Nicastrin is required for cleavage of Notch and β-APP transmembrane domains.
Notch pathway

Flies heterozygous for N (N/+)

= entaille, encoche
Molecular structure Notch

extracellular | intracellular

EGF | LNR | TM | CDC10 | OPA PEST

ubiquitous

αNotch
Notch pathway mutations...

Homozygous Notch die embryos:
expansion of nervous system at the expense of epidermis

Ist instar larvae

\( \alpha \text{Elav: pan neuronal marker} \)

OTHER MUTATIONS WITH SAME PHENOTYPE

Delta
(Serrate): ligands
Notch: receptor

Kuzbanian: cleavage S2?
Presenilin: intramembrane
Nicastrin: cleavage

mam
Su(H): (vertebrates: CSL) transcription
neu: RING domain ubiquitin ligase or E3 endocytosis Delta
mindbomb: E3 boundary specific function?
Notch involved 3 distinct processes:

Lateral inhibition
Lineage decisions
Boundary formation
Lateral inhibition: how 1 cell chosen from a group of equivalent precursors, e.g. sensory bristle precursors.

Proneural cells/genes

N active

Single Neurons etc.

N-

More neurons
Feedback mechanism

Slight difference in receptor ligand

Becomes amplified
Notch in lineage decisions: in muscle and neuronal cell lineages

Unequal segregation of Numb inhibits N signalling
Notch and DV boundary: wing

Wing imaginal disc

A

Wing blade

WT

B C D

wg AS-C↑ SMCs
Expression of N components & targets in wing

Margin wing

Early 3rd instar

WgLacZ
Ap dorsal

Mid 3rd instar

Cut

DI

late 3rd instar

DI

Cut
Notch targets boundary

$N^-$ clones (GFP-): no \textit{wg} expression

\textit{Su(H)}$^-$ similar

+ \textit{hsflp} (in some cells)

$N^-$ clones cross the DV boundary \textbf{after} decision D V made
**DI Ser clones loss of Cut wg**

- DV margin

- Ser required dorsally

- DI required ventrally
Ectopic expression of genes (e.g. Di) in clones (Flip out technique)
Delta expressing clones induce targets (Cut wg) in WT cells Abutting DI cells

ventral

But only dorsally
Notch target genes
Notch signalling to target genes

N receptor made as a single Transmembrane Protein

Cleavage

Target genes $bHLH \ (E(Spl) \ complex), \ vestigial$
N requires a selector gene for target activation

Scalloped (SD) required
For *wg* & *cut* expression margin
Constitutive N activity
Ectopic $\text{UASN}^{\text{intra}}$ clones: $\alpha$Cut: Cut in $\text{N}^{\text{intra}}$ (green + red = yellow) expressing cells and WT cells surrounding/abutting clone

Nonautonomous effect because DI ectopic
Regulating Notch activity
**Fringe modulates Notch-ligand interactions**

Vladislav M. Panin*, Venizelos Papayannopoulos*, Richa Wilson* & Kenneth D. Irvine

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Ser DI feedback regulation in wing

\[\alpha\text{Ser} \quad \alpha\text{DI}\]

- **ptcGal4**
  - DI in ventral
  - Activates Ser
  - In dorsal

Ser in dorsal
- Activates DI
- In ventral

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Ser and DI induce each others expression
- Dorsal ventral assymetrical
- Positive feedback loop restricted to DV boundary

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\[N_{\text{intra}}\]
Fringe is responsible for differential response of N to its ligands

1) Frg only dorsal
2) Ectopic frg induces ectopic DV boundary
3) Frg affects expression Ser and DI
Could result from influence on their signalling activity

ptcGal4 UAS

αDI
Effects of Frg on DI and Ser activity: N targets \( wg, \) Ser in ventral cells

\[ \alpha \text{Ser} \quad \alpha \text{Wg} \]

- \( fng^{D4} \) ectopic Frg in ventral
- Ventral \( frg \) inhibits
- Effects of Ser ventrally

Frg inhibits Ser

- Misexpression of DI
- In \( frg^{D4} \) induces Ser ventrally

Frg potentiates DI
Ser active dorsally in absence of frg

Frg inhibits Ser
Frg detected at cell surface & away from producing cells

FrgHA N

UASfrgHA UASLacZ ptcGal4
Fringe Serrate in dorsal cells, Delta in ventral cells (late)

Fringe prevents N activation
By Serrate in dorsal cells

Fringe in dorsal cells
Allows N to respond to Delta

Serrate signals to N in Ventral cells

wg cut
Glycosyltransferase activity of Fringe modulates Notch–Delta interactions

Katja Brückner†, Lidia Perez*, Henrik Clausen‡ & Stephen Cohen*

Fringe glycolysates N for DV boundary formation

O-linked fucose modification*
Complex N cleavage and signalling

DI endocytosis
With N extracellular domain

N receptor made as a single
Transmembrane Protein
undergoes series cleavages

\( \gamma \) secretase
Intramembrane cleavage

Su(H)

DI

N DI interaction accompanied
By metalloprotease cleavage

Furin mediated cleavage
Kuzbanian? metalloendopeptidase

TACE mediated cleavage

Target genes \( bHLH \) (\( E(Spl) \) complex),
vestigial
Spatial regulation of signal

Fringe glycolysation of N D wing
Nubbin repression of target genes

\[ \text{DI} \rightarrow \text{N} \] (lateral inhibition)

