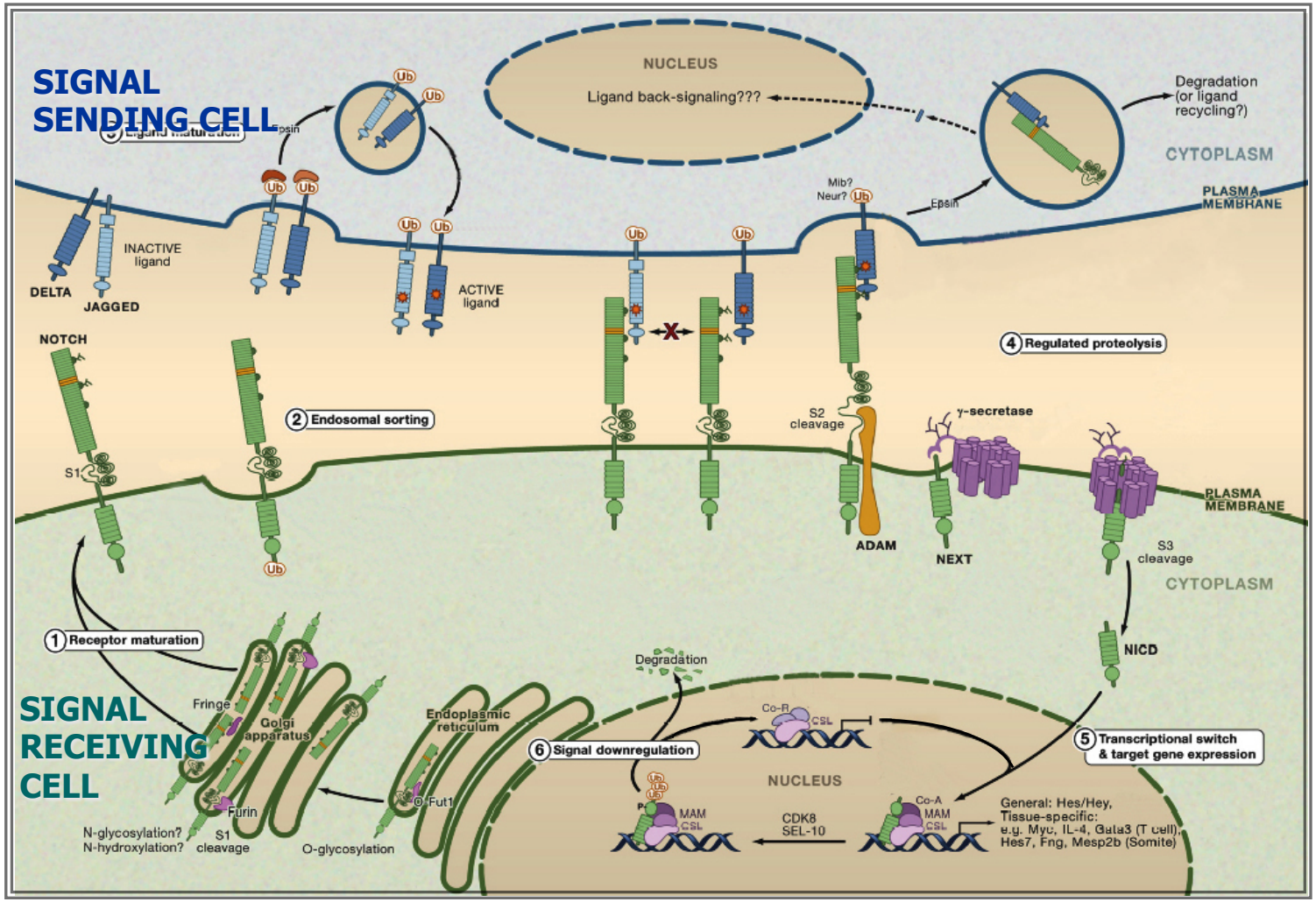
Notch Signaling Pathway

A brief history

- In 1917, Thomas Hunt Morgan described a strain of *Drosophila* with notches at the end of their wing blades, which result from haploinsufficiency
- Notch gene was cloned in the mid-1980s

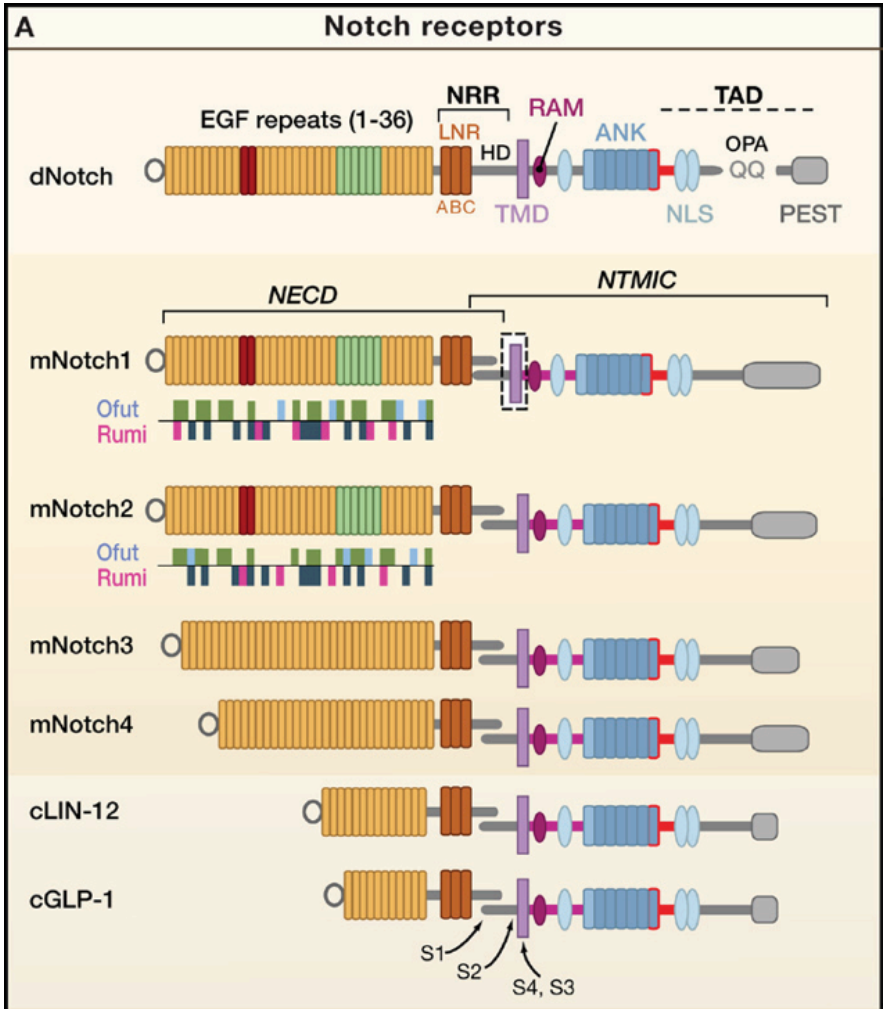
The Notch signalling pathway

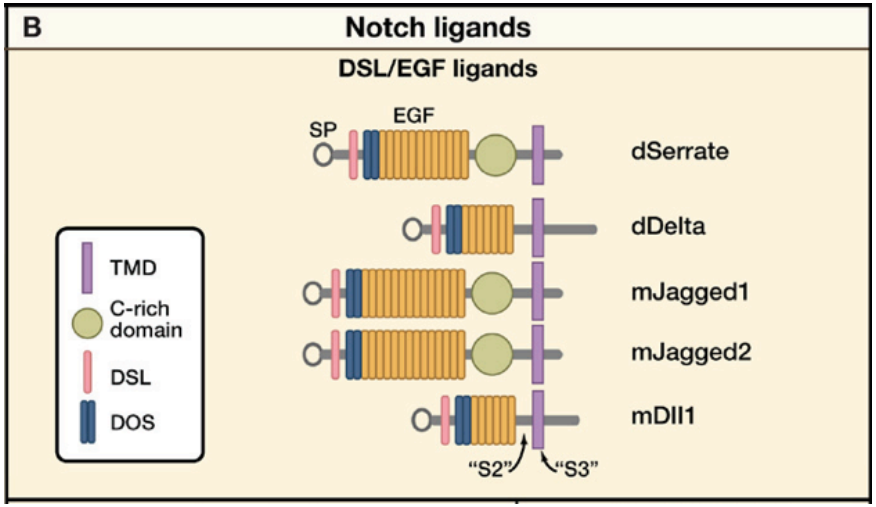


<u>UNIQUE FEATURES</u>:

