# *Notch* and the Awesome Power of Genetics

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**ABSTRACT** Notch is a receptor that mediates cell–cell interactions in animal development, and aberrations in Notch signal transduction can cause cancer and other human diseases. Here, I describe the major advances in the Notch field from the identification of the first mutant in *Drosophila* almost a century ago through the elucidation of the unusual mechanism of signal transduction a little over a decade ago. As an essay for the GENETICS *Perspectives* series, it is my personal and critical commentary as well as an historical account of discovery.

THE overarching theme of this essay is how genetic analysis illuminated the role of Notch in mediating cell–cell interactions during development, identified the core components of the signaling system, and elucidated the mechanism of signal transduction. However, I also found myself developing three other themes, which I will return to at the end in *Concluding Remarks*. One theme is the remarkable synergy that occurred between genetics and molecular biology—something that we take for granted today but had a revelatory feeling when the two approaches converged on animal development in the 1980s. Another theme is how scientific understanding is achieved. Finally, there is a coming-of-age theme about how model organisms came to occupy a prominent place in modern biology research, with an emphasis on *Caenorhabditis elegans*, my personal favorite.

## 1930s–1970s

The earliest alleles of *Notch* arose as spontaneous dominant mutations in fly stocks (see Mohr 1919). It was relatively easy to recover them because *Notch* is haploinsufficient in *Drosophila*: a deletion that removes *Notch* causes the eponymous notch-like indentations of the wing margin. Continuing work on *Notch*—beginning with one of the first characterized chromosomal deficiencies (Mohr 1919) through the 1970s—occurred primarily in the context of advancing concepts of the nature of genes. The many different kinds of alleles of *Notch* generated during this era became a treasure trove for molecular biologists when cloning and sequencing became possible in the 1980s.

Donald F. Poulson is generally regarded as the founding father of the Notch field in *Drosophila*, as he first described the hallmark phenotype of dying homozygous null *Notch* mutant embryos. These embryos display hypertrophy of the nervous system at the expense of ectoderm (later called the "neurogenic phenotype"; see Figure 1) as well as many abnormalities in non-neural tissues (Poulson 1939, 1940). It was a major advance of general significance when Poulson looked carefully at the anatomy of dead embryos and saw that smaller and smaller cytological deficiencies, and even mutations that did not result in cytological deficiency, caused discrete cell-fate transformations. Poulson may have been the first *Drosophila* geneticist forging important connections between genes and embryogenesis at a time when most *Drosophila* workers were focused on adult morphological mutants.

Because effects on wing morphology and bristle number and spacing were an easy mark for early geneticists, many different kinds of alleles of *Notch* were identified, resulting in different effects on these adult traits. In the 1960s and 1970s, fly geneticists were focused on understanding the apparent complexity of the locus through studying the nature and interactions of different alleles, generally by describing the phenotypes of various *trans*-heterozygotes (Welshons and Von Halle 1962; Foster 1975; Portin 1975). These studies were more concerned with the genetic properties of alleles than with their normal roles in development, reflecting the prevalent preoccupation of that era—how the structure and organization of genes in animals could be related to those in microorganisms—at a time when

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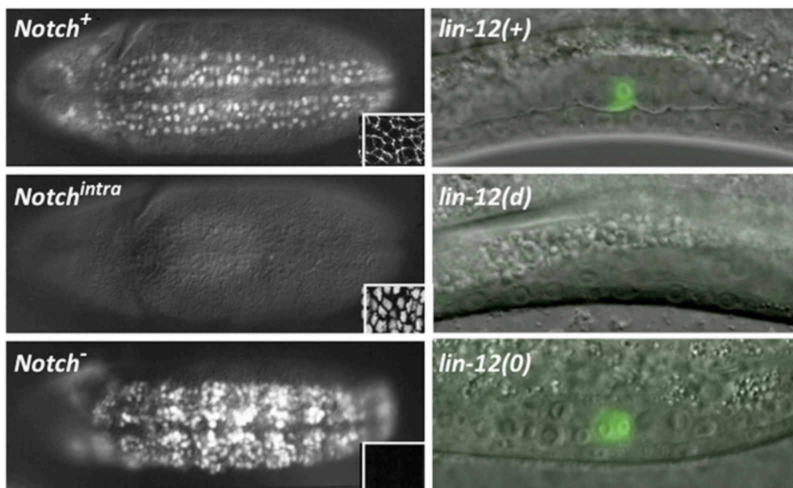


**Figure 1** Photomicrographs showing wild-type and *lin-12/Notch* mutant phenotypes. (Left) *Drosophila* embryos. Neuroblasts are marked by anti-Hunchback staining, and insets show the results of staining with an antibody to the intracellular domain of Notch. (Top Left) A wild-type embryo showing the normal pattern of neuroblast segregation from the ventral ectoderm and Notch protein predominantly at the surface of ectodermal cells. (Middle Left) An embryo expressing Notch$^{intra}$ protein ubiquitously under heat-shock control: all ventral ectodermal cells remain ectodermal, and Notch$^{intra}$ protein accumulates predominantly in nuclei. (Bottom Left) A *Notch*$^{-}$ embryo: all ventral ectodermal cells segregate as neuroblasts, the classic "neurogenic phenotype." (Right) *C. elegans* hermaphrodite gonads. Green fluorescent protein marks the anchor cell (AC) in a wild-type hermaphrodite, two ACs in a *lin-12(0)* hermaphrodite, and the lack of an AC in a *lin-12(d)* hermaphrodite. *Drosophila* photomicrographs courtesy of Gary Struhl; *C. elegans* photomicrographs courtesy of Maria Sallee.

the operon concept was still relatively new and enhancers and introns had not yet been discovered. One article from this era that stands out as more modern in its sensibility is that of Shellenbarger and Mohler (1975), who interpreted the results of temperature-shift experiments as indicating that some of the genetic complexity reflected different spatial and temporal functions for *Notch* during development.

## 1980s

The 1980s were shaped by the tremendous impact of molecular biology on the concept of the gene and how knowing gene products could lead to mechanistic insights. Some critical advances in the understanding of *Notch* that occurred during this decade were made using a new experimental organism, *C. elegans*. I will describe the major developments in the *Notch* story during this decade after a brief digression to introduce *C. elegans* and my own entry into the field during this period.

### *Enter C. elegans and the identification of lin-12/Notch*

I was very fortunate to enter graduate school at the Massachusetts Institute of Technology (MIT) in 1977. Both the venue and timing were propitious. An MIT education meant being steeped in the classics of molecular biology. And, at that time, developmental genetics was beginning a remarkable and sustained log phase of growth and discovery: the impact of the instant-classic *Drosophila* articles such as Lewis (1978) and Nusslein-Volhard and Wieschaus (1980) was profound and increased immeasurably as the power of molecular biology began to be harnessed to genetics. And, fatefully, Bob Horvitz joined the MIT faculty during my first year, captivating me with the prospect of approaching developmental biology with the sensibility of a phage geneticist: *C. elegans* as an experimental system was expressly chosen as the metazoan analog of phage by Sydney Brenner (Brenner 1974), who was already a personal hero of mine from the classic molecular genetics articles that we read in class. Bob soon established an active group that included Victor Ambros, Edwin (Chip) Ferguson, Bill Fixsen, Paul Sternberg, and, a little later, Gary Ruvkun—fantastic colleagues as well as supportive friends.

