Notch signaling:
control of cell communication and cell fate

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A DEMONSTRATION OF GENES MODIFYING THE CHARACTER "NOTCH."

By T. H. Morgan.

Two main topics are dealt with in the following pages from the standpoint of the experimental results obtained. One of them concerns the demonstration of modifying genes that were involved in the results of a selection experiment. The other topic is a discussion of the possibility of contamination of genes as a method that has been appealed to as an influence vitiating the regularity of Mendelian phenomena.

The claim of the Mendelians that genes have been found to be stable in successive generations wherever a critical test of them was made has been challenged both on the grounds of empiric observation and on the more sentimental grounds that such hard and fast rules do not apply to living things which are rather to be thought of as variable quantities. In the following pages an account is given of a character that changed in the course of selection and a demonstration that the result was due to a modifying gene and not to contamination between the notch gene and its normal allelomorph, despite the fact that an exceptional opportunity was given to contaminate the gene, if contamination is a possible process.

In 1915, Dexter described a mutant type of *Drosophila* called Notch or "perfect Notch," and made out the main points in the heredity of the character. The gene is sex-linked, and dominant for the serration that it produces in the wings, but recessive in its lethal effect.

Notch bursts onto the scientific scene in 1915.

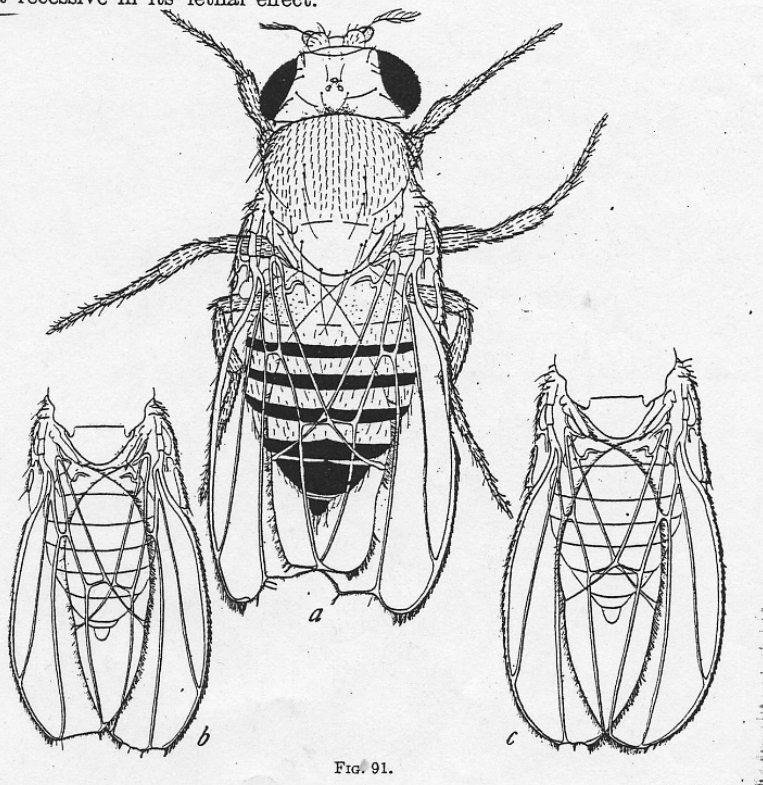


Fig. 91.

Dexter obtained his mutant in a cross between beaded and wild. The Notch that I used arose independently in descendants of vestigial flies, in which stock the factor may have already existed. This mutant has, however, originated several times in other cultures in the laboratory. It is by no means one of the rarer mutations.

Why study Notch signaling?

- although discovered in flies, Notch operates throughout all animals to determine cell fates and pattern tissues

- because of its fundamental roles in development, aberrant/dysfunctional N pathway activity underlies many diseases

- in humans, N pathway mutations cause Alagille syndrome (affects liver,skeleton, eye…) and CADASIL (mutations which predispose individuals to dementia, migraines and strokes.)

Notch pathway mutations also linked to various cancers, including T-ALL (T cell acute lymphoblastic leukemias), cervical, mammary, skin, prostate.

Table 1 **POTENTIAL ROLES OF NOTCH SIGNALING IN HUMAN CANCERS**

Mechanisms of Tumor Progpagation	Potential Tumor Examples	Potential Therapies
Gain of function mutations	T-ALL, mouse mammary carcinomas	Intracellular inhibitors of the NOTCH pathway (disrupt ICN nuclear complex, activate Notch inhibitors
Ligand-mediated activation of the Notch pathway	Lymphoproliferative disorders (CLL, Hodgkin's lymphoma)	Intracellular or extracellular inhibitors of the Notch pathway (block ligand-Notch binding or same targets as above).
Downregulation of the Notch pathway	SCLC, prostate adenocarcinomas, cervical carcinomas, basal cell cancer, neuroblastomas	Activate the Notch pathway (soluble ligands, antibody activation of Notch signaling).

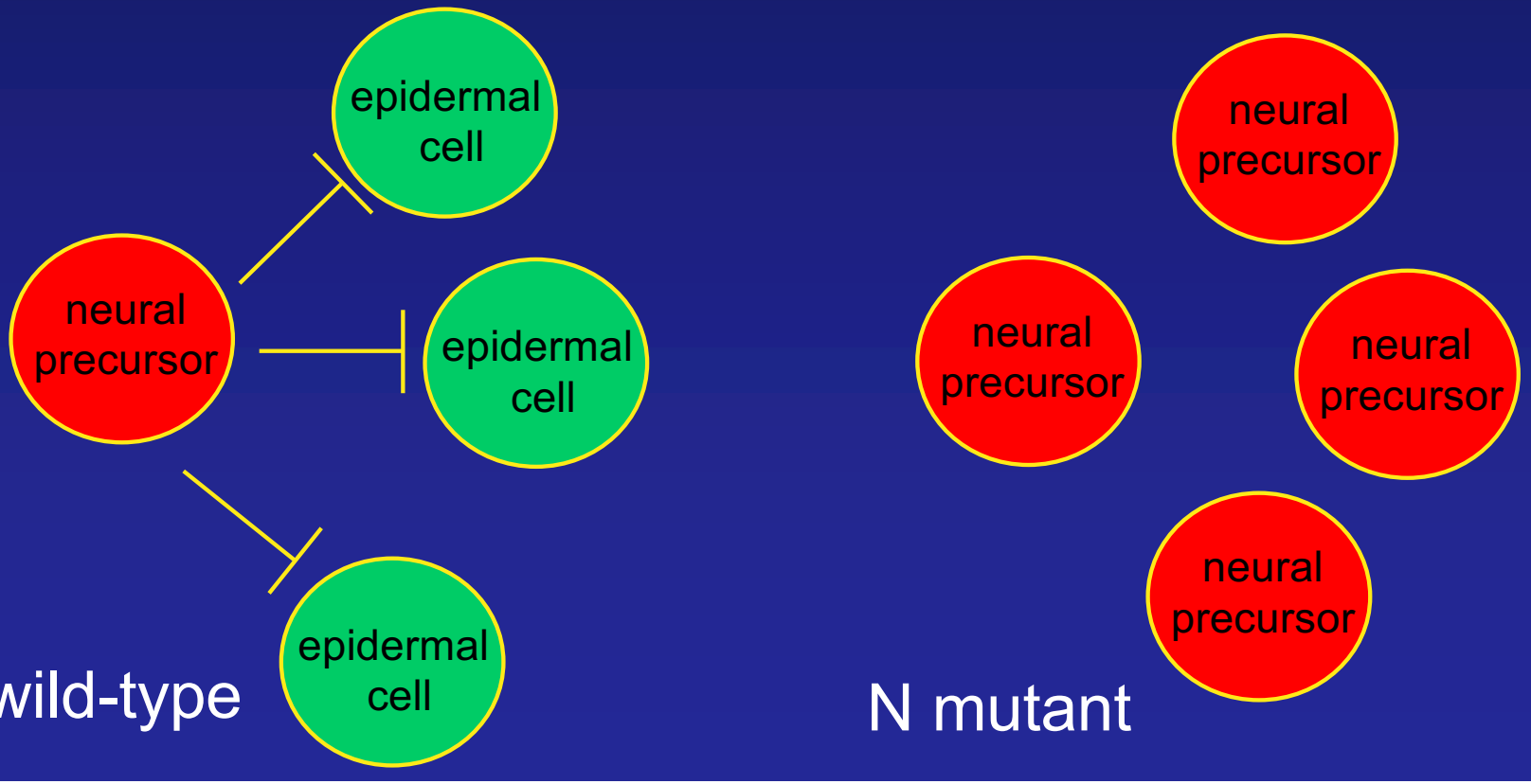
Today's menu:

1. What are the “core” components of the Notch signaling pathway?
   - Notch is a system for cell communication
2. How does this pathway transmit a signal?
   - Notch as a membrane-bound transcriptional coactivator
   - CSL repressor->activator “switch” model
3. What does activation of this pathway tell the cell?
   - inhibition of cell fates
   - inductive signaling
   - consequences of aberrant signaling for disease and cancer

1. What are the "core" components of the Notch signaling pathway?

Many key N pathway factors were recognized genetically,
due to their similar phenotypes

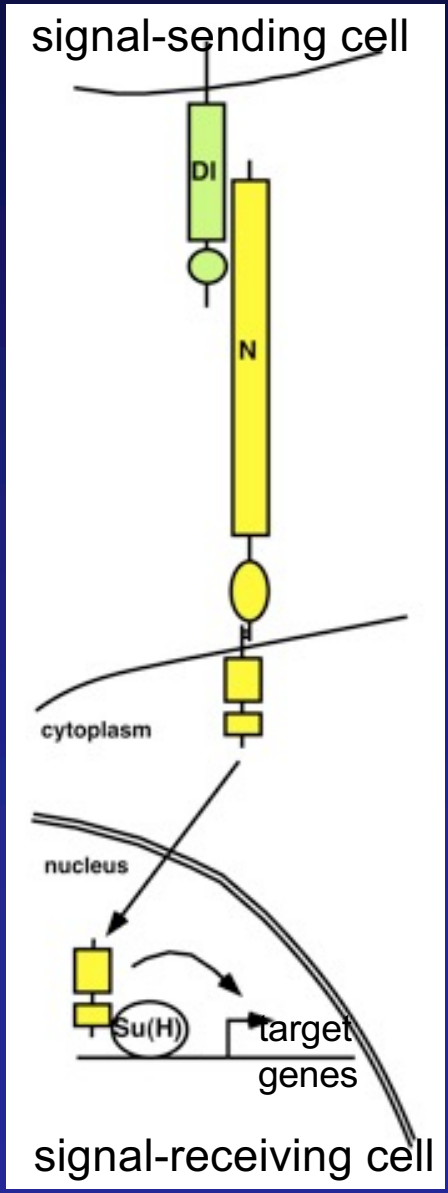
a classic setting is during fly neurogenesis: “neurogenic” mutants
develop excess neurons at expense of epidermis