1) each Notch molecule is **irreversibly activated** by proteolysis

2) **signals only once without amplification** by secondary messenger cascades

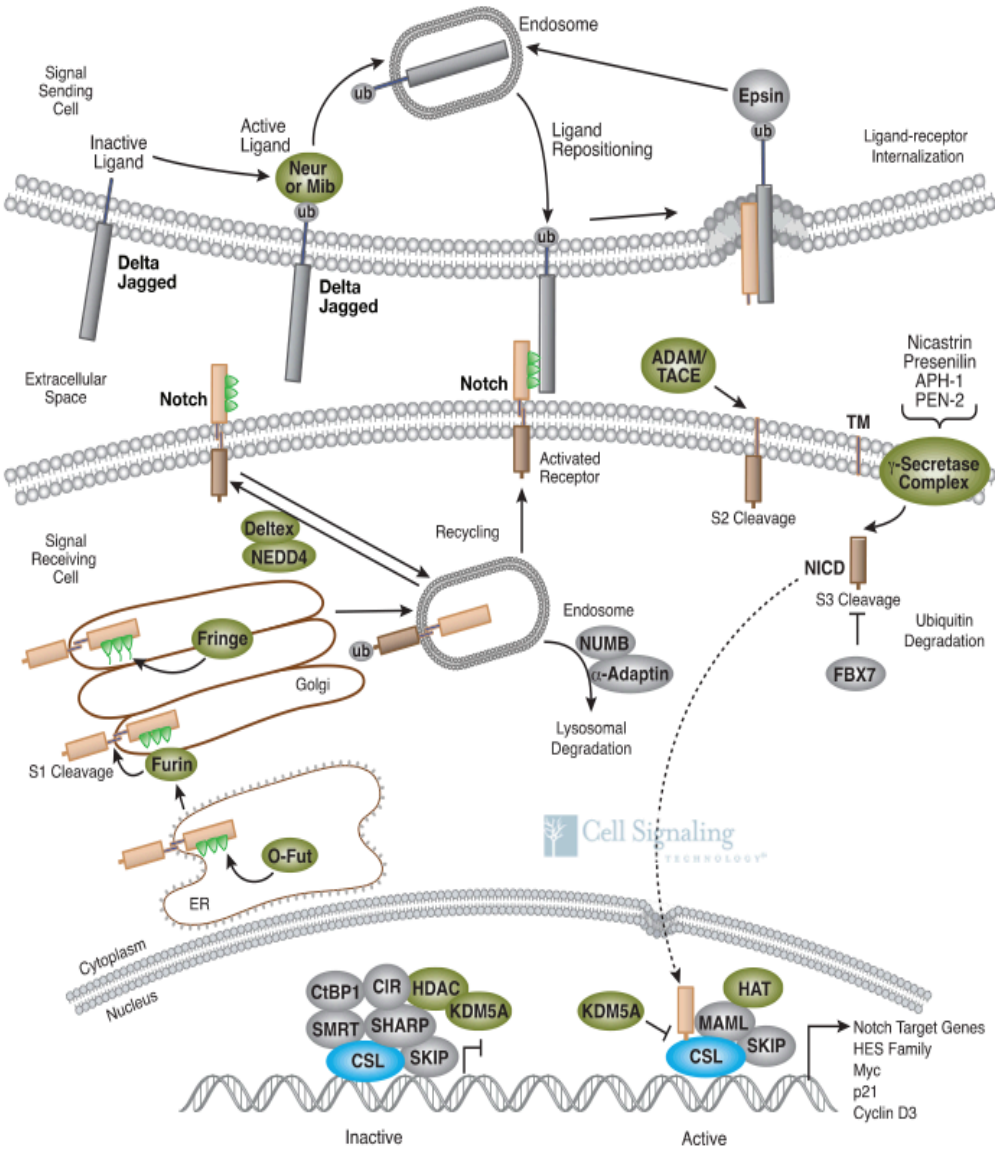




(A) Notch receptors are transmembrane proteins that contain multiple EGF-like repeats, involved in ligand interactions, fucosylation and glucosylation. They also contains a transmembrane domain (TMD), a RAM (RBPjκ association module) domain, nuclear localization sequences (NLSs), seven ankyrin repeats (ANK) domain, and a transactivation domain (TAD) that habors a PEST domain.

(B) Known and putative ligands of Notch receptors can be divided into several groups on the basis of their domain composition.

The Notch Signaling Pathway Is Mediated by Regulated Proteolysis



1. The mature Notch receptor is produced after glycosylation and proteolytic cleavage by PC5/ furin at (S1) site and is targeted to the cell surface as a heterodimer.
2. Notch is activated by binding to the ligand presented by a neighboring cell. Ligand endocytosis is thought to generate mechanical force to promote a conformational change in the bound Notch receptor.
3. This conformational change exposes site S2 in Notch for cleavage by ADAM metalloproteases.
4. Juxtamembrane Notch cleavage at site 2 generates the NEXT fragment, which is cleaved by the γ-secretase complex progressively from site 3 (S3) to site 4 (S4) to release the Notch intracellular domain (NICD) and Nβ peptide. NICD enters the nucleus where it associates with the DNA-binding protein CSL (CBF1/RBPjk in vertebrates, Suppressor of Hairless in Drosophila, Lag-1 in C. elegans). The coactivator Mastermind (MAM) recognizes the NICD/CSL interface, and this triprotein complex recruits additional coactivators (Co-A) to activate transcription.
5. In the absence of NICD,CSL may associate with ubiquitous corepressors (Co-R) and HDACs to repress transcription of the target genes.

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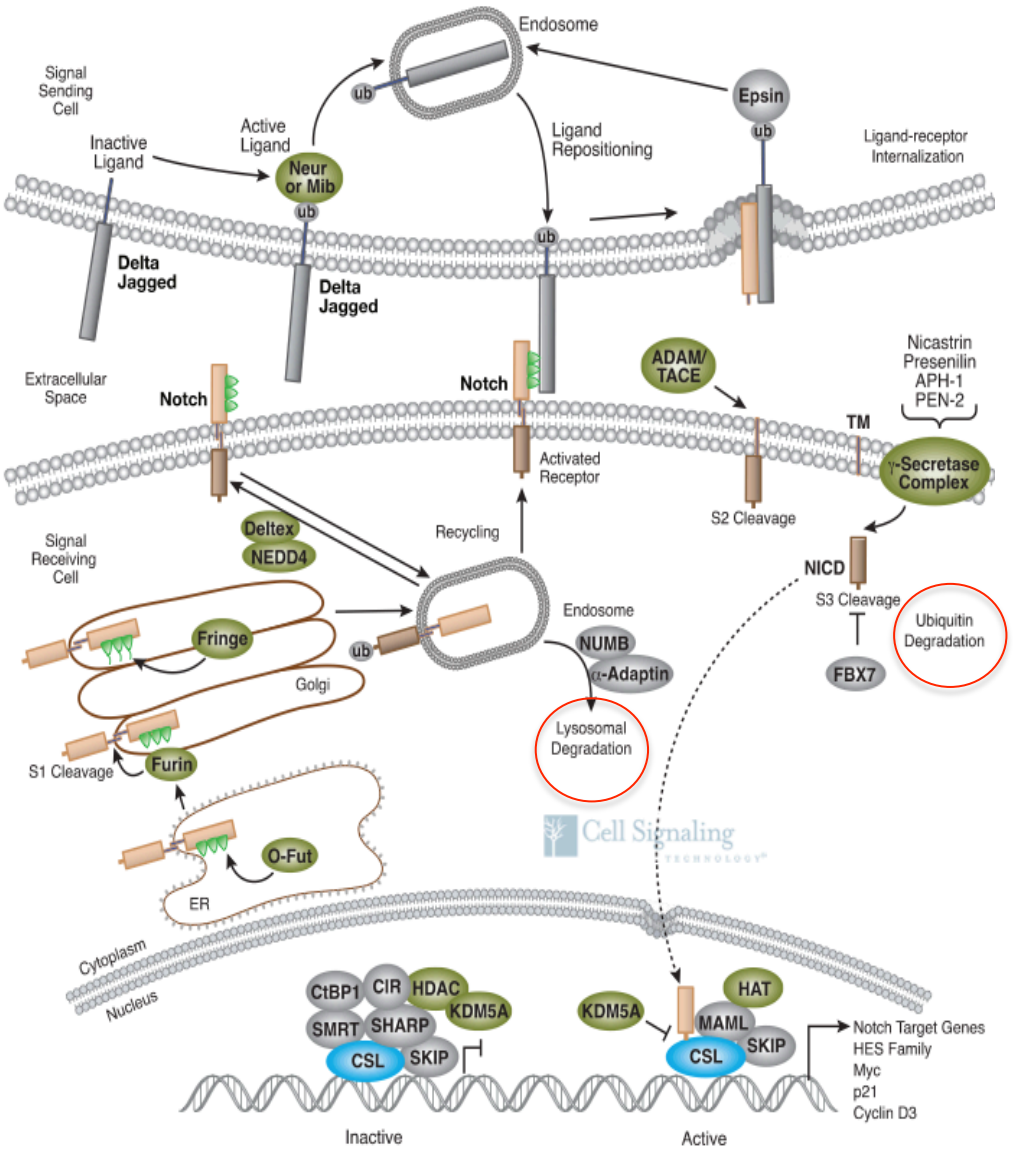

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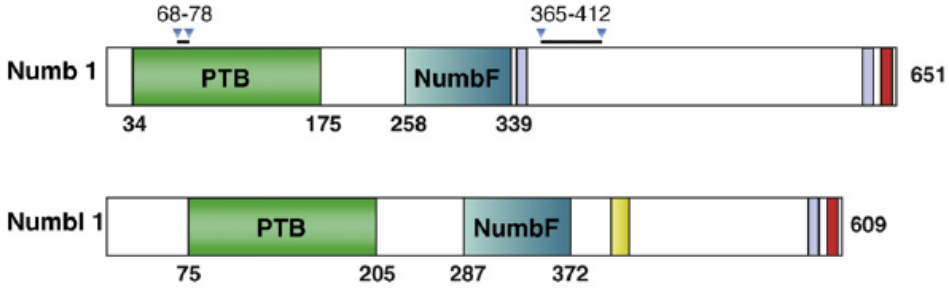
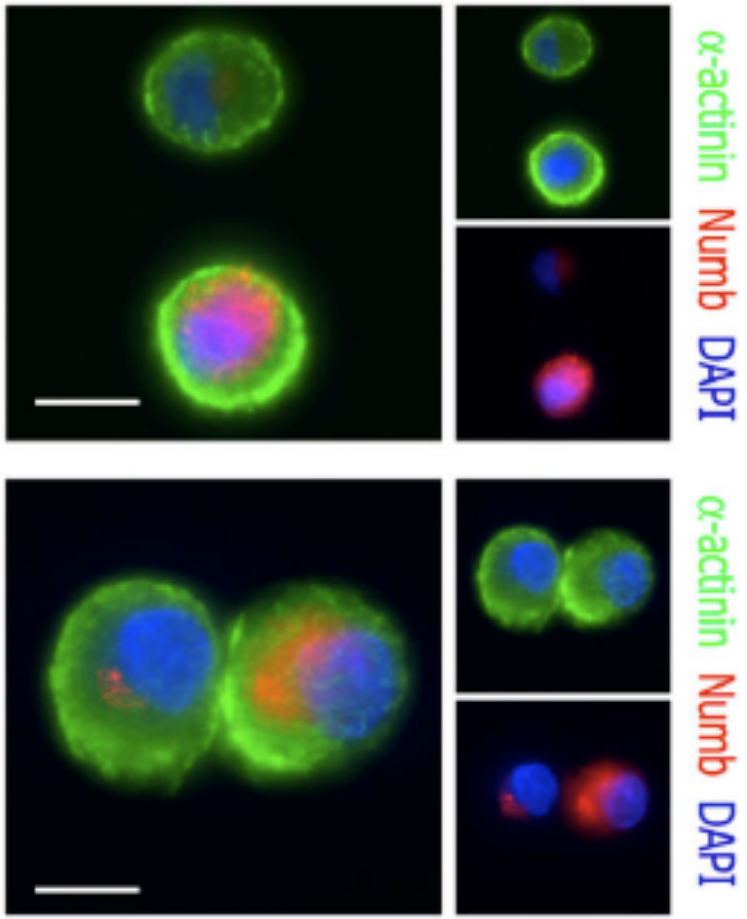
Modulators of Notch signalling: Numb

