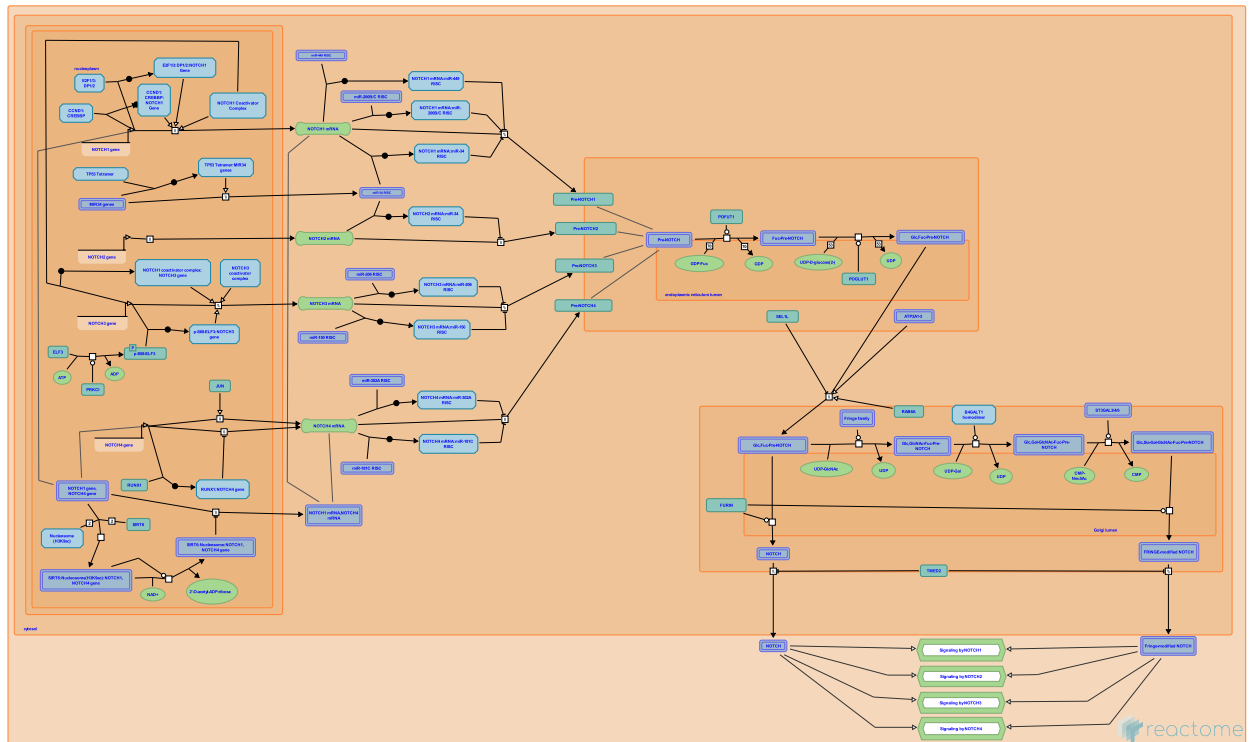


Pre-NOTCH Expression and Processing



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26/04/2024

Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

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Literature references

- Fabregat, A., Sidiropoulos, K., Viteri, G., Forner, O., Marin-Garcia, P., Arnau, V. et al. (2017). Reactome pathway analysis: a high-performance in-memory approach. *BMC bioinformatics*, 18, 142. [↗](#)
- Sidiropoulos, K., Viteri, G., Sevilla, C., Jupe, S., Webber, M., Orlic-Milacic, M. et al. (2017). Reactome enhanced pathway visualization. *Bioinformatics*, 33, 3461-3467. [↗](#)
- Fabregat, A., Jupe, S., Matthews, L., Sidiropoulos, K., Gillespie, M., Garapati, P. et al. (2018). The Reactome Pathway Knowledgebase. *Nucleic Acids Res*, 46, D649-D655. [↗](#)
- Fabregat, A., Korninger, F., Viteri, G., Sidiropoulos, K., Marin-Garcia, P., Ping, P. et al. (2018). Reactome graph database: Efficient access to complex pathway data. *PLoS computational biology*, 14, e1005968. [↗](#)