I spent much of my graduate career honing my skills as a geneticist on intellectually pleasurable but rather arcane aspects of functional redundancy (Greenwald and Horvitz 1980, 1982, 1986). However, everyone else in the lab was studying cell-lineage mutants, and I wanted to do so, too.

At the time, the *C. elegans* larval lineage had been completed and found to be largely invariant (Sulston and Horvitz 1977; Kimble and Hirsh 1979). The lineage of the vulva, one of the main organs that develops in the larva, seemed to be exceptionally tractable to genetic analysis: the ability to cultivate *C. elegans* as a self-fertile hermaphrodite allowed mutants with vulval abnormalities or even lacking a vulva altogether to be readily obtained (Horvitz and Sulston 1980; see also the *Perspectives* by Horvitz and Sulston 1990). In addition, laser microbeam ablation experiments, using a system invented by John White, revealed that cell–cell interactions played a role in vulval development by showing that ablation of certain cells changed the fate of neighboring cells (Sulston and White 1980; Kimble 1981). These features made vulval development a powerful paradigm for genetic analysis of signaling systems—although I do not know if any of us realized just how spectacularly successful it would prove to be at the time.

The first alleles of *lin-12* were dominant mutations that were isolated as part of Chip's epic analysis of a large number of mutations affecting vulval development (Ferguson and Horvitz 1985; Ferguson *et al.* 1987). Chip had mapped several dominant mutations with different vulval phenotypes to a single chromosomal region. Provisionally, these mutations were assigned to a single locus, *lin-12* (*lin*: abnormal cell *lin*eage). When I was contemplating a lineage project, I was attracted to *lin-12* because of its potential genetic complexity—like many of the fly geneticists who studied *Notch*.

### *Notch as a binary switch for cell-fate decisions mediated by cell–cell interactions*

When I began to work on *lin-12*, I first generated null alleles [*lin-12(0)*] for phenotypic analysis as well as for classical gene dosage analysis to gain insight into the nature of the dominant mutations (Muller 1932). The gene dosage analysis revealed that the *lin-12(d)* mutations were hypermorphs, *i.e.*, mutations that result in elevated gene activity. Thus, I had alleles in hand with opposite effects on gene activity to test the exciting prospect that *lin-12* functioned as a "genetic switch" as did the genes that I had loved learning about for λ and the yeast mating type, and a paradigm that was also applied to the *Drosophila* homeotic genes (Lewis 1978; Struhl 1981).

The simple cellular anatomy and invariant cell lineage of wild-type hermaphrodites allows mutants to be understood in terms of altered cell-fate decisions by individual cells (Horvitz and Sulston 1980; Sulston and Horvitz 1981). When Paul Sternberg, with his encyclopedic knowledge of postembryonic worm anatomy and cell lineage, examined the different kinds of *lin-12* mutants, he observed that the hypermorphic and null alleles had the opposite effect on cell fate in many different cell-fate decisions. Because opposite alterations in the level of *lin-12* activity had opposite effects on these cell-fate decisions, we inferred that *lin-12* indeed functions as a genetic switch (Greenwald *et al.* 1983)—the first switch gene described in the worm.

Paul identified many different cell-lineage alterations. Looking strictly at the lineage trees, it was interesting that *lin-12* was acting late in a hierarchy to diversify it, making otherwise similar lineages different (Horvitz *et al.* 1983). However, in examining the lineage alterations of *lin-12* mutants, what seemed most striking to me was that many of the cell-fate decisions altered in *lin-12* mutants involved cell–cell interactions.

I will conclude this section by providing as an example a decision that I will come back to later. This decision occurs during hermaphrodite gonadogenesis and is the most striking exception to the general rule of the invariant lineage: in wild-type hermaphrodites, there are two cells in the hermaphrodite gonad, defined by their lineage history, with variable fates, suggesting that cell–cell interactions play a role in their specification (Kimble and Hirsh 1979). Each cell has the potential to be either an anchor cell (AC) or a ventral uterine precursor cell (VU); every wild-type hermaphrodite has a single AC (Kimble and Hirsh 1979) (Figure 1). When all other gonadal cells are ablated except for one of these two, the solitary cell always becomes an AC, indicating that cell–cell interactions are necessary for one of these cells to become a VU (Kimble 1981).

The role of the AC is to induce the vulva (Kimble 1981). In the *lin-12(d)* hypermorphic mutants, both of these cells become VUs, so the vulva is not induced. In null mutants, both become ACs (Figure 1). Similarly, many other cell-fate decisions displayed such reciprocal behavior, with the *lin-12(d)* hypermorphic and the *lin-12(0)* null alleles having opposite effects on cell fate (Greenwald *et al.* 1983) (Figure 1). Although we did not know at the time that LIN-12 was a Notch ortholog, these genetic data were the first demonstration of the binary nature of Notch-mediated decisions. Indeed, the equivalent experiment of oppositely altering *Notch* activity in *Drosophila* was achieved only when constitutively active forms could be engineered a decade later.

### *"Neurogenic genes" and a potential Notch pathway in Drosophila*

While I was studying what turned out to be a *C. elegans Notch*, Jose Campos-Ortega and colleagues were screening the collection of Nusslein-Volhard and Wieschaus for other mutants with the classic *Notch* neurogenic phenotype. In a seminal article, Lehmann *et al.* (1983) described six other genes, including two that were critical to the elucidation of the Notch signal transduction pathway: *Delta*, subsequently shown to encode a ligand, and *Enhancer of split* [*E(spl)*], subsequently shown to be a direct transcriptional target.

Soon thereafter, landmark laser ablation experiments in the grasshopper demonstrated that cell–cell interactions influence neurogenesis in the embryonic ectoderm (Taghert *et al.* 1984; Doe and Goodman 1985). These observations, along with the molecular identification of Notch as a transmembrane protein (see below), suggested that the neurogenic genes together mediate these cell–cell interactions. However, because neuroblasts delaminate, another idea was that these genes mediate cell adhesion within the ectoderm, a view that I will return to below.

The screen of Lehmann *et al.* (1983) was the first of many other successful screens in *Drosophila* and *C. elegans* specifically for additional components of the Notch signaling system. I do not have enough space to describe them all, but suffice it to say that virtually every core component of the signaling system was first identified or first linked to Notch through powerful genetic screens in flies and worms, including, of course, Notch itself. This point will be apparent in the sections below on ligands, CSL proteins, and γ-secretase.

### *Linking lin-12 and Notch: the awesome power of molecular biology*

Molecular biology revolutionized developmental genetics in the 1980s; the change was rapid and profound. Now we could learn the effect of mutation on the gene product itself and combine genetic approaches with the tools of molecular biology to test and understand mechanism as well as process. And only now would the extent of the conservation of developmental control genes become apparent.

The *Drosophila Notch* gene was a particularly attractive candidate for molecular analysis for both its genetic complexity and its involvement in neurogenesis. The cloning of *Notch* by "chromosomal walking" was reported by two groups (Artavanis-Tsakonas *et al.* 1983; Kidd *et al.* 1983) contemporaneously with the publication of cloning of members of the two most famous developmental gene complexes,

the *Antp* and *Ubx* homeotic genes (Bender *et al.* 1983; Garber *et al.* 1983; Scott *et al.* 1983). The starting point for the walk (or "jump") to *Notch* was an inversion breakpoint that had been characterized cytologically as juxtaposing *Notch* sequences with a previously cloned gene. *Notch* was subsequently sequenced independently by both groups and found to encode a transmembrane protein with repeated epidermal growth factor (EGF)-like motifs in the predicted extracellular domain as well as other repeated motifs (Wharton *et al.* 1985; Kidd *et al.* 1986).