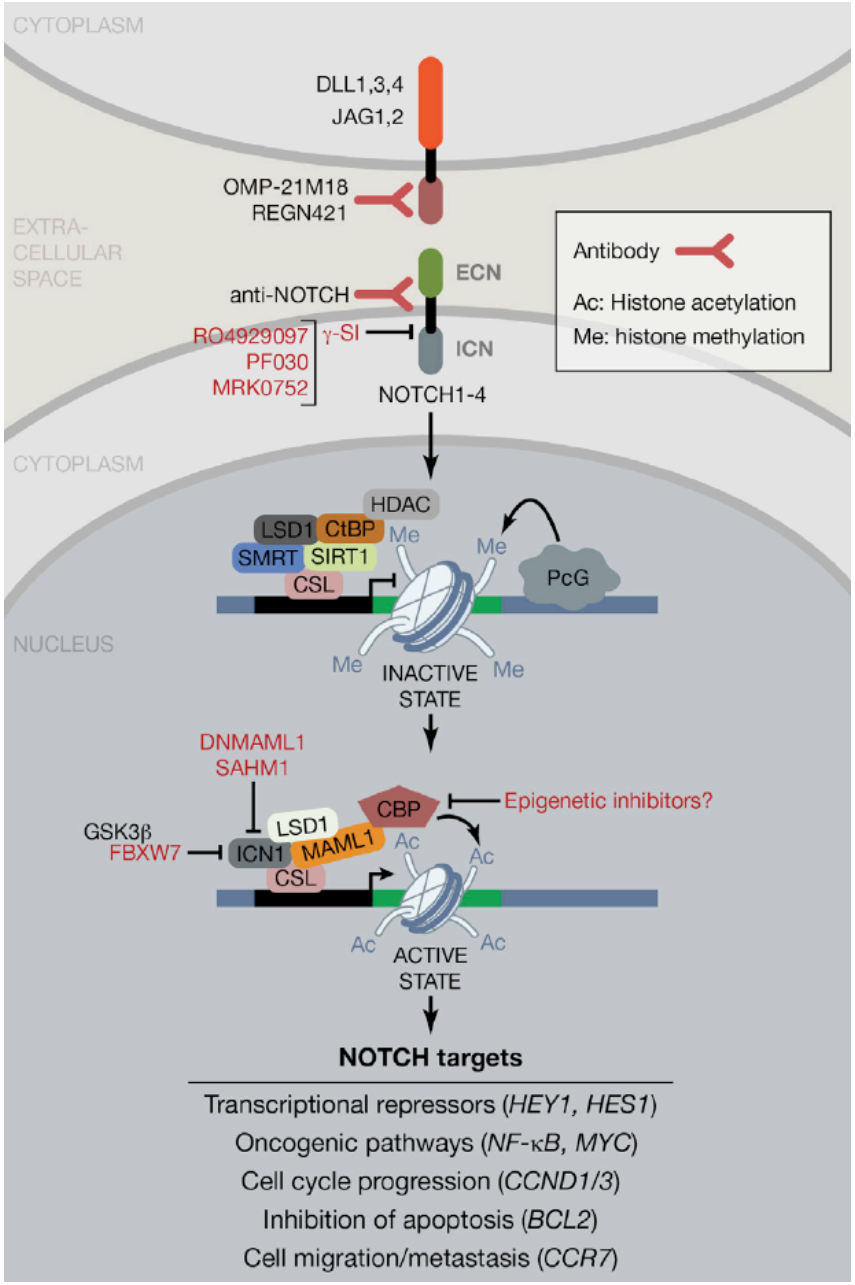


Pece et al., 2011

- Numb domains:
  - 1. PTB domain; N-terminal phosphotyrosine binding domain
  - 2. proline-rich C-terminal region.
- Numb binds directly to NICD. The Cterminal half of the PTB domain and the N-terminus of Numb are required to inhibit Notch. Numb also has two motifs associated with endocytic proteins.
- mammalian Numb (mNumb) localizes to clathrin coated pits and early endosomes, might target endocytosed NICD for proteosomal destruction.
- Numb acts either upstream of S3 cleavage site of Notch or inhibit the endocytosis of membrane-bound activated Notch.

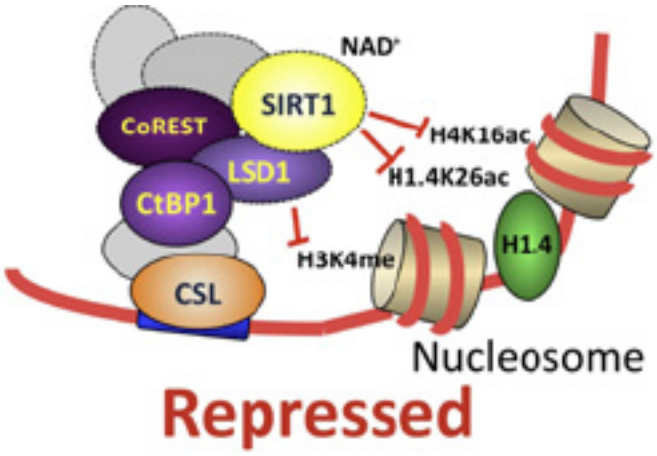


Overview of the Notch Signaling Pathway



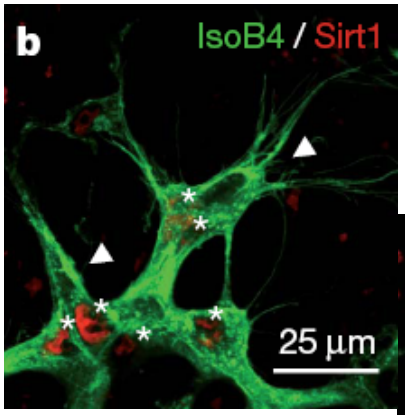
HDAC, histone deacetylase; ICN1, intracellular part of NOTCH1; LSD1, lysine-specific demethylase 1; SMRT, Silencing-Mediator for Retinoid/Thyroid hormone receptors; GSK3b, glycogen synthase kinase 3 beta; DNMAML1, dominant-negative MAML1

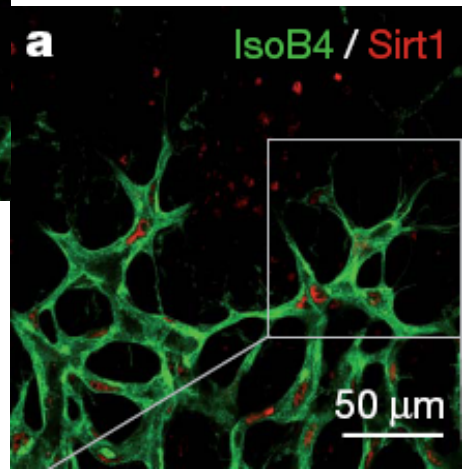
SirT'N repression for Notch



SIRT1 deacetylase acts in concert with the LSD1 demethylase to repress Notch-induced transcription

Mulligan P. et al., 2011

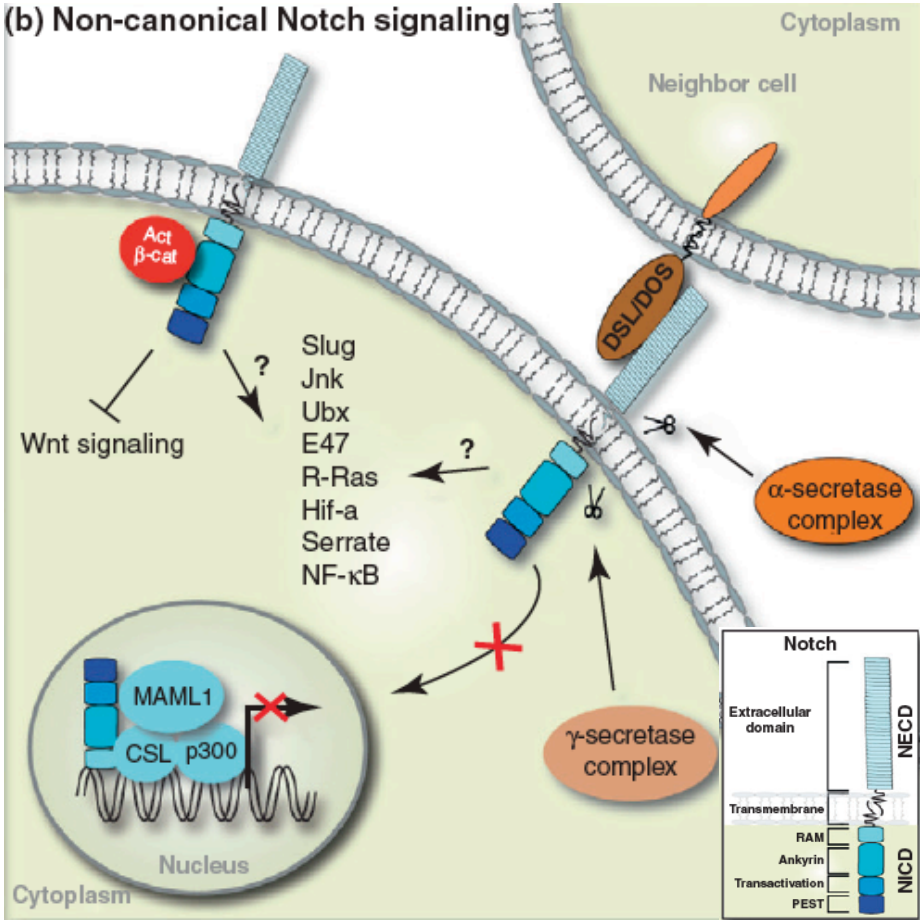




SIRT1 inhibits endothelial cell Notch signaling during angiogenesis in zebrafish and mice.

Guarani V. et al., 2012

Non-canonical Notch Signaling Pathway



Non-canonical Notch signaling is CSL-independent and can be either ligand-dependent or independent. In most cases the mediators of non-canonical Notch signaling are unknown.

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 active β-catenin. Therefore, active β-catenin activity is a readout for non-canonical Notch signals.

