Invagination centers within the *Drosophila* stomatogastric nervous system anlage are positioned by *Notch*-mediated signaling which is spatially controlled through *wingless*

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**SUMMARY**

The gut-innervating stomatogastric nervous system of *Drosophila*, unlike the central and the peripheral nervous system, derives from a compact, single layered epithelial anlage. Here we report how this anlage is initially defined during embryogenesis by the expression of proneural genes of the *achaete-scute complex* in response to the maternal terminal pattern forming system. Within the stomatogastric nervous system anlage, the *wingless*-dependent intercellular communication system adjusts the cellular range of *Notch*-dependent lateral inhibition to single-out three *achaete*-expressing cells. Those cells define distinct invagination centers which orchestrate the behavior of neighboring cells to form epithelial infoldings, each headed by an *achaete*-expressing tip cell. Our results suggest that the *wingless* pathway acts not as an instructive signal, but as a permissive factor which coordinates the spatial activity of morphoregulatory signals within the stomatogastric nervous system anlage.

Key words: cell signaling, cell selection, *Drosophila* neurogenesis, stomatogastric nervous system, tip cell

**INTRODUCTION**

Neurons are generated by the proliferation of progenitor cells that develop from undifferentiated and pluripotent ectodermal cells at an early stage of embryogenesis. In insects, recent work has focused on the origin of the central (CNS) and the peripheral nervous system (PNS) (reviewed by Campos-Ortega, 1993; Jan and Jan, 1993). Cell ablation experiments in the grasshopper embryo highlighted two important features of neural precursor formation in insects (Doe and Goodman, 1985). First, distinct groups of ectodermal epithelial cells display the potential to form the precursor cells, and second, cell interactions result in the selection of a single cell within each cell group that can potentially form a neural precursor. Mutagenesis screenings in *Drosophila* identified two classes of genes that regulate each of these processes. The ‘proneural genes’ (Romani et al., 1987; Ghysen and Dambly-Chaudiere, 1987) control competence of ectodermal cells to become neural precursor cells, and the activities of the ‘neurogenic genes’ (Poulson, 1937; Lehmann et al., 1981) prevent more than one among the competent group of cells from adopting a neural fate. Mutant analyses in *Drosophila* suggested that the proneural genes influence the activity of the neurogenic genes and are required during later stages of embryogenesis to specify the neural fate (reviewed by Campos-Ortega, 1993; Goodman and Doe, 1993; Jan and Jan, 1993).

Proneural genes include the three transcription units of the *achaete-scute complex* (*AS-C*) (Garcia-Bellido, 1979; Romani et al., 1987). The *AS-C* genes encode HLH-type transcription factors (Villares and Cabrera, 1987) which are thought to control the expression of neural-specific developmental genes. In mutants that fail to express the *AS-C* genes, neuroectodermal cells lose their ability to follow a neural fate and contribute instead to the epidermis (Garcia-Bellido, 1979; Jiménez and Campos-Ortega, 1979; Dambly-Chaudiere and Ghysen, 1987; Jiménez and Campos-Ortega, 1990). The *AS-C* genes are initially expressed in small clusters of ectodermal cells (Cubas et al., 1991; Skeath and Carroll, 1992), the proneural cell clusters. From this cell cluster, the neural precursor cell is singled-out and continues *AS-C* gene expression (Cabrera et al., 1987). The singling-out of neural precursor cells within the proneural cell clusters is caused by a process of cell-cell communication referred to as lateral inhibition (Simpson, 1990) and results in a concomitant loss of *AS-C* gene expression in the other cells of the proneural cluster, which thereby restores an epidermal cell fate.

