

This is an electronic reprint of the original article. This reprint may differ from the original in pagination and typographic detail.

Decoding the PTM-switchboard of Notch

Antfolk, Daniel; Antila, Christian; Kemppainen, Kati; Landor, Sebastian K.-J.; Sahlgren, Cecilia

Published in:
Biochimica et biophysica acta. Molecular cell research

DOI:
[10.1016/j.bbamcr.2019.07.002](https://doi.org/10.1016/j.bbamcr.2019.07.002)

Published: 01/01/2019

Document Version
Final published version

Document License
CC BY

[Link to publication](#)

Please cite the original version:

Antfolk, D., Antila, C., Kemppainen, K., Landor, S. K.-J., & Sahlgren, C. (2019). Decoding the PTM-switchboard of Notch. *Biochimica et biophysica acta. Molecular cell research*, 1866, –. <https://doi.org/10.1016/j.bbamcr.2019.07.002>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



Review

Decoding the PTM-switchboard of Notch

Daniel Antfolk^a, Christian Antila^a, Kati Kemppainen^a, Sebastian K.-J. Landor^{a,*},
Cecilia Sahlgren^{a,b,*}

^a Faculty of Science and Engineering, Cell Biology, Åbo Akademi University, Turku, Finland

^b Department of Biomedical Engineering, Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, the Netherlands

ARTICLE INFO

Keywords:

Notch
Post-translational modifications
PTM
Phosphorylation
Glycosylation

ABSTRACT

The developmentally indispensable Notch pathway exhibits a high grade of pleiotropism in its biological output. Emerging evidence supports the notion of post-translational modifications (PTMs) as a *modus operandi* controlling dynamic fine-tuning of Notch activity. Although, the intricacy of Notch post-translational regulation, as well as how these modifications lead to multiples of divergent Notch phenotypes is still largely unknown, numerous studies show a correlation between the site of modification and the output. These include glycosylation of the extracellular domain of Notch modulating ligand binding, and phosphorylation of the PEST domain controlling half-life of the intracellular domain of Notch. Furthermore, several reports show that multiple PTMs can act in concert, or compete for the same sites to drive opposite outputs. However, further investigation of the complex PTM crosstalk is required for a complete understanding of the PTM-mediated Notch switchboard. In this review, we aim to provide a consistent and up-to-date summary of the currently known PTMs acting on the Notch signaling pathway, their functions in different contexts, as well as explore their implications in physiology and disease. Furthermore, we give an overview of the present state of PTM research methodology, and allude to a future with PTM-targeted Notch therapeutics.

1. Introduction

The Notch signaling pathway is an evolutionarily conserved cell-cell contact dependent signaling mediator with pivotal roles in development, including orchestration of complex tissue patterning, and determination of cell fate. A Notch signal is initiated when transmembrane ligands and receptors on signal-sending and signal-receiving cells interact, leading to a chain of sequential cleavages of the receptors (reviewed in [1]). Initial pull of the ligand-receptor pair opens up the S2 cleavage site for ADAM metalloproteases (ADAM-10 and ADAM-17), yielding the truncated membrane-bound form of Notch called Notch extracellular truncation (NEXT). This in turn reveals the S3 site to be cleaved by γ -secretase, which ultimately leads to the release of the Notch intracellular domain (NICD) fragment. NICD can then translocate to the nucleus where it binds the Notch transcriptional regulator CSL (CBF1, Suppressor of Hairless, Lag-1), also known as RBPJ (Recombination signal binding protein for immunoglobulin kappa J region), which together with cofactor MAML forms a trimeric activation complex to initiate Notch downstream target gene expression.

Despite its deceptively simple linear stoichiometry with one transcriptional regulator (CSL), the Notch pathway exhibits an immense

pleiotropism, demonstrated by its vast involvement in different developmental settings as well as its involvement in all of the hallmarks of cancer (reviewed in [2]). The multifaceted character of Notch is partially explained by the many paralogs of Notch receptors and ligands that are expressed in the metazoan organisms as well as by differential binding of the receptors intracellular domains to the transcriptional regulator CSL. In metazoans, the Notch pathway consists of four receptors (Notch1–4) and five ligands (Jagged1, 2 and Dll1, 3, and 4) with paralog-specific outputs, and due to the context-dependent character in the developmental cell, Notch mutations and deregulations are often implicated in different cancers (reviewed in [295]).

An additional layer of complexity comes from numerous reports of post-translational modifications (PTMs) of Notch further broadening the seemingly simple core signaling pathway [3]. PTMs are a set of reversible modifications of proteins that modulate their activity, localization and stability in a cell. The PTMs include both enzymatic and non-enzymatic additions of different groups to target protein amino acid side chains. These PTMs are intricate modifiers of protein function and signaling output, with current predictions indicating that as much as 5% of our proteome are enzymes that perform these modifications [287].

* Corresponding authors at: Faculty of Science and Engineering, Cell Biology, Åbo Akademi University, Turku, Finland.

E-mail addresses: sebastian.landor@abo.fi (S.K.-J. Landor), cecilia.sahlgren@abo.fi (C. Sahlgren).

Table 1
 Post-translational modifications of Notch components lead to differential regulation of Notch output. This Table summarizes previously documented PTMs of the Notch pathway components aligned to Uniprot.org amino acid sequence data: NOTCH1 human (P46531), NOTCH1 mouse (Q01705), Notch1 *Xenopus laevis* (P21783), Notch *Drosophila melanogaster* (P07207), NOTCH2 human (Q04721), NOTCH2 mouse (O35516), NOTCH3 human (Q9UM47), NOTCH3 mouse (Q61982), NOTCH4 human (Q99466), RBPJk human (Q06330) Su(H) *Drosophila melanogaster* (P28159).

PTM	Protein	Function	Discovered sites	Corresponding sites in human	Effect on Notch signaling	Implicated in cancer/disease	Notch pathway component	Reference(s)		
Acetylation	PCAF/KAT2B	(Histone) acetyltransferase	Mouse: K1764, K1770, K1771, K1772, K1785, K1935, K2050, K2068, K2146, K2147, K2150, K2154, K2161, K2164	Human: K1774, K1780, K1781, K1782, K1795, K1945, R2060, K2078, K2156, K2157, K2160, K2164, K2171, K2174	Stabilizes the N1-ICD by inhibiting its ubiquitination	ND	NOTCH1	[88]		
			Mouse: K1764, K1770, K1771, K1772, K1785, K1935, K2050, K2068, K2146, K2147, K2150, K2154, K2161, K2164	Human: K1774, K1780, K1781, K1782, K1795, K1945, R2060, K2078, K2150, K2156, K2157, K2160, K2164, K2171, K2174, K2177, K2181, K2182	Stabilizes the N1-ICD by inhibiting its ubiquitination	ND	NOTCH1	[88,90]		
			Mouse: K1692, K1731	Human: K1691, K1730	De-stabilizes the N3-ICD by promoting its ubiquitination	T-cell acute lympho-blastic leukemia (T-ALL)	NOTCH3	[89]		
			Human: K138, K139, K188, K189	Human: K138, K139, K188, K189	Promotes Notch transcription complex function and target gene induction by recruiting co-activator NACK	Oesophageal adenocarcinoma	MAML1	[175]		
			Human: K67/K69/K73, K188, K189	Human: K67/K69/K73, K188, K189	Promotes Notch transcription complex function and target gene induction by recruiting co-activator NACK	Oesophageal adenocarcinoma	MAML1	[176]		
			Mouse: K1764, K1770, K1771, K1772, K1785, K1788, K1811, K2044, K2068, K2146, K2147, K2150, K2154, K2161, K2164, K2167, K2171, K2172	Human: K67/K69/K73, K188, K189	Stabilizes the N1-ICD	Heart regeneration	NOTCH1	[100]		
			Human: K67/K69/K73, K188, K189	Human: K67/K69/K73, K188, K189	Promotes Notch transcription complex function and target gene induction by recruiting co-activator NACK	Oesophageal adenocarcinoma	MAML1	[176]		
			Mouse, <i>Drosophila</i>	Mouse, <i>Drosophila</i>	Promotes N1-ICD transcriptional activity	ND	NOTCH1	[86]		
			Mouse: K1764, K1770, K1771, K1772, K1785, K1935, K2050, K2068, K2146, K2147, K2150, K2154, K2161, K2164	Human: K1774, K1780, K1781, K1782, K1795, K1945, R2060, K2078, K2156, K2157, K2160, K2164, K2171, K2174	De-stabilizes the N1-ICD by promoting its ubiquitination	ND	NOTCH1	[88]		
			SIRT1	(Histone) acetyltransferase	Mouse: K1692, K1731	Human: K1691, K1730	Promotes Notch signaling	ND	CSL	[102]
Glycosylation	HDAC1	(Histone) deacetylase	Mouse: K1692, K1731	Human: K1691, K1730	Stabilizes the N3-ICD by inhibiting its ubiquitination	T-ALL, urothelial cancer	NOTCH3	[89]		
			Mouse (based on consensus seq.): EGF2, 3, 5, 6, 8, 9, 12, 16, 18, 20, 21, 23, 24, 26, 27, 30, 31, 32, 35, 36	Mapped for mouse NOTCH1 (same in humans); EGF2, 3, 5, 6, 8, 9, 12, 16, 18, 20, 21, 26, 27, 30, 31, 35, 36/(T73, T116, T194, T232, T311, T349,	Essential for Notch function, Chaperone activity, Stabilizes EGF repeats, Enhances ligand-receptor binding	Brain tumor, breast cancer, liver cancer, oral squamous cell carcinoma, colorectal cancer, Dowling-Degos disease	NOTCH1	[9,11,24,38,5-7,64,72,159,2-18-220,288]		
			POFUT1	fucosyltransferase						
			POFUT1							

(continued on next page)

Table 1 (continued)

PTM	Protein	Function	Discovered sites	Corresponding sites in human	Effect on Notch signaling	Implicated in cancer/disease	Notch pathway component	Reference(s)
		<i>O</i> -fucosyltransferase	Mouse (consensus seq.): EGF1-6, 8, 9, 12, 18, 20, 21, 24-28, 30, 31, 34, 36	S458, T466, T617, T692, S759, I767, S784, S797, T805, T900, S921, T997, T1035, T1159, T1197, T1362, T1379, T1402)			NOTCH3	
	POFUT1	<i>O</i> -fucosyltransferase	Mouse (consensus seq.): EGF4-8, 11, 17, 19, 21, 22, 24, 28-30, 34				NOTCH4	
	POGLUT1	<i>O</i> -glucosyltransferase	Mouse (consensus seq.): EGF1, 2, 5-9, 11, 12, 15, 18-21, 24, 25, 27, 29	Mapped for mouse NOTCH1 (same in humans): EGF2, 4, 9, 10, 12-14, 16, 17, 19-21, 25, 27, 28, 31, 33	Essential for Notch function. Mediates ligand-receptor binding, promoting Delta activation, Chaperone activity for proper folding of Notch. Stabilizes EGF repeats	Myeloid leukemia, T-ALL, Dowling-Degos disease	NOTCH1	[9,10,15,22,2-4,63,68,72,28-9]
	POGLUT1	<i>O</i> -glucosyltransferase	Mouse (consensus seq.): EGF4, 10, 12-14, 16-21, 23, 25, 27, 30, 33, 36	S341, S378, S458, S496, S534, S609, S647, S722, S759, S797, S951, S1027, S1065, S1189, S1273)			NOTCH2	
	POGLUT1	<i>O</i> -glucosyltransferase	Mouse (consensus seq.): EGF2, 3, 9, 11-13, 15-17, 19, 23, 25, 29, 32, 34				NOTCH3	
	POGLUT1	<i>O</i> -glucosyltransferase	Mouse (consensus seq.): EGF10, 12-15, 18, 19, 20, 25, 27				NOTCH4	
	POGLUT2	<i>O</i> -glucosyltransferase	Mouse: Notch1: S435 on EGF11 Mouse: Notch3: S414 on EGF10	Same in humans	O-glucose at NOTCH1 S435 in the middle of binding interface with DLL4. Potential regulation of folding and quality control of Notch as well as Notch-ligand interactions in combination with EGF8 or EGF12	ND	NOTCH1 NOTCH3	[30,31]
	POGLUT3	<i>O</i> -glucosyltransferase						
	FRINGE: lunatic, manic, radical	β 1,3 N-acetylglucosaminyltransferase	O-fucosylated EGF-repeats of Notch	Mapped for mouse NOTCH1* (same in humans): Lfng: EGF6, 8, 9, 12, 26, 27, 30, 35, 36 Mfng: EGF6, 8, 9, 26, 27, 30, 35, 36 Rfng: EGF 8, 12, 26	Glycosylation of <i>O</i> -fucose linked EGF-repeats on Notch. Differentially regulates ligand binding. Lfng & Mfng enhance DLL1-Notch, but reduce JAGGED1-Notch, activity. Rfng activates Notch signaling from both DLL1 and JAGGED1.	Breast cancer, spondylocostal dysostosis	NOTCH1-4	[8,16,38,51-5,3,72,159,221,-288]
	GYXL1/2	Glucoside xylosyltransferase	O-glucoylated EGF-repeats of Notch	Mapped for mouse NOTCH1 (same in humans): EGF2, 4, 9, 10, 12-14, 16, 17, 19-21, 25, 27, 28, 31, 33/(S65, S146, S341, S378, S458, S496, S534, S609, S647, S722, S759, S797, S951, S1027, S1065, S1189, S1273)	Negatively regulates Notch activity	ND	NOTCH1-4	[15,18,23,26-28,72]
	XXYL1	Xyloside xylosyltransferase	Xylosylated EGF-repeats of Notch	Mapped for mouse NOTCH1 (same in humans): EGF2, 4, 9, 10, 12-14, 16, 17, 19-21, 25, 27, 28, 31, 33/(S65, S146, S341, S378, S458, S496,	Negatively regulates Notch activity	Squamous cell carcinoma (head and neck, lung)	NOTCH1-4	

(continued on next page)

Table 1 (continued)

PTM	Protein	Function	Discovered sites	Corresponding sites in human	Effect on Notch signaling	Implicated in cancer/disease	Notch pathway component	Reference(s)
	EOGT1	EGF-domain specific O-linked N-acetylglucosamine transferase	Mouse (consensus seq.): EGF2, 3, 7, 8, 9, 10, 11, 14, 15, 17, 19, 20, 21, 23, 26, 27, 28, 29, 35	Mapped in mouse NOTCH1 (same in humans): EGF2, 10, 11, 14, 15, 20, 21, 23, 27, 28, 35. Mapping in <i>Drosophila</i> identified only 5 sites (EGF4, 11, 12, 14 and 20) modified out of 18 predicted Same in humans	Potentiate Dll1 and Dll4-mediated Notch1 interactions. Important for vascular development. EGF2, 10, 17 and 20 in mouse suggested important for Dll1/4-NOTCH1 interactions	Adams-Oliver syndrome	NOTCH1	[68-72,77,79,80,222]
	OST	Oligosaccharyl-transferase (N-glycans)	Human (consensus seq.): N41 (EGF1), N959 (EGF26), N1179 (EGF30), N1241 (EGF32), N1489 (LNR1), N1587 (12 aa C-terminal of LNR3)	Human: N1955, N2022	ND	ND	NOTCH1	[9,12]
Hydroxylation	FIH	Asparaginyl hydroxylase	Mouse: N1945, N2012	Human: N1955, N2022	NA mutants of the hydroxylated asparagines inhibit N1-ICD transcriptional activity	ND	NOTCH1	[82,84]
	FIH	Asparaginyl hydroxylase	Mouse: N1902, N1969	Human: N1904, N1971	ND	ND	NOTCH2	[83]
	FIH	Asparaginyl hydroxylase	Mouse: N1867, N1934	Human: N1866, N1933	ND	ND	NOTCH3	[83]
Methylation	CARM1/PRMT4	Methyl transferase	Mouse: R2253, R2262, R2303, R2317, R2361	Human: R2263, R2272, R2313, R2327, R2372	Drives punctuated Notch-response and ubiquitin-mediated proteasomal degradation of N1-ICD	ND	NOTCH1	[105]
	ND	Methyl transferase	Human: R2174	Human: R2174	ND	Detected in colon cancer cells	NOTCH3	[106]
	ND	Methyl transferase	Mouse: R1850	Human: 1871	ND	ND	NOTCH4	[106]
Phosphorylation	AKT	Ser/Thr kinase	ND	ND	Downregulate Notch nuclear localization and transcription	ND	NOTCH1	[118]
	AKT	Ser/Thr kinase	Human NOTCH4: S1495, S1847, S1865, S1917	Human NOTCH4: S1495, S1847, S1865, S1917	Serve as binding site for 14-3-3 which sequesters N4-ICD in the cytoplasm	ND	NOTCH4	[119]
	CDK8	Ser/Thr kinase	Xenopus laevis: S2482, S2485, S2507	Human: S2513, S2516, S2538	Enhances FBXW7/SEL-10 dependent N1-ICD polyubiquitination and degradation.	ND	NOTCH1	[110]
	Cyclin C — CDK1, 2, 3, 8, 19	Ser/Thr kinase	Human in vitro: T1861, T1963, S2183, T2511, S2513, S2516, S2524	Human in vitro: T1861, T1963, S2183, T2511, S2513, S2516, S2524	Enhances FBXW7 dependent N1-ICD polyubiquitination and degradation.	T cell-acute lymphoblastic leukemia	NOTCH1	[111,112,290]
	CK2	Ser/Thr kinase	Human in vivo: S1801, S1856, T1861, S2121, S2198, S2202, S2205, S2211, S2215, T2483, T2486, S2492, T2497, T2511, S2513, S2516, S2521, S2522, S2523, S2527, S2530	Human in vivo: S1801, S1856, T1861, S2121, S2198, S2202, S2205, S2211, S2215, T2483, T2486, S2492, T2497, T2511, S2513, S2516, S2521, S2522, S2523, S2527, S2530	ND	ND	NOTCH1	[113,276]

(continued on next page)

Table 1 (continued)

PTM	Protein	Function	Discovered sites	Corresponding sites in human	Effect on Notch signaling	Implicated in cancer/disease	Notch pathway component	Reference(s)
			Mouse: T1887, S1890 Human: S1900, T1897		Decreases binding of N1-ICD to Notch-Mastermind-CSL ternary complex.			
	DYRK1A	Ser/Thr kinase	18 RPXS/TP consensus sites in ANK Human: 2078	ND	Reduces capacity to maintain transcription	ND	NOTCH1	[126]
	G-CSF	Ser/Thr kinase	Human: 2078	Human: 2078	N2-ICD inactivation	ND	NOTCH2	[125]
	GSK3β	Ser/Thr kinase	Notch1 Mouse: T1851, T2122, T2124 Notch2 Mouse: T2069, S2071, T2075, S2094 Human: T2068, S2070, T2074, S2093	Human NOTCH1: T1861, T2132, T2134 NOTCH2 Human: T2068, S2070, T2074, S2093	Protects from proteasomal degradation and/or negatively regulates NOTCH1/N1-ICD. Inhibits transcriptional activity of NOTCH2.	ND	NOTCH1/2	[121–123]
	ILK	Ser/Thr kinase	Mouse: S2173	Human: S2183	Enhances FBXW7 dependent N1-ICD polyubiquitination and degradation.	ND	NOTCH1	[114]
	NLK	Ser/Thr kinase	Xenopus laevis: S2118, S2132, S2137, S2194, S2211, S2217, S2222 Mouse: S2152	Human: S2121, S2136, S2142, S2198, S2215, S2221, S2226	Inhibits formation of transcriptionally active ternary complex.	ND	NOTCH1	[116]
	PIM	Ser/Thr kinase	Mouse: S2152	Human NOTCH1: S2162	Enhances N1-ICD nuclear localization and transactivation.	Breast cancer, prostate cancer	NOTCH1	[117]
	PKCζ	Ser/Thr kinase	Mouse: S1791 Human: S1801	Human: S21791, T1861	Regulates NOTCH1 trafficking		NOTCH1	[120]
	SRC	Tyrosine kinase	Human: Y2074, Y2116, Y2145, Y1938	Human: Y2074, Y2116, Y2145, Y1938	Inhibits MAML binding, decreases N1-ICD half-life	ND	NOTCH1	[115]
	Unknown	Tyrosine kinase	Drosophila: Y1850, Y1860, Y2097 Mouse: Y2064	Human NOTCH1: Y2074	Mediates non-canonical Notch/Abi signaling	ND	dNotch, NOTCH1	[127,223]
	EYA1	Threonine phosphatase	Mouse Notch1: T2122	Human: T2132	Increases stability of N1-ICD	ND	NOTCH1	[124]
	ND	ND	Mouse: S1781, T1851 Human: S1791, T1861	Human: S1791, T1861	ND	ND	NOTCH1	[224–226]
	ND	ND	Mouse: T1718, S1724, S1780, T1803, S1805, T1809, T1831, S1837, S1842, S1843, S1846, T2069, S2071, T2075, S2079, S2082, S2091, S2094, T2098, S2116, T2298, Y2342, T2387, S2390, Y2472 Human: S1708, T1716, S1722, S1778, T1802, S1804, T1808, T1830, S1836, S1841, S1842, S1845, S1854, S1855, T1860, S1864, S1868, T1873, T1876, S2010, S2078, S2081, S2090, T2097, S2115, T2296, Y2340, T2385, S2388, Y2470	Human: S1708, T1716, S1722, S1778, T1802, S1804, T1808, T1830, S1836, S1841, S1842, S1845, S1854, S1855, T1860, S1864, S1868, T1873, T1876, S2070, S2078, S2081, S2090, T2097, S2115, T2296, Y2340, T2385, S2388, Y2470	ND	ND	NOTCH2	[135,136,223,227,229–241]
	ND	ND	Mouse: S2033 Human: S1681, S2032	Human: S1681, S2032	ND	ND	NOTCH3	[235]
	ND/AKT?	Ser/Thr kinase		Human: S694, S697		ND	DLL1	[165]