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

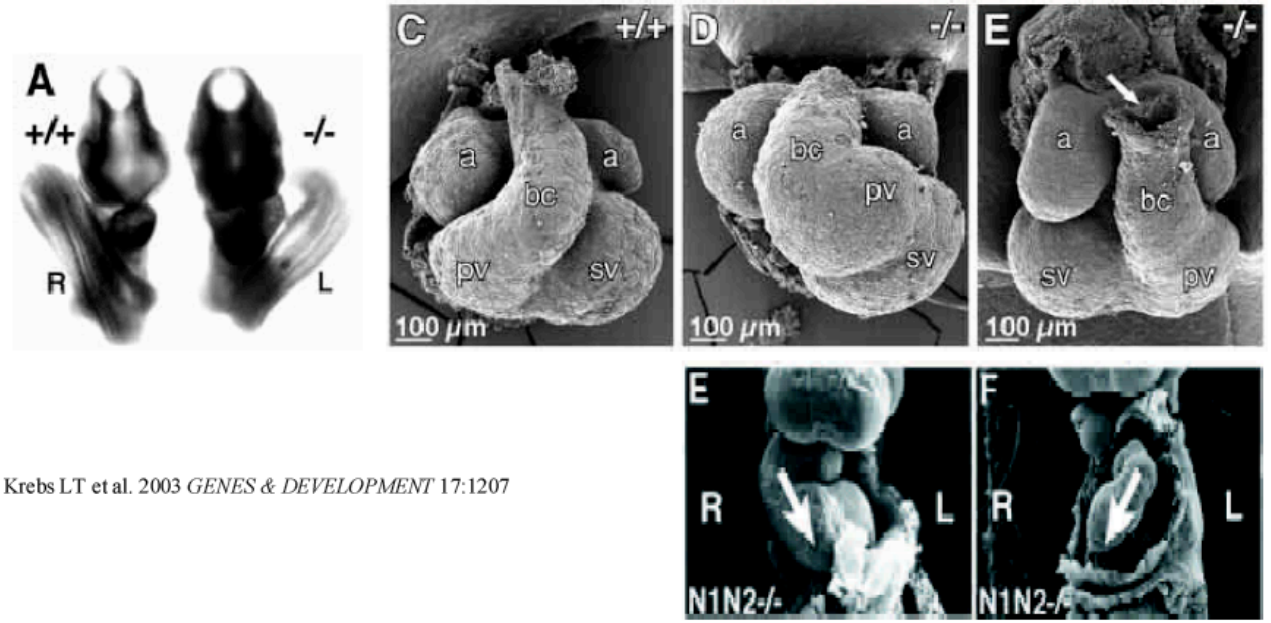
Species	Cell type	System	Independence	Function	Interacting molecule/ signaling (direct or indirect)	Refs
Human	Stem cells (hESCs), Cancer	*in vitro*	Ligand, CSL	Negative regulation of Wnt signaling	Active β-catenin/ Wnt signaling	[5]
Rodent	Stem cells (mESCs, NSCs, MSCs), Progenitors (CPCs)	*in vivo, 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.

Notch signaling has effects in many different organs

- Notch signalling can maintain stem cells or precursor populations in an undifferentiated state
- Notch signalling influences binary cell-fate decisions via lateral or inductive signalling
- A third property of Notch is its ability to influence differentiation and cell-cycle progression

Notch and left-right asymmetry

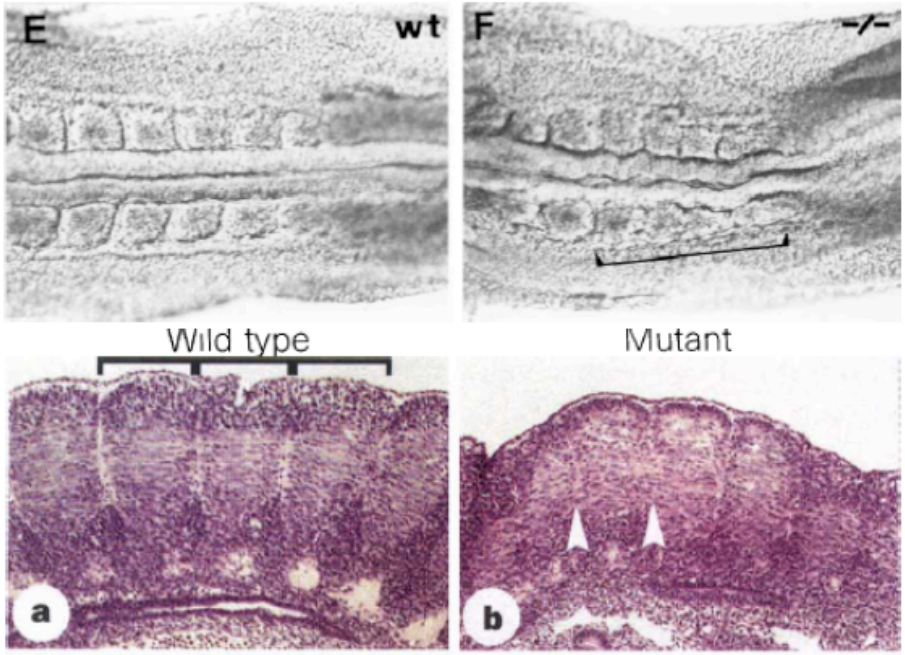


Krebs LT et al. 2003 *GENES & DEVELOPMENT* 17:1207

Embryos mutant for the Notch ligand Dll1 or doubly mutant for the Notch1 and Notch2 receptors exhibit multiple defects in left–right asymmetry.

The Notch signaling pathway plays a primary role in the establishment of left–right asymmetry in mice by directly regulating expression of the *Nodal* gene.

Notch and somitogenesis



Notch1-/-

Conlon RA et al. 1995
Development 121: 1533–45

Dll1-/-

Hrabe de Angelis M et al. 1997
Nature 386:717–21

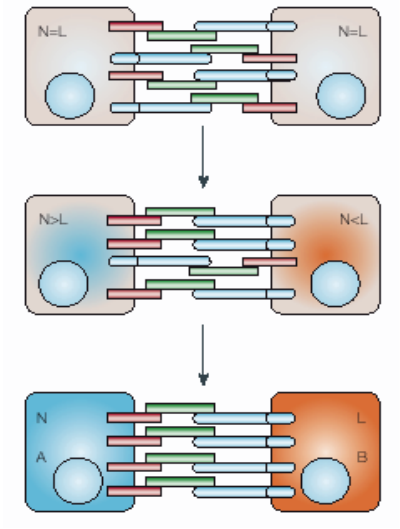
The positioning of segmental boundaries is chaotic, resulting in a large variation in somite size.

Notch signaling and cell-fate decisions

Notch signaling can have many different, if not opposite effects depending on the **timing** and the **tissue context**.

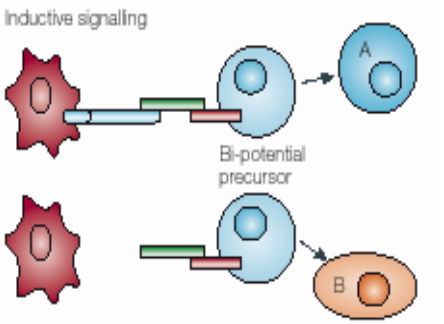
Notch signaling is acting on cell fate decisions either through **lateral signaling** or through **inductive signaling** .

In **lateral signaling**, equipotent cells initially express both Notch receptors and their ligands, but the concentrations of these proteins start to differ between neighboring cells. Small differences in receptor and/or ligand concentrations in cells are amplified over time, leading to cells that exclusively express either the receptors or their ligands, thus guiding the specification of the cell fate and cell differentiation.



lateral signaling

In **inductive signaling**, two distinct cells express exclusively either the receptor or the ligand. The fate of the bi-potential precursor cell is decided by the occurrence of this interaction. The cell expressing the receptor, and therefore the recipient of the Notch signal, is induced to differentiate into a particular cell lineage.



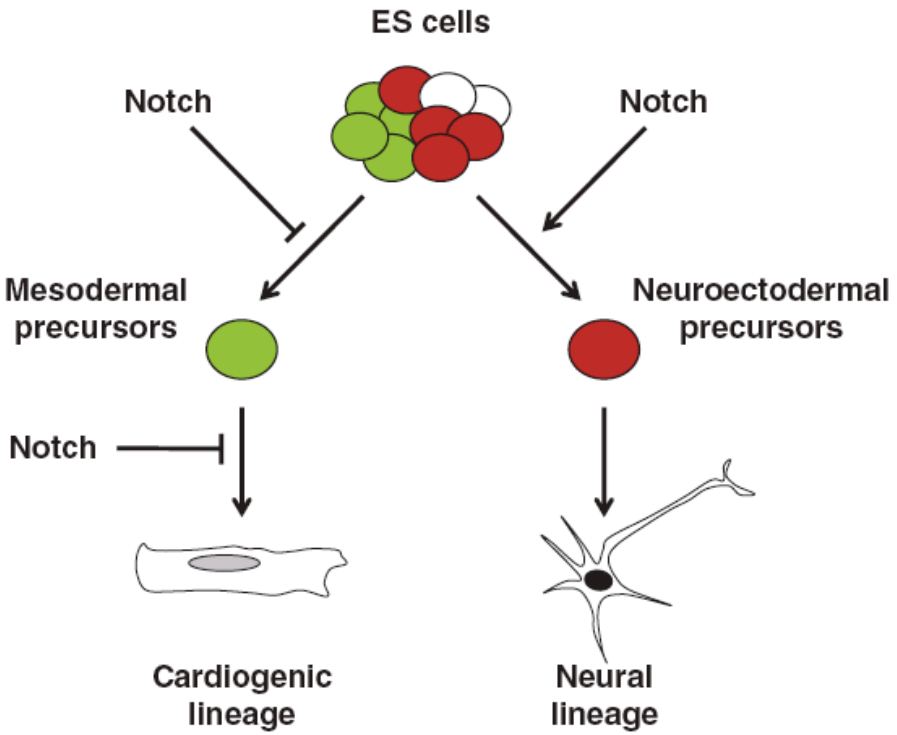
inductive signaling

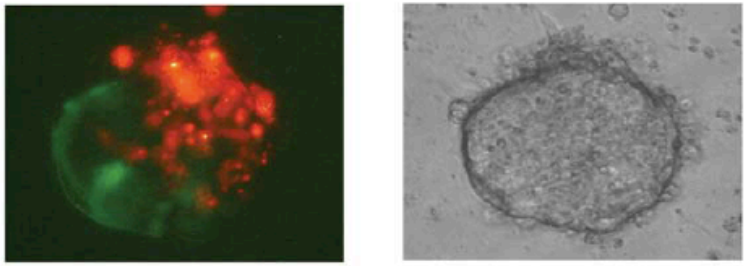
LINEAGE DECISION

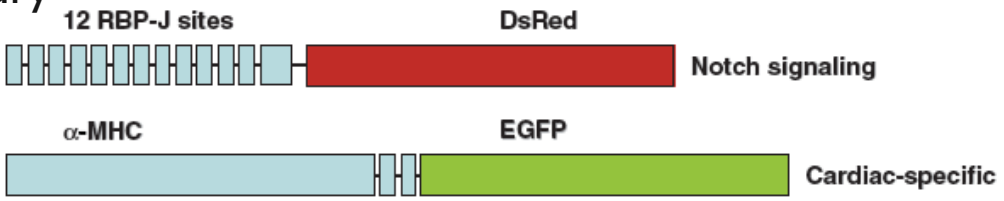
- The NICD-RBP-Jk complex up-regulates expression of target genes of Notch signaling such as HES and HERP in mammals.