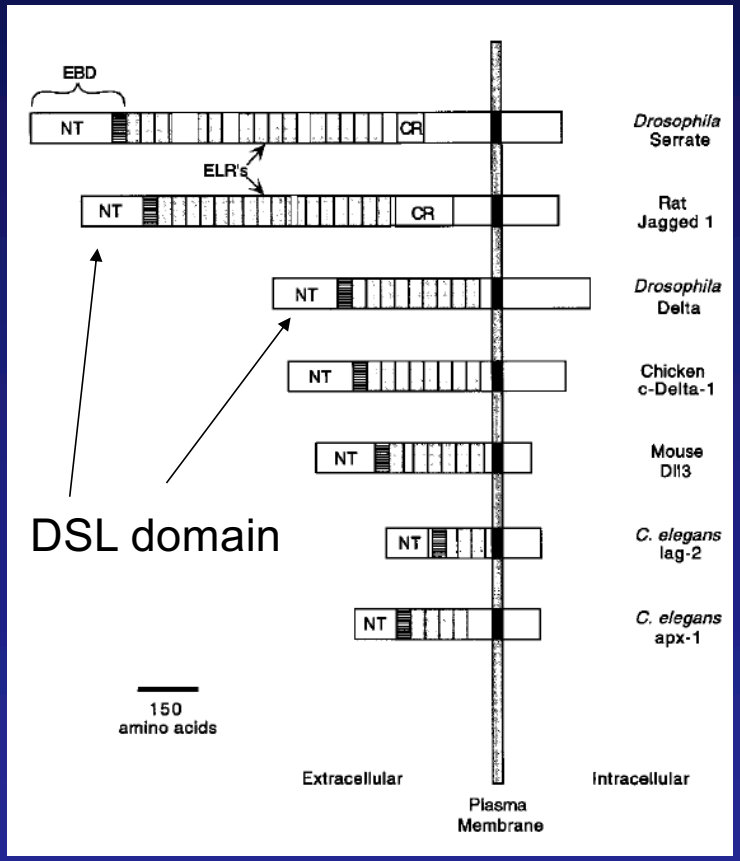
Core Notch Signaling Components

Table 1. Names of core components of Notch signaling (ligand, receptor and transcription factor) in different species

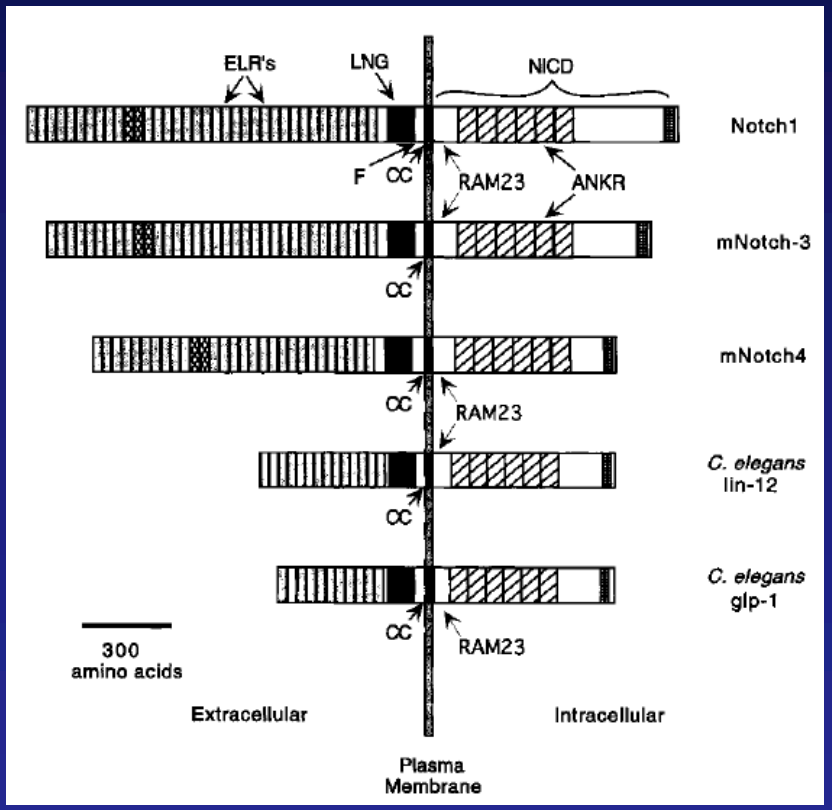
Core component	*C. elegans*	*D. melanogaster*	Mammals
Ligand	LAG-2 APX-1 ARG-2 F16B12.2	Delta Serrate	Delta-like1 (DLL1) Delta-like2 (DLL2) Delta-like3 (DLL3) Jagged 1 (JAG1) Jagged 2 (JAG2)
Receptor (Notch)	LIN-12 GLP-1	Notch	Notch1 Notch2 Notch3 Notch4
Transcription factor (CSL)	LAG-1	Suppressor of Hairless [Su(H)]	CBF1/RBPJκ RBPL



Ligand (eg Delta) Structure



Notch Receptor Structure



both ligand and receptor are single pass TM proteins with large arrays of extracellular EGF repeats

Evidence for Delta and Notch as a ligand-receptor pair?

Phenotypes of *Delta* and *Notch* LOF mutants suggest they function in a common pathway

Also, *Dl* and *N* are two of the very small # of morphologically haploinsufficient genes in flies

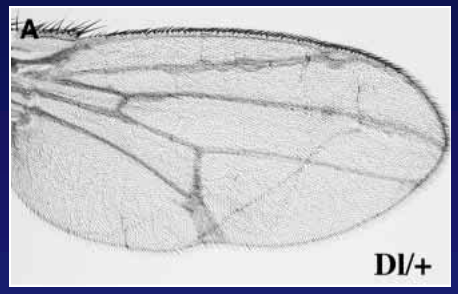
Dosage experiments suggested
N and Dl might be a receptor/ligand pair:

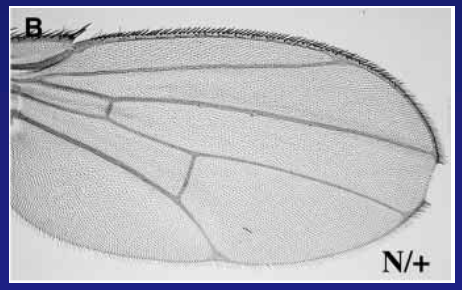
N/+ = wing nicks

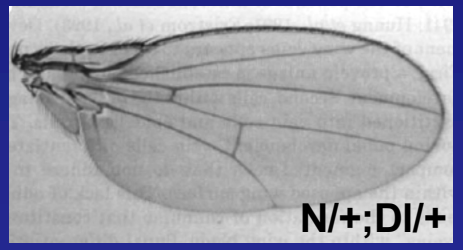
Dl/+ = wing deltas

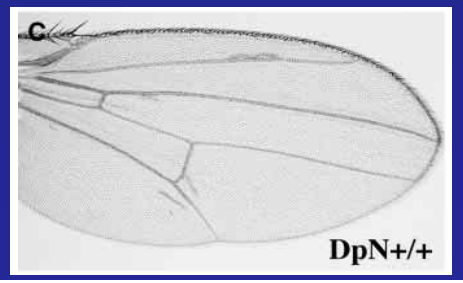
N/+; Dl/+ = wildtype wings

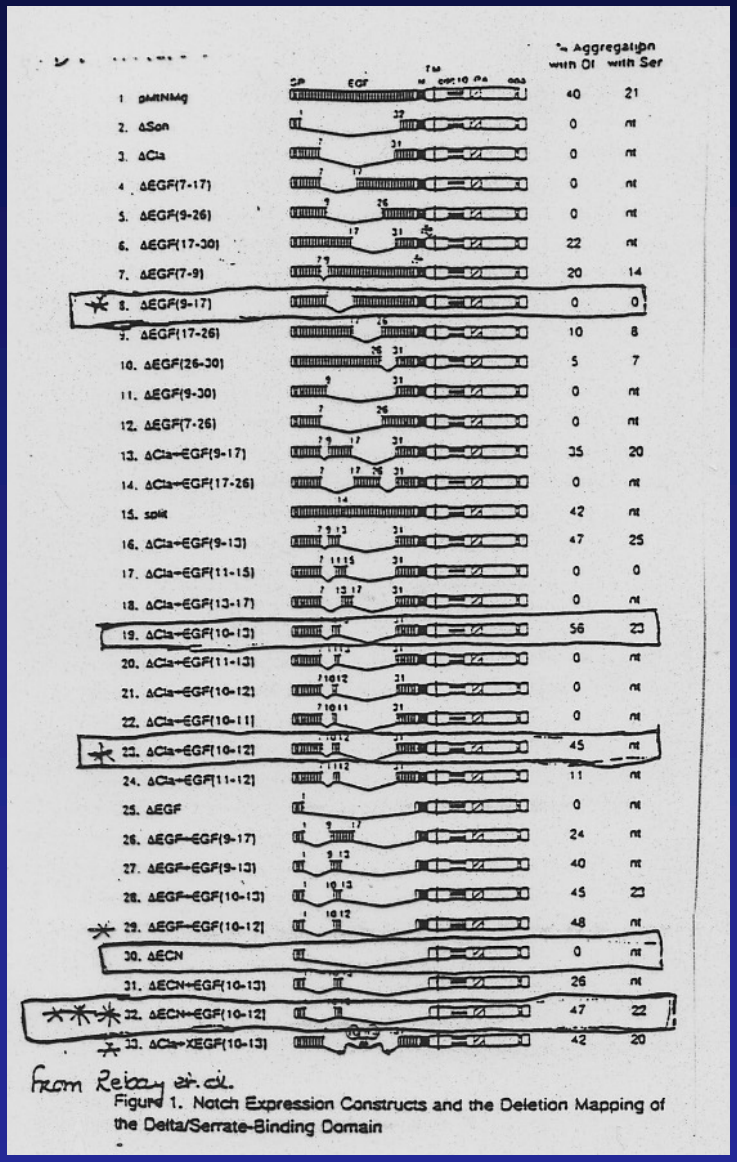
More paradoxical genetics:
an extra copy of *N* looks like *Dl* heterozygote





