Review

Non-canonical Notch signaling: emerging role and mechanism

Peter Andersen*, Hideki Uosaki*, Lincoln T Shenje* and Chulan Kwon

Division of Cardiology, Department of Medicine, Johns Hopkins University, 720 Rutland Avenue, Baltimore, MD 21205, USA

Notch is an ancient transmembrane receptor with crucial roles in cell-fate choices. Although the 'canonical' Notch pathway and its core members are well established – involving ligand-induced cleavage of Notch for transcriptional regulation – it has been unclear whether Notch can also function independently of ligand and transcription ('non-canonically') through a common mechanism. Recent studies suggest that Notch can non-canonically exert its biological functions by post-translationally targeting Wnt/β-catenin signaling, an important cellular and developmental regulator. The non-canonical Notch pathway appears to be highly conserved from flies to mammals. Here, we discuss the emerging conserved mechanism and role of ligand/transcription-independent Notch signaling in cell and developmental biology.

Canonical versus non-canonical Notch signaling

Nearly a century ago, the name Notch was given to an allele found to cause notched fly wings; since this time, the gene encoding the transmembrane protein Notch has been extensively investigated for its function and mechanisms [1–4]. The investigation led to the identification of key members of Notch signaling including ligands, proteases and transcriptional co-factors, forming the dogma of the canonical Notch signal transduction pathway (Box 1). Although Notch mediates a number of biological processes through the canonical pathway, a ligand- or transcription-independent (non-canonical) function of Notch has also been reported [5–29]. However, owing to lack of mechanistic understanding, it was uncertain whether the non-canonical function represented a general role for Notch. Over the past several years, multiple laboratories have reported a novel non-canonical role for Notch [5,15,17,30]: antagonizing Wnt/β-catenin signaling (Box 2) – a crucial regulator of development and disease – independent of Notch ligand-dependent cleavage or nuclear localization. Given the considerable reciprocal involvement of Notch and Wnt/β-catenin signaling in fundamental cellular processes such as expansion and differentiation, understanding the non-canonical role of Notch could provide invaluable insight into regenerative medicine and disease therapeutics. In this review the current understanding of non-canonical Notch biology is summarized, and the emerging role and mechanism of non-canonical Notch regulation of Wnt/β-catenin signaling is discussed in detail.

Corresponding author: Kwon, C. (ckwon13@jhmi.edu)

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* These authors contributed equally to this work.

Early evidence of non-canonical Notch function

Some of the earliest evidence for non-canonical Notch signaling came from *in vitro* studies, in which increased Notch1 levels inhibited the differentiation of myoblast (C2C12) cells into muscle cells [8–10]. The authors reported that, unlike conventional Notch signaling, the inhibition of myoblast differentiation did not require the CSL interacting domain of Notch1 and was not mediated by CSL or known Notch target genes, suggesting the existence of a CSL-independent Notch pathway [9,10]. *In vivo*, Notch loss-of-function studies in *Drosophila* revealed that Notch exerts its inhibitory effect to select muscle progenitors from the mesoderm even in the absence of ligand and/or CSL [19]. This finding provided compelling evidence that ligand/CSL-independent function of Notch is present and active during development. Since then, ligand/CSL-independent Notch functions have been reported in various systems across species (Table 1). However, in most cases, the key mediators of non-canonical Notch signals are unclear and the proposed mechanisms appear to vary with context. Could there be a conserved mechanism? Whereas CSL-independent Notch activity could come from interactions of Notch with non-CSL transcription factors in the nucleus [31,32], this does not explain ligand-independent functions of Notch. Moreover, endogenous Notch protein is mostly detected in the cell membrane and cytoplasm and is rarely observed in the nucleus [33], suggesting Notch may interact in the cytoplasm with other molecules, affecting their function post-translationally. It is worth noting that the described non-canonical Notch functions have mostly been identified in stem/progenitor cells or embryonic/primordial cells across species which are capable of expansion and/or differentiation. This suggests that non-canonical Notch signals might play an important role in undifferentiated early cell populations and might interact with conserved cell regulators. Wnt/β-catenin signaling is one such regulator with which Notch frequently interacts throughout development; their functional and molecular interactions are discussed in the following sections.

Functional interaction of non-canonical Notch and Wnt/β-catenin signaling

Notch exhibits recurrent crosstalk with Wnt/β-catenin signaling in numerous cell types and contexts during development (summarized in Table 1 in [34]). The interaction of Notch and Wnt signaling was first uncovered in the *Drosophila* wing imaginal disc, where Notch is co-expressed with Wingless (*Drosophila* Wnt-1) and enforces Wingless

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Box 1. Canonical versus non-canonical Notch signaling

