

Development 133, 4793 (2006) doi:10.1242/dev.02713

A garden of Notch-ly delights Gerry Weinmaster and Raphael Kopan

There was an error published in *Development* **133**, 3277-3282.

There was a mistake in the first paragraph of this meeting review, which should read as follows:

Hosted by the Cantoblanco Workshops on Biology and organized by José Luis de la Pompa, Tom Gridley and Juan Carlos Izpisúa Belmonte, the meeting covered diverse aspects of this important signaling pathway.

The authors apologize to readers for this mistake.

A garden of Notch-ly delights

Gerry Weinmaster^{1,*} and Raphael Kopan²

Over the past decade, the Notch signaling pathway has been shown to be crucially important for normal metazoan development and to be associated with several human inherited and late onset diseases. The realization that altered Notch signaling contributes at various levels to human disease lead in May to the first meeting dedicated solely to Notch signaling in vertebrate development and disease in Madrid, Spain. Hosted by the Cantoblanco Workshops on Biology and organized by Tom Gridley, José Luis de la Pompa and Juan Carlos Izpisua Belmonte, the meeting covered diverse aspects of this important signaling pathway.

Introduction

Given that Notch signaling (see Fig. 1) appears to be involved in almost every developmental decision and process, it is probably not surprising that Notch, one of the oldest *Drosophila* developmental mutants, still generates much interest. During the 1990s, four distinct Notch receptors (Notch1-4) have been identified in humans and in mouse, including a human ortholog (NOTCH1) that is associated with T-cell acute lymphoblastic leukemia (T-ALL) (Ellisen et al., 1991) and a mouse ortholog (Notch4) that is associated with mammary tumors (Jhappan et al., 1992). Likewise, there are multiple vertebrate Notch ligands (Delta-like 1-4, jagged 1 and jagged 2), the so-called DSL (Delta, serrate, Lag2) ligands, that activate and regulate Notch signaling (reviewed by Lai, 2004). Mechanisms regulating Notch expression and signaling, as well as the various roles for Notch receptors in vertebrate development and disease, were the main focus of this meeting.

Ubiquitination and Notch signaling regulation

Activation of the Notch signaling pathway involves the ligand-regulated proteolysis of the Notch receptor by ADAM proteases, such as Adam17 (also known as TNF α converting enzyme, Tace), followed by the γ -secretase-mediated cleavage of the intramembrane part of the receptor (the so-called S3 cleavage, see Fig. 1) to release its intracellular domain (NICD). NICD functions as the signal transducer and it directly regulates Notch target gene transcription through its interactions with the DNA-binding protein CSL [for Cbf1, Su(H), Lag1], which is known as Rbpsuh in the mouse (previously RBPj κ). As ligand binding leads to Notch proteolysis, it has been assumed that these activating proteolytic events occur at the cell surface. However, closer inspection of the amino acids required for proteolysis reveal that the ubiquitination of Notch and its targeting to an endocytic vesicle are necessary prior to the γ -secretase cleavage of Notch (Gupta-Rossi et al., 2004). Alain Israel (CNRS, Paris, France) reported on his more recent studies on the regulation of Notch proteolysis that suggest that endocytosis

precedes Notch ubiquitination. Moreover, although ubiquitination of many proteins involves Lys48 in the formation of the ubiquitin chain, Israel reported that Notch1 and Deltex (one of the E3 ligases that regulates the level of cell surface Notch) are polyubiquitinated via the less commonly used Lys29. Israel's finding suggests that levels of Deltex, and possibly of Notch, are regulated via their ubiquitination by the Itch/AIP4/Su(Dx) E3 ligase, followed by their lysosomal degradation. These studies provide insight into how ubiquitination regulates both the basal levels of Notch, as well as its proteolytic activation for signaling.