Singling-out by lateral inhibition is mediated by the activity of the neurogenic genes which are integrated into the *Notch*-mediated signaling pathway (Lehmann et al., 1981; Artavanis-Tsakonas et al., 1991; Fortini and Artavanis-Tsakonas, 1993). Within this intercellular communication system, the gene products of *Delta* (*Dl*) and *Notch* (*N*) seem to act as ligand and receptor, respectively (Heitzler and Simpson, 1991). Their activities are mediated by downstream components such as *Enhancer of split* (*E(spl)*) or *mastermind* (*mam*; reviewed by Campos-
Ortega, 1993). Loss-of-function mutants in any one of these genes fail to single-out AS-C gene-expressing cells and thereby show an overproduction of neural precursor cells both in the central and the peripheral nervous system (Brand and Campos-Ortega, 1988; Gorielly et al., 1991).

Here we describe the early development of the *Drosophila* stomatogastric nervous system (SNS), a system which coordinates ingestion, swallowing and peristaltic movements of the gut (reviewed by Penzlin, 1985). The SNS is composed of four ganglia which derive from a compact epithelial anlage within the dorsal roof of the stomodeal invagination. Once the SNS anlage is formed in response to positional information provided by the maternal terminal system (Perrimon, 1993), three distinct AS-C-expressing cells are single-out under the control of the N-mediated signaling pathway. The range of N-mediated signaling depends on wingless (wg) activity. Our results suggest that wg acts as a permissive rather than instructive component within the SNS anlage.

**MATERIALS AND METHODS**

*Drosophila* strains and mutant embryos

*Drosophila* strains were kept under standard conditions. Mutant alleles are described in Lindsley and Zimm (1992). Embryos were collected from stocks balanced with FM7 (Notch55c and Df(1)B57, ac–sc– lsc), CyO (mastermindBD49 and winglessG23) or TM3, Sb, Sh (Delta8, Enhancer of split1, forkheadBD76, huckebeinA82 and Df(3R)null1/2). The balancer chromosomes carried a lacZ reporter gene containing the *fushi tarazu* (FTZ) or the *hunchback* promoter (CyO and TM3) which allow homozygous mutant embryos to be unambiguously identified on the basis of the lack of hunchback or *fushi tarazu* staining patterns.

*tor*PPM embryos derived from homozygous *tor*PPM females. *Df(1)B57: Kr*1 double mutant embryos were distinguished on the basis of the lack of external sense organs in the dorsal epidermis of the trunk and on the basis of the segmentation phenotype of *Kr* (Gaul and Jäckle, 1987). *dsh*28, *arm*M19 and *sgg*M11 mutant embryos derived from females bearing homozygous mutant germ line clones. Germ line clones were generated by the ‘FLP-DFS’ technique as described by Siegfried et al. (1994). These females were mated to FM7, *fz-lacZ* males. This allows one to distinguish the respective mutant embryos that have received neither the maternal nor the zygotic wild-type product by the absence of *fz-lacZ* reporter gene expression.

Staining and embedding procedures

Antibody stainings were performed as previously described (Macdonald and Struhl, 1986). Antibodies were diluted at the following concentrations: 1:50 mAb22C10 (Fujita et al., 1982), 1:50 anti-fasciclin II (González-Gaitán et al., 1994), 1:50 anti-IhK (Weigel et al., 1989b), 1:500 anti-Kr (Gaul and Jäckle, 1987), 1:3 anti-ac (Skeath and Carroll, 1992), 1:20 anti-er-crb (Tepass and Knust, 1993), 1:1000 rabbit anti-β-galactosidase (Cappel) and 1:200 mouse anti-β-galactosidase (Promega). Whole-mount in situ hybridizations using digoxigenin-labeled probes for *lsc, sc* and *wg* cDNAs were performed according to the method of Tautz and Pfeifle (1989). After antibody staining or whole-mount in situ hybridization, embryos were embedded in glass capillaries to allow rotation under the compound microscope (Prokop and Techmann, 1993).

