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Review

Cell-cell signaling during neurogenesis: some answers and many questions

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Introduction

Like other developmental processes, generation of a nervous system occurs in stages. It begins with the specification of neural potential to a group of embryonic cells and culminates with the cyto-differentiation of post-mitotic neurons. These series of developmental steps are controlled by both cell autonomous and cell non-autonomous mechanisms and the interaction between the two. This review article focuses on cell non-autonomous pathways which regulate embryonic neurogenesis in the fruitfly *Drosophila melanogaster*.

There are two common ways by which cells signal each other in order to differentiate into specific cell types during development. The first one is the contact signaling. This signaling, also termed lateral signaling (see Cabrera, 1992 and ref. thereof) allows a cell (or a group of cells) to be singled out from a cluster of equivalent cells. The signal is typically transmitted back and forth between these equivalent cells and at some point, for reasons not entirely clear, one cell (or a small number of cells) becomes different from the rest and begins to distinguish itself from the remaining cells. The second is the inductive signaling. This involves a short range signaling molecule and its receptor and functions between two different types of "non-equivalent" cells (it must be pointed out that the distinction between the two is not necessarily of exclusive nature). A temporal and spatial difference in the expression of a signaling molecule and/or its receptor leads to different cells assuming distinct identities within a field of heterogeneous cells.

In each of the steps during neurogenesis (i.e., formation of a neuroblast, specification of identity of a neuroblast and elaboration of a neuroblast lineage), both lateral and inductive signaling inputs play a crucial role in ultimately generating a complex nervous system consisting of many different types of neurons and glial cells. In terms of elucidating the role of lateral signaling in neuroblast formation, significant progress has been made using two neurogenic genes *Notch (N)* and *Delta (Dl)* (reviewed in Artavanis-Tsakonas *et al.*, 1995). Definitive progress has been made recently in understanding the role of inductive signaling in neuroblast formation and identity specification by studying several of the genes which function during *Drosophila* body patterning (see Patel

Abbreviations used in this paper: CNS, central nervous system; NB, neuroblast; NE, neuroectoderm; ML, medio-lateral; AP, anterio-posterior; GMC, ganglion mother cell; MP2, midline precursor 2; MP3, midline precursor 3; EGFR, epidermal growth factor receptor; RP2, raw prawn 2.

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et al., 1989; Skeath *et al.*, 1995; Bhat, 1996; Matsuzaki and Saigo, 1996; Bhat and Schedl, 1997; McDonald and Doe, 1997). These include the signaling molecules *wingless (wg)* and *hedgehog (hh)*, the transmembrane receptor *patched (ptc)* and genes which interact with these signaling pathways {such as *gooseberry (gsb), engrailed (en), invected (inv), armadillo (arm)}*. This review will focus on the role of lateral and inductive signaling during 1) neuroblast formation, 2) neuroblast identity specification, and 3) neuroblast lineage elaboration. Only the recent developments will be discussed with an objective to identify open questions that need to be addressed to further our understanding of the functioning of these signaling pathways during neurogenesis. Since a number of excellent reviews have been published on the role of several of these genes on body patterning (c.f., Klingensmith and Nusse, 1994; Ingham, 1995; Perrimon, 1996), this article will entirely focus on the role of these signaling pathways during neurogenesis.

Drosophila neurogenesis: a brief overview

CNS development in Drosophila: In the *Drosophila* embryo, the central nervous system consists of 28 hemineuromeres, one in each hemisegment (16 abdominal, 6 thoracic and 6 gnathal), forming the ventral nervous system and four supraesophageal neuromeres, forming the two brain hemispheres. The ventral hemineuromeres are similar in different hemisegments and each of the hemineuromeres has about 350 cells, of which ~320 are distinct and highly specialized neurons while the rest are glia (see Bossing *et al.*, 1996). The complex array of neurons in a hemineuromere is generated by ~30 progenitor cells, the neuroblast (NB) stem cells (see Bate, 1976; Campos-Ortega and Hartenstein, 1985; Cabrera, 1992; Doe, 1992; Bossing *et al.*, 1996). These neuroblasts are derived from the pluripotential (neural/epidermal/glial) neuroectoderm (NE) in successive waves and are distributed as seven rows in mediolateral (M-L) columns along the anterior-posterior (A-P) axis of the hemisegment (see Bate, 1976; Doe, 1992). Each neuroblast has a distinct identity and typically it undergoes several asymmetric divisions to produce an invariant and highly specific array of ganglion mother cells (GMCs). Each GMC then divides to generate two daughter cells which generally assume two different identities and differentiate into distinct post-mitotic neurons. While a majority of the neuroblasts gives rise exclusively to neurons, there are some neural precursor cells called glioblasts and neuroglioblasts. While the glioblasts give rise exclusively to glial cells, the other principal cell type in the CNS, the neuroglioblasts, generates both neurons and glia (see Udolph *et al.*, 1993; Prokop and Technau, 1994; Bossing *et al.*, 1996; see also Hosoya *et al.*, 1995; Jones *et al.*, 1995; Vincent *et al.*, 1996).

The brain hemispheres are also generated by neural stem cells in a manner analogous to that of ventral ganglia (in Campos-Ortega and Hartenstein, 1985). The post-mitotic neurons in both the ventral ganglia and the supraesophageal ganglia then undergo cytodifferentiation, i.e., begin expressing their specific genetic programs and find their synaptic targets (i.e., muscle in the case of motoneurons and other neurons in the case of interneurons). Chemotropism (Tosney, 1987; Okamoto and Kuwada, 1991a,b; Chang et al., 1992; Guthrie and Lumsden, 1992), repulsion (Tosney, 1987; Davies *et al.*, 1990) and differential adhesion (c.f., Tang *et al.*, 1992) provide a guidance mechanism for neurons to find their appropriate targets (see Van Vactor *et al.*, 1993).

Cell-cell signaling during Drosophila Neuroblast formation

As discussed in the previous section, during *Drosophila* neurogenesis neuroblasts are formed in ~5 different waves over a period of about 3 h in a stereotypic manner in each hemisegment. One cell from an equivalence group of bipotential NE cells (the number of NE cells in an "equivalence group" varies depending on position within the neuroectoderm along the anterior-posterior and medio-lateral axes) delaminate as a large neuroblast cell. This process of neuroblast formation appears to be under the control of both lateral and inductive signaling. This conclusion can be drawn based on the studies on two neurogenic genes *N* and *Dl* (see Cabrera, 1992; Artavanis-Tsakonas *et al.*, 1995) which are components of a lateral signaling and studies on such segmentation genes as *wg*, *ptc* and *hh* (see Chu-LaGraff and Doe, 1993; Bhat, 1996; Matsuzaki and Saigo, 1996; Bhat and Schedl, 1997) which are components of an inductive signaling. These results and many of the open questions related to neuroblast formation are discussed in the next two sections.

The contribution of lateral signaling to neuroblast formation

The Notch - Delta pathway:

The two genes *N* and *Dl* function as counter-parts in a cell-cell communication cascade during the formation of neuroblasts. The *N* gene encodes a transmembrane receptor while *Dl* encodes one of its ligands. Loss of function for *N* or *Dl* leads to a neurogenic phenotype —formation of an excess number of neuroblasts (see review by Artavanis-Tsakonas *et al.*, 1995). During neuroblast formation a group of genes called the proneural genes (e.g., genes from the *achaete-scute* complex or the *daughterless* gene, which encode transcription factors) are responsible for instructing a cluster of neuroepithelial cells to assume a neural fate. The way these proneural genes confer neural fate to the NE cells is through promoting the accumulation of Dl in all of the cells of a proneural cluster or equivalence group (Hinz *et al.*, 1994; Kunisch *et al.*, 1994). The Dl ligand, however, interacts with N to inhibit the expression of proneural genes in all but one cell in a proneural cluster. The cell which has the highest levels of proneural gene activity then delaminates as a large distinct neuroblast cell. Thus the commitment to neuroblast fate by one of the cells in an equivalence group must necessarily involve the blocking of the *Dl-N* signaling pathway in that cell. Consistent with this conclusion, the expression of a constitutively active form of N prevents neuroblast formation (Rebay *et al.*, 1993; Struhl *et al.*, 1993; reviewed in Artavanis-Tsakonas *et al.*, 1995).