- The HES/E(spl) family is a basic helix-loop-helix (bHLH) type trancriptional repressor and acts as Notch effectors by negatively regulating expression of downstream target genes such as tissue-specific transcription factors.

- HES1 and HES5, for instance, were shown to be upregulated by NICD and necessary to prevent neuronal differentiation of neural precursor cells from mouse embryos







Pedrazzini et al., 2007

Box 3. Notch signaling during vascular development – artery specification

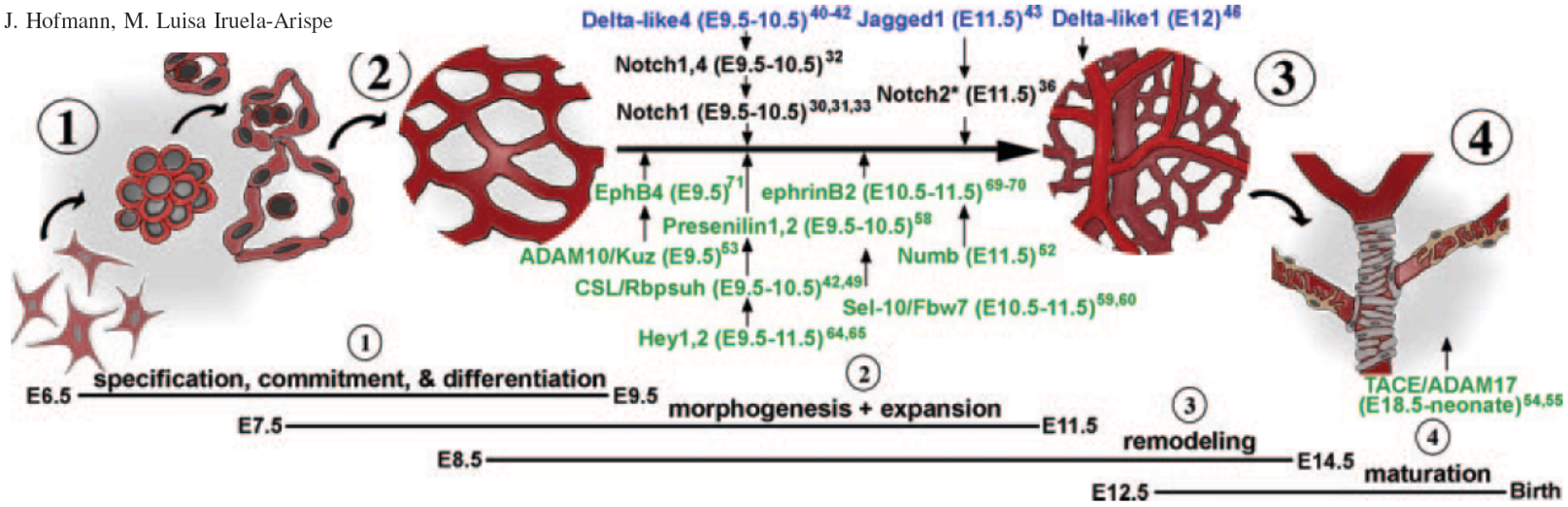
Studies in zebrafish already indicate a crucial role for Notch activity not only in embryonic hematopoietic stem cell (HSC) development, but also in vascular/angiogenic development. Given that arterial specification can be viewed as an important prerequisite for HSC emergence (Bertrand et al., 2010; Boisset et al., 2010; Kissa and Herbomel, 2010), Notch activity in the vascular system may also have an impact on hematopoiesis. A novel Notch signaling activity in zebrafish was shown to modulate fate specification of endothelial progenitors in the mesoderm, such that inhibition of Notch activity at an early stage promoted endothelial cell production at the expense of hematopoietic lineages (Lee et al., 2009). Moreover, genetic analysis has revealed a crucial role for Notch activity in mammalian vascular morphogenesis and artery specification (Krebs et al., 2000): both Notch1-deficient mouse embryos and compound-deficient embryos lacking Notch1 and Notch4 have severe vascular defects and die *in utero* prior to embryonic day 10.5. These findings have been recapitulated with specific conditional inactivation of Notch1 in endothelium (Limbourg et al., 2005). Both studies highlight the essential role of Notch signaling in the endothelium during vascular development and indicate a cell-autonomous function. Targeted deletion of several other players in the Notch signaling pathway, such as RPB-J (Krebs et al., 2004), mindbomb 1 (Koo et al., 2005), or Hey1 and Hey2 (Fischer et al., 2004), as well as Delta-like 4 (Duarte et al., 2004) were also shown to be essential for arterial specification. Moreover, the Notch-gridlock (Hey2 in mice) signaling axis regulates arterial versus venous cell fate choice in zebrafish. Gridlock, which is normally considered to be downstream of Notch signaling, was recently shown to act upstream of Notch in this context: overexpression of the Notch intracellular domain in gridlock mutants rescues the arterial phenotype (Rowlinson and Gering, 2010).

Circulation Research June 8, 2007

Notch Signaling in Blood Vessels

Who Is Talking to Whom About What?

Jennifer J. Hofmann, M. Luisa Iruela-Arispe



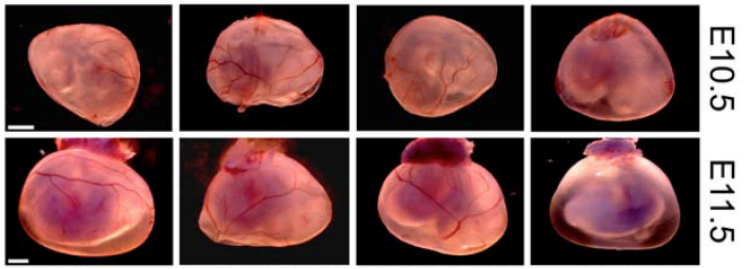
Genetic inactivation of Notch ligands receptors, downstream effectors, and modulators leads to embryonic lethality as a result of vascular defects. The vascular system develops from mesenchymal progenitor cells that differentiate into hemangioblasts (1) and subsequently form the primitive vascular plexus. (2) Later, this uniform network remodels into a hierarchical vascular system. (3) It is at this stage that the functional consequences of Notch signaling are most notable.

Inactivation of several Notch receptors, ligands, and genes associated with Notch signaling result in embryonic lethality at the developmental stages indicated in parenthesis.

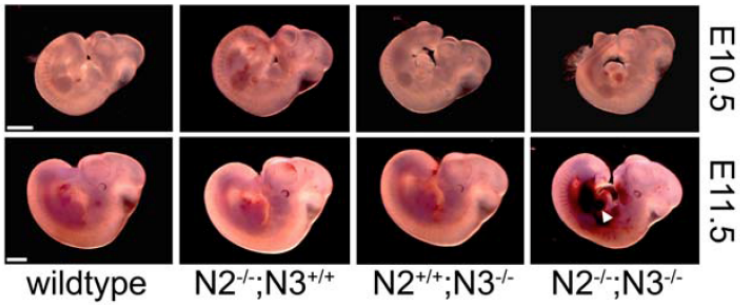
The stages of vascular development are: (1) specification, commitment, and differentiation of endothelial cells (E6.5 to E9.5); (2) morphogenesis and expansion of the vasculature (E7.5 to 11.5); (3) remodeling (E8.5 to E14.5); and (4) maturation (E12.5 to birth).

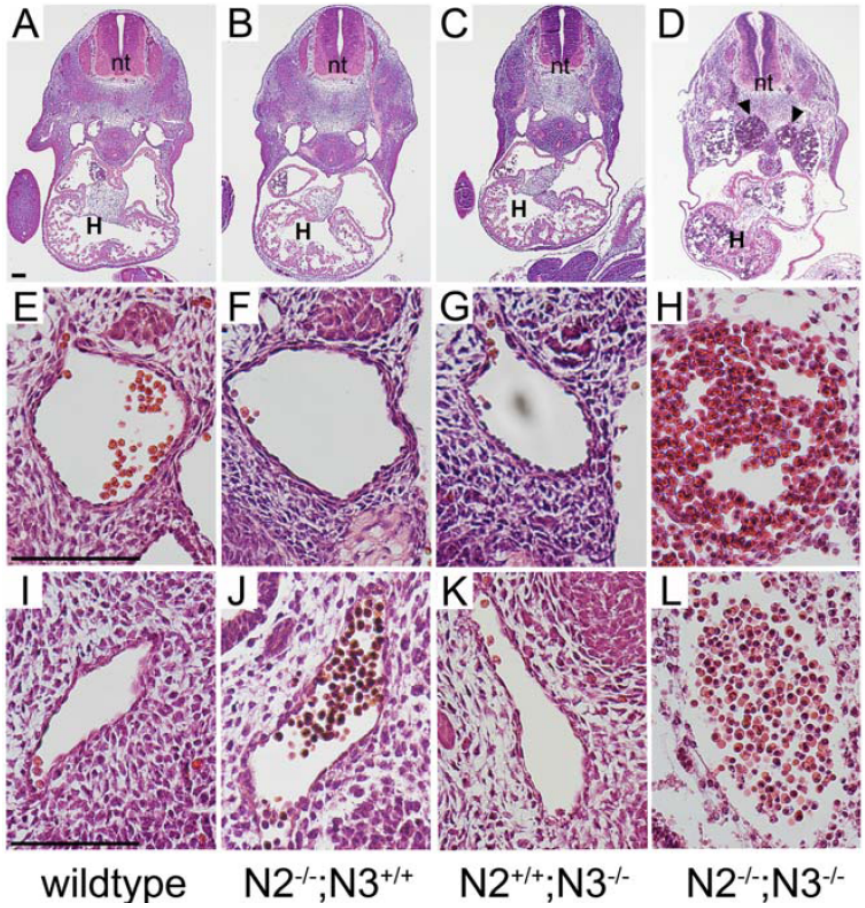
At E10.5, Notch2-/-;Notch3-/-; (N2-/-;N3-/-) embryos exhibit a decrease in yolk sac blood vessels, while the embryo is relatively normal in appearance.
At E11.5, Notch2-/-;Notch3-/- mice show severe vascular defects in both yolk sac and embryo. Yolk sac blood vessels are not visible and extensive hemorrhaging is seen in the embryo (arrowhead).