**RESULTS**

Morphology and origin of the *Drosophila* SNS

The SNS is located in the head region close to the embryonic brain. It consists of four prominent interconnected ganglia (Fig. 1A; overview), i.e. the frontal ganglion (FG), the esophageal ganglia (EG1 and EG2) and the proventricular ganglion (PG) (González-Gaitán et al., 1994). They can be visualized by mAb22C10 (Fujita et al., 1982) (Fig. 1B-D,G,H) or by anti-fasciclin II (Grenningloh et al., 1991) antibody staining (Fig. 1E,F) at late stages of embryogenesis (González-Gaitán et al., 1994; Hartenstein et al., 1994).
The FG is composed of two groups of neurons, one on either side of the pharynx (Fig. 1A,B). They are connected by axon bundles contributing to the frontal commissure (Fig. 1B). They also connect to the subesophageal ganglion of the CNS via the frontal connectives (Fig. 1E,F). EG1 and EG2 are associated with the esophagus (Fig. 1A,D,F). EG1 extends as a row of seven pairs of neurons apposed to the esophagus (Fig. 1C); EG2 is composed of about 10 tightly clustered neurons (Fig. 1B,D). Both ganglia are connected to the FG via the recurrent nerve (Fig. 1B,D,F). The PG is formed by a cluster of about 10 neurons which are apposed to the proventriculus (Fig. 1A,D); Skaer, 1993). The PG is connected to the EG2 via the proventricular nerve (Fig. 1D). Axons projecting from different SNS ganglia innervate distinct portions of the gut, e.g. the FG innervates the dorsal pharyngeal muscles via the frontal nerve (Fig. 1B), the EG1 innervates the esophagus (Fig. 1C), and the PG innervates both the proventriculus and the midgut (Fig. 1G,H).

The developmental origin of the SNS ganglia can be traced back to a compact, single-layered epithelial anlage at the dorsal roof of the stomodeal invagination (Fig. 2A; Poulson, 1937; Schoeller, 1964; Campos-Ortega and Hartenstein, 1985). During stage 10 (stages according to Campos-Ortega and Hartenstein, 1985), the stomodeal invagination is characterized by the expression of forkhead (fkh; Fig. 2B), a gene involved in gut development (Weigel et al., 1989a,b). At this stage, the SNS anlage is spatially defined by the localized expression of the AS-C proneural genes achaete (ac), scute (sc) and lethal of scute (l’sc) (Romani et al., 1987), and by the expression of the gap gene Krüppel (Kr; Gaul and Jäckle, 1987) (Fig. 2C-G).

SNS development becomes morphologically distinct by the appearance of three evenly spaced dorsal invaginations (Schoeller, 1964; Campos-Ortega and Hartenstein, 1985; Hartenstein et al., 1994; Fig. 3A), which can be visualized by anti-crumbs (crb) antibody staining (Tepass and Knust, 1993), by fkh and by Kr expression (Fig. 3C-F). At stage 14, the invaginations detach from the developing esophagus, and each forms a vesicular structure (Fig. 3B; see also Hartenstein et al., 1994) which continues to express fkh (Fig. 3C-F) until the stereotyped pattern of the SNS ganglia emerges (cf. Fig 1B,C and Fig. 3G,H; see González-Gaitán et al., 1994; Hartenstein et al., 1994).

SNS anlage formation requires activity of the maternal terminal system
The functional significance of fkh, Kr and AS-C gene expression for SNS development is apparent from the mutant phenotypes (Figs 4,5). fkh mutant embryos lack the SNS (Fig. 4A,B) and fail to express Kr and the AS-C gene expression in the SNS anlage (Fig. 5A,B). In the absence of AS-C gene activities, remnants of SNS structures can be observed (Fig. 5C). In the absence of Kr, SNS ganglia are formed (Fig. 5D). Thus, AS-C gene expression is required for the normal SNS development, whereas Kr activity is not necessary. However, the AS-C mutant phenotype is strongly enhanced in embryos lacking both Kr and AS-C gene activities (Fig. 5E). This observation suggests that Kr and AS-C genes may function in a common developmental pathway, implying a proneural-like function of Kr during an early stage of SNS development.

