Supplementary Data

Methods

Plasmid construction was done by ET cloning using homologous recombination in E. coli (Zhang et al., 1998) and standard cloning procedure. For the generation of APP fusion construct, a DNA fragment coding for amino acids 2-87 of LexA and amino acids 410-489 of VP16 (LV) together with a chloramphenicol resistance gene was amplified by PCR with primers containing homologies to the C-terminus of APP and its 3'UTR. To ensure that the released IC is translocated to the nucleus, we inserted the NLS from the SV 40 T-antigen between APP and LV. This fragment was inserted into a cDNA of APP695 or SPA4CT-DA (Lichtenthaler et al., 1999), fusing LV directly to the coding sequence of APP.

The Notch constructs were made by fusing amino acids 410-489 of VP16 to N^{LexA} and $N^{\Delta EGF1\text{-}36\text{-}LexA}$ (Kidd et al., 1998) again using recombination and selection for chloramphenicol resistance. For generation of $N^{S2\Delta CT/LV}$, the remaining extracellular domain until amino acids 1725 and the intracellular domain starting from amino acids 1771 of $N^{\Delta EGF/LV}$ were deleted by recombination. After restriction enzyme mediated excision of the selection marker, all fusion constructs were subcloned into pUAST.

The reporter LexA-hrGFP was made by replacing the LacZ gene in LexA-lacZ (Kidd et al., 1998) with the hrGFP gene amplified from the vector phrGFP-C (Stratagene). pWIZ-PS/RNAi was made by cloning a PCR amplified fragment containing nucleotides 1281-1781 of the *Drosophila* PS cDNA into the pWIZ vector as suggested by Lee and Carthew (2003).

Primer sequences

Italic characters represent nucleotides homologues to APP or Notch, underlined characters the PCR primer and **bold** characters the inserted NLS.

APP^{LV} and $SPA4CT^{LV}$

up:

*GGCTACGAAAATCCAACCTACAAGTTCTTTGAGCAGATGCAGAAC***CCTCCAAAAAA GAAGAGAAAGGTA**ATGAAAGCGTTAACGGCCAGGCAACAAG

down:

*CCGATGGGTAGTGAAGCAATGGTTTTGCTGTCCAACTTCAGAGGCTGCTG*TTACGC CCCGCCCTGCCACTCATC

N^{LV} and $N^{\Delta EGF/LV}$

up:

*GTTTGTCCAATTATGTCACACCACAGAAGTAAGGTTCCTTCACAAAGATC*GGCGCG CCTTACGCCCCGCCCTGCCACTCATC

down:

*AAGAGGAAGAAGGGTTGCCGCTGGTAGGTCGTGTGGCTGCCGGTGAACCT*CTGTC GACGGCCCCCCCGAC

$N^{S2\Delta CT/LV}$

Deletion of the EC

up:

*GCCGGCGGCTCACCATTGTCCTCATCCCCGGGATTCTTGATGCCCCTAAC*CGCAA ACGCCAGCGTCAACAGCAG

down:

*CTGAATAGGGAATTGGGAATTCGTTAACAGATCTGCGGCCGCGGTCTAGA*GGTAC

CTTACGCCCCGCCCTGCCACTCATC

Deletion of N^{ICD}

up:

*GCATTGGCCTTCTTTGGCATGGTCTTGAGTACGCAAAGAAAGCGG***CCTCCAAAAA**

AGAAGAGAAAGGTAATGAAAGCGTTAACGGCCAGGCAACAAG

down:

*AAGAGGAAGAAGGGTTGCCGCTGGTAGGTCGTGTGGCTGCCGGTGAACC*TCTGTC

GACGGCCCCCCCGAC

lexA-hrGFP

up: GACGGTACCACCATGGTGAGCAAGCAGATCCTGAAG

down: TACCTGGTACCCTATTACACCCACTCG

PsnRNAi

up: GACTCAGACTGAGGCTGCGGGTTTCACGCAAG

down: GACTCTAGAGGATTACAAAAAGCTTTGTTAGACTATGATACTG

Supplementary Table I. Partial amino acid sequence of the LexA-VP16 fusion constructs

Construct	Amino acid sequence
N^{LV}	…NNLYISGGHQANKGSEAIYI - **MKALTARQQEVFDLIRDHIS**…
$N^{\Delta EGF/LV}$	…LLTLAFANLPNTVRGTDTAL - IYDANYPGWNGGSGSGNDRY…NNLYISGGHQANKGSEAIYI - **MKALTARQQEVFDLIRDHIS…**
$N^{S2\Delta CT/LV}$	…LPASLPLLLLTLAFA – VRGIKNPGDEDNGEPPANVKYVITGIILVIIALAFFGMVLSTQRKR – *PPKKKRKV* - **MKALTARQQEVFDLIRDHIS…**
APP^{LV}	…NPTYKFFEQMQN - *PPKKKRKV* - **MKALTARQQEVFDLIRDHIS…**
$SPA4CT^{LV}$	MLPGLALLLLAAWTARADA - DAEFRHDSGYEVHHQKLVFF…KMQQNGYENPTYKFFEQMQN - *PPKKKRKV* - **MKALTARQQEVFDLIRDHIS…**

The partial sequences of the Notch- and APP-LV fusions are shown with signal sequences (underlined), the inserted nuclear localization sequences (*italic*) and LexA sequences (**bold**). For $N^{S2\Delta CT/LV}$ the complete sequence of the remaining extracellular and transmembrane domain is shown.

References

Kidd, S., Lieber, T. and Young, M.W. (1998). Ligand-induced cleavage and regulation of nuclear entry of Notch in *Drosophila melanogaster* embryos. *Genes Dev.*, **12**:3728-3740.

Lee, Y.S. and Carthew, R.W. (2003). Making a better RNAi vector for *Drosophila*: use of intron spacers. *Methods*, **30**:322-329.

Lichtenthaler, S.F., Multhaup, G., Masters, C.L. and Beyreuther, K. (1999). A novel substrate for analyzing Alzheimer's disease gamma-secretase. *FEBS Lett.,* **453**:288-292.

Zhang, Y., Buchholz, F., Muyrers, J.P. and Stewart, A.F. (1998). A new logic for DNA engineering using recombination in *Escherichia coli*. *Nat. Genet.*, **20**:123-128.