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CORRECTION

The Notch meeting: an odyssey from structure to function

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There was an error published in Development **143**, 547-553.

The name of the second author was spelt incorrectly. This error has now been corrected in the print, online and PDF versions of the paper.

We apologise to the authors for this mistake.

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MEETING REVIEW

The Notch meeting: an odyssey from structure to function

Ajay Chitnis[1,*] and Laure Bally-Cuif[2]

ABSTRACT

The Notch signaling pathway plays fundamental roles in diverse developmental processes. Studies of the basic biology of Notch function have provided insights into how its dysfunction contributes to multi-systemic diseases and cancer. In addition, our understanding of Notch signaling in maintaining stem/progenitor cell populations is revealing new avenues for rekindling regeneration. The Notch IX meeting, which was held in Athens, Greece in October 2015, brought together scientists working on different model systems and studying Notch signaling in various contexts. Here, we provide a summary of the key points that were presented at the meeting. Although we focus on the molecular mechanisms that determine Notch signaling and its role in development, we also cover talks describing roles for Notch in adulthood. Together, the talks revealed how interactions between adjacent cells mediated by Notch regulate development and physiology at multiple levels.

KEY WORDS: Molecular characterization, Structure-function, Systems biology

Introduction

In the 100 or so years since its initial discovery, and 30 years since its molecular characterization by Spyros Artavanis-Tsakonas and Michael Young, Notch has come to be recognized as a key receptor in a highly conserved metazoan signaling pathway (see Box 1) used by cells to influence fate, behavior, proliferation and morphology. Notch signaling thus plays fundamental roles in diverse developmental contexts, from the self-organization of cell diversity to the differentiation and proliferation of stem/progenitor cells. Dysfunction of this pathway contributes to multi-systemic developmental defects, and mutations that result in inappropriate activation of Notch signaling contribute to the development of cancer.

In recognition of the growing diversity of contexts in which Notch signaling plays a role and the multiple levels at which its function is examined, a group of scientists spearheaded by Spyros Artavanis-Tsakonas initiated *The Notch Meeting*: a series of annual conferences held in Athens, Greece, that bring together and foster collaborations between researchers addressing diverse aspects of Notch signaling. In October 2015, researchers came together for the ninth Notch Meeting, which covered broad inter-related subjects, including structural and physical mechanisms of Notch activation, trafficking mechanisms, and transcriptional and epigenetic controls that regulate Notch signaling output. Additional sessions addressed the diverse roles of Notch in regulating the proliferation, fate and differentiation of progenitor cells and the patterning of tissues. Concluding sessions examined Notch signaling at a systems level and discussed its complex integration with diverse regulatory networks. Recounting the highlights of these four intense and intellectually stimulating days is a challenge in itself. Here, we attempt to focus on insights that emerged from an examination of Notch signaling at different levels – from the mechanisms that activate and regulate Notch signaling to those defining new roles for the pathway in stem cell biology, development and organ function (Fig. 1).

Notch-ligand interactions and the mechanics of Notch activation

The meeting began with a discussion of the structure of Notch and its ligands, how they interact and what determines the efficacy of this interaction. Examining these interactions at high resolution has proven difficult, notably because Notch has an intrinsically low affinity for its ligand. Vincent Luca (Stanford University, USA) described how this issue was overcome by using *in vitro* evolution to engineer mutations in DLL4 that enhance its affinity for Notch1. The EGF repeats 11 and 12 in Notch, as well as the module at the N-terminus of Notch ligands (MNNL) domain and the conserved DSL domains, are crucial for DSL ligand interactions with Notch. The crystal structure of interacting regions now reveals how antiparallel binding of Notch EGF repeats 11 and 12 to the DSL and MNNL domains of DLL4, respectively, determines interaction (Luca et al., 2015). Calcium binding in combination with a conserved packing interaction ensures the rigidity of many EGF modules, including EGF repeats 11 and 12. Penny Handford (University of Oxford, UK) discussed how the interface between EGF repeat 10, which does not have calcium-binding consensus sequences, and EGF repeat 9 is flexible. As also discussed in Luca et al. (2015), Handford suggested that this organization probably helps Notch and its DSL ligands bind in a similar antiparallel manner both when they interact in *trans* and in *cis*. The analysis by Luca and colleagues also revealed how the addition of O-fucose and O-glucose to threonine and serine residues on Notch1 functionalizes the receptor by making specific and essential contacts with residues on DLL4. Collaborative work from Robert Haltiwanger (Stony Brook University, USA) and Handford further showed how Fringe-mediated addition of GlcNAc to O-fucose increases binding of Notch1 to Notch ligands (Taylor et al., 2014). These observations illustrate how post-translational modifications of ligand-binding sites on Notch proteins that are regulated by specific biosynthetic pathways provide a strategy for regulating Notch function during development.