How does the Dl -N pathway allow precisely *one* and only *one* cell from a group of cells in a proneural cluster (rather than between a pair of cells as in the AC/VU decision in nematode vulva development, see Greenwald and Rubin, 1992) to accumulate high enough levels of proneural gene products ? One possibility is that since N-Dl signaling occurs both ways between cells within an equivalence group, a small random difference in signaling activity controlled by N-Dl interaction becomes amplified in one cell, which eventually delaminates as a neuroblast (see Artavanis-Tsakonas *et al.*, 1995). However, as yet no definite evidence exists to support this hypothesis. In addition, the remaining cells within an equivalence group of NE cells retain their bipotentiality for a short period of time following the delamination of a cell as neuroblast. This

conclusion is based upon the result that ablation of a newly formed neuroblast leads to the delamination of another NE cell from that equivalence group as a neuroblast (Doe and Goodman, 1985; reviewed in Cabrera, 1992). This result also suggests that soon after the ablation of a newly formed neuroblast, the N-Dl pathway must be re-initiated to promote proneural gene expression in another cell. A re-setting of the dynamic interaction between N and Dl must therefore necessarily involve certain reading into the cell number and/or the cell size (given that the neuroblast is larger than a NE cell) and therefore that the process is unlikely to be controlled entirely by a random fluctuation in the signaling activity (see above; reviewed in Artavanis-Tsakonas *et al.*, 1995). Identifying the ultimate target genes of the *N-Dl* pathway during neuroblast formation will help solve this issue.

If the *Dl -N* pathway is responsible for ensuring that only one cell in a proneural cluster assumes neuroblast identity, then all of the cells in the cluster or equivalence group would be expected to become neuroblasts in *Dl* or *N* mutants. Indeed, this appears to be the case (c. f., Simpson and Carteret, 1989; Spana and Doe, 1996). A recent study on the Midline Precursor 2 (MP2) lineage {MP2 asymmetrically divides to give rise to two interneurons, dorsal MP2 (dMP2) and ventral MP2 (vMP2)} provides further insight into this problem (Spana and Doe, 1996). In a *Dl* mutant, between 5-6 MP2s per hemisegment were observed. This observation suggests that the equivalence group from which MP2- neuroblast is formed, consists of not a constant number but a variable number of 5 or 6 equivalent cells between different hemisegments. Thus, the number of cells in an equivalence group can not only vary for different neuroblasts, but for a given neuroblast *between* different hemisegments. Alternatively, it is also possible that the "extra" MP2s could arise from a fate transformation of neuroblasts from a neighboring equivalence group. If this is correct, it would be argued that *Dl* and *N* are required not only for the formation of neuroblasts but also for the specification of neuroblast identities (see under "neuroblast lineage elaboration" section; see Table 1).

The N protein has an extra cellular domain containing Epidermal Growth Factor-like motifs and an intracellular domain which contains tandem cdc10/SWI6 repeats (also called ankyrin repeats). The results of Rebay *et al.* (1993) and Struhl *et al.* (1993) using transgenic lines carrying various N deletion constructs provide several interesting insights into the functioning of the N signaling pathway. For instance, Struhl *et al.* (1993) showed that ectopic expression of only the intracellular domain of N (N^{intra}) resulted in a gain of function phenotype and prevented the formation of neuroblasts. This suggests that the extracellular domain of N normally functions to prevent the activity of intracellular domain and removal of the extracellular domain (and also the membrane spanning region) results in a constitutively active N protein. Furthermore, N^{intra} suppressed the transformation of cell fate from epidermis to neural (neurogenic phenotype) in a *Dl* mutant background. Thus instead of exhibiting a neurogenic phenotype, *Dl; N^{intra}* embryos showed a proneural phenotype (hypoplasic CNS)

TABLE 1

EFFECTS OF MUTATIONS IN GENES INVOLVED IN THE LATERAL AND INDUCTIVE SIGNALING PATHWAYS DURING NEUROGENESIS

Genes	Signaling mech. (Lateral/Inductive)	Neuroblast formation	Neuroblast specification	Neuroblast lineage elaboration	Comments
Notch-Delta	L	+	?	+	Similarly reguired in the PNS[1,2] May also function in an inductive signaling parhway[3]
brainiac	?	+	?	?	Interacts with EGFR durning oogenesis[4]
pecanex	?	+	?	?	Has sequence similarity to N[5]
big brain	?	+	?	?	Has homolgy to channel proteins[6]
wingless	I	+	+	-	Affects proneural expression negatively in the eye and positively in the wing durning bristle formation?
patched	I	+	+	-	Forms a receptor complex with Smo; recieves the Hh signal[8,9]; tumor suppressor gen in humans [10,11]
hedgehog	I	+	+	-	Affects NB formation also outside of the Ptc domain[12-14]
shaggy/zw3	I	+	+	-	Has opposing effect on NB formation to that of *wg*
armadillo	I	? (+)	? (+)	-	May function by directly activating nuclear genes[15-17], besides functioning in cell adhesion[18]

Abbreviations: PNS, peripheral nervous system; EGFR, epidermal growth factor receptor; N, Notch; Smo, Smoothened; Hh, Hedgehog; Ptc, Patched. References: 1. Hartenstein and Posakony (1990); 2. Parks and Muskavitch (1993); 3. reviewed in Artavanis-Tsakonas et al. (1995); 4. Goode et al. (1992); 5. Labonne et al. (1992); 6. Rao et al. (1990); 7. Cadigan and Nusse (1996); 8. Marigo et al. (1996); 9. Stone et al. (1996); 10. Hahn et al. (1996); 11. Johnson et al (1996); 12. Bhat (1996); 13. Bhat and Schedl (1997); 14. Matsuzaki and Saigo (1996); 15. Behrens et al. (1996); 16. Molenaar et al. (1996); 17. Brunner et al. (1997); 18. reviewed in Miller and Moon (1996).

similar to the CNSs of proneural mutants. This indicates that NE cells in *Dl; N^{intra}* embryos assume an epidermal fate. Taken together, these above results also indicate that loss of *Dl* leads to a loss of inhibition by N on proneural gene expression and that N blocks proneural gene expression (or function of proneural genes) during neuroblast formation.

Since the N^{intra} protein was found to be localized to the nucleus (Struhl *et al.*, 1993), it must be that during neurogenesis N suppresses proneural gene expression by directly interacting with modifiers of transcription of proneural genes. However, in the *Drosophila* eye both truncated nuclear N and membrane bound N produce the same phenotype (Fortini *et al.*, 1993), indicating that nuclear localization is not necessary to produce activated *N* phenotypes. However, this appears to be true only during eye development and membrane bound N did not produce activated *N* phenotypes during embryonic neurogenesis. Identification of interacting genes with N both during neurogenesis and eye development using this activated N in enhancer and suppressor screens will be useful to understand this difference in N signaling in these two different lineages.