As the fly people began succeeding in cloning their developmental genes, it became imperative that we *C. elegans* people had to clone ours, too. Victor Ambros and Gary Ruvkun encouraged me to try to clone *lin-12* for my postdoctoral work at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England. Again, I was fortunate in where I was training: there was no better place to do molecular biology, especially DNA sequencing and analysis. So, with the support and encouragement of my postdoctoral sponsor, Jonathan Hodgkin, and with a lot of help from many people, including my main molecular biology gurus, Bob Holmgren and Andy Fire, I began my quest to clone *lin-12*.

That I would succeed in cloning *lin-12* was not a given. To give some context for the times: when I started my postdoctoral work in 1983, the remarkable *C. elegans* genome project was still at the stage of gridding the cosmid clones for physical mapping, and no one had yet cloned a *C. elegans* gene that had only been defined genetically.

Ideas of how to clone worm genes were widely being discussed in the field; most strategies were based on Tc1, an element with sequence features of a transposable element (Emmons *et al.* 1983; Liao *et al.* 1983). Tc1 is present in >300 copies in the Bergerac strain but in only ~30 copies in the canonical Bristol strain; thus, one approach to cloning genes was to use Tc1 as a restriction-fragment length polymorphism (Files *et al.* 1983) to provide an entry point into a chromosomal walk. Another strategy was transposon tagging, based on the as-yet-unverified proposal that Tc1 transposition might be the molecular basis for a high spontaneous mutation rate in the Bergerac strain (Moerman and Waterston 1984). I tried both strategies in parallel; transposon-tagging, accomplished through the reversion of *lin-12(d)* by insertion of Tc1 into the locus, worked first. I decided not to publish the cloning on its own, instead waiting to see if sequence information might reveal something interesting. And it sure did—the presence of EGF-like motifs.

I reported the cloning and partial sequence analysis of *lin-12* (Greenwald 1985) in the same issue of *Cell* as one of the two reports of the sequence of Notch (Wharton *et al.* 1985; Kidd *et al.* 1986). The copublication of sequence information about LIN-12 and Notch in *Cell* was no coincidence. As I recall, the sequence of *Notch* had been kept tightly under wraps, but the presence of EGF-like motifs in LIN-12 had been big news for several months and presented not just by me in several venues—I was on the job market!—but also by others at many meetings because it showed that worm developmental genes could be cloned and that "our" gene products had elements of homology to human proteins, too. I suppose that is how Benjamin Lewin, the founding editor of *Cell*, knew about my work and why he invited me to submit my article so as to come out back-to-back with Notch.

It is hard to capture the excitement that the EGF homology engendered, but it was truly thrilling. The homeobox had been identified only the year before (McGinnis *et al.* 1984; Scott and Weiner 1984), and the potential importance of vertebrate Hox genes for development was only just beginning to emerge through studying their expression (Carrasco *et al.* 1984; Hart *et al.* 1985). I think discovery of the EGF-like motifs in LIN-12/Notch was only the second time that sequence similarity had been reported between an important regulator of invertebrate development and a vertebrate gene. The fact that EGF had already been implicated as a key gene in mammalian development gave it particular resonance at a time when the meaning of the homeobox conservation was still mysterious.

The domain organization of Notch proteins in animals from hydra to human is shown in Figure 2. Fortunately, Kathleen Weston, a graduate student working on cytomegalovirus and a sequencer par excellence, became interested in *lin-12* and rapidly sequenced and analyzed a draft sequence of much of the remaining coding region. From her work, we knew early on that LIN-12, like Notch, was a transmembrane protein as well and that the homology to Notch extended throughout the protein, even though we did not complete the full genomic and cDNA sequences until later (Yochem *et al.* 1988).

### *Cell autonomy of lin-12 and Notch and feedback mechanisms in lateral "specification"*

In 1986, I joined the Biology Department at Princeton, where I was treated as an honorary member of the incredible fly community; everyone, especially my senior colleagues, Eric Wieschaus, Tom Cline, and Paul Schedl, not only made me feel very welcome personally but also accepted the worm as a legitimate model organism without reservation and, in particular, accepted the relevance of my work on *lin-12* to *Notch*. And compounding my great fortune, some outstanding students were willing to take the risk of joining my lab.

EGF had been discovered in the 1960s as a signal important in development; molecular cloning revealed that it was generated from a transmembrane protein precursor that also included multiple EGF-like motifs as well as bona fide EGF (Gray *et al.* 1983; Scott *et al.* 1983a). Thus, the presence of multiple EGF-like motifs in the extracellular domain made it conceivable that Notch functioned as a signal between cells. Alternatively, Notch might function as a receptor with its large, conserved intracellular domain mediating signal transduction. In addition, because neuroblasts delaminate from an ectodermal monolayer, some in the *Drosophila*

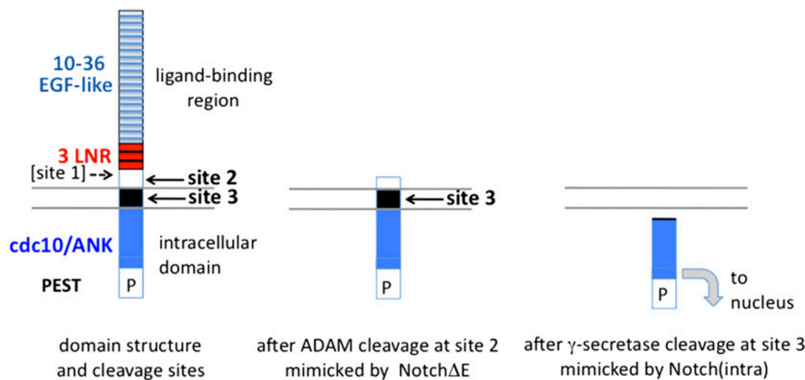


**Figure 2** Notch domain organization, intermediates in signal transduction, and mimics that result in constitutive activity. Schematic domain structure of Notch proteins, showing epidermal growth factor (EGF)-like and LNR (**L**IN-12/**N**otch **R**epeat) motifs in the ectodomain and the cdc10/Ankyrin (ANK) repeat motifs in the intracellular domain. The PEST sequence influences the stability of the intracellular domain. The text details how the mechanism of signal transduction was determined. Cleavages at sites 2 and 3 are part of the mechanism of signal transduction. After ligand binding, exposure of site 2 allows for ADAM protease to cleave, resulting in ectodomain shedding. Site 2 cleavage can be mimicked by a truncated product that deletes much of the extracellular domain (center). Virtually any type 1 transmembrane protein with a short ectodomain can serve as a substrate for γ-secretase (Struhl and Adachi 2000). Thus, the cleavage of Notch at site 2 creates a substrate for γ-secretase cleavage at site 3, thereby releasing the intracellular domain for translocation to the nucleus and association with CSL for target gene activation. Site 3 cleavage can be mimicked by expression of the intracellular domain alone. Mammalian Notch is cleaved by Furin at site 1 during secretory trafficking, resulting in a heterodimer between the amino- and carboxy-terminal fragments (Blaumueller *et al.* 1997; Logeat *et al.* 1998). *Drosophila* Notch is not cleaved by Furin (Kidd and Lieber 2002), and it is not known whether the *C. elegans* Notch proteins LIN-12 and GLP-1 are cleaved. Site 1 cleavage is not regulated by ligand and hence is not part of the signal transduction mechanism per se.

community favored a model for Notch as a cell adhesion molecule, with reduction in *Notch* activity precipitating extrusion of neural precursor cells to allow them to receive signals inducing neural differentiation. Determining whether *Notch* functions nonautonomously in the signaling cell, autonomously in the receiving cell, or possibly in both cells would help differentiate among these possibilities.