(continued on next page)

Table 1 (continued)

PTM	Protein	Function	Discovered sites	Corresponding sites in human	Effect on Notch signaling	Implicated in cancer/disease	Notch pathway component	Reference(s)
			Mouse <i>in vitro</i> : T638, S693, S696 Mouse <i>in vivo</i> : T693 CSL/RBPJκ Human : T339	Human : T339	Required for full ligand activity		CSL	[172]
	MAPK p38	Ser/Thr kinase			Induces Ubiquitination and degradation of CSL/RBPJ κ	ND	CSL	[172]
	MAPK	Ser/Thr kinase	Su(H) <i>Drosophila</i> : T426	Human : T352	May slow down activator complex formation	ND	CSL	[173]
	<i>Unknown</i>	Ser/Thr kinase	Su(H) <i>Drosophila</i> : S269	Human : S195	Inhibits DNA binding of Su(H)	ND	CSL	[174]
Sumoylation	ND	E3 ligase	Human : K1780	Human : K1780	Represses expression of Notch target genes	ND	NOTCH1	[130]
Ubiquitination	Mindbomb 1,2	E3 ligase	ND	ND	Required for Notch activation, DLL internalization, Mib1 KO mice are not viable, Mib2 is dispensable for mouse development, but can compensate for loss of Mib1 in zebrafish	ND	DLL1,2,3,4, JAGGED1,2	[169-171,242,291]
	Skeletrophin/Mib2	E3 ligase	ND	ND	JAGGED2 internalization	ND	JAGGED2	[243]
	Neutralized 1,2	E3 ligase	<i>Drosophila</i> : K742	ND	Required for Notch activation in <i>Drosophila</i> /Neur1-Neur2 double knock out mice are viable. Regulates DLL1 trafficking	ND	dDelta, JAGGED1, DLL1	[244-249]
	Deltex 1-4	E3 ligase	ND	ND	Promotes ligand-independent Notch receptor endocytic trafficking and activation in <i>Drosophila</i> . NOTCH1 proteasome degradation	ND	dNotch, NOTCH1	[250-252,292]
	Nedd4	E3 ligase	PPSY motif	ND	Notch loss-of-function wing phenotypes in <i>Drosophila</i> . Promotes Notch receptor endocytosis before cleavage by γ -secretase in mammals	ND	dNotch, NEXT	[253-255]
	Suppressor of Deltex/ITCH/AIP4	E3 ligase	ND	ND	Mediates lysosomal degradation of non-activated Notch receptors and NOTCH1 proteasomal degradation	ND	NOTCH1	[140,141,256]
	FBXW7/SEL-10/Archipelago	E3 ligase	Mouse : Phosphodegdon at T2486 Human : Phosphodegdon at T2511	Human : Phosphodegdon at T2511	Induces Ubiquitination and degradation of N1- and N4-ICD. Notch proteasomal degradation.	T cell-acute lymphoblastic leukemia	NOTCH1	[146-148,151,257]
	Cbl	E3 ligase	ND	ND	NOTCH1 ligand independent lysosomal degradation	ND	NOTCH1	[258]
	Mdm2	E3 ligase	ND	ND	Increases NUMB degradation and thereby Notch activity. NOTCH1 stability.	ND	NUMB, NOTCH1, NOTCH4	[154,155,259]
	USP12	Deubiquitinating enzyme (DUB)	ND	ND	Promotes Notch degradation	ND	NOTCH1	[145]
	eiF3f	DUB	ND	ND	Promotes Notch activation	ND	NOTCH1	[260]

(continued on next page)

Table 1 (continued)

PTM	Protein	Function	Discovered sites	Corresponding sites in human	Effect on Notch signaling	Implicated in cancer/ disease	Notch pathway component	Reference(s)
	ND	E3 ligase	Mouse: K1785	Human: K1795	ND	ND	NOTCH1	[261]
	ND	E3 ligase	Human: K1821	Human: K1821	ND	ND	NOTCH1	[262]
	ND	E3 ligase	Human: K1705	Human: K1705	ND	ND	NOTCH2	[263]
	ND	E3 ligase	Human: K1684	Human: K1684	ND	ND	NOTCH3	[263]
	ND	E3 ligase	Mouse: K1749	Human: K1759	Required for γ -secretase cleavage of Notch	ND	NOTCH1	[264]

* Modified at high stoichiometries by Fringes.

Research into PTMs of Notch is a developing field of study with the potential to shed new light on the pathway's still poorly understood multitude of context-dependent phenotypic effects. Advanced developments in mass spectrometry (MS) with high sensitivity, now allows for global profiling of the proteome. In recent years analysis of PTMs have generated hope in therapy with the identification of several PTM-linked biomarkers for different diseases, including several types of cancer [5,6]. In this review we aim to summarize PTMs of the Notch pathway and present clues to the wide implications of these on the status and function of the Notch as well as the potential therapeutic targetability of the pathway. Indeed, much of the still unexplained Notch pleiotropism is believed to lie in the PTM-mediated spatio-temporal control of Notch signaling. However, what impact each modification has on the output of Notch signaling in different cell contexts and epigenetic states, and whether it is to fine-tune signal strength or to mediate larger signaling crosstalk is still largely unknown and still being unraveled.

2. PTMs of the Notch receptors

Notch receptors are single-pass transmembrane proteins expressed at the plasma membrane. Synthesis of the monomeric Notch-receptors takes place in the endoplasmic reticulum (ER) followed by cleavage in the trans-golgi at S1 [7]. The two components subsequently form a non-covalent heterodimer representing the active Notch-receptor, which is then transported to the cell surface. The Notch extracellular domain (NECD) is modified by glycans (or sugar residues) [8,9] and the intracellular domain is modified by a multitude of other PTMs [3]. In the last few decades many individual sites have been identified as potential targets of PTMs as shown in the list of identified sites in Table 1. Nevertheless, the various PTMs of Notch do not modulate the signaling output in a vacuum. Instead, the different modifications present on the receptor, work together to determine the final output. In this sense, the different modifications can be seen as a computing unit where the input of all modifications together generates a specific output based on their combinatorial effect (Fig. 1). The PTMs can therefore be seen as modifiers of signaling which convert an activated signal to a specific output. In the following sections we dig deeper into each individual type of PTM and its impact on Notch function.

2.1. Glycosylation of the NECD

During synthesis and processing in the ER, the NECD domain is modified by O-linked glycans [10,11]. These are sugar modifications of hydroxy groups on specific serines or threonines that alter the structure of the Notch receptor and its specificity for different ligands. There are three types of O-glycosylation of EGF repeats of Notch: O-fucosylation, O-glucosylation and O-GlcNAcylation [12]. In addition, an O-GalNAc glycan modification has been shown to modify Notch outside of the EGF-repeats near the S2 cleavage site [13].

In mammals the Notch receptors consists of up to 36 EGF-like repeats in their extracellular domain. The EGF repeats are approximately 40 amino acid long sequences, including six conserved cysteine residues, which form three disulfide bridges [1]. The O-linked glycans can be added at specific consensus sequences between these cysteines. O-glucosylation by POGLUT1 can occur between the first and the second cysteine residues, O-fucosylation by POFUT1 can occur between the second and the third cysteines and O-GlcNAcylation by EOGT between the fifth and sixth conserved cysteine residues [12]. The Notch receptors contain the most O-fucose and O-glucose consensus sites, in a comparison to other proteins with EGF-like repeats [14,15]. The O-linked glycans can be further modified by other sugars, notably: O-fucose by GlcNAc (Fringe enzymes) [8,16,17], and O-glucose by xyloses (xylosyltransferases) [18,26].

The role of glycosylation has been studied with detail in *Drosophila melanogaster*, partly because of the simplicity of having only one Notch

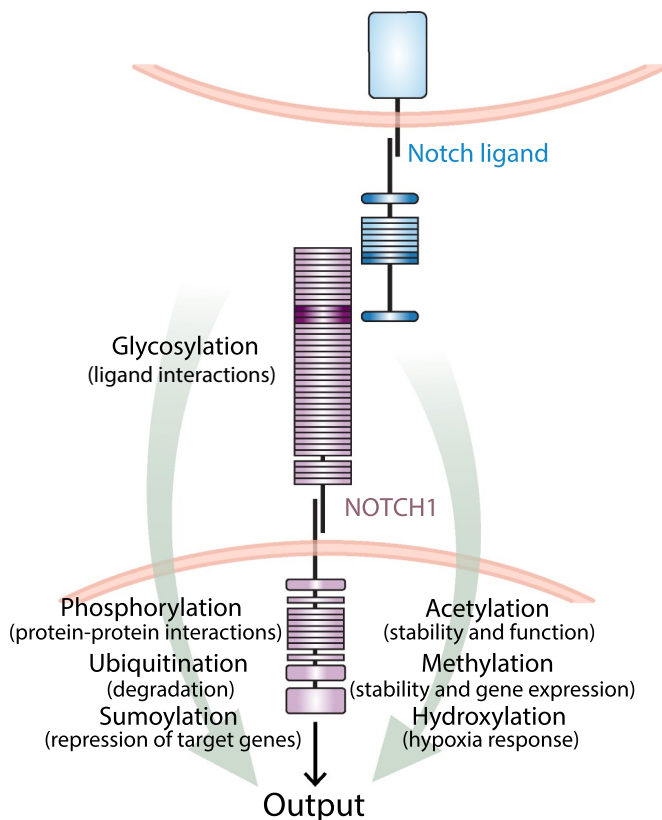


Fig. 1. The multitude of post-translational modifications on the Notch receptor determines the output of the signal.

The effects of many single PTMs are already well known, but the interplay between different modifications is mostly lacking within the field. Some of the main modifications of Notch and their key effects are illustrated above, but to truly understand Notch output, the crosstalk between PTMs and which ratios of different modifications produce certain outcomes.

receptor, two ligands and one Fringe protein [19]. Mammals are more complex with four different Notch receptors (NOTCH1–4) and five ligands (JAGGED1–2, DLL1, 3–4) as well as three mammalian Fringe homologs: Lunatic Fringe (LFNG), Radical Fringe (RFNG) and Manic Fringe (MFNG) [20].

There are also reports of *N*-linked glycan modifications of Notch in mammals, and experiments in mutant Chinese Hamster Ovary (CHO) cell lines have so far indicated that complex-type *N*-glycans are not essential for Notch signaling [9,21]. The locations of these *N*-linked and *O*-linked glycans can be found in Table 1.

2.1.1. *O*-glucosylation by POGLUT1

The *O*-glucosylation of Notch receptors occurs at C^1 -X-S-X-(P/A)- C^2 (where X is any amino acid) and is mediated by *O*-glucosyltransferases called Rumi in *Drosophila melanogaster* and POGLUT1 in mammals [10,15,22,23] (see Fig. 2).

Mice lacking *Poglut1* display embryonic lethality with phenotypes linked to Notch, such as defects in vascular remodeling, cardiogenesis and somitogenesis. Loss of Rumi/POGLUT1 is essential for Notch function in both *Drosophila melanogaster* and mice, and *Poglut1* mutants also show defects in trafficking of the receptor [10,24]. Loss of POGLUT1 in mice has also displayed phenotypes that are not linked to Notch activity, such as defects in neural tube development, thereby implying that not all effects of the loss of POGLUT1 are mediated through Notch [10,22]. Furthermore, loss of POGLUT1 in mice also causes the accumulation of the apical transmembrane protein CRUMBS2 (CRB2) in the ER, resulting in defects in gastrulation with earlier embryonic defects than those from Notch [25]. Two xylose

residues to form a glucose-xylose disaccharide or a glucose-xylose-xylose trisaccharide can further elongate the *O*-glucose on Notch EGF-repeats. The first xylose is added by Glucoside xylosyltransferases, which in mammals are catalyzed by GXYL1 and GXYL2. The second xylose can be added by a xyloside xylosyltransferase termed XXYL1 in mammals [18,26]. Rana et al. showed that all glucosylated EGF-repeats could form the *O*-glucose trisaccharide, consisting of Glc-Xyl-Xyl. The trisaccharide was formed at high stoichiometries and was the most common form of *O*-glucose modification in their samples in all cases except on EGF27 [15]. It has been reported that xylose extensions of *Drosophila melanogaster* Notch glycans inhibit Notch signaling, although xylose modifications in flies are limited to only a few EGF repeats [27]. Downregulation of the *Drosophila melanogaster* glucoside xylosyltransferase was shown to increase Notch activity while overexpression of the human GXYL1 decreased Notch activation in developing fly wings by reducing cell surface expression of Notch [27]. It is therefore thought that xylose modifications could interfere with endocytosis of Notch receptors, but more work still needs to be done in this area. Interestingly, the retaining glycosyltransferase XXYL1 can greatly change the conformation of the EGF repeats of the acceptor substrate and mutations in XXYL1 have been linked to several different cancers, notably squamous cell carcinomas [28]. Matsumoto and colleagues have also recently shown that the dixylose modification of *O*-glucose can play redundant roles with the single *O*-fucose modification in Notch trafficking and activation. It was therefore proposed that previous studies focusing on knock out or mutations of individual glycans may have led to misinterpretation or an underestimation of the function of glycans in Notch signaling [29].

Recent structural studies showed that serine 435 at EGF11 of NOTCH1, a previously unidentified *O*-glucose site, forms interactions with the DSL domain of DLL4 [30]. Interestingly, S435 on EGF11 is not part of a POGLUT1 consensus motif, even though the crystal structure of the modified DLL4-NOTCH1 complex showed that the EGF11-DSL interface is a major point for ligand binding, with *O*-glucosylated S435 being a key part of the interface [30]. Even more recently work from the Haltiwanger lab showed that S435 is indeed glucosylated *in vitro* [31], not by POGLUT1, but by previously identified POGLUT1 homologs named KDELC1 and KDELC2 with previously unknown functions [23]. The identification of these enzymes modifying S435 on EGF11 of NOTCH1 as well as the corresponding site S414 on EGF10 of NOTCH3 led the authors to rename these enzymes, to POGLUT2 and POGLUT3 [31]. Both POGLUT2 and POGLUT3 were able to add an *O*-glucose modification on S435 of NOTCH1 and corresponding site S414 of NOTCH3 in their *in vitro* studies, but with different specificity in a way that POGLUT2 slightly prefers EGF10 of Notch3, while POGLUT3 has a strong preference for EGF11 of NOTCH1. A mutation of S435 alone did not have significant impact on Notch activity but mutations of EGF8 and S435 together significantly decreased NOTCH1 at the cell surface while EGF12 and S435 reduced activation by DLL1, showing that the modification at EGF11 is an additional step of finetuning for Notch folding and activation [31]. The proposed consensus site based on the new data for POGLUT2/POGLUT3 modification is C^3 -X-N-T-X-G-S-F-X- C^4 .

2.1.2. *O*-fucosylation by POFUT1

The *O*-fucosyltransferase Ofut1 in *Drosophila melanogaster* and POFUT1 in mammals mediates *O*-fucosylation, which adds a fucose to a serine or threonine site in the consensus sequence C^2 -X-X-X-(S/T)- C^3 (see Fig. 2). *Pofut1* knock out in mice leads to embryonic lethality with similar phenotypes as in mice lacking CSL [32]. The effect of POFUT1 in modifying all Notch receptors is also implied from its phenotype being more severe than the phenotype resulting from elimination of any individual Notch receptor [32]. The *O*-fucosylation of Notch can be extended with GlcNAc monosaccharides, mediated by the Fringe family homologs. *Fringe* deletion in *Drosophila melanogaster* leads to a Notched phenotype of its wings, which quite early suggested Notch as a key

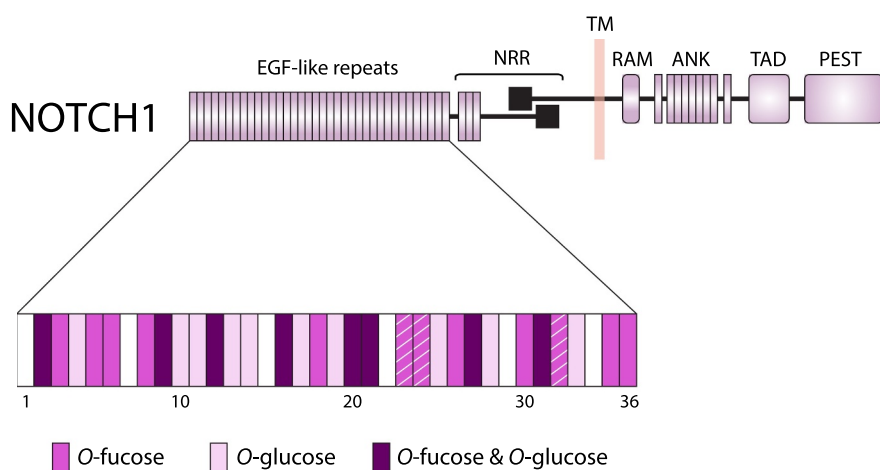


Fig. 2. Glycosylation sites on mammalian Notch1 EGF-repeats (see Table 1).

Notch is a transmembrane receptor with the extracellular domain of Notch (NECD) being a target for glycosylation, which affects structure and ligand binding. A representation of predicted sites of *O*-fucosylation (C²-X-X-X-X-(S/T)-C³) and *O*-glucosylation (C¹-X-S-X-(P/A)-C²) based on current consensus sequences for mouse NOTCH1. The same sites have all been mapped by glycoproteomic methods, except for the *O*-fucosylation sites EGF23, EGF24 and EGF32 (diagonal white lines) [15,38]. The consensus sequence for the newly discovered POGlut2/3-site (C³-X-N-T-X-G-S-F-X-C⁴) can be found only on EGF11 of NOTCH1 (and the corresponding EGF10 of NOTCH3) [31].

target [33]. The effect of Fringes on the fucose residues depend on their catalytic activity, and elimination of the catalytic domain interferes with modification of the Notch receptor [8,16]. *In vitro* studies have shown that the Fringe modifications alter ligand binding [34–36].