Notch proteins and their targets in normal and malignant T cells

The maturation of vertebrate Notch proteins involves its proteolytic processing by furin in the trans-Golgi to produce a stable intramolecular heterodimeric structure that maintains the proteolysis resistance of Notch proteins in the absence of a ligand. Jon Aster (Brigham and Women's Hospital, Boston, MA, USA) presented analysis by his group of T cells from individuals with sporadic T-cell acute lymphoblastic leukemia (T-ALL). Their work identified two

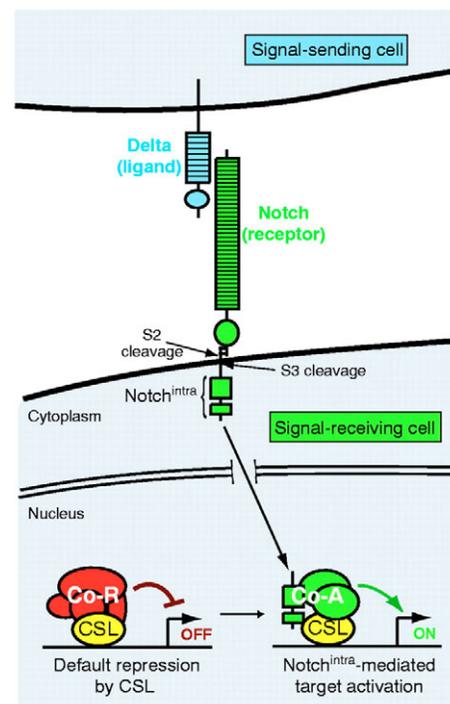


Fig. 1. The Notch signaling pathway. The key players in this pathway are shown and consist of a Delta-type ligand, the receptor Notch and the CSL transcription factor. Delta and Notch are transmembrane proteins that contain extracellular arrays of EGF repeats (rectangles). The activation of Notch by its ligand triggers two proteolytic cleavages of Notch (S2 and S3). S3 cleavage releases the Notch intracellular domain (NICD, Notch^{intra}), which translocates to the nucleus and then activates CSL. The CSL co-repressor complex is displaced by a co-activator complex, containing NICD (Co-A, green), which mediates Notch target gene activation. In the absence of nuclear NICD, CSL associates with a co-repressor complex (Co-R, red), which actively represses the transcription of Notch target genes. Reproduced, with permission, from Lai (Lai, 2004).

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hot spots for Notch1-activating mutations. In collaboration with Steven Blacklow's group (Harvard Medical School, Boston, MA, USA), they found that one mutational cluster maps to the heterodimerization domain (HD) of NOTCH1; most of the identified HD mutations appear to destabilize the non-covalent interactions that are required for the NOTCH1 heterodimeric structure to form. These mutations result in receptor dissociation and in the ligand-independent activation of NOTCH1 (Malecki et al., 2006). Interestingly, a duplication of 14 amino acids near the ADAM cleavage site did not destabilize the HD but instead increased the distance between the ADAM site and the rest of the NOTCH1 extracellular domain, resulting in constitutive proteolysis. These observations confirm that the resistance to proteolysis of Notch1 is based on preventing access to the ADAM cleavage site and that ligand binding can somehow expose this region. The second hotspot stabilizes NOTCH1 by eliminating a novel phosphorylation site within the PEST motif, which is associated with protein turnover. Alison Miyamoto (UCLA School of Medicine, Los Angeles, CA, USA), from Gerry Weinmaster's group, also highlighted the importance of heterodimeric dissociation for Notch activation. She presented recently published findings on Notch dissociation and activation induced by the secreted extracellular matrix protein Magp2 (microfibril-associated glycoprotein 2), which indicate that mechanical forces have a role in Notch dissociation (Miyamoto et al., 2006).

Aster also reported exciting new data, produced in collaboration with Warren Pear (University of Pennsylvania, Pittsburgh, PA, USA), that identify Myc as a key downstream target of Notch1 in the T-cell lineage. In elegant experiments, these researchers demonstrated that Myc overexpression can bypass a pharmacological block of γ -secretase (see Box 1), and that NICD can restore tumour growth when tetracycline-regulated Myc expression is extinguished, by activating the endogenous Myc gene. Alfredo Ferrando (Columbia University, New York, NY, USA) presented a detailed ChIP on chip analysis that identified a large cohort of co-regulated Notch/Myc targets, most involved in cell growth and metabolism. The ability of Notch to upregulate Myc in T-ALL cells appears to reflect a normal developmental relationship, as Pear has observed that Myc expression in thymocytes at the DN3a stage also depends on Notch activation. Of interest, DN3a cells are poised to undergo a proliferative burst that depends on expression of the pre-T cell receptor, a process referred to as β -selection. The normal upregulation of Myc by Notch probably primes DN3a cells for several rapid rounds of cell division, while persistent aberrant Notch signaling gives T-ALL cells a metabolic license to divide indefinitely.