In the *Drosophila* community, the question of *Notch* autonomy was controversial. Before my arrival at Princeton, my colleagues Hoppe and Greenspan (1986) analyzed gynandromorphs and suggested that Notch acts cell-autonomously in the embryonic neurectoderm, but the resolution of their mosaic analysis was severely limited by the technical constraints of available markers at that time, particularly the reliance on cuticular markers that did not allow a clear conclusion at the level of individual cells and their immediate neighbors. In contrast, in what initially seemed to be a more definitive test offering single-cell resolution, Technau and Campos-Ortega (1987) performed transplantation experiments using markers for neural differentiation that could be scored on a cell-by-cell basis; they concluded that *Notch* function was nonautonomous, reporting that cells that were transplanted from the neurogenic ectoderm of mutant donor embryos into wild-type host embryos could give rise to either neurons or epidermis. However, they lacked an independent marker for the genotype of the *Notch(−)* donor embryos produced from crossing heterozygotes, which now appears to have led to errors in inferring the donor genotypes. At the time, the limitations of the two studies and their contradicting conclusions left the question of *Notch* autonomy unsettled.

The time was ripe for addressing this question in *C. elegans*. Bob Herman had recently developed an elegant method for genetic mosaic analysis based on the spontaneous loss of free duplications (Herman 1984); Ed Hedgecock had identified a mutation that altered nucleolar morphology and allowed the genotype of individual cells in mosaics to be deduced (Hedgecock and Herman 1995); and Judith Austin and Judith Kimble had generated a free duplication that contained wild-type sequences for both *lin-12* and *ncl-1* (Austin and Kimble 1987; see also below).

Thus, my student Geraldine Seydoux had the means to generate genetic mosaics affecting a pair of adjacent cells of the somatic gonad that have a *lin-12*-mediated choice between the AC and VU fates, with *lin-12* activity promoting the VU fate as described above (Greenwald *et al.* 1983). These two cells can be distinguished on the basis of their lineage histories and positions in the gonad primordium; each has a 50% chance of becoming the AC, and signaling between them specifies their fates (Kimble and Hirsh 1979; Kimble 1981; Seydoux and Greenwald 1989). The "AC/VU decision" is therefore an example of a process that has been classically called "lateral inhibition," originally the neuroscience term for when an excited neuron reduces the excitability of its neighbors (see Meinhardt and Gierer 2000).

Geraldine screened for genetic mosaics in which one of the two cells was *lin-12(0)* and the other was *lin-12(+)* and examined how the fate of each cell correlated with its genotype. She found that the *lin-12(0)* cell always became an AC, indicating that *lin-12* functions cell-autonomously to promote the VU fate (Figure 3).

With hindsight, it is clear that the more profound observation was that there is a nonautonomous aspect to *lin-12* function as well. Geraldine found that in the mosaic situation the *lin-12(+)* cell no longer had a choice of fate and always became a VU (Figure 3). We were initially surprised by this observation because, in thinking of the AC/VU decision as lateral *inhibition*, we might have expected that 50% of the time the *lin-12(+)* cell would still become an AC through its intrinsic propensity to do so, regardless of its neighbor's inability to be inhibited. However, the *lin-12(+)* cell always became a VU, suggesting that its fate was biased by the decision of its *lin-12(0)* neighbor, which would not be able to receive the VU-promoting signal and hence had no choice

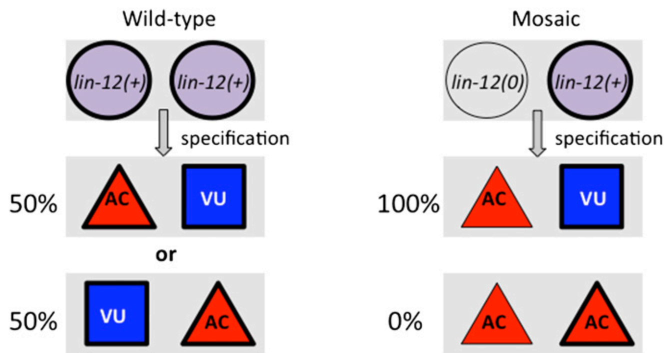


**Figure 3** The anchor cell (AC)/ventral uterine precursor cell (VU) decision, cell autonomy, and bias in cell-fate choice in genetic mosaics. See text for description.

but to be an AC. From this bias in cell-fate choice of the *lin-12(+)* cell in these mosaics, we inferred the existence of a feedback mechanism that amplifies small stochastic initial differences in the level of *lin-12* activity. Subsequently, my students Hilary Wilkinson and Kevin Fitzgerald found that the feedback mechanism involves transcriptional regulation of both *lin-12* and the gene encoding its ligand in this decision, *lag-2*, in response to *lin-12* activity, amplifying the small, stochastic initial difference (Wilkinson *et al.* 1994).

Although the "hot" issue at the time was the question of autonomy, the bias in cell-fate choice for the *lin-12(+)* cell, reflected in the nonautonomous effect on its fate, was more important in terms of a more general understanding of the decision-making process. Indeed, the key point is that the process is not simple "inhibition" because the cells communicate with each other to reach a consensus about which fates to adopt—the reason that I prefer the term lateral "specification" (Greenwald and Rubin 1992), although, alas, the term never caught on. The bias in cell-fate choice in mosaics also suggested that Notch functions in reception of a signal rather than as a passive cell adhesion molecule that facilitates other signaling events: if Notch were simply mediating adhesion, then the degree of adhesion between two cells should be reduced without causing a bias.

Findings in the worm were not regarded as general until Heitzler and Simpson (1991) published a beautiful mosaic analysis in flies that reached conclusions similar to ours. They analyzed mosaics affecting bristles on the notum, rather than the classic embryonic neurogenic phenotype. Bristle patterning was another classic paradigm for lateral inhibition: Curt Stern had shown that, when an *achaete* mutant clone eliminated a landmark bristle, if the clone border was near the bristle site, the nearby adjacent wild-type tissue could form a bristle, slightly displaced from the landmark site, suggesting that loss of the landmark bristle released a nearby epidermal cell from inhibition (Stern 1954). Subsequent studies led to the concept of a proneural cluster of cells, each having the potential to generate a sense organ precursor (SOP) for a bristle, with single SOPs specified as a result of lateral inhibition (Simpson 1990).

