Numb suppresses the negative complementation at the Notch locus of Drosophila melanogaster, suggesting a putative mechanism for negative complementation

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Summary

The mutant form of the intracellular asymmetrically localized Numb membrane-bound protein of Drosophila melanogaster suppresses the negative complementation of certain Abruptex (Ax) mutations of the Notch (N) locus encoding a transmembrane receptor protein in which the Ax mutations are mutations in the epidermal growth factor (EGF)-like repeats of the extracellular domain of the receptor. One model for how Ax mutants affect N function is that they are refractory to an antagonistic signal generated by an excess of N ligands. Genetically numb (nb) is an antagonist of N. In the absence of nb, cells follow the same fate as they would in the presence of a gain-of-function N allele, such as Ax. Numb has been shown to interact with the cytoplasmic domain of Notch. It is therefore suggested that numb counteracts the effect of Abruptex on Notch ligand binding, i.e. that Numb is an antagonist to the activation of the Notch signal generated by Notch ligands. Numb might accomplish this by interfering with the proteolytic cleavage of the Notch intracellular domain at the cell membrane. Thus, it seems possible that the mechanism of negative complementation of certain Ax mutants is the failure of this cleavage. Other possible mechanisms for negative complementation are also discussed.

1. Introduction

Notch is a transmembrane receptor protein that participates in a highly conserved cell-to-cell signalling pathway that regulates morphogenesis in metazoan animals (Simpson, 1994; Artavanis-Tsakonas et al., 1995). Ligands of the Notch protein in Drosophila melanogaster include the products of the Delta (Dl, 3-66.2) and Serrate (Ser, 3-91.9) genes (reviewed by Simpson, 1994; Artavanis-Tsakonas et al., 1995), and their binding sites in the Notch protein are in the epidermal growth factor (EGF) motif (Rebay et al., 1991). Delta and Serrate have a positive effect on Notch by activating its function (Fehon et al., 1991; Kooh et al., 1993).

In Drosophila melanogaster the Abruptex (Ax, 1-3.0) mutations are a particular type of mutation occurring at the Notch (N, 1-3.0) locus. They are point mutations in the EGF-like repeats of the extracellular domain of the receptor protein (Wharton et al., 1985; Kidd et al., 1986). The Ax mutations are characterized by lack of bristles on the head and thorax and interruption of wing veins, and can be divided into recessive lethals and viable alleles. The viable alleles, for their part, can be divided into suppressors (Ax$^{56N}$) and enhancers (Ax$^{E0X}$) of the Notch mutations. The enhancers and suppressors of Notch show an interesting type of allelic interaction, namely negative complementation; in other words, heteroallelic combinations of these viable alleles are lethal or semilethal (Ax$^{28}$/Ax$^{E7}$) (Foster, 1975; Portin, 1975). Mutations of the Delta gene act as suppressors of the negative complementation (Xu et al., 1990). One model for how Ax mutants affect N function is that they are refractory to an antagonistic signal generated by an excess of N ligands (De Celis & Bray, 2000).

The Notch signal is mediated from the cell membrane to the nucleus by proteolytic cleavage of the intracellular domain from the extracellular domain and is, together with the product of the Suppressor of Hairless gene [Su(H), 2-50.8], moved to the nucleus, where they constitute a transcription factor (Le-courtois & Schweisguth, 1997, 1998; Kidd et al.,...
Ax28 studied were Notch Abruptex mutants. The mechanism of negative complementation of certain Notch mutations with the cell membrane. Thus, it seems possible that the teolytic cleavage of the Notch intracellular domain at a specific site might accomplish this by interfering with the pro-teolytic cleavage of the Notch intracellular domain at the cell membrane. This suggests that Numb counteracts the effect of Abruptex on Notch ligand binding, in other words, suggesting that Numb antagonizes the transport of Notch signal from the cell surface into the nucleus.