A further interesting observation with respect to the functioning of N comes from the studies of Rebay *et al.* (1993). They observed that ectopic expression of a full length N has no significant effect on development. This would argue that Dl is able to prevent the "excess" N in a cell from activating proneural genes. This result also argues against the possibility that a random fluctuation in the level of Dl within an equivalence group leads to selection of one cell as neuroblast (by inhibiting N activity thereby promoting proneural gene activity in that cell).

Previous studies indicate that binding of N to Dl both activates (Heitzler and Simpson, 1991; Parody and Muskavitch, 1993; reviewed in Artavanis-Tsakonas, 1995) and antagonises N activity (Vassein et *al.*, 1985; Heitzler and Simpson, 1993). Genetic mosaic analysis indicate that in the case of activation of N by Dl, Dl appears to function non-autonomously, whereas in the case of antagonistic interaction, Dl appears to function cell autonomously (Heitzler and Simpson, 1993), perhaps by binding to one another in the same cell at the surface. Indeed, tissue culture experiments indicate that N and Dl binds to each other on the surface of the same cell (see Artavanis-Tsakonas *et al.*, 1995 and ref. therein), although it is not clear why such interaction is prevented from taking place during neurogenesis. Elucidating this would be most useful to understand the N signaling pathway during neurogenesis (and in other tissues).

The N protein has another ligand, Serrate, which functions during wing development. While a loss of function for Serrate has no neurogenic phenotype, a hypermorphic situation (ectopic expression at high levels) for *Serrate* suppresses achaete expression in proneural clusters in *Dl* mutants in a N-dependent manner (Gu *et al.*, 1995) thus rescuing the neurogenic phenotype of *Dl* mutants. This rescuing activity of Serrate, however, appears to be partial indicating that *Serrate* can not fully complement the loss of *Dl* function even when expressed at high levels. While the *Serrate* gene product appears to function cell-autonomously during wing development (perhaps by antagonising N), it is not clear how the ectopic Serrate is able to rescue (to any extent) the neurogenic phenotype in *Dl* mutants. The partial rescue situation however suggests that Serrate may interact with N both cell-autonomously and non-autonomously, acting simultaneously as an activator and an antagonist of N. It might be that where N-Serrate interaction is cell non-autonomous, only one cell within the equivalence group delaminates as neuroblast as in wild type. Whereas in those equivalence groups where this interaction is cell-autonomous (and antagonistic), all the cells delaminate as neuroblasts. Further experiments are required to determine whether this possibility is correct.

The other "signaling" neurogenic genes:

N and Dl are not the only "signaling" neurogenic genes which affect neuroblast formation. Three other neurogenic genes that could be directly involved in cell-cell signaling during neuroblast formation are *brainiac* (Perrimon *et al.*, 1989), *pecanex* (Labonne *et al.*, 1992) and *big brain* (Rao *et al.*, 1990). *brainiac* (*brc*) mutations appear to interact with Epidermal Growth Factor Receptor (EGFR) during oogenesis (Goode *et al.*, 1992). Therefore it is conceivable that *brc* encodes a ligand for EGFR. It remains to be determined whether a similar relationship between *brc* and EGFR exists during neuroblast formation. Cloning of *brc* gene would be most helpful to determine whether brc indeed functions directly in a signaling cascade. The gene *pecanex* (*pcx*) also encodes a transmembrane protein with sequence similarity to N (Labonne *et al.*, 1992) and it is possible that Brc and Pcx could function in a manner analogous to Dl and N during neuroblast formation. In this regard, it would be helpful to determine whether *brc* and *pcx* mutations show genetic interaction with each other. A genetic interaction study between *brc* and *N* and *pcx* and *Dl* will also be informative to understand the contribution of these various signaling pathways to neuroblast formation.

The third gene which might potentially function in the cell-cell communication during neuroblast formation is big brain (*bib*). *bib* encodes a transmembrane molecule and has homology to channel proteins (Rao *et al.*, 1990). Given this topology, it seems unlikely that Bib transduces signals in a manner similar to that of *N-Dl* pathway. Moreover, in contrast to *Dl* and *N*, *bib* does not show genetic interactions with the known neurogenic genes. Thus it will be necessary to determine how *bib*, *pcx* and *brc* function at the molecular level to assess the overall contribution of these genes to neuroblast formation as well as their relationship to the *N-Dl* signaling pathway.

Inductive signaling during neuroblast formation

Several recent studies indicate that many of the genes which belong to the segment polarity group play a crucial role in neuroblast formation via an inductive signaling mechanism (Chu-LaGraff and Doe, 1993; Skeath *et al.*, 1995; Bhat, 1996; Bhat and Schedl, 1997). These include the secreted signaling molecules *Wingless (Wg)* and *Hedgehog (Hh)*, the transmembrane protein Patched (Ptc) and the transcription factor *Gooseberry (Gsb)*. Like the proneural genes, mutations in these segment polarity genes result in a failure in neuroblast formation (see below). Additionally, these segment polarity genes are also required for the specification of the identity of a subset of neuroblasts (see under "neuroblast identity specification").

The wingless pathway:

During neurogenesis, Wg is expressed in row 5 cells (neuroblasts and their precursor cells) and is required non-autonomously for the formation of neuroblasts in the rows (4 and 6) located to either side