Notch is an evolutionarily-conserved single-pass transmembrane receptor that affects numerous cell fate decisions through short-range cell–cell interactions. Notch protein (cLIN-12 and cGLP-1 in *Caenorhabditis elegans*, Notch in *Drosophila*, Notch1–4 in mammals) consists of the extracellular domain (NECD) with 29-36 epidermal growth factor (EGF) repeats for ligand binding, the transmembrane domain (TM), and the intracellular domain (NICD) with transcriptional activity [1,76]. The canonical Notch pathway initiates when Notch ligands – transmembrane proteins characterized by three motifs: DSL (Delta, Serrate, LAG-2), DOS (Delta and OSM-11 like) and EGF repeats – bind to the EGF repeats 11-12 and 24-29 of NECD from adjacent cells (Figure Ia). The ligand-NECD interaction allows members of the α-secretase/metalloprotease family (ADAM10/Kuzmanian, ADAM17/TACE) to shed NECD, leading to sequential cytoplasmic cleavage of NICD by γ-secretase – a multi-subunit protease complex composed of presenilin (PS), nicastrin (NCT), Aph-1, Pen-2 and others [77–79]. The resulting NICD translocates to the nucleus, where the RAM domain of NICD interacts with the DNA-binding transcription factor CSL (CBF1/RBPjk in vertebrates, Suppressor of Hairless in *Drosophila*, Lag-1 in *C. elegans*). NICD functions as a coactivator for CSL, Mastermind-like proteins (Mastermind in *Drosophila*, MAML1 in mammals, Lag-3 in *C. elegans*) and other cofactors such as CBP/p300 to transcriptionally activate Notch target genes [80–82]. In the absence of NICD, CSL functions as a sequence-specific repressor [83]. In addition to the RAM domain, NICD consists of an Ankyrin repeat domain, which is involved in protein interactions, a transactivation domain and a PEST domain rich in proline, glutamate, serine and threonine residues.

Non-canonical Notch signaling is CSL-independent and can be either ligand-dependent or independent (Figure Ib). Although some genes are affected by non-canonical Notch function, in most cases the mediators of non-canonical Notch signaling are unknown (summarized in Table 1). The most well-studied and conserved effect of non-canonical Notch function is regulation of Wnt/β-catenin signaling: Notch binds and titrate levels of the obligate Wnt-signaling component active β-catenin. Therefore, active β-catenin activity may serve as a useful readout for non-canonical Notch signals. Currently, at least in mammals, there is no simple genetic approach or tool available to test non-canonical Notch function *in vivo*; testing probably requires combinatorial deletion/overexpression of Notch members including Notch, NICD, CSL, Mastermind, Ligands, and Presenilin.

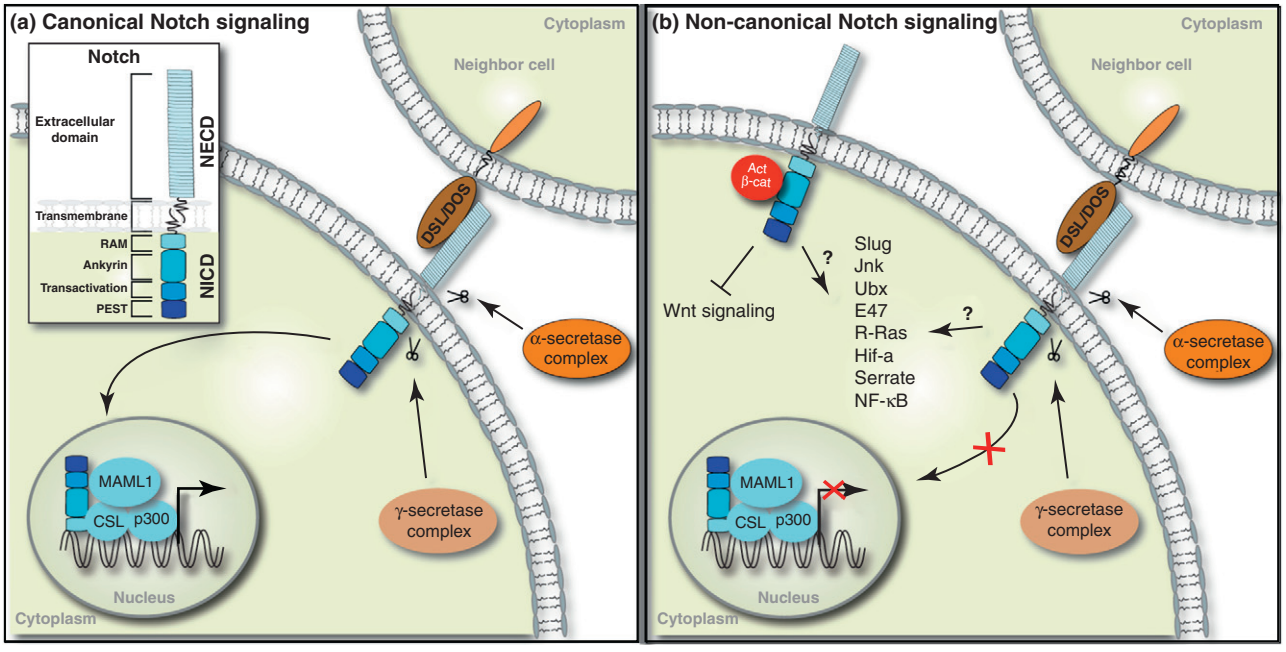

Figure I. Canonical versus non-canonical Notch signaling.

signaling [29,35]. Notch interacts with Wnt/β-catenin signaling in synergic or antagonistic ways, depending on the context [24,27]. The synergistic interactions generally involve ligand/CSL-dependent Notch signaling. For instance, Notch and β-catenin synergistically act to induce arterial endothelial cells and gene expression in an RBP-J dependent manner. [36]. The synergistic activity of Notch and Wnt/β-catenin signaling is also observed in early intestinal precursors and adenomas [37].