At least two ADAM metalloproteases have been implicated in vertebrate Notch signaling. The Adam17 (Tace)-mediated cleavage of Notch is thought to facilitate efficient γ -secretase cleavage in the generation of the NICD. Although the phenotypes generated through the targeted deletion of ADAM10 (also called kuzbanian or Kuz) in mice (Hartmann et al., 2002) suggest a role for this protease in Notch signaling, its essential functions in this pathway remain a mystery. Ellen Robey (University of California, Berkeley, CA, USA) presented findings that show that the expression of a dominant-negative (DN) form of Kuz in T-cell progenitors produces defects in T-cell fate decisions that are reminiscent of those caused by the loss of Notch (Manilay et al., 2005). She reported that T-cells are unable to activate Notch on their neighbors when grown in the absence of stromal cells (see below), while the overexpression of Delta-like1 (Dll1) on neighboring thymocytes can suppress T-cell fate defects within the thymus, indicating that cell-cell communication within

this organ differs from the interactions that occur between isolated thymocytes. Co-expression of Dll1 with DN-Kuz in thymocytes could not suppress the cell fate defects, similar to the non-autonomous cell effects that have been identified for Kuz in flies.

Elegant studies presented by Juan Carlos Zuniga-Pflucker (Sunnybrook and Women's Research Institute, Ontario, Canada) demonstrated that the Notch ligand Dll1 (and more recently Dll4) are sufficient to block B cell development and are necessary for thymic stromal cells to direct progenitors into T-cell differentiation. Although jagged ligands are expressed in the thymus, they are not as efficient, most probably owing to the expression of lunatic fringe (Lfng) by thymic progenitors and stem cells (Lfng negatively regulates the Jag1-mediated activation of Notch). Interestingly, Dll ligands presented by 3T3 cells can only block B cell development but cannot induce T cells from stem cells or lymphoid progenitors. Bone marrow stromal cells lacking Dll1 expression can complement this missing activity, which was suggested to be due to Wnt signals. Consistent with this result, mice deficient for Tcf, the transcription factor that drives Wnt target gene expression, have defective T-cell development, and the Wnt antagonist dickkopf 1 (Dkk1) prevents T-cell development. Importantly, hematopoietic stem cells (HSCs) can also respond to the Notch/Wnt combination and adopt a T-cell fate. Pear closed this session by demonstrating that adult murine HSCs do not require Notch signaling. He reported that in the presence of a

Box 1. A quick guide to Notch inhibitors

The use of pharmacological reagents to inhibit Notch signaling is expanding, and discussions at this meeting arose regarding the selection of the correct inhibitor and regimen. Before performing such experiments, the IC_{50} of an inhibitor should always be determined (the concentration that inhibits 50% of Notch cleavage, which can vary between cell types and when used in vivo). Researchers should familiarize themselves with the pharmacokinetics of the drug before using it in vivo and/or use a protocol tested for their chosen system, examples of which abound in the γ -secretase inhibitor literature. In selecting the drug, selectivity wins over efficacy.

Popular inhibitors

DAPT, a recommended Notch antagonist (Dovey et al., 2001), has an IC_{50} in most cells is in the low μ M range. It has remarkably low activity against the γ -secretase-related SPP (signal peptide peptidase) family members at all concentrations (Weihofen et al., 2002) and has been used extensively in cell and organ culture, and in model organisms (Cheng et al., 2003). It is not an active site-directed molecule (Morohashi et al., 2006).

LY411575, a DAPT derivative (Searfoss et al., 2003), is less effective against SPP than it is against γ -secretase in some cells, but may be effective against SPP when used at higher concentrations. In animal studies, when toxicity must be avoided, the degree of Notch signaling reduction is uncertain without detailed biochemical analysis of target tissues.

L685458 and L852647 (Merck) (Li et al., 2000; Shearman et al., 2000) are highly effective, active-site-directed inhibitors with IC_{50} values in the nM range, making them cheaper to use for long-term or animal studies, but they can also inhibit SPP in the same range that they inhibit γ -secretase, which can have phenotypic consequences (Krawitz et al., 2005).