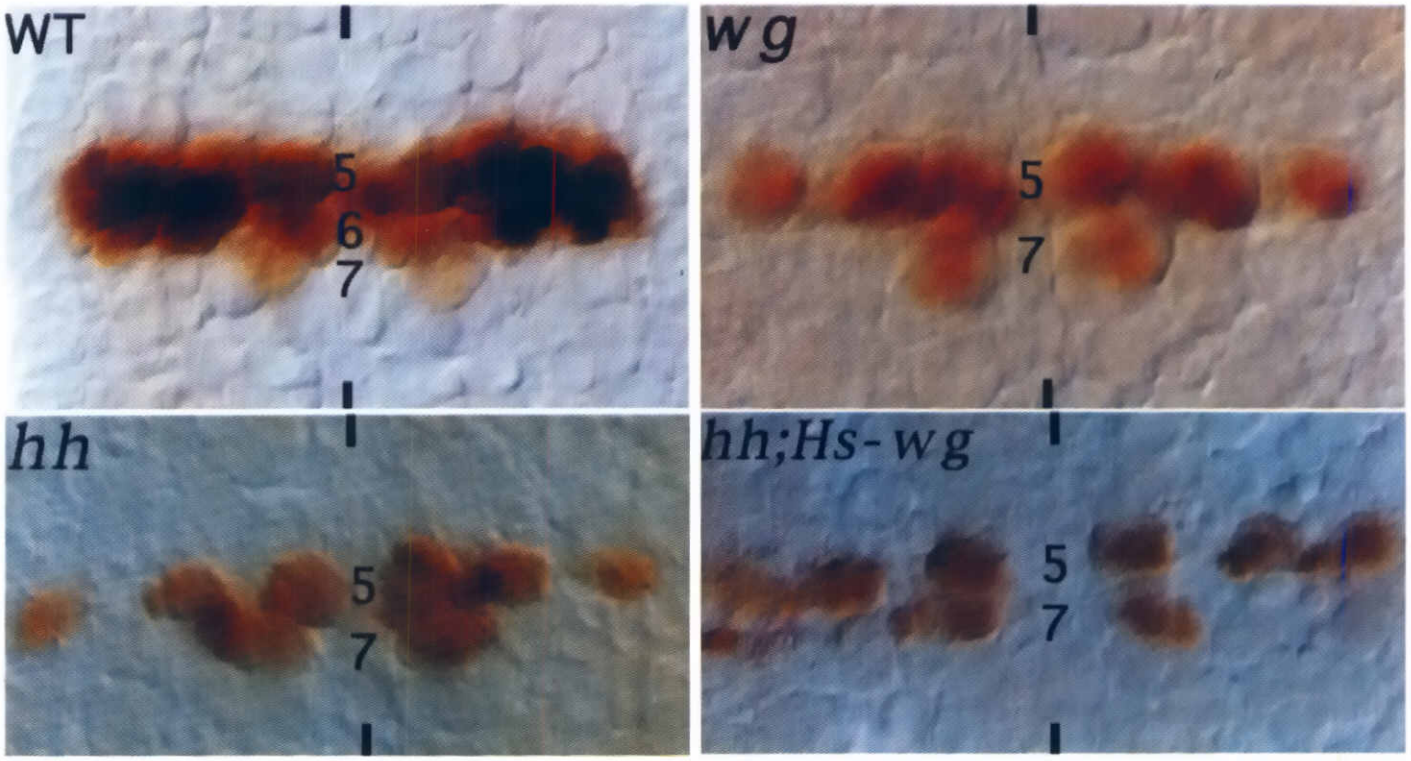

Fig. 1. *Wg* and *Hh* signaling pathways are required for neuroblast formation. *These panels show Gsb protein expression in wild type, wg, hh and hh Hs-wg embryos (~late stage 10, ~6 h old). In wild type embryos Gsb protein is observed in neuroblast rows 5, 6 and one cell in row 7 (NB7-1). In wg and hh mutant embryos, row 6 neuroblasts are missing (see text). The hh Hs-wg embryo was heat shocked to induce ubiquitous expression of Wg (the Hs-wg line was described in Noordermeer et al., 1992; see Bhat, 1996, for heat shock and staining procedures; the hh Hs-wg line was constructed by recombining a hh point mutant with the chromosome carrying the transgenic wg gene under the control of heat shock 70 gene promoter). In hh, Hs-wg embryos, no rescue of the row 6 neuroblasts is observed. Ectopic expression of wg at any time during neurogenesis failed to rescue the loss of row 6 neuroblasts in hh mutants. These results indicate that the hh and wg pathways function either independently or hh is downstream of wg during row 6 neuroblast formation. The anterior end of the embryos is towards the top and the midline is marked by a vertical bar. The numbers along the midline indicate cell rows.*

of row 5 (see also Chu-LaGraff and Doe, 1993; see Fig. 1). The results of Hartenstein *et al.* (1994) indicate that *wg* is also required for the formation of NB5-2, a row 5 neuroblast often fails to form in *wg* mutants. This indicates that *wg* may also be required autonomously for neuroblast formation. However, the loss of neuroblasts in *wg* mutants is variable — sometimes row 4 or row 6 neuroblasts are formed in several hemisegments (see Chu-LaGraff and Doe, 1993; K.B., unpublished results). In general, row 6 neuroblasts (NBs (NB6-2, NB6-4, and NB6-5) are more sensitive to the loss of *wg* than row 4 neuroblasts. In addition, within a row there are differences along the M-L axis. For instance, while the row 6 neuroblast, NB6-1, is occasionally formed in *wg* mutants, the remaining row 6 neuroblasts are almost never observed. Why neuroblasts are differentially sensitive to the loss of *wg* and why the sensitivity depends upon the row or column are still open questions.

One possible explanation for the variable and position dependent effects of *wg* mutations on neuroblast formation is that the Wg signaling pathway may be partially redundant. For example, it is conceivable that other *wnt* genes also play a role in neuroblast formation and can partially compensate for the loss of *wg*. A good candidate would be *wnt 4*. *wnt 4* is located very close to *wg* on the second chromosome and presumably arose by a gene duplication event (see Graba *et al.*, 1995). Since mutations in either gene have a segment polarity phenotype (in Graba *et al.*, 1995), it is clear that one gene can not fully substitute for the loss of the other. However, since they have an overlapping pattern of expression (Graba *et al.*, 1995), it is possible that they could have an overlapping function in a developmental process such as neuroblast formation or they could have an additive effect in this process. The possible contribution of *wnt 4* to neuroblast formation (and therefore to this aspect of the Wg signaling pathway) could be determined by examining the effect of *wnt 4* single mutant and *wnt 4, wg* double mutants on the formation of row 4 and row 6 neuroblasts. It would be very informative to determine whether a *wnt 4* transgene can rescue the loss of neuroblasts in *wg* mutants.

While it remains a possibility that *wnt 4* could participate with *wg* in the formation of row 4 and row 6 neuroblasts, the results from the analysis of *wnt 3*, another member of the Wnt family, argue that this *wg* relative probably does not play a role in neuroblast formation. This is based on the finding that a small deficiency which removes *wnt 3* has no apparent effect on the formation of row 4 or row 6 neuroblasts (K.B., unpublished results). There are, however, defects in other aspects of neurogenesis which are distinct from those produced by *wg* mutations (see Fradkin *et al.*, 1995; K.B., unpublished results). Also, it must be pointed out that the lack of effect on

the formation of row 4 and row 6 neuroblasts in embryos deficient for *wnt3* could not be due to a maternal deposition since *wnt3* is not deposited (either RNA or protein) into an egg during oogenesis and the gene is expressed only zygotically.

Answers to some of the questions raised above will require determining how the Wg signaling pathway contributes to neuroblast formation. In the ectoderm, a number of components of the Wg signaling pathway have been identified (reviewed in Klingensmith and Nusse, 1994). These include: Porcupine (Porc), which is required for the secretion of Wg (see review by Klingensmith and Nusse, 1994), *Drosophila* Frizzled 2 (DFz2), the presumptive receptor for Wg (Bhanot *et al.*, 1996), Disheveled (Dsh), which is required for transducing the Wg signal, Shaggy/Zeste White3 kinase (Sgg/ZW3) which is involved in constitutively phosphorylating Armadillo, and the Armadillo (Arm) protein which is the *Drosophila* beta-catenin (see review by Klingensmith and Nusse, 1994). It is thought that the Wg signal prevents Sgg from phosphorylating Arm, leading to the intracellular accumulation of Arm (Noordermeer *et al.*, 1994; Peifer *et al.*, 1994; Siegfried *et al.*, 1994; reviewed in Miller and Moon, 1996). In mammalian tissue culture experiments, the hypo-phosphorylated Arm interacts with HMG box factors such as LEF-1 and gets translocated translocated into the nucleus (Behrens *et al.*, 1996). Similarly in the *Xenopus* embryo, the Xtcf-3 transcription factor mediates β-catenin (the vertebrate homolog of Arm) induced axis formation (Molenaar *et al.*, 1996) presumably by translocating β-catenin into nucleus and activating target gene expression. That a similar phenomenon may also be occurring in the *Drosophila* embryo is indicated by the recent *in vivo* result that the *Drosophila* LEF-1 homolog, Pangolin, functions downstream of Arm to transduce the *wg* signal (Brunner *et al.*, 1997; Riese *et al.*, 1997; van de Wetering *et al.*, 1997). It is, however, not known whether any of these downstream components of the Wg signaling pathway are known to play a role in neuroblast formation. Since the intracellular accumulation of Arm appears to be one of the last steps in the Wg signal transduction pathway, a crucial experiment would be to determine the requirement for Arm in neuroblast formation. While the RP2 neuronal lineage, a lineage produced by a row 4 neuroblast, NB4-2 (Thomas *et al.*, 1984; see Doe, 1992), is missing in *arm* mutants (Loureiro and Peifer, pers. commun.), it is not yet clear whether this is due to a failure in the formation of NB4-2 or a failure in its specification (since Wg is also required for the specification of this neuroblast) or both.