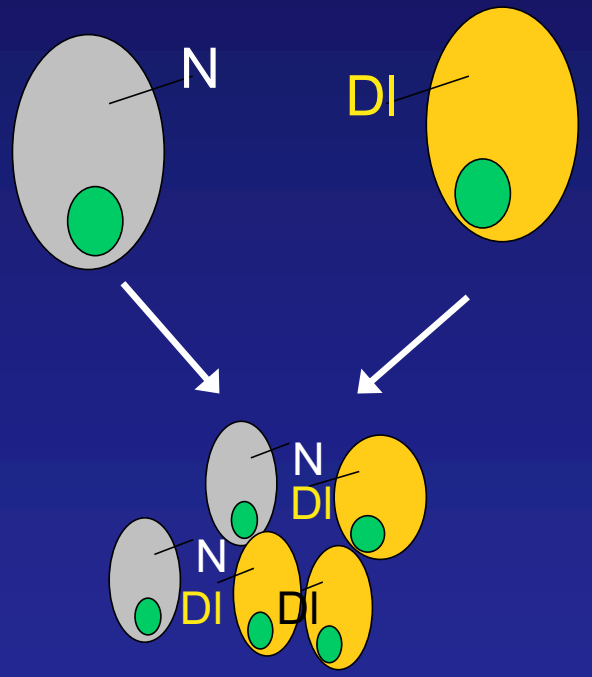






Rebay and Artavanis-Tsakonas Cell 1991

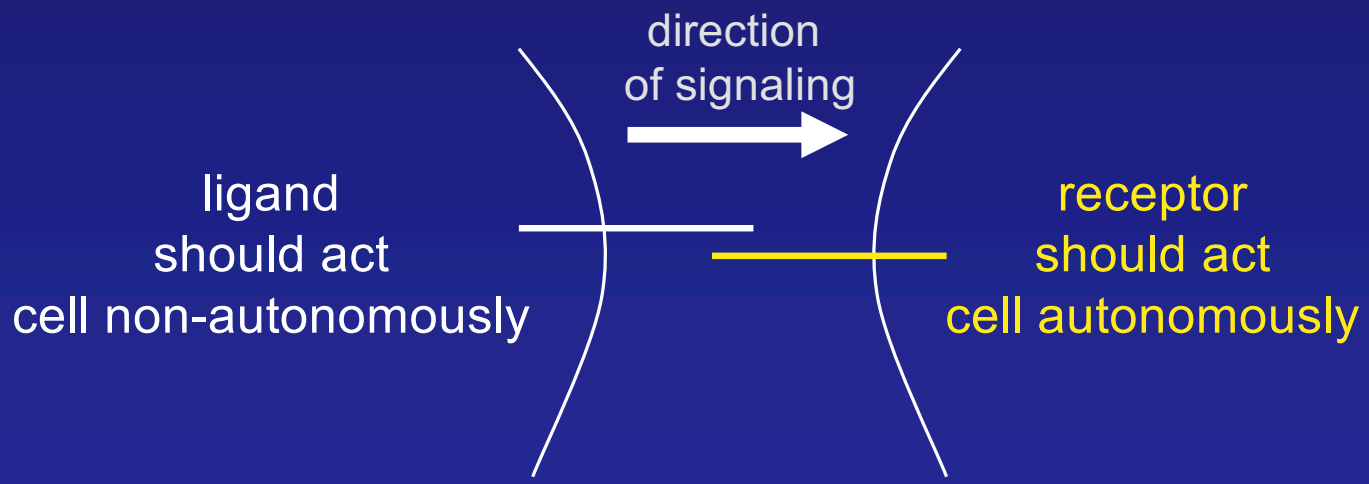
Cell aggregation studies of Notch+Delta define domains of interaction: Dl-DSL domain binds N-EGF repeats 11-12



mixing Dl+ and N+ cells gives clumping

How to distinguish the ligand from the receptor?

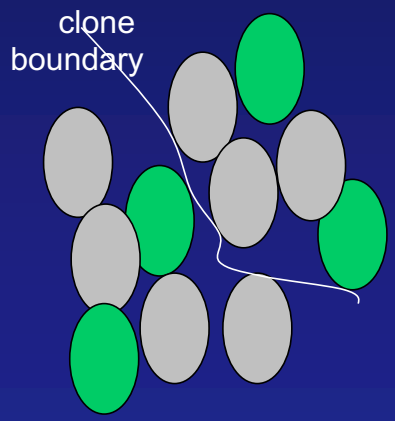
- analyze cell autonomy of signal activation



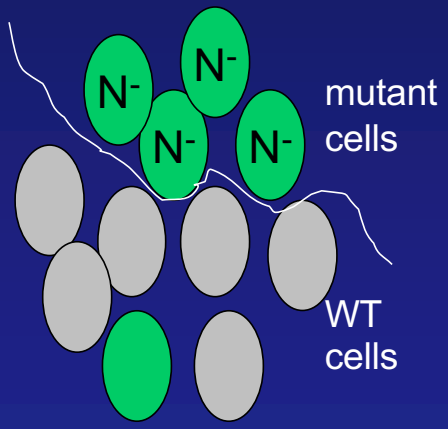
Cell Autonomy experiments:

remember that N signaling represses neural fate

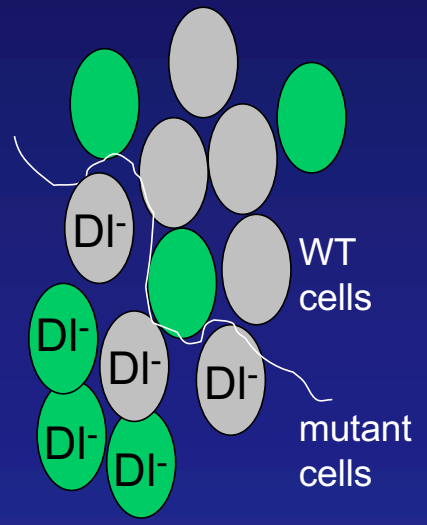
experiment: analyze whether mutant cells at clone borders adopt neural or epithelial fate



green: neural
gray: epidermal



N-/- cells are always neural, bordering WT cells always epidermal
N mutant cells act "autonomously", b/c cant be inhibited by neighboring WT

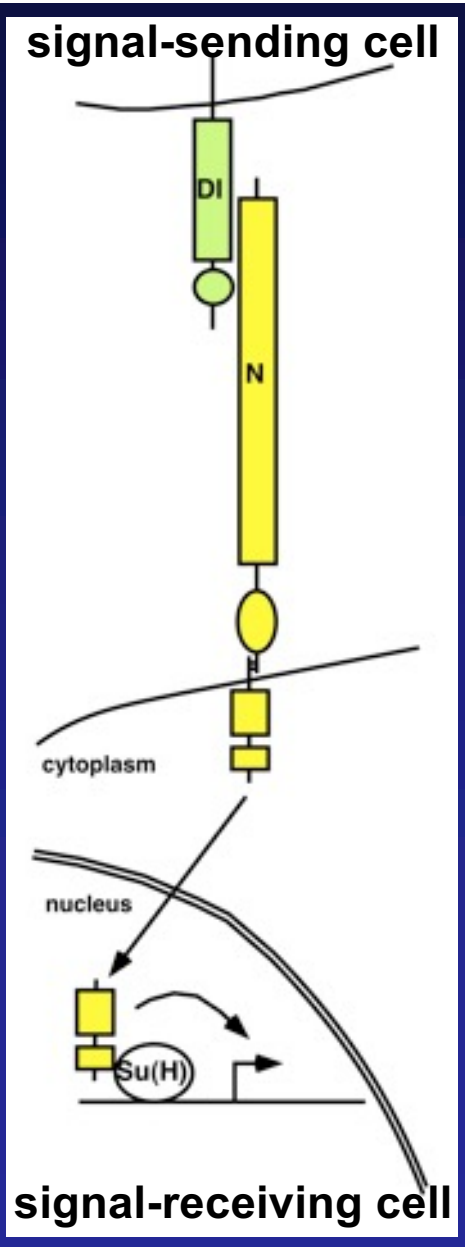


Dl-/- cells at border are epidermal
(i.e. they retain ability to activate N signaling; thus Dl acts non-autonomously)

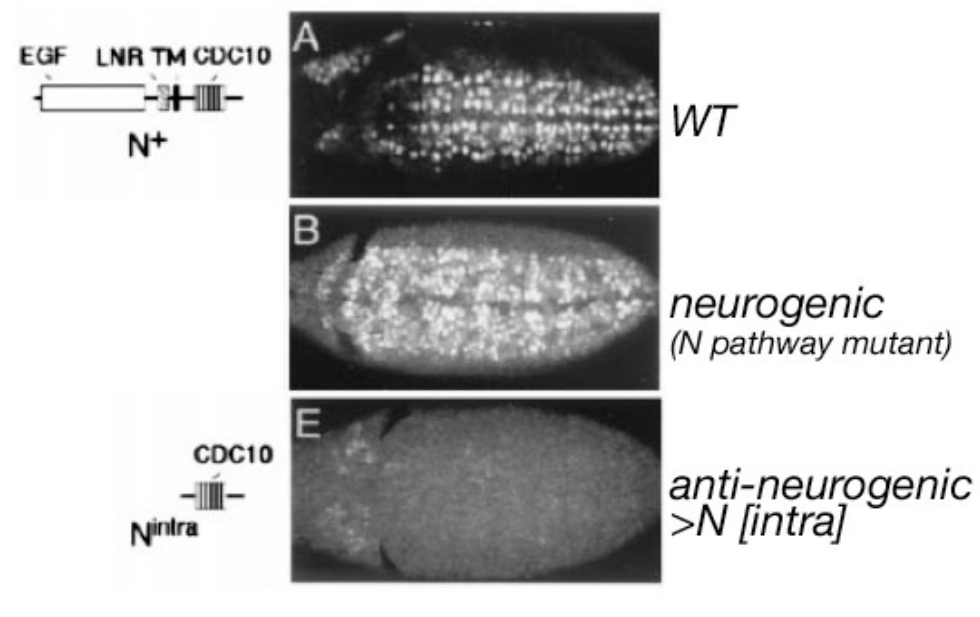
Dl --> N --| neural fate
↘ epidermal fate

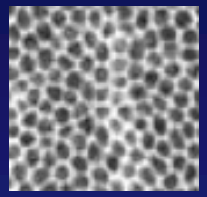
Heitzler and Simpson Cell 1991

Notch processing and signal activation



Evidence for "nuclear" Notch and its role in transcriptional regulation





endogenous N at
plasma membrane

N[intra] in nucleus

- Engineered N[intra] acts as constitutive GOF and localizes to nucleus
- But, endogenous Notch never seen in the nucleus

