# Notch signalling in context

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Abstract | The highly conserved Notch signalling pathway functions in many different developmental and homeostatic processes, which raises the question of how this pathway can achieve such diverse outcomes. With a direct route from the membrane to the nucleus, the Notch pathway has fewer opportunities for regulation than do many other signalling pathways, yet it generates exquisitely patterned structures, including sensory hair cells and branched arterial networks. More confusingly, its activity promotes tissue growth and cancers in some circumstances but cell death and tumour suppression in others. Many different regulatory mechanisms help to shape the activity of the Notch pathway, generating functional outputs that are appropriate for each context. These mechanisms include the receptor–ligand landscape, the tissue topology, the nuclear environment and the connectivity of the regulatory networks.

Paralogues
Sequences, or genes, that have originated from a common ancestral sequence, or gene, by a duplication event.

Lateral inhibition
The process by which a cell with a particular fate interacts with its immediate neighbours to prevent them from adopting the same fate.

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Despite the fact that the core Notch pathway operates in vastly different developmental and disease contexts, from stem cell regulation and heart morphogenesis to cancers and cardiomyopathies, it is relatively simple in its operation. Ligand-mediated activation induces a series of proteolytic cleavages in members of the Notch family of receptors, which release the Notch intracellular domain (NICD) (FIG. 1; BOX 1). Once released, the NICD enters the nucleus and, together with the DNA-binding protein CBF1–Suppressor of Hairless–LAG1 (CSL; also known as RBPJ) and the co-activator Mastermind (Mam; Mastermind-like transcriptional co-activator 1 (MAML1) in human), stimulates transcription of target genes[1–4] (FIG. 1). No intermediates are present between the membrane and the nucleus, and thus no amplification of the signal can occur, unlike in many other pathways — NICD takes responsibility for implementing pathway activation.

Given this relative simplicity — receptor–ligand interactions release the bioactive NICD — how can the canonical Notch pathway coordinate so many diverse biological outcomes? *Drosophila melanogaster* has one Notch receptor and two ligands, which are the transmembrane proteins Delta and Serrate (Ser), whereas mammals have four Notch paralogues (Notch1–4) and various ligands in the Delta-like (DLL1, DLL3 and DLL4) and Jagged (JAG1 and JAG2) protein families. This limited repertoire clearly cannot account for the diversity of Notch pathway signalling outcomes, indicating that other mechanisms of regulation exist.

In this Review, we discuss features that enable the Notch pathway to function differently according to its setting and that help to explain the myriad of roles that the pathway has in development and disease. These features range from the patterned deployment of key components to the composition of the nuclear milieu. The types and extent of cell–cell contacts are also emerging as important factors, along with the wiring of regulatory circuits that shape the response. It is only possible to draw on a small subset of examples to illustrate these properties owing to space limitations, but these examples highlight fundamental principles that should be widely applicable across the different contexts in which the Notch pathway operates.

## Ligand–receptor landscapes

Early models suggested that Notch-mediated developmental patterning occurred when stochastic differences in ligand–receptor interactions directed signalling in fields of cells with near-uniform levels of ligand expression. It now seems that this scenario is the exception rather than the rule: the expression profiles of ligands, receptors and several modifying enzymes have important roles in defining whether Notch signalling is activated and its functional outcome. Furthermore, other signalling pathways can regulate these expression patterns to augment, inhibit or modulate Notch pathway activity, providing an important mechanism of crosstalk.

***Expression of canonical ligands and receptors.*** The classic paradigm of lateral inhibition, which is a common phenomenon during selection of neural and muscle precursor cells, assumed that the driving force for signalling arose through stochastic differences in ligand levels. The initial bias would be reinforced by a negative-feedback loop so that ligand expression became repressed in signal-receiving cells, generating a spaced distribution of signalling cells and receiving cells that explained the distribution of precursor cells formed[5]. It now seems that signalling rarely relies on uniformly distributed ligands;

rather, their spatial and temporal regulation directs Notch signalling profiles in many contexts and is an important mode for regulation by other signalling pathways. Although regulation of ligand expression mostly occurs at the transcriptional level, microRNAs also contribute. For example, members of the miR-200 family target the 3′ untranslated regions of Ser and JAG1 to fine-tune the levels of protein produced in *D. melanogaster* and in human tissues, respectively[6,7].

One example of how ligand expression dictates the spatial pattern of signalling occurs during the formation of the growth organizer in *D. melanogaster* wing imaginal discs. Here, Ser is produced exclusively in the dorsal territory owing to its regulation by the spatially restricted Apterous transcription factor[8,9], and it specifically generates a stripe of Notch activity in adjacent ventral cells to create a boundary that organizes tissue growth[8]. Dynamic changes in expression of both ligand and receptor also help to drive oscillations in Notch signalling[10], such as those that occur during somitogenesis in the presomitic mesoderm. Here, Notch1 expression is dependent on its pathway activity, which might help to reinforce signalling, whereas ligand expression is regulated by WNT signalling, thus ensuring that the pattern of Notch activity is instructed by another main component involved in the somite clock[11,12].

In addition, interplay between different ligands is frequently required to set the correct number and spacing of precursor cells in many situations. For example, in the zebrafish ear, a broad 'inductive' signal by Ser (JAG1 in mammals) sustains the neurogenic potential of the placode[13,14] before subsequent dispersed expression of Delta (DLLs in mammals) in incipient precursors inhibits the surrounding cells to ensure that a single precursor cell is specified at each position[15–18]. Likewise, to establish the branching pattern of blood vessels, the production of DLL4 in tip cells prevents neighbouring cells from adopting the same fate[19,20], whereas JAG1 acts as a potent pro-angiogenic regulator that antagonizes DLL4–Notch signalling to favour new sprouting.

Although the fundamental consequences of ligand binding are the same — cleavage of Notch receptors to produce NICD — they nevertheless often elicit different outcomes[21]. One possibility is that different ligands bring about different strengths (or durations) of intracellular signal. For example, JAG1 and DLL1 have lower measured affinities for Notch1 than does DLL4, possibly owing to differences in the orientation of the amino-terminal and Delta–Ser–LAG2 (DSL) domains involved in the binding interface[22]. Indeed, in the mouse haemangiogenic endothelium, specification of haematopoietic stem cells involves a low-strength JAG1-dependent signal, whereas specification of endothelial arterial cells requires a high-strength DLL4 signal[23]. Strikingly, cells that received the low-strength JAG1-induced Notch activity seemed to be unresponsive to a high-strength DLL4 signal. Although it is unclear what causes this lack of response, if the expression of JAG1 precedes that of DLL4, it could switch on an inhibitory programme or cause cell re-arrangements to disrupt contacts with DLL4. Similarly, in the inner ear JAG1 might elicit a lower level of Notch1 activation than DLL1 does, as JAG1 can induce the expression of Hairy/Enhancer of split related with YRPW motif protein 1 (HEY1), which requires a low threshold, but not Hairy and Enhancer of split 5 (HES5), which requires a higher level of signalling[24].

