNAc is attached to Ser/Thr between the fifth and sixth cysteines (green). Note that the consensus sequence of *O*-GlcNAc modification has not yet been proposed. Conserved cysteines are shown in light green. Disulfide bonds are shown by pink bars. Lower panel shows fully extended structures of *O*-fucose, *O*-glucose, and *O*-GlcNAc glycans and the glycosyltransferases responsible for their syntheses. Fucose (red triangle), GlcNAc (blue square), Galactose (yellow circle), Sialic acid (purple diamond), Glucose (blue circle), and Xylose (orange star). *O*-Fucose on *Drosophila* Notch has only been found as a disaccharide to date [35]. Xylosyltransferase(s) which adds a terminal xylose on *O*-glucose has not been cloned yet. GlcNAc-transferase(s) responsible for *O*-GlcNAc modfification of EGF repeats has not been cloned yet [32].

2. Regulation of Notch function with glycosylation

The discovery that Fringe, a known modulator of Notch activity, is a glycosyltransferase modifying *O*-fucose glycans on Notch EGF repeats [27,28], brought the study of Notch into the field of Glycobiology [29]. The EGF repeats of Notch are modified with three different types of *O*-linked glycosylation: *O*-fucosylation, *O*-glucosylation, and *O*-GlcNAc'ylation (Fig. 1) [30–32]. Addition of *O*-fucose to Ser/Thr occurs within the consensus sequence C^2-X-X-X-X-$(\underline{S/T})$-C^3 (C, cysteine; X, any amino acid; S, serine; T, threonine) between the second and the third cysteines conserved in EGF repeats [33]. *O*-Fucose can be elongated by the addition of an *N*-acetylglucosamine (GlcNAc) [27,28,34]. Further elongation with a galactose and sialic acid occurs on mammalian Notch, but not in *Drosophila* (Fig. 1) [30,35]. Notch ligands also have numerous EGF repeats in their ECDs which are modified with *O*-fucose glycans, but the functional significance of ligand *O*-fucosylation is unclear [36]. Similarly, addition of *O*-glucose occurs only at serine within the *O*-glucose consensus sequence C^1-X-$\underline{S}$-X-P-C^2 (C, cysteine; X, any amino acid; S, serine; P, proline) between the first and the second cysteines conserved in EGF repeats [30,37]. *O*-Glucose on the EGF repeats of mammalian Notch1 is elongated with two α1,3-linked xyloses [30,31], but our preliminary data suggest that *O*-glucose on *Drosophila* Notch may only be modified with a single xylose (Rana and Haltiwanger, unpublished observation). In contrast, *O*-GlcNAc seems to be a monosaccharide on the EGF repeats of Notch [32].

Most EGF repeats of Notch proteins contain consensus sequences for *O*-fucose and/or *O*-glucose (Fig. 2). Mutations in Notch-related glycosyltransferase genes lead to aberrant Notch signaling, which clearly suggests that glycosylation is essential for Notch function. Although many other proteins bear, or are predicted to bear, these modifications [17,37], given the abundance of potential modification sites for these *O*-linked glycans on Notch