N[intra] works at “subdetectable” levels

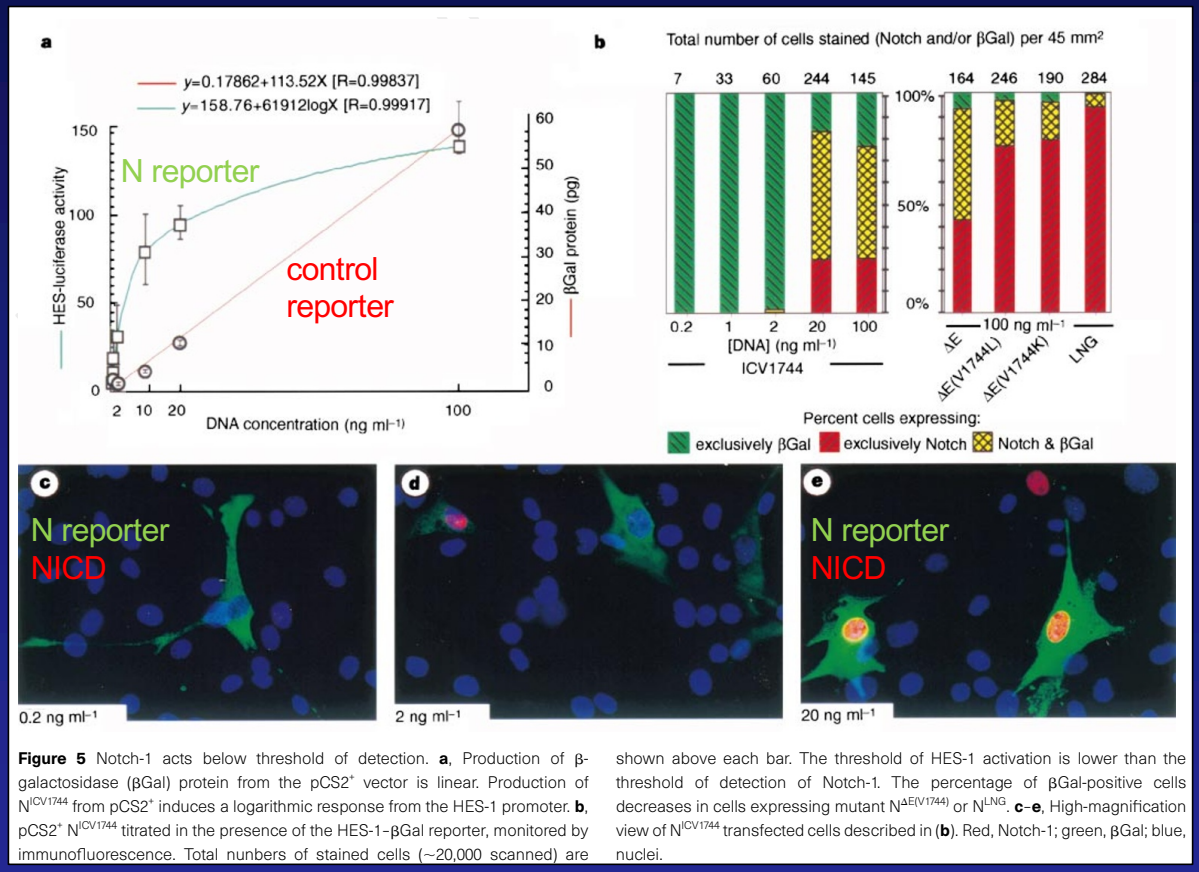
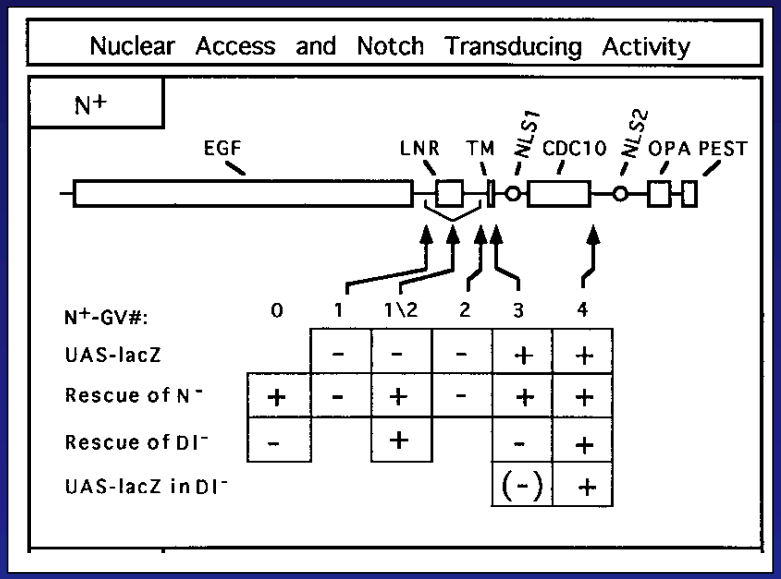


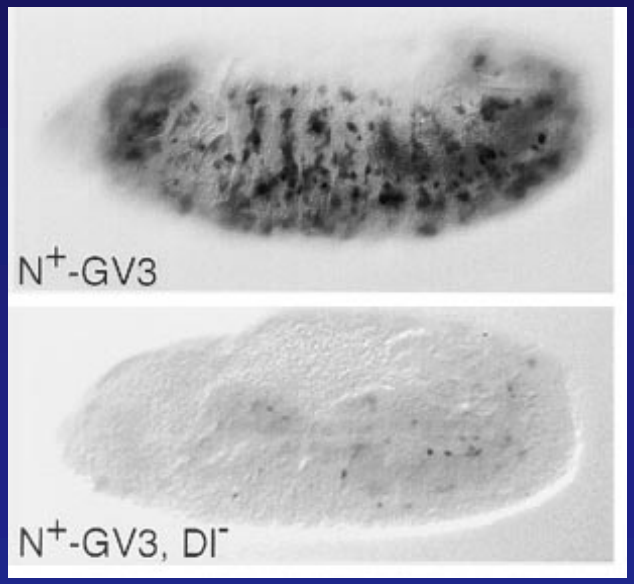
Figure 5 Notch-1 acts below threshold of detection. **a**, Production of β-galactosidase (βGal) protein from the pCS2$^+$ vector is linear. Production of N^{ICV1744} from pCS2$^+$ induces a logarithmic response from the HES-1 promoter. **b**, pCS2$^+$ N^{ICV1744} titrated in the presence of the HES-1–βGal reporter, monitored by immunofluorescence. Total nunbers of stained cells (~20,000 scanned) are shown above each bar. The threshold of HES-1 activation is lower than the threshold of detection of Notch-1. The percentage of βGal-positive cells decreases in cells expressing mutant N$^{\Delta E(V1744)}$ or N^{LNG}. **c**–**e**, High-magnification view of N^{ICV1744} transfected cells described in (**b**). Red, Notch-1; green, βGal; blue, nuclei.

Schroeter and Kopan
Nature 1998

green: activation of N reporter
red: staining for N[intra]
titration shows that reporter is turned on long before you can “see” N[intra]

Visualizing nuclear access of N[intra] produced from full-length Notch



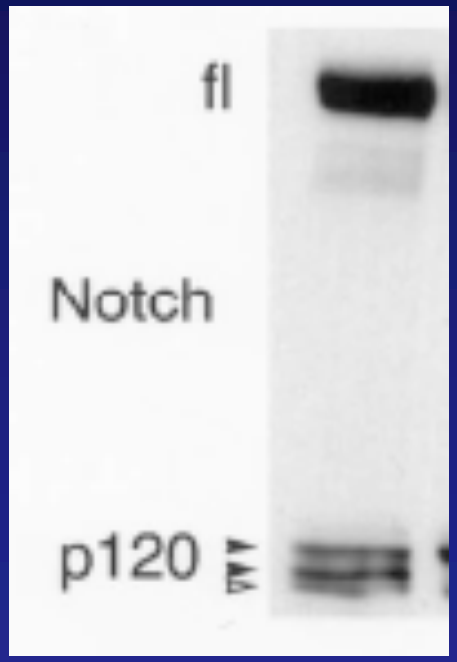


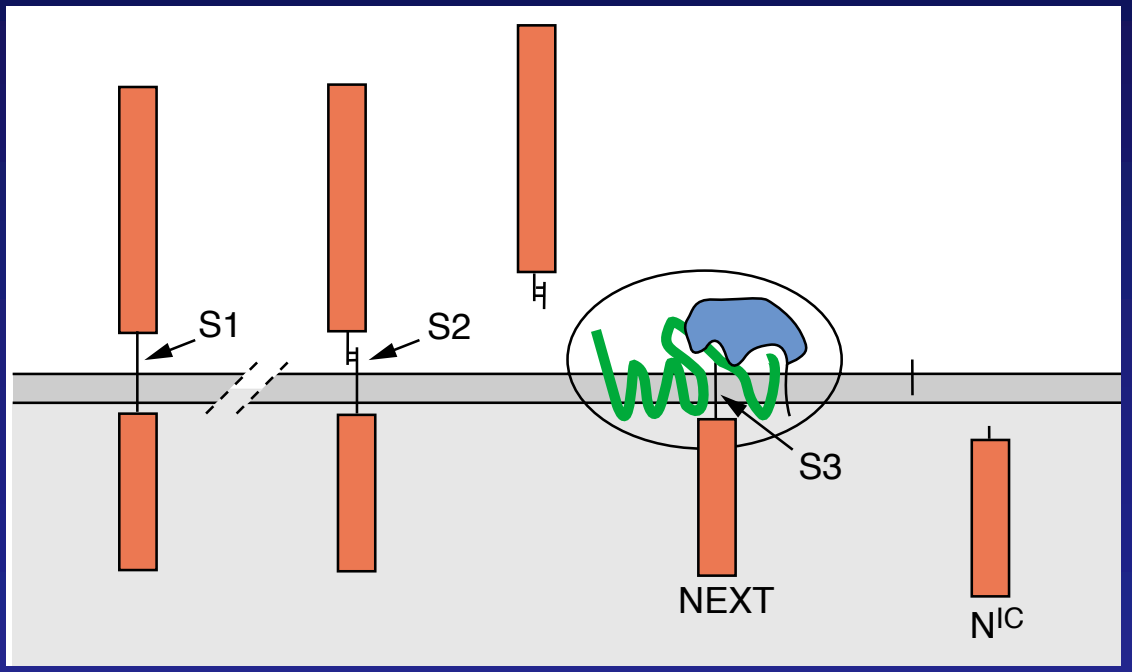
Gary Struhl
Cell 1998

GV= insertion of Gal4-VP16 into the N protein
N-GV constructs introduced into UAS-lacZ background

GV #3 insertion into intracellular domain results in *lacZ* activation,
in a ligand-dependent fashion

Membrane localized Notch is cleaved--how?





S2: ADAM metalloprotease (Kuzbanian)

N[intra]
N[ICD]

we'll focus on "S3" cleavage by "gamma secretase complex"

Making the final cut: gamma-secretase complex

originally defined as an activity that cleaves APP;
aberrant cleavage underlies accumulation of APP
in neural plaques and tangles in Alzheimer's patients

pharmacological studies suggest gamma-secretase
has an aspartyl protease activity

mutations in presenilin (PS) 1 or 2 are most common cause of
autosomal dominant Alzheimers' disease

** PS mutation in worms suppresses a GOF N receptor
** PS mutations in flies and mice phenocopy N

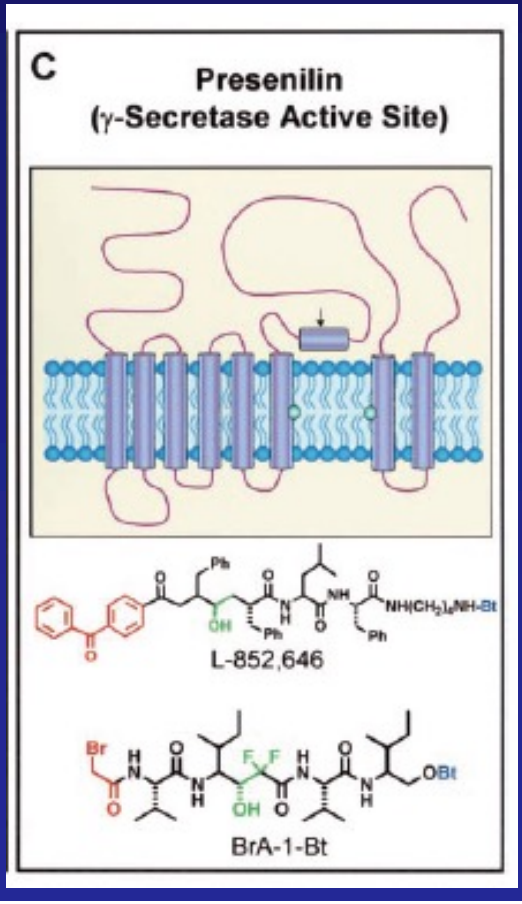
Identity of gamma-secretase protease was controversial