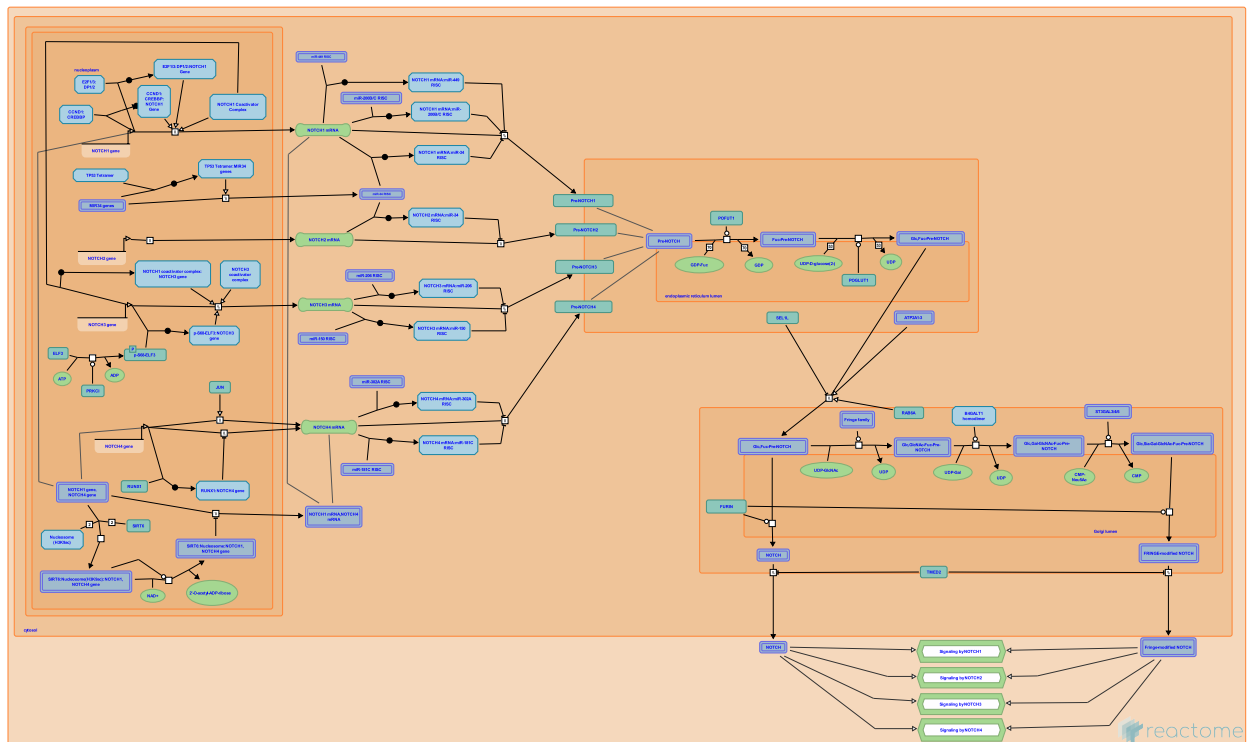
Reactome database release: 88

This document contains 4 pathways ([see Table of Contents](#))

Pre-NOTCH Expression and Processing ↗

Stable identifier: R-HSA-1912422

Compartments: nucleoplasm, cytosol, endoplasmic reticulum membrane, endoplasmic reticulum lumen, Golgi membrane, Golgi lumen, plasma membrane



In humans and other mammals the NOTCH gene family has four members, NOTCH1, NOTCH2, NOTCH3 and NOTCH4, encoded on four different chromosomes. Their transcription is developmentally regulated and tissue specific, but very little information exists on molecular mechanisms of transcriptional regulation. Translation of NOTCH mRNAs is negatively regulated by a number of recently discovered microRNAs (Li et al. 2009, Pang et al. 2010, Ji et al. 2009, Kong et al. 2010, Marcet et al. 2011, Ghisi et al. 2011, Song et al. 2009, Hashimoto et al. 2010, Costa et al. 2009).