Determining the requirement of Arm (in neuroblast formation and neuroblast identity specification) becomes more relevant when we consider the effect of loss of function for *sgg*. During the patterning of the ectoderm, Wg is thought to function by antagonizing Sgg (see Siegfried *et al.*, 1994). If Wg also functions to antagonize Sgg during neuroblast formation, then *sgg* mutants might be expected to have extra row 4 and row 6 neuroblasts. Indeed, it has been reported that *sgg* has a neurogenic phenotype and has excess numbers of neuroblasts (see Cabrera, 1992; see also Heitzler and Simpson, 1991). However, it is not clear whether these extra neuroblasts are actually row 4 and row 6 neuroblasts. One would have to determine if the defects in neuroblast formation in *wg* mutants can be suppressed by the loss of *sgg* to draw further conclusions.

The Hh signaling pathway in neuroblast formation:

Loss of function in the Hh signaling molecule also affects the formation of neuroblasts. As in *wg* mutants, row 6 neuroblasts usually fail to form in *hh* mutants (Fig. 1; see also Matsuzaki and Saigo, 1996). Moreover, like *wg* mutants, the different row 6 neuroblasts are not equally sensitive to the loss of *hh*; NB6-1 is occasionally found in some hemisegments, while the other row 6 neuroblasts NBs (NB6-2, NB6-4 and NB6-5) appear to be absent. These findings would suggest that Hh and Wg participate in the same (or parallel) pathway during row 6 formation. On the other hand, this cannot be true for row 4 neuroblasts since the formation of this row is not affected in *hh* mutants (Patel *et al.*, 1989; K.B., unpublished results). Similarly, unlike in *wg* mutants, in *hh* mutants row 2 neuroblasts also fail to form (Matsuzaki and Saigo, 1996).

Previous results indicate that in the epidermis Hh is required for sustaining *wg* expression in stage 10 and beyond (Bejsovec and Wieschaus, 1993). Since most row 6 neuroblasts are formed during stage 10 (see Doe, 1992), it was possible that the loss of row 6 neuroblasts in *hh* mutants may be indirectly due to a decay in Wg expression. If this were true, it should be possible to rescue the loss of row 6 neuroblasts in *hh* mutants by ectopically expressing *wg* during neurogenesis. However, as shown in Figure 1, *Hs-wg* failed to rescue the loss of row 6 neuroblasts in *hh* mutants. This result suggests that the loss of row 6 neuroblasts in *hh* mutants is not due to a premature decay of Wg. In addition, it suggests that *hh* is either downstream of or parallel to *wg* in the signaling cascade which controls row 6 formation. Distinguishing between which of these possibilities is correct will require identification of additional mutations which would cause loss of row 6 neuroblasts. Also, determining why the formation of row 4 neuroblasts depends on Wg but not on Hh will be very informative to understand the differential signaling cascade during neurogenesis.

Based on the phenotypic similarity between loss of function *hh* and another mutant, *smoothened (smo)*, and the fact that *smo* encodes a transmembrane protein, it was proposed that Smo is the receptor for Hh (Alcedo *et al.*, 1996; van den Heuvel and Ingham, 1996). However, the recent findings that Ptc, but not Smo interacts with Hh *in vitro* (Marigo *et al.*, 1996; Stone *et al.*, 1996) and that Ptc is involved in sequestering and transducing Hh signal in imaginal disc (Chen and Struhl, 1996) indicate that Ptc, not Smo is the receptor for Hh. This issue is complicated by the finding that in the CNS of *hh* mutant embryos row 6 neuroblasts are not formed (Fig. 1; see also Matsuzaki and Saigo, 1996; Bhat and Schedl, 1997). Since Ptc protein is not detected in row 6 cells nor is row 6 neuroblast formation affected by *ptc* mutations (Bhat, 1996; see below), it would appear that Ptc can not be the receptor for Hh at least in these cells. It remains to be determined which molecule functions as the Hh receptor during row 6 neuroblast formation (and whether this same molecule also functions in other Hh dependent signaling pathways).

Ptc and gsb function in neuroblast formation:

Two other segment polarity genes which play a role in neuroblast formation are the transmembrane protein Ptc (Hooper and Scott, 1989; Nakano et al., 1989) and the transcription factor *gsb* (Baumgartner *et al.*, 1987). In *ptc*⁻ embryos, there are defects in the formation of neuroblasts in rows 2-5 (Bhat, 1996). This is best documented for NB4-2 which is missing in ~30% of the *ptc*⁻ hemisegments. Since this phenotype is only partially penetrant, it seems likely that there are mechanisms which can compensate for the loss of Ptc in these rows. Like *ptc*, there are partially penetrant defects in neuroblast formation in *gsb* mutants. For instance, NB5-

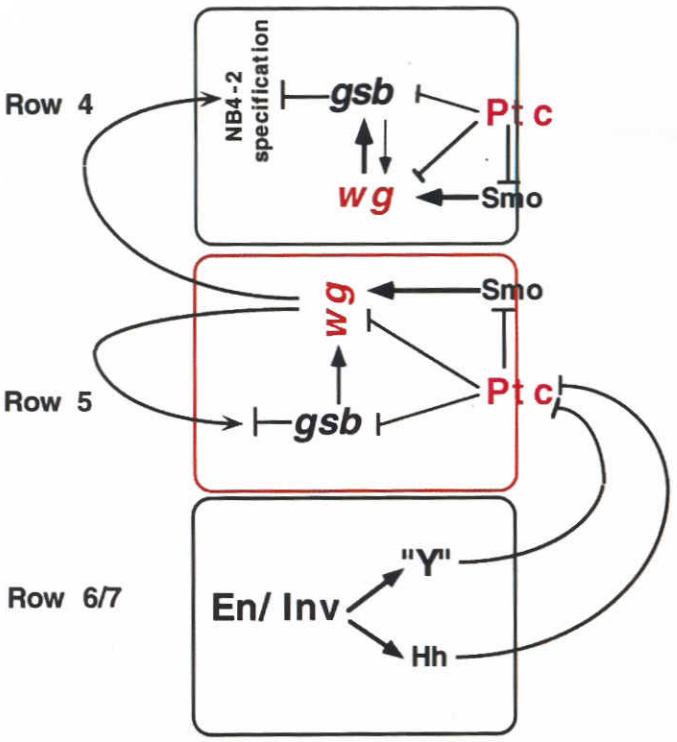

Fig. 2. Interaction of signaling pathways controlled by *wg, ptc, hh, gsb* and *en/inv* during neuroblast identity specification (between late stage 8 and late stage 9). *In row 4 cells, Ptc blocks the expression of gsb in a wg-dependent manner (see Bhat and Schedl., 1997) (via Smo, assuming that smo is expressed in row 4). Since the level of Gsb in rows 4-7 in ptc mutant appears to be higher compared to the level of Gsb in rows 5-7 in wild type, Ptc may also repress gsb expression which does not involve wg (or smo). The repression of gsb in the neuroectodermal cells from which NB4-2 is derived permits the specification of NB4-2 identity by a Wg signal emanating from the adjacent row 5 cells. Gsb is also involved in maintaining a wild type level of wg expression (in row 4 and 5 in ptc mutants and in row 5 in wild type; wg is expressed only in row 5 in wild type). In row 5 cells, the ptc-gsb (and ptc-wg or ptc-smo-wg) negative regulatory circuit is uncoupled by an en/inv dependent mechanism. Since en and inv are expressed in row 6/7 but not in row 5, they must antagonize Ptc activity by some type of non-autonomous mechanism, likely through an as yet unidentified short range signaling molecule «Y» that is distinct from (but perhaps redundant to) hh. The uncoupling of the ptc-gsb regulatory circuit by en/inv induced signal also allows Gsb to block an autocrine Wg signal from specifying NB4-2 identity to presumptive NB5-3 neuroblast precursor. The uncoupling of the ptc-gsb regulatory circuit by "Y" has another function. The Gsb protein produced in response to the Y-Ptc interaction activates wg expression (see Bhat and Schedl., 1997). The Hh signaling pathway is also required to generate wild type levels of Wg expression. hh is activated in row 6/7 cells by en/inv and the Hh protein secreted from these cells interacts with Ptc allowing the activation of wg expression by Smo. Interaction of Hh (and "Y") with Ptc also relieves the repression of wg by Ptc (see Bezsovec and Wieschaus., 1993; Bhat and Schedl., 1997). These results indicate that the identity of neuroblasts are determined by a signaling cascade from posterior row to anterior row.*