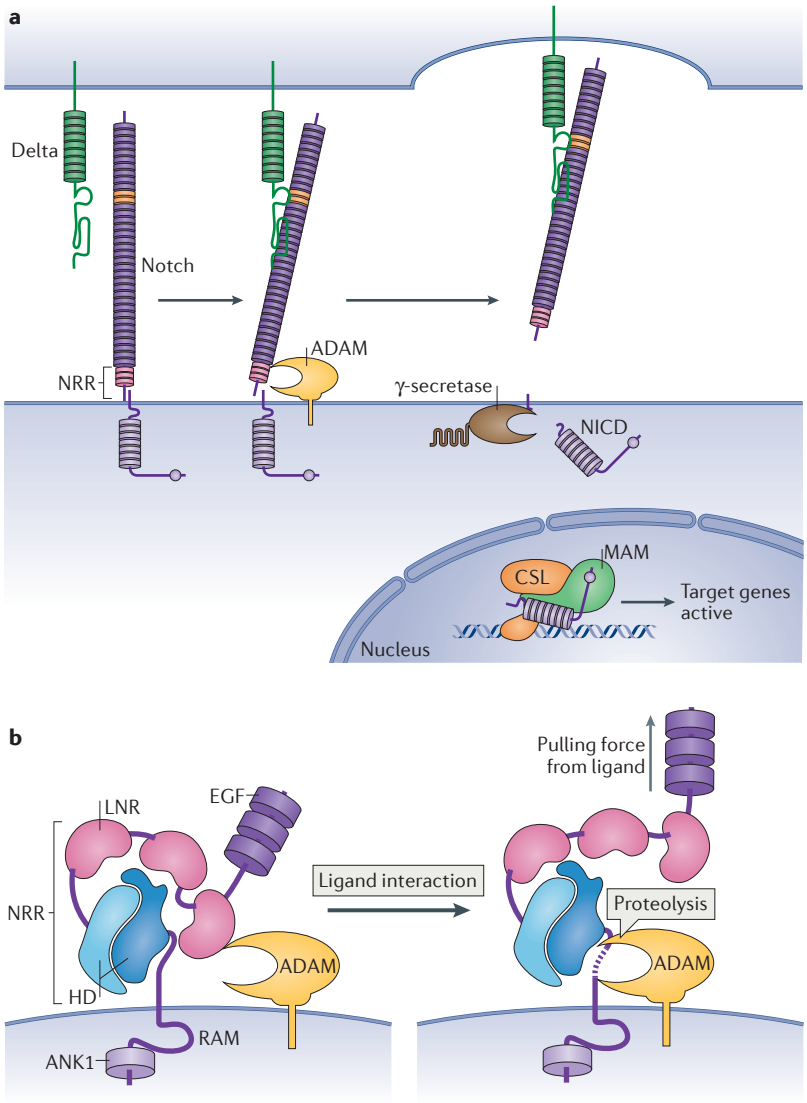
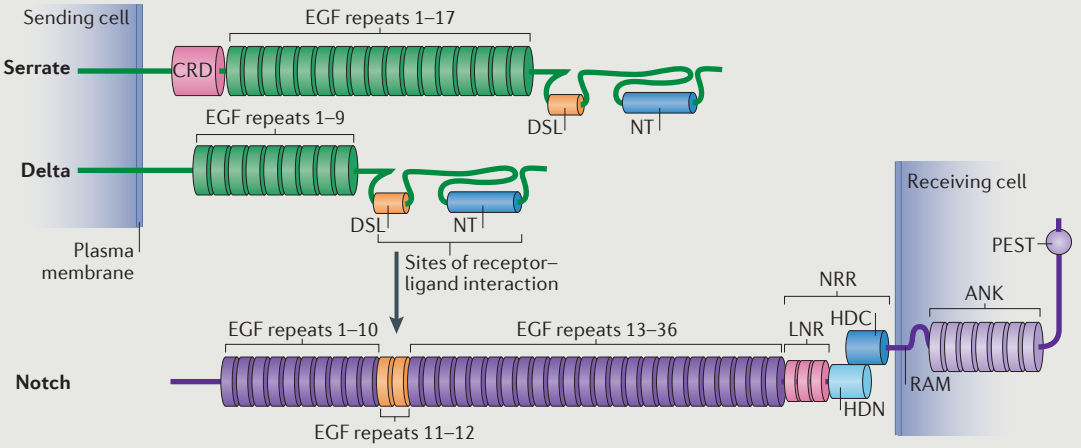



Figure 1 | **Ligand binding leads to exposure of the cleavage site in Notch. a** | Summary of core pathway: when canonical Notch ligands (green) bind to Notch receptors (purple; orange indicates EGF repeats 11–12, pink indicates the negative regulatory region (NRR)) on the adjacent cell surface, they elicit two proteolytic cleavage events, the first by ADAM10 and the second by γ-secretase, that release the Notch intracellular domain (NICD). In the nucleus, NICD interacts with the DNA-binding protein CBF1–Suppressor of Hairless–LAG1 (CSL)[3,171–173] and the co-activator Mastermind (MAM) to promote gene transcription[172,173]. **b** | Schematic based on the crystal structure[56,57], illustrating how the Notch NRR occludes the cleavage site for ADAM proteases until it is exposed by forces generated through ligand binding. The NRR comprises three Lin12/Notch repeats (LNRs; pink) and the heterodimerization domain (HD; dark blue and light blue), which surround the recognition site, making it inaccessible to ADAM proteases. Also represented are three EGF repeats from the Notch extracellular domain (ECD), the RBPJ-associated module (RAM) and first ankyrin repeat domain (ANK1) in NICD (left panel). Ligand binding exerts a force on the receptor (right panel), which displaces the LNRs, exposing the site for cleavage by ADAM10 (right panel). This cleavage renders the residual transmembrane Notch fragment as a substrate for proteolysis by the γ-secretase complex, to release the NICD (as in part **a**). Part **b** is adapted with permission from REF. 57, Elsevier.

Box 1 | **Mechanistic features of Notch signalling**

All canonical Notch ligands are transmembrane proteins (apart from some unusual relatives in *Caenorhabditis elegans*) that share a largely similar structure, with an extracellular domain comprised primarily of multiple EGF repeats (see the figure, which shows *Drosophila melanogaster* Notch and its ligands, Serrate and Delta). Serrate and its Jagged orthologues also contain a cysteine-rich domain (CRD). Binding by canonical Notch ligands involves the extracellular Delta–Serrate–LAG2 (DSL) domain and amino-terminal (NT) domain, which contact EGF repeats 11–12 in the extracellular domain of Notch[22,168]. As the NT domains have phospholipid-binding characteristics[169], interactions with the adjacent cell membranes might also be involved. Notch receptors on the cell surface are heterodimers: the two heterodimeric portions (HDN and HDC) interact and, together with the cysteine-rich Lin12/Notch repeats (LNRs), form the negative regulatory region (NRR), which occludes the cleavage site for ADAM proteases[56]. The key step induced by ligand binding is the exposure of this cleavage site, which allows access by proteases. ADAM10 is likely to be the main protease responsible for cleavage under physiological conditions[170]. Cleavage renders the remaining transmembrane–intracellular fragment a substrate for the γ-secretase complex, which catalyses intramembrane proteolysis to release the Notch intracellular domain (NICD). NICD is characterized by an RBPJ-associated module (RAM) domain and ankyrin (ANK) repeats, both of which are required for interactions with the DNA-binding complex CBF1–Suppressor of Hairless–LAG1 (CSL)[3,171–173]. Near the carboxyl terminus is a PEST domain, which regulates NICD degradation. Between the ANK repeats and PEST, NICD also contains several nuclear localization signals and a region that can confer transactivation. The association of NICD with CSL forms an interface to which the N terminus of the co-activator Mastermind (Mam) binds, locking the complex into its active conformation and promoting gene transcription[172,173].




Growth organizer
Group of cells that produces signals necessary to promote growth of a tissue.

Somitogenesis
The process by which somites, blocks of mesoderm that give rise to axial muscles, bones and dermis in vertebrates, are formed.

Somite clock
Oscillatory mechanism that ensures periodic formation of somites.

Placode
Ectodermal thickening from which a sense organ or ganglion develops.

Tip cells
Leading cells of sprouting blood vessels that sense their environment for guidance cues.

Sprouting
Initiation of a branch from blood vessels during angiogenesis.

Haemangiogenic endothelium
A subset of endothelial cells that can give rise to haematopoietic stem cells as well as to blood vessels.

In animals with multiple Notch paralogues, the presence of different receptors will also influence signalling outcomes. In humans, mutations in different paralogues have different disease consequences: for example, only defects in Notch3 cause cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), emphasizing different receptor roles[21,25,26]. Although these differences might be partly explained by variations in their expression patterns, examples exist in which individual paralogues — for example, Notch1 and Notch2 — make different contributions even when expressed in identical patterns. Indeed, Notch1 and Notch2 were found to have opposite effects on the growth of one specific tumour cell type[27]. When the intracellular domains of Notch1 and Notch2 were swapped in each normal gene context, their intrinsic activities were similar[28,29], but in some contexts the amount or stability of the NICD moieties differed[29]. This may reflect differences in their potential for post-translational modifications. These observations emphasize the possibility that, ultimately, the amount or duration of the NICD 'signal' produced will depend on the specific ligand–receptor pairs that are engaged, as well as the availability of the metalloproteinases that are essential for the activating cleavages. Whether such NICD differences would result in quantitative or in qualitative differences in the downstream signalling output is an important question that remains to be resolved.

***Contributions from* cis *inhibition.*** Notch signalling is highly sensitive to the relative levels of ligands and receptors owing to *cis* inhibition, which is an inhibitory interaction that occurs when ligand and receptor are present in the same cell[30] (FIG. 2). First identified through genetic experiments in *D. melanogaster*[31,32], the precise mechanism for *cis* inhibition remains elusive, although some studies suggest that Notch molecules are targeted for degradation after they have undergone ligand interaction[33,34].