In the past, the proteasome inhibitor MG132 (Z-LLL-CHO) has been used to inhibit NICD formation (Kopan et al., 1996). A modified form of MG132, GSI1, is now available, but its similarity to MG132 has not been disclosed. It is a broad chymotryptic and aspartyl protease inhibitor, which cannot be considered to be selective. It is not recommended for use when biological outcome is monitored, but it can be used for some biochemical studies.

DN form of the Notch co-activator protein mastermind (DN-Maml), which functions as a pan-Notch inhibitor when expressed in HSCs, irradiated mice can still reconstitute their immune system after two rounds of sequential transplantation, with only loss of the T-cell lineage.

All the data reviewed thus far have focused on the canonical role of Notch1 in T cells. Isabella Screpanti (La Sapienza University, Rome, Italy) presented evidence that Notch3 acts downstream of Notch1 in T-cell development, and presented data suggesting that some Notch activity may be derived via modulation of the transcription factor NF κ B. Barbara Osborne (University of Massachusetts, MA, USA) has further investigated this possibility by interrogating the time line of Notch and NF κ B activation. Although early NF κ B responses occur in the presence of a γ secretase inhibitor (GSI), maintaining the NF κ B response required Notch activation. Mechanistically, some controversy remains, as Osborne presented data that support a model in which Notch binding to NF κ B may regulate the nuclear half-life of NF κ B. However the domain required for Notch to interact with NF κ B is the same as the one that is required to stabilize the interactions between CSL and NICD (Wang et al., 2001); thus, it is unclear if the observed losses in activity are due to a loss of CSL-mediated transcription and an indirect effect on NF κ B localization.

Roles for Notch in multiple tissues and in disease Kidney development

The embryonic lethality caused by the loss of either *Notch1* or *Notch2* in mice has previously indicated that these Notch receptors have non-redundant roles during development (Swiatek et al., 1994; Conlon et al., 1995; Hamada et al., 1999). However, it was assumed that, when co-expressed, these proteins have redundant functions (Pan et al., 2005). Rafi Kopan (Washington University, St Louis, MO, USA) presented interesting data regarding the non-redundant function of these Notch receptors during kidney development. Kopan reported that in the absence of *Notch2*, proximal tubule and podocyte precursor differentiation fails to occur in mutant mouse embryos, despite clear evidence that Notch1 is activated in the right cells at the right time and that the signal is Rbpsuh dependent. This provides evidence that, in the same cell, each receptor has a unique function. In collaboration with Doug Barrick (Johns Hopkins University, Baltimore, MD, USA), Kopan's group found that the affinity of Notch1 and Notch2 proteins for Rbpsuh was identical. Given this, the inability of activated Notch1 to rescue the Notch2 kidney defects suggests that different functional thresholds for different Notch paralogs are present within the same nucleus that rely on qualitative, rather than quantitative, differences between these two conserved proteins.

Liver development

Stacey Huppert (Vanderbilt University Medical Center, Vanderbilt, TN, USA) reported preliminary findings describing the affects of Notch1 and Notch2 loss on liver development in mice. She reported that loss of *Notch2* impedes mature bile duct and hepatic artery formation, while loss of *Notch1* on a *Notch2*-null background enhances bile duct loss and arterial defects. This loss of bile duct structures is reminiscent of the paucity of bile ducts present in individuals with Alagille, a syndrome that is associated with *JAG1* mutations. Her findings are also consistent with a recent report that *Notch2* mutations can produce Alagille (McDaniell et al., 2006). Together with a mouse model of Alagille that has been generated by Tom Gridley, in which a single copy of both *Jag1* and *Notch2* have been targeted (McCright et al., 2002), these findings suggest that

Notch2 signaling induced by *Jag1* regulates bile duct formation. Whether Notch plays a role in the formation of the whole biliary tree, or of just particular segments, is currently being investigated by Huppert.