The nascent forms of NOTCH precursors, Pre-NOTCH1, Pre-NOTCH2, Pre-NOTCH3 and Pre-NOTCH4, undergo extensive posttranslational modifications in the endoplasmic reticulum and Golgi apparatus to become functional. In the endoplasmic reticulum, conserved serine and threonine residues in the EGF repeats of NOTCH extracellular domain are fucosylated and glucosylated by POFUT1 and POGLUT1, respectively (Yao et al. 2011, Stahl et al. 2008, Wang et al. 2001, Shao et al. 2003, Acar et al. 2008, Fernandez Valdivia et al. 2011).

In the Golgi apparatus, fucose groups attached to NOTCH EGF repeats can be elongated by additional glycosylation steps initiated by fringe enzymes (Bruckner et al. 2000, Moloney et al. 2000, Cohen et al. 1997, Johnston et al. 1997, Chen et al. 2001). Fringe-mediated modification modulates NOTCH signaling but is not an obligatory step in Pre-NOTCH processing. Typically, processing of Pre-NOTCH in the Golgi involves cleavage by FURIN convertase (Blamueller et al. 1997, Logeat et al. 1998, Gordon et al. 2009, Rand et al. 2000, Chan et al. 1998). The cleavage of NOTCH results in formation of mature NOTCH heterodimers that consist of NOTCH extracellular domain (NEC i.e. NECD) and NOTCH transmembrane and intracellular domain (NTM i.e. NTMICD). NOTCH heterodimers translocate to the cell surface where they function in cell to cell signaling.

Literature references

Wei, L., Lowe, JB., Gerson, S., Xin, W., Zhou, L., Yao, D. et al. (2011). Protein O-fucosyltransferase 1 (Pofut1) regulates lymphoid and myeloid homeostasis through modulation of Notch receptor ligand interactions. *Blood*, 117, 5652-62. ↗

Blacklow, SC., Grimm, LM., Sklar, J., Artavanis-Tsakonas, S., Patriub, V., Rand, MD. (2000). Calcium depletion dissociates and activates heterodimeric notch receptors. *Mol Cell Biol*, 20, 1825-35. ↗

Jan, YN., Chan, YM. (1998). Roles for proteolysis and trafficking in notch maturation and signal transduction. *Cell*, 94, 423-6. [↗](#)

Shao, L., Haltiwanger, RS., Moloney, DJ. (2003). Fringe modifies O-fucose on mouse Notch1 at epidermal growth factor-like repeats within the ligand-binding site and the Abruption region. *J Biol Chem*, 278, 7775-82. [↗](#)

Rajan, A., Pan, H., Takeuchi, H., Acar, M., Rana, NA., Haltiwanger, RS. et al. (2008). Rumi is a CAP10 domain glycosyltransferase that modifies Notch and is required for Notch signaling. *Cell*, 132, 247-58. [↗](#)