In contrast, ligand/CSL-independent Notch signaling is frequently associated with antagonism of Wnt/β-catenin signaling. In *Drosophila*, Wingless is required for the induction of Slouch $(S59)^{+}$ muscle progenitors and its gain-of-function causes their expansion [18]. *Notch*-null mutation also leads to excessive numbers of muscle progenitors independent of ligand/CSL-mediated lateral inhibition and deficiency of Notch – but not ligands or CSL – restores the induction of $Slouch^{+}$ progenitors in the absence of Wingless signals [18]. Similarly, decreased Notch or increased Wnt signaling promotes expansion of $Evenskipped^{+}$ cardiac progenitors during development [38], although interaction of Notch and Wnt signaling was undetermined. This repressive role of Notch was also identified at the gene promoter level, where Notch inhibits Wingless activity on a mesodermal enhancer independent of CSL function in the peripheral nervous system as well as in epithelial cells [17,24–26].

Interestingly, Notch antagonism of Wnt signaling that controls progenitor cell numbers appears to be conserved in mammalian stem and progenitor cells. In mouse embryos, *Notch1* ablation in $Islet1^{+}$ cardiac progenitor cells (CPCs) results in an expansion of CPCs with increased levels of

Box 2. Wnt/β-catenin signal transduction pathway [50,84,85]

The Wnt signaling pathway is a conserved cascade that regulates a number of crucial developmental and stem cell processes (Figure II). The central signaling component is β-catenin, an obligatory transcriptional mediator. Wnt/β-catenin signaling is initiated when the secreted glycoprotein Wnt binds to the cognate receptor complex of Frizzled and Lrp. This interaction activates the cytoplasmic protein Dishevelled, which stabilizes β-catenin by inhibiting the kinase activity of the destruction complex of adenomatous polyposis coli (APC), axin, and glycogen synthase kinase-3β (GSK3β). Active (unphosphorylated at Ser37/Thr41) β-catenin translocates to the nucleus where it binds to the TCF/lymphoid enhancer factor (LEF) transcription factors to activate Wnt target genes. In the absence of Wnt, the destruction complex phosphorylates the N-terminal of β-catenin to lead to ubiquitin-mediated proteolytic degradation.

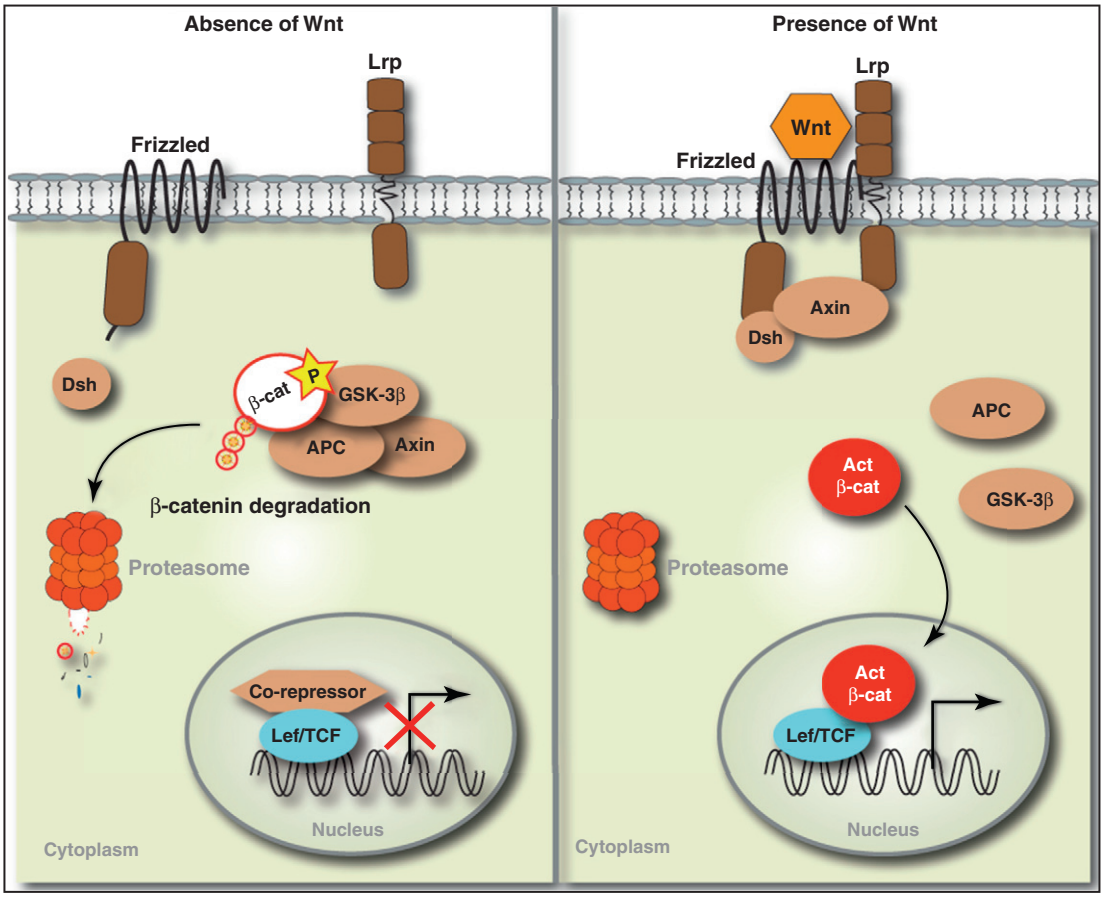

Figure II. Proposed model for Numb regulation of Notch and β-catenin.