In *Drosophila melanogaster* Fringe modifies the *O*-fucose of a Notch receptor to potentiate Delta-mediated signaling while inhibiting the Jagged equivalent Serrate-mediated signaling [8,16,37]. The role of Fringes in mammals is similar, but slightly more complicated. Consistent with their function in *Drosophila melanogaster*, modification by Fringes in mammals always seems to potentiate Dll signaling and for the most part is reported to inhibit Jagged signaling [16,21]. There are exceptions, however, as Radical fringe has been shown to also increase JAGGED1 signaling to NOTCH1 [36,38,39]. In all cases relating to NOTCH1 receptor studies, both MFNG and LFNG promoted DLL1-NOTCH1 signaling while decreasing JAGGED1-NOTCH1 signaling [36]. In signaling to NOTCH2 on the other hand, LFNG also leads to enhanced JAGGED1 signaling in addition to DLL1 signaling [40]. In a recent comparison of the three Fringes in NOTCH1 signaling assays, Kakuda and Haltiwanger showed that out of 17 EGF-like repeats which were modified by *O*-fucose, only a handful were further elongated by Fringes [38]. The different Fringe homologs selectively modified different fucose sites, showing that modifications of fucose sites at EGF 6 and EGF 36 are the ones that inhibit JAGGED1 to Notch signaling. RFNG only modifies three EGF repeats: EGF8, EGF12 and EGF26. The RFNG modification of EGF12 was shown to be the major site that increases Jagged to Notch signaling. All three fringes increase Notch activity from DLL1, and the key EGF repeats seem to be EGF8 and EGF12 with the fucose of EGF12 extending the interaction to the ligands [38]. The fucose modification at EGF12 was also shown to bind directly to ligands, in addition to *O*-glucose on EGF11, based on the co-crystal structure of DLL4 and NOTCH1 mentioned above in Section 2.1.1 [30]. Further structural binding studies, using high affinity ligands, have demonstrated that JAGGED1 binds EGF8–12 on NOTCH1 with high affinity, while measurements with DLL4 showed that even though it does bind to EGF8–12 of NOTCH1, the majority of the affinity comes from EGF11 and 12 [41]. Molecular force measurements showed that Notch receptor-ligand interactions form so called catch bonds, where tensile forces can increase the time of the bond interactions and induce activation under low affinity conditions. As JAGGED1 and DLL4 have different thresholds for activation, the catch bond formation under tensile force could work to further fine-tune the output of different ligands [41].

During development, only LFNG elimination has adverse effect on viability and fertility with disorganization of the axial skeleton [42–44]. LFNG also seem to have a role in the context of angiogenesis, hematopoiesis and kidney development [45–47]. Although MFNG and RFNG are expressed in various tissues during development neither are

required for viability and fertility of mice [48–50]. LFNG mutations have also been reported to cause spondylocostal dysostosis in humans, which gives rise to abnormalities similar to those in mice lacking LFNG [51]. A lack of LFNG has also been found to lead to basal-like breast cancer [52]. In another study, modulation of Notch receptors by LFNG had a tumor-suppressive function in prostate cancer [66]. On the contrary, expression of MFNG has been implied to have an oncogenic role in claudin-low breast cancer [53]. It was recently shown that all Fringes are required to modulate Notch activity during B- and T-cell differentiation [54]. After the Fringe enzymes add the *N*-acetylglucosamine (GlcNAc) to an *O*-fucose sugar, the subsequent addition of a galactose and sialic acid residues can occur, forming a tetrasaccharide [9]. The analysis of *O*-fucose function based on *Pofut1* knock out has been complicated by the fact that POFUT1 also has crucial functions related to Notch signaling that are independent of its *O*-fucosyltransferase function. POFUT1 has been shown to work as a chaperone needed for correct Notch protein folding, independently of its *O*-fucosyltransferase function [55,56]. Overexpression of *Ofut1*, the *Drosophila melanogaster* homologue of POFUT1, has also been shown to promote Notch endocytosis nonautonomously [57]. Based on a mutant of *Ofut1* lacking the fucosyltransferase activity (*Ofut*^{R245A}), but retaining the chaperone activity, that was able to rescue the loss of wild type *Ofut1* during embryonic neurogenesis in flies, it was deduced that the actual *O*-fucosylation of Notch does not have a key function on its own. Furthermore the phenotype of *Ofut*^{R245A} was the same as Fringe mutant clones indicating that the single monofucosylation of Notch, without GlcNAc added by Fringe, is not necessary for proper function of Notch [55]. More recent evidence, however, illustrates that the function may not be quite that simple. By using a knock-in version of the mutant *Ofut1*, lacking *O*-fucosyltransferase activity, *Ofut*^{R245A knock-in}, Ishio and co-workers could show that the monofucosylation by *Ofut* in *Drosophila melanogaster* has a clear role for Notch signaling during embryonal development in a temperature specific manner, where the loss of monosaccharide modification by *Ofut1* leads to the neurogenic phenotype in embryos at 30 °C but not at 25 °C, with no differences in the protein levels of *Ofut1*-mutants between the different temperatures and in a Fringe-independent manner [58]. With the same setup they also showed that monofucosylation is required for correct localisation of Notch at 30 °C. Even more interestingly, by using a double mutant of *Ofut*^{R245A} and a null mutation of the glucosyltransferase *rumi* called *rumi*ⁱ⁴⁴, they further showed that the *O*-glucose modification of Notch could function redundantly to the monofucosylation during development, and not only at 30 °C. Similarly, these double mutants hindering *O*-fucose and *O*-glucose monosaccharides resulted in less Notch at the cell surface and instead accumulated in intracellular compartments whereas either *Ofut*^{R245A knock in} or *rumi*ⁱ⁴⁴ cells had no effects on Notch at the cell membrane, indicating a redundant role also in trafficking of

Notch to the cell membrane [58]. Even more recently it was shown that *O*-fucose functions redundantly with both monosaccharide glucose and the already mentioned dixylosylated *O*-glucose. The monosaccharide *O*-glucose was required for the transport of Notch from the apical plasma membrane to adherens junctions while the dixylose of *O*-glucose regulated Notch export from the ER. Loss of these could, however, both be compensated for by monosaccharide *O*-fucose [29]. These studies highlight the importance of analysis that deciphers the function of several glycans and other PTMs as a combination on output. Focusing on one site at a time may be overlooking key pieces of information about the true function of the modification.

Dysfunctional glycosylation has been shown to lead to developmental disorders and diseases in humans. There have been numerous studies reporting an overexpression of glycosyltransferases in various cancer types. POFUT1 has been found overexpressed in brain tumors, colorectal cancers, oral squamous cell carcinoma, and hepatocellular carcinoma [59–62]. In a microarray screening of glioblastomas POFUT1 was found overexpressed [59]. In another study, POFUT1 was introduced as a potential diagnostic marker for human oral cancer [62]. Heterozygous mutations of both *POFUT1* and *POGLUT1* have also been identified in the rare skin disease Dowling-Degos Disease (DDD) in humans [63,64]. Similar defects of the skin have not been reported in mice heterozygous for either *Pofut1* or *Poglut1* [22,32]. Elimination of *Pofut1* has also been related to the development of myeloid hyperplasia [65].

2.1.3. *O*-GlcNAcylation by EOGT

Notch has also been shown to be modified by the EGF domain specific *O*-GlcNAc transferase (EOGT) [67–70]. EOGT can add a *O*-linked GlcNAc between the fifth and the sixth conserved cysteine at the consensus sequence C⁵-X-X-X-(F/W/Y)-(T/S)-G-X-X-C⁶ [71], although this sequence could yet be further specified in the future. When comparing the modified sequences identified in *Drosophila melanogaster* [72] with the ones in mammals [71], Ogawa and Okajima present a broad consensus sequence of C⁵-X-X-X-X-(T/S)-G-X₂₋₃-C⁶ [73]. The functional effects of *O*-GlcNAc on Notch signaling are not immediately clear in *Drosophila melanogaster* as *Eogt* mutants lack obvious Notch phenotypes [70]. Harvey and colleagues only found 5 sites modified by *O*-GlcNAc in a MS analysis, out of 18 predicted *Drosophila melanogaster* consensus sites [72]. Unlike in flies, *O*-GlcNAc is further modified by additional glycans (galactose and sialic acid) in mammals, implying that *O*-GlcNAc could be distinct between flies and mammals [38,71]. Specifically, Ogawa and colleagues showed (with highly variable stoichiometries) monosaccharide *O*-GlcNAc modifications on EGF14, EGF27 and EGF28, mono- or disaccharide *O*-GlcNAc-Gal on EGF11, EGF15, EGF21, EGF23 and EGF35, as well as mono-, di- or trisaccharide form *O*-GlcNAc-Gal-NeuAc on EGF2, EGF10 and EGF20 [71]. T673 on EGF17 of Notch2 might also be modified by *O*-GlcNAc [67]. Mutations in human *EOGT* and Notch components have been linked to Adams-Oliver syndrome, which is a rare congenital disorder characterized by missing or underdeveloped distal limbs, combined with abnormal skin development of the scalp that may extend to abnormalities in the skull bone. Sometimes Adams-Oliver syndrome is accompanied by blood vessel and heart defects [74–78]. All *EOGT* mutations found in Adams-Oliver syndrome affect the enzyme activity of EOGT in distinct ways [77,79]. It was also recently shown that binding of DLL1 and DLL4 to NOTCH1 was reduced in *EOGT*-deficient mice while JAG1-NOTCH1 signaling was not affected [80]. The majority of *O*-GlcNAcylation was related to EGF2, EGF10, EGF17 and EGF20, although their individual contributions were not specifically determined. In the same study loss of *EOGT* reduced Notch signaling and was shown to regulate angiogenesis and vessel integrity in the mouse retina [80].

2.2. Hydroxylation of NICD

Hydroxylation is the addition of a hydroxyl group to amino acid side

chains, most often proline and this occurs frequently under hypoxic conditions. Hypoxia-inducible factor (HIF) is a target for hydroxylation by both prolyl hydroxylases (PHD) and the asparaginyl hydroxylase Factor Inhibiting HIF (FIH) (reviewed in [81]). FIH also hydroxylates conserved asparagines on the ankyrin repeat domain (ANK) of N1-ICD, N2-ICD and N3-ICD. In contrast, the N4-ICD-ANK lacks the necessary binding motif and the second asparagine residue and is not a FIH substrate [82–84]. FIH overexpression negatively regulates Notch1 signaling [4,82,84]. Surprisingly, this effect is independent of FIH enzymatic activity [82,84]. One function of the FIH-N1-ICD interaction appears to be modulation of HIF-1 signaling and the hypoxia response as FIH has higher affinity for N1-ICD than HIF-1 α [83,84]. N1-ICD promotes HIF signaling through competition for FIH binding and subsequent decreased HIF asparagine hydroxylation [82,84]. N1-ICD hydroxylation decreases FIH affinity for N1-ICD and may fine tune the N1-ICD/HIF competition [82,84]. Based on proteomics screens, asparaginyl hydroxylation by FIH is common for ANK-containing proteins [85].

2.3. Acetylation of NICD

Acetylation involves the transfer of acetyl groups from acetyl coenzyme A to protein lysine residues regulating the protein function and stability. Several acetyltransferases have been shown to associate with the Notch intracellular domain or the CSL-NICD-MAML ternary complex. They are not only involved in regulating Notch transcriptional activity through histone acetylation and consequent effects on chromatin, but also by targeting the individual components of the ternary complex itself. The (histone) acetyltransferases p300, p300/CREB-binding protein-associated factor (PCAF) and GCN5 have been shown to interact with and directly acetylate certain Notch ICDs [86,87] whereas the (histone) deacetylases sirtuin 1 (SIRT1) and histone deacetylase 1 (HDAC1) have been found to deacetylate them [88,89]. p300 and/or PCAF acetylate numerous lysines in the mammalian N1-ICD and many of these sites are deacetylated by the NAD⁺-dependent deacetylase SIRT1. Acetylation attenuates ubiquitination and subsequently degradation of the N1-ICD [88,90]. MAML1 may have a central role in promoting the acetylation through recruitment of p300 to the N1-ICD and stimulation of autoacetylation and acetyltransferase activity of p300 [90–93]. The acetylation-dependent stabilization of N1-ICD is counteracted by SIRT1 activity. Loss of SIRT1 function promotes anti-angiogenic DLL4-Notch signaling in endothelial cells [88,94–96]. Accordingly, in zebrafish and mouse retinas decreased Sirt1 activity leads to an attenuation of vascular growth which is rescued by treatment with the γ -secretase inhibitor DAPT [88]. Inhibitory effects of SIRT1 function on N1-ICD level, localization and activity has additionally been reported in murine neural stem and progenitor cells [97], Treg cells [98] and macrophages [99] as well as in neonatal rat cardiomyocytes [100]. In Ewing's sarcoma cells NOTCH1 has been shown to repress SIRT1 levels through *HEY1* in a negative feedback loop [101].

Interestingly, in *Drosophila melanogaster* Sirt1 activity on the contrary promotes Notch signaling and the phenotype of *Sirt1* mutant flies is similar to flies with a weak loss of Notch function. However, it is not certain if this is due to an effect on the acetylation status of Notch or e.g. Su(H) which has also been identified as a Sirt1 deacetylation target in *Drosophila melanogaster* [102]. The NOTCH3 ICD is acetylated by p300, and deacetylated by HDAC1 [89]. By contrast to the stabilizing effect of p300-mediated acetylation on N1-ICD, acetylation of N3-ICD promotes its ubiquitination and degradation [89]. Furthermore, non-acetylatable lysine-to-arginine mutants of the identified N3-ICD acetylation sites promote proliferation in T-cells whereas treatment with the (histone) deacetylase inhibitor (HDACi) trichostatin A (TSA) has the opposite effect [89]. Importantly, in NOTCH3-overexpressing transgenic mice which typically develop T-cell acute lymphoblastic leukemia (T-ALL), inhibition of N3-ICD deacetylation via TSA treatment prevents T-ALL formation [89]. Similarly, in urothelial cancer cells another HDACi, suberoylanilide hydroxamic acid (SAHA), has been shown to also

increase Notch3 ubiquitination and degradation as well as inhibit proliferation [103]. On the other hand, a recent study attributed the effect of TSA on NOTCH3 signaling in T-ALL cells to increased acetylation of α -tubulin and consequent lysosomal trafficking of full length NOTCH3 [104].

2.4. Methylation of NICD

Methylation refers to the transfer of a methyl group to lysine or arginine residues in proteins, and beyond maintaining epigenetic control and impacting histone proteins, methylation also regulates non-histone protein expression and stability. The first methylation events modulating NICD function have only recently been discovered. Methyltransferase coactivator-associated arginine methyltransferase 1 (CARM1), a member of the protein arginine methyltransferase (PRMT) family, methylates N1-ICD on five conserved arginine residues within the C-terminal transactivation domain (TAD) [105]. Methylated N1-ICD was found to occur primarily in the cell nucleus [105]. CARM1 was also shown to physically interact with both N1-ICD and CSL and to be present at Notch target gene enhancer elements together with N1-ICD [105]. Interestingly, methylation on one hand stimulates Notch transcriptional activity and on the other increases ubiquitin-mediated degradation of the N1-ICD [105]. Based on mathematical modeling N1-ICD methylation regulates the duration and strength of its activity and mediates a full, but transient activation [105]. This example demonstrates the complexity of PTMs and the need to decipher functional consequences of specific PTMs in detail. Additionally, MS screen of methylated proteins identified NOTCH3 as a target of arginine methylation in HCT116 colon cancer cells [106].

2.5. Phosphorylation of NICD

Phosphorylation is the addition of phosphate groups from ATP to specific serine, threonine, and tyrosine residues. It is one of the key mechanisms for tight dynamic regulation of protein activity in eukaryotic cells as one third of all eukaryotic proteins undergo reversible phosphorylation. Indeed, Notch is included in the phospho-protein family containing a myriad of paralog-specific phosphorylation sites impacting on both activity and stability. So far, both the membrane-tethered furin S2-cleaved NEXT as well as NICD have been shown to undergo phosphorylation while the full-length Notch does so only to a lesser extent [107]. This could also be a consequence of challenges in identifying sites on full-length Notch. Subsequently many kinases have already been identified as acting on the Notch pathway (reviewed in [3,108]). However, within the Notch field notation of phosphorylation sites varies and is inconsistent with protein sequence data from UniProt [109]. This is mostly due to the use of the 2556 aa long sequence conflict variant NOTCH1 sequence instead of the canonical 2555 aa NOTCH1 in the UniProt database. In this review we have corrected phosphosite locations to match the canonical 2555 aa NOTCH1 UniProt-protein sequence.

2.5.1. Cyclin-dependent kinases (CDKs)

A classical example of Notch-targeted phosphorylation is that of cyclin-dependent kinase 8 (CDK8) in the article by Fryer et al., where in N1-ICD isolated from the African clawed frog *Xenopus laevis* *in vitro* assays show CDK8 binding directly to MAML and phosphorylating N1-ICD at S2482, S2485, and S2507 of the PEST domain (corresponding to S2513, S2516, and S2538 in human) leading to enhanced PEST-dependent degradation of N1-ICD by FBXW7/Sel10 ubiquitin ligase [110]. However, the impact of CDK-mediated phosphorylation on N1-ICD has evolved considerably after the emergence of Cyclin C as an arbiter of CDK-driven phosphorylation of Notch [111]. In their study, Li et al. unveiled the tumor suppressor Cyclin C as a potent activator of CDK3, 8 and 19 with a myriad of targets identified both *in vitro* and *in vivo* on different domains of Notch corroborating the evidence of CDK-

phosphorylation mediated control of N1-ICD turnover. The sites identified *in vitro* were T1861, T1963, S2183, T2511, T2513, S2516, and S2524. The *in vivo* sites concluded S1801, S1856, T1861, S2121, S2198, S2202, S2205, S2211, S2215, T2483, S2486, S2492, T2497, T2511, S2513, S2516, S2521, S2522, S2523, S2527, and S2530 [111]. From these S2513 and S2516 align with the previously found sites S2482 and 2485 on *Xenopus laevis* [110]. Furthermore, also CDK1 and CDK2 have been shown to phosphorylate S2513 leading to FBXW7-mediated degradation of N1-ICD, impacting the somite segmentation clock [112]. Taken together, the evidence points to an important role for Cyclin C and CDKs especially in controlling NICD turnover.

2.5.2. CK2, ILK, SRC, and NLK

Many kinases also directly control transcriptional activity. Casein kinase 2 (CK2) initially targets N1-ICD on S1900 subsequently revealing another site for CK2 at T1897 [113]. The collective effect of phosphorylation at both sites leads to decreased binding of the N1-ICD-Mastermind-CSL complex thus lowering transcriptional activity [113]. Based on sequence conservation the authors hypothesize that also NOTCH2 may be similarly regulated by CK2. Likewise, Integrin-signaling is modulating Notch as integrin-linked kinase (ILK) phosphorylates N1-ICD at 2173 in mouse (corresponding to S2183 in human) thus inhibiting transcriptional activity and leading to FBXW7-mediated proteasomal degradation [114]. Recently also SRC kinases, acting downstream of Integrins, have been identified to phosphorylate N1-ICD on several tyrosine residues in the ANK domain (Y2074, Y2116, Y2145, Y1938), leading to decreased MAML recruitment and N1-ICD half-life [115]. Interestingly, by sequence alignment the authors also find the phosphorylation sites conserved across many species and present in several Notch paralogs. In a similar fashion in *Xenopus laevis*, Nemo like kinase (NLK) phosphorylates N1-ICD C-terminally of the ANK domain potentially on seven sites at S2118, S2132, S2137, S2194, S2211, S2217, and S2222 decreasing transcriptional activity by interfering with formation of the transcriptionally active ternary complex [116]. These sites in human correspond to S2121, S2136, S2142, S2198, S2215, S2221, and S2226. The authors found the inhibition of transcription to roughly correspond to the number of residues phosphorylated. By contrast, in the same study N3-ICD activity was found to be increased by NLK phosphorylation [116].