Since fkh expression requires the maternal terminal signal transduction pathway (Gaul and Weigel, 1990; Weigel et al., 1990), we examined the SNS of embryos lacking the activity of torso (tor). tor encodes a membrane-spanning tyrosine kinase receptor activity (Casanova and Struhl, 1989; Sprenger et al., 1989). Upon local activation, tor initiates the terminal transduction pathway, activating downstream signaling cascades that mediate the establishment of the rostral-caudal axis, the establishment of segmental identities, and the differentiation of neuronal lineages. Tor signaling is also involved in the regulation of cell proliferation and apoptosis, as well as in the control of cell fate decisions during embryonic development. The precise mechanisms by which Tor signaling regulates SNS development remain to be elucidated.
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signal transduction pathway (reviewed by Perrimon, 1993). Fig. 4C shows that embryos fail to develop SNS structures when tor activity is absent.

The activated tor signaling pathway eventually leads to localized zygotic target gene expression including the terminal gap genes tailless (tll) and huckebein (hkb) (Weigel et al., 1990). The activities of both genes are required for fkh expression (Gaul and Weigel, 1990; Weigel et al., 1990). tll or hkb embryos fail to develop normal SNS ganglia, although remnants can be observed (Fig. 4D,E). This suggests that hkb and tll act through fkh and that the remaining fkh activity observed in their absence (Gaul and Weigel, 1990; Weigel et al., 1990) is sufficient to generate some SNS remnants.

**Invagination centers are selected by N-mediated signaling**

ac, sc and l’sc are initially expressed in all cells of the SNS anlage (Fig. 2E-G). While l’sc expression continues during the
subsequent invagination process (Fig. 6A,B), both ac and sc expression become restricted to three cells (Fig. 6C,E). The singled-out ac- and sc-expressing cells represent invagination centers in the SNS anlage and they characterize the tip cells of each invagination (Fig. 6G,H). Once the folds are maximally extended, ac and sc become re-expressed throughout the epithelial folds and consequently all SNS cells undergo a second round of proneural gene expression (Fig. 6B,D,F).

In order to establish the genetic requirement for the singling-out of invagination centers, we used ac expression as a marker to examine the selection of invagination centers in neurogenic mutant embryos that lack integral components of the N-mediated signaling pathway (Lehmann et al., 1981; Artavanis-Tsakonas et al., 1991; Fortini and Artavanis-Tsakonas, 1993). In N mutant embryos, initial ac expression covers the SNS anlage as in wild type (not shown). During late stage 10, however, when the three ac-expressing cells are normally singled-out, ac expression continues throughout the SNS anlage (Fig. 7A). Instead of forming distinct invagination folds, the N mutant SNS anlage invaginates en masse (Fig. 7E). In Dl, E(spl) or mam homozygous mutant embryos, ac expression is also not restricted (Fig. 7B-D), and the SNS anlage invaginates as observed in the N mutants (Fig. 7F-H). These results indicate that the singling-out of ac-expressing cells in the wild-type SNS anlage is dependent on N-mediated lateral inhibition as observed for early CNS and PNS development. However, instead of delaminating, the ac-expressing cells of the SNS anlage define the position of invagination centers.

The range of N-mediated signaling in the SNS depends on the wg pathway

When analysing wingless (wg) mutant embryos, we noted an SNS phenotype (see below) which suggested that the range of N-mediated signaling in the SNS anlage may depend on a second intercellular communication system which depends on wg activity (reviewed by Perrimon, 1994). The essential components of the wg signaling pathway include wg itself, dishev-
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elled (dsh) (Klingensmith et al., 1994), shaggy/zeste white 3 (sgg; Siegfried et al., 1992) and armadillo (arm; Peifer and Wieschaus, 1990). dsh, sgg and arm are expressed in all Drosophila cells, while the expression of the secreted wg protein is spatially and temporally restricted (van den Heuvel et al., 1989; Siegfried et al., 1992; Li and Noll, 1993; Peifer et al., 1993; Klingensmith et al., 1994). Analysis of their epistatic relationships showed that wg activates dsh-dependent inhibition of sgg, which in turn acts negatively on arm (Noordermeer et al., 1994; Perrimon, 1994; Siegfried et al., 1994).