Heitzler and Simpson (1991) found that, in genetic mosaics containing *Notch*$^{-}$ and *Notch*$^{+}$ cells within a proneural cluster, a *Notch*$^{-}$ cell always became an SOP—indicating autonomy of *Notch* function in epidermal specification—and a *Notch*$^{+}$ cell always became epidermal, a bias in cell-fate choice similar to what we observed for the AC/VU decision. They went further by analyzing mosaic proneural clusters juxtaposing cells that differed only in the number of copies of *Notch*. They found that, when a cell with one copy of *Notch*$^{+}$ and a cell with two copies of *Notch*$^{+}$, or even two copies of *Notch*$^{+}$ *vs.* three copies of *Notch*$^{+}$, were juxtaposed, the cell with fewer copies generally became an SOP and the cell with more copies became epidermal. This result was quite astonishing and suggested that the feedback mechanism is both sensitive enough to detect small initial differences and accurate enough to ensure that the outcome invariably tracks with the relative level of *Notch* activity.

### *glp-1*

During this decade, *glp-1*, a second *C. elegans Notch* gene, was also identified. *glp-1* was defined genetically in two different screens: loss of zygotic function dramatically reduces germline proliferation and loss of maternal function alters early embryonic cell fate (Austin and Kimble 1987; Priess *et al.* 1987). These phenotypes, like the *lin-12* phenotypes, reflect a failure of cell–cell interactions: germline proliferation depends on a signal from the soma (reviewed in Kimble and Crittenden 2005) and early embryonic development involves numerous successive *glp-1*-mediated inductive signaling events (reviewed in Priess 2005). Genetic mosaic analysis established that *glp-1* functions autonomously in the germline to promote its proliferation (Austin and Kimble 1987), so when we identified another *C. elegans Notch* gene by low-stringency hybridization and found that it corresponded to *glp-1*, the interpretation that *Notch* functions in receiving cells was reinforced (Yochem and Greenwald 1989).

*lin-12* and *glp-1* appear to have arisen by a gene duplication event and are located very close together in the genome. When Lambie and Kimble (1991) made the heroic effort of constructing the *lin-12 glp-1* double mutant, they found that concomitant loss of zygotic *lin-12* and *glp-1* activity causes larval arrest with novel cell-fate transformations, defining the "Lag" phenotype (for **L**in-12 **a**nd **G**lp-1). Their results suggested that the two Notch proteins are functionally redundant, an inference supported by the finding that GLP-1 is able to substitute for LIN-12 in cell-fate decisions when expressed using *lin-12* regulatory sequences (Fitzgerald *et al.* 1993). These studies together implied that different roles for *Notch*, as the mediator of inductive interactions or lateral specification, reflect different regulatory mechanisms rather than intrinsic differences in the Notch proteins themselves. The results of Lambie and Kimble (1991) also prefigured findings in vertebrates, where there is substantial functional redundancy among the four *Notch* proteins (*e.g.*, Krebs *et al.* 2000).

Understanding that zygotic loss of both *lin-12* and *glp-1* causes a synthetic phenotype also allowed Lambie and

Kimble (1991) to isolate strong alleles of two critical core components, *lag-1* and *lag-2* in a genetic screen analogous to the neurogenic screen of Lehmann *et al.* (1983). The Lag phenotype has also been important for assessing the roles of other potential core components in *C. elegans*, as it represents the loss of all zygotic *Notch* activity in *C. elegans*.

#### *Ligands*

Although much of the remainder of this essay concerns the elucidation of the mechanism of signal transduction by activated Notch, the process of signal transduction is initiated by the binding of a ligand. Thus, I will briefly describe another important development during this period—the genetic and molecular characterization of Delta, the first member of the DSL protein family of ligands for Notch.

*Delta*, like *Notch*, was defined in the early days of *Drosophila* genetics; it is also haploinsufficient, although deficiency heterozygotes show thickening of the wing veins, called "deltas," rather than notching. *Delta* was clearly implicated in the *Notch* pathway via the neurogenic phenotype of homozygous null mutants (Lehmann *et al.* 1983). Cloning and sequence analysis revealed that *Delta* encodes a transmembrane protein with multiple EGF-like motifs (Vassin *et al.* 1987; Kopczynski *et al.* 1988); this basic structure, which also includes an N-terminal DSL domain, is the hallmark of this family. When Delta was expressed in cultured cells, it promoted aggregation with Notch-expressing cells, suggesting a physical interaction between the two proteins then mainly thought to be suggestive of a passive role in mediating cell–cell adhesion (Fehon *et al.* 1990).

Compelling evidence for a role of *Delta* in the signal-sending cell came from the mosaic analysis of Heitzler and Simpson (1991), who showed that *Delta* functions nonautonomously to promote the epidermal fate in neighboring cells, Furthermore, mosaics juxtaposing cells with different copy numbers of wild-type *Delta* genes displayed a bias in cell-fate choice, opposite to that displayed for *Notch*: the cell with the lower level of *Delta* activity was biased toward the epidermal fate, further underscoring the intimate relationship between *Delta* and *Notch* activity. Finally, the bias in cell-fate choice reveals that regulation of *Delta* activity is part of the feedback mechanism operating during lateral specification. Heitzler *et al.* (1996) subsequently found genetic circuitry consistent with this mechanism operating at the level of transcription of the *Delta* gene.

As noted above, the bias in cell-fate choice in *lin-12* mosaics argued for a role of Notch as a receptor rather than a simple passive adhesion molecule. The finding that *Delta* mosaics displayed the opposite bias further supported the view that there is an instructive role, which, in view of the physical interaction between Delta and Notch, was consistent with its role as a ligand for Notch.

### 1990s

The basic Notch story as we think of it today emerged in the 1990s. The discovery of a new role for Notch as an oncogene in mammals started off the decade with éclat (Ellisen *et al.* 1991). This finding not only gave urgency to understanding more about this signaling system for potential insights into disease, but also provided the first clue as to the mechanism of signal transduction. By the end of a very active decade of research with parallel studies in *C. elegans*, *Drosophila*, and mammalian cultured cells, the key steps in the unusual mechanism of Notch signal transduction had been elucidated and accepted.

#### *Notch as an oncogene and the first clue as to the mechanism of signal transduction*

Ellisen *et al.* (1991) reported that three patients with T-cell acute lymphoblastic leukemia (T-ALL) had chromosomal translocations involving the Notch1 and the β-T-cell receptor gene. The breakpoints were all similar, resulting in a high level of expression of a message predicted to encode a truncated Notch1 product (then called "TAN1") beginning near the last EGF-like motif.

This study was a major advance for two reasons. First, the association of T-ALL with similar translocations in different patients suggested a potential role for Notch as an oncogene. This possibility was supported by the finding that a mouse mammary tumor virus insertion called "int3" produced transcripts predicted to encode truncated Notch4 proteins and caused carcinomas and hyperplasia in mice (Gallahan *et al.* 1987; Jhappan *et al.* 1992; Robbins *et al.* 1992). It must be remembered that, at the time, forging connections between genes that control normal development and genes that contribute to cancer was still fairly new, and the extent to which development and oncogenesis were related mechanistically could only be conjectured. So at the time of publication the connection of Notch to cancer was very exciting news.

Second, for those of us puzzling over how Notch worked in development, the Ellisen *et al.* (1991) findings immediately suggested that analyzing the activity of engineered truncated forms of Notch would be a genetic approach to elucidating the mechanism of signal transduction and, by extension, cancer.