Somitogenesis

The involvement of the Notch pathway in somitogenesis was first indicated by defects in somite morphology in mice bearing targeted mutations in either Notch1 (Swiatek et al., 1994; Conlon et al., 1995) or Rbpsuh (Oka et al., 1995). The presomitic mesoderm (PSM) is unique in maintaining a precise pattern of periodic oscillations in gene expression that lead to the formation of bilaterally symmetrical somites. Julian Lewis (Cancer Research UK, London, UK) presented data refining and testing a mathematical model of how the oscillations are generated through negative feedback loops and built-in delays that are inherent to the processes of transcription, translation and protein export. The data provide quantitative support for a theory of the timing mechanism governing the periodicity in zebrafish, based on the idea that auto-inhibition of Notch target genes creates an intracellular oscillator in each cell and that cell-cell communication via Notch signaling maintains synchrony among neighboring cells.

The Notch signaling pathway integrates with additional pathways (such as the Wnt, Fgf and retinoic acid pathways) to regulate the oscillations and thus pattern the vertebrate musculoskeletal system in space and time. Olivier Pourquie (Stower Institute, Kansas City, MO, USA) was the first to observe Notch target mRNA oscillation in the chick PSM, and at the meeting he showed real-time images of oscillation in live mouse embryos expressing short-lived GFP under the control of the cycling gene *Lfng*. The Wnt pathway also oscillates in the PSM, and opposing gradients of Fgf (low in the anterior) and retinoic acid (high in the anterior) act to define a zone within the PSM in which cells are competent to respond to Notch activation by acquiring rostral and caudal somite identities. To identify additional molecular components of this 'clock and wavefront' mechanism, Olivier's group painstakingly collected and staged 17 embryos for a caudal transcriptome comparison at all phases within one clock cycle. This analysis identified two major clusters containing Notch- and Wnt-related genes, and increased by one order of magnitude the expression of additional genes that cycle during somitogenesis.

The dorsolateral cells of the somites go on to form the myotome, where axial muscle precursors are born. Achim Gossler (Institute for Molecular Biology, Hannover, Germany) reported his group's characterization of a new hypomorphic allele of *Dll1*. When placed on *Dll1*-null background, this allele results in mice that develop with very few skeletal muscle fibers. In these mutants, the myogenic program initiates normally, as does precursor migration into the limb, but too many cells differentiate early, depleting the muscle stem cell/progenitor pool prematurely. As a result, no additional muscle cells can form during later stages of development, accounting for the observed motionless phenotype of these mutants.

Cardiovascular development

Notch signaling is required for the development and maintenance of multiple cardiovascular structures. Jose Luis de la Pompa (Universidad de Autonoma, Madrid, Spain) reported his analysis of Notch signaling during cardiogenesis by following the appearance of activated Notch1. Notch is involved in regulating ventricular differentiation via regulation of ephrin B2 and neuregulin, and, at the same time, in balancing proliferation via Bmp10 modulation.

Vascular defects account for the embryonic lethality of mouse embryos defective in Notch signaling. Of all the Notch pathway mutations associated with vascular defects, the *Dll4*-null mutation is the most severe, causing embryonic lethality in heterozygotes because of vascular system malformation, even though the DSL ligands Jag1 and Dll1 are present. Extending these observations, Tom Gridley (The Jackson Laboratory, Bar Harbor, ME, USA) reported that complete deletion of *Rbpsuh* in endothelial cells produced the same vascular phenotype as loss of a single *Dll4* allele, resulting in loss of ephrin B2, a marker of arterial identity. In addition to defects in vascular remodeling, branching and sprouting, Gridley also identified characteristic differences in the type of arteriovenous malformations (AVM) associated with gain versus loss of Notch function, by following the distribution of ink after its injection into the outflow tract of mutant hearts (see Fig. 2). Interestingly, although both gain and loss of *Dll4* function cause AVM, they appear to do so in an anatomically distinct manner, producing distinct ink flow patterns. Nick Gale (Regneron Pharmaceuticals, Tarrytown, NY, USA) and colleagues are also characterizing *Dll4* mutant vascular defects based on their identification of *Dll4* as a target of *Vegf* signaling. When *Dll4* mutant mice mated to ICR outbred mice, their viability is restored, allowing Gale to characterize vascular development in the retinas of *Dll4* heterozygotes. He found these mutants display increases in branching and sprouting at the growing edge of the arteries, coupled with a delay in arterial and capillary remodeling, processes that are regulated by *Vegf*.