Modelling experiments suggest that an ultrasensitive switch between two mutually exclusive cell states, signal-sending and signal-receiving, could be generated by Notch having a sharp ligand threshold for *cis* inhibition combined with a graded response to *trans*-acting ligands[35]. One example of a *cis*-inhibitory interaction affecting the signalling outcome occurs during photoreceptor specification in *D. melanogaster*: here, loss of Delta-mediated *cis* inhibition reversed the direction of lateral signalling, thereby generating

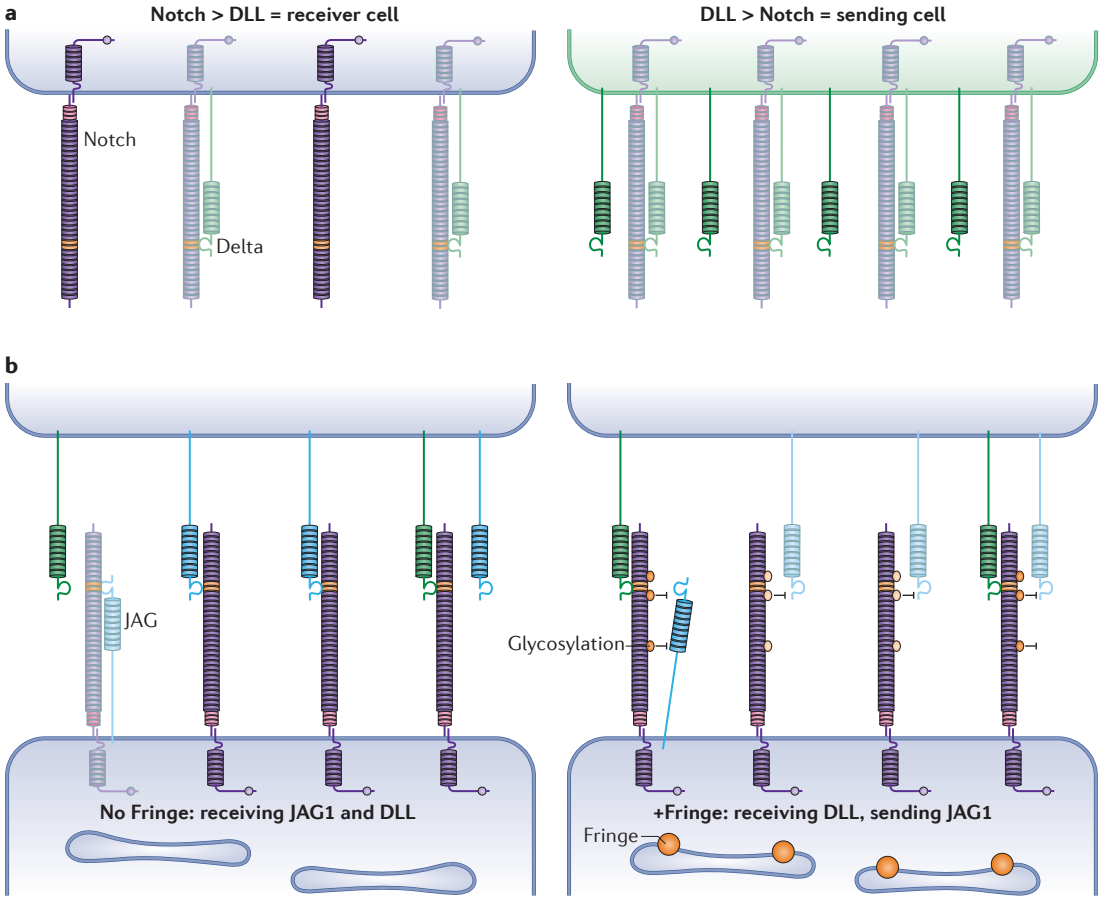



Figure 2 | **The consequences of *cis* inhibition and Fringe expression on Notch signalling. a** | Relative levels of ligands (green) (Delta in *Drosophila melanogaster*, Delta-like (DLL) in mammals) and Notch receptors (purple) determine whether cells send or receive signals because *cis* interactions between ligands and receptors present on the same cells are inhibitory (light shading). A receiver cell (left) expresses more Notch than DLL (Notch > DLL); some Notch molecules are *cis*-inhibited by DLL, but sufficient Notch remains available to interact with ligands from neighbouring cells, making the cell capable of receiving signals. A sending cell (right) expresses more DLL than Notch (DLL > Notch); all Notch molecules are *cis*-inhibited by DLL, and sufficient DLL remains available to interact with receptors on neighbouring cells, making the cell capable of sending a signal. **b** | Fringe proteins (orange), located in the Golgi, glycosylate the Notch extracellular domain and modulate both *cis* and *trans* interactions with ligands. In the absence of Fringe (left), Jagged 1 (JAG1) (turquoise) preferentially *cis*-inhibits Notch, so that none of the ligand present in this cell is available for signalling. Any uninhibited Notch is competent to interact with either DLL (green) or JAG1 ligands from neighbouring cells. When Fringe is present (right), glycosylation of Notch interferes with JAG1 *cis* and *trans* interactions, with the result that the cell can only receive signals from DLL ligands (green), but can now send JAG1 signals to neighbouring cells.

the wrong complement of photoreceptors[36]. Likewise, *cis* inhibition helps to stabilize tip and stalk cell fates during angiogenesis, and hence prevents hybrid tip–stalk cells forming[37]. In addition, different Notch receptors might also *cis* inhibit one another, adding another potential mechanism for fine-tuning signal reception[38]. The balance between *cis* interactions and *trans* interactions is thus likely to be important in determining signalling outcomes (FIG. 2a).

Intriguingly, the mammalian ligand DLL3 might operate only in a *cis*-inhibitory mode. In cell-based assays, DLL3 was unable to activate signalling in *trans* but, when co-expressed with Notch1, it could prevent Notch1 responding to other ligands on neighbouring cells[39]. *In vivo*, the loss of DLL3 led to increased Notch activity during T cell development[40] and to defects in Notch1 signalling in the presomitic mesoderm[41]. It is not fully clear why DLL3 might have a uniquely inhibitory role, although its highly divergent DSL domain might be a contributory factor. However, this inhibitory function adds another strand of pathway regulation in vertebrates.

***Deployment of Fringes and other modifying enzymes.*** Further influencing Notch signalling is the presence of enzymes that modify the extracellular domains of ligands and receptors and modulate their ability to signal (FIG. 2b). Although modifications to ligands might be important, most focus has been on receptors, the activity of which is profoundly affected by glycosylation of EGF repeats in the extracellular domain. *O*-fucosyltransferases, which add fucose to serine and threonine residues, and *O*-glucosyltransferases,

EGF repeats
Protein domains, commonly found in the extracellular domain of membrane-bound proteins, that are related to a sequence in EGF and include cysteine residues involved in disulfide bonds. EGF-like domains frequently occur in numerous tandem copies in proteins, as in Notch.

which add glucose to serine residues, are essential for optimal Notch signalling[42,43]. Many *O*-fucose monosaccharides on Notch can subsequently be extended (with *N*-acetylglucosamine) by the Fringe proteins, with differing effects on Notch, depending on the sites modified and the ligands present[42].

Fringe-mediated modifications could influence specific ligand–receptor interactions. Structural studies indicate that elongation of an *O*-fucose on EGF repeat 12 of Notch receptors could provide additional energetic contributions to Notch–ligand interfaces[22]. This modification enhanced binding of DLL1 and JAG1 to a greater extent than binding to DLL4, possibly because the inherent affinity of DLL4 is relatively high even in the absence of glycosylation[22]. Whether or not the enhanced ligand binding translates into increased pathway activity is uncertain. In some cases in which the affinity of JAG1 for the receptor was increased by the presence of Fringe proteins, the effects on transcriptional output were reduced[44]. Concomitant changes to *cis* inhibition, to competition with other ligands or to other sites in the receptor are possible explanations for this apparent anomaly.

Fringe proteins are thus likely to modulate the ability of cells to send and receive signals in a manner that is highly dependent on the cocktail of ligands (and Fringe proteins) present (FIG. 2b). For example, in *D. melanogaster* wing imaginal discs, modifications by Fringe render Notch insensitive to Ser[45]. Here, co-expression of Ser and Fringe in dorsal cells guarantees that Ser can only signal to the adjacent ventral territory[8]. At the same time, Delta is enriched in ventral cells, and its binding is enhanced by Fringe modifications[45,46]. The combined effects generate a stripe of cells with Notch activity that straddles the boundary. Likewise, when high levels of both JAG1 and Fringe are present in stalk cells, Fringe is thought to make JAG1 into an effective competitor for the more signalling-proficient DLL4, which prevents DLL4 from signalling between adjacent stalk cells, and thereby inhibits excessive sprouting[47]. Similarly, during ventricular development, temporal modulation of Manic Fringe (MFNG) enables sequential Notch activation to drive different morphological processes; MFNG and DLL4 downregulation in the endocardium allows these cells to respond to myocardial JAG1 and/or JAG2 and generate a functional ventricular wall[48]. Finally, in the spinal cord, where Fringe enzymes enhance DLL-activated Notch signalling and block that of JAG1, the consequence of their patterned expression (controlled by homeodomain proteins) is domain-wide Notch activation by either DLL1 or JAG1, and a suppression of signalling across progenitor domain boundaries[49].