Editions

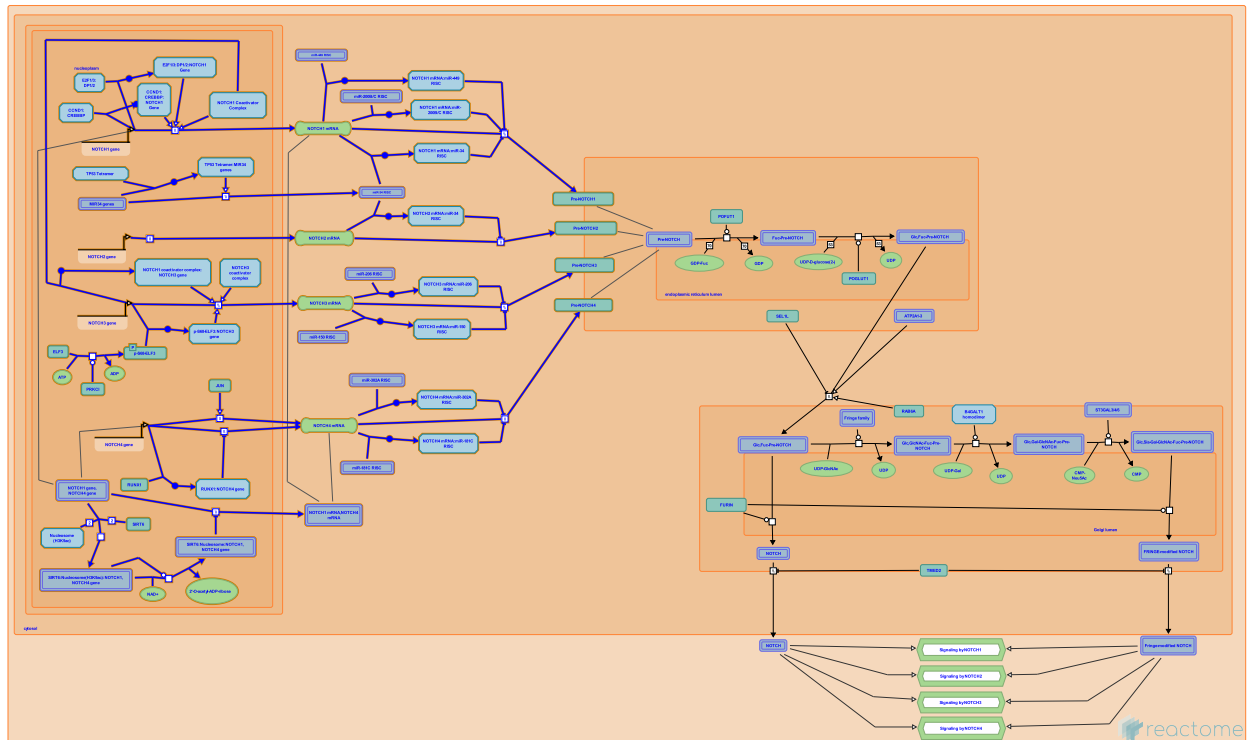
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Pre-NOTCH Transcription and Translation ↗

Location: Pre-NOTCH Expression and Processing

Stable identifier: R-HSA-1912408

Compartments: cytosol, nucleoplasm, endoplasmic reticulum membrane



In humans, the NOTCH protein family has four members: NOTCH1, NOTCH2, NOTCH3 and NOTCH4. NOTCH1 protein was identified first, as the product of a chromosome 9 gene translocated in T-cell acute lymphoblastic leukemia that was homologous to *Drosophila* Notch (Ellisen et al. 1991). At the same time, rat Notch1 was cloned (Weinmaster et al. 1991), followed by cloning of mouse Notch1, named *Motch* (Del Amo et al. 1992). NOTCH2 protein is the product of a gene on chromosome 1 (Larsson et al. 1994). NOTCH2 expression is differentially regulated during B-cell development (Bertrand et al. 2000). NOTCH2 mutations are a rare cause of Alagille syndrome (McDaniell et al. 2006). NOTCH3 is the product of a gene on chromosome 19. NOTCH3 mutations are the underlying cause of CADASIL, cerebral arteriopathy with subcortical infarcts and leukoencephalopathy (Joutel et al. 1996). NOTCH4, the last NOTCH protein discovered, is the product of a gene on chromosome 6 (Li et al. 1998).

MicroRNAs play an important negative role in translation and/or stability of NOTCH mRNAs. MicroRNAs miR-34 (miR-34A, miR-34B and mi-R34C), whose transcription is directly induced by the tumor suppressor protein p53 (Chang et al. 2007, Raver-Shapira et al. 2007, He et al. 2007, Corney et al. 2007) bind and negatively regulate translation of NOTCH1 mRNA (Li et al. 2009, Pang et al. 2010, Ji et al. 2009) and NOTCH2 mRNA (Li et al. 2009). NOTCH1 mRNA translation is also negatively regulated by microRNAs miR-200B and miR-200C (Kong et al. 2010), as well as miR-449A, miR-449B and miR-449C (Marcet et al. 2011). Translation of NOTCH3 mRNA is negatively regulated by microRNAs miR-150 (Ghisi et al. 2011) and miR-206 (Song et al. 2009). Translation of NOTCH4 mRNA is negatively regulated by microRNAs miR-181C (Hashimoto et al. 2010) and miR-302A (Costa et al. 2009).