Fig. 5. Krüppel and the achaete-scute complex genes act synergistically during SNS development. (A) Anti-Kr and (B) anti-ac antibody stainings of fkh^{27a} mutant embryos at stage 10. No expression of these genes is detected in the roof (arrows) of the stomodeum which is substantially reduced. (A,B) Orientation of the embryos is anterior to the left and dorsal up (lateral views). (C) mAb22C10 staining at stage 17 of Df(1)B57 mutant embryo, lacking the AS-C genes. Remnants of the SNS ganglia can be detected in Df(1)B57 homozygous mutant embryos. Most frequently the EG1 (arrowhead) is seen in a position apposed to the esophagus (es); remnants of the other SNS ganglia are occasionally detected. Orientation of the embryo is anterior to the left and dorsal up (lateral view). (D) mAb22C10 staining at stage 17 of Kr^{2} mutant embryo. Note that the SNS structures are present (FG, EG1, EG2, fn; other nerves and the PG, out of focus). The abnormal appearance of some structures is caused by the Kr segmentation defect, which affects the head involution. Orientation of the embryo is anterior to the left (dorsal view). (E) mAb22C10 staining at stage 17 of Df(1)B57; Kr^{1} double mutant embryo. Note that all SNS ganglia are lacking when both Kr and the AS-C genes are absent. Remnants of what appear to be SNS structures were observed infrequently. Orientation of the embryo is anterior to the left (dorsal view). Abbreviation: bh, brain hemisphere.
At stage 10, \(wg\) expression encompasses the SNS anlage of wild-type embryos (Fig. 8A). In the absence of \(wg\) activity, initial \(ac\) expression in the SNS anlage is normal (not shown), but only one \(ac\)-expressing cell was observed in the center of the SNS anlage of the \(wg\) mutant embryos (compare Figs 6C, 8D), and only one corresponding central invagination fold was formed (Fig. 8E). The same observations were made in embryos lacking \(dsh\) or \(arm\) activity (Fig. 8F-I). In contrast, more than three \(ac\)-expressing cells (Fig. 8J) and corresponding numbers of invaginations were observed in \(sgg\) mutant embryos (Fig. 8K-M). The opposite effects seen in \(sgg\) mutants as compared to the \(wg, dsh\) or \(arm\) mutants reflect a restriction versus an expansion of the range of \(N\)-mediated signaling. This implies that the \(wg\) pathway functions to spatially control the range of \(N\)-mediated signaling in the SNS anlage to allow for the selection of the three invaginations (Fig. 9).

**DISCUSSION**

We show that the larval SNS originates from a single-layered epithelium in which distinct invagination centers are selected.
The selection process depends on lateral inhibition by \(\text{wg}\)-dependent \(\text{N}\)-mediated signaling and results in three singled-out \(\text{ac}\)-expressing cells from which distinct invagination folds are formed, each headed by an \(\text{ac}\)-expressing tip cell. Thus, unlike PNS and CNS development, SNS morphogenesis is not initiated by delamination of single neural precursor cells in a scattered pattern but rather resembles the process of vertebrate neurulation from a compact epithelium (reviewed by Gilbert, 1994).

**SNS anlage formation and neural fate**

The genetic circuitry that establishes the SNS anlage includes the known maternal and zygotic components necessary to control pattern formation in the anterior terminal region of the embryo (Perrimon, 1993). Of those, \(\text{fkh}\) activity is required for the expression of \(\text{Kr}\) and the genes of the \(\text{AS-C}\) complex. While the SNS is absent in \(\text{fkh}\) mutant embryos, the lack of the \(\text{AS-C}\) gene activities causes only a reduction of neurons, as observed during CNS development (Jiménez and Campos-Ortega, 1990), and the absence of \(\text{Kr}\) activity has no significant effect on SNS development. However, the absence of both \(\text{Kr}\) and \(\text{AS-C}\) gene activities causes a dramatic reduction of the SNS which exceeds the degree of reduction in the absence of \(\text{AS-C}\) gene activities. This observation suggests that \(\text{Kr}\) provides proneural-like function, which can be compensated for by \(\text{AS-C}\) gene activities. Since remnants of SNS ganglia can still be detected in the absence of \(\text{AS-C}\) and \(\text{Kr}\) gene activities, other components with proneural activity must act in the SNS anlage of these embryos.