active β-catenin protein and inhibits their cardiac differentiation [39]. The phenotype can be recapitulated, not by CSL deletion, but by stabilizing β-catenin or administering Wnt3a [5,39,40]. Conversely, the β-catenin-mediated expansion is rescued when Notch intracellular domain (NICD) is co-expressed in CPCs [5], implying Notch negatively regulates β-catenin activity. In epithelial progenitor cells, Notch1 deletion causes epidermal hyperplasia, whereas increased levels of activated Notch1 leads to growth arrest and induction of early differentiation markers through a CSL-independent mechanism [41]. Similarly, Notch-mediated antagonism of Wnt/β-catenin signaling is also observed in embryonic stem cells (ESCs), neural stem cells and mesenchymal stem cells [5].

These findings may reveal an evolutionarily conserved role of non-canonical Notch signals in controlling stem/progenitor cell expansion mediated by canonical Wnt signaling and support the notion that Notch may function as a tumor suppressor [42]. For instance, Notch1 deletion in the epidermis causes epidermal and corneal hyperplasia, leading to skin carcinogenesis [43]. The hyperplasia is accompanied by increased Wnt/β-catenin signaling in epidermis, which can be reduced upon NICD overexpression [43]. In addition, although interaction with Wnt signaling has not been determined, Notch1-deficiency leads to high incidence and progression of pancreatic cancer, when the GTPase K-ras is activated [44]. Curiously Notch was proposed as an oncogene in a few other cancers [45]. However, Notch-mediated tumorigenesis requires activation of another oncoprotein; therefore this discrepancy might represent a context-dependent nature of Notch signaling.

Together, these findings suggest that non-canonical Notch function could be closely associated with inhibition of canonical Wnt signaling during stem/progenitor cell development and oncogenesis. Nevertheless, the mechanisms by which Notch negatively regulates Wnt/β-catenin signaling through a ligand/CSL-independent pathway were not understood until recently.

Table 1. Evidence of CSL/ligand-independent Notch signaling

Species	Cell type	System	Independence	Function	Interacting molecule/ signaling (direct or indirect)	Refs
Human	Stem cells (hESCs), Cancer	*in vitro*	Ligand, CSL	Negative regulation of Wnt signaling	Active β-catenin/ Wnt signaling	[5]
Rodent	Stem cells (mESCs, NSCs, MSCs), Progenitors (CPCs)	*in vivo, in vitro*	Ligand, CSL	Negative regulation of Wnt signaling	Active β-catenin/ Wnt signaling	[5]
	T cells	*In vitro*	CSL	Notch-1 stimulates NF-κB	NF-κB pathway	[28]
	Primary embryonic cells	*in vitro*	PS, Ligand	HES1 activation and MCK inhibition	HES1 and MCK	[6]
	Skin progenitors	*in vivo*	CSL	Leukocytosis, longevity	nd	[7]
	Muscle stem cells (C2C12)	*in vitro*	CSL	Inhibition of muscle cell differentiation	nd	[8–10]
	Fibroblasts (3T3)	*in vitro*	CSL	Inhibition of E47	E47	[11]
	CHO cell line	*in vitro*	CSL	b1 integrin activation	R-Ras	[12]
Avian	Neural crest (stem cells)	*in vivo*	CSL	Slug expression	Slug	[13,14]
Frog	Embryo	*in vivo*	CSL	Negative regulation of Wnt signaling	β-catenin/ Wnt signaling	[15]
Fly	Wing primordium	*in vivo*	Ligand, CSL	Negative regulation of Wnt signaling	Active β-catenin/ Wnt signaling	[16,17,27]
	Muscle progenitors	*in vivo*	Ligand, CSL	Muscle precursor selection	Wnt signaling	[18,19]
	Neural progenitors	*in vivo*	Ligand, CSL	Neuronal Cell (MP2) selection	nd	[20]
	Blood cells	*in vivo*	Ligand	Hemocyte survival	Hif-a	[21]
	Wing primordium	*in vivo, in vitro*	CSL	Inhibition of ligand function	Serrate	[22]
	Embryo	*in vivo*	CSL	Dorsal epidermis patterning (closure)	JNK pathway	[23]
	Visceral mesoderm progenitors	*in vivo*	CSL	Inhibition of Wnt signaling	Ubx	[24]
	Neural precursors	*in vivo*	CSL	Repression of neural fate	Wnt signaling	[25,26]

Abbreviations: hESC, human embryonic stem cells; mESC, mouse embryonic stem cells); NSCs, neural stem cells; MSCs, mesenchymal stem cells); CPCs, cardiac progenitor cells); PS, presenilin; nd, not determined.