2.5.3. PIM, AKT, and PKC zeta

PIM-kinases have been found to mediate phosphorylation of N1-ICD at the second NLS (S2152 in mouse, S2162 in human) thus increasing nuclear localization and transcriptional activity [117]. Moreover, in the same study, also N3-ICD was discovered as a target of PIM-mediated phosphorylation. By contrast, AKT-mediated phosphorylation of N1-ICD has been shown to inhibit proper nuclear localization and transcriptional activity [118]. Similarly on NOTCH4, four distinct AKT phosphorylation sites (S1495, S1847, S1865, and S1917) were found to mediate N4-ICD association with 14-3-3 thus restricting nuclear translocation of N4-ICD [119]. Surprisingly, despite the similarities in output between N1-ICD and N4-ICD upon Akt-mediated phosphorylation, only S1847 on N4-ICD is conserved on N1-ICD. Phosphorylation also impacts receptor recycling as shown by Sjöqvist et al., where PKC zeta was observed to phosphorylate NOTCH1 as both membrane bound NEXT and full-length NOTCH1 receptor form at S1791 in mouse (S1801 in human). This phosphorylation was found to regulate Notch endocytosis which depending on activation state, either enhanced N1-ICD formation or triggered Notch receptor recycling [120].

2.5.4. GSK3- α/β and the dephosphatase EYA1

Findings regarding the effects of glycogen synthase kinase 3 α/β (GSK3- α/β) on Notch activity have revealed conflicting results. In one study, GSK-3 β /Shaggy has been shown to enhance the stability and half-life of N1-ICD and support NOTCH1-mediated *HES1* expression in embryonal fibroblasts and neuroblastoma [121]. However, another

group reported GSK3- α/β as a negative regulator of NOTCH1/N1-ICD in mouse embryonic fibroblasts and HEK 293 cells with suggested phosphorylation sites in mouse at T1851, T2122, and T2124 (corresponds to T1861, T2132, and T2134 in human) [122]. Supportive of this, GSK-3 β has also been described to target N2-ICD at four specific sites C-terminally of the ANK domain (T2068, S2070, T2074, and S2093) thus negatively regulating the transcriptional activity of N2-ICD [123].

Recently the phosphatase EYA1, previously shown to be important for craniofacial morphogenesis was found to be acting on the Notch pathway through dephosphorylation of T2122 on mouse NOTCH1 (corresponding to T2132 in human) [124]. The proposed mechanism involves dephosphorylation-mediated mitigation of FBXW7-activity on N1-ICD and thus lowered ubiquitination and the subsequent proteasomal degradation leading to enhanced N1-ICD stability [124].

2.5.5. G-CSF, DYRK1A, and ABL

Similarly, the serine-threonine rich region at 2067–2099 of NOTCH2 has also been reported to contain at least three phosphorylation sites including S2078 for Granulocyte colony-stimulating factor (G-CSF) [125]. Phosphorylation at these sites, on cleaved N2-ICD is shown to negatively regulate myeloid differentiation in 32D cells [125].

The Down-syndrome associated kinase (DYRK1A) is co-expressed with Notch in many tissues during embryonic development. Fernandez-Martinez et al. have shown DYRK1A to phosphorylate NOTCH1 on up to 18 different Serines and Threonines in the Ankyrin-domain thereby attenuating Notch-mediated transcription [126]. Also AKT has been shown to phosphorylate NOTCH1 leading to down-regulated Notch-dependent transcription [118], however, as with DYRK1A the exact sites remain obscure.

During development in *Drosophila melanogaster*, the Notch protein undergoes tyrosine-phosphorylation on three sites (Y1850, Y1860, Y2097) yielding a phosphorylated population selectively associated with ABL-kinase components Disabled and Trio [127]. Despite the phosphorylation event not being required for complex formation, the authors hypothesize the importance of the phosphorylation in mediating downstream Notch/Abl signaling during axon patterning [127].

2.5.6. Sites targeted by unknown kinases

Several more phosphorylation sites have been discovered in phosphoproteomic studies with yet undetermined kinases acting on them. A complete list of discovered phosphosites is presented in Table 1, as well as reviewed in Borggreffe et al. [3]. It is clear that although mapping of the phosphorylation sites on NOTCH1 has yielded significant headway, the marathon to identify all the players phosphorylating the other Notch paralogs is only beginning. As a harbinger of cell fate decisions exhibiting both context and temporal dependency understanding dynamic Notch phosphorylation at different times in different physiological situations will be of crucial importance in the future.

2.6. Sumoylation of NICD

The PTM mediated by small-ubiquitin like modifier proteins (SUMO) regulates a wide variety of cellular functions including transcription, macromolecular assembly, chromatin organization, protein homeostasis, signal transduction, trafficking and DNA repair [128]. Sumoylation has already been shown to have a key role in nuclear function [129].

The roles of sumoylation within the Notch pathway, however, have only started to be uncovered in the last few years as we and others have shown that SUMO regulates critical aspects of Notch activity. We recently showed that heat stress and proteotoxic stress induce direct interaction between SUMO and N1-ICD in the nucleus and represses the expression of the classical Notch1 target genes *HES1*, *HEY1* and *HEY2* [130].

However, it had already previously been indicated over a decade

ago that sumoylation inhibits one or more components in the LIN-12/Notch signaling pathway in *Caenorhabditis elegans* [131]. More indications of SUMO modification regulating Notch signaling was obtained when it was shown that the LIM domain protein KyoT2, which negatively regulates Notch signaling by interacting with RBPJ, is a target protein for SUMO in a protein inhibitor of activated STAT1 (PIAS1)-catalyzed reaction [132]. The growth of NOTCH1-activated breast epithelial cells decreases by mitigation of global sumoylation by inhibition of the E1-activating complex SAE1/UBA2 and knockdown of the E2-conjugating enzyme UBC9 [133]. It has also been shown that N1-ICD sumoylation is increased in mesenchymal stem cells (MSCs) and HEK293T cells with stabilized hypoxia-inducible factor 1 alpha (HIF-1 α) expression [134].

A role and mechanism for NOTCH1 sumoylation has been proposed to occur in the endothelium. According to recent data, deletion of endothelial sentrin-specific protease 1 (SENPI1), which is the primary protease in sumoylation, enhances endothelial Notch sumoylation and prolongs NOTCH1 signaling, which suppresses vascular endothelial growth factor (VEGF) receptor signaling and angiogenesis. Thus, by targeting endothelial Notch signaling, sumoylation negatively regulates angiogenesis [228]. PIAS1 catalyzes the oxidative stress-induced sumoylation of the Notch signaling factor hairy and enhancer of split 1 (HES1), which represses the expression of GADD45 α and consequently enhances cell survival [137]. Interestingly, a SUMO proteomics strategy has recently indicated NOTCH2 to undergo conjugation by SUMO at K1353 and NOTCH3 at K2061 [138]. The physiological functions of these sumoylations are still to be discovered.

2.7. Ubiquitination of NICD

Ubiquitination adds ubiquitin groups to lysine residues modulating cellular localization and protein stability, with polyubiquitination being the standard tag for degradation by proteasomes. The lifespan of Notch is fairly short and its degradation occurs predominantly through polyubiquitin conjugation. Although ubiquitination of Notch does not always cause repressed Notch levels, ubiquitination-mediated proteasomal degradation of Notch is crucial to fine-tune the half-life of NICD. Failure in preventing prolonged Notch signaling causes serious diseases [139].

Many different ubiquitin E3 ligases have been discovered to ubiquitinate NICD. Ubiquitin conjugation of the membrane-tethered NOTCH1 by Itch [140] is facilitated by the mammalian protein NUMB, which also promotes the degradation of N1-ICD [141]. Recently it was shown that Shootin1 enhances the activity of the Notch pathway by interacting with LNX1/2 and stimulating NUMB ubiquitination, or by forming a complex with Itch and debilitating NICD ubiquitination [142]. Ligand activated NOTCH1 is prior to ADAM10 processing ubiquitinated by the E3 ubiquitin ligase DTX4 resulting in the endocytosis of the NOTCH1 extracellular domain by the ligand expressing cell, whereas the membrane attached fragment of Notch and Dtx4 are internalized by the NOTCH1 expressing cell [143]. In *Caenorhabditis elegans*, the E3 ubiquitin ligase Ubiquitin protein ligase E3 component n-recogin 5 (UBR5) negatively regulates GLP-1 and LIN12 activity, the two Notch receptors expressed by *Caenorhabditis elegans* [144]. The Ubiquitin-specific Protease 12 (USP12) is a negative regulator of Notch signaling as USP12 directly targets Notch and directs it to lysosomal degradation. Accordingly, USP12 silencing leads to less lysosomal degradation of Notch and consequently to a greater number of Notch at the cell membrane and consequently to higher activity of Notch [145].

Phosphorylation of the C-terminal PEST domain of N1-ICD has been shown to lead to subsequent ubiquitination by F-box/WD40 domain-containing protein 7 (FBXW7/SEL-10), which negatively regulates Notch signaling by targeting Notch for proteasome-mediated degradation [146–148]. Flaws in the N1-ICD-FBXW7 interaction are associated with leukemia and many solid cancers [149]. For instance, in T-cell leukemia (ATL) FBXW7 mutant proteins are defective to interact with

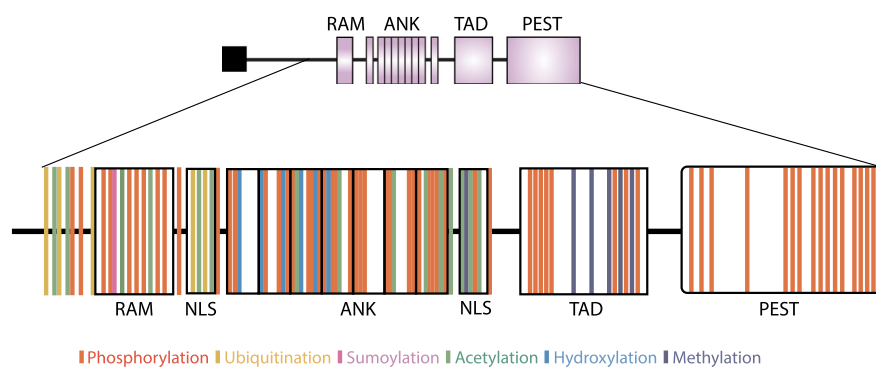


Fig. 3. Illustration of post-translational modifications of mammalian NICD (see Table 1). NICD is the active part of the Notch receptor, which can translocate to the nucleus after S3 cleavage and thereby regulate Notch target genes through RBPJ and co-activators. The NICD is composed of an RBPJ-associated molecule (RAM) domain, two nuclear localisation signals (NLS), six ankyrin (ANK) repeats, a transactivation domain (TAD) and a Pro-Glu-Ser-Thr (PEST) domain associated with NICD degradation.

N1-ICD resulting in increased N1-ICD levels and prolonged NOTCH1 signaling [150]. Interestingly, *FBXW7* gene mutations in leukemic cells do not only enhance the signaling efficiency of NOTCH1, but also mediate resistance to γ -secretase inhibitors [151]. Recently it was shown that also the E3 ubiquitin ligase ring finger protein 8 (RNF8) ubiquitinates N1-ICD resulting classically in the degradation of N1-ICD. In addition, low expression of full-length RNF8 correlates with bad prognosis for breast cancer patients [152].

However, ubiquitination does not always result in decreased N1-ICD levels. Ubiquitination of N1-ICD by the RING ubiquitin ligase RNF4 stabilizes and enhances the transcriptional activity of N1-ICD [153]. Similarly, N1-ICD ubiquitination by murine double minute 2 (MDM2) leads to activation and stimulation of N1-ICD transcriptional activity [154]. MDM2 also ubiquitinates N4-ICD, which stimulates an N4-ICD-Trp53 interaction, which in turn represses N4-ICD-induced anchorage-independent growth in mammary epithelial cells [155].

Ubiquitination is balanced to some extent by deubiquitinases (DUBs). The utilization of an *in vivo* RNA interference (RNAi) screen led to the discovery of several DUBs that regulate Notch signaling [156]. The loss of the deubiquitinating protein Ubiquitin carboxyl-terminal hydrolase 5 (Usp5) leads to the upregulation of Notch during *Drosophila melanogaster* eye development [157]. HES1 is a target for the E3 ubiquitin ligase SCFFBXL14 complex resulting in HES1 proteolysis, which further enhances neuronal differentiation [158].

3. PTMs of Notch ligands

Similarly to Notch receptors, the Notch ligands also have EGF-repeats in their ECD, which can be modified by glycosylation, but the functional effects are not immediately evident [14,159]. Ofut, the *O*-fucosylation protein crucial for correct Notch receptor activation in *Drosophila melanogaster* does not seem to be required for signal sending cells expressing Notch ligands [160]. Signal assays from mice lacking *Pofut1* also only show a need for POFUT1 in the signal receiving cells [161]. DLL1 harboring mutations in the *O*-fucosylation consensus sequences still activates Notch signaling, but does show some additional intracellular accumulation [162]. By contrast, recent evidence shows Fringe elongation of fucosylated sites on DLL3 affecting somitogenesis in mice. Here DLL3-mutants lacking the POFUT1 target site are unable to rescue the somitogenesis defects of mice lacking DLL3 [163]. *Drosophila melanogaster* Delta and Serrate have also been shown to be targets for *O*-GlcNAcylation by EOGT [68,164]. DLL1 has been shown to be phosphorylated at its intracellular domain. Three sites in mouse at T638, S693, and S696 were detected *in vitro* (two sites corresponding to S694 and S697 in human) while only S693 was found to be phosphorylated *in vivo* [165]. Interestingly, phosphorylation of S693 and S696 occurred sequentially and although the phosphorylations were required for full ligand activity *in vitro*, the authors found them dispensable for normal embryonic development in mice. All DSL ligands are potential targets of ubiquitination by E3 ligases and all mammalian Notch ligands have been reported to undergo ubiquitination (see

Table 1) [166,171]. Two notable E3 ligase families affecting endocytosis of Notch ligands through monoubiquitination have been documented in multiple different species, Neuralized (NEUR1–2 in mammals) and Mind Bomb (Mib) 1 and 2 [166,296]. The ubiquitin E3 ligase Neuralized (Neur) interacts with the Notch ligand Delta1 and represses its levels in *Xenopus laevis* [167] and *Drosophila melanogaster* [168]. NEUR1 and NEUR2 are dispensable for normal development in mice, even in *Neur1/2* double knock outs [169]. Removal of MIB1 results in embryonic lethality with Notch phenotypes [170]. MIB2 is not required for normal mouse development [171], but Mib2 in zebrafish has been able to partially rescue neurogenic and vasculogenic phenotypes of *Mib1* knock outs [297]. For reviews on Notch ligand ubiquitination and its effects on ligand endocytosis, see [166,293,294].

4. PTMs of the Notch transcriptional complex (CSL and MAML)

4.1. Acetylation, phosphorylation and ubiquitination of CSL

In *Drosophila melanogaster*, The Suppressor of Hairless (Su(H)), the homologue of mammalian CSL has been shown to be acetylated *in vivo* and to be at least partially deacetylated by the *Drosophila melanogaster* Sirt1 [102]. Sirt1 activity in *Drosophila melanogaster* has a stimulatory effect on Notch signaling, but it is uncertain if this is due to deacetylation of Su(H) or effects on other components of the Notch transcription complex [102]. Similarly phosphorylation of human CSL by the MAPK p38 at T339 induces ubiquitination and degradation of the CSL protein [172].

However, the phosphorylation of Su(H) can also modulate Su(H) activity in *Drosophila melanogaster*, depending on the phosphorylated residue. MAPK-mediated phosphorylation has been documented to occur both *in vitro* and *in vivo* at the beginning of the C-terminal domain (CTD) on T426 (corresponding to T352 in humans) slowing down the activator complex formation, possibly favoring the formation of the repressor complex and thus inhibiting transcriptional activity [173]. The authors also hypothesize this phosphorylation to be plausible in humans. Similarly another site in the beta-trefoil domain (BTD), on S269 (corresponding to S195 in humans) was also discovered in *Drosophila melanogaster* and is shown to inhibit the binding of BTD to DNA [174].

4.2. Acetylation, phosphorylation and ubiquitination of MAML

p300 interacts with MAML1 [91,93] and acetylates it on several lysine residues in the N-terminus, most prominently on K188/K189 [175]. Another acetyltransferase, CBP, has also been shown to acetylate MAML1 [176]. MAML1 promotes autoacetylation of p300 and its acetyltransferase activity [92] and the p300-MAML1 complex acetylates histone (H3/4) tails [175] as well as the N1-ICD [90]. Acetylation of MAML1 increases transcription of Notch target genes by promoting binding of the Notch co-activator PEAK1 related kinase-activating pseudokinase 1 (NACK) to the Notch transcription complex [176].

NACK recruits RNA polymerase II to the complex, which leads to initiation of transcription [176]. Importantly, inhibition of both p300/CBP (with C464) and Notch (with DAPT) has a synergistic inhibitory effect on the viability of oesophageal adenocarcinoma (EAC) cell lines and the size of EAC xenograft tumors [176]. As MAML3 is able to bind NACK through a lysine (K245) corresponding to MAML1 K188/189 it is possibly also a target for similar acetylation as MAML1 [176]. Interestingly, MAML2 does not contain a corresponding lysine or area based on amino acid sequence and does not bind NACK [176].

In addition, MAML induces phosphorylation of CBP/p300 via its second transcriptional activation domain (TAD2) during assembly of MAML into the Notch enhancer complex, however the direct implications of this phosphorylation on Notch signaling are unknown [91]. Likewise, NLK has been observed to phosphorylate MAML, yet also the function of this phosphorylation remains unknown [116].

MAML1 in the N1-ICD transcriptional complex is a subject for SUMO conjugation, which enhances the MAML1-HDAC7 interaction causing decreased MAML1 transcriptional activity [177]. Ubiquitin conjugation to MAML1 is inhibited by N1-ICD, but enhanced by p300, and decreased MAML1 ubiquitination causes increased transcriptional output in a Hes1 reporter assay [178].

5. Interplay between PTMs

One of the challenges of Notch-PTM research is the study of interplay between different dynamic modifications. Although direct impact of singular PTM-events has been established to influence protein-protein interactions and creating new binding sites on their own, a largely untouched aspect of post-translational modulation of Notch is the interactions between PTMs and their cumulative effects on protein function. How can the addition of a singular dynamic PTM affect the addition of another PTM in the same domain? How do the PTMs differ within the entire family of Notch paralogs? How do competitive modifiers targeting the same site affect PTM-saturation? What is the crosstalk between PTM “writers” and “erasers”? What is the effect of specific PTM-signatures? Although deciphering this complex map of interactions is still in its infancy, there is mounting evidence pointing towards a much more complex “molecular switchboard” than any modification on its own could achieve. These crosstalk mechanisms generate multiple different functional forms of a protein and provide a complex fine-tuning necessary for rapid responses within the cell. There already exists examples of this crosstalk on Notch, which we will highlight below, but there is also recent data on modification interactions from other fields that we will use to discuss future possibilities within the Notch-field.

A common form of post-translational crosstalk is when one PTM generates a signal for the addition or removal of another PTM, or when the first PTM simply allows a binding protein to carry out another modification. CK2 for example initially targets one site on N1-ICD for phosphorylation and subsequently another in a sequential manner ultimately leading to inhibited transcriptional complex activation [113]. A recent example of phosphorylation-mediated recognition of a binding protein comes from NOTCH4 phosphorylation mediating 14-3-3 association [119]. Phosphorylation can also create a signal that an E3 ligase can recognize for ubiquitin-mediated degradation. Short sequences that are targets for phosphorylation-dependent ubiquitination are called phosphodegrons. N1-ICD contains two phosphodegrons located in both of its terminal ends, although the C-terminal PEST domain contains what is considered the main phosphodegron of Notch [179]. Many kinases are known to enable docking for FBXW7 leading to subsequent ubiquitination and degradation of proteins [108]. In the case of Notch, CDK-mediated phosphorylation of NICD is one of the enablers for FBXW7-mediated degradation [110,111].

