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Cell commitment and cell interactions in the ectoderm of *Drosophila melanogaster*

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Summary

The separation of neural from epidermal progenitor cells in the ventral neuroectoderm of *Drosophila* is thought to be mediated by cellular interactions. In order to verify the occurrence of regulatory signals and to test the neurogenic capabilities of cells from various regions of the ectoderm, we have carried out homotopic and heterotopic transplantations of single ectodermal cells. We found that cells from any of the tested regions, with the exception of the proctodeal anlage, are capable of developing as neuroblasts following their transplantation into the ventral neuroectoderm. These neurogenic capabilities are gradually distributed. Cells from the procephalic and ventral neurogenic regions exhibit maximal capabilities, as shown by their behavior in heterotopic transplantations. However, the two neurogenic regions differ from each other in that no epidermalising signals can be demonstrated to occur within the procephalic neuroectoderm, whereas such signals are strong within the ventral neuroectoderm; in addition, neuralising signals from neighbouring cells seem to be necessary for neuroectodermal cells to develop as neuroblasts. Other ectodermal regions whose cells exhibit weaker neurogenic capabilities are, in decreasing order of capability, the dorsal epidermal anlage, the anterolateral region of the procephalic lobe, comprising the anlage of the pharynx, and the anterior pole of the embryo, corresponding to the anlagen of the stomodeum and ectodermal anterior midgut. We assume that, during development *in situ*, the neurogenic capabilities of all these cells are suppressed by inhibitory signals, which are released upon heterotopic transplantation into the neuroectoderm. A community effect which prevents groups of dorsal epidermal cells from taking on a neural fate upon their transplantation into the ventral neuroectoderm, is shown. Finally, we hypothesize that the lack of neurogenic capability in the cells from the proctodeal anlage is due to the absence of products of the proneural genes.

Key words: *Drosophila*, cell transplantation, cell interaction, cell commitment.

Introduction

The ectoderm of *Drosophila* is subdivided into several regions, the cells of which generate a variety of larval organs. Thus, the ventral neurogenic region (VNR) generates both the ventral nerve cord and the ventral half of the epidermal sheath; the procephalic neurogenic region (PNR) generates the brain hemispheres; the dorsal epidermal anlage (DEA) gives rise to the dorsal half of the epidermal sheath, whereas the larval foregut and hindgut develop from the stomodeal and proctodeal anlagen respectively (see Hartenstein *et al.* 1985; Jürgens *et al.* 1986). Very little is known about how the ectoderm becomes subdivided into these different territories and how the ectodermal cells become committed to their various developmental fates. Nevertheless, some data are available about the mechanisms of commitment of the cells of the VNR of insects (see Campos-Ortega, 1991, for a recent review). Here, neighbouring cells can develop either as epidermal or as neural progenitor cells, and two pieces of evidence indicate that how they develop is mediated by cell–cell interactions. On the one hand, laser ablation experiments carried out in grasshoppers show that the cells remaining in the VNR after the neuroblasts have segregated from the ectoderm are not firmly committed to their epidermal fate (Taghert *et al.* 1984; Doe and Goodman, 1985): cells that normally would develop as epidermoblasts may adopt the neural fate if a neuroblast in their immediate vicinity is ablated. These results suggest that the prospective epidermoblasts are inhibited by the neuroblasts from adopting the neural fate (Doe and Goodman, 1985). On the other hand, heterotopic, heterochronic and interspecific cell transplantations in members of the genus *Drosophila* suggest that regulatory signals with epidermalising and neuralising character are involved in the ectodermal cell commitment to one of the two developmental fates

(Technau and Campos-Ortega, 1986*a*, 1987; Technau *et al.* 1988; Becker and Technau, 1990).

In this report, we discuss results of further transplantation experiments that were carried out in *Drosophila melanogaster* during the early gastrula stage, using single cells from various ectodermal regions, to investigate the role played by cellular interactions in providing the cells with a given developmental fate. We also discuss results of transplanting groups of cells from the DEA into the VNR in order to compare their behaviour with that of single cells and thus assay any kind of 'community effect' on the cells behaviour in ectopic positions (see Gurdon, 1988). Our results suggest that signals with neuralising influences occur within the VNR, and that inhibitory signals impede neurogenesis within the non-neurogenic regions of the ectoderm.