Nascent NOTCH peptides are co-translationally targeted to the endoplasmic reticulum for further processing, followed by modification in the Golgi apparatus, before trafficking to the plasma membrane. Endoplasmic reticulum calcium ATPases, positively regulate NOTCH trafficking, possibly by contributing to accurate folding of NOTCH precursors (Periz et al. 1999).

Literature references

Meiri, E., Raver-Shapira, N., Moskovits, N., Spector, Y., Rosenfeld, N., Bentwich, Z. et al. (2007). Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Mol Cell*, 26, 731-43. ↗

Yeung, WS., Lee, KF., Pang, RT., Chiu, PCN., Leung, CON., Liu, W. et al. (2010). MicroRNA-34a suppresses invasion through downregulation of Notch1 and Jagged1 in cervical carcinoma and choriocarcinoma cells. *Carcinogenesis*, 31, 1037-44. [↗](#)

Arndt, K., Costa, FF., Bischof, JM., Seftor, EA., Soares, MB., Hendrix, MJC. et al. (2009). Epigenetically reprogramming metastatic tumor cells with an embryonic microenvironment. *Epigenomics*, 1, 387-398. [↗](#)

Friedman, C., Banta, AB., Chen, L., Deng, Y., Hood, L., Huang, GM. et al. (1998). Cloning, characterization, and the complete 56.8-kilobase DNA sequence of the human NOTCH4 gene. *Genomics*, 51, 45-58. [↗](#)

Gridley, T., Del Amo, FF., Greenspan, RJ., Gendron-Maguire, M., Smith, DE., McMahon, AP. et al. (1992). Expression pattern of Motch, a mouse homolog of Drosophila Notch, suggests an important role in early postimplantation mouse development. *Development*, 115, 737-44. [↗](#)

Editions

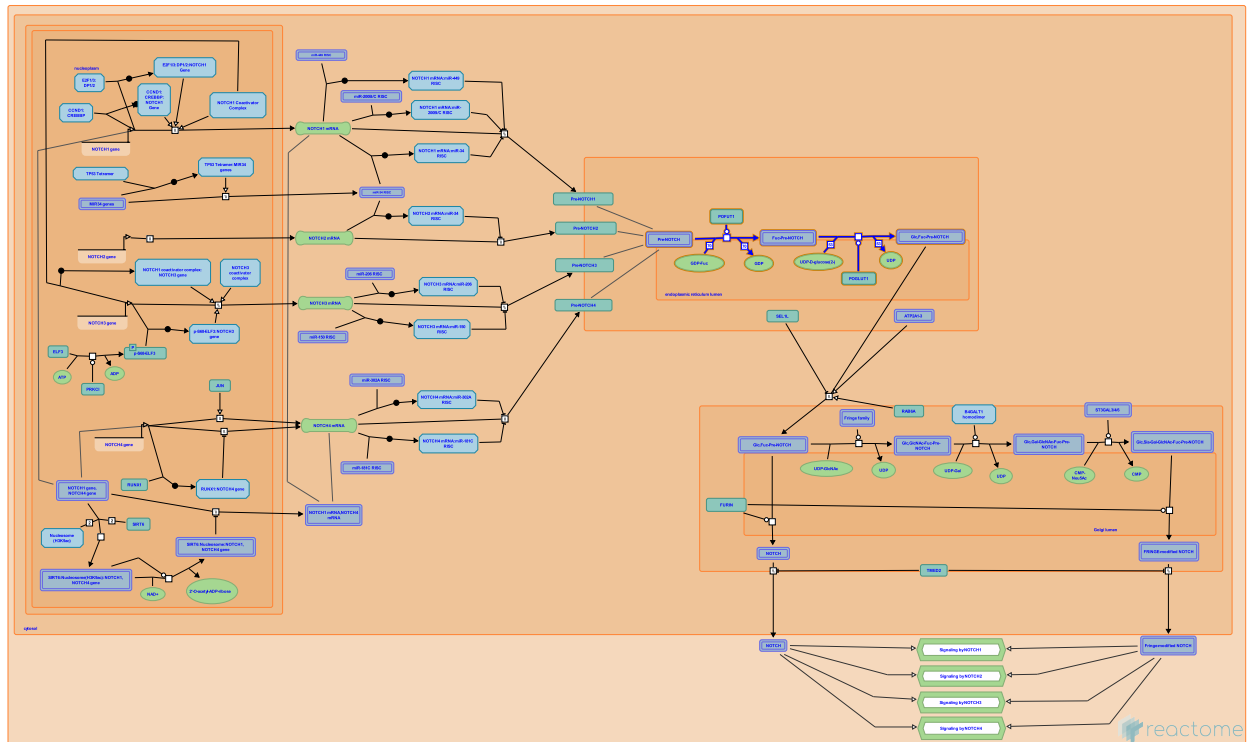
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Pre-NOTCH Processing in the Endoplasmic Reticulum ↗