PS was an attractive candidate,
but not possible to show that PS cuts N or APP by in vitro reconstitution

active site chemical inhibitors of gamma-secretase
bind directly to presenilin

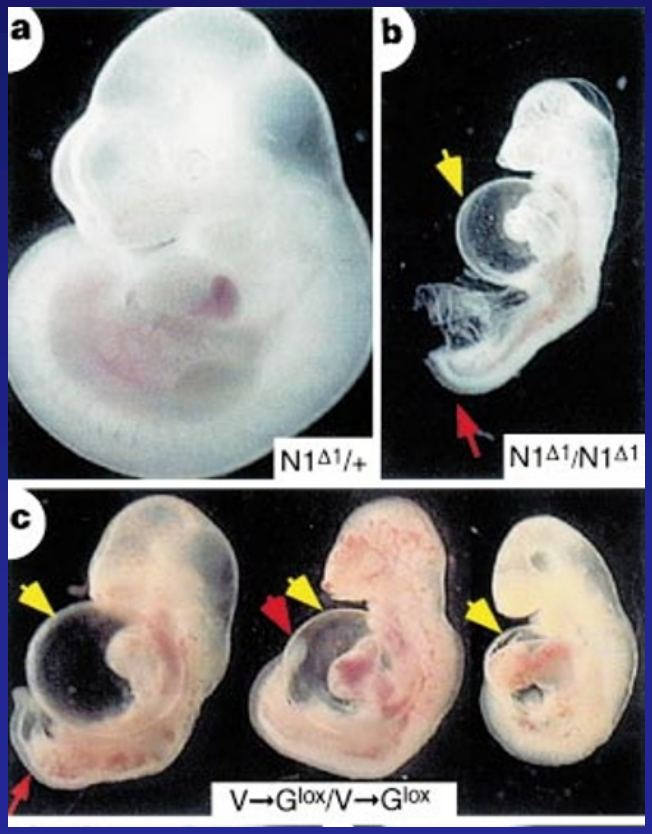
identification of PS active site allowed its
recognition as an atypical aspartyl protease

4 factors needed for functional gamma-secretase
Presenilin, Nicastrin, Aph-1, Pen-2



Genetic demonstration of the importance of N cleavage

- knock in point mutation of the S3 cleavage site: what happens?

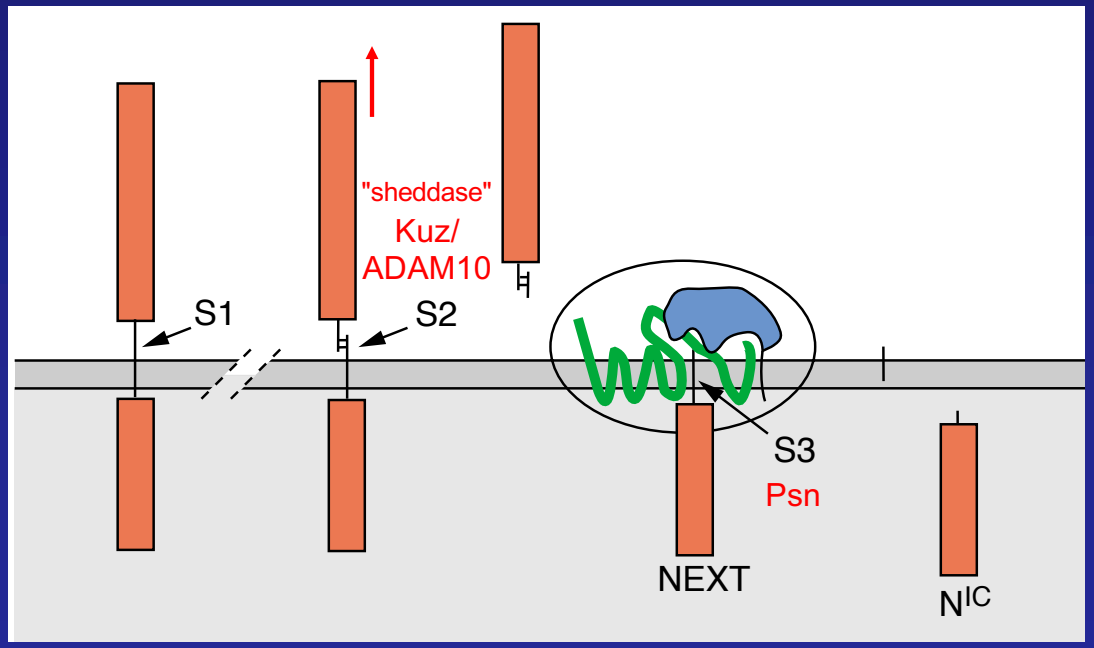


N point mutant at cleavage site (V1744G) almost phenocopies N null

de Strooper and Kopan Nature 1999

Signaling by RIP (regulated intramembranous proteolysis) – considerations

1. Not just Notch: APP (amyloid precursor protein), N-cadherin, and others do it.
2. Irreversible: the ligand binding domain is dissociated from the intracellular signaling domain, hence each receptor can signal once
3. Signaling is direct; no second messengers necessary
4. Sometimes (eg Notch) requires "pulling force" to expose the cleavage site (mechanobiology)
5. Can release of extracellular domain could regulate ligands? (titration)



And now to the nucleus...

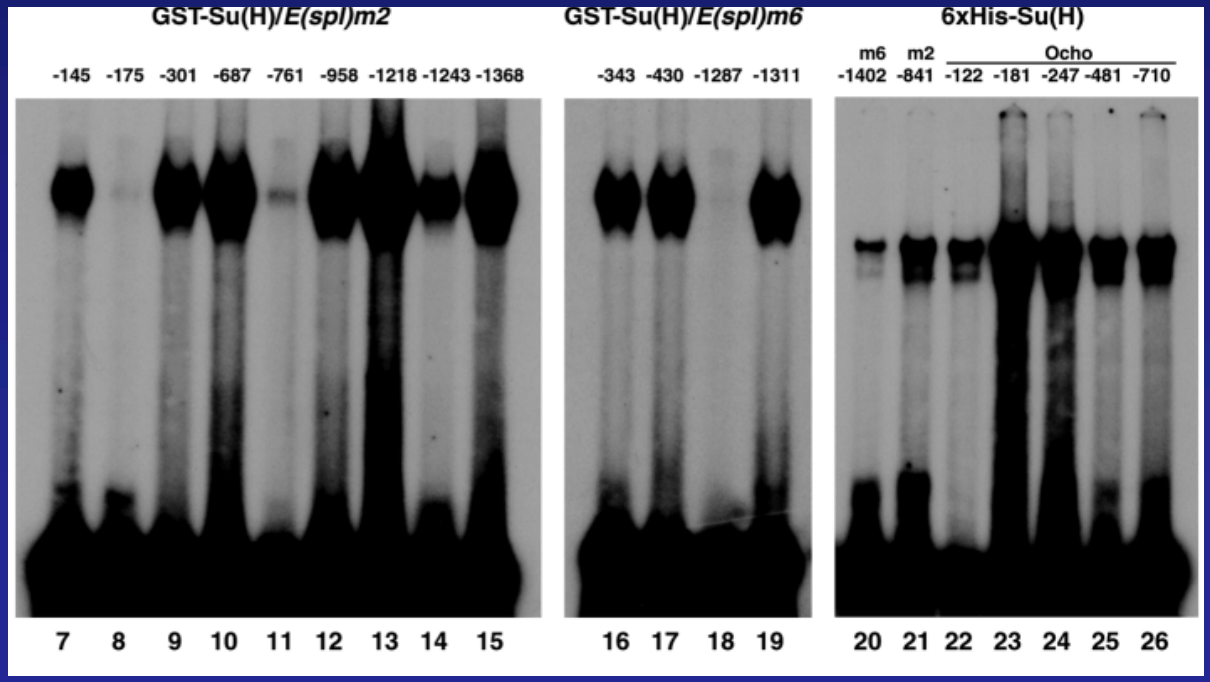
...linking N activation at cell surface to nuclear transcriptional changes

transcriptional regulation by CSL transcription factors,
before and after N activation

CSL = CBF1 (mammalian), Suppressor of Hairless (fly), Lag-1 (worm)

fly Su(H) and worm Lag-1 genetically required for N signaling

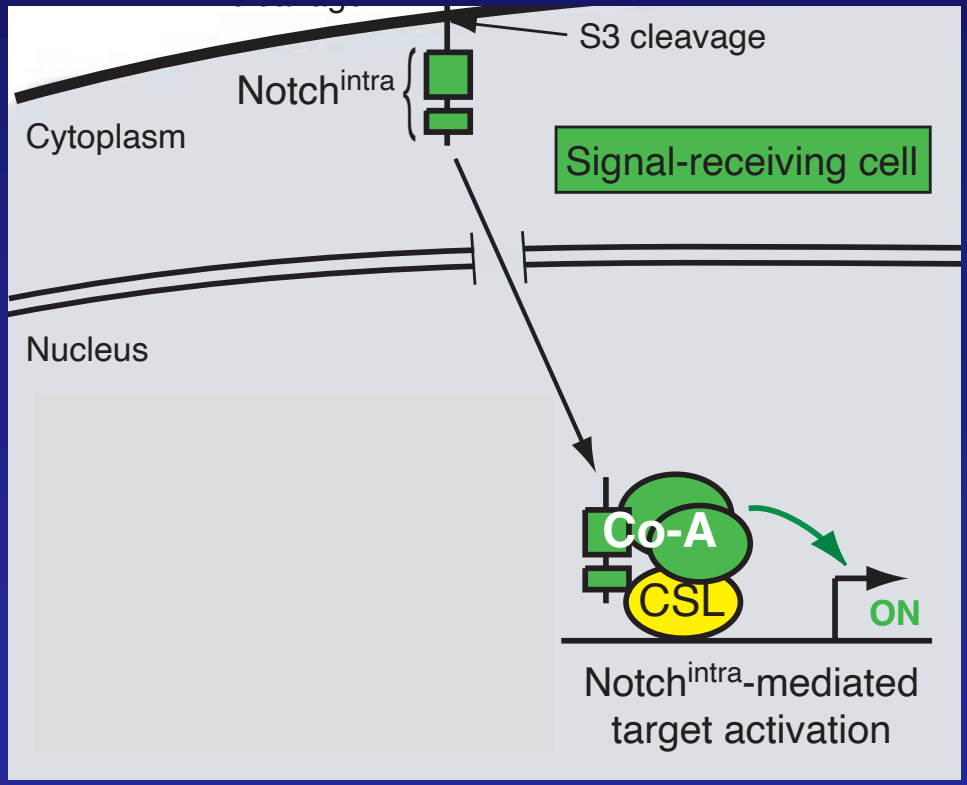
- Su(H) binds to N[intra]
- Su(H) binds to YGTGDGAA motifs located in N target gene enhancers