The Notch S2 cleavage site is masked by Lin12 Notch repeats (LNRs) in the Notch regulatory region (NRR). It has long been thought that the endocytosis of DSL ligands, following their interaction with Notch in *trans*, provides a pulling force that helps expose the S2 cleavage site to ADAM proteases. Wendy Gordon (University of Minnesota, USA) described elegant *in vitro* assays to demonstrate that forces associated with endocytosis are adequate to unmask the S2 cleavage site and drive Notch activation (Gordon et al., 2015). First, a single-molecule magnetic tweezer assay showed that a

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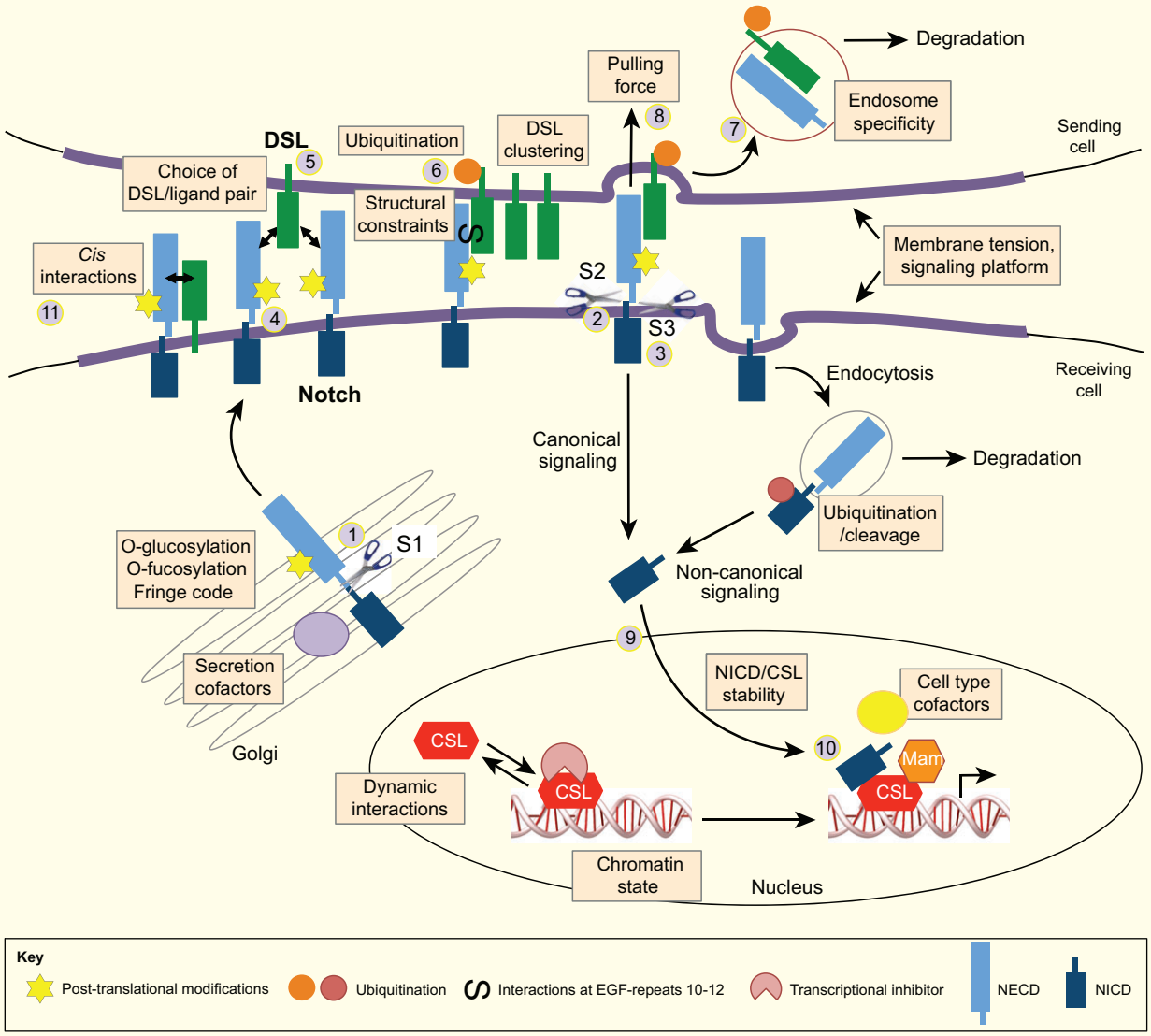
Development (2016) 143, 547-553 doi:10.1242/dev.131086