DI triggers transendocytosis of \( \text{N}^{\text{ECN}} \)

Neuralised: membrane associated protein
2 novel repeats + C-terminal RING
(E3 ubiquitin ligase activity)
DI is detected: cell membrane and in particles inside cell

Expression of Neu coincides with intracellular DI particles

EYE

WING (24h APF Neu in central provein)

αDI
αNeu

αDI
αNeu

DI accumulation in SOPs (neuZ)

Most wing disc cells
No Neu, DI pericellular Only in SOPs DI intra

Reduced DI apically
Loss of *neu* clones: DI expression

Increase in pericellular DI

Control E(Spl) clones
No effect on DI

Neu blocks DI internalisation
Neu blocks DI internalisation

Ectopic Neu (GFP +) in clones = internalised DI

Not transcriptional as DI LacZ NOT affected

Where N alone NeCN NOT internalised

NeCN and DI internalise together
In ectopic Neu clones
(Not transcription of N as NLacZ unaffected)
DI internal particles are in endocytic vescicles

Particles endocytic compartment
As colocalise with Dextran
In late endosomes
Neuralised decreases DI posttranscriptionally

Two forms of DI: FL and extracellular

ombaGal4 (broad wing disc expression) UASDI

With UASGFP (control) or UASneu

MUST BE POSTTRANSCRIPTIONAL
As driven by omb

WT ombGal4 ombGal4
UASDI UASDI
UASGFP UASNeu

Neu downregulates levels DI especially cleaved isoform
Does Neu affect ability of DI/N to signal?

Looked target genes in wing pouch

Ectopic DI clones with GFP (control) or Neu: looked at *wingless* target

Nonautonomous Wg
DI alone
Only dorsal close to margin

Same when DL + Neu
But irrespective of position
Non autonomous effects
For lateral inhibition

*neu* cells not defective in receiving signal
From neighbouring wild type cells
Ubiquitinisation required for endocytic events

Is Neu lacking RING domain defective in internalisation?

NeuΔRINGGFP

Like WT Neu, ΔRINGNeu apical

Coexpression With DI: DI still apical

RING domain required for internalisation But not apical localisation

RING domain NOT required for signalling
NeuΔRINGGFP: intracellular peripheral protein
Complex N cleavage

DI endocytosis
With N extracellular domain

N DI interaction accompanied by metalloprotease cleavage

N receptor made as a single Transmembrane Protein undergoes series cleavages

Furin mediated cleavage
Kuzbanian? metalloendopeptidase

TACE mediated cleavage

γ secretase Intramembrane cleavage

Su(H) Target genes bHLH (E(Spl) complex), vestigial
Demonstration that Nintra regulates targets in nucleus following cleavage

Difficult to see nuclear N unless express Nintra alone

Adachi & Struhl

Heat shock promoter

Gal4 VP16 GV3

Gal4 VP16 GV4

X UASLacZ
Ligand dependence of NGal4VP16 transgenes

$D^+_1$

$N^+/GV3$

$N^+/GV4$

$N^+/GV3, D^+_1$

$N^+/GV4, D^+_1$
Presenilin is required for activity and nuclear access of Notch in *Drosophila*

Gary Struhl*† & Iva Greenwald†‡

*Nature (1999)*

Presenilin membrane protein multiple transmembrane domains contribute to Alzheimer’s disease by affecting processing of β-Amyloid precursor protein.

Also required for N processing

[Diagram showing β-secretase and γ-secretase with Presenilin.]
N/ Ps function in embryo

**Neuroblasts** more in ps⁻ or N⁻

**Ventral midline cells** reduction in ps⁻ or N⁻

**Wing margin clones** loss of margin and thicker veins in ps⁻ or N⁻

**Notch protein** unaffected in ps⁻
Lacks extracellular part

Active in absence of ligand
Presenilin is required for nuclear access of Notch

X UASLacZ

No ligand

No Presinilin
Nicastrin  Chung and Struhl Nat Cell Biol (2001)

Transmembrane glycoprotein identified in a complex with Presenilin
Nicastin is required for N activity

Wing margin: loss margin and thicker veins in nct or N-

Neuroblasts: more in nct or N-

Notch protein: unaffected in nct
Nicastrin required for transducing activity of transmembrane forms of Notch

\[ nct^- \text{ cells GFP}^+ \]

Expressing \( N^{ECN}, N^{intra} \) or DI

\( \alpha \text{Cut} \quad \alpha \text{Senseless} \)

Nct not required for Cut or Wg (\( \alpha \text{Ssl} \)) by Nintr

Nct not required to send DI signal

Nicastrin required for \( N^{ECN} \) signalling: no ectopic Cut; \( nct^- \) required for Cut boundary
Nicastrin is required for accumulation of Presenilin at plasma membrane

\[
\frac{F_{1}^{-32}}{F_{1}^{-32}} \quad nct \quad \frac{\text{Tub}\alpha_{1}-\text{PS-HA}}{\text{ubiGFP}}
\]

\[
\begin{aligned}
nct & \quad \text{GFP}^- \\
nct^+ & \quad 2 \times \text{GFP} \\
* & \quad \text{Lack Tubulin PS HA transgene}
\end{aligned}
\]

Tubulin\(\alpha_{1}\)-PS-HA transgene