The numb (nb, 3-35) gene for its part encodes a membrane-associated intracellular protein that is asymmetrically localized in the cell (Rhyu et al., 1994; Posakony, 1994) and antagonizes Notch in the development of the central and peripheral nervous system (Spana et al., 1995; Campos-Ortega, 1996; Frise et al., 1996; Sapa & Doe, 1996; Guo et al., 1996; Park et al., 1998; Wai et al., 1999). The known mutations of the numb gene are recessive embryonic lethals, because in these mutants the neurons of the peripheral nervous system of the embryo cannot acquire their correct identity (Lindsley & Zimm, 1992). In the absence of nb, cells follow the same fate as they would in the presence of a gain-of-function N allele, such as Ax (De Celis & Bray, 2000). Therefore, there is the possibility that numb antagonizes Ax, and thus the study of their interaction might elucidate the mechanism of the negative complementation of certain Ax mutations.

Here I show that numb suppresses negative complementation between the viable Abruptex mutations, suggesting that Numb counteracts the effect of Abruptex on Notch ligand binding, in other words, that Numb is an antagonist to the activation of the Notch signal generated by Notch ligands. Numb might accomplish this by interfering with the proteolytic cleavage of the Notch intracellular domain at the cell membrane. Thus, it seems possible that the mechanism of negative complementation of certain Ax mutants is the failure of this cleavage, even though other mechanisms are also possible.

2. Materials and methods

The suppressors of the Notch Abruptex mutations studied were Ax28 and Ax8b2, and the enhancers of the Notch mutations were Ax1d4, Ax82, and Ax6d72. In the experimental crosses all the pairwise combinations on the nb2 pr ch Bc/SM6B background of these mutations were studied for female viability as compared with their Abruptex brothers by crossing homozygous Abruptex females carrying nb2 pr ch Bc/SM6B autosomes with the respective males (except in the case of the female sterile Ax902 allele, where Ax902/Basic; nb2 pr ch Bc/SM5B females were crossed to Ax902/Y males carrying the same autosomal marker combination: numb-2, nb2, 2-35; purple, pr, 2-54.5; chubby, ch, 2-73.8; Black cells, Bc, 2-80.6). The control crosses were otherwise identical with the experimental crosses, but the autosomes both in female and male parents were of wild type.

The crosses were made on a standard Drosophila medium at 25 °C.

3. Results

The initial rationale of this study was the fact that in the absence of numb, cells follow the same fate as they would in the presence of a gain-of-function N allele, such as Ax (De Celis & Bray, 2000). Therefore, there is the possibility that numb antagonizes Ax, and thus the study of their interaction might elucidate the mechanism of the negative complementation of certain Ax mutations.

In the experimental crosses all the progenies were non-purple, non-chubby and curled winged, showing that they carried one copy of the numb gene, the nb2/nb2 genotypes being lethal.

In the control crosses all the females carrying suppressor of Notch/enhancer of Notch of the Abruptex mutations were either lethal or semilethal (Ax28/Ax82) (Table 1).

| Suppressors of Notch | Enhancers of Notch |
|----------------------|---------------------|
| 28                   | 9B2                 |
| 100                  | 100                 | 54.4 | 0 | 0.7 |
| (248)                | (356)               | (1752) | (155) | (441) |
| 9B2                  | E2                  |
| 100                  | 0                   | 0 | 0 |
| (543)                | (362)               | (656) | (367) |
| E2                   | 16172               | 100 | 100 |
| 100                  | (703)               | (1119) | (1905) |
| 16172                | 100                 | 100 |
| (1520)               | (2261)              |
| 71d                  |                     |
| 100                  | (526)               |

Viabilities in percentages of homo- and heteroallelic combinations of certain Abruptex mutations on the wild-type autosomal background were calculated by dividing the number of female progeny by the number of their Abruptex brothers and multiplying by 100. In parentheses are given the total number of flies.
4. Discussion

The lethal crisis of the negative complementation between the Abruptex mutations occurs at the late pupal stage (Foster, 1975; Portin, 1975). The phenocritical period is at the transition between the third instar larval and pupal stages (Portin & Sirén, 1976). On the basis of the analysis of gynandromorphs, the lethal focus is near the ventral structures of the thorax, and is a single focus (Portin, 1977). Immunolabelling of the proteins has shown that the negative interaction of Abruptex proteins most likely occurs within a single cell and not between cells (Fehon et al., 1990).