#### *Notch and oncogenesis: Binary switch or differentiation block?*

The exciting connection between activated Notch and cancer also began to influence how people thought about the normal role of Notch in vertebrate development. In particular, Jhappan *et al.* (1992) generated transgenic mice expressing the truncated form of Notch defined by the int-3 oncogenic insertion and found evidence of developmental arrest in mammary glands and other glands in which transcription of this form occurred. In addition, Coffman *et al.* (1993) expressed a truncated form of Notch in *Xenopus* and found that expression of some differentiation markers were delayed while animal caps displayed an extended period of competence to neural or mesodermal induction. From these studies, a view began to emerge that Notch activation delays

or blocks differentiation, thereby maintaining the competence of cells to respond to other signaling events.

This view initially appeared to be supported by *Drosophila* studies in the Artavanis-Tsakonas lab when expression of activated Notch forms (see below) expressed under control of the *sevenless* promoter was interpreted as blocking the ability of the presumptive photoreceptor cells to differentiate until expression subsided, at which time the cells would choose an inappropriate fate because they were now exposed to inappropriate signals (Fortini *et al.* 1993). However, the experimental design, lacking mosaic analysis, did not allow the proper identification of which cells adopted which cell fates. Indeed, subsequent studies by several groups showed that Notch activation directly specifies the fates of the R3, R4, and R7 photoreceptors, whose differentiation was purportedly blocked, in lateral specification (R3/R4) and inductive signaling (R7) events (Cooper and Bray 1999, 2000; Fanto and Mlodzik 1999; Tomlinson and Struhl 1999, 2001).

At the time, I suggested that the binary decision of some cells was to choose between differentiating or remaining a stem cell and that *Notch* promoted the stem-cell choice rather than blocking differentiation per se (Greenwald 1994). My concern was essentially that thinking of a "block" would imply that the problem was in the execution of a fate, not at the level of a cell-fate decision. Now it is clear that *Notch* mediates many binary cell-fate decisions in mammalian development, including choices that promote maintenance of progenitor cells. Furthermore, altered cell-fate choices impacting progenitor cells caused by aberrations in Notch activity may contribute to its oncogenic effect. For example, *Notch* controls a binary decision between T- and B-cell progenitors (Tanigaki and Honjo 2007); one way that aberrant *Notch* activation contributes to cancer is to increase the number of T-cell progenitors (see Ferrando 2009). *Notch* activity also appears to control binary cell-fate decisions between stem cell and differentiated cell fate that go the "other way" in other cell types and thus can act as a tumor suppressor (see Lobry *et al.* 2011).

### *The cleavage model for Notch signal transduction*

Many different groups recognized that the analysis of truncated forms of Notch might reveal the mechanism by which Notch transduces signals. At this time, in addition to studies in model organisms, studies of the Notch-signaling system in cell culture became an active area as well.

My own excitement about testing the effect of truncated forms, however, was tempered by the limitations of transgenic technology in *C. elegans* at the time. We simply did not know how to express proteins in the right time and place for our purposes. Fortunately—and not just for my work!—I am married to Gary Struhl, who was the first person to use the heat-shock promoter to create gain-of-function forms of developmental switch genes (Struhl 1985) and who had just developed "Flp-out" technology to express genes in specific tissues (Struhl and Basler 1993). Both of these technologies would prove to be useful for expressing truncated forms of Notch. Gary was attracted by what seemed likely to be an interesting mechanism for an important patterning gene and, for me, expressing a truncated form in the fly in parallel offered the promise of an answer even if the worm experiments did not work. And if we could show similar behavior of equivalent truncated forms in two systems, then it would underscore the universality of whatever we found individually.

It also was fun for us to be doing a project together while our ultimate collaboration, our daughter Abigail, was gestating. My student Kevin Fitzgerald graciously joined in while pursuing other valuable structure–function studies of Notch and DSL ligands (Fitzgerald *et al.* 1993; Wilkinson *et al.* 1994; Fitzgerald and Greenwald 1995), and after beginning in Princeton, we completed the project after moving to Columbia in 1993.

We reasoned that the predicted truncated protein associated with T-ALL would lack the signal sequence, suggesting that such a protein, if indeed produced and stable, would be cytosolic, so we decided to express just the intracellular domain ("intra") as the most extreme case. Gary easily made flies expressing the Notch intracellular domain, and he and Kevin successfully implemented a novel expression strategy for worms to produce LIN-12(intra) using *lin-12* regulatory sequences. As we had hoped, the results using the two systems were concordant: in worms, expression of LIN-12(intra) caused the phenotype associated with *lin-12(d)* alleles, and in flies, expression of Notch (intra) promoted epidermal differentiation opposite to the neurogenic phenotype of null alleles (Struhl *et al.* 1993). Further genetic analysis showed that the apparent signaling activity of the intracellular domain was constitutive and acting at the time of the respective cell fate decisions.

When Gary used an antibody to stain flies expressing Notch(intra), he had a big surprise: the protein was in the nucleus. This observation led us to formulate the cleavage model with a directness that surprises me now: "Our finding that Notch(intra) protein causes a gain-of-function phenotype and accumulates in the nucleus makes it worth considering the possibility that signal transduction mediated by the wild-type protein involves cleavage and transport of the intracellular domain to the nucleus and even the possibility that the intracellular domain of Notch may be directly involved in transcriptional regulation" (Struhl *et al.* 1993, p. 340).

Soon after our article was published, Toby Lieber, Simon Kidd, and Michael Young published a comprehensive study of many different truncated forms, including a comparable Notch(intra) form (Lieber *et al.* 1993). They also reported that Notch(intra) causes phenotypes consistent with Notch activation and localizes to the nucleus and considered a cleavage model and a potential role for Notch in transcriptional activation. However, they also generated other constitutively active truncated forms that could not be detected in the nucleus. Although Lieber *et al.* (1993) proposed reasonable ways to reconcile such observations with a cleavage

model, the apparent lack of correlation between constitutive activity and nuclear localization in this study and several others became a major line of evidence advanced against the cleavage model by others. I will come back to this point below.

Several articles also described another informative truncated form, which lacked much of the extracellular domain yet contained a signal sequence, so that the resulting protein was targeted to the membrane (unlike the T-ALL leukemic truncation). This form, NotchΔE, was reported to have constitutive activity in *Xenopus* (Coffman *et al.* 1993), *Drosophila* (Lieber *et al.* 1993; Rebay *et al.* 1993), and *C. elegans* (referred to in Struhl *et al.* 1993), again underscoring that the mechanism of signal transduction was conserved in all animals.

Notch(intra) and NotchΔE, the key constitutively active forms used in subsequent genetic analysis, are diagrammed in Figure 2 as what we now know they are: mimics of the cleavage products of successive proteolytic processing events that ensue upon ligand binding. I note that the constitutive activities of these forms were observed to promote epidermal fate at the expense of neurogenesis, the opposite of the neurogenic phenotype caused by loss of *Notch* activity, thereby formally demonstrating the binary nature of Notch-mediated cell-fate choice in *Drosophila* (Lieber *et al.* 1993; Rebay *et al.* 1993; Struhl *et al.* 1993), as we had demonstrated using the *lin-12(d)* forms in *C. elegans* (Greenwald *et al.* 1983).