Box 1. An overview of Notch signaling



The mature Notch receptor is a heterodimer of two fragments generated by S1 site cleavage during their maturation (1). One fragment, tethered to the cell surface by a membrane-spanning domain, includes the Notch intracellular domain (NICD). NICD can be released via ADAM (A disintegrin and metalloproteinase) protease-dependent cleavage at an S2 site in the extracellular portion of the membrane-spanning domain (2). This cleavage facilitates subsequent intramembranous γ-secretase-dependent cleavage at an S3 site (3). S2 cleavage in the inactive Notch receptor is prevented by the Notch extracellular domain (NECD). NECD binds to the extracellular stub of the membrane-tethered Notch fragment via its negative regulatory region (NRR), preventing access to the S2 site (4). The remaining extracellular domain, consisting primarily of modular epidermal growth factor (EGF)-like repeats, determines interactions with the extracellular domain of Delta serrate Lag2 (DSL) family ligands, which also contain EGF repeats and are transmembrane proteins (5). When Notch interacts with a DSL ligand in a neighboring cell (in *trans*), it stimulates E3 ligase-mediated ubiquitination of the intracellular domain of the ligand (6), and this serves as a signal for endocytosis of the NECD-bound ligand (7). It is thought that this endocytosis provides a force that pulls the bound NECD away from the extracellular stub, unmasking the S2 site and allowing cleavage by ADAM proteases (8). This permits S3 cleavage and 'activates' Notch by releasing NICD from the cell membrane. NICD then translocates to the nucleus (9), where, with Mastermind (Mam), it forms a ternary complex with CSL [CBF-1/RBP-jκ, Su(H), Lag-1] proteins that bind Notch target genes to promote their transcription (10). By contrast, when a DSL ligand interacts with NECD on the surface of the same cell (in *cis*), endocytosis cannot produce a pulling force to activate Notch (11). Instead, internalization removes both Notch and its bound ligand from the cell surface; interaction in *cis* thus inhibits Notch signaling. Boxes highlight the mechanisms for which significant progress was reported at the meeting. The purple membrane region depicts the Notch signaling platform between adjacent cells.

force between 3 and 5.4 pN, consistent with that generated by endocytosis, is adequate to determine ADAM17-dependent S2 cleavage of isolated Notch1 NRR. A similar conclusion was obtained in a cell-based reporter assay directly measuring Notch activation in response to force. In this assay, a synthetic ligand-receptor system could also effectively substitute the Dll4-Notch1 interaction domains. Thus, pulling forces are adequate for Notch activation, and no specific allosteric changes are required for