Another form of crosstalk comes from the competitive inhibition of a single site by different PTMs. Single lysines have been shown to be targets for both ubiquitination and sumoylation. Similarly acetylation,

methylation and ubiquitination of the same site have been identified in other proteins like ER α [180]. Acetylation has previously been shown to be able to stabilize proteins [181]. As such, lysines that can be modified by both acetylation and ubiquitination are likely to be competing in a form of negative crosstalk where acetylation stabilizes and ubiquitination can mark the protein for degradation.

The complex protein fine-tuning mediated by ubiquitination originates from the different ways ubiquitin chains can be generated. The ubiquitination events recognized by the proteasome for degradation depend on Lys48-linked chains, while Lys63 chains are used for various purposes including endocytotic recycling and sorting as well as activation of protein kinases [182]. In addition monoubiquitination events can regulate protein function independently [182]. Thereby the crosstalk between phosphorylation and ubiquitination can affect function in several ways starting from the fact that phosphorylation can both promote and inhibit ubiquitination.

PTMs may also function as mediators of coordinated regulation of signaling pathways. This applies in particular for SUMO conjugation. In some cases several proteins of the same pathway can be simultaneously multisumoylated, stabilizing the interaction between the modified proteins [183]. Furthermore, protein complexes may be held together by several SUMO-SUMO interacting motifs (SIMs) [184], and elimination of one SUMO site may thus have no major physiological consequences. Proteins engaged in interrelated complexes have been demonstrated to be subjects to synchronous SUMO2 modification in heat-shocked cells [185]. However, strong signaling network control may not be exclusive to SUMO-mediated regulation, but may extend to other PTMs as well, causing challenges in elucidating the physiological relevance of PTMs of a single target protein. Indeed, coordinated control of the activity of different components in the Notch signaling mechanism would constitute a dynamic, but robust cell-intrinsic system to regulate cellular responses to Notch activation.

In a more obscure form of crosstalk there is also a report on how both stem cells and cancer stem cells can be modified by the cis/trans isomerization protein prolyl-isomerase PIN1 in a way where NOTCH1 and NOTCH4 are able to escape proteasomal degradation by blocking FBXW7a-mediated ubiquitin ligation [186]. Most amino acids have a strong preference for trans peptide bond conformation, but proline with its cyclic structure can be catalyzed to form a cis bond which in the case of NOTCH1 and NOTCH4 leads to a steric hindrance for FBXW7a and in this case promotes tumor growth and metastasis *in vivo* [186].

While methylation of N1-ICD promotes its ubiquitination and proteasomal degradation, it also coincides with phosphorylation of N1-ICD [105]. It has also been shown that acetylation of N1-ICD by p300 inhibits its ubiquitination and *vice versa*, phosphorylation of N1-ICD by CDK8 in turn inhibits the acetylation and subsequently promotes ubiquitination and degradation of N1-ICD [90]. Both p300 and CDK8 are recruited to N1-ICD by MAML1 [91,93,110]. By contrast, another study has shown that acetylation promotes N3-ICD ubiquitination [89].

The dynamic regulation of phosphorylation is a complex entity on its own, and is mediated not by single actors but by a myriad of kinases and phosphatases acting in concert. Indeed, as published data shows us, different phosphorylation sites are often intertwined in complex arrangements and can sometimes overlap. The identified sites of GSK3 (T1861), ILK (2183), and NLK (S2121, S2198, S2215) all overlap with identified CDK-sites. In a similar fashion more complex relationships can form for example as the identified EYA1 phosphatase targets a GSK3 β -site (T2132). These discoveries imply that the system exhibits a fair share of redundancy with the arguably same functional modification being implemented by different mediators, however, the exact biological relevance of this overlap in sites is still not clear.

In a screen that identified over 2500 proteins being targets for SUMO E3 ligase-dependent sumoylation, it was revealed that protein kinases were particularly often conjugated by SUMO, indicating crosstalk between phosphorylation and sumoylation [188]. In addition, a site-specific mapping identified over 800 sumoylated peptides that

were co-modified with phosphorylation [138]. In another recent screen, which focused on the crosstalk between sumoylation and ubiquitination, the authors describe an improved method for enrichment of co-modified proteins. The screen identified 498 proteins which are modified by both sumoylation and ubiquitination, but the method can be applied to other combinations of PTMs [189]. The improvement in purification of proteins simultaneously modified by different PTMs could help reveal the network of Notch crosstalk as well.

6. Discussion

In this review we have glimpsed beneath the surface of the Notch-PTMome and shown that the devil is indeed in the details as many deceptively small dynamic modifications (Fig. 3) can have dramatic effects on Notch protein output. While certain PTMs of Notch have been described only in single publications, others have been more meticulously researched (Table 1). One of the most studied Notch modifications is glycosylation by POFUT1, POGLUT1 and FRINGE, where the effects of glycosylation and the embryonic lethality of glycosylation enzyme knockouts has been corroborated between several research groups. Also, the CDK-mediated phosphorylation of NICD and subsequent ubiquitination by FBXW7 represents one of the most robust examples of PTM-driven Notch fine-tuning. Many diseases are also linked to mutations of Notch-modulators (Table 2) and although in some cases (e.g. Dowling-Degos disease) the effects have been directly linked to Notch-modulation (POFUT1/POGLUT1/NOTCH1/DLL4), in others (e.g. Down's Syndrome) the dysregulated genes impact a larger proportion of the proteome with Notch being just one of the targets (DYRK1A). Thus certain large scale modulators e.g. AKT likely impacts many signaling pathways including Notch, via PTMs that can have implications in different disease contexts.

The complete post-translational roadmap of Notch is however far from finished. In the case of phosphorylation, as shown in Table 1, and by Borggreffe et al. [3] many novel phosphorylation sites have already been discovered in phosphoproteomic studies although info on kinases *i.e.* writers acting on these sites as well as their physiological relevance and output are yet to be determined. Furthermore, several *in silico* phosphosite finder tools are available, including The Human Protein Reference Database, where algorithms can be applied to specific protein sequences to find consensus motifs for all known kinases in specific proteins of interest. The effects of PTMs can also be increasingly studied *in silico*, with novel advanced protein-modeling programs. Already used

in drug development, modeling software packages can predict unknown structures of proteins based on other related proteins and provide information on protein-protein docking as well as how a PTM affects the interface of the protein and its docking capabilities with other proteins [190–194]. These kinds of modeling programs can then predict what kind of molecules might block specific PTM-sites to affect intermolecular interactions for therapeutic effects.

The current PTM data in the Notch-field across scientific publications sometimes contains unorganized and inconsistent data, which interfere with data utilization. Challenges arise with the use of multiple non-human protein species due to sequence homology mismatches, and with phosphosite discrepancies in published journals due to the use of different protein variants. A new accurate repository for Notch PTM sites, especially for human PTMs would certainly help analysis of data. We also urge the Notch field to implement the canonical 2555 aa NOTCH1, *i.e.* the most common polymorphic sequence variant presented by uniprot.org, for data consistency. This would allow for more meaningful utilization of the data.

Today, methods relying on MS and phospho-specific antibodies are the standard ways of detecting and analyzing PTMs. MS is the large-scale PTM tool for mapping *in vitro* sites, despite certain shortcomings for identifying actual physiologically relevant sites *in vivo*. These include the inability to determine the stoichiometry of a phosphorylation event and the fact that the increasingly higher detection sensitivity today enables detection of phosphorylated peptides at such low stoichiometry that the results may become irrelevant on a cellular scale [195]. This is especially true for *in vitro* phosphorylation studies that combine high kinase concentrations with long reaction times in the absence of phosphatases that are normally active in the cell. Luckily, quantitative MS is able to home in on physiologically relevant sites by also deciphering the stoichiometry of a phosphorylation reaction. When quantitative MS is not convenient or accessible, comparing the *in vitro* sites with *in vivo* data highly increases the relevance of the identified sites. Sites discovered *in vitro* that have been conserved through evolution also imply a functional role for the identified modification. Utilization of MS to gauge PTM levels in cancer patient samples for diagnostic and prognostic purposes has already shown potential [196]. Similarly, PTM levels in certain plasma biomarkers of glioblastoma were found changed using MS analysis, a finding that could provide diagnostic value in the future [197].

Despite these advances in PTM analyses, an obvious need still exists to both develop and utilize new methods in enrichment and analysis of

Table 2

Diseases associated with mutation of Notch-modifiers. Overview of diseases linked to mutations of Notch PTM writers or erasers. Although in certain diseases, PTM-modifier mutations link directly to Notch, e.g. POFUT1/POGLUT1 in Dowling-Degos disease (DDD), in others the impact on proteome function is broader, e.g. DYRK1A in Down's Syndrome, where Notch is only one of the many proteins affected.

Gene	PTM on Notch	Mutation	Disease	Reference
<i>AKT1</i>	Phosphorylation	Hyperactivation	Proteus syndrome	[265]
<i>CBP</i>	Acetylation	Loss of function	Rubinstein-Taybi syndrome	[266]
<i>CK2</i>	Phosphorylation	Loss of function	Intellectual disability and neurodevelopmental disorders	[267]
<i>Cyclin C — CDK3, 8, 19</i>	Phosphorylation	Loss of function	T-ALL (T-cell acute lymphoblastic leukemia)	[111]
<i>CDK8</i>	Phosphorylation	Loss of function	Syndromic developmental disorder	[268]
<i>DYRK1A</i>	Phosphorylation	Overexpression	Intellectual disability, especially in Down's Syndrome	[126,269]
<i>E3 ligases</i>	Ubiquitination	Loss of function	Fanconi's anemia, neurological diseases including ALS, Alzheimer's, multiple sclerosis, autism <i>etc.</i> (reviewed in [270])	[270,271]
<i>EOGT</i>	O-GlcNacylation	Loss of function	Adams-Oliver syndrome	[79,222]
<i>EYA1</i>	Dephosphorylation	Loss of function	Branchio-oto and branchio-oto-renal syndrome, congenital cataracts, ocular anterior segment anomalies	[272]
<i>FBXW7</i>	Ubiquitination	Loss of function	T-ALL (T-cell acute lymphoblastic leukemia)	[273]
<i>G-CSF</i>	Phosphorylation	Loss of function	Severe congenital neutropenia (SCN), acute myelogenous leukemia (AML)	[280,281,286]
<i>GSK3β</i>	Phosphorylation	Hyperactivation	Psychiatric diseases (bipolar disorder, depression, anxiety, schizophrenia), and neurological diseases (Alzheimer's, Parkinson's, multiple sclerosis)	[274,275]
<i>Lunatic FRINGE</i>	O-fucosylation	Loss of function	Spondylocostal dysostosis	[51]
<i>POFUT1</i>	O-fucosylation	Loss of function	Dowling-Degos disease (DDD), or reticular pigmented anomaly of the flexures	[64]
<i>POGLUT1</i>	O-glycosylation	Loss of function	DDD	[63]

PTM crosstalk and functionality. Today, several additional tools to study combinations of PTMs are already being developed. An interesting emerging toolset is the combination of sensitive MS with computational modeling allowing for analysis of systems level dynamics. Combinatorial bioinformatics such as multiple clustering analysis methodology (MCAM) could provide data on how the whole PTM network behaves following different input stimuli off the cells [198]. MCAM has already been used to study how receptor tyrosine kinases behave following EGF stimulation. In a similar fashion Gajadhar et al., used systems biology to analyze the effects of increased HER2 expression on the RTK network by quantifying tyrosine phosphorylation at different timepoints following HER2 stimulation. Using partial least squares regression algorithms (PLSR) on the signal-response data they identified the phosphorylation sites correlating with the cell response at each time point [199]. Another interesting method comes in the form of novel signal-seeker PTM kits that have shown promise in utilizing phospho-specific antibodies conjugated to affinity beads to detect endogenous levels of different PTMs [200,201]. This toolkit allows for simultaneous detection of multiple PTMs using a single lysis system, and thus enables an easy methodology for a more comprehensive snapshot of the proteome at any given time. Furthermore, the use of PTM-specific antibodies in combination with high-resolution immunofluorescence imaging can be utilized to reveal the location of modified proteins and can thereby also hint to their function in the cell. Simultaneous improvements in live-cell imaging can also make it possible to study modification events in real time. This kind of information could help decode when the modifications happen, where and as a consequence to which stimuli.

In the realm of therapeutics the promise of PTM-specific antibodies has yet to come into fruition, although concepts already exist. Ever since the development of the first therapeutic monoclonal antibody (mAb) in 1985 (muromonab-CD3) as an anti-rejection agent for organ transplantation, mAbs have been successfully used to treat several conditions including autoimmune-diseases, cardiovascular- and infectious diseases as well as cancer (reviewed in [202]). The use of mAbs in therapeutic use for blocking of plasma membrane proteins to inhibit complete signaling pathways or parts of them is a well-established concept that has already shown potential. Examples include the CTLA-4 and PD-1 mAbs utilized in modern immunotherapy [203] or the mAb 2F1 inhibiting the Wnt pathway coreceptor LRP6 [204] in the treatment of diabetic retinopathy. Indeed, one therapeutic avenue of PTM-specific mAb therapeutics may be to block PTM-specific signaling pathway interactions. However, as most PTMs occur intracellularly new methodology is required for targeting. Administration of so called intracellular antibodies, or intrabodies would require modification of the antibodies making them tolerant to cytosolic conditions and permeable to the cell membrane. Alternatively, a type of gene therapy could be used enabling vectors to produce the necessary antibodies within the cell [205].

The design and generation of PTM-specific antibodies on their own is however still difficult. This includes difficulties in molecular recognition of PTMs, however, recent engineering advances may allow for highly functional anti-PTM antibodies with exquisite specificity to be developed in the future (reviewed in [206]). PTM-antibodies have so far mostly been utilized in research and whether these will develop into a therapeutic modality remains to be seen. This futuristic vision would however allow for the selection of expected changes in the PTM makeup of a selected protein and the utilization of this information for the design of specific PTM-antibodies for different needs, for example to block certain PTM-specific outputs. These “PTM-epitopes” would thus enable another layer of specificity and allow for tailored therapeutic approaches.

In contrast to issues with low stoichiometries of phosphorylation sites, the *O*-glycans are present at high stoichiometries on NECD, which has enabled evaluation of relative levels of modification by recent mass spectral glycoproteomics [38,72]. As glycosylation regulates Notch

activity through extracellular NECD-modifications it might also be more amendable to therapeutics than targeting specific intracellular modifications. To this end, Schneider et al. recently introduced synthetic GDP-fucose analogs as substrates for POFUT1, which were added onto Notch EGF-repeats. These sugar analogs were able to inhibit DLL signaling with no significant effect on signaling from JAGGED1 [207]. It has also been suggested that the recent structural insights for XXYL1 [28], POGUT1 [208] and POFUT1 [209] could facilitate the development of inhibitors for these enzymes [210]. As xylosyltransferases, in contrast to *O*-glycosyltransferases, have been shown to reduce Notch signaling, there is an opportunity to increase or reduce Notch signaling depending on enzyme target.

The technological breakthrough of CRISPR (clustered regularly interspaced short palindromic repeats)/Cas (CRISPR-associated protein) has introduced a platform for efficient and specific genome editing. The RNA-guided nuclease Cas9 can be targeted to specific sequences in the genome upstream of a NGG or PAM-sequence making genetic editing easier than ever before. Introducing double-strand breaks (DSBs) with CRISPR allows for insertion of specific mutations that knock-out a gene of interest, a method adopted widely today. Indeed in the case of Notch, knocking out specific PTM writers and/or erasers can give useful information about the effects of PTMs on certain phenotypic effects related to Notch. However, in order to pinpoint the exact site for a particular modification and for deciphering the mechanism more meticulous examination is required. Although basic mutagenesis of expression plasmids in cells *in vitro* will give an overview of the effects, in order to study it on the endogenous level CRISPR can again be utilized. By taking advantage of homology-directed repair (HDR) with single-stranded oligo-donor nucleotides (ssODNs) as templates its now possible to make single amino acid substitutions in the form of serine to alanine phospho-dead mutants ($S \rightarrow A$) for probing the effects of PTMs [211]. Although CRISPR/Cas9 is advancing the field of biology on a big scale, the more advanced CRISPR techniques have been suffering from low efficiency. However, as the methods and procedures for single amino-acid substitutions and knock-in insertions are improved [212–214] so will our abilities to probe the functional effects of multiple modified sites. With that said, the PTMs occurring in the cell can be very rapid and are highly dynamic in order to accommodate constantly changing signals in the cell. In the case of phosphorylation for example, an amino acid substitution can be utilized to mimic a phosphorylated form, e.g. serine to glutamate ($S \rightarrow E$). These types of PTM-mutants where the protein is locked in a certain position mimicking phosphorylation can give rise to phenotypes similar to in a phosphorylated situation. These mimics may however not behave as their true physiological counterparts not only because of the biochemical difference in glutamate compared to phosphorylated serine but also as *in vivo* phosphorylations are often rapidly reversed. This is another highlight of the complexity and difficulty of accurately registering and studying such rapid and dynamic effects. Nevertheless, new emerging technologies continue to push the envelope on what is technologically possible and will in the future allow for decoding the complexity associated with Notch output and help us devise new therapeutic strategies targeting PTMs.

A largely unstudied layer of regulation of Notch is by the PTMs of the auxiliary proteins of the Notch pathway, i.e. of proteins that bind to the trimeric-complex of NICD/MAML/CSL and facilitate either gene expression or repression. Indeed, many contact-dependent signaling pathways rely on dynamic switching between activation and repression states mediated by co-activators and co-repressors leading to changes in specific chromatin and histone tail modifications. In the case for Notch certain examples of PTM-mediated co-mediator control exist, such as that of the p21-activated kinase PAK1 that has been shown to phosphorylate SHARP thus enhancing the SHARP-mediated repression of Notch target gene expression [215]. Similarly, the Notch-repressor Nuclear hormone receptor corepressor (NCoR) is phosphorylated by CK2 α [216] and this phosphorylated NCoR can bind SHARP together

with HDAC thus replacing the Notch-activating Lysine methyltransferase 2D (KMT2D) [217]. These complicated relationships between Notch-complex cofactors and chromatin modifying proteins are outside the scope of this review, however the examples herein aid in illuminating the importance of this research area, which will hopefully lead us closer to a complete understanding of the harmonious symphony of PTMs in the Notch pathway.

7. Conclusion

PTMs add a crucial layer of fine-tuning and regulation of cellular processes. PTMs can provide an immediate response to cell cues to modulate protein function, including stability, protein-protein interactions, localization and activity. The highly dynamic nature of many PTMs makes the characterization and functional relationship between different modifications a big challenge. Furthermore the latest development of techniques have not been fully utilized within the Notch field to understand the intricate effects that PTMs can have on the cellular decisions and their contribution to pathophysiology. Advancements in enrichment methods and in high-resolution MS have led to an enormous increase in data, but the biological relevance and function of the identified sites as well as their combinatorial effects are largely unknown. Generating multiple phosphospecific antibodies by for instance using phage display libraries could show the status of many individual phosphosites during different timepoints and reveal the activation state of Notch components. *In silico* methods such as applying computational algorithms to combine different datasets, provides a promise for untangling the highly dynamic processes of PTM crosstalk, protein-protein interactions and their combined effect on cellular output. Combining the latest advancements in PTM identification with functional predictions of their output on function could help explain a great deal of the pleiotropism of Notch signaling.

Author contributions

D.A., C.A, K.K. and S.K.-J.L. and C.S. researched data for the article and wrote the manuscript. All authors contributed to discussion of content and reviewed the manuscript before submission.

Transparency document

The [Transparency document](#) associated with this article can be found, in online version.

Declaration of Competing Interest

The authors declare no conflict of interest. Funding agencies have been disclosed in the manuscript and in this document.

Acknowledgements

The authors wish to thank Käthe M. Dahlström for assistance and helpful discussions, Kalen Lin for work on the figures and Oscar Stassen for comments on the manuscript.