### CSL: a sequence-specific DNA-binding protein and core component of the Notch-signaling system

Before discussing further why the cleavage model was initially resisted and the path by which it gained general acceptance, I must introduce a core component now generally called "CSL," an acronym coined from some of the names it had been called in different systems: CBF1, Su(H), and LAG-1 (Christensen *et al.* 1996). CSL is now established as the sequence-specific DNA-binding protein with which Notch(intra) associates to promote target gene expression. Attaining an understanding of that role was also critical in the path to acceptance of the cleavage model and an important development in its own right during this time period as well.

The functional connection of CSL to Notch initially came from genetic studies of *Drosophila Suppressor of Hairless* [*Su(H)*], a classic modifier (see Nash 1970). Genetic analysis in several labs had connected *Su(H)* to the neurogenic genes and peripheral nervous system development. Molecular cloning established that Su(H) is the ortholog of CBF1 (also called RBP-J or KBF1) (Furukawa *et al.* 1992; Schweisguth and Posakony 1992). CBF1 had been found as a sequence-specific DNA-binding protein through association with many different cellular and viral promoters (Yano *et al.* 1987; Hamaguchi *et al.* 1989; Ling *et al.* 1993), so this orthology provided a crucial potential link between Notch signal transduction and transcription. This link was strengthened when Su(H) was found to associate physically with the intracellular domain of Notch (Fortini and Artavanis-Tsakonas 1994; Tamura *et al.* 1995) and by a combination of genetic and biochemical evidence establishing Su(H) as a direct transcriptional activator of genes of the *Drosophila E(spl)* complex (Jennings *et al.* 1994; Bailey and Posakony 1995; Lecourtois and Schweisguth 1995; Schweisguth 1995).

I note that, in the absence of the *Drosophila* genetic data, the broad expression and promiscuous binding of CSL would have obscured its critical role in Notch activity. Indeed, its various mammalian names underscore this point; *e.g.*, CBF1 (C Binding Factor 1) refers to its being a cellular factor that binds to the Epstein-Barr virus "C" promoter, and "RBP-J" refers to its binding to the recombination signal sequence of immunoglobulin J κ gene.

### Challenges to and acceptance of the cleavage model

A reasonable argument advanced against the cleavage model was that the intracellular domain of Notch could not be detected in nuclei *in vivo* in many contexts where Notch signal transduction was known to be active: nuclear Notch was not evident in wild-type animals or in transgenic animals carrying transmembrane constitutively active forms such as NotchΔE (*e.g.*, Johansen *et al.* 1989; Fehon *et al.* 1990; Lieber *et al.* 1993; Roehl and Kimble 1993). Although plausible reasons for this lack were also postulated (see, *e.g.*, Lieber *et al.* 1993), ultimately it would have to be reckoned with for the model to be validated and accepted.

Resistance to the cleavage model gained momentum when Fortini and Artavanis-Tsakonas (1994) claimed that, in *Drosophila* tissue culture, Su(H) protein is sequestered in the cytoplasm when coexpressed with Notch protein and is translocated to the nucleus when Notch binds to its ligand Delta. They proposed that the role of Notch was to sequester Su(H) from the nucleus in the absence of ligand binding. This simple tethering model was puzzling to Gary and me at the time because it did not seem to account for basic genetic observations *in vivo*, such as the fact that loss of *Notch* [which should release Su(H) from the proposed tether] had similar phenotypic effects as loss of *Su(H)*. Nevertheless, the tethering model continued to exert a strong hold on the field, even after the central claim that the subcellular localization of Su(H) depends on the activity of Notch was shown to be wrong *in vivo* in *Drosophila* (Gho *et al.* 1996).

In contrast to the *Drosophila* cell culture findings, a growing body of work in mammalian cell culture supported the cleavage model. An important article by Jarriault *et al.* (1995) showed that CBF1 directly stimulates transcription of the Notch target gene *HES1* in the presence of the ΔE truncated, constitutively active form of Notch, but not alone or in the presence of full-length (inactive) Notch. This cell culture study was possible only because both CBF1 and *HES1* had been placed in the Notch pathway through *Drosophila* genetics by using *Su(H)*, as described above, and the target gene *E(spl)* (Klambt *et al.* 1989; Jennings *et al.* 1994).

In addition, in compelling studies inspired by viral proteins that interact with the host CBF1 protein, essentially

acting as Notch mimics, S. Diane Hayward and colleagues found that the Notch intracellular domain appears to abrogate repression by CBF1 and to recruit coactivators (Hsieh *et al.* 1996, 1997). Other contemporaneous studies reinforced these mechanistic insights.

Even though biochemists at the time were much more receptive than geneticists to the cleavage model, the experimental evidence available at that time was not yet compelling. There was no unequivocal evidence that proteolytic processing was necessary for transcriptional activation. Furthermore, the tissue culture work was performed using gain-of-function alleles and therefore could not address whether processing and transcriptional activation are normally regulated by ligand. Indeed, ligand inducibility is essential for a signaling system to have an instructive role.

Both Struhl and Adachi (1998) and Lecourtois and Schweisguth (1998) approached the question of ligand-dependent nuclear access by a similar approach to increase the sensitivity of detection of the Notch intracellular domain: they inserted a GAL4 DNA- binding domain moiety into the intracellular domain of an otherwise intact Notch protein and looked for transcriptional activation of a GAL4 target-*lacZ* reporter. Both groups found that β-galactosidase activity, which indicates nuclear access of the Notch intracellular domain, depends on ligand.

Struhl and Adachi (1998) also comprehensively assessed the relationship between nuclear access and signal transduction for constitutively active forms, providing compelling evidence that nuclear access is functionally relevant for signal transduction and transcriptional activation *in vivo*. In one set of experiments, they showed that signal transduction depended on nuclear access of the intracellular domain by targeting a derivative of Notch(intra) to the membrane using myristylation, thereby eliminating activity, or to the nucleus by adding nuclear localization sequences, thereby potentiating activity. In another set of experiments, they showed that signal transduction reflects transcriptional activation by the intracellular domain because adding a heterologous transcriptional activator further increased activity whereas adding a heterologous transcriptional repressor domain blocked signal transduction.

At the same time, Schroeter *et al.* (1998) assessed cleavage using transient transfection followed by immunoprecipitation in cultured mammalian cells. The immunoprecipitation step allowed them to obtain evidence for a scarce cleavage product, and two key experiments supported the hypothesis that this product was a bona fide intermediate in the signal transduction mechanism. First, Schroeter *et al.* (1998) identified and mutated a valine residue at the cleavage site in the transmembrane domain and reported that this mutation results in less cleavage product and reduced signaling ability in the NotchΔE but not Notch(intra) context, suggesting that cleavage and signaling are correlated. Second, they reported that ligand cotransfection was necessary for the detection of the cleavage product from the wild-type form, suggesting that cleavage was ligand-dependent.

Given the theme of this essay, I want to make a general comment about the contribution of *in vivo* approaches to assessing molecular mechanism, as there is a large community of investigators who put a premium on biochemical approaches in mammalian tissue culture over genetic experiments in model organisms. In particular, I want to note that there are virtues of *in vivo* genetic experiments that are difficult to achieve *ex vivo*. In the *Drosophila* experiments, transgenes were expressed at approximately endogenous levels; they were expressed in normal cells in their normal context, *i.e.*, as part of epithelia, with their normal contacts, and receiving any other signals that they normally do; there were few experimental variables to control; and it was possible to remove endogenous components cleanly for assessing properties such as constitutive activity or ligand dependence, as well as the functional consequences of introducing tags and other probes of molecular function into the native ligands and receptors.