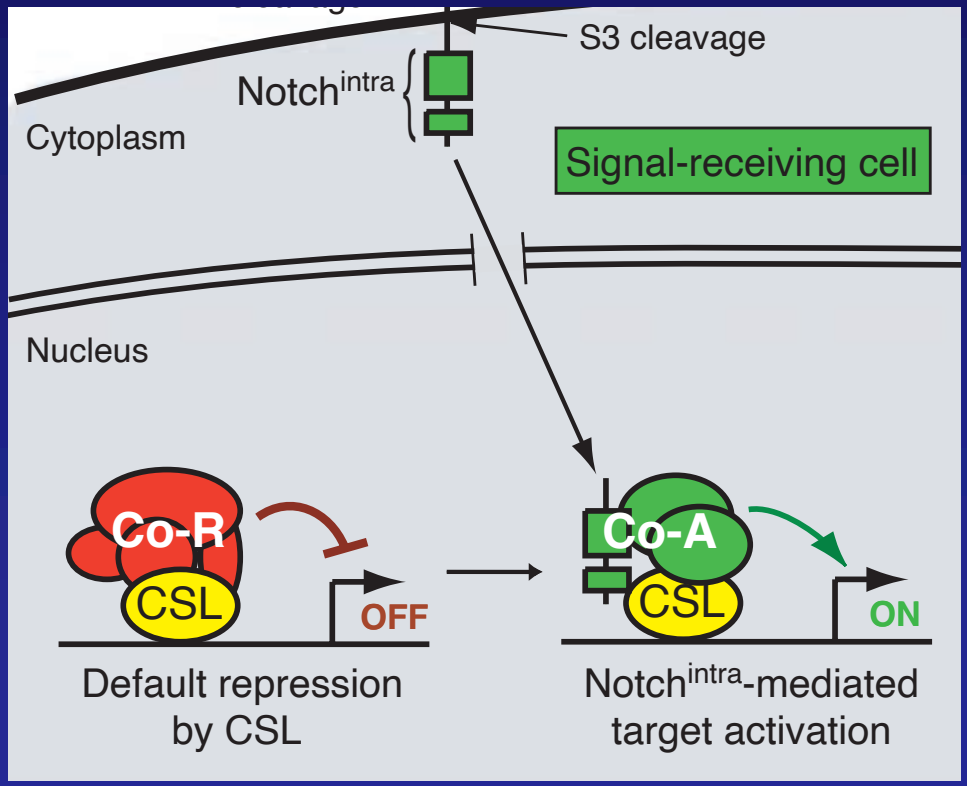
Lai Development 2000

A discrepancy in activity:

- fly Su(H) and worm Lag-1 required for Notch signaling
- however, vertebrate ortholog (CBF) originally characterized as a transcriptional repressor in tissue culture



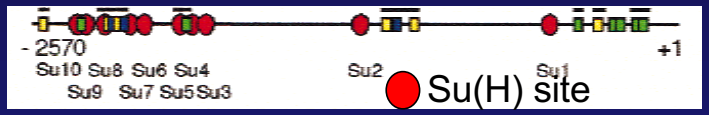
Dual activity of CSL TFs may explain N target specificity: CSL proteins act as repressors AND as activators of transcription

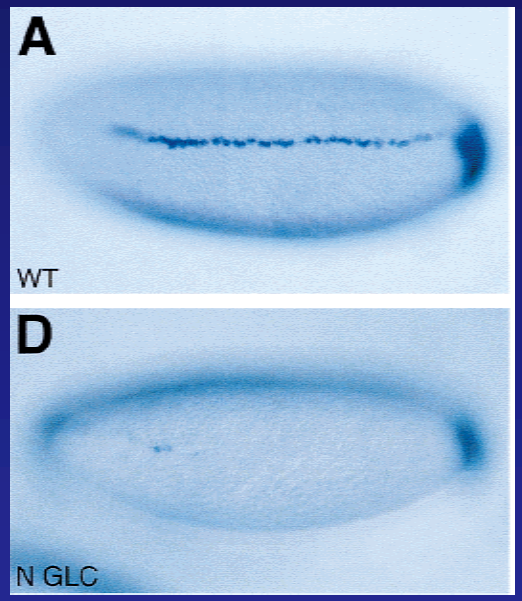


(dynamic vs static genomic occupancy by CSL still remains to be clarified)

An example: N signaling activates single-minded (sim)

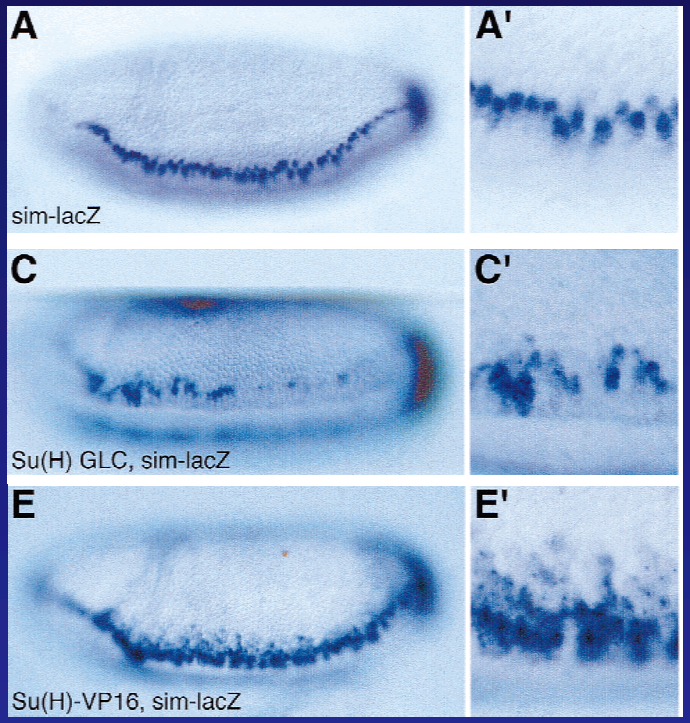
- in mesectoderm, a single line of cells at mesodermal/ectodermal boundary
- sim is a direct N target; has a ton of Su(H) sites in its regulatory region





Morel and Schweisguth
Genes Dev 2000

wt: single line of *sim*
N mutant: loss of *sim*



- Su(H) mutants: *sim* expression is patchy, and ectopic staining seen (>single line)
- Su(H)VP16: *sim* in several 2-3 rows of cells

loss of Su(H) causes weakened but broadened activation of some N targets

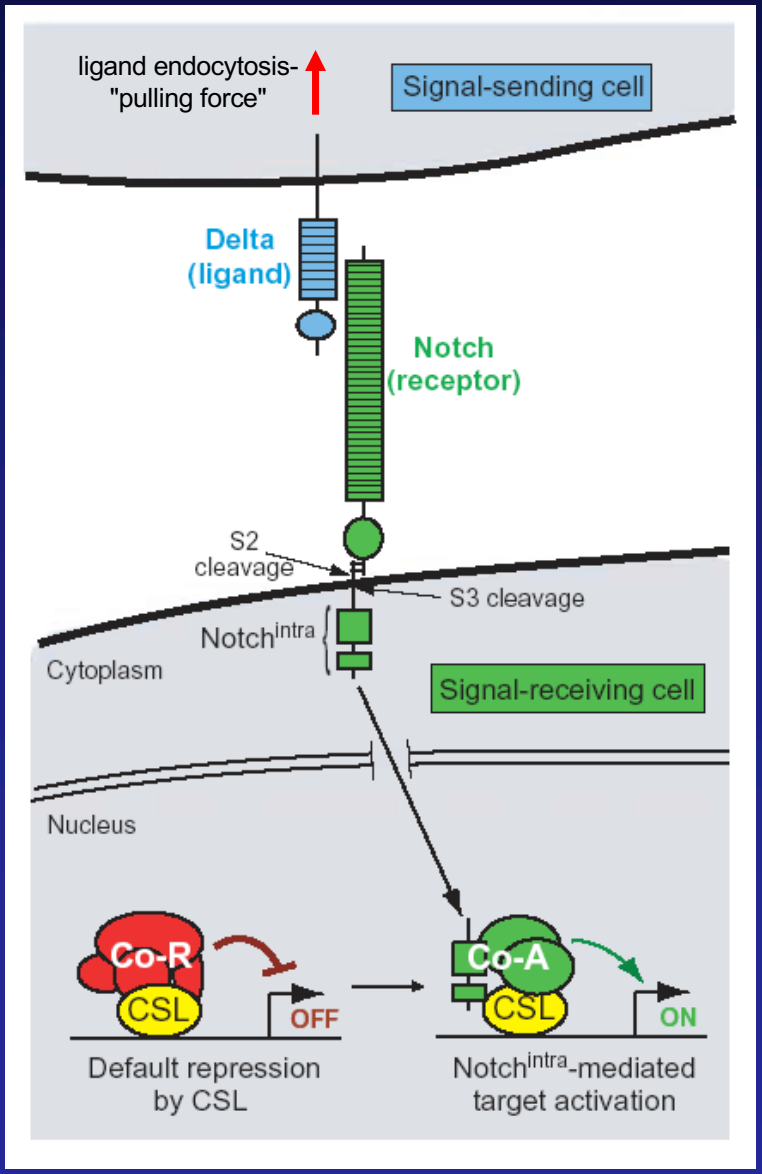
Dual activity of CSL solves a genetic puzzle

loss of Su(H) is not as “bad” as loss of N

- evidence for Su(H)-independent N signaling?

- or, reflects that Su(H) mutants get rid of both
  Su(H)-mediated repression and activation
  whereas, N mutants get rid only of activation

- N target genes are actively repressed in the absence of signaling,
  - what is this good for?



Summary of key points in N signaling

1. Delta (ligand) requires ubiquitination/endocytosis to be active for signaling (generates "pulling force")

2. Activated Delta physically interacts with Notch

3. Activated N is cleaved to release N[intra], which goes to the nucleus

4. N[intra] converts CSL from a repressor into an activator of target gene expression

3. What does N signaling tell a cell?

CHROMOSOMAL CONTROL OF EMBRYOGENESIS IN DROSOPHILA

DR. D. F. POULSON

OSBORN ZOOLOGICAL LABORATORY, YALE UNIVERSITY

1945

The facet, or Notch, deficiencies, Fig. 7, all lead to an earlier and more drastic series of disturbances in which each of the germ layers is involved. The most conspicu-

All in all, a kind of hopeless monster is produced which can not develop beyond the embryonic stage, although its constituent cells and parts remain alive for some hours after normal hatching time. Since the results are the

it is difficult to name a tissue or developmental process that N signaling does NOT regulate...