Experiments modelling the outcome of Fringe modifications have shown how these could affect the relative signalling capabilities of cells. For example, cells expressing Lunatic Fringe (LFNG) or MFNG in combination with JAG1 and Notch1 acquired the ability to send and receive signals simultaneously, but only using different ligands[50]. This observation could be explained if Fringe modifications weaken *cis* interactions between JAG1 and Notch1, consequently making high surface levels of both available, and also prevent Notch1 from being *trans* activated by JAG1 (FIG. 2b). Notch1 could thus only receive signals from DLL ligands on adjacent cells, but JAG1 would itself be free to signal to neighbouring cells. Such a model fits well with the observations at the dorsal–ventral boundary of the *D. melanogaster* wing and with the tip/stalk decision in angiogenesis, and highlights the profound effect of the patterned deployment of these molecules on signalling outcomes.

## Topological context

Notch receptors and their ligands are transmembrane proteins. Furthermore, endocytosis of receptors and ligands is known to be required for ligand activity[51,52]. Mechanisms that transport the proteins to and from the correct places in the cell are therefore likely to have an important impact on signalling. Although it has largely been assumed that ligand–receptor interactions occur at sites where cells are tightly opposed, several observations are challenging this assumption, and highlight that the dimensions and stability of the contact area might have important roles.

***Endocytic trafficking and ubiquitin ligases.*** Factors that modulate endocytosis and trafficking of receptors and ligands have a number of important consequences on pathway activity[53,54]. First, endocytosis of the ligand after it has engaged with its Notch receptor is thought to generate the force on the receptor that exposes the protease cleavage site in the negative regulatory region (NRR) 'cage' (REFS 55–57) (FIG. 1b). In the absence of certain E3 ubiquitin ligases of the Mindbomb (MIB) or Neuralized (NEUR) families, ligand endocytosis is prevented with concomitant loss of signalling[52,58–64]. Because ubiquitylation of the ligand intracellular domains by these E3 enzymes is critical for ligand activity, the presence of MIB and/or NEUR proteins determines which cells can send a signal. During asymmetric division of SOP cells, for example, directional Notch signalling is achieved, in part, by the polarized segregation of these E3 ligases into one of two daughter cells, depending on the underlying cell polarity[65–67].

Second, receptor trafficking affects pathway activity not only by determining receptor levels on the cell surface but also because ligand-independent activation can occur during this process[53,54,68]. Some of the endocytosed Notch is likely to be recycled to the membrane, whereas a large fraction is targeted for degradation. When this latter fraction of the receptor fails to be properly routed into the inner luminal vesicles of maturing lysosomes — for example, in response to mutations that affect the endosomal sorting complex required for transport (ESCRT) complex[69–72] — this can result in ligand-independent Notch activation. The precise mechanisms that underlie this phenomenon are unknown, but it is likely that the conditions encountered in the lysosome promote ligand-independent activation by destabilizing the NRR. Proteins that regulate endolysosomal transport have a concomitant effect on Notch trafficking that can, in some cases, result in receptor activation in normal physiological conditions. These include the E3 ligases Deltex and Itch (the mammalian protein; known as Suppressor

SOP cells
(Sensory organ precursor cells). Cells that give rise to all of the cells in a *Drosophila* species sensory organ.

Endosomal sorting complex required for transport
(ESCRT). The multiprotein ESCRT machinery (ESCRT-I, -II and -III) promotes inward vesiculation at the limiting membrane of the sorting endosome, and selects cargo proteins for delivery to the intra-luminal vesicles of multivesicular bodies.

of Deltex in *Drosophila*), which, by ubiquitylating Notch, alter both the receptor levels on the membrane and the amount of ligand-independent activation[73–75].

Numb is another factor that regulates cell-fate decisions through its powerful effects on receptor trafficking; its depletion in several lineages results in ectopic Notch activity. During sensory organ development, Numb is differentially segregated into one of two daughter cells, in which it inhibits Notch activity to bring about specific 'Notch off' fates[76–80]. Numb achieves this inhibition either by promoting Notch internalization or by altering the route taken after Notch is endocytosed[81,82]. Notably, this phenomenon only occurs in certain contexts, despite Numb being present more widely. In *D. melanogaster*, this specificity depends on the adaptor Sanpodo, which couples Numb localization with Notch trafficking[82–84]. Adaptors analogous to Sanpodo are likely to perform a similar task in other species.

Third, spatial regulation of trafficking also has the potential to affect the geometry of signalling. For example, endocytic depletion of Notch from the cleavage furrow at the division of sensory organ precursor cells in *D. melanogaster* is important to enable unidirectional signalling in the progeny[85]. The asymmetrical distribution of endosomes containing receptors and/or ligands is also associated with a bias in signalling to promote specific cell fates in neural lineages[86,87]. In addition, by affecting the activity and/or localization of the γ-secretase complex, which is required for the release of the NICD, proteins that organize cellular polarity (for example, Crumbs[88] and Hibris[89]) can consequently affect Notch signalling. Finally, beyond regulating the activity of the ligands and receptor, endocytic trafficking might also be important for localizing functional pools of ligand and receptor to the appropriate cellular subdomains, although many questions remain about whether such subdomains exist and where they might be located.

***Tissue architecture and morphology.*** Because Notch signalling occurs between cells that are in contact with one another, the organization of the tissue is likely to influence the levels or patterns of signalling, such that the strength and periodicity of signalling could depend on the extent or durability of the adhesive contacts between cells. For example, models suggest that, under conditions in which the extent of cell–cell contact is greater than the diffusion range of the ligand within the membrane, the signal generated will be proportional to the contact area[90]. Signalling might also be mediated by dynamic cellular protrusions such as filopodia, which, importantly, could extend the distance over which signalling occurs[91–94]. The nature of the cell–cell contacts could therefore have important consequences for the functional outcomes of signalling (FIG. 3a).

Adherens junctions mediate cell–cell adhesion and are important for effective Notch signalling in some contexts. Thus, during vertebrate neurogenesis, the dimensions of the contacts made by the 'end feet' and the integrity of their adherens junctions are important for effective DLL–Notch signalling between nascent neurons and their progenitors to prevent the latter from differentiating. Disrupting the adherens junctions downregulated Notch signalling and caused precocious neurogenesis[95], whereas manipulations that expanded the size of the apical domain enhanced signalling and reduced neurogenesis[96].

Strong adhesion between posterior lateral mesoderm and somite cells, mediated by junctional adhesion molecule 1a (Jam1a) and Jam2a, is also important for generating a sufficiently high Notch signal to specify the haematopoietic lineage in zebrafish[97]. The contact surface area between migrating lateral mesoderm cells and the somite was decreased when *jam1a* or *jam2a* was depleted, and correlated with decreased activation of Notch signalling. As this phenotype could be rescued by widespread expression of ligands, the transduction pathway remained functional, leading to the model that dimensions or stability of contacts was important. The deployment of specific adhesion molecules can thus create a unique topological opportunity for signalling to occur.

Signalling might not always require stable cell–cell contacts: transient interactions can be sufficient to deliver Notch activity and switch cells to a specific cell fate. Neural crest cells expressing DLL proteins could elicit signalling in myotome Notch1-containing cells that they contacted, in a 'kiss and run' mode[98]. Likewise, during angiogenic sprouting cells constantly shuffle their positions, which means that a presumptive stalk cell might encounter high levels of Notch for only a brief period before losing contact[99] (FIG. 3b). Transient structures may also extend the range over which a cell can signal. For example, filopodia extend from Delta-producing cells on the *D. melanogaster* notum, and their disruption (by specific genetic mutations) perturbed the spacing of sensory organ precursor cells[91] (FIG. 3b). Similarly, dynamic filopodia project between neurogenic progenitors and radial glial cells during signalling in the mammalian neocortical progenitor cell niche, although their functional relevance has not yet been tested[100]. And during the formation and maintenance of pigmented stripes in zebrafish, signalling is mediated through long cellular protrusions that extend between xanthophores and melanophores. Interestingly, the geometry switches: first the protrusions carry vesicles of DLL from xanthophores to promote melanophore stripe consolidation[93] (FIG. 3b). Later, in adults, the melanophores extend protrusions towards inter-stripe, DLL-expressing xanthophores to receive signals that are necessary for their own survival[94].