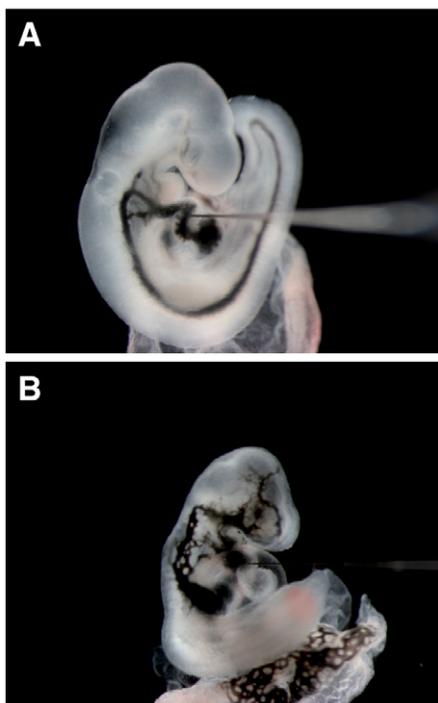


Fig. 2. Notch pathway mutations and arteriovenous malformations. (A) In a wild-type mouse embryo, India ink injected into the heart in order to visualize blood flow exits through the aortic arch arteries and enters the descending dorsal aorta. (B) In an embryo with an endothelial cell-specific deletion of the *Notch1* gene, the injected ink leaks directly into the venous system via arteriovenous malformations. Images courtesy of Luke Krebs and Tom Gridley.

CADASIL

Anne Joutel (INSERM, Paris, France) continued her relentless pursuit of the etiology of CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), which is the most frequent cause of inherited stroke in humans. *Notch3* is expressed by vascular smooth muscle cells (SMCs) associated with arteries, which are the very cells affected in CADASIL. Although all CADASIL mutations involve loss or gain of a single cysteine residue in the extracellular domain of Notch3, how these mutations affect Notch3 activity and vascular SMC function has not been easy to resolve. *Notch3*-null mice are viable but display specific defects in arterial vascular SMCs. As none of the traditional Notch/CSL targets were decreased in *Notch3* nulls, unique targets may be required in mouse arterial SMCs. However, these vascular defects do not phenocopy those associated with CADASIL mutations, and by using several genetic strategies, Joutel was able to demonstrate that the CADASIL-encoding mutations in Notch3 retain signaling activity *in vivo*. Therefore, a novel Notch3 function is most likely to be responsible for the CADASIL phenotype.

Keratinocyte differentiation and oncogenesis

Paolo Dotto (University of Lausanne, Epalinges, Switzerland) discussed his recently published findings of interactions between Notch signaling and p63 during keratinocyte differentiation (Nguyen et al., 2006). This interaction seems to be mediated by the ability of Notch signaling to affect a subset of interferon-responsive genes, which in turn regulate p63, independently of NF κ B. Lucio Miele (Loyola University Medical Center, Chicago, IL, USA) reported his studies of transformed cervical keratinocytes, which lack functional Rb/p53 activity and have an overactive PI3K pathway. In this context, Notch receptors play a positive oncogenic role. Notch receptor reduction via siRNA or the use of GSIs reduced the viability of these cells. Mechanistically, this effect is mediated by enhancing sub-optimal NF κ B complexes. Exactly how this occurs is still controversial, but Miele confirms Osborne's observations that Notch/p50 and Notch/Ikk α complexes can form. Silencing either Notch or Ikk α enhanced the activity of DNA damaging agents. These responses seem to depend on the tumor cell context; such interactions are not seen in normal keratinocytes or lymphocytes. In addition, they disagree with previous findings from Paolo Dotto's laboratory, showing a tumor suppressor function for Notch in several cervical keratinocytes cell lines, underscoring the context-dependent nature of Notch signaling. Sudhir Krishna (National Center for Biological Sciences, Bangalore, India) presented data suggesting that activated receptor tyrosine kinases can sustain Notch signaling in some epithelial tumors through phosphorylation of NICD; interestingly, the target sequence on Notch1 is the same one identified by Aster. Determining which biochemical interactions reported in Notch-overexpressing cells are physiologically relevant from those that are not will require more selective pharmacological and genetic tools.

