Notch Responds Differently to Delta and Wingless in Cultured Drosophila Cells*

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Notch, a cell surface receptor, is required for producing different types of cells during development of Drosophila melanogaster. Notch activates expression of one set of genes in response to ligand Delta and another set of genes in response to ligand Wingless. The means by which Notch initiates these different intracellular activities was examined in this study. Cultured cells expressing Notch were treated with Delta or Wingless, and the effect on Notch was examined by Western blotting. Treatment of cells with Delta resulted in accumulation of ~120-kDa Notch intracellular domain molecules in the cytoplasmic fraction. This form of Notch did not accumulate in cells treated with Wingless, but the ~350-kDa full-length Notch molecules accumulated. These results indicate that N responds differently to binding by Delta and Wingless, and suggest that although the Delta signal is transduced by the Notch intracellular domain released from the plasma membrane, the Wingless signal is transduced by the Notch intracellular domain associated with the plasma membrane.

Notch (N) is required for the specification of different cell types during development of Drosophila melanogaster (1). It is a cell surface receptor, the intracellular activities of which are regulated by ligands binding to the extracellular domain. Delta (Dl) is the ligand for the well known N functions associated with lateral inhibition. During lateral inhibition, N and Dl produce the neuronal precursor cells that differentiate the cuticle (1–4). Wingless is the ligand for some N functions whose products specify the sensory bristles (5–7). N binds both Dl and Wg, in vivo and in vitro (7). It regulates expression of the Enhancer of split Complex and wingless in response to Dl (1, 11–14). In response to Wg, it regulates expression of patched, shaggy, and hairy, but not Enhancer of split Complex and wingless (7). Thus, the same receptor regulates different sets of genes in response to Dl and Wg.

N intracellular signals in response to Dl are mediated by the Suppressor of Hairless (Su(H)) signal transduction pathway (11–13, 15–16). The signaling pathway used by N to transduce the intracellular signals in response to Wg is unknown. Because Wg binding does not activate expression of Enhancer of split Complex and wingless (7), it is unlikely to be the same Su(H) pathway used with Dl. Furthermore, Wg-dependent functions of N during development are distinct from Dl-dependent N functions (5–10). These observations indicate that full-length N generates different intracellular signals in response to Dl and Wg. However, the same N receptor generates one signal after binding Dl and a different signal after binding Wg.

We treated N-expressing cultured cells with Dl and Wg and found out that N responds differently to binding by these two ligands. This differential response likely initiates transduction of different signals to the nucleus.

MATERIALS AND METHODS

S2-N and S2-Dl cells are Schneider (S2) cells transfected with the Notch and Delta genes, respectively, for expression of their proteins under the control of heat shock promoter (17). S2-Notch is an S2 cell engineered to co-express the Notch and Delta genes. Delta and Wingless, Wingless and Notch, and Notch cells were treated with Delta or Wingless, and the effect on Notch was examined by Western blotting. Treatment of cells with Delta resulted in accumulation of ~120-kDa Notch intracellular domain molecules in the cytoplasmic fraction. This form of Notch did not accumulate in cells treated with Wingless, but the ~350-kDa full-length Notch molecules accumulated. These results indicate that N responds differently to binding by Delta and Wingless, and suggest that although the Delta signal is transduced by the Notch intracellular domain released from the plasma membrane, the Wingless signal is transduced by the Notch intracellular domain associated with the plasma membrane.

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The abbreviations used are: N, Notch; Dl, Delta; Wg, Wingless; Su(H), Suppressor of Hairless; EGF, epidermal growth factor.
**Fig. 1. N responds differently to treatments with DI and Wg. a, S2-N cells treated with DI accumulated N120 and N55 molecules (lanes 1 and 2 and 7 and 8) whereas S2-N cells treated with Wg accumulated the co-linear N350 and N55 molecules (lanes 5 and 6, 9 and 10, and 11–13). N120 molecules did not accumulate in S2-N cells treated with DI (lanes 3 and 4). Co-linear molecules also accumulated in S2-N cells treated with Wg (lanes 17 and 18) but did not accumulate in NΔEGF1–18 cells (lanes 15 and 16). S2-N cells treated with DI was used in lane 14 for alignment of lanes 14–18 with the other lanes. Lanes 1–13 can be aligned using minor N bands. Lanes 14–16 and lanes 17 and 18 are from the same gel exposed to autoradiographic film for different periods due to different levels of N expression. The molecules marked by an asterisk are variably produced in these experiments. The blots containing lanes 11–18 were reprobed with an anti-heat shock protein 70 antibody (Sigma) to show that the same amount of total proteins is present in these lanes. b, Clone-8 cells show similar responses to treatments with DI and Wg. c, N120 molecules accumulate in the cytoplasmic fraction whereas N350 molecules accumulate in the membrane fraction. Cells in b and c were treated for 2 h. Cells: N, NΔEGF1–18, and NΔEGF1–36 = S2 cells expressing these molecules; S2 = untransfected S2 cells; DI = S2-DI cells; Wg = media conditioned by growth of S2-Wg cells.