Molecular link between non-canonical Notch and Wnt signals: active β-catenin

Cleaved NICD has long been thought to be the activated form of Notch, whereas uncleaved membrane-bound Notch is thought to be biologically inactive and constantly internalized for recycling or degradation through an endo-lysosomal pathway [46]. Interestingly, uncleaved full-length Notch1 in the plasma membrane, generated by inactivating the Notch-processing protease Furin or site-specific mutagenesis of Furin target sequence in Notch, potently inhibits myogenesis of C2C12 myoblasts [8], which is also mediated by canonical Notch signaling [47]. However, unlike canonical Notch signaling, the uncleaved Notch mediates this event without affecting expression of the myogenic master transcription factor MyoD. These studies suggest that Notch can affect cell fates and differentiation in a non-canonical fashion. In 2005, it was demonstrated that a membrane-bound form of Notch physically interacts with β-catenin and modulates Wnt signaling by negatively regulating β-catenin activity in flies [30]. This study provided the first mechanistic clue *in vivo* for the antagonism of Wnt signaling by uncleaved Notch without involving Notch ligands and CSL.

Recent *in vivo* and *in vitro* studies provided further insight into how Notch functions not only as a membrane-tethered transcription factor, but also post-translationally by lowering levels of the transcriptionally active form of β-catenin as a membrane-bound regulator [5,15,17]. This form of β-catenin is dephosphorylated at Ser37 and Thr41 and normally constitutes a small fraction of total β-catenin [48]. In many mammalian stem and progenitor cell populations Notch levels seem to be inversely correlated with active β-catenin; increased levels of membrane Notch decrease active β-catenin levels and decreased levels of Notch increase active β-catenin levels. Notch regulation, however, does not appear to affect total β-catenin protein or transcript levels, but rather targets active β-catenin [5,30,39]. In agreement with this, the physical association of Notch and β-catenin is mostly notable in cells with high levels of active β-catenin [5,39]. The CSL binding domain of Notch, the RAM domain, was also required for the physical interaction and regulation of active β-catenin [5,49], implying a dual role for canonical and non-canonical Notch function.

It was unexpected, however, that the membrane Notch regulation of active β-catenin occurred independently of GSK3β, a major component of the destruction complex, which acts through the ubiquitin–proteasome system [50,51]. Genetic analyses revealed that membrane Notch was still able to oppose increased β-catenin activity resulting from GSK3β loss-of-function [30]. Similarly, membrane Notch could efficiently lower active β-catenin levels in stem cells treated with the GSK3β inhibitor, 6-bromoindirubin-3′-oxime (BIO) [5]. However, a newer study suggested that Axin and Apc – other key components of the destruction complex – participate in this regulation by modulating endocytosis and trafficking of membrane Notch [16]. In this process, Axin or Apc was necessary for normal trafficking of membrane Notch, which might contribute to the Notch-dependent lowering of active β-catenin levels in addition to the β-catenin destruction complex-mediated degradation [16]. It is unknown if Axin and APC are also

involved in Notch endocytosis and trafficking in vertebrates.

Similar to the inhibitory role of uncleaved membrane Notch in differentiation of C2C12 myoblasts, increased levels of a membrane-tethered form of Notch in differentiating ESCs were shown to suppress induction of Brachyury+ mesodermal cells, dependent of Wnt/β-catenin signaling [52]. This may indicate that membrane-bound Notch modulates a Wnt/β-catenin-mediated cellular response in stem cells. Intriguingly, the phenotype was recapitulated when Notch endoproteolysis was blocked by treating cells with the γ-secretase inhibitor (GSI), N-[N-(3,5-difluorophenacetyl-L-alanyl)]-S-phenylglycine τ-butyl ester (DAPT), suggesting increased levels of endogenous membrane-bound Notch may negatively regulate active β-catenin levels [5,53]. Indeed, DAPT treatment lowered active β-catenin levels and activity in various stem, progenitor and cancer cells [5]. Consistently, blocking α-secretase activity, required for ligand-mediated cleavage of NECD, showed a similar outcome [5]. This is surprising because γ-secretase inhibitors are widely used as a potent inhibitor of canonical Notch signaling, but paradoxically result in opposite biological effects: Wnt/β-catenin signaling is increased by Notch deficiency but decreased by DAPT. This might provide an explanation for some aspects of phenotypic differences between DAPT and other Notch loss-of-function mutations described earlier.

Multiple clinical studies demonstrated that a subset of non-steroidal anti-inflammatory drugs (NSAIDs) possess significant GSI activity and their chronic use is associated with lowering the risk of developing various cancer cell types – including human colorectal cancers – whose tumorigenesis is initiated by an upregulation of active β-catenin [54–56]. Although their anti-cancer effects were generally attributed to anti-inflammatory function, NSAIDs with GSI activity are also likely to contribute to the beneficial effect [57,58]. In fact, treating human colorectal cancer cells with ibuprofen, a common NSAID with GSI activity, lowered active β-catenin levels and activity in a Notch1-dependent manner [5], which agrees with the *in vivo* report that the number of intestinal adenomas is reduced by GSI treatment [59,60]. Curiously, deleting CSL also results in reduction of intestinal adenomas [60], implying canonical Notch signaling can have oncogenic function in this context. If this holds true, although CSL can independently function as a transcriptional repressor [61,62], GSI treatment might simultaneously inhibit the canonical Notch pathway and activate non-canonical Notch function, which might have increased protective effects on tumorigenesis linked with high levels of Notch/CSL signaling and active β-catenin. Indeed, GSI treatment was shown to suppress expansion of intestinal adenoma cells caused by APC mutations *in vivo* and *in vitro* [5,60].