Intriguing parallels between CNS/PNS and SNS development are not only apparent with respect to the initial expression patterns of the proneural genes, but also with respect to the singling-out of \(\text{ac}\)-expressing cells in response to neurogenic gene activities. However, while the selected cells already represent the CNS and PNS neural precursors (reviewed by Campos-Ortega, 1993; Goodman and Doe, 1993; Jan and Jan, 1993), the corresponding three cells of the SNS anlage define the invagination centers from which...
epithelial folds are generated. Furthermore, l'sc expression is not part of the singling-out process in response to N-mediated signaling and ac and sc expressions become reinitiated. Thus, the cells of the developing SNS combine ac, sc and l'sc activities during a second round of AS-C gene expression. In view of this second round of AS-C gene expression, the first one may not determine neural fate, but rather sets a morphoregulatory process into motion that causes epithelial invaginations. The second round of AS-C expression may therefore represent the functional equivalent to the proneural gene expression during CNS and PNS development. At present we do not know how the N-dependent singling-out of l'sc-expressing cells in the SNS anlage is circumvented and how the reinitiation of ac and sc gene expression is controlled.

Fig. 8. The number of ac-expressing cells and invagination folds depends on the wg pathway. (A) wg expression encompasses the SNS anlage of stage 10 wild-type embryos as revealed by whole-mount in situ hybridization; lateral view, anterior to the left, dorsal up. (B) Anti-achaete and (C) anti-crumbs antibody staining of wild-type SNS primordia at stage 11; dorsal view, anterior to the left. (D-M) Anti-achaete (D,F,H,J) and anti-crumbs (E,G,I,K-M) antibody stainings of wgG23 (D,E), dshv26 (F,G), armXM19 (H,I) and sggM11/L mutant embryos at stage 11; (D-L,L,M) lateral view; (J,K) dorsal view. Note a single ac-expressing cell (arrows) and the corresponding single invagination fold (arrowhead) in wgG23 (D,E), dshv26 (F,G) and armXM19 (H,I). In contrast, five ac-expressing cells (arrows) are found in sggM11/L (J, compare with the wild-type ac pattern in B). Note that the corresponding number of invagination folds appears in sggM11/L mutants (K-M). (K) A sggM11/L embryo with four invagination folds (compare with C). (L,M) Different focal planes of a lateral view of the same embryo to demonstrate the four invagination folds. Note that the number of both ac-expressing cells and the corresponding invaginations in sggM11/L embryos is variable; there are more than three and up to five. The dshv26, armXM19 and sggM11/L mutant embryos lack the respective gene activity both maternally and zygotically. For a description of the generation of such embryos see Materials and Methods. Note that during late stage 11, when the invagination process is completed, wild-type wg expression is restricted to a ring in the inner part of the esophagus that will contribute to the proventriculus development (not shown).
Singling-out of invagination centers

*N*-mediated signaling is both necessary and sufficient to provide lateral inhibition throughout the SNS anlage in a manner analogous to that seen in the proneural cell clusters initiating both PNS and CNS development (Campos-Ortega, 1993). This argument is supported by the finding that in the absence of *wg* activity, one *ac*-expressing invagination center is selected. The selection of three invagination centers in the wild-type SNS anlage therefore depends upon the functional range of *N*-mediated signaling, which is controlled by *wg* activity (Fig. 9A).