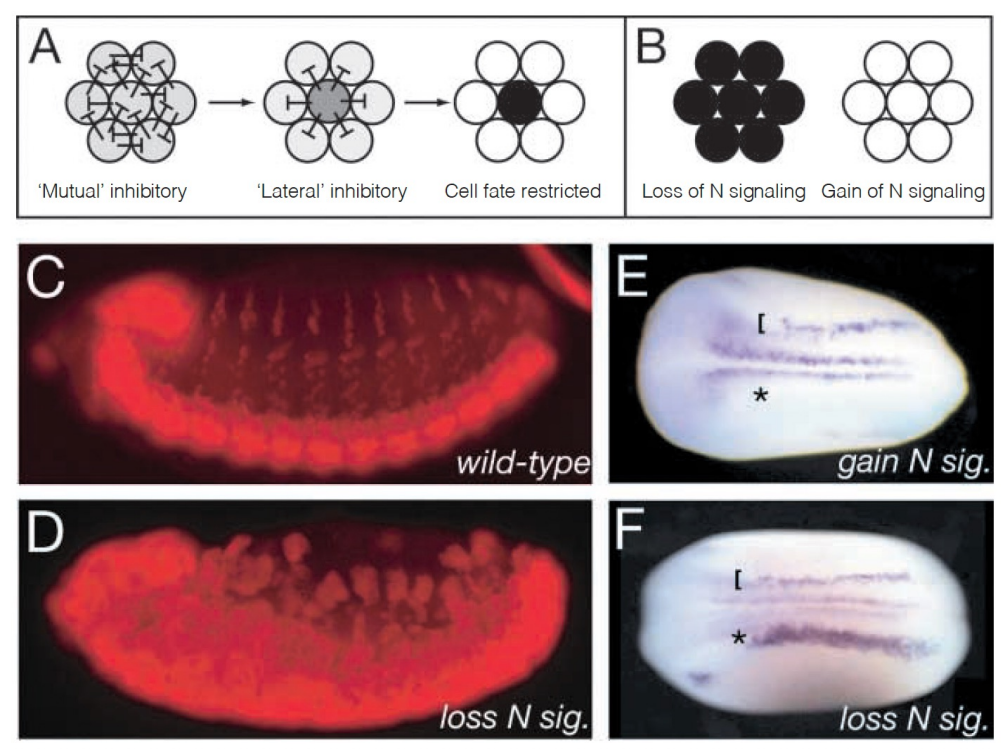
Spyros Artavanis-Tsakonas: "There are two kinds of scientists: those that study Notch and those that don't yet know they are studying Notch"

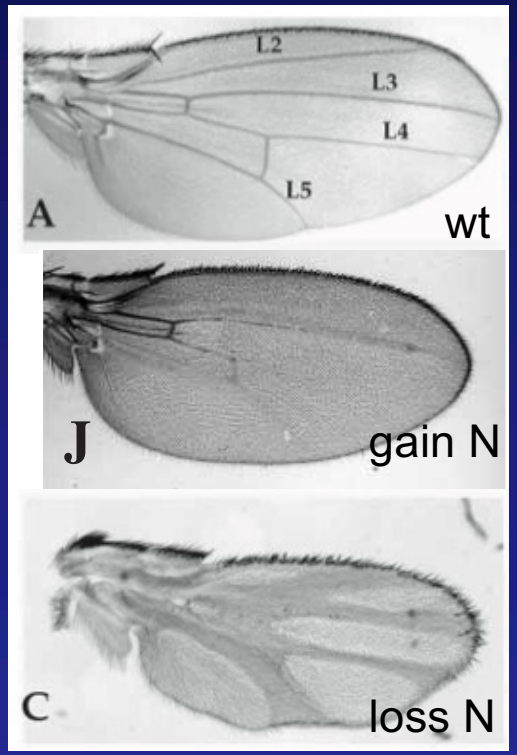
Table 2. A non-exhaustive list of developmental processes that are regulated by Notch signaling in different species

C. elegans	*D. melanogaster*	Vertebrates
Regulation of early blastomere specification	Inhibition of neurogenesis	Inhibition of neurogenesis
Regulation of AC/VU decision	Regulation of gliogenesis, neural lineage fates	Regulation of fate choices in the inner ear
Regulation of vulval precursor fates	Inhibition of wing venation	Inhibition of non-neural ectodermal derivates
Induction of left-right asymmetry	Inhibition of myogenesis, cardiogenesis	(*Xenopus* ciliated cells, chick feather buds)
Induction of germline proliferation	Inhibition of midgut precursors	Inhibition of myogenesis, cardiogenesis
	Induction of mesectoderm	Induction of left-right asymmetry
	Induction of wing margin	Regulation of limb bud development
	Induction of leg segments	Regulation of somitogenesis
	Induction of dorsoventral eye polarity	Regulation of lymphopoiesis
	Induction of cone cells in the eye	Regulation of vascular development
	Regulation of hematopoiesis	Regulation of kidney development

these diverse N-regulated processes can be broadly grouped into two general categories: inhibitory and inductive

Lateral Inhibition : Restriction of Cell FATE

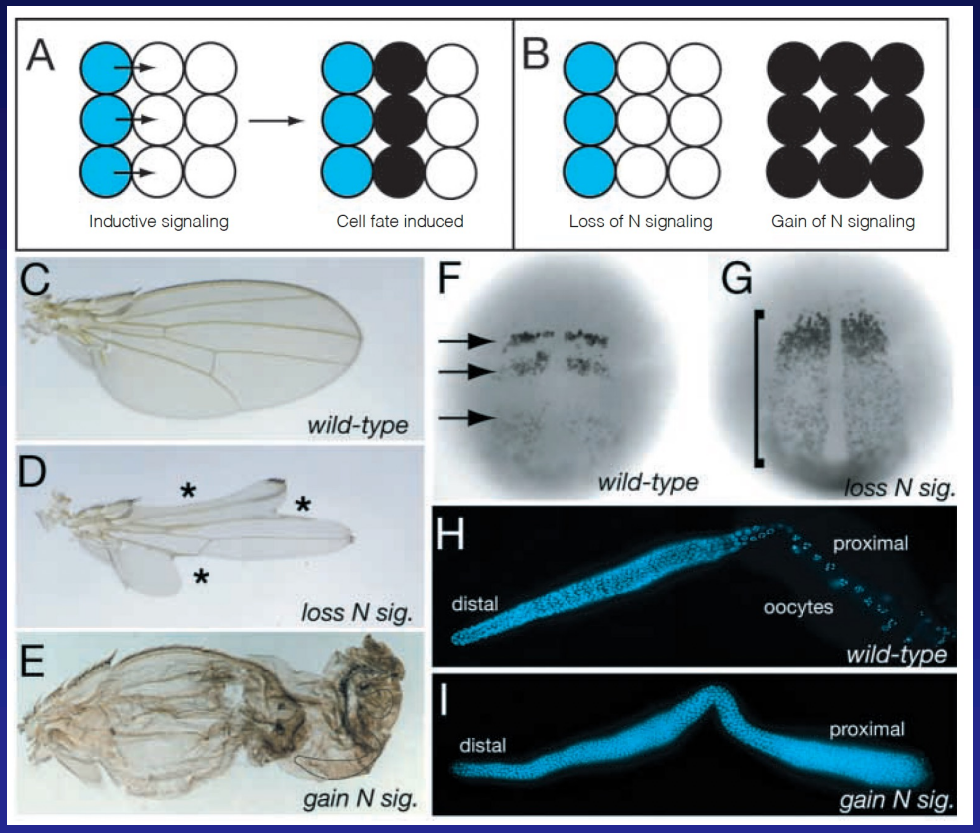




Important N target genes for inhibitory signaling include bHLH repressor genes

example: Dl --> N/CSL --> E(spl)bHLH --| proneural bHLH repressor

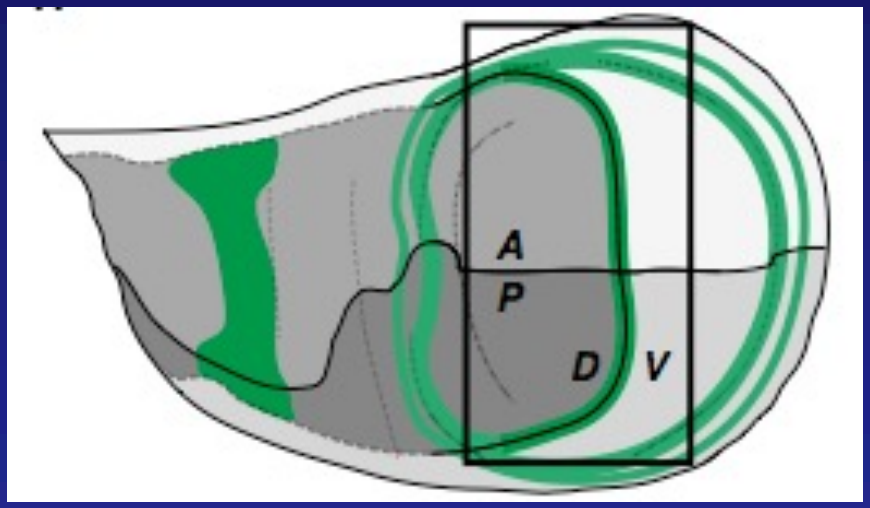
Inductive N signaling: making new cell types and tissues



Important N targets for inductive signaling include transcriptional activators and signaling molecules

example: Dl > N/CSL > vestigial (nuclear factor, wing development)

Inductive N signaling often at borders b/w distinct cell populations

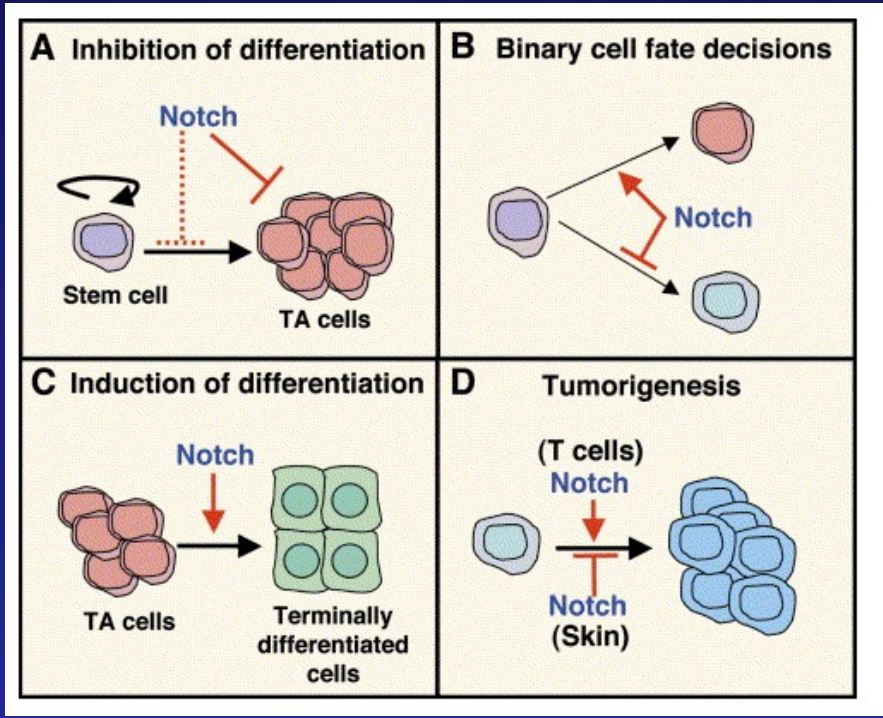


wing margin develops between
dorsal and ventral compartments of wing disc

pleiotropic effects of Notch signaling mean that aberrant Notch signaling in self-renewing tissues is dangerous