Development (2016) 143, 547-553 doi:10.1242/dev.131086

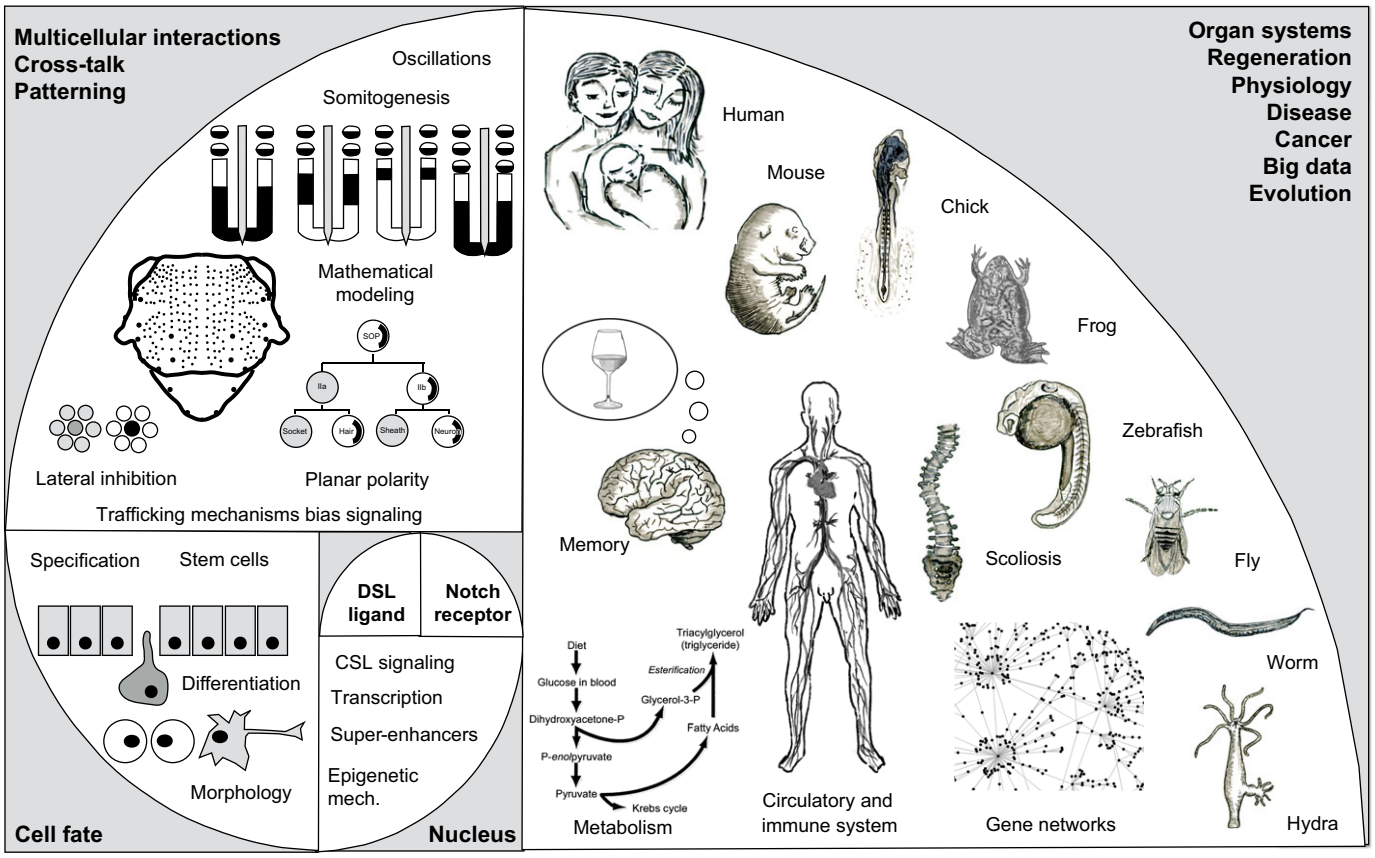


Fig. 1. A summary of the Notch IX meeting. This image attempts to capture the spirit of the Notch meeting by emphasizing the many levels (ranging from signaling mechanisms within the nucleus and within cells, to organ and organismal development and regeneration) at which this signaling system was discussed.

unmasking the S2 site. As suggested by Roland Le Borgne (University of Rennes, France), an important parameter of pulling strength is in-plane plasma membrane tension. In the *Drosophila* epithelium of the pupal notum, the asymmetrical division of sensory organ precursor (SOP) cells involves Notch signaling at cell contacts. Using laser nano-ablation of these contacts, Le Borgne demonstrated that, in striking contrast to neighboring non-signaling epithelial cells, the Notch signaling interface is subjected to low tensile forces. This is favorable for membrane deformation induced by ligand or receptor endocytosis. These results suggest that the capacity to activate Notch *in vivo* might rely on specific mechanical properties.

The interaction of DSL ligands with Notch determines ubiquitination by RING E3 ligases such as Mind bomb (Mib) or Neuralized, marking the ligands for Epsin-dependent endocytosis. Brian McMillan (from the Blacklow lab, Harvard University, USA) described recent structural analyses showing that MZM and REP domains, near the Mib1 N-terminus, interact independently with two intracellular regions of Jagged1, raising the possibility of combinatorial binding schemes that might facilitate Mib1 interactions with individual or clustered substrates (McMillan et al., 2015). As Mib1 has substrates other than DSL ligands, such combinatorial binding could also facilitate simultaneous binding to distinct substrates in a complex. These intriguing speculations and their implications will need to be investigated in the future. Overall, the mechanisms that coordinate Notch-DSL ligand interactions with ubiquitin ligases and endocytosis factors remain poorly understood. In this context, Motoyuki Itoh (Chiba University, Japan) showed that the ubiquitin ligase activity of Mib1 is induced by Notch-ligand interactions. Furthermore, Mib1 promotes the interaction between dynamin 2 and Snx18. In this manner, Mib1 modulates dynamin recruitment by regulating the interaction between Snx18 and dynamin 2 to ensure efficient signaling. As DSL ligands intrinsically have low affinity for Notch, their clustering is thought to increase avidity; however, it remained unclear whether Delta clustering by itself contributes to Notch activation. Stefano de Renzis and his colleague Aleksander Nekakov (both at EMBL Heidelberg, Germany) investigated this issue using a functional, optogenetic allele of the *Drosophila* Notch ligand Delta (Opto-Delta); although light-mediated clustering of Opto-Delta induced rapid clustering of the Notch receptor in an adjacent cell, this could not activate Notch in the absence of effective endocytosis.