Funding

This work was supported by Jane and Aatos Erkko Foundation (S.K.-J.L., C.S), K. Albin Johansson Foundation (S.K.-J.L., C.A, D.A), The Swedish Cultural foundation of Finland (S.K.-J.L., C.A, D.A), The Finnish Cultural Foundation (C.A, D.A), Ida Montin Foundation (C.A, D.A), Victoriastiftelsen (C.A, D.A) and Åbo Akademi University MolBio (D.A). C.S is also supported by European Research Council – ForceMorph #771168, Academy of Finland # 307133, Sigrid Juselius Foundation, Liv och Hälsa Foundation and the Cancer Society of Finland.

References

- [1] R.A. Kovall, et al., The canonical notch signaling pathway: structural and biochemical insights into shape, sugar, and force, *Dev. Cell* 41 (3) (2017) 228–241.
- [2] S.J. Bray, Notch signalling in context. *Nature Reviews, Molecular Cell Biology* 17 (11) (2016) 722–735.
- [3] T. Borggrefe, et al., The Notch intracellular domain integrates signals from Wnt, Hedgehog, TGF β /BMP and hypoxia pathways, *Biochim. Biophys. Acta Mol. Cell Res.* 1863 (2) (2016) 303–313.
- [4] M.V. Gustafsson, et al., Hypoxia requires notch signaling to maintain the undifferentiated cell state, *Dev. Cell* 9 (5) (2005) 617–628.
- [5] Z. Kyselova, et al., Alterations in the serum glycome due to metastatic prostate cancer, *J. Proteome Res.* 6 (5) (2007) 1822–1832.
- [6] J. Zhao, et al., N-linked glycosylation profiling of pancreatic cancer serum using capillary liquid phase separation coupled with mass spectrometric analysis, *J. Proteome Res.* 6 (3) (2007) 1126–1138.
- [7] C.M. Blauemueller, et al., Intracellular cleavage of Notch leads to a heterodimeric receptor on the plasma membrane, *Cell* 90 (2) (1997) 281–291.
- [8] K. Brückner, et al., Glycosyltransferase activity of fringe modulates Notch-Delta interactions, *Nature* 406 (6794) (2000) 411–415.
- [9] D.J. Moloney, et al., Mammalian Notch1 is modified with two unusual forms of O-linked glycosylation found on epidermal growth factor-like modules, *J. Biol. Chem.* 275 (13) (2000) 9604–9611.
- [10] M. Acar, et al., Rumi is a CAP10 domain glycosyltransferase that modifies notch and is required for notch signaling, *Cell* 132 (2) (2008) 247–258.
- [11] Y. Luo, R.S. Haltiwanger, O-fucosylation of Notch occurs in the endoplasmic reticulum, *J. Biol. Chem.* 280 (12) (2005) 11289–11294.
- [12] H. Takeuchi, R.S. Haltiwanger, Significance of glycosylation in Notch signaling, *Biochem. Biophys. Res. Commun.* 453 (2) (2014) 235–242.
- [13] M.T. Boskovski, et al., The heterotaxy gene GALNT11 glycosylates Notch to orchestrate cilia type and laterality, *Nature* 504 (7480) (2013) 456–459.
- [14] R. Rampal, K.B. Luther, R.S. Haltiwanger, Notch signaling in normal and disease states: possible therapies related to glycosylation, *Curr. Mol. Med.* 7 (4) (2007) 427–445.
- [15] N.A. Rana, et al., O-glucose trisaccharide is present at high but variable stoichiometry at multiple sites on mouse Notch1, *J. Biol. Chem.* 286 (36) (2011) 31623–31637.
- [16] D.J. Moloney, et al., Fringe is a glycosyltransferase that modifies Notch, *Nature* 406 (6794) (2000) 369–375.
- [17] R. Rampal, et al., Lunatic fringe, manic fringe, and radical fringe recognize similar specificity determinants in O-fucosylated epidermal growth factor-like repeats, *J. Biol. Chem.* 280 (51) (2005) 42454–42463.
- [18] M.K. Sethi, et al., Identification of glycosyltransferase 8 family members as xylosyltransferases acting on O-glucosylated notch epidermal growth factor repeats, *J. Biol. Chem.* 285 (3) (2010) 1582–1586.
- [19] S. Artavanis-Tsakonas, M.D. Rand, R.J. Lake, Notch signaling: cell fate control and signal integration in development, *Science* 284 (5415) (1999) 770–776.
- [20] R. Kopan, M.X.G. Ilagan, The canonical Notch signaling pathway: unfolding the activation mechanism, *Cell* 137 (2) (2009) 216–233.
- [21] J. Chen, D.J. Moloney, P. Stanley, Fringe modulation of Jagged1-induced Notch signaling requires the action of beta 4galactosyltransferase-1, *Proc. Natl. Acad. Sci. U. S. A.* 98 (24) (2001) 13716–13721.
- [22] R. Fernandez-Valdivia, et al., Regulation of mammalian Notch signaling and embryonic development by the protein O-glycosyltransferase Rumi, *Development* 138 (10) (2011) 1925–1934.
- [23] H. Takeuchi, et al., Rumi functions as both a protein O-glycosyltransferase and a protein O-xylosyltransferase, *Proc. Natl. Acad. Sci. U. S. A.* 108 (40) (2011) 16600–16605.
- [24] H. Takeuchi, et al., O-glycosylation modulates the stability of epidermal growth factor-like repeats and thereby regulates Notch trafficking, *J. Biol. Chem.* 292 (38) (2017) 15964–15973.
- [25] N. Ramkumar, et al., Protein O-glycosyltransferase 1 (POGLUT1) promotes mouse gastrulation through modification of the apical polarity protein CRUMBS2, in: A. Sutherland (Ed.), *PLOS Genetics*, 11(10) 2015, p. e1005551.
- [26] M.K. Sethi, et al., Molecular cloning of a xylosyltransferase that transfers the second xylose to O-glucosylated epidermal growth factor repeats of notch, *J. Biol. Chem.* 287 (4) (2012) 2739–2748.
- [27] T.V. Lee, et al., Negative regulation of notch signaling by xylose, in: N. Perrimon (Ed.), *PLoS Genetics*, 9(6) 2013, p. e1003547.
- [28] H. Yu, et al., Notch-modifying xylosyltransferase structures support an SNI-like retaining mechanism, *Nat. Chem. Biol.* 11 (11) (2015) 847–854.
- [29] K. Matsumoto, et al., Dual roles of O-glucose glycans redundant with monosaccharide O-Fucose on Notch in Notch trafficking, *J. Biol. Chem.* 291 (26) (2016) 13743–13752.
- [30] V.C. Luca, et al., Structural biology. Structural basis for Notch1 engagement of Delta-like 4, *Science* 347 (6224) (2015) 847–853.
- [31] H. Takeuchi, et al., Two novel protein O-glycosyltransferases that modify sites distinct from POGLUT1 and affect Notch trafficking and signaling, *Proc. Natl. Acad. Sci. U. S. A.* 115 (36) (2018) E8395–E8402.
- [32] S. Shi, P. Stanley, Protein O-fucosyltransferase 1 is an essential component of Notch signaling pathways, *Proc. Natl. Acad. Sci. U. S. A.* 100 (9) (2003) 5234–5239.
- [33] K.D. Irvine, E. Wieschaus, Fringe, a boundary-specific signaling molecule, mediates interactions between dorsal and ventral cells during *Drosophila* wing development, *Cell* 79 (4) (1994) 595–606.

- [34] X. Hou, Y. Tashima, P. Stanley, Galactose differentially modulates lunatic and manic fringe effects on Delta1-induced NOTCH signaling, *J. Biol. Chem.* 287 (1) (2012) 474–483.
- [35] P. Taylor, et al., Fringe-mediated extension of O-linked fucose in the ligand-binding region of Notch1 increases binding to mammalian Notch ligands, *Proc. Natl. Acad. Sci. U. S. A.* 111 (20) (2014) 7290–7295.
- [36] L.-T. Yang, et al., Fringe glycosyltransferases differentially modulate Notch1 proteolysis induced by Delta1 and Jagged1, *Mol. Biol. Cell* 16 (2) (2005) 927–942.
- [37] V.M. Panin, et al., Fringe modulates Notch-ligand interactions, *Nature* 387 (6636) (1997) 908–912.
- [38] S. Kakuda, R.S. Haltiwanger, Deciphering the fringe-mediated notch code: identification of activating and inhibiting sites allowing discrimination between ligands, *Dev. Cell* 40 (2) (2017) 193–201.
- [39] L. LeBon, et al., Fringe proteins modulate Notch-ligand cis and trans interactions to specify signaling states, *eLife* 3 (2014) e02950.
- [40] C. Hicks, et al., Fringe differentially modulates Jagged1 and Delta1 signalling through Notch1 and Notch2, *Nat. Cell Biol.* 2 (8) (2000) 515–520.
- [41] V.C. Luca, et al., Notch-jagged complex structure implicates a catch bond in tuning ligand sensitivity, *Science* 355 (6331) (2017) 1320–1324.
- [42] Y.A. Ervard, et al., Lunatic fringe is an essential mediator of somite segmentation and patterning, *Nature* 394 (6691) (1998) 377–381.
- [43] K. Wahi, M.S. Bochter, S.E. Cole, The many roles of notch signaling during vertebrate somitogenesis, *Semin. Cell Dev. Biol.* 49 (2016) 68–75.
- [44] N. Zhang, T. Gridley, Defects in somite formation in lunatic fringe-deficient mice, *Nature* 394 (6691) (1998) 374–377.
- [45] R. Benedetto, et al., The notch ligands Dll4 and Jagged1 have opposing effects on angiogenesis, *Cell* 137 (6) (2009) 1124–1135.
- [46] Z. Liu, et al., The extracellular domain of Notch2 increases its cell-surface abundance and ligand responsiveness during kidney development, *Dev. Cell* 25 (6) (2013) 585–598.
- [47] P. Stanley, C.J. Guidos, Regulation of Notch signaling during T- and B-cell development by O-fucose glycans, *Immunol. Rev.* 230 (1) (2009) 201–215.
- [48] J.L. Moran, et al., Manic fringe is not required for embryonic development, and fringe family members do not exhibit redundant functions in the axial skeleton, limb, or hindbrain, *Developmental Dynamics: an official publication of the American Association of Anatomists* 238 (7) (2009) 1803–1812.
- [49] J.L. Moran, J.M. Levorse, T.F. Vogt, Limbs move beyond the radical fringe, *Nature* 399 (6738) (1999) 742–743.
- [50] N. Zhang, C.R. Norton, T. Gridley, Segmentation defects of notch pathway mutants and absence of a synergistic phenotype in lunatic fringe/radical fringe double mutant mice, *Genesis* 33 (1) (2002) 21–28.
- [51] D.B. Sparrow, et al., Mutation of the LUNATIC FRINGE gene in humans causes spondylocostal dysostosis with a severe vertebral phenotype, *Am. J. Hum. Genet.* 78 (1) (2006) 28–37.
- [52] K. Xu, et al., Lunatic fringe deficiency cooperates with the Met/Caveolin gene amplicon to induce basal-like breast cancer, *Cancer Cell* 21 (5) (2012) 626–641.
- [53] S. Zhang, et al., Manic fringe promotes a claudin-low breast cancer phenotype through notch-mediated PIK3CG induction, *Cancer Res.* 75 (10) (2015) 1936–1943.
- [54] Y. Song, et al., Lunatic, manic, and radical fringe each promote T and B cell development, *J. Immunol.* 196 (1) (2016) 232–243.
- [55] T. Okajima, et al., Contributions of chaperone and glycosyltransferase activities of O-fucosyltransferase 1 to Notch signaling, *BMC Biol.* 6 (1) (2008) 1.
- [56] T. Okajima, A. Xu, K.D. Irvine, Modulation of Notch-ligand binding by protein O-fucosyltransferase 1 and fringe, *J. Biol. Chem.* 278 (43) (2003) 42340–42345.
- [57] T. Sasamura, et al., The O-fucosyltransferase O-fut1 is an extracellular component that is essential for the constitutive endocytic trafficking of Notch in *Drosophila*, *Development* 134 (7) (2007) 1347–1356.
- [58] A. Ishio, et al., O-fucose monosaccharide of *Drosophila* Notch has a temperature-sensitive function and cooperates with O-glucose glycan in Notch transport and Notch signaling activation, *J. Biol. Chem.* 290 (1) (2015) 505–519.
- [59] R.A. Kroes, G. Dawson, J.R. Moskal, Focused microarray analysis of glyco-gene expression in human glioblastomas, *Journal of Neurochemistry*, 103 Suppl 1 (s1) (2007) 14–24.
- [60] L.W.M. Loo, et al., Integrated analysis of genome-wide copy number alterations and gene expression in microsatellite stable, CpG island methylator phenotype-negative colon cancer, *Genes, Chromosomes & Cancer* 52 (5) (2013) 450–466.
- [61] E.T. Sawey, et al., Identification of a therapeutic strategy targeting amplified FGF19 in liver cancer by oncogenomic screening, *Cancer Cell* 19 (3) (2011) 347–358.
- [62] S. Yokota, et al., Protein O-fucosyltransferase 1: a potential diagnostic marker and therapeutic target for human oral cancer, *Int. J. Oncol.* 43 (6) (2013) 1864–1870.
- [63] F.B. Basmanav, et al., Mutations in POGUT1, encoding protein O-glycosyltransferase 1, cause autosomal-dominant Dowling-Degos disease, *Am. J. Hum. Genet.* 94 (1) (2014) 135–143.
- [64] M. Li, et al., Mutations in POFUT1, encoding protein O-fucosyltransferase 1, cause generalized Dowling-Degos disease, *Am. J. Hum. Genet.* 92 (6) (2013) 895–903.
- [65] D. Yao, et al., Protein O-fucosyltransferase 1 (Pofut1) regulates lymphoid and myeloid homeostasis through modulation of Notch receptor ligand interactions, *Blood* 117 (21) (2011) 5652–5662.
- [66] S. Zhang, et al., Tumor-suppressive activity of lunatic fringe in prostate through differential modulation of notch receptor activation, *Neoplasia* 16 (2) (2014) 158–167.
- [67] J.F. Alfaro, et al., Tandem mass spectrometry identifies many mouse brain O-GlcNAcylated proteins including EGF domain-specific O-GlcNAc transferase targets, *Proc. Natl. Acad. Sci.* 109 (19) (2012) 7280–7285.
- [68] A. Matsuura, et al., O-linked N-Acetylglucosamine is present on the extracellular domain of Notch receptors, *J. Biol. Chem.* 283 (51) (2008) 35486–35495.
- [69] Y. Sakaidani, et al., O-linked-N-acetylglucosamine modification of mammalian Notch receptors by an atypical O-GlcNAc transferase Eogt1, *Biochem. Biophys. Res. Commun.* 419 (1) (2012) 14–19.
- [70] Y. Sakaidani, et al., O-linked-N-acetylglucosamine on extracellular protein domains mediates epithelial cell–matrix interactions, *Nat. Commun.* 2 (1) (2011) 583.
- [71] M. Ogawa, et al., Structural divergence in O-GlcNAc glycans displayed on epidermal growth factor-like repeats of mammalian Notch1, *Molecules* 23 (7) (2018) 1745.
- [72] B.M. Harvey, et al., Mapping sites of O-glycosylation and fringe elongation on *Drosophila* Notch, *J. Biol. Chem.* 291 (31) (2016) 16348–16360.
- [73] M. Ogawa, T. Okajima, Structure and function of extracellular O-GlcNAc, *Curr. Opin. Struct. Biol.* 56 (2019) 72–77.
- [74] S. Hased, et al., Adams-Oliver syndrome review of the literature: refining the diagnostic phenotype, *Am. J. Med. Genet. A* 173 (3) (2017) 790–800.
- [75] S.J. Hased, et al., RBPJ mutations identified in two families affected by Adams-Oliver syndrome, *Am. J. Hum. Genet.* 91 (2) (2012) 391–395.
- [76] J.A.N. Meester, et al., Heterozygous loss-of-function mutations in DLL4 cause Adams-Oliver syndrome, *Am. J. Hum. Genet.* 97 (3) (2015) 475–482.
- [77] M. Ogawa, et al., Impaired O-linked N-acetylglucosaminylation in the endoplasmic reticulum by mutated epidermal growth factor (EGF) domain-specific N-acetylglucosamine transferase found in Adams-Oliver syndrome, *J. Biol. Chem.* 290 (4) (2015) 2137–2149.
- [78] A.-B. Stittrich, et al., Mutations in NOTCH1 cause Adams-Oliver syndrome, *Am. J. Hum. Genet.* 95 (3) (2014) 275–284.
- [79] R. Shaheen, et al., Mutations in EOGT confirm the genetic heterogeneity of autosomal-recessive Adams-Oliver syndrome, *Am. J. Hum. Genet.* 92 (4) (2013) 598–604.
- [80] S. Sawaguchi, et al., O-GlcNAc on NOTCH1 EGF repeats regulates ligand-induced Notch signaling and vascular development in mammals, *eLife* 6 (2017).
- [81] C.W. Pugh, P.J. Ratcliffe, New horizons in hypoxia signaling pathways, *Exp. Cell Res.* 356 (2) (2017) 116–121.
- [82] M.L. Coleman, et al., Asparaginyl hydroxylation of the Notch ankyrin repeat domain by factor inhibiting hypoxia-inducible factor, *J. Biol. Chem.* 282 (33) (2007) 24027–24038.
- [83] S.E. Wilkins, et al., Differences in hydroxylation and binding of Notch and HIF-1 α demonstrate substrate selectivity for factor inhibiting HIF-1 (FIH-1), *Int. J. Biochem. Cell Biol.* 41 (7) (2009) 1563–1571.
- [84] X. Zheng, et al., Interaction with factor inhibiting HIF-1 defines an additional mode of cross-coupling between the Notch and hypoxia signaling pathways, *Proc. Natl. Acad. Sci. U. S. A.* 105 (9) (2008) 3368–3373.
- [85] M.E. Cockman, et al., Proteomics-based identification of novel factor inhibiting hypoxia-inducible factor (FIH) substrates indicates widespread asparaginyl hydroxylation of ankyrin repeat domain-containing proteins, *Molecular & Cellular Proteomics: MCP* 8 (3) (2009) 535–546.
- [86] H. Kurooka, T. Honjo, Functional interaction between the mouse notch1 intracellular region and histone acetyltransferases PCAF and GCN5, *J. Biol. Chem.* 275 (22) (2000) 17211–17220.
- [87] F. Oswald, et al., p300 acts as a transcriptional coactivator for mammalian Notch-1, *Mol. Cell. Biol.* 21 (22) (2001) 7761–7774.
- [88] V. Guarani, et al., Acetylation-dependent regulation of endothelial Notch signaling by the SIRT1 deacetylase, *Nature* 473 (7346) (2011) 234–238.
- [89] R. Palermo, et al., Acetylation controls Notch3 stability and function in T-cell leukemia, *Oncogene* 31 (33) (2012) 3807–3817.
- [90] A.E. Popko-Scibor, et al., Ubiquitination of Notch1 is regulated by MAML1-mediated p300 acetylation of Notch1, *Biochem. Biophys. Res. Commun.* 416 (3–4) (2011) 300–306.
- [91] C.J. Fryer, et al., Mastermind mediates chromatin-specific transcription and turnover of the Notch enhancer complex, *Genes Dev.* 16 (11) (2002) 1397–1411.
- [92] M.L. Hansson, et al., The transcriptional coactivator MAML1 regulates p300 acetylation and HAT activity, *Nucleic Acids Res.* 37 (9) (2009) 2996–3006.
- [93] A.E. Wallberg, et al., p300 and PCAF act cooperatively to mediate transcriptional activation from chromatin templates by notch intracellular domains in vitro, *Mol. Cell. Biol.* 22 (22) (2002) 7812–7819.
- [94] Y. Kida, J.A. Zullo, M.S. Goligorsky, Endothelial sirtuin 1 inactivation enhances capillary rarefaction and fibrosis following kidney injury through Notch activation, *Biochem. Biophys. Res. Commun.* 478 (3) (2016) 1074–1079.
- [95] P. Wang, et al., Intracellular NAMPT-NAD⁺-SIRT1 cascade improves post-ischaemic vascular repair by modulating Notch signalling in endothelial progenitors, *Cardiovasc. Res.* 104 (3) (2014) 477–488.
- [96] M. Xie, M. Liu, C.-S. He, SIRT1 regulates endothelial Notch signaling in lung cancer, in: N.A. Hotchin (Ed.), *PLoS One*, 7(9) 2012, p. e45331.
- [97] C.-Y. Ma, et al., SIRT1 suppresses self-renewal of adult hippocampal neural stem cells, *Development (Cambridge, England)* 141 (24) (2014) 4697–4709.
- [98] N. Marcel, et al., The lysine deacetylase Sirtuin 1 modulates the localization and function of the Notch1 receptor in regulatory T cells, *Sci. Signal.* 10 (473) (2017) eah4679.
- [99] X. Bai, et al., Acetylation-dependent regulation of Notch signaling in macrophages by SIRT1 affects sepsis development, *Front. Immunol.* 9 (2018) 762.
- [100] C. Collesi, et al., Reversible Notch1 acetylation tunes proliferative signalling in cardiomyocytes, *Cardiovasc. Res.* 114 (1) (2018) 103–122.
- [101] J. Ban, et al., Suppression of deacetylase SIRT1 mediates tumor-suppressive NOTCH response and offers a novel treatment option in metastatic Ewing sarcoma, *Cancer Res.* 74 (22) (2014) 6578–6588.