in stem cells, N maintains the undifferentiated state

in transit-amplifying cells, (eg skin) N induces terminal differentiation

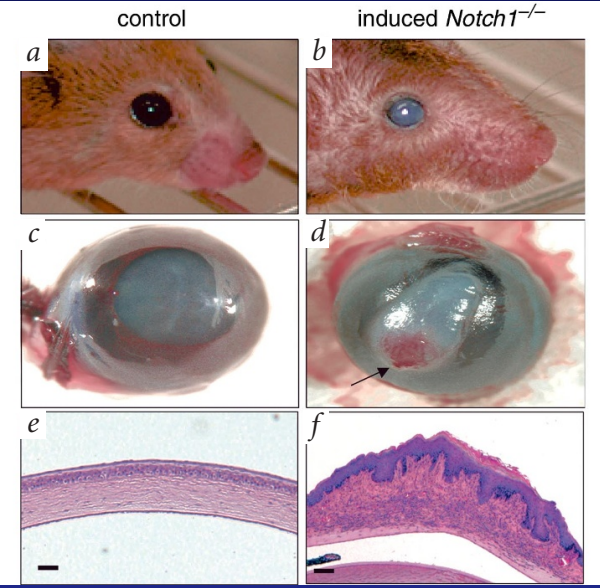


N controls cell fate determination

both gain and loss of N signaling can induce tumorigenesis

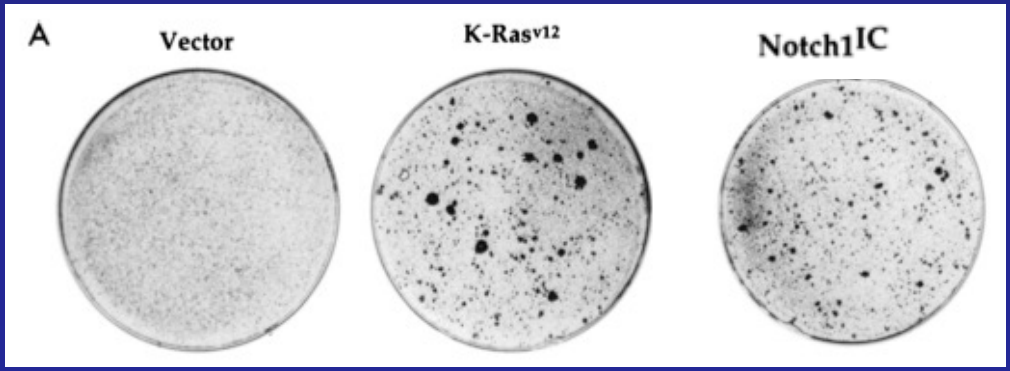
- in different settings, Notch can also suppress proliferation OR induce proliferation OR induce apoptosis...context is everything
- what are implications for doing experiments only in cultured cells?

pleiotropic effects of Notch signaling mean that aberrant Notch signaling in self-renewing tissues is dangerous



loss of N signaling
can be tumorigenic

N -/- clones induce epithelial tumors



gain of N signaling can be
pro-growth and/or tumorigenic

Recurrent GOF mutations in Notch in various cancers

Activating Mutations of *NOTCH1* in Human T Cell Acute Lymphoblastic Leukemia

Andrew P. Weng,[1]*† Adolfo A. Ferrando,[2]* Woojoong Lee,[1] John P. Morris IV,[2] Lewis B. Silverman,[2] Cheryll Sanchez-Irizarry,[1] Stephen C. Blacklow,[1] A. Thomas Look,[2] Jon C. Aster[1]‡

SCIENCE VOL 306 8 OCTOBER 2004

that more than 50% of human T-ALLs, including tumors from all major molecular oncogenic subtypes, have activating mutations that involve the extracellular heterodimerization domain and/or the C-terminal PEST domain of NOTCH1. These findings greatly expand the role of activated NOTCH1 in the molecular pathogenesis of human T-ALL and provide a strong rationale for targeted therapies that interfere with NOTCH signaling.

Leukaemogenesis induced by an activating β-catenin mutation in osteoblasts

Aruna Kode[1], John S. Manavalan[1], Ioanna Mosialou[1], Govind Bhagat[2], Chozha V. Rathinam[3], Na Luo[1], Hossein Khiabanian[4], Albert Lee[4], Vundavalli V. Murty[5], Richard Friedman[6], Andrea Brum[1,7], David Park[8], Naomi Galili[9], Siddhartha Mukherjee[10], Julie Teruya-Feldstein[8], Azra Raza[9], Raul Rabadan[4], Ellin Berman[11] & Stavroula Kousteni[1,12]

NATURE | VOL 506 | 13 FEBRUARY 2014

Activated β-catenin stimulates expression of the Notch ligand jagged 1 in osteoblasts. Subsequent activation of Notch signalling in haematopoietic stem cell progenitors induces the malignant changes. Genetic or pharmacological inhibition of Notch signalling ameliorates acute myeloid leukaemia and demonstrates the pathogenic role of the Notch pathway. In 38% of patients with myelodysplastic syndromes or acute myeloid leukaemia, increased β-catenin signalling and nuclear accumulation was identified in osteoblasts and these patients showed increased Notch signalling in haematopoietic cells.

Recurrent LOF mutations in Notch in various cancers

Exome Sequencing of Head and Neck Squamous Cell Carcinoma Reveals Inactivating Mutations in *NOTCH1*

Nishant Agrawal,[1,2*†] Mitchell J. Frederick,[3*] Curtis R. Pickering,[3*] Chetan Bettegowda,[2,4*] Kyle Chang,[5] Ryan J. Li,[1] Carole Fakhry,[1] Tong-Xin Xie,[3] Jiexin Zhang,[6] Jing Wang,[6] Nianxiang Zhang,[6] Adel K. El-Naggar,[7] Samar A. Jasser,[3] John N. Weinstein,[6] Lisa Treviño,[5] Jennifer A. Drummond,[5] Donna M. Muzny,[5] Yuanqing Wu,[5] Laura D. Wood,[8] Ralph H. Hruban,[8] William H. Westra,[8] Wayne M. Koch,[1] Joseph A. Califano,[2,9] Richard A. Gibbs,[5,9] David Sidransky,[1] Bert Vogelstein,[2] Victor E. Velculescu,[2†] Nickolas Papadopoulos,[2] David A. Wheeler,[5] Kenneth W. Kinzler,[2†] Jeffrey N. Myers[3†]

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The Mutational Landscape of Head and Neck Squamous Cell Carcinoma

Nicolas Stransky,[1*] Ann Marie Egloff,[2*] Aaron D. Tward,[1,3,4*] Aleksandar D. Kostic,[1,5] Kristian Cibulskis,[1] Andrey Sivachenko,[1] Gregory V. Kryukov,[1,5] Michael S. Lawrence,[1] Carrie Sougnez,[1] Aaron McKenna,[1] Erica Shefler,[1] Alex H. Ramos,[1] Petar Stojanov,[1] Scott L. Carter,[1] Douglas Voet,[1] Maria L. Cortés,[1] Daniel Auclair,[1] Michael F. Berger,[1] Gordon Saksena,[1] Candace Guiducci,[1] Robert C. Onofrio,[1] Melissa Parkin,[1] Marjorie Romkes,[6] Joel L. Weissfeld,[7] Raja R. Seethala,[8] Lin Wang,[8] Claudia Rangel-Escareño,[9] Juan Carlos Fernandez-Lopez,[9] Alfredo Hidalgo-Miranda,[9] Jorge Melendez-Zajgla,[9] Wendy Winckler,[1] Kristin Ardlie,[1] Stacey B. Gabriel,[1] Matthew Meyerson,[1,5,10,11] Eric S. Lander,[1,5,12] Gad Getz,[1] Todd R. Golub,[1,5,11,13,14†] Levi A. Garraway,[1,5,10,11†‡] Jennifer R. Grandis[2,15†‡]

SCIENCE VOL 333 26 AUGUST 2011

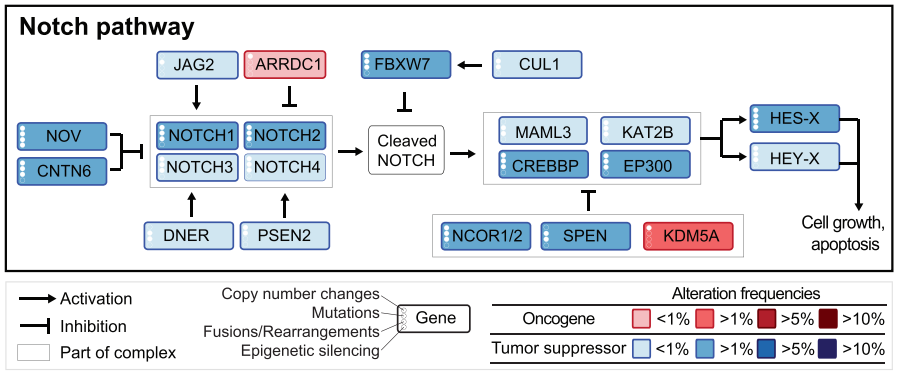
identified mutations in *FBXW7* and *NOTCH1*. Nearly 40% of the 28 mutations identified in *NOTCH1* were predicted to truncate the gene product, suggesting that *NOTCH1* may function as a tumor suppressor gene rather than an oncogene in this tumor type.

also 25% *NOTCH1* LOF in SCLC, Nature 2015

Cell

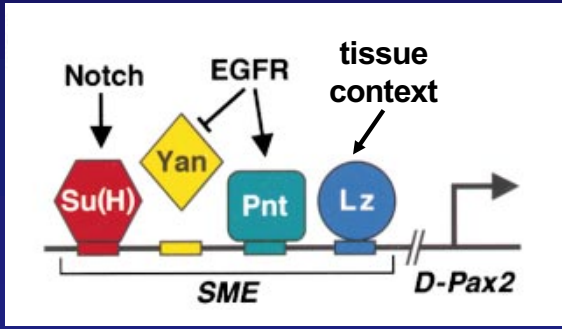
Sanchez-Vega et al., 2018, Cell *173*, 321–337

Oncogenic Signaling Pathways in The Cancer Genome Atlas

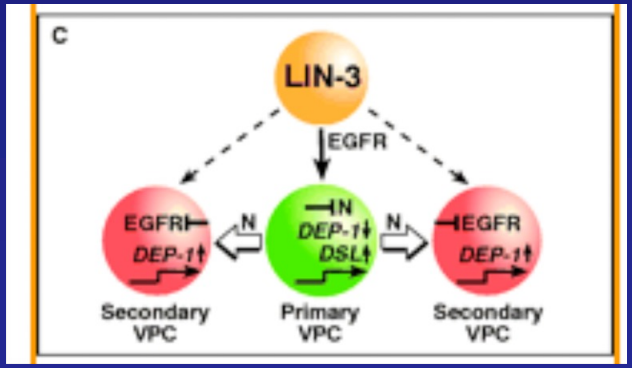


To better understand what N "does" in normal and disease conditions, we need to think about pathway crosstalk, synergism, antagonism...

N can synergize or act antagonistically with other pathways
for example: EGFR signaling



EGFR and N cooperate to turn on Dpax2
in cone cells of the fly eye
• induction of N signaling by oncogenic Ras maintains a neoplastic state



antagonism between N and EGFR
during worm vulval precursor selection
• many cases of N / EGFR antagonism in the fly

• there are also functional interactions between N and Wg/Wnt signaling

Many unresolved questions about Notch signaling

How are Notch ligands activated by ubiquitination?

How does Notch function as a mechanoreceptor?

Mechanism of ligand-receptor interactions causing "cis-inhibition" in same cell

Other “components” of Notch pathway? (genetic/biochemical hits)

How does vesicular trafficking control the activity of N and Dl?

Is the genomic occupancy of CSL TFs regulated by N activation?

How do other pathways crosstalk with Notch signaling?

If Notch is used “everywhere” to do “everything”, how are setting-specific outputs achieved?

Do the 4 mammalian Notch receptors elicit any distinct effects?

Do additional ligands control N signaling?

Is there CSL-independent N signaling?