Genetic studies have indicated that numb acts upstream of Notch, and biochemical studies have revealed that Numb can bind Notch (Guo et al., 1996). For a functional assay of the action of Numb on Notch signalling, these proteins have been expressed in cultured Drosophila cells. Nuclear translocation of Suppressor of Hairless [Su(H)] was used as a reporter for Notch activity. It was found that Numb interfered with the ability of Notch to cause nuclear translocation of Su(H) (Frise et al., 1996).

The mechanism of negative complementation at the Notch locus is not known, and therefore the very aim of this study is to try find hints for the elucidation of this mechanism in spite of the fact that the results of this study are rather surprising, and biochemical data are lacking.

How is it possible that the mutant membrane-bound intracellular Numb protein suppresses the negative interaction of Notch transmembrane proteins that carry point mutations at the EGF-like repeats on their extracellular domains? The best putative answer I can see is that Numb is involved in the proteolytic cleavage of the Notch receptor at the cell membrane. This proposition, however, needs careful explanation. The Notch receptor has 36 EGF-like repeats (Wharton et al., 1985; Kidd et al., 1986). The Ax mutations map to repeats 25–30 (Kelley et al., 1987) while Delta binds to EGF-like repeats 11–12 (Rebay et al., 1991). There could, however, be interaction between these distantly located EGF-like repeats. In fact, De Cels & Bray (2000) proposed this possibility when they explained how the Abruptex phenotype might arise, and on the basis of the results of Brennan et al. (1999) it can be concluded that Abruptex inhibits the transport of the Notch signal from the cell membrane into the nucleus. Numb could possibly counteract the effect of Abruptex on Notch ligand binding, that is, Numb may be an antagonist to the activation of the Notch signal generated by Notch ligands (Spana et al., 1995; Campos-Ortega, 1996; Frise et al., 1996; Spana & Doe, 1996; Guo et al., 1996; Park et al., 1998; Wai et al., 1999). Numb might accomplish this by interfering with the proteolytic cleavage of the Notch intracellular domain at the cell membrane. In fact, the available evidence indicates that Numb acts by inhibiting nuclear accumulation of the Notch intracellular domain (Frise et al., 1996; Wakamatsu et al., 1999). However, it is not yet known if Numb also affects proteolytic processing of Notch. However, I would not in particular favour impairment of nuclear accumulation as a mechanism of negative complementation, even though this remains an alternative, because negative complementation most likely involves interaction of Notch receptors in the EGF-like motif of the extracellular domain. This suggestion

Table 2. Results of the experimental crosses

| Suppressors of Notch | Enhancers of Notch |
|----------------------|--------------------|
| 28                   | E2                 | 16172  | 71d    |
| 100                  | 100                | 100    | 100    |
| (623)                | (692)              | (1029) | (969)  |
| 9B2                  | 100                | 100    | 100    |
| (870)                | (385)              | (1055) | (883)  |
| E2                   | 100                | 100    | 100    |
| (368)                | (1055)             | (883)  |        |
| 16172                | 100                | 100    |        |
| (802)                | (860)              |        |        |
| 71d                  | 100                |        |        |
| (547)                |                    |        |        |

Viabilities in percentages of homo- and heteroallelic combinations of certain Abruptex mutations on the nb2 pr ch Bc/SM6B background calculated by dividing the number of female progenies by the number of their Abruptex brothers and multiplying by 100. In parentheses are given the total number of flies.
naturally needs experimental evidence with biochemical methods at the cellular and molecular levels.