Indeed, these features of *in vivo* genetic analysis continue to be relevant for investigations into the mechanism of any biological process, and while *ex vivo* evidence has its own set of virtues, it is the combination of both approaches that is ultimately compelling.

For Notch, the two approaches synergized beautifully, and together the *in vivo* analysis of Struhl and Adachi (1998) and the *ex vivo* analysis of Schroeter *et al.* (1998) made a compelling case for the cleavage model, which became widely accepted at that time. However, there were still pockets of resistance, as can be seen from continued arguments against the cleavage model that were still being advanced afterward (Artavanis-Tsakonas *et al.* 1999). As described in the next section, elucidating the role of Presenilin in Notch signal transduction helped silence any remaining skepticism while adding a fascinating twist to the mechanism.

### *γ-Secretase and the release of the intracellular domain of Notch*

All of the core components of the Notch signal transduction system were implicated as such through genetic analysis in worms and flies, and γ-secretase is no exception.

γ-Secretase was originally an inferred enzyme activity based on the proteolytic processing pattern of a transmembrane protein called β-APP. The peptide produced when β -APP is cleaved at the β site in its ectodomain and the γ site in its transmembrane domain can form β-amyloid plaques in the brain and cause Alzheimer's disease. However, despite tremendous efforts in industry—where γ-secretase inhibitors were potentially valuable drugs—the identity of γ-secretase had been refractory to biochemical approaches. Genetic studies of familial early-onset Alzheimer's disease (Sherrington *et al.* 1995) and of *lin-12/Notch* signaling in *C. elegans* (Levitan and Greenwald 1995) identified what proved to be the catalytic component of γ-secretase, Presenilin. Genetic analysis in *C. elegans* was later successful in identifying the other three core components of γ-secretase (Goutte *et al.* 2000, 2002; Francis *et al.* 2002).

My postdoctoral fellow Diane Levitan had been studying *sel-12* (coincidentally, the 12th **s**uppressor/**e**nhancer of ***l**in-12*), a suppressor of the Multivulva phenotype caused by a *lin-12(d)* mutation. We were on the verge of submitting a manuscript when an astute colleague, Steve L'Hernault, called to tell us that SEL-12 was highly similar to the early-onset Alzheimer's disease gene then called S182 (Sherrington *et al.* 1995) and now known as Presenilin (PS) 1. Although Sherrington *et al.* had not deposited the S182 sequence in public databases, the article included an alignment showing weak homology with the product of a *C. elegans* gene that Steve studied, SPE-4 (L'Hernault and Arduengo 1992). Aware that SEL-12 was also weakly homologous to SPE-4, Steve did the sequence analysis and discovered that SEL-12 and S182 are highly similar to each other, and we quickly retitled our manuscript to reflect that fact. Remarkably, Diane subsequently found that human PS1 could functionally replace SEL-12 in *C. elegans* (Levitan *et al.* 1996).

Although our genetic analysis indicated that Presenilin influences Notch signal transduction, it was not yet apparent that it was a core component of the signaling system, since *sel-12* null mutants did not have the hallmark phenotypes associated with loss of *Notch* activity. However, the essential role of Presenilin became apparent when my student Xiajun Li identified a second *C. elegans* Presenilin gene, *hop-1* (**h**omolog **o**f **p**resenilin), and used RNA interference—still misunderstood at that time as antisense RNA—to demonstrate that depletion of *hop-1* in a *sel-12* null mutant background caused hallmark phenotypes associated with loss of *Notch* activity (Li and Greenwald 1997). Null alleles of the single *Drosophila Presenilin* gene were subsequently shown to cause Notch phenotypes, affirming that Presenilin is a conserved core component of the Notch signaling system (Struhl and Greenwald 1999; Ye *et al.* 1999).

In the intervening time, Schroeter *et al.* (1998) and Struhl and Adachi (1998) had provided compelling evidence for the cleavage model, and De Strooper *et al.* (1998) had found that Presenilin was required for transmembrane cleavage of β-APP. Given the parallels between β-APP processing and Notch processing, the next step was to assess the role of Presenilin in Notch cleavage. Three articles published together in *Nature* in 1999 did just that.

De Strooper *et al.* (1999) found that processing of NotchΔE was reduced in PS1-null cells; Gary and I found that nuclear access of NotchΔE-GAL-4 was blocked in PS null mutant clones in *Drosophila* (Struhl and Greenwald 1999). Both studies concluded that Presenilin promoted the transmembrane cleavage of Notch, consistent with a function as a protease itself or in facilitating protease function.

In contrast, Ye *et al.* (1999) examined processing and signal transduction of Notch in PS null mutant *Drosophila* embryos and reached different conclusions. The key genetic result that led to considerable commentary at the time was that Ye *et al.* (1999) claimed that *NotchΔE* signal transduction, as assessed by suppression of neurogenesis, was not affected in the absence of *PS*. Taken at face value, their results would indicate that signal transduction is not correlated with transmembrane cleavage (the assay used in the other two studies), thus challenging the cleavage model. However, Gary and I immediately recognized that Ye *et al.* (1999) had used a phenotypic assay for signal transduction that depended on the imaginal disks being "old enough" for neurogenesis to have occurred, but without independent markers for timing. Therefore, the apparent lack of neurogenesis might instead have been observed if the disks examined were too young or developmentally delayed by the manipulations used to express NotchΔE.

To address this direct challenge to the cleavage model, we re-examined the relationship between transmembrane cleavage and signal transduction in the same cellular context using internal controls for timing and found that they were strictly correlated, again validating the cleavage model and assigning Presenilin to the transmembrane cleavage step (Struhl and Greenwald 2001). There were no significant challenges to the cleavage model after that.

## Concluding remarks

I think it is timely to remember that the history of *Notch* shows that molecular biology synergized with genetics but did not replace it. The current gadarene rush to "systems biology" has created a sense that classical genetics is being superseded. There was a similar feeling when molecular biology was first becoming a powerful force in fields that had previously been accessible only through genetics. While it remains to be seen whether "systems biology" will have a high and lasting impact, I think for it to arrive at its full potential, it will need to achieve some synergy with traditional genetics—at a minimum, for testing *in vivo* the models that emerge from genome-wide approaches.

I also think that it is interesting to see how the Notch story, as has been true for most scientific advances, did not develop as a neat linear narrative, but instead had its share of wrong directions and parallel paths, synergies and antagonisms, and restriction by and liberation from trends and expectations. With the intense pressure these days to make every story seem simple and tidy, I think it is important to remember (and for students to learn) that scientific understanding does not usually happen that way.

Finally, I think the Notch story offers a prime example of how and why flies and worms became such incredible systems for studying animal development. The major credit for these model systems, of course, belongs to others. But, with distance, I see that my work had some role in the acceptance of the worm, at least, as there were several "firsts" in the Notch field that came from studies in the worm, and first developments in the worm field that came from studies of Notch. I had no idea at the time that I was part of this larger story. I was just having a great time.

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