Yolk sac



Embryos





Embryos lacking both Notch2 and Notch3 have disrupted blood vessels

May 2012 | Volume 7 | Issue 5 | e37365

Notch2 and Notch3 Function Together to Regulate Vascular Smooth Muscle Development

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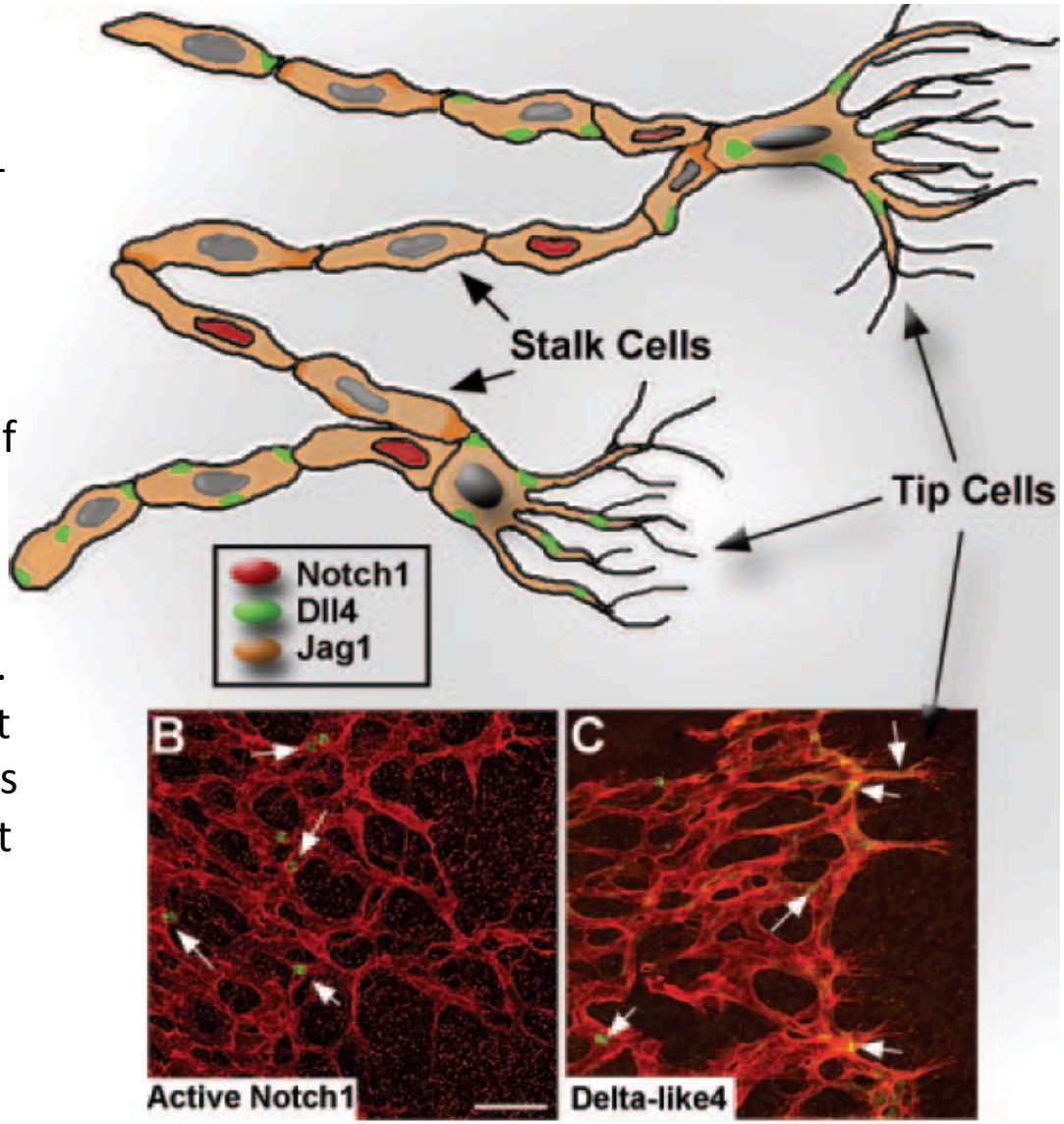
Active Notch signaling during angiogenesis

A. Relative distribution of active Notch1 (red), Delta-like4 (Dll4) (green), and Jagged1 (Jag1) (orange) in tip and stalk cells of angiogenic sprouts.
B. The pattern of active Notch (green, arrows) in the developing vasculature of postnatal day 7 mouse retinas, stained with platelet endothelial cell adhesion molecule (PECAM) (red), is scattered throughout the plexus and in stalk cells.
C, In contrast, Dll4 expression (green) at postnatal day 7 solely marks the tip cells at the leading edge of the vascular front but is also found throughout the homogeneous vascular plexus before remodeling (arrows). Scale bar, 50 m (B and C).



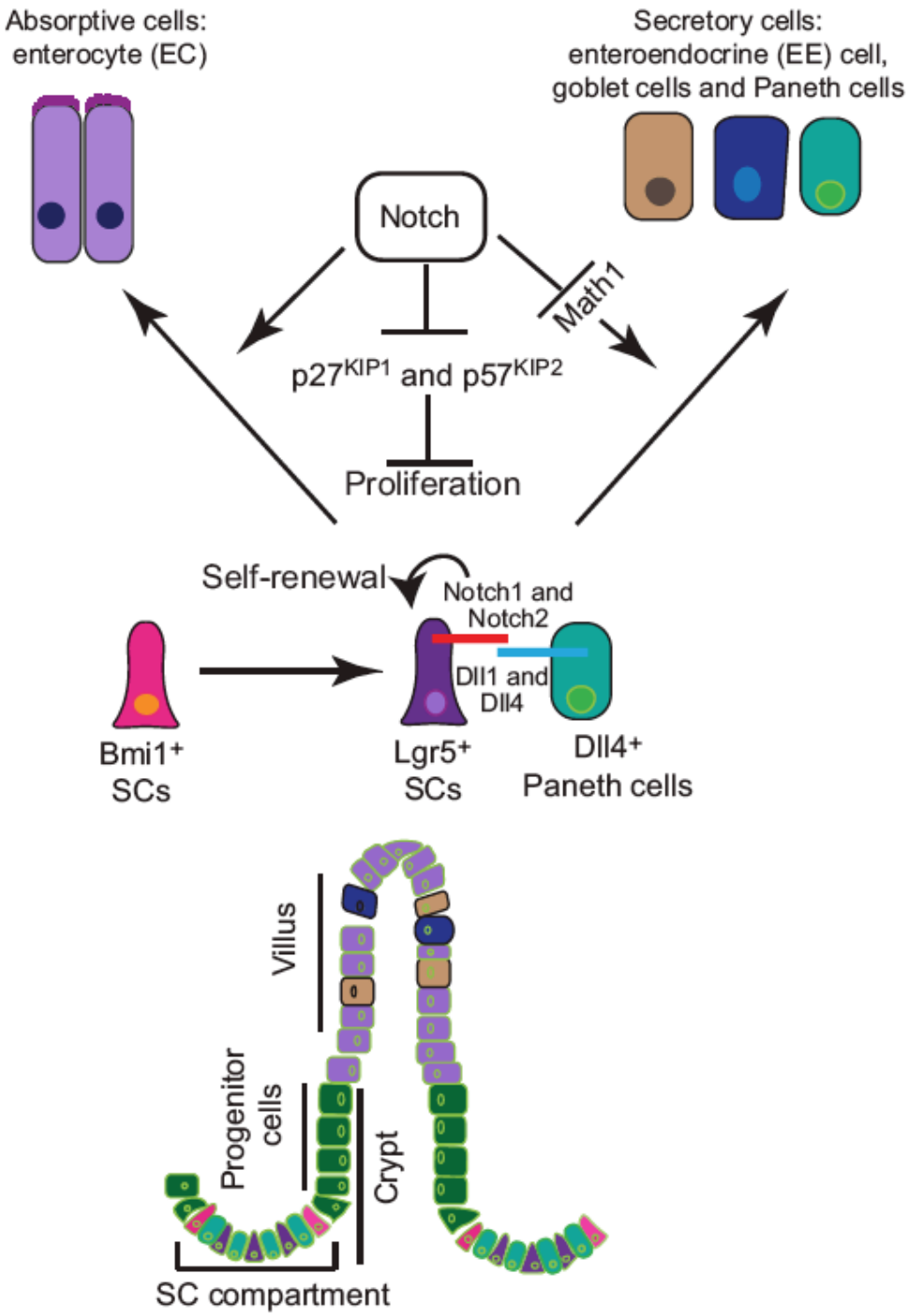
Notch in murine intestine

Long-lived Bmi1^{+} SCs give rise to mitotically active Lgr5^{+} SCs.

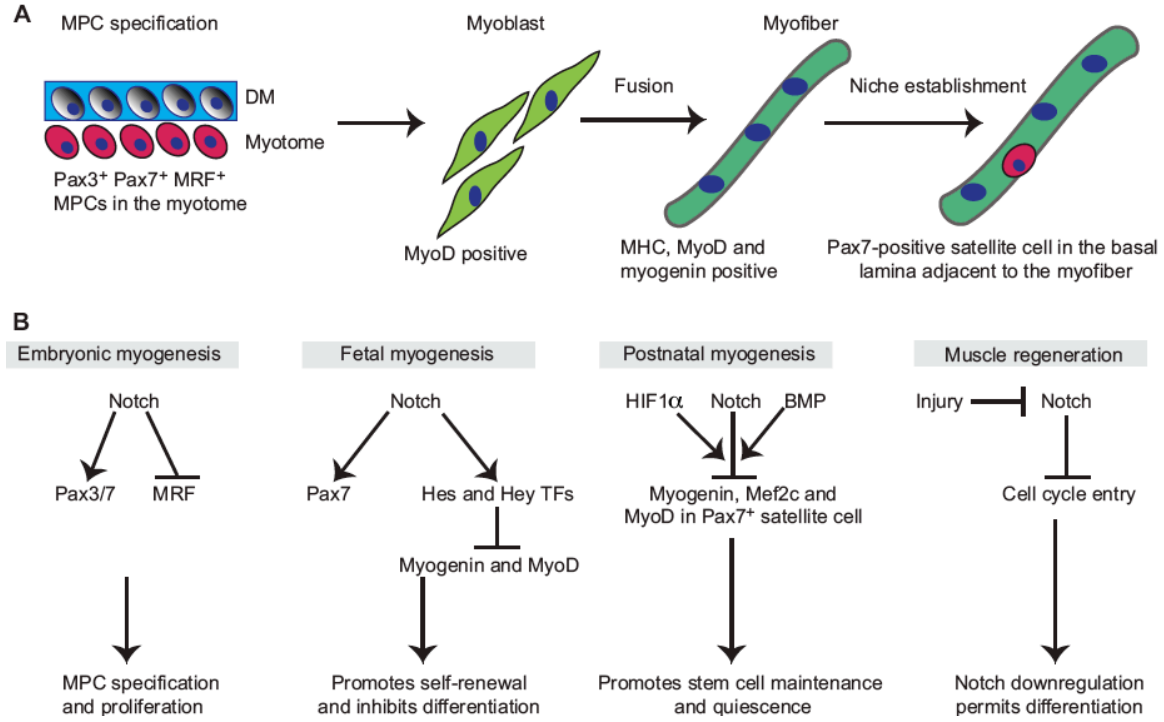
In the crypt compartment, Notch expressing Lgr5^{+} SCs are sandwiched between Delta-like 4^{+} Paneth cells.