3 fails to form in ~50% of the hemisegments (see Skeath *et al.*, 1995; Bhat, 1996). Since *gsb* and *ptc* are also components of a signaling pathway that specifies neuroblast identity (see below) an important question is whether the defects in neuroblast formation observed in mutants in these two genes are related to a failure in neuroblast specification, or represent some type of independent pathway(s). (There is a suggestion from genetic epistasis experiments that the latter possibility may be correct. As described below in more detail, it is possible to rescue the defects in NB4-2 specification in *ptc* mutant embryos by simultaneously eliminating *gsb*; however, the elimination of *gsb* does not appear to rescue the partially penetrant defects in the formation of NB4-2 that is normally observed in *ptc* mutants; see Bhat, 1996)

Although defects in neuroblast formation are evident in several different segment polarity mutants, it is not entirely clear what sort of role this group of genes plays in this process. A trivial explanation for their "proneural"-like phenotype is that the segment polarity genes are required for the formation and/or survival of ectodermal cells from which the affected neuroblasts are normally derived. While *wg* embryos are smaller than wild type (which would be consistent with this possibility) the size reduction is only evident at stages after most neuroblasts normally have formed. Additionally, there is no apparent size reduction in *hh*, *ptc* or *gsb* mutant embryos. Furthermore, embryos from these mutants do not show evidence of any apoptotic or necrotic cell death prior to or during early neurogenesis (K.B., unpublished results). The results of Hartenstein *et al.* (1994) are rather significant from the point of role of segmentation genes such as *wg* in neuroblast formation. These authors have shown that a) neuroblast delamination and mitosis are closely related to one another, that neuroblast delamination occurs just before the mitosis of ventral neuroectodermal cells, and b) mutation in *wg* gene affects mitosis of the cells in the neuroectoderm —the mitosis in these cells is postponed. Given these results, the failure in neuroblast delamination in *wg* mutants could be due to the postponement of mitosis of the ventral neuroectoderm. However, given the fact that the neuroblasts in the affected rows in *wg* mutants fails to delaminate, the role of *wg* in neuroblast formation appears to be much more complex (see below).

One intriguing possibility is that the segment polarity genes promote neuroblast formation by modulating the activity of the neurogenic and/or proneural genes. In this context, several recent findings may be of interest. First, during the formation of bristles in the adult thorax the N pathway appears to repress *ac-sc* expression by activating Sgg (Ruel *et al.*, 1993). Second, in the wing disc, Wg is required to activate *ac-sc* genes (Philips and Whittle, 1993). Third, there is evidence for a direct interaction between the segment polarity protein Dsh and N (Axelrod *et al.*, 1996). These findings suggest that the segment polarity genes in the Wg pathway could promote neuroblast formation in the embryonic CNS in two ways. In the first, *wg—>dsh* would inhibit Sgg activity leading to the activation of proneural genes such as *ac/sc* (presumably via Arm). Second, *wg—>dsh* would prevent N from repressing *ac/sc* (Axelrod *et al.*, 1996; see below). Although a model of this type could potentially explain why mutations in segment polarity genes affect neuroblast formation, a link between the segment polarity genes and the neurogenic/proneural gene has not yet been demonstrated in the embryonic CNS. Establishing the relationship between these genes would help understand how segment polarity genes (and the inductive signaling pathways) contribute to neuroblast formation.

Cell-cell signaling during neuroblast identity specification

Each newly delaminated neuroblast in the CNS of the *Drosophila* embryo has a specific identity, and this identity determines the array of GMCs (and neurons) that the cell will ultimately produce. A question that has drawn considerable interest is whether neuroblast identity is established prior to or after neuroblast formation/delamination (Doe and Goodman, 1985). Some recent studies indicate that neuroblast identity is most likely determined just prior to delamination (see Chu-LaGraff and Doe, 1993; Skeath *et al.*, 1995; Udolph *et al.*, 1995; Bhat, 1996; Bhat and Schedl, 1997). As described in more detail below, a cell-cell signaling network that includes several segment polarity genes plays a critical role in the specification of neuroblast identity (Chu-LaGraff and Doe, 1993; Bhat, 1996; Bhat and Schedl, 1997; McDonald and Doe, 1997). Temperature shift experiments with *temperature sensitive* alleles and ectopic expression experiments suggest that these genes probably act to specify neuroblast identity prior to the delamination of neuroblasts from the NE. For instance, Wg is required for the specification of the identity of a row 4 neuroblast, NB4-2. Thus in *wg* mutants this neuroblast (when it forms) is misspecified (Chu-LaGraff and Doe, 1993; Bhat, 1996). Temperature shift experiments with a wg^{ts} allele indicates that the temperature sensitive period for specification of NB4-2 identity is just prior to the delamination of this neuroblast from the NE. Conversely, ectopic expression of the transcription factor *gsb* prevents NB4-2 specification by *wg*. In order to block NB4-2 specification, the *gsb* gene must be expressed prior to the formation of this neuroblast (Bhat, 1996). While these and other experiments (see Udolph *et al.*, 1995) indicate that a subset of the neuroblasts are likely to be specified prior to delamination, it is not yet clear whether this is a general rule. The recent tracing of all the neuroblasts in the embryonic ventral ganglia (Bossing *et al.*, 1996; Technau, pers. commun.) will be very useful in solving this question.

A second question is whether the identity of a neuroblast changes following each division. This seems to make sense since a different GMC is produced following each neuroblast division. This would imply that the identity of a neuroblast is specified continuously following each division. Consistent with this view is the observation that a neuroblast can often change its gene expression pattern during its history (see Doe, 1992). However, it could also be convincingly argued that a particular chain of different GMCs to be produced by a given neuroblast is determined during its "initial specification" itself and thus while the initial specification of a neuroblast identity depends on cell-cell signaling cascade in the NE, subsequent development of the lineage is quite autonomous. This is supported by a recent result obtained by Prokop and Technau (1994) in the neuroblast, NB1-1. The development of NB1-1 differs between abdominal and thoracic segments: in the abdominal segments, NB1-1 gives rise to only neurons, whereas in the thoracic segments, it gives rise also to glial cells (therefore a neuroglioblast). Transplantation of the NB1-1 parental NE cell from the abdomen to the thorax leads to the generation of abdomen-specific NB1-1 lineage. However, it remains to be seen whether the entire lineage development of each and every neuroblasts is determined in the NE when the initial identity of a neuroblast is specified, or if this phenomenon is true for only a subset of neuroblast lineages such as NB1-1.