Trafficking mechanisms that bias and regulate Notch activation

Factors regulating endocytosis, recycling and other trafficking mechanisms that determine the localization of Notch signaling components play a key role in determining the outcome of Notch signaling. In particular, the mechanisms that link the asymmetric distribution of such factors to cell polarity or to other spatial patterning cues play a key role in coordinating cell fate following division. Previously, Marcos Gonzalez-Gaitan described how Sara endosomes carrying internalized Delta and Notch, move to the central spindle in SOPs during mitosis. The endosomes are then segregated preferentially to the pIIa daughter, whose fate depends on active Notch signaling. The Gonzalez-Gaitan lab have now shown that Sara endosomes are targeted to the central spindle via a plus-end kinesin motor, and that they get preferentially delivered to

Development (2016) 143, 547-553 doi:10.1242/dev.131086

pIIa because of the asymmetric polymerization/de-polymerization of microtubules (Derivery et al., 2015).

Regulated trafficking also controls non-canonical Notch signaling pathways, in which Notch endocytosis, independent of ligand interaction, can result in Notch activation or inhibition. In this context, Notch is endocytosed in response to ubiquitination by Deltex or by Supressor of deltex [Su(dx)]. Following this, lysosomal enzymes either release NICD into the cytoplasm (activation) or degrade Notch (inhibition). At the meeting, Martin Baron (University of Manchester, UK) described how Dx-mediated Notch activation helps increase the size of the stem cell niche in the *Drosophila* ovary. He further identified a novel RING finger protein, Murashka, which binds to Notch in the Golgi and prevents it from reaching the cell surface. This reduces stem cell niche size. More generally, this work illustrates how the balance of secretion/endocytosis routes can control the amplitude of Notch signaling in physiological contexts.

The specificity of Notch signaling outputs

Canonical Notch signaling converges towards a single transduction cascade: NICD interacts in the nucleus with cell-specific factors, CSL and the coactivator Mastermind to drive target gene expression. Hence, a major aim in the field is to understand how Notch signaling triggers different outcomes in different contexts. Different DSL ligands, for example, can activate the same Notch receptor with differential effects on cell fate. As reported at the meeting by Anna Bigas (Institut Hospital del Mar d'Investigacions Mèdiques, Spain), Jag1- and Dll4-mediated Notch1 signaling in mouse aorta, gonad or mesonephros progenitors drives different transcriptomic and differentiation programs – hematopoietic stem cell or arterial specification (Gama-Norton et al., 2015). As presented by David Traver (University of California San Diego, USA), work in the zebrafish suggests that these distinct ligand-Notch interactions are conserved across species (e.g. Kim et al., 2014). In these examples, specificity could be achieved at several levels, including via the signaling 'strength' provided by a given receptor-ligand interaction or through enhancer choice by NICD fragments.

Nagarajan Nandagopal, from the Elowitz lab (California Institute of Technology, USA), found that Dll1 and Dll4 activate distinct target genes and cell fates through differences in NICD activation dynamics. In movies of single cells, Dll4 produced sustained Notch1 activation, but Dll1 unexpectedly activated Notch1 in discrete, stochastic, stereotyped pulses. Although these pulses selectively induced Hes1, only sustained NICD induced Hey1/L. Moreover, in somite myogenesis, Dll1 promoted differentiation through Hes1 (Rios et al., 2011), but Dll4 inhibited differentiation, inducing Hey1/L. These results are provoking further investigation of the mechanisms responsible for ligand-specific differences in activation dynamics (encoding) and selective target activation by these dynamics (decoding).

To test for functional differences in the intracellular domains of distinct Notch receptors, Raphael Kopan's lab (University of Cincinnati, USA) swapped NICD domains between mouse Notch1 and Notch2 receptors using knock-in techniques. Careful analysis of the kidney and skin failed to reveal phenotypes, suggesting that signal composition does not impart specificity (Liu et al., 2015b). Rather, signal 'strength', including the amount of NICD in the nucleus and the half-life of DNA-binding complexes, might account for some of the contextual differences observed *in vivo*. Other contexts and receptor combinations remain to be tested in such a rigorous way. Emily J. Capra, in a collaboration between the Elowitz and Bernstein labs, used different fluorescent protein readouts for Notch1 and Notch2 signaling to explore how specificity might arise from distinct abilities of DSL ligands to determine *cis* inhibition of Notch.