- [102] M. Horvath, et al., The silent information regulator 1 (Sirt1) is a positive regulator of the Notch pathway in *Drosophila*, *The Biochemical Journal* 473 (22) (2016) 4129–4143.
- [103] H. Zhang, et al., Notch3 overexpression enhances progression and chemoresistance of urothelial carcinoma, *Oncotarget* 8 (21) (2017) 34362–34373.
- [104] M. Pinazza, et al., Histone deacetylase 6 controls Notch3 trafficking and degradation in T-cell acute lymphoblastic leukemia cells, *Oncogene* 37 (28) (2018) 3839–3851.
- [105] K. Hein, et al., Site-specific methylation of Notch1 controls the amplitude and duration of the Notch1 response, *Sci. Signal.* 8 (369) (2015) ra30.
- [106] A. Guo, et al., Immunoaffinity enrichment and mass spectrometry analysis of protein methylation, *Molecular & Cellular Proteomics: MCP* 13 (1) (2014) 372–387.
- [107] D.R. Foltz, J.S. Nye, Hyperphosphorylation and association with RBP of the intracellular domain of Notch1, *Biochem. Biophys. Res. Commun.* 286 (3) (2001) 484–492.
- [108] H.-J. Lee, M.-Y. Kim, H.-S. Park, Phosphorylation-dependent regulation of Notch1 signaling: the fulcrum of Notch1 signaling, *BMB Rep.* 48 (8) (2015) 431–437.
- [109] The UniProt Consortium, UniProt: the universal protein knowledgebase, *Nucleic Acids Res.* 45 (D1) (2017) D158–D169.
- [110] C.J. Fryer, J.B. White, K.A. Jones, Mastermind recruits CycC:CDK8 to phosphorylate the notch ICD and coordinate activation with turnover, *Mol. Cell* 16 (4) (2004) 509–520.
- [111] N. Li, et al., Cyclin C is a haploinsufficient tumour suppressor, *Nat. Cell Biol.* 16 (11) (2014) 1080–1091.
- [112] F.A. Carrieri, et al., CDK1 and CDK2 regulate NICD1 turnover and the periodicity of the segmentation clock, *EMBO Rep.* (2019) e46436.
- [113] P. Ranganathan, et al., Hierarchical phosphorylation within the ankyrin repeat domain defines a phosphoregulatory loop that regulates notch transcriptional activity, *J. Biol. Chem.* 286 (33) (2011) 28844–28857.
- [114] J.-S. Mo, et al., Integrin-linked kinase controls Notch1 signaling by Down-regulation of protein stability through Fbw7 ubiquitin ligase, *Mol. Cell Biol.* 27 (15) (2007) 5565–5574.
- [115] B. LaFoya, et al., Src kinase phosphorylates Notch1 to inhibit MAML binding, *Sci. Rep.* 8 (1) (2018) 15515.
- [116] T. Ishitani, et al., Nemo-like kinase suppresses Notch signalling by interfering with formation of the Notch active transcriptional complex, *Nat. Cell Biol.* 12 (3) (2010) 278–285.
- [117] N.M. Santio, et al., Phosphorylation of Notch1 by Pim kinases promotes oncogenic signaling in breast and prostate cancer cells, *Oncotarget* 7 (28) (2016) 43220–43238.
- [118] J. Song, et al., Down-regulation of Notch-dependent transcription by Akt in vitro, *FEBS Lett.* 582 (12) (2008) 1693–1699.
- [119] G. Ramakrishnan, et al., AKT and 14-3-3 regulate Notch4 nuclear localization, *Sci. Rep.* 5 (1) (2015) 8782.
- [120] M. Sjöqvist, et al., PKC ζ regulates Notch receptor routing and activity in a Notch signaling-dependent manner, *Cell Res.* 24 (4) (2014) 433–450.
- [121] D.R. Foltz, et al., Glycogen synthase kinase-3 β modulates notch signaling and stability, *Current Biology: CB* 12 (12) (2002) 1006–1011.
- [122] Y.H. Jin, et al., Regulation of Notch1/NICD and Hes1 expressions by GSK-3 α /beta, *Molecules and Cells* 27 (1) (2009) 15–19.
- [123] L. Espinosa, et al., Phosphorylation by glycogen synthase kinase-3 beta down-regulates Notch activity, a link for Notch and Wnt pathways, *J. Biol. Chem.* 278 (34) (2003) 32227–32235.
- [124] H. Zhang, et al., An Eya1-Notch axis specifies bipotential epibranchial differentiation in mammalian craniofacial morphogenesis, *eLife* 6 (2017).
- [125] J. Ingles-Esteve, et al., Phosphorylation of Ser2078 modulates the Notch2 function in 32D cell differentiation, *J. Biol. Chem.* 276 (48) (2001) 44873–44880.
- [126] J. Fernandez-Martinez, et al., Attenuation of Notch signaling by the Down-syndrome-associated kinase DYRK1A, *J. Cell Sci.* 122 (10) (2009) 1574–1583.
- [127] R. Kannan, et al., Tyrosine phosphorylation and proteolytic cleavage of Notch are required for non-canonical Notch/Abl signaling in *Drosophila* axon guidance, *Development* (2017) dev.151548.
- [128] A. Flotho, F. Melchior, Sumoylation: a regulatory protein modification in health and disease, *Annu. Rev. Biochem.* 82 (1) (2013) 357–385.
- [129] J.M. Henley, T.J. Craig, K.A. Wilkinson, Neuronal SUMOylation: mechanisms, physiology, and roles in neuronal dysfunction, *Physiol. Rev.* 94 (4) (2014) 1249–1285.
- [130] C.J.M. Antila, et al., Sumoylation of Notch1 represses its target gene expression during cell stress, *Cell Death & Differentiation* 25 (3) (2018) 600–615.
- [131] G. Poulin, et al., Chromatin regulation and sumoylation in the inhibition of Ras-induced vulval development in *Caenorhabditis elegans*, *EMBO J.* 24 (14) (2005) 2613–2623.
- [132] J. Wang, et al., The transcriptional repression activity of KyoT2 on the notch/RBP-J pathway is regulated by PIAS1-catalyzed SUMOylation, *J. Mol. Biol.* 370 (1) (2007) 27–38.
- [133] M.P. Licciardello, et al., NOTCH1 activation in breast cancer confers sensitivity to inhibition of SUMOylation, *Oncogene* 34 (29) (2015) 3780–3790.
- [134] M. Ciria, et al., Mesenchymal stem cell migration and proliferation are mediated by hypoxia-inducible factor-1 α upstream of notch and SUMO pathways, *Stem Cells Dev.* 26 (13) (2017) 973–985.
- [135] Y. Yu, et al., Phosphoproteomic analysis identifies Grb10 as an mTORC1 substrate that negatively regulates insulin signaling, *Science* 332 (6035) (2011) 1322–1326.
- [136] Y. Zhang, Y. Zhang, Y. Yu, Global phosphoproteomic analysis of insulin/Akt/mTORC1/S6K signaling in rat hepatocytes, *J. Proteome Res.* 16 (8) (2017) 2825–2835.
- [137] H.-Y.C. Chiou, et al., Hes-1 SUMOylation by protein inhibitor of activated STAT1 enhances the suppressing effect of Hes-1 on GADD45 α expression to increase cell survival, *J. Biomed. Sci.* 21 (1) (2014) 53.
- [138] I.A. Hendriks, et al., Site-specific mapping of the human SUMO proteome reveals co-modification with phosphorylation, *Nat. Struct. Mol. Biol.* 24 (3) (2017) 325–336.
- [139] K. Bielskienė, et al., E3 ubiquitin ligases as drug targets and prognostic biomarkers in melanoma, *Medicina* 51 (1) (2015) 1–9.
- [140] L. Qiu, et al., Recognition and ubiquitination of Notch by Itch, a hect-type E3 ubiquitin ligase, *J. Biol. Chem.* 275 (46) (2000) 35734–35737.
- [141] M.A. McGil, C.J. McGlade, Mammalian Numb proteins promote Notch1 receptor ubiquitination and degradation of the Notch1 intracellular domain, *J. Biol. Chem.* 278 (25) (2003) 23196–23203.
- [142] T. Sapir, et al., Notch activation by Shootin1 opposing activities on 2 ubiquitin ligases, *Cerebral Cortex*, pp. (2017) 1–14.
- [143] P. Chastagner, E. Rubinstein, C. Brou, Ligand-activated Notch undergoes DTX4-mediated ubiquitylation and bilateral endocytosis before ADAM10 processing, *Sci. Signal.* 10 (483) (2017) eaag2989.
- [144] K. Safdar, et al., UBR-5, a conserved HECT-type E3 ubiquitin ligase, negatively regulates Notch-type signaling in *Caenorhabditis elegans*, *G3 Genes/Genomes/Genetics* 6 (7) (2016) 2125–2134.
- [145] J. Moretti, et al., The ubiquitin-specific protease 12 (USP12) is a negative regulator of notch signaling acting on notch receptor trafficking toward degradation, *J. Biol. Chem.* 287 (35) (2012) 29429–29441.
- [146] E.J. Hubbard, et al., sel-10, a negative regulator of lin-12 activity in *Caenorhabditis elegans*, encodes a member of the CDC4 family of proteins, *Genes Dev.* 11 (23) (1997) 3182–3193.
- [147] C. Oberg, et al., The Notch intracellular domain is ubiquitinated and negatively regulated by the mammalian Sel-10 homolog, *J. Biol. Chem.* 276 (38) (2001) 35847–35853.
- [148] G. Wu, et al., SEL-10 is an inhibitor of Notch signaling that targets Notch for ubiquitin-mediated protein degradation, *Mol. Cell Biol.* 21 (21) (2001) 7403–7415.
- [149] F.A. Carrieri, J.K. Dale, Turn it down a Notch, *Frontiers in Cell and Developmental Biology* 4 (2017) 151.
- [150] C.-H. Yeh, et al., Oncogenic mutations in the FBXW7 gene of adult T-cell leukemia patients, *Proc. Natl. Acad. Sci.* 113 (24) (2016) 6731–6736.
- [151] J. O’Neil, et al., FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors, *J. Exp. Med.* 204 (8) (2007) 1813–1824.
- [152] L. Li, et al., Ubiquitin ligase RNF8 suppresses Notch signaling to regulate mammary development and tumorigenesis, *J. Clin. Investig.* 128 (10) (2018) 4525–4542.
- [153] J.J. Thomas, et al., RNF4-dependent oncogene activation by protein stabilization, *Cell Rep.* 16 (12) (2016) 3388–3400.
- [154] S. Pettersson, et al., Non-degradative ubiquitination of the Notch1 receptor by the E3 ligase MDM2 activates the Notch signalling pathway, *The Biochemical Journal* 450 (3) (2013) 523–536.
- [155] Y. Sun, et al., Trp53 regulates Notch 4 signaling through Mdm2, *J. Cell Sci.* 124 (7) (2011) 1067–1076.
- [156] J. Zhang, et al., A targeted in vivo RNAi screen reveals deubiquitinases as new regulators of Notch signaling, *G3 (Bethesda, Md.)* 2 (12) (2012) 1563–1575.
- [157] X. Ling, et al., The deubiquitinating enzyme Usp5 regulates Notch and RTK signaling during *Drosophila* eye development, *FEBS Lett.* 591 (6) (2017) 875–888.
- [158] F. Chen, et al., The E3 ubiquitin ligase SCF^{FBXL14} complex stimulates neuronal differentiation by targeting the Notch signaling factor HES1 for proteolysis, *J. Biol. Chem.* (2017) jbc.M117.815001.
- [159] V.M. Panin, et al., Notch ligands are substrates for protein O-fucosyltransferase-1 and fringe, *J. Biol. Chem.* 277 (33) (2002) 29945–29952.
- [160] T. Okajima, K.D. Irvine, Regulation of notch signaling by o-linked fucose, *Cell* 111 (6) (2002) 893–904.
- [161] M. Stahl, et al., Roles of Pofut1 and O-fucose in mammalian Notch signaling, *J. Biol. Chem.* 283 (20) (2008) 13638–13651.
- [162] J. Müller, et al., O-fucosylation of the notch ligand mDLL1 by POFUT1 is dispensable for ligand function. T. Brand, ed. *PLoS One*, 9(2), p.e88571, 2014.
- [163] K. Serth, et al., O-fucosylation of DLL3 is required for its function during somitogenesis, in: D. Henrique (Ed.), *PLoS One*, 10(4) 2015, p. e0123776.
- [164] R. Müller, A. Jenny, P. Stanley, The EGF repeat-specific O-GlcNAc-transferase Eogt interacts with notch signaling and pyrimidine metabolism pathways in *Drosophila*, in: A. Singh (Ed.), *PLoS One*, 8(5) 2013, p. e62835.
- [165] E.-B. Braune, et al., S/T phosphorylation of DLL1 is required for full ligand activity in vitro but dispensable for DLL1 function in vivo during embryonic patterning and marginal zone B cell development, *Mol. Cell Biol.* 34 (7) (2014) 1221–1233.
- [166] G. Weinmaster, J.A. Fischer, Notch ligand ubiquitylation: what is it good for? *Dev. Cell* 21 (1) (2011) 134–144.
- [167] G.A. Deblandre, E.C. Lai, C. Kintner, *Xenopus* neuralized is a ubiquitin ligase that interacts with Δ Delta1 and regulates Notch signaling, *Dev. Cell* 1 (6) (2001) 795–806.
- [168] E.C. Lai, et al., *Drosophila* neuralized is a ubiquitin ligase that promotes the internalization and degradation of delta, *Dev. Cell* 1 (6) (2001) 783–794.
- [169] B.-K. Koo, et al., An obligatory role of mind bomb-1 in Notch signaling of mammalian development, in: F. Schweisguth (Ed.), *PLoS ONE*, 2(11) 2007, p. e1221.
- [170] B.-K. Koo, et al., Mind bomb-2 is an E3 ligase for Notch ligand, *J. Biol. Chem.* 280 (23) (2005) 22335–22342.
- [171] B.-K. Koo, Mind bomb 1 is essential for generating functional Notch ligands to activate Notch, *Development* 132 (15) (2005) 3459–3470.