Specification of neuroblast identity by inductive signaling

Recent studies on the specification of two neuroblasts, NB4-2 in row 4 and NB5-3 in row 5, indicate that identities of these neuroblasts are determined by a cell-cell signaling network (Fig. 2). Two of the key cell autonomous players in this network are the segment polarity genes *ptc* and *gsb* which have antagonistic activities in the specification of NB4-2 and NB5-3 identity. In order to specify NB4-2 identity, Ptc must repress Gsb in the NB4-2 precursor cell (Fig. 3). In *ptc* mutants, Gsb is expressed in the NB4-2 precursor cell preventing this cell from assuming NB4-2 identity. The misspecification of NB4-2 in *ptc* can be rescued by also removing *gsb*. In contrast to NB4-2, *gsb* must be expressed in the NB5-3 precursor cell to properly specify this NB (Fig. 2) and in *gsb* mutants, NB5-3 assumes an NB4-2 identity (Skeath *et al.*, 1995; Bhat, 1996; Bhat and Schedl, 1997).

Significantly, Ptc is expressed not only in row 4, but also in row 5. Hence, there must be a mechanism which enables Ptc to repress *gsb* in row 4 but prevents it from repressing *it* in row 5. This mechanism appears to involve a cell-cell signal emanating from the adjacent cells in row 6/7 (Bhat and Schedl, 1997). This signal depends upon the *en* and *inv* transcription factors which are expressed in row 6/7 cells (Fig. 2). In *en, inv* double mutant embryos, *gsb* expression in NB5-3 and/or its precursor cell prematurely decays and NB5-3 is misspecified as NB4-2. The premature decay of *gsb* in the NB5-3 precursor cell seems to be mediated by Ptc. Thus, *gsb* expression in *en, inv* double mutant embryos can be restored by the removal of *ptc*.

While NB5-3 identity depends upon an *en/inv* activated signal, this signal must be short range. Otherwise, the En/Inv activated signal would inappropriately block Ptc activity in the precursor to NB4-2 and cause the misspecification of this neuroblast. The specification of NB4-2 does, however, depend upon a cell-cell signal. This signal is Wg which is expressed in row 5 NE cells (see Fig. 2). In the absence of *wg*, NB4-2 is not correctly specified. Like the En/Inv-activated signal, the Wg signal must also be short ranged; otherwise, a row 3 neuroblast would be misspecified as NB4-2 Bhat, 1996).

What is the nature of the non-autonomous interaction of En/Inv with Ptc?

From studies on ectodermal patterning a very good candidate for the *en/inv* dependent signal would be the secreted protein Hh. Hh is in row 6/7 cells under the control of *en* (and *inv*?) and is thought to be a ligand for Ptc. Surprisingly, however, *hh* mutations have no apparent effect on NB5-3 specification. This finding implies that there must be some other *en/inv* dependent signaling molecule, "Y", which is capable of antagonizing Ptc activity in row 5 cells. On the other hand, the observation that *hh* mutations have no effect on NB5-3 specification does not exclude the possibility that "Y" and *hh* may perform redundant functions with respect to the specification of this neuroblast identity. Resolution of the relationship between "Y" and *hh* will require the identification and characterization of the gene encoding the "Y" signaling factor.

Gsb and the selective reception of Wg signal:

As indicated in Figure 2, one of the functions of Gsb in the NB5-3 precursor cell is to prevent an autocrine Wg signal from specifying NB4-2 identity to this cell (Bhat, 1996; Bhat and Schedl, 1997). However, there is evidence that Wg autoregulates its own expres-

sion in row 5 cells (Li and Noll, 1993; Hooper, 1995). Taken together, these results argue that while Gsb must block the Wg signaling pathway from specifying NB4-2 identity to a row 5 cell, it must not block the Wg autoregulatory pathway in this cell. How is it possible to selectively block one response but not the other? Since Gsb is a transcription factor, it could, for example, prevent the expression of a component of the Wg reception pathway which is specific to NB4-2 specification. Furthering of this hypothesis requires identification of a component of this type by genetic screens.

The En/Inv ->"Y"—>Ptc pathway is cell specific and depends on the position of the cell along the M-L axis:
While *en/inv* are required to sustain Gsb expression in row 5 NE cells, the loss of Gsb expression in *en, inv* mutant embryos is not uniform along the M-L axis (Bhat and Schedl, 1997). This has important consequences for neuroblast specification. Gsb expression disappears early in neurogenesis in the NE cells giving rise to NB5-3, and this neuroblast is not properly specified in *en, inv* double mutant embryos. In contrast, the NE cells close to the ventral midline which give rise to NB5-2 typically retain Gsb until somewhat later in neurogenesis in *en, inv* mutants, permitting the proper specification of NB5-2. This difference indicates that the postulated *en/inv —>»y»—>ptc—>gsb* pathway is critical in the specification of only a subset of the row 5 NBs. It remains to be determined why *gsb* expression can be sustained in some cells through the critical period in the absence of *en* and *inv* while it can not in other cells. Likely this has to do with differences in patterning along the ML axis (see also Bhat and Schedl, 1997; McDonald and Doe, 1997). The nature and mechanism of these differential regulatory interactions along the M-L axis remains to be explored.

The Wg pathway which specifies neuroblast identity may be different from the Wg pathway which promotes neuroblast formation:
As discussed in previous sections, Wg is required for both the formation and identity specification of neuroblasts. For instance, in *wg* mutants whenever NB4-2 is formed, its identity is not specified correctly (Chu-LaGraff and Doe, 1993). By contrast, Wg does not appear to be required for the specification of NB6-1, a row 6 neuroblast since this neuroblast, when it is formed in *wg* mutants, maintains NB6-1 specific gene expression (K.B., unpublished results). (Thus the formation of row 6 neuroblasts appears to be more sensitive to loss of *wg* than row 4, specification of identities of row 4 neuroblasts is more sensitive to loss of *wg*.) These results raise the possibility that the *wg* signaling pathway which specifies neuroblast identity could be different from the one which contributes to the formation of neuroblasts. How Wg directs these two processes during neurogenesis is an open question. In this context, determining the effects of loss of other component genes of the *wg* signaling pathway will be informative. It will also provide some insight on the conservation / divergence in these signaling pathway between ectoderm and CNS.

Separation of segmentation requirements from neurogenesis requirements

Analysis of phenotypic defects associated with mutations in genes which are expressed (and presumably functions) during different stages of development can be often problematic because of difficulty in distinguishing between the primary and secondary effects. For instance, it can be argued whether the CNS defects in embryos mutant for segmentation genes are due to an indirect consequence of the earlier disruptions in segmentation. However, several lines of evidence indicate that the CNS defects observed in mutants for segmentation genes are due to the disruption of neurogenesis and that these segmentation genes have distinct CNS requirement (Duffy *et al.*, 1991; Chu-LaGraff and Doe, 1993; Bhat and Schedl, 1994; Bhat, 1996). The best evidence to support this conclusion comes from the studies on temperature sensitive mutant alleles in several of the segmentation genes (Duffy *et al.*, 1991; Chu-LaGraff and Doe, 1993; Bhat and Schedl, 1994). For instance, using a *temperature sensitive* allele of *wg*, it has been shown that the RP2 lineage defect in *wg* mutants is separable from the segmentation defects (Chu-LaGraff and Doe, 1993). Consistent with this conclusion, ectopic expression of *wg* from a heat shock promoter (*Hs-wg*) just prior to the formation of S1 neuroblasts causes another neuroblast, NB3-2, to change its identity into NB4-2 (Bhat, 1996). Based on these results one can argue that the CNS defects observed in mutants for the other components of the *wg* signaling pathway (such as *arm*) are also indicative of their CNS requirement and not due to the disruption of segmentation process. A similar conclusion was also reached in the case of another segmentation gene *fushi tarazu* (*ftz*) by selectively removing the expression of *ftz* in the developing CNS but not during segmentation by creating promoter mutations (Doe *et al.*, 1988).