Filopodia-like protrusions are more transient than adherens junctions, indicating that a prolonged stable contact point might not be essential for ligand–receptor signalling, although the extent of their contribution to Notch signalling remains to be determined. Modelling experiments suggest that the strength of the signal mediated through filopodia will depend principally on the diffusion of ligands or receptors[90], so that factors that regulate their trafficking would be influential. Furthermore, it is possible that the types of downstream response might differ according to the way that the ligand is presented — for example, filopodia might provide a burst of signal, whereas more stable cellular junctions could generate a more sustained signal.

Filopodia
Thin cellular processes containing long, unbranched, parallel bundles of actin filaments.

Adherens junctions
Actin-filament-associated, epithelial cell–cell junctions that have classical cadherins as their core component.

End feet
The name given to the apical membrane surface as a consequence of cortical neuroepithelial progenitors becoming very tall and thin over the course of development.

Notum
Structure that is part of the back of an animal; in insects, the back of the thorax.

Xanthophores
Yellow chromatophores, pigment-containing and light-reflecting cells, of a fish, amphibian or reptile.

Melanophores
Melanin-containing cells of a fish, amphibian or reptile that appear black or dark-brown because of melanin's light-absorbing qualities.

### Nuclear context

The output of Notch signalling primarily relies on NICD entering the nucleus; mechanisms that set the nuclear context are therefore crucially important because they determine the gene expression programme and consequent physiological outcome. For example, in the outer proliferation centre of the *D. melanogaster* optic lobes, Notch signalling induces neurons to die or to survive, depending on the transcription factors present[101]. Likewise, the contrasting oncogenic and tumour suppressor roles of Notch activity that occur in different tissue contexts are likely to be the consequences of differential cell-type-specific transcriptional programmes[102]. Nuclear mechanisms that confer such context-specific Notch responses are potentially of broad relevance to other signalling pathways, although the precise components might differ.

***Key features underpinning the nuclear response.*** A key aspect of specificity will logically result from the presence of cell-type-specific or stage-type-specific transcription factors that alter the selection of responsive

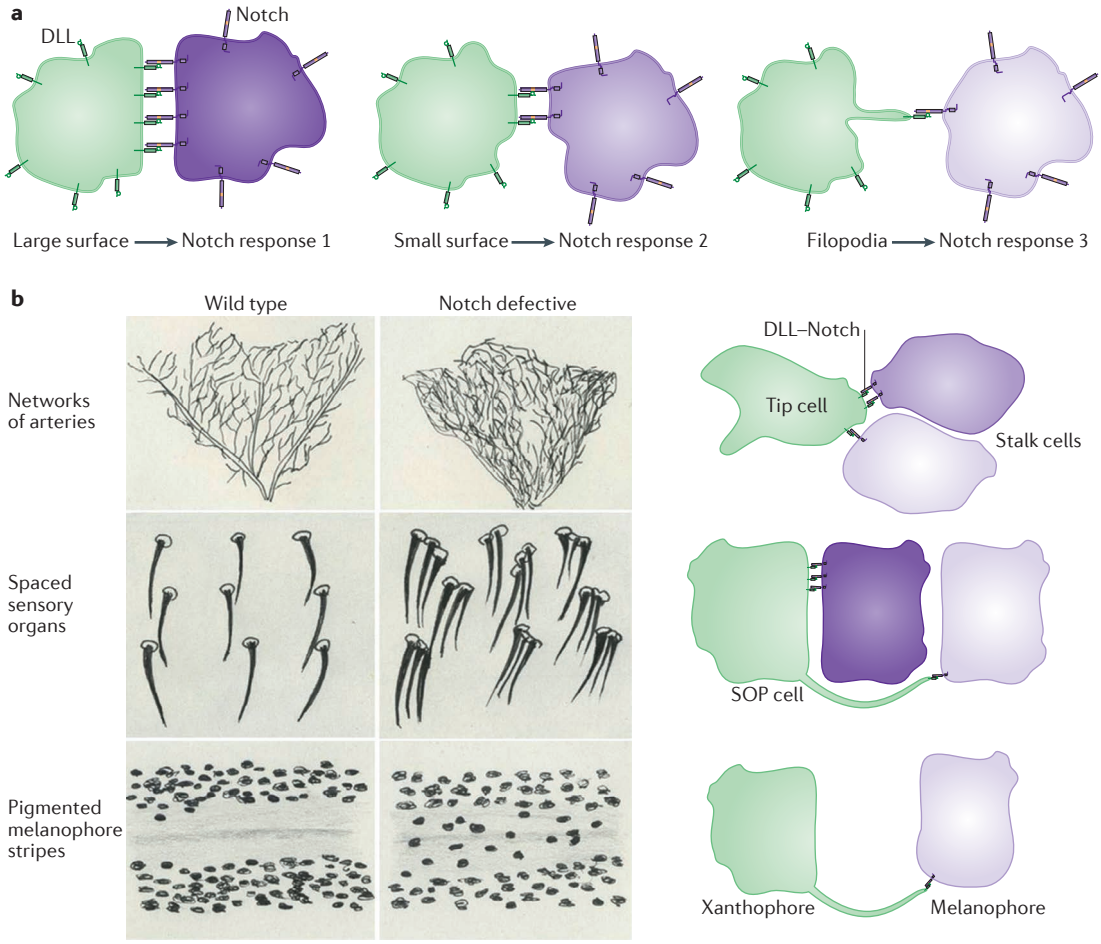



Figure 3 | **Influence of cell contacts and tissue architecture on signalling. a** | Models illustrating how contact dimensions between Delta-like- (DLL; green) and Notch-expressing cells (purple) could alter Notch responses. The left panel shows that when the contact region is large, many ligand–receptor interactions occur, generating high levels of Notch intracellular domain (NICD) (Notch response 1, dark purple). The middle panel shows that when the contact surface is smaller, fewer ligand–receptor interactions occur, less NICD is available for signalling and different and/or fewer target genes are activated (Notch response 2, mid-purple). The right panel shows that cell contacts through filopodia mediate a limited number of transient receptor–ligand interactions, generating low, transient levels of NICD and response (Notch response 3, pale purple). **b** | Notch signalling associated with different cell architectures. Angiogenic branching and extension of blood vessels involves the formation of dynamic contacts between tip cells (green; ligand-producing) and adjacent stalk cells (purple; Notch-expressing), during which a presumptive stalk cell might receive high levels of Notch for a brief period before losing contact. When Notch signalling is perturbed, excess tip cells generate a densely branched, compacted network. Selection of sensory organ precursor (SOP) cells involves direct contact between signal-sending cells (green) and signal-receiving cells (purple), but might also require filopodia to transduce the signal across a longer range. Perturbations to Notch give tufts of bristles, as all neighbouring cells become SOP cells. Disruptions to filopodia result in extra SOP cells and altered bristle spacing. During the formation of pigmented stripes in zebrafish, melanophores (purple) are restricted to stripes via signals conveyed by long cellular protrusions extending from xanthophores (green). Perturbations to Notch result in expansion of melanophores into the inter-stripe region.