The degradation of active β-catenin protein by the destruction complex is well understood and involves phosphorylation of the N-terminus of β-catenin leading to proteasome-mediated degradation [50,51]. Compromising the activity of the degradation complex did not prevent membrane Notch from suppressing active β-catenin protein levels and activity [5,30], implying Notch shuttles active β-catenin to the proteasome in some other manner or may lead to lysosomal degradation. However, DAPT treatment effectively decreased active β-catenin in the presence of proteasome inhibitors, suggesting a proteasome-independent mechanism in which stem and cancer cells post-transcriptionally titrate the dosage of active β-catenin. A pulse-chase experiment supports the idea of lysosomal pathway-mediated degradation of active β-catenin; the authors show in the developing fly wing disc that membrane-bound Notch is actively endocytosed into the endosomal compartment in a ligand-independent fashion and that some of the internalized Notch molecules colocalize with β-catenin in endocytic vesicles [17]. The endocytosis and trafficking required the RAM-ANK domain, which was also important for the physical interaction of Notch and β-catenin in ESCs [5,17]. However, it is unclear if high levels of active β-catenin actively trigger the endocytosis and trafficking. A similar finding was reported in APC-mutated human colorectal cells, where a membrane-tethered form of Notch colocalizes with active β-catenin and the lysosomal protein Lamp1 [5]. Moreover, compromising lysosomal activity with bafilomycin A1, a specific inhibitor of vacuolar proton ATPases [63], abrogated the DAPT-induced reduction of active β-catenin levels in mouse ESCs [5]. Thus, convincing evidence exists that membrane-bound Notch controls the pools of active β-catenin by endo-lysosomal degradation (Figure 1).

Although uncleaved Notch seems to modulate the levels and activity of active β-catenin through a lysosomal pathway, it is ambiguous if cleaved NICD also mediates the event through a similar mechanism. Several NICD overexpression studies suggest that NICD can also antagonize Wnt/β-catenin signaling by targeting active β-catenin and thereby affect cellular processes [15,17]. In vertebrates, active β-catenin activity specifies dorsal cell fates during early embryogenesis, which is essential for establishing the dorso-ventral axis, and ventral overexpression of β-catenin causes dorsalization of ventral cells [64,65]. It was reported that increased NICD levels ventralize frog embryos by opposing the dorsalizing activity of active β-catenin [15]. As in the case of membrane-bound Notch, increased levels of NICD decrease levels of β-catenin in a manner that is insensitive to GSK3β activity [15]; this is also observed in other cell types including ESCs, CPCs and ST-2 stromal cells [5,66]. By contrast, GSK3β was shown to protect NICD from proteasomal degradation [67]. The nuclear localization of NICD appears to depend on β-catenin levels in the *Xenopus* blastula cells and cancer cells; overexpressed NICD localizes in the cytosol and nuclei, but when coexpressed with β-catenin, NICD is not found in the nucleus but in cell–cell junctions [15], resembling the process mediated by membrane-bound Notch [17].

The bulk of these findings indicate that increased levels of NICD may inhibit active β-catenin levels in a similar mechanism to that of membrane-bound Notch. However, under endogenous conditions NICD might mostly translocate to the nucleus after membrane cleavage, whereas overexpression of NICD results in aberrant localization of significant NICD in the cytosol. Thus, although NICD interacts with active β-catenin for lysosome-mediated degradation in the cytosol, this may not be its normal function under physiologic conditions.