Downstream of NICD, cell type-specific co-factors also impart specificity. In the vascular and hematopoietic systems, the transcription factor Runx/Lozenge potentiates Notch signaling through an unknown mechanism. Mark Chiang (University of Michigan, USA) identified a novel cell type-specific Notch cofactor, Zmiz1 [a protein inhibitor of activated STAT (PIAS) family member] (Pinnell et al., 2015), that directly interacts with the N1ICD RAM domain and co-binds the *Myc* enhancer to drive its expression in leukemic cells. Zmiz1, which is expressed tissue-specifically, is dispensable for Notch activity in the intestine. The identification of other such cofactors will permit targeting of the Notch pathway with some degree of cell specificity.

The dynamics of Notch signaling at the nucleus

The mechanisms and dynamics of enhancer recognition and occupancy were addressed in several talks, which suggested that NICD/CSL binding to DNA can both respond to and influence epigenetic/structural changes in chromatin organization. In the context of T-lymphoblastic leukemia, only a subset of NICD/RBPj binding sites is occupied reversibly by NICD (Wang et al., 2014). These sites are generally located at or close to super enhancers carrying active epigenetic marks. In a provocative talk, Warren Pear (University of Pennsylvania, USA) showed how a Myc super enhancer carrying NICD/RBPj and Brd4 binding sites in its proximity toggles between the NICD- and Brd4-responsive states based on the presence of NICD or Brd4, through dynamic changes of the chromatin landscape (Yashiro-Ohtani et al., 2014). Hao Yuan Kueh from the lab of Ellen Rothenberg (California Institute of Technology, USA) also described data suggesting that NICD modifies chromatin state: through specific tagging, he could quantify the transcription of each allele of the *Bcl11b* gene in cultured hematopoietic progenitors and demonstrate that Notch signaling increases the probability of *Bcl11b* gene activation, potentially through its major enhancer.

The more extreme suggestion that Notch complexes act as pioneer factors was recently proposed. To address this hypothesis, Kopan's lab used the split Dam-Id technique to map DNA loci jointly bound by N1ICD/RBPj and the transcriptional co-activator p300 in mouse kidney progenitor cells (Hass et al., 2015). FAIRE (formaldehyde-assisted isolation of regulatory elements) analysis, comparing control and Notch1-overexpressing states, further showed that chromatin accessibility changes upon Notch activation, and that FAIRE sites overlap with N1ICD/p300-bound loci. This suggests that N1ICD binding induces *de novo* chromatin opening at binding sites. Although appealing, this conclusion nevertheless remains to be reconciled with the observed cell-type specificity of Notch signaling targets, which suggests some degree of upstream control in the accessibility of NICD/CSL sites. Along these lines, Maria Gomez-Lamarca from Sarah Bray's lab (University of Cambridge, UK) used live imaging to monitor the redistribution of Su(H) complexes on chromatin upon Notch signaling in *Drosophila* salivary glands. She highlighted the very fast dynamics of Su(H) on and off DNA, in a pattern that remained unchanged upon Notch activation except for the increased recruitment of Su(H) at specific chromosomal loci [e.g. *E(spl)* genes]. This can be interpreted as a longer time spent on DNA, or increased concentration of NICD/Su(H) molecules close to target sites, in line with the recent suggestion that Su(H) binds and

Development (2016) 143, 547-553 doi:10.1242/dev.131086

modifies predefined (open) loci upon Notch signaling (Skalska et al., 2015).

The mechanisms driving the inhibition of RBPj/Su(H) target genes in the absence of Notch signaling appear to be just as complex and differ between species. In the fly, Su(H) recruits the co-repressor Hairless (H), itself binding general repressor proteins. Analyzing the X-ray structure of Su(H)/H complexes bound to DNA, Rhett Kovall (University of Cincinnati, USA) showed that H binds the CTD domain of Su(H) and induces a conformational change, rendering this domain incompatible with NICD binding. Overall, the different modes of transcriptional repression of Notch target genes remain to be dissected in detail. This will involve elucidating the functions of Su(H)/RBPj itself – a daunting task that is beginning to be addressed through elegant conditional rescue experiments, as reported by Juan Carlos Zúñiga-Pflücker (University of Toronto, Canada).