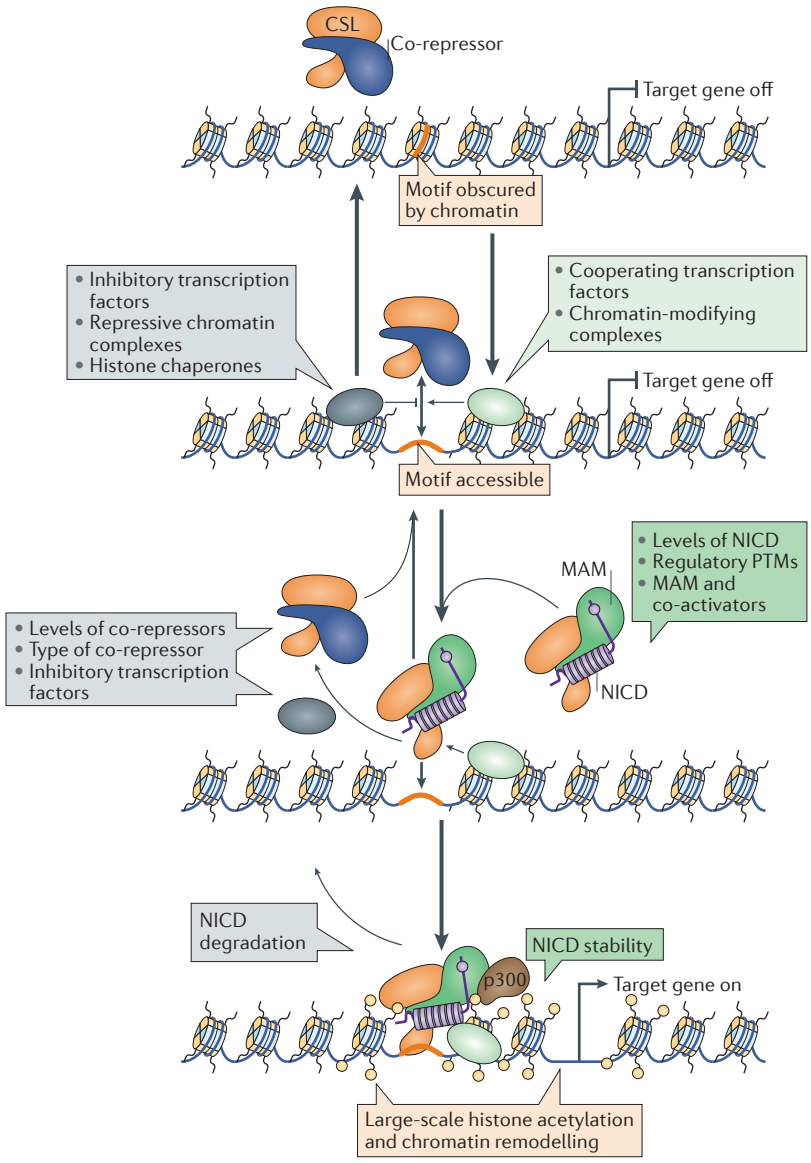



Figure 4 | **Regulation of the nuclear context.** Steps involved in Notch intracellular domain (NICD)-mediated activation at target enhancers, indicating the types of regulation occurring at each. Left side, inhibitory mechanisms (grey); right side, activating mechanisms (green). In the absence of cooperating transcription factors (light green), CBF1–Suppressor of Hairless–LAG1 (CSL) motifs (orange) are obscured by nucleosomes; this may also be driven by specific inhibitory transcription factors (for example, Hamlet or Ikaros, grey) acting in combination with chromatin-modifying enzymes, chromatin-remodelling enzymes and histone chaperones. Cooperating transcription factors (for example RUNT-related transcription factor (RUNX), GATA or ETS factors, light green) promote chromatin remodelling to expose CSL motifs. In many contexts, CSL binding is unstable and remains transient under these conditions, possibly due to the influence of its co-repressors (dark blue). The presence and stability of NICD dictates the outcome of Notch signalling: a tertiary complex containing CSL, NICD and Mastermind (MAM) is formed and resides at the CSL-binding sites, where it recruits co-activators including the histone acetylase p300 (or the closely related CREB-binding protein (CBP)). This leads to a widespread increase in histone acetylation across target enhancers and stimulates transcription. Levels and duration of transcription will depend on the levels and stability of NICD, which may be regulated by post-translational modifications (PTMs). Ubiquitylation directed by E3 ligases, through intermediaries such as F-box and WD repeat domain-containing 7 (FBW7), may promote degradation to terminate NICD activity. Note that some target genes may already be transcribed in the absence of Notch, so that Notch binding will augment rather than initiate expression.

genes. Indeed, if CSL itself could function as a 'pioneer factor', binding to sites in dense nucleosome-covered 'closed' enhancers, it would be difficult to account for the cell-type specificity of responsive genes — how, then, could cell-death genes be selected only in specific neuronal progeny to correctly programme neural networks[101], for example? In agreement with this notion that CSL functions in a cell-specific manner, it has a higher affinity for motifs at the edge of nucleosomes, suggesting that it binds preferentially to more open chromatin[103]. Furthermore, the motifs bound by CSL in *D. melanogaster* cells are located preferentially within regions of primed or active chromatin, making it likely that response specificity is aided by many motifs being hidden in chromatin environments that are not accessible to CSL[104] (FIG. 4). However, even though the NICD–CSL complex prefers ready-primed chromatin regions, it is not just an idle passenger — the consequences of its recruitment include large-scale changes in histone acetylation, removal of repressive complexes and enhanced accessibility of the DNA[104–108] (FIG. 4).

The original 'switch' model proposed that, before Notch activation, CSL was present at enhancers in a complex with co-repressors that kept the enhancer silenced by recruiting histone deacetylases or other modifying enzymes[109]. NICD was thought to displace the co-repressors from CSL to render the target enhancers active. However, this model in its simplest form has been challenged. First, the co-repressors KyoT2 (a LIM domain-containing protein) and MSX2-interacting protein (MINT; also known as SHARP) bind to CSL with similar affinities to those of NICD, making it hard to explain how NICD could displace them[110,111]. Resolving this question will, however, also require detailed knowledge of the relative stoichiometries of all the proteins concerned, including NICD. Second, CSL binding dramatically increases at target enhancers after Notch activation, which suggests that this event is dynamic[105,112–114]. Thus, it is likely that an exchange of entire complexes (rather than co-activators substituting for co-repressors on DNA-resident CSL) occurs, similar to the model for hormone receptors, with the NICD–CSL complex being 'captured' to increase the amount or duration of its binding (FIG. 4).

Although further studies are needed to determine the extent to which enhancers are 'marked' by CSL before Notch activation, CSL-binding co-repressors are nevertheless likely to contribute to the regulatory landscapes. First, loss or down-modulation of CSL leads to de-repression of tumour-promoting genes in several contexts[115,116]. Second, because several co-repressors bind directly to CSL in a manner that would preclude concomitant NICD binding, they will titrate the availability of CSL and hence set a threshold that NICD would need to exceed in order to activate transcription[110,111,117–119]. Finally, the different types of co-repressor might give rise to different types of repression complex, especially if they differ in their ability to recruit chromatin-modifying complexes (BOX 2). Thus, by binding to their target sites transiently, certain types of CSL–repressor complex might help to make the enhancers more refractory to

Box 2 | **Players in the nuclear arena**

**Nuclear activation complexes**

The CBF1–Suppressor of Hairless–LAG1 (CSL)–Notch intracellular domain (NICD)–Mastermind (MAM) complex recruits p300 (or the closely related CREB-binding protein (CBP)), which modifies chromatin at target enhancers. Notch-dependent histone modifications include a widespread increase in histone H3 Lys27 acetylation (H3K27ac) and a decrease in H3K27 trimethylation (H3K27me3)[104,105,107]. Other components that have been associated with the co-activator complex include the demethylase Jumonji domain-containing protein 3 (JMJD3; also known as KDM6B)[107], the RNA helicase DDX5 and the long non-coding RNA steroid receptor co-activator (SRA)[174,175]. Chromatin modifiers that enhance Notch transcriptional activity include BRG1 (also known as Brahma) complexes, BRD4 and BRE1 (REF. 176).

**Nuclear co-repressor complexes**

CSL-binding co-repressors for which direct interactions have been mapped include MSX2-interacting protein (MINT; mammals)[110], KyoT2 (mammals)[111] and Hairless (*Drosophila melanogaster*)[118]. Silencing mediator of retinoic acid and thyroid hormone receptor (SMRT; also known as NCOR2) and CBF1-interacting repressor (CIR) interactions have also been detected[119]. Co-repressors recruit enzyme complexes that modify chromatin at target enhancers, including class 1 histone deacetylases (HDACs) and the histone demethylases Kdm5 (also known as Lid) and LSD1 (REFS 117,119,157,177,178). Histone chaperones including chromatin assembly factor 1 (CAF1) complex, nucleosome assembly protein 1 (NAP1) and antisilencing factor 1 (ASF1) are also implicated in CSL-dependent repression of target enhancers[119]. In some cases, co-repressor-recruitment of modifying enzymes relies on intermediaries, including Groucho (also known as TLE) and CtBP[179].

**Additional examples of cooperating transcription factors**

- TEA domain family member 4 (TEAD4; also known as TEF3): a DNA-binding protein that is regulated by the Hippo pathway. TEAD4 co-binds the enhancer of Cdx2 with Notch/CSL in early mouse embryos[180].
- SoxF: combinatorial regulation by SoxF transcription factors is necessary for Delta-like 4 (DLL4) expression during arterial specification, and co-binding is needed to give full enhancer activity[181].
- TCF: TCF sites are present in enhancers that are collaboratively regulated by Notch1 and WNT activity in intestinal crypts[182].
- ETS: ETS1-binding motif and ETS1 occupancy was enriched near NICD–CSL-bound regions in T cell acute lymphoblastic leukaemia cells[176].

the effects of NICD. Uncovering their contributions will be important for deciphering the regulatory landscapes that NICD encounters.