The divergence of pathways mediated by several of the genes during segmentation and neurogenesis also indicates a separate requirement for these genes in these two processes. For example, during segmentation the *wg* signaling pathway ultimately controls *engrailed* (*en*) expression via *arm* (see review by Klingensmith and Nusse, 1994). During neurogenesis, while an intact *wg*-signaling pathway is required for the specification of NB4-2 identity, *en/inv* is not required for the specification of this neuroblast (Bhat and Schedl, 1997). Similarly, loss of function for *wg* results in row 6 neuroblasts failing to delaminate (Chu-LaGraff and Doe, 1993), whereas row 6 neuroblast formation is unaffected in *en, inv* double mutants (Bhat and Schedl, 1997). Furthermore, loss of function for *wg*, *ptc* or *hh* genes causes a fully penetrant segmentation phenotype, while the CNS defects in these mutants are generally partially penetrant (indicative of a partial complementation of their function during neurogenesis). Since *wg* is shown to have a distinct CNS requirement (Chu-LaGraff and Doe., 1993; Bhat, 1996) and the signaling cascade mediated by *wg* is intertwined with the pathways regulated by *ptc*, *hh* and *gsb* (see Bhat, 1996; Bhat and Schedl, 1997), it follows from these studies that the CNS defect in mutation in genes such a *ptc*, *hh* and *gsb* reflect their requirement during neurogenesis.

Cell-cell signaling during neuroblast lineage elaboration

The elaboration of a neuroblast lineage in *Drosophila* is in essence governed by the asymmetric cell division and asymmetric cell fate specification of neuroblasts and GMCs. (These asymmetric divisions also present an interesting problem since their division not only leads to asymmetric cell fate specification, but also cells of asymmetric sizes; a GMC cell is always smaller than its parent neuroblast and often, one of the two progeny of a GMC cell are of unequal sizes.) Molecular genetic studies in *Drosophila* and selec-

tive ablations of identified cells at different developmental stages in *grasshopper* indicate that extrinsic pathways interact with intrinsic factors to control asymmetric mitosis and cell fate specification (Kuwada and Goodman, 1985; Guo *et al.*, 1996; Spana and Doe, 1996). Thus determination of the identity of a GMC and a neuron appears to be dependent on mitotic ancestry and cell-cell interactions with neighboring cells. I will discuss two specific examples in this context here, first is the interaction between N and Numb during the asymmetric division of the MP2 cell (Spana and Doe, 1996). The MP2 cell is a special type of neuroblast. It is formed as a neuroblast (under the control of proneural and neurogenic genes) whereas it behaves as a GMC. That is, it divides asymmetrically without self-renewing itself, into two distinct interneurons —dMP2 and vMP2. During the asymmetric division of MP2, Numb becomes localized to the apical region of the neuroblast (Knoblich et *al.*, 1995; Spana et al., 1995) by a process which involves the cytoplasmic adaptor protein Inscuteable (Kraut and Campos-Ortega, 1996; Kraut *et al.*, 1996). The localized Numb (to one of the two cells produced in the division of MP2) negatively interacts with N. This interaction prevents N from activating the pathway which specifies dMP2 identity, thus this progeny assumes a vMP2 identity (Spana and Doe, 1996). A loss of *N* or *Dl* activity eliminates asymmetric cell fate specification and both of the MP2-NB daughters assume dMP2 fate. On the other hand, loss of *numb* causes both progeny to assume vMP2 fate.

The results of Zhong *et al.* (1996) indicate that Numb is also asymmetrically localized during mouse cortical neurogenesis and that m-Numb interacts with Notch-1. It is possible that the two interact negatively in a subset of precursor cells in the mouse cortex to generate daughter cells of asymmetric identities. This is consistent with the observation that asymmetric divisions are common in vertebrate cerebral cortex and that Notch 1 is asymmetrically inherited in one of the two daughter cells (Chenn and McConnell, 1995). It remains to be determined whether the asymmetric distribution of Numb (or similar factors) involves inputs from the surrounding cells and whether this type of mechanism operates in other lineages as well (see below).

The second example concerns with the role of extrinsic factors (cell-cell interactions) in asymmetric cell fate specification during the elaboration of GMC-1 of NB1-1 and Midline Precursor 3 (MP3; which gives rise to H cell and H cell sibling) in *grasshopper* embryonic CNS (Kuwada and Goodman, 1985). In the NB1-1—>GMC-1->aCC/pCC lineage, while the two progeny of GMC-1 appears to be initially non-committed to either of the two sibling cell identities (thus equivalent), the identity of pCC appears to be determined first and the pCC cell then instructs the specification of aCC identity. Similarly during the elaboration of the MP3 lineage, the H-cell sib appears to be specified first which then instructs H cell identity to the other sibling cell. These ablation studies (Kuwada and Goodman, 1985; see also ref. therein) also indicate that either MP3 or GMC-1—>aCC/pCC cells receive no input from adjacent cells during this process, indicating that both mitotic ancestry and cell-cell communication between sibling cells are involved in this cell fate specification. The molecular mechanism by which pCC instructs aCC identity or H cell sib instructs H cell identity to sibling cells or how the identity of pCC or H cell sib is determined in the first place are still open questions. It is also important to determine whether this rule for the MP3 and aCC/pCC lineage elaboration (see above; Kuwada and Goodman, 1985) holds good a) in *Drosophila*, and b) for other lineages (in both *grasshopper* and in *Drosophila*). As for the MP2 lineage elaboration is concerned, as discussed above, these results indicate that the two progeny cells (vMP2 and dMP2) are non-equivalent when they are born from MP2 (see above). Resolving these questions would indeed contribute much to our understanding of asymmetric fate specification to two daughter cells during neural development.

Concluding remarks

In this review, I have discussed recent results on cell-cell signaling during CNS development in the *Drosophila* embryo, with an emphasis to identify open questions. However, I have not discussed a number of issues such as the role of Dorsal-Ventral patterning genes on neurogenesis, which play an important role in specifying the identity of neuroblasts (see Udolph *et al.*, 1995), or the role of cell-cell communication during PNS development and axonal pathfinding. In addition, I have also not addressed how cell-cell signaling controls vertebrate neurogenesis (see Calof, 1995). A number of studies indicate that several of the signaling pathways are conserved between insects and vertebrates (c.f., Chenn and McConnell, 1995; Chitnis *et al.*, 1995; Goodrich *et al.*, 1996; Guo *et al.*, 1996) and that the knowledge gained by studying neurogenesis can be applied to studying vertebrate neurogenesis. The studies discussed in this article also show that *Drosophila* is an excellent organism to address questions regarding how a complex CNS is generated during embryogenesis. The ease with which one can gain insight into these pathways in *Drosophila* using not only knock out mutants but also hypomorphs, conditional mutants and gain of function mutants makes *Drosophila* an organism of choice. One can anticipate that many of the open questions raised in this article will be solved in the near future.

Summary

Development of a multicellular organism requires that cells communicate with each other in order to regulate their growth, organize into tissues and coordinate their function. This cell-cell communication is mediated by signals cells receive (or send) between each other and from the environment. The signaling can be a short range remote signaling (through secreted signaling molecules), contact signaling (via plasma membrane bound molecules, gap junctions) or a long range signaling (through hormones). In this article, I have reviewed the recent advances on the role of cell-cell signaling in the development of the embryonic nervous system of the fruitfly *Drosophila melanogaster* and discussed some of the open questions raised by these studies. It discusses the contributions of the neurogenic genes *Notch* and *Delta* and the signaling pathways controlled by *wingless*, *patched* and *hedgehog* in neuroblast formation, neuroblast identity specification and neuroblast lineage elaboration.

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