Notch-mediated regulation of cell state and fate

The pleiotropic functions of Notch, and its activity as a cell-cell cross-talk molecule, make it a prime component of the mechanisms controlling fate specification. A few general observations were consolidated at the meeting. The first is the reiterative use of Notch to control successive fate decisions along given developmental pathways. Differential ligand/receptor usage (as mentioned above), or different levels of Notch activity, can add specificity to each step. For example Ben Ohlstein (Columbia University, USA) illustrated the effect of Notch activity levels, showing how high and low Notch signaling drives enterocyte versus intestinal stem cell (ISC) fate in the *Drosophila* intestine. Most interestingly, this involves bidirectional signaling: a high Notch signal is emitted from the intestinal stem cell to specify enteroblasts, and a low Notch signal is emitted from another ISC daughter, the entero-endocrine cell, to maintain ISC multipotency (Guo and Ohlstein, 2015). The second general feature is the role of Notch in modulating the activity of other cell fate signaling pathways. A beautiful example of this was provided by Kim Dale (University of Dundee, UK), who showed how Notch modulates the cellular response to Hh signaling by regulating cilia architecture and ciliary localization of the key Hh signaling components Smoothened and Patched 1 (Stasiulewicz et al., 2015).

Although an effect of Notch signaling on cell state (e.g. proliferation initiation or arrest) can result secondarily from changes in cell fate, several talks highlighted cases in which Notch activity directly impacts proliferation. In the ventral nerve cord of the *Drosophila* embryo, neuroblasts switch over time from a 'type I' proliferation mode (generating daughter cells that divide once to generate two differentiated progeny cells) to a 'type 0' mode (generating daughters that directly differentiate). Previous data from Stefan Thor's group (Linköping University, Sweden) demonstrated that the cyclin-dependent kinase inhibitor Dacapo (Dap) controls this switch, counterbalancing Cyclin E. At the meeting, Thor illustrated a direct contribution of Notch to the I>0 switch, Cyclin E being directly inhibited by E(spl) factors. Notch signaling – specifically Notch3 in zebrafish and Notch2 in mice – also promotes adult neural stem cell quiescence in the vertebrate brain, as reported by Laure Bally-Cuif (Paris-Saclay Institute for Neuroscience, France) and by Anna Engler (from Verdon Taylor's lab, University of Basel, Switzerland). The downstream effectors of Notch in this process remain to be discovered. Diana Ho (from the Artavanis-Tsakonas lab, Harvard University, USA) identified synergistic interactions between Notch and Src, and Notch and Mef2, as part of proliferation-regulating circuitries controlling *Drosophila* eye size.

Dissecting these multiple functions of Notch will strongly benefit from the precise and exhaustive identification of signal-receiving cells across time and tissues. This daunting task is being undertaken by Angeliki Louvi (Yale University, USA) in collaboration with Artavanis-Tsakonas, who are mapping the lineages arising from Notch1-4-expressing cells in the mouse central nervous system. This is being complemented by lineage tracing analyses performed by Silvia Fre (Institute Curie, France) in the mammary gland. The recently generated Notch1 activity trap mouse line (Liu et al., 2015a) will further help to map signal-receiving cells.

Notch and cell morphology

Notch signaling determines distinct fates in adjacent cells, and these cells also often adopt distinct morphologies, raising questions about how cell morphology can affect Notch-mediated fate decision processes. In this context, David Sprinzak (Tel Aviv University, Israel) discussed how modifying cell shape can alter the contact area with the neighboring cell. This, in turn, is expected to affect the efficacy with which the cell is able to deliver or receive Notch signals. Sprinzak discussed theoretical models and *in vitro* experiments to explore the consequences of changes in cell shape on the dynamics of Notch signaling.

In the zebrafish neural epithelium, progenitor cells selected for a neuronal fate by lateral inhibition delaminate prior to differentiating as neurons. Ajay Chitnis (National Institutes of Health, USA) described work, started by Miho Matsuda while in his lab and being continued in her own lab (Rutgers, USA), on how the band 4.1 superfamily protein EPB41l5, which facilitates neuron delamination and differentiation by promoting apical junctional complex disassembly, is regulated by Notch signaling; Mib1 promotes EPB41l5 degradation but Delta competes with EPB41l5 as a substrate for Mib1. Chitnis suggested that high levels of Delta expression in progenitors selected to become neurons could spare EPB41l5 from degradation, facilitating the disassembly of apical junctional complexes and, in turn, promoting the delamination of committed cells.

Marek Mlodzik (Icahn School of Medicine at Mount Sinai, USA) discussed how, in the *Drosophila* eye, Wnt-Frizzled-PCP pathways inhibit Notch activation and promote the delivery of Notch signals by the prospective R3 cell, whereas Notch activation promotes R4 photoreceptor cell fate in an adjacent cell. A Su(H)-independent pathway then affects cell adhesion and cytoskeletal elements to mediate ommatidial rotation.