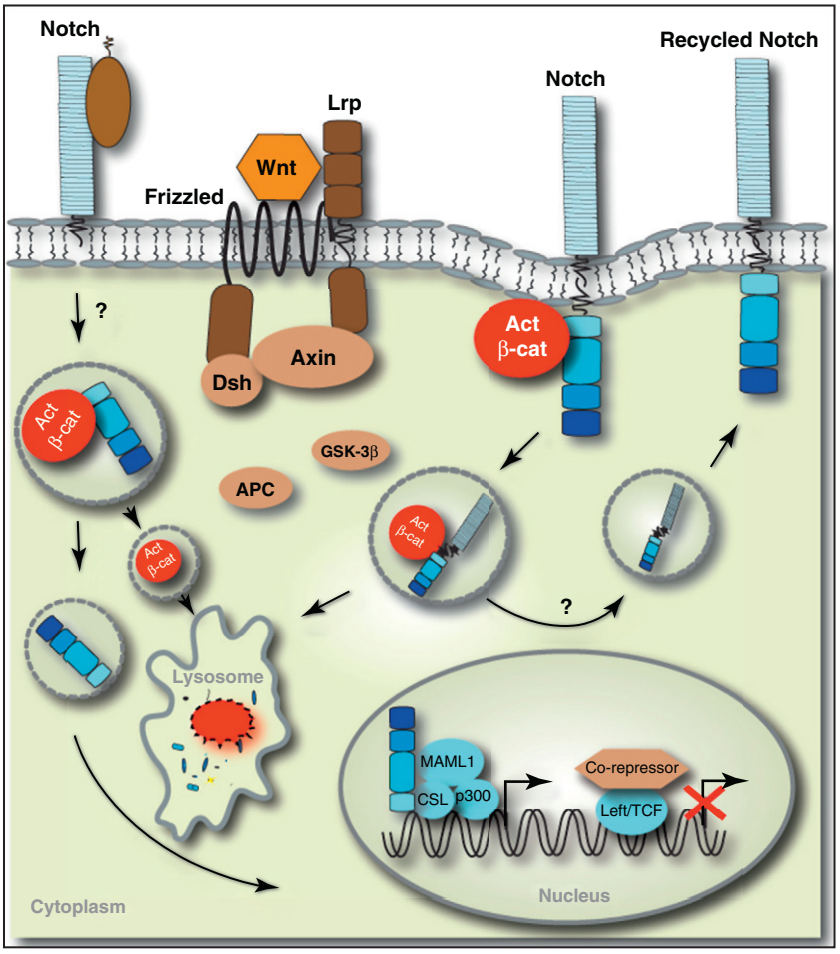

Figure 1. Post-translational regulation of β-catenin protein by Notch. Notch can negatively regulate active β-catenin levels in a non-canonical fashion. In the presence of Wnts, membrane-bound Notch forms a complex with active β-catenin and degrades active β-catenin through an endo-lysosomal pathway. The degradation is independent of GSK3β-dependent destruction complex. Whether Notch is recycled back to the membrane is unclear. NICD can also regulate active β-catenin levels in a similar mechanism, although it is unknown whether endogenously processed NICD regulates active β-catenin protein. Protein interactions can be either direct or indirect.

Curiously, Notch was also shown to physically associate with the endocytic protein Numb and required Numb and its homolog Numblike to regulate active β-catenin activity in ES cells [5,68]. These findings indicate Numb may be a key component of the non-canonical Notch pathway. The potential role and mechanisms of Numb will be discussed in the next section.

Potential role of Numb in Notch and β-catenin regulation

While Notch had been defined as a fundamental mediator of extrinsic factors for cell-fate specification, Numb was identified as the primary intrinsic factor that antagonized Notch in classic studies in *Drosophila* [68]. This interaction depends on the spatio-temporal distribution of Numb during cell division to one pole of the cell resulting in asymmetric cell division in which daughter cells retained distinct properties and different fates [69,70].

Numb might inhibit canonical Notch activity by direct interaction or as a mediator recruiting other factors to prevent the nuclear translocation of Notch protein [69,71]. One mechanism of inhibition requires Numb to bind the NICD of membrane-bound Notch with a third party to sequester Notch [72]. For instance, α-adaptin, a component of adaptor complex 2, is asymmetrically distributed with Numb and these proteins interact to induce endocytosis of Notch at specific sites [72]. Numb-dependent regulation of Notch may also occur via endosome-independent pathways [73]. For example, Numb interacts with E3 ligases to promote ubiquitination of membrane-bound Notch, leading to its subsequent degradation [74].

Membrane-bound Notch is constantly internalized through endocytosis and then sorted for endosome-mediated recycling to the membrane or for lysosomal degradation by Numb [75]. Because Numb and its homolog Numblike appear to be necessary for degradation of active β-catenin by membrane Notch [5], it is reasonable to consider that the Notch-β-catenin complex is being trafficked into lysosomes for degradation. This is in agreement with the recent finding that Notch associates with active β-catenin and together are endocytosed into the endosomal compartment [17]. It remains to be determined if Numb shares common molecular machinery for the regulation of Notch and active β-catenin and, if so, how Numb selectively affects the activity and levels of Notch and active β-catenin (Figure 2).