Location: Pre-NOTCH Expression and Processing

Stable identifier: R-HSA-1912399

Compartments: endoplasmic reticulum membrane, endoplasmic reticulum lumen



In the endoplasmic reticulum, glycosyl transferases modify NOTCH precursors by glycosylating conserved serine and threonine residues in EGF repeats of NOTCH.

O-fucosyl transferase POFUT1 fucosylates NOTCH serine and threonine residues that conform to the consensus sequence C2-X(4-5)-S/T-C3, where C2 and C3 are the second and third cysteine residue within the EGF repeat, and X(4-5) is four to five amino acid residues of any type (Yao et al. 2011, Stahl et al. 2008, Wang et al. 2001, Shao et al. 2003).

O-glucosyl transferase POGLUT1, mammalian homolog of the *Drosophila* enzyme Rumi, adds a glucosyl group to conserved serine residues within the EGF repeats of NOTCH. The consensus sequence for POGLUT1-mediated glucosylation is C1-X-S-X-P-C2, where C1 and C2 are the first and second cysteine residue in the EGF repeat, respectively, while X represents any amino acid (Acar et al. 2008, Fernandez-Valdivia et al. 2011). Both fucosylation and glucosylation of NOTCH receptor precursors are essential for functionality.

Literature references

Lopez, M., Fernandez-Valdivia, R., Takeuchi, H., Leonardi, J., Haltiwanger, RS., Jafar-Nejad, H. et al. (2011). Regulation of mammalian Notch signaling and embryonic development by the protein O-glucosyltransferase Rumi. *Development*, 138, 1925-34. ↗

Shao, L., Harris, RJ., Stanley, P., Spellman, MW., Haltiwanger, RS., Shi, S. et al. (2001). Modification of epidermal growth factor-like repeats with O-fucose. Molecular cloning and expression of a novel GDP-fucose protein O-fucosyltransferase. *J Biol Chem*, 276, 40338-45. ↗

Tashima, Y., Stanley, P., Stahl, M., Shi, S., Ge, C., Uemura, K. (2008). Roles of Pofut1 and O-fucose in mammalian Notch signaling. *J Biol Chem*, 283, 13638-51. ↗

Rajan, A., Pan, H., Takeuchi, H., Acar, M., Rana, NA., Haltiwanger, RS. et al. (2008). Rumi is a CAP10 domain glycosyltransferase that modifies Notch and is required for Notch signaling. *Cell*, 132, 247-58. ↗

Shao, L., Haltiwanger, RS., Moloney, DJ. (2003). Fringe modifies O-fucose on mouse Notch1 at epidermal growth factor-like repeats within the ligand-binding site and the Abruptex region. *J Biol Chem*, 278, 7775-82. [↗](#)