Activation of Notch signaling in Lgr5^{+} cells maintains SC self-renewal and proliferation by negative regulation of the cyclin-dependent kinase inhibitors p27 and p57, and the transcription factor Math1, which is necessary for differentiation of the secretory cell lineages.

Notch thus biases cell fate choice towards the absorptive lineage.



Notch in muscle development and stem cell maintenance



Notch signaling contributes to muscle development by regulating the muscle stem/ progenitor cells during embryonic, fetal and postnatal myogenesis.

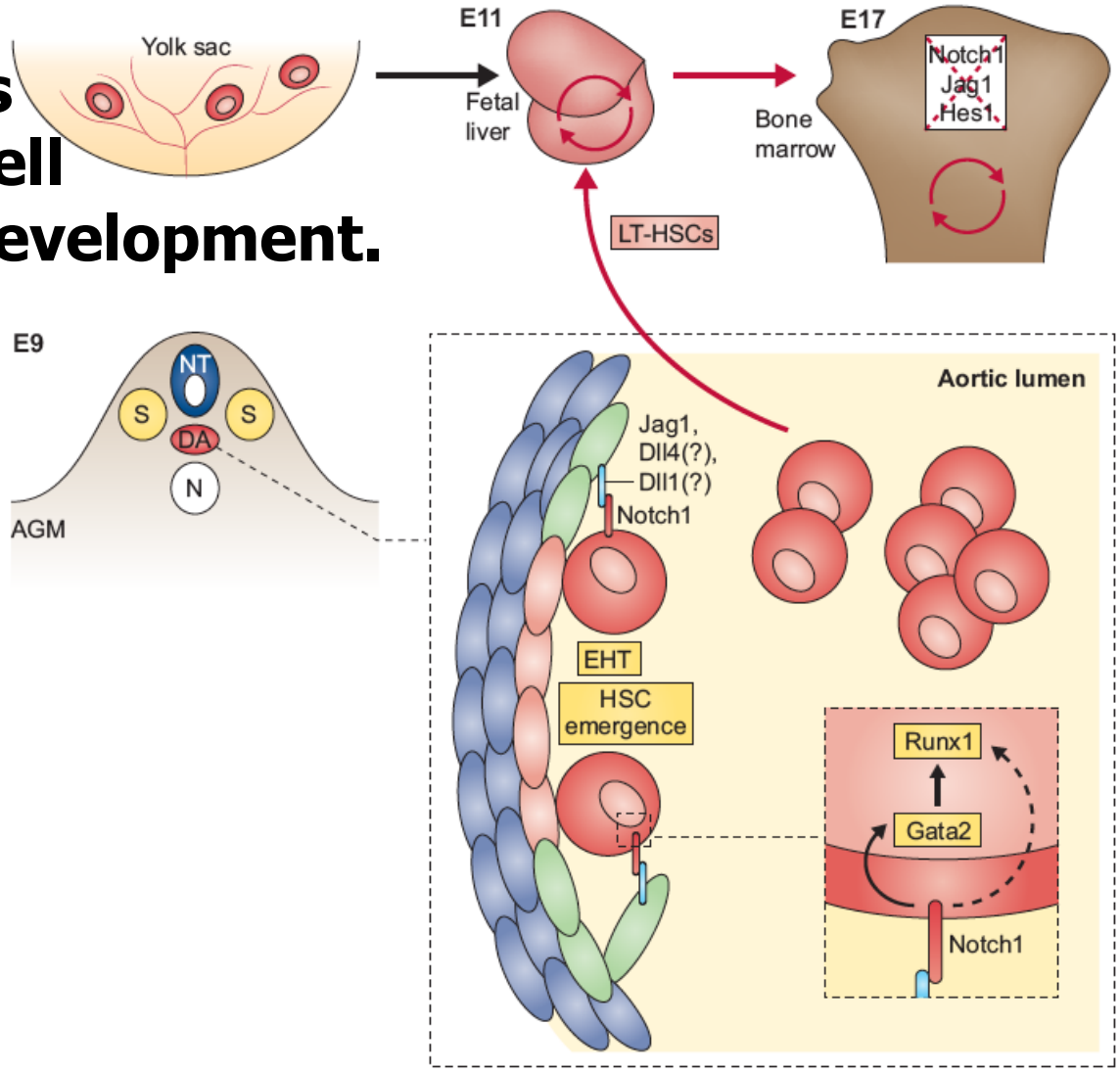
During embryonic myogenesis, Notch signaling determines the number of muscle stem/progenitor cells in the DM and inhibits lineage commitment by repression of muscle regulatory factors (MRFs).

During fetal and postnatal myogenesis, Notch-driven transcriptional activation of Pax7 ensures self-renewal of MPCs and satellite cells. Notch signaling also inhibits their premature terminal differentiation into skeletal muscle via transcriptional repression of myogenic genes. Notch signaling cooperates with HIF1α and BMP to induce expression of Notch targets Hes and Hey, which further repress the transcriptional activation of myogenic genes, thus blocking the terminal differentiation of MPCs and satellite cells.

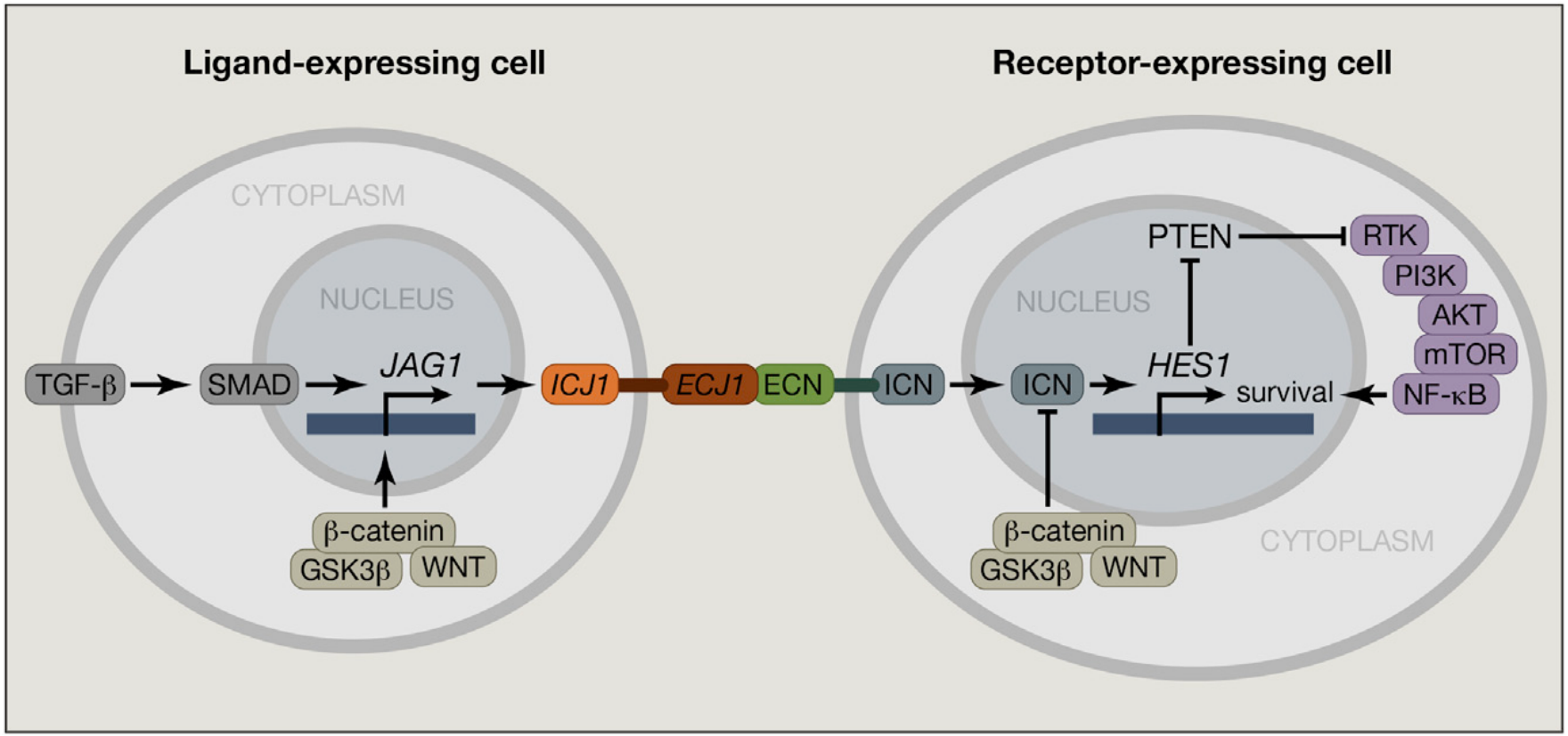
In case of muscle regeneration, Notch signaling is downregulated, accompanied by an upregulation of Wnt signaling. This allows the satellite cells to enter the cell cycle; activation of myogenic genes such as MyoD, myogenin and MHC further drive their terminal differentiation into multinucleated muscle fibers.

Notch signaling drives hematopoietic stem cell specification during development.