If the suggestion presented above is correct, it could be proposed that the mechanism of negative complementation would be failure of the proteolytic cleavage of the Notch receptor at the cell membrane due to an impairment of the interaction of the Ax mutant sites and ligand binding sites among the EGF-like repeats of the Notch receptor. It is worth noticing that in a recent article De Celis & Bray (2000), on the basis of immunocytochemical studies, proposed that the Abruptex phenotype results from blocking of the inhibitory activity of high concentrations of Notch ligands. Moreover, Kadesch (2000) in his review article suggested that the Notch extracellular domain actively inhibits terminal proteolytic events, and that the ligand serves to neutralize the extracellular domain. Thus, if now Abruptex mutants interfere with the ligand binding, and in this way inhibit the cleavage of the Notch receptor, the results of this study become comprehensible.

Other plausible mechanisms of the negative complementation include blocking of Notch transport to the cell surface, processing of Notch to form a heterodimer, delivery of the ligand to the cell surface, transport of the Notch signal from the cell membrane into the nucleus, and downregulation of the receptor prior to ligand binding. I, however, favour the alternative of the blocking of intracellular cleavage, since this alternative is most compatible with the results of this study and present knowledge on the genetics of the Notch signalling pathway. Unfortunately, the discussion of the interesting results of this study necessarily remain speculative. Therefore this paper is mainly a starting point of work that addresses the problem of negative complementation.

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References

Artavanis-Tsakonas, S., Matsuno, K. & Fortini, M. E. (1995). Notch signaling. Science 268, 225–232.

Brennan, K., Tateson, R., Lieber, T., Couso, J. P., Zecchini, V. & Arias, A. M. (1999). The Abruptex mutations of Notch disrupt the establishment of proneural clusters in Drosophila. Developmental Biology 216, 230–242.

Campos-Ortega, J. A. (1996). Numb diverts Notch pathway off the tramtrack. Neuron 17, 1–4.

de Celis, J. F. & Bray, S. J. (2000). The Abruptex domain of Notch regulates negative interactions between Notch, its ligands and Fringe. Development 127, 1291–1302.

Fehon, R., Kooh, P., Rebay, I., Regan, C. L., Xu, T., Muskavitch, M. A. T. & Artavanis-Tsakonas, S. (1990). Molecular interaction between the protein products of the neurogenic loci Notch and Delta, two EGF-homologous genes in Drosophila. Cell 61, 523–534.

Fehon, R. G., Johansen, K., Rebay, I. & Artavanis-Tsakonas, S. (1991). Complex cellular and subcellular regulation of Notch expression during embryonic and imaginal development of Drosophila: implication for Notch function. Journal of Cell Biology 113, 657–669.

Frise, E., Knoblich, J. A., Younger-Shepherd, S., Jan, L. Y. & Jan, Y. N. (1996). The Drosophila Numb protein inhibits signaling of the Notch receptor during cell-cell interaction in sensory organ lineage. Proceedings of the National Academy of Sciences of the USA 93, 11925–11932.

Foster, G. G. (1975). Negative complementation at the Notch locus of Drosophila melanogaster. Genetics 81, 99–120.

Guo, M., Jan, L. Y. & Jan, Y. N. (1996). Control of daughter cell fates during asymmetric division: interaction of Numb and Notch. Neuron 17, 27–41.

Jan, W.-C., Gawantha, V., Poller, N., Niehrs, C. & Kintner, C. (1999). Periodic repression of Notch pathway genes governs the segmentation of Xenopus embryos. Genes & Development 13, 1486–1499.

Kadesch, T. (2000). Notch signaling: a dance of proteins changing partners. Experimental Cell Research 260, 1–8.

Kelley, M. R., K Sidd, S., Deutsch, W. A. & Young, M. W. (1987). Mutations altering the structure of epidermal growth factor-like coding sequences at the Drosophila Notch locus. Cell 51, 539–548.