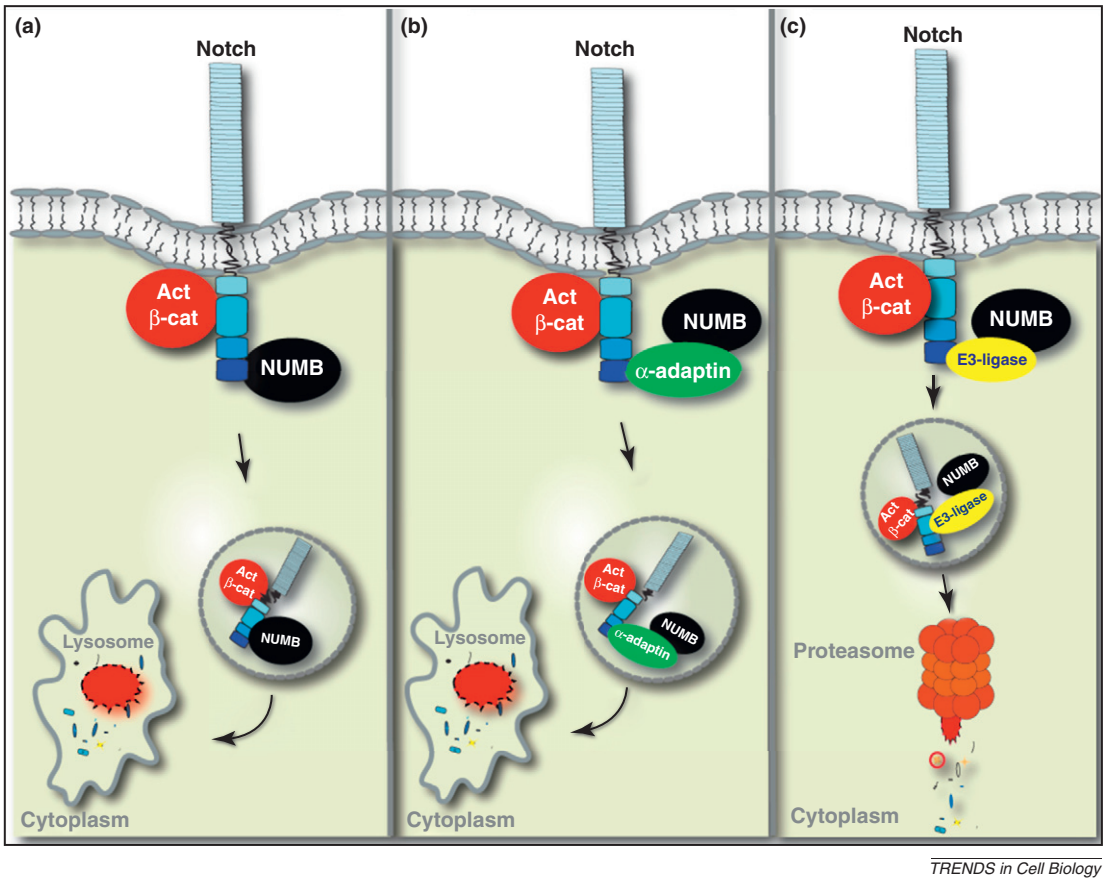

Figure 2. Proposed model for Numb regulation of Notch and β-catenin. Numb could bind directly to Notch independently of α-adaptin **(a)** or could bind via an α-adaptin-dependent mechanism **(b)** with subsequent targeting of the Numb-Notch complex for lysosomal degradation. In both cases it might be possible that activated β-catenin could also be targeted for lysosomal destruction either as an innocent bystander or through an active process with unknown partners. Downregulation of Notch may occur through Numb-mediated targeting via ubiquitination intermediaries, such as E3-ligase, for proteasome-mediated degradation **(c)**.

Physiological significance

During the past decade increasing evidence has suggested that a complex functional relation exists between Notch and Wnt signaling, particularly during establishment of stem and progenitor cell fate determination and cancer formation. The recent findings of how membrane-bound Notch post-translationally regulates Wnt/β-catenin signaling provide novel insight into this complex relation during fundamental biological and disease processes, such as proliferation, differentiation, lineage decisions and tumorigenesis. Although increased levels of membrane Notch were shown to significantly affect key cellular events including proliferation and differentiation, the physiological role of membrane Notch remains to be elucidated. Further investigation is now required to understand the endogenous function of the membrane Notch/β-catenin pathway.

Concluding remarks

It has been puzzling that endogenous Notch protein is mostly detected at the cell membrane and/or cytoplasm but rarely seen in the nucleus. With accumulating evidence it is now becoming apparent that Notch can function in non-nuclear environments, where it affects canonical Wnt signaling by titrating active β-catenin levels. Although active β-catenin has emerged as a conserved mediator of a ligand/CSL-independent Notch pathway across species, it is probable that Notch interacts with additional key players, such as Numb and Numblike, to control cellular processes outside the nucleus. Thus, it will be crucial to identify these molecules and determine their roles in the non-canonical pathway (Box 3). Nevertheless, the functional and molecular interactions of Notch and active β-catenin provide a potential explanation for many aspects of non-canonical Notch effects described, and make active β-catenin levels and activity useful readouts for non-canonical Notch activity.

GSIs and NICD have been widely used to mimic canonical Notch loss-of-function and gain-of-function mutations respectively; however, it is important to acknowledge the fact that both can act as potent inhibitors of active β-catenin. Although this observation could provide the foundation for novel therapeutic targets, caution regarding

Box 3. Outstanding questions

- What proteins are associated with the membrane Notch–β-catenin complex and what are their roles?
- What is the role of Numb in Notch regulation of active β-catenin?
- What is the biological role of the membrane Notch/β-catenin pathway in stem/progenitor cell maintenance and lineage-specific differentiation?
- Can the membrane Notch/β-catenin pathway be targeted for cancer therapeutics?
- Is there a β-catenin-independent function and mechanism of membrane Notch?

their effects on Wnt/β-catenin signaling is warranted when they are used for experimental or therapeutic purposes.

At present, the biology of membrane Notch has been minimally explored in the field of stem, progenitor and cancer cells. Notch and Wnt/β-catenin signaling are directly involved in, and essential to, nearly all known stem/progenitor cell self-renewal and differentiation processes, and in oncogenesis. As such, future investigation of the biological function and mechanism of the membrane Notch/β-catenin pathway will greatly expand our fundamental knowledge of stem, progenitor and cancer cell biology, and could eventually be leveraged for regenerative and therapeutic approaches.

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