Notch signaling drives cell-autonomous hematopoietic stem cell (HSC) specification in the dorsal aorta (DA).
During vertebrate embryonic development, aortic endothelial cells of the DA express Jagged1, Delta-like 1 and Delta-like 4, whereas cells destined to emerge as HSCs
express the Notch1 receptor.
Interaction between Jag1 and Notch1 is essential for HSC specification.
Notch signaling is dispensable for HSC maintenance in the bone marrow.



Notch Interactions with Other Signaling Pathways



The TGFβ, PI3K, NFkB, and WNT pathways are some of the most important pathways that interact with NOTCH

Jagged 1 is activated by the TGFβ pathway and in turn activates NOTCH receptors in neighboring cells.

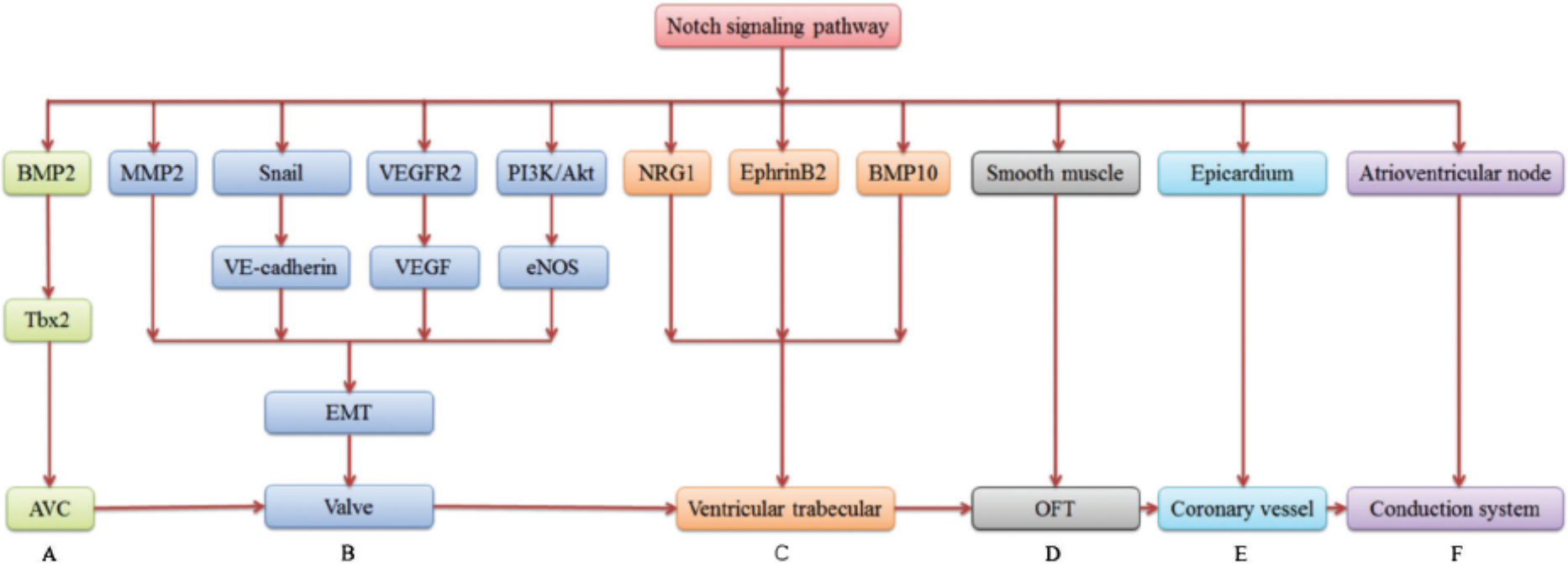
Phosphorylation of NOTCH from the WNT-induced GSK3β leads to ubiquitination through FBXW7 and final degradation.

A classical NOTCH target, HES1, represses PTEN, a competitor of PI3K pathway, which in turn activates NFkB, important for leukemia progression.

Notch in genetic disorders

disease	target	description
Tetralogy of Fallot	JAG1	heart malformation: ventricular septal defect, pulmonary stenosis, displaced aorta, right ventricular hypertrophy
Alagille syndrome	JAG1	arteriohepatic dysplasia: paucity of biliary ducts in the liver, cardiovascular abnormalities affecting the great vessels
Spondylocostal dysostosis	DLL3	Jarcho-Levin syndrome: vertebrae and rib malformations
CADASIL	NTC3	cerebral autosomal dominant arteriopathy with subcortical infarcts, dementia
T-cell acute lymphoblastic leukemia	NTC1 NTC3	chromosomal translocation: TCRβ promoter – truncated Notch; mutations
Mucoepidermoid (salivary gland) carcinoma	MECT1 MAML2	chromosomal translocation: mect1-mastermind

Notch signaling and heart



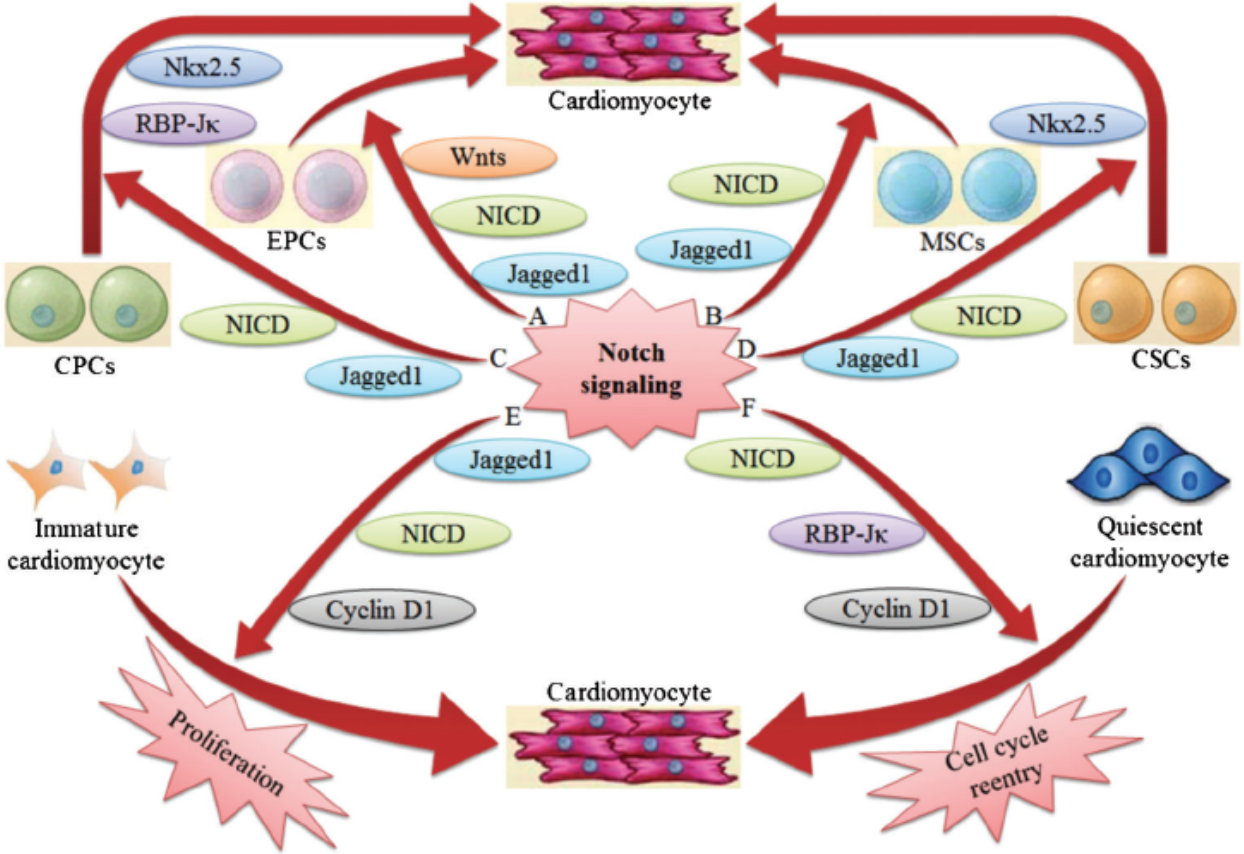
Summary of cardiac developmental aspects of Notch signaling.
A, Notch signaling affects AVC development via BMP 2/Tbx 2 pathway.
B, Notch signaling initiates EMT via MMP-2, snail/VE-cadherin, VEGFR2/VEGF, and PI3K/Akt/eNOS pathways.
C, Notch signaling promotes ventricular trabecular formation dependent on NRG1-ErbB2/4, Ephrin B2/Eph B4, and BMP-10 signaling pathways.
D, Notch signaling stimulates smooth muscle differentiation during OFT development.
E, Notch signaling modulates coronary vessel morphogenesis in which the embryonic epicardium actively participates.
F, Notch signaling regulates cardiac conduction system function via effects on the atrioventricular node.

Table 1. Relationship between Notch signaling and congenital heart disease.

Congenital heart disease	Gene mutation
Aortic valve degenerative disease	RBP-Jκ
Left ventricular outflow tract defects	Notch 1
Bicuspid aortic valve disease	Notch 1-4, Jagged 1, Hes 1, Hey 1, Hey 2
Aortic valve calcification	Notch 1, Hey 1, Hey 2
Pulmonic stenosis	Jagged 1
Tetralogy of Fallot	Jagged 1
Mitral valve disease	HRT 2
Tricuspid valve disease	HRT 2
Ventricular septal defect	HRT 2
Atrial septal defect	HRT 2
Pericardial distension	Notch 1, RBP-Jκ
Alagille syndrome	Notch 2, Jagged 1, HRT 2, Hey 2

RBP-Jκ: recombination signal binding protein for immunoglobulin Jκ region; Hes: hairy and enhancer of split: Hey: hairy/enhancer of split-related with YRPW motif; HRT: hairy-related transcription.

Regulatory role of Notch signaling for myocardial regeneration.



A, Notch signaling amplifies EPC differentiation into cardiomyocytes through Jagged 1, NICD and Wnts.
B, MSCs enhance cardiomyocyte proliferative capacity through Jagged 1 and NICD.
C, Notch signaling promotes the differentiation of CPCs into cardiomyocytes through Jagged 1, NICD, RBP-Jk, and Nkx2.5.
D, Notch signaling expands the proportion of CSCs differentiating into cardiomyocytes through Jagged 1, NICD, and Nkx2.5.
E, Notch 1 signaling stimulates proliferation of immature cardiomyocytes through Jagged1, NICD, and cyclin D1.
F, Notch signaling activates cell cycle reentry of quiescent cardiomyocytes through NICD, RBP-Jk, and cyclin D1.