Notch in pattern formation

Notch is part of an ancient evolutionarily conserved metazoan signaling system that allows one cell to regulate transcriptional activity in an adjacent cell. As a consequence, it is best known for its role in determining distinct fates in adjacent cells. Nevertheless, this mode of cell-cell communication has been co-opted in the course of evolution to coordinate cell fate and pattern formation at many different scales. Angelika Böttger (Ludwig Maximilians University, Germany) described a relatively ancient role for Notch in regulating regeneration in Hydra. Removal of the head in Hydra results in the re-establishment of a Wnt-dependent organizer region that facilitates the regeneration of the mouth or hypostome and surrounding evenly spaced tentacles. Böttger reported that Notch signaling is essential to block tentacle fate in cells that form the new hypostome and express Wnt-3. Similarly, Lindsey Mork (from the Gage Crump lab at the University of Southern California, USA) described how, by regulating the timing of differentiation, Notch

Development (2016) 143, 547-553 doi:10.1242/dev.131086

signaling helps determine distinct fates in dorsal versus ventral domains of the developing branchial arches, regulating when and where condensations of skeletogenic precursors form during development of the zebrafish facial skeleton.

Notch sometimes activates the expression of transcriptional repressors that inhibit their own expression and/or that of positive Notch targets. This delayed negative feedback leads to oscillations in gene expression. Previous studies suggested that Notch- and Delta-mediated cell interactions in the presomitic mesoderm help synchronize such oscillations, which coordinate sequential formation of somites. Olivier Pourquié (Harvard University, USA) challenged this notion and suggested a provocative alternative model: oscillations of the Notch pathway in mouse can be described in terms of an excitable system. This provides a new view on the emergence of collective oscillations in the presomitic mesoderm. In a related talk, Sally Dunwoodie (Victor Chang Cardiac Research Institute, Australia) described how gestational hypoxia in mice interferes with Notch-dependent gene oscillations that are crucial for somitogenesis, giving rise to vertebral defects such as scoliosis.

François Schweisguth (Institut Pasteur, France) provided another example in which local interactions mediated by Notch signaling determine pattern at a much larger multicellular scale. He discussed a mathematical model that suggests how the regulation of achaete-scute expression by Notch signaling initially determines proneural gene expression in longitudinal stripes on the *Drosophila* dorsal thorax, within which SOP cells are subsequently specified by lateral inhibition.

Understanding the mechanisms that regulate the function of transcription factors encoded by proneural genes like *achaete* and *scute* is essential for understanding their evolving spatiotemporal patterns of expression. In this context, Nick Baker (Albert Einstein College of Medicine, USA) described simple mechanisms by which the proneural factor Atonal (Ato) is regulated by interactions between the Id protein Extramachrochaete (Emc) and the E protein Daughterless (Da), whose competition allows cells to toggle between states that either promote or inhibit neurogenesis.

A role for Notch beyond development

Notch signaling has traditionally been studied in the context of development. However, a number of talks discussed its role in the ongoing regulation of cellular responses in a variety of organ systems. As examples, Andreas Fischer (German Cancer Research Center DKFZ, Germany) described its role in endothelial cells, where hyperglycemia promotes Notch signaling and balances glucose and fatty acid transport from blood plasma to muscle cells. Karla Kaun (Brown University, USA) described a potential role for Scabrous, previously described as an inhibitor of Notch signaling, in establishing the memory of an olfactory cue associated with an alcohol reward.

Future directions

In two years, this diverse group of Notch researchers will reconvene in Athens to share what the growing tool chest for examining Notch has revealed about various outstanding issues. This includes the functional significance of the differential affinity of Notch-DSL ligand pairs, the clustering of ligand receptor complexes and the pulsatile versus graded modes of Notch activation. Insights into the trafficking mechanisms that regulate and bias Notch signaling at many levels, and their coordination with other regulatory mechanisms, from the cell surface to nucleus and chromatin structure, will also hopefully be gained. Computational models will play a progressively bigger role in providing a framework for understanding how local interactions mediated by Notch pattern development in time and space at a multicellular scale. Ultimately, these studies will provide insight into the development, function and regeneration of organ systems and will open avenues for therapeutic approaches, while big data approaches promise to reveal levels of coordination that would not have been possible with traditional reductionist approaches.

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Competing interests

The authors declare no competing or financial interests.

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