Editions

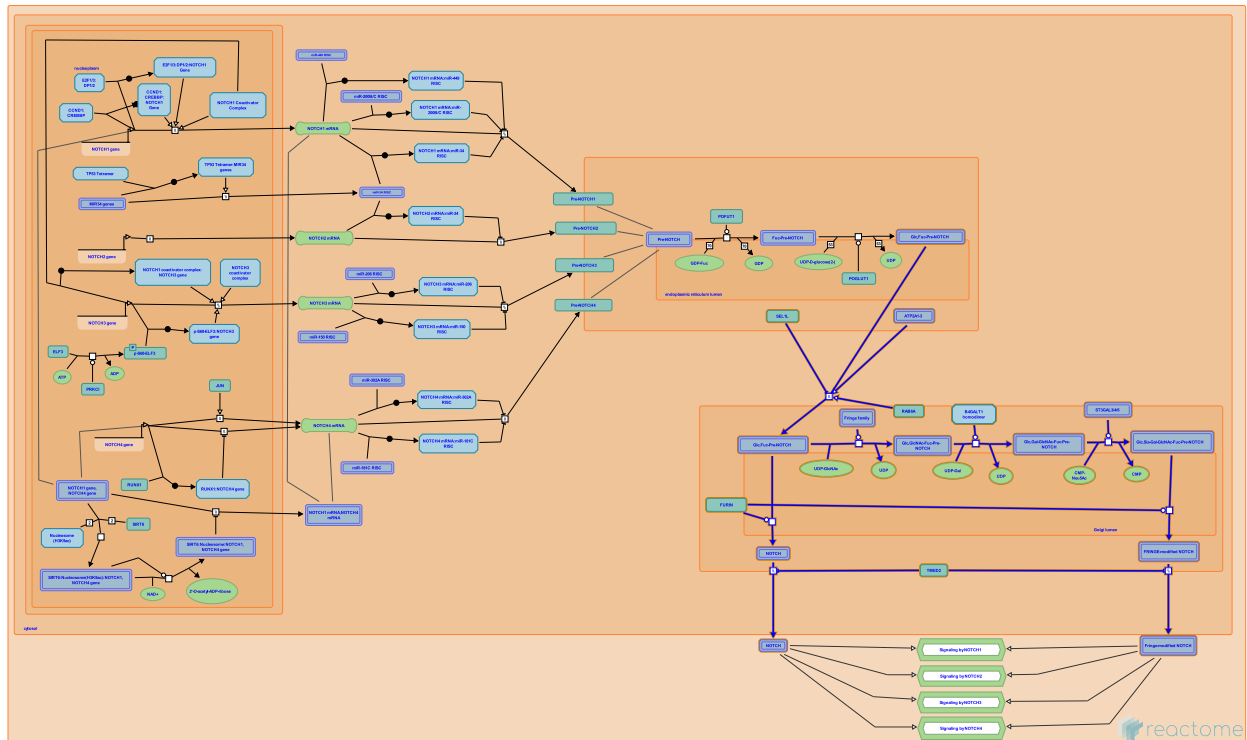
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Pre-NOTCH Processing in Golgi ↗

Location: Pre-NOTCH Expression and Processing

Stable identifier: R-HSA-1912420

Compartments: Golgi membrane, Golgi lumen, plasma membrane, endoplasmic reticulum membrane



NOTCH undergoes final posttranslational processing in the Golgi apparatus (Lardelli et al. 1994, Blaumueller et al. 1997, Weinmaster et al. 1991, Weinmaster et al. 1992, Uyttendaele et al. 1996). Movement of NOTCH precursors from the endoplasmic reticulum to Golgi is controlled by SEL1L protein, a homolog of *C. elegans* sel-1. SEL1L localizes to the endoplasmic reticulum membrane and prevents translocation of misfolded proteins, therefore serving as a quality control check (Li et al. 2010, Sundaram et al. 1993, Francisco et al. 2010). Similarly, *C. elegans* sel-9 and its mammalian homolog TMED2 are Golgi membrane proteins that participate in quality control of proteins transported from Golgi to the plasma membrane. Translocation of a mutant *C. elegans* NOTCH homolog lin-12 from the Golgi to the plasma membrane is negatively regulated by sel-9 (Wen et al. 1999). A GTPase RAB6 positively controls NOTCH trafficking through Golgi (Purcell et al. 1999).