**DISCUSSION**

In **in vivo**, the complete N intracellular domain (−120 kDa) is released from the plasma membrane in response to DI. This domain translocates to the nucleus with Su(H) and activates expression of target genes (13, 15, 16, 22, 23). In our experiments, DI treatment results in accumulation of −120-kDa N intracellular domain molecules (N120) in the cytoplasm. N120 7–10). N55 contains only the amino terminus half of the intracellular domain, requires about 2 h to accumulate, and is variably recovered after about 3 h of treatment.

To determine whether the responses observed in S2 cells are general N responses to treatments with DI and Wg, the experiments were repeated with clone-8 cells that express N endogenously (Fig. 1b). The results showed that N in clone-8 cells responded similarly to N in S2 cells. Treatment with DI resulted in accumulation of N120 and not N350, whereas treatment with Wg resulted in accumulation of N350 and not N120; both DI and Wg treatments resulted in accumulation of N55 molecules (Fig. 1b). The difference in levels of N350 between DI-treated and Wg-treated cells is obvious here after just 2 h of treatment. Clone-8 cells express a higher level of N55 molecules in the absence of any treatment, presumably because they also express DI endogenously.2

When DI binds N in **in vivo**, the −120-kDa N intracellular domain is released into the cytoplasm (1, 15, 16, 22, 23). To determine whether the N120 in our **in vitro** experiments with DI also accumulated in the cytoplasm, S2-N cells were fractionated and analyzed following treatments with DI and Wg. Following treatment with DI, N120 molecules accumulated in the cytoplasmic fraction (Fig. 1c). In contrast, N350 molecules accumulated in the membrane fraction following treatment with Wg (Fig. 1c). N55 molecules are not consistently detected in these experiments as they are very unstable in this fractionation and extraction procedure (not shown).

We do not know whether the N120 molecules that accumulate in the cytoplasm in response to DI are the same as those present in the membranes (Fig. 1c) or whether they are different molecules migrating in the same region of the gel. Membrane-tethered N intracellular domain (Nintratr), un tethered Nintrat, and N120 migrate along side each other in these gels.2 N120 molecules associated with the membranes or with the cytoplasm are probably the membrane-tethered or released N intracellular domain, respectively. Accumulation of N350 molecules in response to Wg is likely to be in the intracellular membranes associated with production of the heterodimeric cell surface receptor (see Ref. 24–26). N55 is derived from N350 upon activation of Notch signaling by a ligand.3

**Fig. 2. A model for N action in response to DI and Wg.** DI binds N to shunt N120 and Su(H) to the nucleus for turning on expression of DI-related genes. Wg binds N to send an activator to the nucleus for turning on expression of Wg-related genes. A = an activator of transcription; E(spl)/C = Enhancer of split Complex gene; wg = wingless gene; ptc = patched gene; sgg = shaggy gene; h = hairy gene; CDC = CDC10/ankyrin repeats.
in our experiments and the ~120-kDa in vivo molecule described by others are likely the same molecules. These molecules themselves act as activators of genes responsive to Dl. In numerous experiments, treatment of S2-N or clone-8 cells with Wg never resulted in accumulation of N120. Thus, it appears that N120 is not the activator of genes responsive to Wg.

The co-linear N350 molecules accumulate to higher levels in Wg-treated cells when compared with both untreated and Dl-treated cells. Co-linear N molecules are proposed to be cut into separate extracellular and intracellular fragments that are covalently linked to produce the heterodimeric cell surface receptor (24–26). N350 accumulates in Wg-treated cells possibly because they are converted into the heterodimeric cell surface receptors at a slower rate compared with the rate in untreated S2-N cells and Dl-treated S2-N cells. This slower rate may be due to non-processing of the intracellular domain of Wg-bound N receptors and availability of limited receptor molecules that interact with the N intracellular domain rather than the N intracellular domain itself. N55 is unlikely to be involved in activation of Dl- or Wg-responsive genes because it is produced by both ligands. N55 is produced in response to Wg-bound N receptors and availability of limited receptor molecules that act as activators of genes responsive to Wg. In numerous experiments, treatment of S2-N or clone-8 cells with Wg results in accumulation of N120. Thus, N120 is not the activator of genes responsive to Wg.

Our results also suggest that the set of molecules involved in transducing N intracellular signals in response to Dl is likely to be different from the set that transduces N intracellular signals in response to Wg. We have identified what may be the initial differences between these two different N intracellular signaling pathways, the ones likely to set in motion different intracellular events. Starting with these differences, it should be possible in the future to identify the molecules that are involved in each N intracellular signaling pathway. This will enable integration and a better understanding of the functions of N, Dl, and Wg during Drosophila development.

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