***Cooperation with transcription factors.*** To ensure that the appropriate tissue- and cell-type response occurs, mechanisms that direct NICD to the appropriate enhancers must exist. Although the execution of some roles of Notch signalling relies on a set of common targets, the HES family of basic helix–loop–helix (bHLH)-containing proteins, other roles depend on diverse transcriptional responses[120]. These responses are likely to be achieved through close cooperation between CSL complexes and other transcription factors (FIG. 5), either because specific configurations of binding motifs allow direct interactions between transcription factors and NICD–CSL or because nearby motifs recruit transcription factors that help to recruit NICD–CSL indirectly by modifying chromatin. At the same time, other transcription factors might block CSL recruitment to specific enhancers.

One well-characterized example of an enhancer 'signature' is the so-called SPS+A site in Notch-regulated genes during neural precursor specification. This signature combines a pair of specifically orientated CSL motifs (known as SPS) with a binding site for the pro-neural bHLH activator proteins (referred to as A), and it allows NICD–CSL to interact directly with the activators, thereby conferring Notch–bHLH synergy[121]. Similarly, in *Caenorhabditis elegans*, the regulator of fusion-1 (*ref-1*) enhancer contains four predicted binding sites for GATA transcription factors that are required for Notch-dependent endodermal expression[122] and that probably facilitate NICD–CSL recruitment through a direct interaction between CSL and the GATA factor. A third example involves the mouse forkhead box p3 (*Foxp3*) gene, in which an overlapping CSL–nuclear factor-κB (NF-κB) binding site in the promoter facilitates cooperative regulation by Notch3 and canonical NF-κB signalling[123].

By contrast, other examples indicate that cooperation can occur without a distinctive enhancer signature. An association between RUNT-related transcription factor (RUNX) proteins and NICD–CSL complexes exists in several cellular contexts, with CSL-bound regions being enriched for RUNX sites[105,108,124] but without any very precise arrangement of their motifs. RUNX proteins are required for CSL to be recruited to these enhancers, and it seems likely that the mechanism involves changes in the local chromatin organization to 'expose' the CSL-binding motifs[104] (FIG. 4). Other examples in which specific transcription factors are important exist (BOX 2), but it is as yet unclear whether direct or indirect mechanisms are involved. It also remains to be determined whether subsets of transcription factors have a special relationship with NICD–CSL because they introduce a specific partner or chromatin conformation or, alternatively, whether any transcription factor binding in proximity to a CSL-binding motif might be sufficient to render an enhancer responsive.

Notably, binding of specific transcription factors can prevent enhancers from responding to Notch. A well-characterized example is the zinc-finger transcription factor Ikaros, which restricts the Notch responsiveness of many T cell targets, including HES1 and MYC, by binding to their enhancers[125–127] (FIG. 4). In the absence of Ikaros, Notch target genes that are normally shut off in thymocytes were persistently expressed, and other normally inactive or weakly upregulated genes became strongly induced by Notch[125]. Conversely, re-expressing Ikaros could repress Notch1 target genes, including *MYC*, in T cell acute lymphoblastic leukaemia (T-ALL) cells[127]. Among other transcription factors that inhibit the activity of NICD–CSL complexes, several do so by binding to the complex rather than by blocking enhancer binding[128,129]. For example, the transcriptional repressor BCL6 inhibits the expression of oestrogen receptor 1 (*ESR1*)[130] either by preventing the recruitment of MAML1 to NICD or by recruiting the histone deacetylase sirtuin 1 (SIRT1) to promote deacetylation of neighbouring histones[131]. Finally, BEN-SOLO proteins can bind to nearby sites on DNA and directly contact CSL to antagonize Notch activity during neurogenesis[132]. Direct and indirect negative regulators are therefore likely to have widespread roles in setting the transcriptional landscape.

Pioneer factor
A subset of transcription factors that are capable of binding to their target-motifs even when located in DNA that is wrapped around nucleosomes, enabling them to initiate changes in regulation at silent enhancers.

Enhancers
DNA segments that increase transcription of a linked promoter if placed in either orientation, upstream or downstream.

HES family
A family of genes related to Hairy and Enhancer of split that encode basic helix–loop–helix nuclear proteins that suppress transcription.

Basic helix–loop–helix
(bHLH). A basic domain adjacent to two α-helices separated by a loop (the HLH domain), which binds DNA in a sequence-specific manner.

GATA transcription factors
A family of transcription factors that contain a zinc-finger motif that was first identified in the vertebrate GATA1 protein. These transcription factors bind the consensus sequence GATA in the regulatory regions of genes.

Zinc finger
A motif in proteins that contains conserved cysteine residues. The sulfydryl groups of the cysteines coordinate a $Zn^{2+}$ ion.

BEN-SOLO proteins
Proteins containing only a BEN domain, a sequence-specific DNA-binding domain that has been identified in some transcription repressors.

Neurogenic transition
Change in the competence of neural precursor cells that enable them to generate different types of neural or glial progeny.

***Chromatin context and indirect mechanisms.*** If target enhancers need to be present in the appropriate chromatin complex to be bound by CSL, epigenetic mechanisms that alter the accessibility of enhancers should have an important influence on gene expression in responding cells[133], especially during developmental (and other) transitions when the Notch-responsive programme needs to change between one state and the next (FIG. 5). Several examples show that such transitions might be coordinated by stage-specific transcription factors in conjunction with chromatin-modifying complexes. For example, Hamlet (also known as Evi1), when recruited to targets in nascent *D. melanogaster* olfactory neurons, seems to enable a modified response in a subsequent round of Notch signalling by altering histone methylation and density to erase the Notch state inherited from the parental cell[134]. BCL6 similarly mediates stable epigenetic repression of HES5 by recruiting SIRT1 during the neurogenic transition in mouse cortical progenitors[131]. Finally, Pax6 (also known as Eyeless) blocks the ability of NICD to promote tumours in older-generation progenitors in some *D. melanogaster* neural stem cell lineages, in which it prevents transcriptional activation of direct target genes, possibly through the BRG1-associated factor (BAF)–SWI/SNF-related chromatin remodelling complex[135].

Polycomb complex-mediated silencing of target enhancers is also likely to shape the Notch response. Although their action is reversible, two multiprotein Polycomb repressive complexes (PRCs) confer heritable repressive states, and their presence at many Notch-regulated genes in embryonic stem cells indicates their potential importance[133]. The inability of Notch to drive cardiac regeneration in adult rat myocardiocytes is also attributed to PRC-mediated repression[136]. Likewise, the activity of PRCs curtailed NICD-mediated activation of target genes in *D. melanogaster* cells and in human T-ALL cells[107,137].

Many other chromatin regulatory complexes have been found to influence Notch activity *in vivo* (BOX 2). Although the functional data indicate that these complexes contribute to the landscape of target genes, it remains challenging to distinguish whether they do so by specific or non-specific mechanisms. Nevertheless, because Notch signalling is sensitive to changes in the activity of several chromatin-regulatory complexes, chromatin organization might be particularly significant for NICD responsiveness.

***Factors that modify the stability or activity of NICD.*** Another potentially potent way to regulate the Notch pathway is by modulating the activity or stability of NICD (FIG. 4). Relatively little is known about these aspects of its regulation post cleavage, but NICD could be subject to post-translational modification or might interact with other proteins that modulate its nuclear levels and/or activity. Yes-associated protein (YAP, a key effector of the Hippo tumour suppressor pathway) and SMAD3 (an intracellular transducer of transforming growth factor-β signals) both augmented the activity of NICD independently of DNA binding[138,139].

In some contexts, the stability of NICD is affected by its interactions with F-box and WD repeat domain-containing 7 (FBW7), the substrate-recognition component in a ubiquitin ligase complex. Cancer-associated mutations in FBW7 were identified in people with γ-secretase insensitive T-ALL and correlated