Processing of mammalian NOTCH precursors in the Golgi typically involves the cleavage by FURIN convertase. Pre-NOTCH is a ~300 kDa protein, and cleavage by FURIN produces two fragments with approximate sizes of 110 kDa and 180 kDa. The 110 kDa fragment contains the transmembrane and intracellular domains of NOTCH and is known as NTM or NTMICD. The 189 kDa fragment contains NOTCH extracellular sequence and is known as NEC or NECD. The NTM and NEC fragments heterodimerize (Blaumueller et al. 1997, Logeat et al. 1998, Chan et al. 1998) and are held together by disulfide bonds and calcium ions (Rand et al. 2000, Gordon et al. 2009).

An optional step in Pre-NOTCH processing in the Golgi is modification by fringe enzymes. Fringe enzymes are glycosyl transferases that initiate elongation of O-linked fucose on fucosylated peptides by addition of a beta 1,3 N-acetylglucosaminyl group, resulting in formation of disaccharide chains on NOTCH EGF repeats (GlcNAc-beta1,3-fucitol). Three fringe enzymes are known in mammals: LFNG (lunatic fringe), MFNG (manic fringe) and RFNG (radical fringe). LFNG shows the highest catalytic activity in modifying NOTCH (Bruckner et al. 2000, Moloney et al. 2000). Fringe-created disaccharide chains on NOTCH EGF repeats are further extended by B4GALT1 (beta-1,4-galactosyltransferase 1), which adds galactose to the N-acetylglucosaminyl group, resulting in formation of trisaccharide Gal-beta1,4-GlcNAc-beta1,3-fucitol chains (Moloney et al. 2000, Chen et al. 2001). Formation of trisaccharide chains is the minimum requirement for fringe-mediated modulation of NOTCH signaling, although fringe-modified NOTCH expressed on the cell surface predominantly contains tetrasaccharide chains on EGF repeats. The tetrasaccharide chains are formed by sialyltransferase(s) that add sialic acid to galactose, resulting in formation of Sia-alpha2,3-Gal-beta1,4-GlcNAc-beta1,3-fucitol (Moloney et al. 2000). Three known Golgi membrane

sialyltransferases could be performing this function: ST3GAL3, ST3GAL4 and ST3GAL6 (Harduin-Lepers et al. 2001). The modification of NOTCH by fringe enzymes modulates NOTCH-signaling by increasing the affinity of NOTCH receptors for delta-like ligands, DLL1 and DLL4, while decreasing affinity for jagged ligands, JAG1 and JAG2.

Literature references

- Blacklow, SC., Grimm, LM., Sklar, J., Artavanis-Tsakonas, S., Patriub, V., Rand, MD. (2000). Calcium depletion dissociates and activates heterodimeric notch receptors. *Mol Cell Biol*, 20, 1825-35. [↗](#)
- Jan, YN., Chan, YM. (1998). Roles for proteolysis and trafficking in notch maturation and signal transduction. *Cell*, 94, 423-6. [↗](#)
- Roberts, VJ., Lemke, G., Weinmaster, G. (1991). A homolog of Drosophila Notch expressed during mammalian development. *Development*, 113, 199-205. [↗](#)
- Prabhakaran, B., Johnston, SH., Irvine, KD., Rauskolb, C., Wilson, R., Vogt, TF. (1997). A family of mammalian Fringe genes implicated in boundary determination and the Notch pathway. *Development*, 124, 2245-54. [↗](#)
- Shao, L., Wang, Y., Johnston, SH., Irvine, KD., Stanley, P., Haltiwanger, RS. et al. (2000). Fringe is a glycosyltransferase that modifies Notch. *Nature*, 406, 369-75. [↗](#)

Editions

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