Keith Brennan (University of Manchester, Manchester, UK) reported that active NICD levels are high in breast cancer cell lines, adding his voice to those suggesting that Notch signaling is important for maintaining the malignant phenotype. In support of this, overexpression of the Notch antagonist Numb blocked growth in soft agar assays. Moreover, he found that NICD expression resulted in the phosphorylation and activation of AKT, which in turn protected NICD-expressing cells from DNA damage-induced apoptosis. Although NICD leads to phosphorylation of AKT, this effect is mediated by pathway targets, as RBP-VP16 (a

Notch-independent, transcriptionally active form of Rbpsuh) can provide tumor cells with the same AKT-dependent protection. This result needs to be considered in the context of the NF κ B/Notch connection, where similar experiments should be performed. Interestingly, Urban Lendahl (Karolinska Institute, Stockholm, Sweden) finds that the balance of NICD and Numb can be tilted based on the level of these proteins, such that the more abundant protein can target the other to proteasome-mediated degradation.

Notch and stem cells

The isolation and cultivation of many different cell types, especially embryonic and neural stem cells, can be enhanced by growth in low oxygen tension. Lendahl presented his published studies that link the effects of oxygen tension on cell growth and differentiation to Notch signaling (Gustafsson et al., 2005). He proposes that interactions between NICD and the well-characterized oxygen-sensing transcription factor Hif1 α explain the impact of hypoxia on cell differentiation. Both Notch signaling and hypoxia prevent myogenesis and neurogenesis. However, the block induced by hypoxia is reversed by Notch inhibition: DAPT-treated myocytes differentiate under hypoxic culture conditions. Hypoxia stabilizes NICD through direct interactions with Hif1 α ; the expression of both Hif1 α target genes and CSL-NICD target genes are enhanced through these interactions. These findings are reminiscent of previous reports of NICD interactions with Smad proteins that enhance the efficacy of Bmp target gene expression. Thus, Hif1 α joins Ikk α , p50 and Smad as possible partners for Notch in the nucleus. Whether these interactions occur under physiological conditions remains an unresolved issue.

Freddie Radtke (Ludwig Institute for Cancer Research, Epalinges, Switzerland) discussed his analysis of Notch signaling in corneal development. Using embryological and genetic strategies, he has uncovered two populations of stem cells that repopulate the cornea; Notch1 is required to prevent these stem cells from defaulting into an epidermal program and to maintain their corneal fate.

Notch and transcriptional repression

Two speakers addressed the functions of Notch signaling in transcriptional repression. David Ish-Horowitz (Cancer Research UK, London, UK) presented crystal structures that show how the Hes/Hey family of transcriptional repressors interact via a WRPW/YRPW motif with the WD motif of Groucho/Grg/Tle co-repressor proteins (Jennings et al., 2006). As the expression of Hes/Hey genes are activated by Notch signaling, and interactions with Groucho co-repressors are required for transcriptional repression, this structural analysis could lead to the development of novel pharmaceuticals that could reverse GRG/TLE-mediated repression and thus the effects of Notch signaling. Daisuke Yabe (Kyoto University, Kyoto, Japan) also reported an elegant series of genetic experiments that establish Spen (previously Mint) as the Hairless homolog in mice and the main mediator of Rbpsuh-mediated transcriptional repression. In several systems (marginal B cells, follicular B cells and during cortical neurogenesis) loss of Spen produced the opposite effect to loss of Rbpsuh, indicating that Notch acts to antagonize the repressive function of Spen and, in its absence, that its repressive task becomes easier. To rule out Mx2-dependent functions in these processes, Spen/Rbpsuh conditional double null animals were also generated, and in all cases, the Spen phenotypes were epistatic to Rbpsuh, providing convincing evidence that Spen phenotypes depend on the presence of Rbpsuh.

Conclusion

At the end of this exciting and intense meeting, many of us escaped to the Museo del Prado to experience some new images. One particular painting displayed in the museum, Bosch's 'The Garden of Earthly Delights', in some ways reflected the myriad effects and roles identified for Notch signaling in vertebrates. Although many new twists and insights have enhanced our understanding of the molecular mechanisms that regulate Notch signaling and its biological consequences, the reality of the adage 'the more we learn the more we don't know', seems incredibly apt. Nonetheless, the importance of Notch signaling to both development and human disease will ensure that we will all continue to discover more Notchly delights.

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