Local *wg* activity causes *dsh*-dependent inhibition of *sgg* which, in turn, acts negatively on *arm*, i.e. *wg* activity eventually causes the increase of active *arm* protein at the receiving end of the pathway (Noordermeer et al., 1994; Perrimon, 1994; Siegfried et al., 1994). The finding of only one selected *ac*-expressing invagination center in *wg*, *dsh* and *arm* mutant embryos indicates that a reduction or loss of *arm* activity leads to an expansion of the range of *N* function in the SNS anlage (Fig. 9C). Conversely, the absence of *sgg* leads to increased *arm* activity which generate supernumerary *ac*-expressing cells and the corresponding number of invaginations (Fig. 9D). This means that critical levels of *sgg* activity must be present at functional levels in the wild-type SNS anlage. *wg* activity, which covers the entire wild-type SNS anlage, does not repress, but rather reduces its level so that the range of lateral inhibition. As a consequence, more than three *ac*-expressing cells are singled-out (see Fig. 8J) and each supernumerary cell leads to the formation of an extra invagination fold (see Fig. 8K-M).

How *wg* and *N* may interact in the SNS anlage

*sgg* encodes a cytoplasmic serine/threonine kinase (Bourouis et al., 1990) which had been proposed to act as an integral component of the *wg* pathway as well as of *N*-mediated signaling (Simpson et al., 1993). In the notum, clonal analysis of *sgg* mutant cells suggested the possibility that *sgg* functions downstream of *N* during lateral inhibition (Ruel et al., 1993;
axis duplication assay and short-range signaling could be integrated into our model only if one assumes mutations (Couso and Martínez Arias, 1994). The latter suggests that arm regulates cellular junctions to control the reception of signaling molecules (reviewed by Peifer et al., 1993; Martínez Arias, 1994). Since wg is expressed throughout the SNS anlage, it may act to affect arm-dependent cell junctions throughout the epithelium. Our findings are consistent with wg acting autonomously to affect the reception of other molecules by controlling a cell adhesion prerequisite for signaling. Moreover, the control of cell adhesion has the potential to modify or prevent the transmission of signals between adjoining cells by either affecting the emission or reception of the signal. This implies that the wg pathway is not necessarily generating an instructive signal in its own right but rather regulates the functional range of other instructive signals (Fig. 9).

Is wg action in the SNS anlage a paradigm?
The proposal that the activated wg pathway modulates instructive signals appears at variance with the orthodox view that wg initiates a signaling cascade to instruct neighboring cell fates nonautonomously (Immerglück et al., 1990; Chu-LaGraff and Doe, 1993; Couso et al., 1993; Li and Noll, 1993; Struhl and Basler, 1993; Couso et al., 1994; Couso and Martínez Arias, 1994; Perrimon, 1994) and with the recent finding that wg may directly interact with the N-encoded transmembrane receptor as suggested by allele-specific combinations of wg and N mutations (Couso and Martínez Arias, 1994). The latter suggestion could be integrated into our model only if one assumes that wg-dependent arm function ultimately controls the effects generated by the proposed interaction of wg and N.

Our proposal is consistent with the observation of short-range effects of the wg activity in different embryonic developmental systems such as epidermal segmentation (reviewed by Perrimon, 1994), gut (Immerglück et al., 1990) and CNS development (Chu-LaGraff and Doe, 1993), and with the recent demonstration that the segment polarity function of wg affects engrailed targeted gene expression only in the adjoining epidermal cells (Vincent and Lawrence, 1994). However, it does not explain the long-range effect of wg activity observed during imaginal development (Struhl and Basler, 1993). This phenomenon could be due to an initial short-range effect of wg, which has been propagated through cell proliferation and by local cascades of cell interactions, as was recently shown for the non-secreted sgg protein (Díaz-Benjumea and Cohen, 1994). Furthermore, the non-diffusible membrane-associated vertebrate homolog of wg, Wnt-1, provides both long-range effects in the Xenopus axis duplication assay and short-range effects on adjoining cells in cell culture transfection assays (Parkin et al., 1993). These findings are consistent with the view that although wg encodes a secreted protein (van den Heuvel et al., 1989), both long-range diffusion and morphogen function may be irrelevant to its action as described here in the SNS anlage. Thus, wg and wg-like molecules of different species may exclusively function to control morphoregulatory signals using an evolutionarily conserved molecular strategy of wg action, i.e. wg activity may serve to regulate morphoregulatory signals and thereby act as a permissive component rather than as an instructive signal (Sampedro et al., 1993).

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