Kidd, S., Kelley, M. R. & Young, M. W. (1986). Sequence of Notch locus of Drosophila melanogaster: relationship of the encoded protein to mammalian clotting and growth factors. Molecular Cell Biology 6, 3094–3108.

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

Kooh, P. J., Fehon, R. G. & Muskavitch, M. A. T. (1993). Implications of dynamic patterns of Delta and Notch expression for cellular interactions during Drosophila development. Development 117, 493–507.

Lecourtois, M. & Schweisguth, F. (1997). Role of Suppressor of Hairless in the Delta-activated Notch signaling pathway. Perspectives of Developmental Neurobiology 4, 305–311.

Lecourtois, M. & Schweisguth, F. (1998). Indirect evidence for Delta-dependent intracellular processing of Notch in Drosophila embryos. Current Biology 18, 771–774.

Lindsley, D. L. & Zimm, G. G. (1992). The Genome of Drosophila melanogaster. San Diego, CA: Academic Press.

Park, M., Yäch, L. E. & Bodmer, R. (1998). Mesodermal cell fate decisions in Drosophila are under the control of the lineage genes numb, Notch, and Sampodo. Mechanism of Development 75, 117–126.

Pavan, P. (1975). Allelic negative complementation at the Abruptex locus of Drosophila melanogaster. Genetics 81, 121–133.

Portin, P. (1977). Analysis of the negative complementation of Abruptex alleles in gynandromorphs of Drosophila melanogaster. Genetics 86, 309–319.

Portin, P. & Sire, M. (1976). Timing of temperature sensitive period for lethality of Abruptex mutations of Drosophila melanogaster. Hereditas 84, 109–118.

Posakony, J. W. (1994). Nature versus nurture: asymmetric cell divisions in Drosophila bristle development. Cell 76, 415–418.

Rebay, I., Fleming, R. J., Fehon, R. G., Cherbas, L., Cherbas, P. & Artavanis-Tsakonas, S. (1991). Specific
EGF repeats of Notch mediate interactions with Delta and Serrate: implications for Notch as a multifunctional receptor. *Cell* 67, 687–699.

Rhyu, M. S., Jan, L. Y. & Jan, Y. N. (1994). Asymmetric distribution of Numb protein during division of the sensory organ precursor cell confers distinct fates to daughter cells. *Cell* 76, 477–491.

Simpson, P. (1994). *The Notch Receptors*. Austin: R. G. Landes Company.

Spana, E. P. & Doe, C. Q. (1996). Numb antagonizes Notch signaling to specify sibling neuron cell fates. *Neuron* 17, 21–26.

Spana, E. P., Kopczynski, C., Goodman, C. S. & Doe, C. Q. (1995). Asymmetric localization of numb autonomously determines sibling neuron identity in the *Drosophila* CNS. *Development* 121, 3489–3494.

Wai, P., Truong, B. & Bhat, K. M. (1999). Cell division genes promote asymmetric interaction between Numb and Notch in the *Drosophila* CNS. *Development* 126, 2759–2770.

Wakamatsu, Y., Maynard, T. M., Jones, S. U. & Weston, J. A. (1999). Numb localizes in the basal cortex of mitotic avian neuroepithelial cells and modulates neuronal differentiation by binding to NOTCH1. *Neuron* 23, 71–81.

Wharton, K. A., Johansen, K. M., Xu, T. & Artavanis-Tsakonas, S. (1985). Nucleotide sequence from the neurogenic locus Notch implies a gene product that shares homology with proteins containing EGF-like repeats. *Cell* 43, 567–581.

Xu, T., Rebay, I., Fleming, R. J., Scottgale, T. N. & Artavanis-Tsakonas, S. (1990). The Notch locus and the genetic circuitry involved in early *Drosophila* neurogenesis. *Genes & Development* 4, 464–475.