- [172] S.-M. Kim, et al., Presenilin-2 regulates the degradation of RBP-Jk protein through p38 mitogen-activated protein kinase, *J. Cell Sci.* 125 (5) (2012) 1296–1308.
- [173] J.S. Auer, et al., MAPK-dependent phosphorylation modulates the activity of suppressor of hairless in *Drosophila*, *Cell. Signal.* 27 (1) (2015) 115–124.
- [174] A.C. Nagel, et al., Phosphorylation of suppressor of hairless impedes its DNA-binding activity, *Sci. Rep.* 7 (1) (2017) 11820.
- [175] M. Saint Just Ribeiro, M.L. Hansson, A.E. Wallberg, A proline repeat domain in the Notch co-activator MAML1 is important for the p300-mediated acetylation of MAML1, *The Biochemical Journal* 404 (2) (2007) 289–298.
- [176] K. Jin, et al., Acetylation of mastermind-like 1 by p300 drives the recruitment of NACK to initiate notch-dependent transcription, *Cancer Res.* 77 (16) (2017) 4228–4237.
- [177] M.J. Lindberg, et al., SUMO modification regulates the transcriptional activity of MAML1, *FASEB J.* 24 (7) (2010) 2396–2404.
- [178] M. Farshbaf, et al., Mastermind-like 1 is ubiquitinated: functional consequences for notch signaling, in: M.A. Sussman (Ed.), *PLOS ONE*, 10(7) 2015, p. e0134013.
- [179] M.R. Broadus, et al., Identification of a paralog-specific Notch1 intracellular domain Degron, *Cell Rep.* 15 (9) (2016) 1920–1929.
- [180] C. Atsriku, et al., Systematic mapping of posttranslational modifications in human estrogen receptor-alpha with emphasis on novel phosphorylation sites, *Molecular & Cellular Proteomics: MCP* 8 (3) (2009) 467–480.
- [181] V. Giandomenico, et al., Coactivator-dependent acetylation stabilizes members of the SREBP family of transcription factors, *Mol. Cell. Biol.* 23 (7) (2003) 2587–2599.
- [182] K.N. Swatek, D. Komander, Ubiquitin modifications, *Cell Res.* 26 (4) (2016) 399–422.
- [183] I. Psakhye, S. Jentsch, Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair, *Cell* 151 (4) (2012) 807–820.
- [184] S. Jentsch, I. Psakhye, Control of nuclear activities by substrate-selective and protein-group SUMOylation, *Annu. Rev. Genet.* 47 (1) (2013) 167–186.
- [185] T. Tammsalu, et al., Proteome-wide identification of SUMO2 modification sites, *Sci. Signal.* 7 (323) (2014) rs2.
- [186] A. Rustighi, et al., Prolyl-isomerase Pin1 controls normal and cancer stem cells of the breast, *EMBO Mol. Med.* 6 (1) (2014) 99–119. A.
- [188] I. Uzoma, et al., Global identification of SUMO substrates reveals crosstalk between SUMOylation and phosphorylation promotes cell migration, *Molecular & Cellular Proteomics, MCP*, 2018p.mcp.RA117.000014.
- [189] S.A.G. Cuijpers, E. Willemstein, A.C.O. Vertegaal, Converging small ubiquitin-like modifier (SUMO) and ubiquitin signaling: improved methodology identifies co-modified target proteins, *Molecular & Cellular Proteomics: MCP* 16 (12) (2017) 2281–2295.
- [190] N. Blom, et al., Prediction of post-translational glycosylation and phosphorylation of proteins from the amino acid sequence, *Proteomics* 4 (6) (2004) 1633–1649.
- [191] R. Chaudhuri, et al., PhosphOrtholog: a web-based tool for cross-species mapping of orthologous protein post-translational modifications, *BMC Genomics* 16 (1) (2015) 617.
- [192] F. Li, et al., GlycoMine: a machine learning-based approach for predicting N-, C- and O-linked glycosylation in the human proteome, *Bioinformatics (Oxford, England)* 31 (9) (2015) 1411–1419.
- [193] C. Steentoft, et al., Precision mapping of the human O-GalNAc glycoproteome through SimpleCell technology, *EMBO J.* 32 (10) (2013) 1478–1488.
- [194] Y.-H. Wong, et al., KinasePhos 2.0: a web server for identifying protein kinase-specific phosphorylation sites based on sequences and coupling patterns, *Nucleic Acids Res.* 35 (Web Server issue) (2007) W588–W594.
- [195] T.C. Walther, M. Mann, Mass spectrometry-based proteomics in cell biology, *J. Cell Biol.* 190 (4) (2010) 491–500.
- [196] H. Jin, R.C. Zangar, Protein modifications as potential biomarkers in breast cancer, *Biomark. Insights* 4 (2009) 191–200.
- [197] N.A. Petushkova, et al., Post-translational modifications of FDA-approved plasma biomarkers in glioblastoma samples, in: F. Lisacek (Ed.), *PloS One*, 12(5) 2017, p. e0177427.
- [198] K.M. Naegle, et al., MCAM: multiple clustering analysis methodology for deriving hypotheses and insights from high-throughput proteomic datasets, in: J.A. Papin (Ed.), *PLoS Computational Biology*, 7(7) 2011, p. e1002119.
- [199] A.S. Gajadhar, F.M. White, System level dynamics of post-translational modifications, *Curr. Opin. Biotechnol.* 28 (2014) 83–87.
- [200] H. Horita, et al., A simple toolset to identify endogenous post-translational modifications for a target protein: a snapshot of the EGFR signaling pathway, *Biosci. Rep.* 37 (4) (2017) BSR20170919.
- [201] H. Horita, A. Law, K. Middleton, Utilizing optimized tools to investigate PTM crosstalk: identifying potential PTM crosstalk of acetylated mitochondrial proteins, *Proteomics* 6 (2) (2018) 24.
- [202] P. Chames, D. Baty, Bispecific antibodies for cancer therapy: the light at the end of the tunnel? *mAbs* 1 (6) (2009) 539–547.
- [203] S.C. Wei, C.R. Duffy, J.P. Allison, Fundamental mechanisms of immune checkpoint blockade therapy, *Cancer Discovery* 8 (9) (2018) 1069–1086.
- [204] K. Lee, et al., Therapeutic potential of a monoclonal antibody blocking the Wnt pathway in diabetic retinopathy, *Diabetes* 61 (11) (2012) 2948–2957.
- [205] I. Trenevskaya, D. Li, A.H. Banham, Therapeutic antibodies against intracellular tumor antigens, *Front. Immunol.* 8 (2017) 1001.
- [206] T. Hattori, S. Koide, Next-generation antibodies for post-translational modifications, *Curr. Opin. Struct. Biol.* 51 (2018) 141–148.
- [207] M. Schneider, et al., Inhibition of Delta-induced Notch signaling using fucose analogs, *Nat. Chem. Biol.* 14 (1) (2018) 65–71.
- [208] H. Yu, et al., Structural analysis of Notch-regulating Rumi reveals basis for pathogenic mutations, *Nat. Chem. Biol.* 12 (9) (2016) 735–740.
- [209] Z. Li, et al., Recognition of EGF-like domains by the Notch-modifying O-fucosyltransferase POFUT1, *Nat. Chem. Biol.* 13 (7) (2017) 757–763.
- [210] B.M. Harvey, R.S. Haltiwanger, Regulation of Notch function by O-glycosylation, *Adv. Exp. Med. Biol.* 1066 (2018) 59–78.
- [211] F.A. Ran, et al., Genome engineering using the CRISPR-Cas9 system, *Nat. Protoc.* 8 (11) (2013) 2281–2308.
- [212] E.J. Aird, et al., Increasing Cas9-mediated homology-directed repair efficiency through covalent tethering of DNA repair template, *Commun. Biol.* 1 (1) (2018) 54.
- [213] J.-B. Renaud, et al., Improved genome editing efficiency and flexibility using modified oligonucleotides with TALEN and CRISPR-Cas9 nucleases, *Cell Rep.* 14 (9) (2016) 2263–2272.
- [214] X. Xu, et al., Efficient homology-directed gene editing by CRISPR/Cas9 in human stem and primary cells using tube electroporation, *Sci. Rep.* 8 (1) (2018) 11649.
- [215] R.K. Vadlamudi, et al., An essential role of Pak1 phosphorylation of SHARP in Notch signaling, *Oncogene* 24 (28) (2005) 4591–4596.
- [216] J.-Y. Yoo, et al., Nuclear hormone receptor corepressor promotes esophageal cancer cell invasion by transcriptional repression of interferon- γ -inducible protein 10 in a casein kinase 2-dependent manner, *Mol. Biol. Cell* 23 (15) (2012) 2943–2954.
- [217] F. Oswald, et al., A phospho-dependent mechanism involving NCoR and KMT2D controls a permissive chromatin state at Notch target genes, *Nucleic Acids Res.* 44 (10) (2016) 4703–4720.
- [218] Y. Du, et al., POFUT1 promotes colorectal cancer development through the activation of Notch1 signaling, *Cell Death Dis.* 9 (10) (2018) 995.
- [219] T. Okajima, et al., Chaperone activity of protein O-fucosyltransferase 1 promotes notch receptor folding, *Science* 307 (5715) (2005) 1599–1603.
- [220] L. Lei, et al., An O-fucose site in the ligand binding domain inhibits Notch activation, *Development (Cambridge, England)* 130 (26) (2003) 6411–6421.
- [221] S. Munro, M. Freeman, The notch signalling regulator fringe acts in the Golgi apparatus and requires the glycosyltransferase signature motif DXD, *Current Biology: CB* 10 (14) (2000) 813–820.
- [222] I. Cohen, et al., Autosomal recessive Adams-Oliver syndrome caused by homozygous mutation in EOGT, encoding an EGF domain-specific O-GlcNAc transferase, *European Journal of Human Genetics: EJHG* 22 (3) (2014) 374–378.
- [223] Y. Bai, et al., Phosphoproteomics identifies driver tyrosine kinases in sarcoma cell lines and tumors, *Cancer Res.* 72 (10) (2012) 2501–2511.
- [224] Y. Bian, et al., An enzyme assisted RP-RPLC approach for in-depth analysis of human liver phosphoproteome, *J. Proteome* 96 (2014) 253–262.
- [225] T. Shimomizu, et al., Identification of missing proteins in the neXtProt database and unregistered phosphopeptides in the PhosphoSitePlus database as part of the Chromosome-centric Human Proteome Project, *J. Proteome Res.* 12 (6) (2013) 2414–2421.
- [226] E.L. Huttlin, et al., A tissue-specific atlas of mouse protein phosphorylation and expression, *Cell* 143 (7) (2010) 1174–1189.
- [227] B. Zhu, et al., Quantitative phosphoproteomic analysis reveals key mechanisms of cellular proliferation in liver cancer cells, *Sci. Rep.* 7 (1) (2017) 10908.
- [228] X. Zhu, et al., SUMOylation negatively regulates angiogenesis by targeting endothelial NOTCH signaling novelty and significance, *Circ. Res.* 121 (6) (2017) 636–649.
- [229] C. Franchin, et al., Quantitative analysis of a phosphoproteome readily altered by the protein kinase CK2 inhibitor quinalizarin in HEK-293T cells, *Biochim. Biophys. Acta* 1854 (6) (2015) 609–623.
- [230] T. Yi, et al., Quantitative phosphoproteomic analysis reveals system-wide signaling pathways downstream of SDF-1/CXCR4 in breast cancer stem cells, *Proc. Natl. Acad. Sci. U. S. A.* 111 (21) (2014) E2182–E2190.
- [231] K. Sharma, et al., Ultradeep human phosphoproteome reveals a distinct regulatory nature of Tyr and Ser/Thr-based signaling, *Cell Rep.* 8 (5) (2014) 1583–1594.
- [232] H. Zhou, et al., Toward a comprehensive characterization of a human cancer cell phosphoproteome, *J. Proteome Res.* 12 (1) (2013) 260–271.
- [233] M. Franz-Wachtel, et al., Global detection of protein kinase D-dependent phosphorylation events in nocodazole-treated human cells, *Molecular & Cellular Proteomics: MCP* 11 (5) (2012) 160–170.
- [234] D.H. Phanstiel, et al., Proteomic and phosphoproteomic comparison of human ES and iPS cells, *Nat. Methods* 8 (10) (2011) 821–827.
- [235] A.N. Kettenbach, et al., Quantitative phosphoproteomics identifies substrates and functional modules of Aurora and Polo-like kinase activities in mitotic cells, *Sci. Signal.* 4 (179) (2011) rs5.
- [236] P.P. Hsu, et al., The mTOR-regulated phosphoproteome reveals a mechanism of mTORC1-mediated inhibition of growth factor signaling, *Science* 332 (6035) (2011) 1317–1322.
- [237] V. Mayya, et al., Quantitative phosphoproteomic analysis of T cell receptor signaling reveals system-wide modulation of protein-protein interactions, *Sci. Signal.* 2 (84) (2009) ra46.
- [238] R. Malik, et al., Quantitative analysis of the human spindle phosphoproteome at distinct mitotic stages, *J. Proteome Res.* 8 (10) (2009) 4553–4563.
- [239] N. Dephoure, et al., A quantitative atlas of mitotic phosphorylation, *Proc. Natl. Acad. Sci. U. S. A.* 105 (31) (2008) 10762–10767.
- [240] J.V. Olsen, et al., Global, in vivo, and site-specific phosphorylation dynamics in signaling networks, *Cell* 127 (3) (2006) 635–648.
- [241] S.A. Beausoleil, et al., Large-scale characterization of HeLa cell nuclear phosphoproteins, *Proc. Natl. Acad. Sci. U. S. A.* 101 (33) (2004) 12130–12135.
- [242] J.C. Barsi, et al., Mind bomb1 is a ubiquitin ligase essential for mouse embryonic development and Notch signaling, *Mech. Dev.* 122 (10) (2005) 1106–1117.
- [243] T. Takeuchi, Y. Adachi, Y. Ohtsuki, Skeletrophin, a novel ubiquitin ligase to the intracellular region of Jagged-2, is aberrantly expressed in multiple myeloma, *Am.*

- J. Pathol. 166 (6) (2005) 1817–1826.
- [244] A. Daskalaki, et al., Distinct intracellular motifs of Delta mediate its ubiquitylation and activation by Mindbomb1 and Neuralized, *J. Cell Biol.* 195 (6) (2011) 1017–1031.
- [245] N. Benhra, et al., Neuralized promotes basal to apical transcytosis of Delta in epithelial cells M. Gonzalez-Gaitan, ed, *Mol. Biol. Cell* 21 (12) (2010) 2078–2086.
- [246] E. Koutelou, et al., Neuralized-like 1 (Neurl1) targeted to the plasma membrane by *N*-myristoylation regulates the notch ligand Jagged1, *J. Biol. Chem.* 283 (7) (2008) 3846–3853.
- [247] R. Song, et al., Neuralized-2 regulates a Notch ligand in cooperation with mind Bomb-1, *J. Biol. Chem.* 281 (47) (2006) 36391–36400.
- [248] B. Vollrath, et al., Isolation of a murine homologue of the *Drosophila* neuralized gene, a gene required for axonemal integrity in spermatozoa and terminal maturation of the mammary gland, *Mol. Cell. Biol.* 21 (21) (2001) 7481–7494.
- [249] Y. Ruan, et al., Ethanol hypersensitivity and olfactory discrimination defect in mice lacking a homolog of *Drosophila* neuralized, *Proc. Natl. Acad. Sci.* 98 (17) (2001) 9907–9912.
- [250] K. Yamada, et al., Roles of *Drosophila* dextex in Notch receptor endocytic trafficking and activation, *Genes to Cells: Devoted to Molecular & Cellular Mechanisms* 16 (3) (2011) 261–272.
- [251] P. Zhang, et al., Regulation of NOTCH signaling by reciprocal inhibition of HES1 and Deltex 1 and its role in osteosarcoma invasiveness, *Oncogene* 29 (20) (2010) 2916–2926.
- [252] P. Chastagner, A. Israël, C. Brou, Itch/AIP4 mediates Deltex degradation through the formation of K29-linked polyubiquitin chains, *EMBO Rep.* 7 (11) (2006) 1147–1153.
- [253] E.B. Sorensen, S.D. Conner, γ -Secretase-dependent cleavage initiates notch signaling from the plasma membrane, *Traffic* 11 (9) (2010) 1234–1245.
- [254] T. Sakata, et al., *Drosophila* Nedd4 regulates endocytosis of notch and suppresses its ligand-independent activation, *Current Biology: CB* 14 (24) (2004) 2228–2236.
- [255] M.B. Wilkin, et al., Regulation of notch endosomal sorting and signaling by *Drosophila* Nedd4 family proteins, *Curr. Biol.* 14 (24) (2004) 2237–2244.
- [256] P. Chastagner, A. Israël, C. Brou, AIP4/Itch regulates Notch receptor degradation in the absence of ligand, in: S. Wölfl (Ed.), *PLoS One*, 3(7) 2008, p. e2735.
- [257] K.H. Moberg, et al., Archipelago regulates Cyclin E levels in *Drosophila* and is mutated in human cancer cell lines, *Nature* 413 (6853) (2001) 311–316.
- [258] B.M. Jehn, et al., C-Cbl binding and ubiquitin-dependent lysosomal degradation of membrane-associated Notch1, *J. Biol. Chem.* 277 (10) (2002) 8033–8040.
- [259] M. Sczaniecka, et al., MDM2 protein-mediated ubiquitination of NUMB protein, *J. Biol. Chem.* 287 (17) (2012) 14052–14068.
- [260] J. Moretti, et al., The translation initiation factor 3f (eIF3f) exhibits a deubiquitinase activity regulating notch activation, in: N. Perrimon (Ed.), *PLoS Biology*, 8(11) 2010, p. e1000545.
- [261] S.A. Wagner, et al., Proteomic analyses reveal divergent ubiquitylation site patterns in murine tissues, *Mol. Cell. Proteomics* 11 (12) (2012) 1578–1585.
- [262] W. Kim, et al., Systematic and quantitative assessment of the ubiquitin-modified proteome, *Mol. Cell* 44 (2) (2011) 325–340.
- [263] S.A. Wagner, et al., A proteome-wide, quantitative survey of in vivo ubiquitylation sites reveals widespread regulatory roles, *Mol. Cell. Proteomics* 10 (10) (2011) M111.013284.
- [264] N. Gupta-Rossi, et al., Monoubiquitination and endocytosis direct gamma-secretase cleavage of activated Notch receptor, *J. Cell Biol.* 166 (1) (2004) 73–83.
- [265] M.J. Lindhurst, et al., A mosaic activating mutation in *AKT1* associated with the Proteus syndrome, *N. Engl. J. Med.* 365 (7) (2011) 611–619.
- [266] F. Petrij, et al., Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP, *Nature* 376 (6538) (1995) 348–351.
- [267] K. Poirier, et al., CSNK2B splice site mutations in patients cause intellectual disability with or without myoclonic epilepsy, *Hum. Mutat.* 38 (8) (2017) 932–941.
- [268] E. Calpena, et al., De novo missense substitutions in the gene encoding CDK8, a regulator of the mediator complex, cause a syndromic developmental disorder, *Am. J. Hum. Genet.* 104 (4) (2019) 709–720.
- [269] A. Feki, Y. Hibaoi, DYRK1A protein, a promising therapeutic target to improve cognitive deficits in down syndrome, *Brain Sci.* 8 (10) (2018) 187.
- [270] A.J. George, et al., A comprehensive atlas of E3 ubiquitin ligase mutations in neurological disorders, *Front. Genet.* 9 (2018) 29.
- [271] G. Atkin, H. Paulson, Ubiquitin pathways in neurodegenerative disease, *Front. Mol. Neurosci.* 7 (2014).
- [272] A. Musharraf, et al., BOR-syndrome-associated Eya1 mutations lead to enhanced proteasomal degradation of Eya1 protein, in: E.E. Schmidt (Ed.), *PLoS One*, 9(1) 2014, p. e87407.
- [273] C.-H. Yeh, M. Bellon, C. Nicot, FBXW7: a critical tumor suppressor of human cancers, *Mol. Cancer* 17 (1) (2018) 115.
- [274] E. Beurel, S.F. Grieco, R.S. Jope, Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases, *Pharmacol. Ther.* 148 (2015) 114–131.
- [275] M.K. King, et al., Glycogen synthase kinase-3 inhibitors: rescuers of cognitive impairments, *Pharmacol. Ther.* 141 (1) (2014) 1–12.
- [276] P. Cheng, et al., Effects of notch signaling on regulation of myeloid cell differentiation in cancer, *Cancer Res.* 74 (1) (2014) 141–152.
- [280] M.G. Hunter, B.R. Avalos, Deletion of a critical internalization domain in the G-CSFR in acute myelogenous leukemia preceded by severe congenital neutropenia, *Blood* 93 (2) (1999) 440–446.
- [281] M.G. Hunter, B.R. Avalos, Granulocyte colony-stimulating factor receptor mutations in severe congenital neutropenia transforming to acute myelogenous leukemia confer resistance to apoptosis and enhance cell survival, *Blood* 95 (6) (2000) 2132–2137.
- [286] J. Skokowa, et al., Severe congenital neutropenias, *Nat. Rev. Dis. Prim.* 3 (1) (2017) 17032.
- [287] C.T. Walsh, S. Garneau-Tsodikova, G.J. Gatto, Protein posttranslational modifications: the chemistry of proteome diversifications. *Angewandte Chemie (International Ed. in English)*, 44 (45) (2005) 7342–7372F.
- [288] A. Xu, N. Haines, M. Dlugosz, N.A. Rana, H. Takeuchi, R.S. Haltiwanger, K.D. Irvine, In vitro reconstitution of the modulation of *Drosophila* Notch-ligand binding by Fringe, *J. Biol. Chem.* 282 (48) (2007) 35153–35162, <https://doi.org/10.1074/jbc.M707040200>.
- [289] H. Jafar-Nejad, J. Leonardi, R. Fernandez-Valdivia, Role of glycans and glycosyltransferases in the regulation of Notch signaling, *Glycobiology* 20 (8) (2010 Aug) 931–949, <https://doi.org/10.1093/glycob/cwq053>.
- [290] Chiang, et al., Identification of a Conserved Negative Regulatory Sequence That Influences the Leukemogenic Activity of NOTCH1, *Mol. Cell Biol.* 26 (16) (2006) 6261–6271.
- [291] M. Itoh, C.H. Kim, G. Palardy, T. Oda, Y.J. Jiang, D. Maust, S.Y. Yeo, K. Lorick, G.J. Wright, S. Ariza-McNaughton, A.M. Weissman, J. Lewis, S.C. Chandrasekharappa, AB. Chitnis, Mind bomb is a ubiquitin ligase that is essential for efficient activation of Notch signaling by Delta, *Dev. Cell* 4 (1) (2003 Jan) 67–82.
- [292] Martin Peter Steinbuck, Susan Winandy, A Review of Notch Processing With New Insights Into Ligand-Independent Notch Signaling in T-Cells, *J. Immunol.* 200 (3) (2018 Feb 1) 997–1007, <https://doi.org/10.4049/jimmunol.1700070>.
- [293] Julien Moretti, Christel Brou, Ubiquitinations in the Notch Signaling Pathway, *Int. J. Mol. Sci.* 14 (3) (2013 Mar) 6359–6381, <https://doi.org/10.3390/ijms14036359> (Published online 2013 Mar 19).
- [294] S. Le Bras, N. Loyer, R. Le Borgne, The multiple facets of ubiquitination in the regulation of notch signaling pathway, *Traffic* 12 (2) (2011 Feb) 149–161, <https://doi.org/10.1111/j.1600-0854.2010.01126>.
- [295] J.C. Aster, W.S. Pear, S.C. Blacklow, The Varied Roles of Notch in Cancer, *Annu. Rev. Pathol.* 12 (2017 Jan 24) 245–275, <https://doi.org/10.1146/annurev-pathol-052016-100127>.
- [296] B. D'Souza, A. Miyamoto, G. Weinmaster, The many facets of Notch ligands, *Oncogene* 27 (38) (2008 Sep 1) 5148–5167, <https://doi.org/10.1038/onc.2008.229>.
- [297] C. Zhang, Q. Li, C.H. Lim, X. Qiu, Y.J. Jiang, The characterization of zebrafish antimorphic mib alleles reveals that Mib and Mind bomb-2 (Mib2) function redundantly, *Dev. Biol.* 305 (1) (2007 May 1) 14–27.