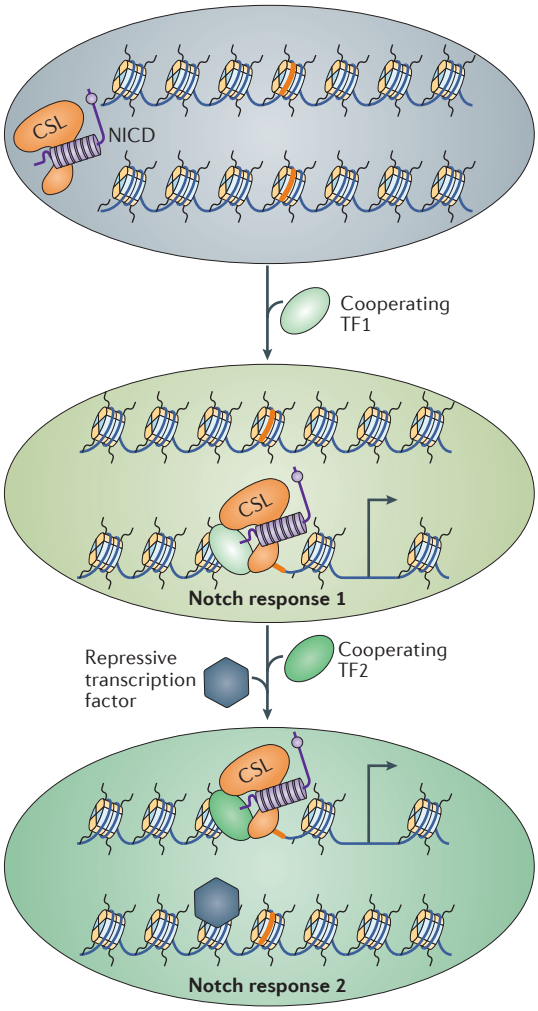



Figure 5 | **Transitions in Notch-responsive programmes.** Requirement for different cooperating factors and repressors to bring about changes in the Notch response at different developmental and physiological transitions. Top: in a nucleus that contains no cooperating transcription factors, CBF1–Suppressor of Hairless–LAG1 (CSL) motifs (orange) are inaccessible. Middle: when an enabling cooperating transcription factor is expressed (TF1, light green), it binds to target sites in enhancers, making them competent to respond to CSL–Notch intracellular domain (NICD) complexes, yielding a specific Notch response 1. When the cell undergoes a subsequent transition (such as following cell division in the olfactory lineage), different transcription factors are expressed, some of which inhibit (grey hexagon) the response 1 class of genes by conferring a non-permissive chromatin context, whereas others act positively (Cooperating TF2, dark green), opening up a new cohort of enhancers to enable a different Notch response (Notch response 2). In this way, nuclei can transition from one response to another, depending on the timing and on the presence of other signals.

with increased levels of NICD activity[140]. FBW7 has been shown to bind directly to NICD, promoting its polyubiquitylation and proteasomal degradation[140,141], and, as this interaction is regulated by CDK8 (a nuclear serine/threonine kinase that functions as a transcriptional regulator), it was thought to terminate NICD activity at its transcriptional targets[142]. However, FBW7 affects many other substrates[143] and regulates the association of CDK8 with the Mediator complex[144], making it tricky to distinguish the significance of direct NICD regulation in many contexts. Furthermore, although FBW7 mutation affects several developmental processes linked to altered Notch1 activity in vertebrates[145,146], mutations in the *D. melanogaster* homologue have not uncovered an equivalent role. Thus, the extent of direct NICD regulation by FBW7 remains to be clarified, and the existence of other E3 ligases that perform similar roles to terminate NICD activity merits further exploration.

SIRT1 and co-activator-associated arginine methyltransferase 1 (CARM1) are speculated to act as rheostats by modulating the activity of NICD. SIRT1 directly associates with NICD and attenuates Notch activity in zebrafish endothelial cells[147], and CARM1 methylates NICD and regulates the duration of transcriptional responses from some target enhancers[148]. Other modifications may attenuate the transcriptional activity of NICD or affect its nuclear localization. These include hydroxylation, mediated by factor inhibiting hypoxia-inducible factor (FIH) in response to changes in oxygen levels during myogenesis[149]; phosphorylation, by glycogen synthase kinase 3 (GSK3)[150] or AKT[151]; and ubiquitylation, conferred by the homologous to E6-AP carboxyl terminus (HECT) ubiquitin ligase WW domain-containing protein 2 (WWP2)[152,153]. Post-translational modifications targeted to positions in the six ankyrin repeats can prevent NICD from forming its tripartite activation complex with CSL and MAM[154–156]. If the relevant enzymes were recruited to specific targets, these modifications could nevertheless lead to differential effects on gene expression. Modifying enzymes potentially provide mechanisms of crosstalk with other pathways, as suggested by the purification of multiple kinase with NICD[157], and are, in some cases, specific to one paralogue. Clearly, more knowledge about when and where NICD is modified and what effects each modification has on its activity will be important for understanding its operations in a given milieu.

## Network context

Interpreting the context-specific effects of Notch will ultimately require that we understand the wiring of the regulatory networks in which it operates. Although this presents an enormous challenge, indications already exist as to how differences between cell types affect Notch pathway outcomes, as illustrated by the examples below.

Feedback regulation of ligand expression is one example in which the regulatory logic has profound consequences. For contexts in which classical lateral inhibition occurs, Notch activation frequently inhibits ligand expression to polarize the signalling (negative feedback). This mechanism relies on the HES family of direct Notch targets, which antagonize the activity of proneural bHLH transcription factors, which themselves promote the expression of ligands and E3 ligases (such as NEUR)[158]. Thus, Notch activation leads to decreased ligand expression, and the fates of the cells become mutually exclusive[5]. For contexts in which signalling is inductive, Notch activity promotes ligand expression (positive feedback). This feedforward positive regulation of ligand expression occurs at the signalling boundary in the *D. melanogaster* wing disc and during the formation of sensory patches in the chick ear[24,31,45,159]. In *D. melanogaster* the evidence points to a direct regulation of Ser expression by NICD–CSL[9,160]. By intensifying and perpetuating ligand expression, such positive feedback can sharpen the boundaries between expressing and non-expressing regions. Alternatively, under some circumstances it could lead to a shutdown if the increased ligand levels blocked signal reception through *cis* inhibition. Differences in the wiring of the regulatory network also account for opposing effects on PTEN that occur in response to Notch activity. In T-ALL cells, Notch activity, by directly targeting HES1, inhibits the expression of PTEN, thereby promoting proliferation[161]. By contrast, in stalk cells, PTEN is itself a direct target of Notch and is upregulated to inhibit proliferation[162].

Differences in the mode of crosstalk between the Notch and epidermal growth factor receptor (EGFR)–RAS pathways lead to them functioning antagonistically in some contexts and cooperatively in others[163]. Such polarized differences partly arise from the regulatory logic of the target enhancers and partly as a consequence of whether Notch regulates the expression of RAS pathway inhibitors or activators (and vice versa). For example, in the *D. melanogaster* eye, EGFR and Notch pathways cooperate to promote the development of cone cells by converging on enhancers of key differentiation genes (for example, Pax2)[164], but antagonize one another at the onset of ommatidial development because EGFR promotes the expression of the proneural protein Atonal, whereas Notch activity inhibits *atonal* expression through HES targets[165]. Similarly, during *C. elegans* vulval development, EGFR activity first initiates Notch activity by upregulating ligand expression to stimulate the receptor on adjacent cells, then Notch activity antagonizes EGFR–RAS signalling by promoting the expression of pathway inhibitors[166]. These examples show how differences in wiring can profoundly influence the consequences of activating the two pathways. Similar differences in crosstalk are likely to underpin many of the context-specific interactions of Notch with WNT, fibroblast growth factor, Hippo and other pathways.

## Conclusions and perspectives

The ability of the Notch pathway to carry out many tasks despite the relative simplicity of its core pathway relies on the deployment of different levels of control that adapt the pathway to each context. For example, the tissue architectures and the expression patterns of Notch and its ligands can determine both the range and strength of Notch signalling, whereas the nuclear

Mediator complex
A multiprotein complex that is required for gene transcription by RNA polymerase II.

Homologous to E6-AP carboxyl terminus
(HECT). The HECT domain is an ~350-amino-acid domain that is highly conserved among a family of E3 enzymes.

context will shape the identity of the target genes regulated and hence the transcriptional outcome. Many of the strategies, especially in the nuclear context, will be relevant for other signalling pathways that similarly induce a diversity of tasks. For example, the context-dependent modifiers of WNT–β-catenin signalling that contribute to its differing effects in stem cells include the cocktail of cooperating transcription factors that are present[167]. However, other strategies are more likely to be unique to Notch. Notably, the fact that Notch ligands are transmembrane proteins constrains the range of the signal and makes the cell architecture and tissue organization particularly important features. The one-to-one interaction between ligand and receptor places more emphasis on the precise relationship between their levels, especially as they can also inhibit one another when present in the same cells. All the levels of regulation can be modulated to enable the pathway to adapt to a changing environment. However, although we can appreciate how the regulation might occur conceptually, many aspects are still poorly understood, making it hard to predict how physiological and environmental differences will influence signalling.

Recent progress has been driven by structural studies of key complexes involved in Notch signalling; the next challenge will be to find ways to view the molecules in action, to find where on or in the cell they interact and to discover the levels, stoichiometries and dynamics of the different complexes. A more quantitative picture will aid predictions about the transcriptional and physiological outcomes. Discovering how these can be modulated by environmental factors will also be important for understanding disease susceptibilities from heterozygous mutations in Notch pathway genes.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### Competing interests statement
The author declares no competing interests.