Materials and methods

Throughout our work we used Technau's procedure (1986) to label the cells to be transplanted. In short, a mixture of rhodamine–dextran and HRP is injected into an embryo before cell formation has taken place; the injected substances are incorporated into the cells as they form during the blastoderm stage. In our experiments, cells were removed at the gastrula stage (stage 7 of Campos-Ortega and Hartenstein, 1985) from various embryonic regions (Fig. 1A–C and below). Single cells were transplanted isochronically into various embryonic regions of unlabelled host embryos, which were allowed to continue development. Transplantation of single cells was controlled by briefly observing the host embryos by fluorescence microscopy immediately after the transplantation had been done; we are confident that only cases of single cell transplantation were further considered in these series. To assess 'community effects' within the *Drosophila* ectoderm, two additional series of experiments were carried out in which we attempted to transplant groups comprising several cells each (3–4 and up to 10) from the DEA into the VNR.

The histochemical reactions to demonstrate HRP activity in the progenies of the transplanted cells were performed on stage 16–17 host embryos. All cell clones obtained from the experiments described below were classified into various histotypes according to location and organisation of the clone, cell shape and size, presence or absence of axonal and dendritic processes in the clone cells, and clone size (see Technau, 1986, 1987; Technau and Campos-Ortega, 1986*a*, 1986*b*; Beer *et al.* 1987, for a description of the diagnostic criteria). In some of the experimental series (see Tables 1–3), we used as donors embryos which, in addition to being labelled with rhodamine–HRP, specifically expressed β-galactosidase either in most of the neuroblasts and their progenies, or in most of the epidermal cells. Therefore, in these series the diagnosis of a cell as neural or epidermal was based on its β-galactosidase content, its HRP activity and the morphological criteria referred to above. Double staining for HRP and β-galactosidase was carried out following the procedure of Prokop and Technau (1991). Clones were denominated neural if they consisted of neurons and/or glia cells, as determined by their morphology and/or β-galactosidase expression, and irrespective of their location in the central nervous system; neural cells have distinct morphological characteristics which permit their unambiguous identification. Clones were denominated epidermal or as muscle, midgut or heart cells, if the cells were located in the corresponding organs and showed morphological features typical of these organs. In cases of ectopic location, clones were generally not assigned to any specific histotype, except in the cases of β-galactosidase expression.

Results

Neuralising signals in the VNR?

Signals with neuralising character in the VNR, contributing a neural fate to those neuroectodermal cells that develop as neuroblasts, were proposed to exist based on the following results of homotopic and heterotopic cell transplantations (Technau and Campos-Ortega, 1986*a*; see also Campos-Ortega, 1988). When single cells from the VNR [from approximately 45–55 % egg length (EL), 0 % at the posterior pole, and 0–50 % ventrodorsal dimension (VD), 0 % at the ventral midline] were transplanted homotopically into the VNR of a host embryo, they developed either as neuroblasts or as epidermoblasts; in some cases, the transplanted cells divided once before the two daughter cells took on the neural and/or the epidermal fate. Consequently, the transplanted cells gave rise to three types of clones: neural, epidermal, and mixed. This demonstrates that single cells of the VNR have neurogenic and epidermogenic capabilities. When single cells from the DEA (from 45–55 % EL, 80–100 % VD) were transplanted into the DEA, they gave rise to epidermal clones only; thus, the DEA appears to lack neurogenic capabilities (experiments 1 and 2 in Fig. 1A, Table 1).

Following heterotopic transplantation, VNR and DEA cells also behaved differently. VNR cells transplanted into the DEA (sites of origin and destination as above) developed according to their site of origin and differentiated both epidermal and neural histotypes (experiment 3 in Fig. 1A, Table 1). This suggests that at least some of the VNR cells are committed to neural fate, since they are capable of differentiating as neuroblasts in ectopic positions. In contrast, DEA cells transplanted into the VNR developed according to their new location, giving rise to either epidermal or neural clones (experiment 4 in Fig. 1A, Table 1).

This behavior of the DEA cells is striking: cells which normally do not develop as neuroblasts may adopt a neural fate upon heterotopic transplantation into the VNR. Two possible interpretations of the above results are: (i) the cells in the DEA are, in principle, able to develop as neuroblasts, but, *in situ*, are prevented by inhibitory processes from taking on neural fate: upon their transplantation into the VNR, this inhibition is relieved; (ii) the transplanted DEA cells are actively induced by their neighbours in the VNR to adopt a neural fate. Since transplanted VNR cells took on neural fate within the DEA in a fairly large number of cases (experiment 3 in Fig. 1A, Table 1), signals which inhibit neurogenesis seem not to exist or, at least, do not act very efficiently in this region of the ectoderm;

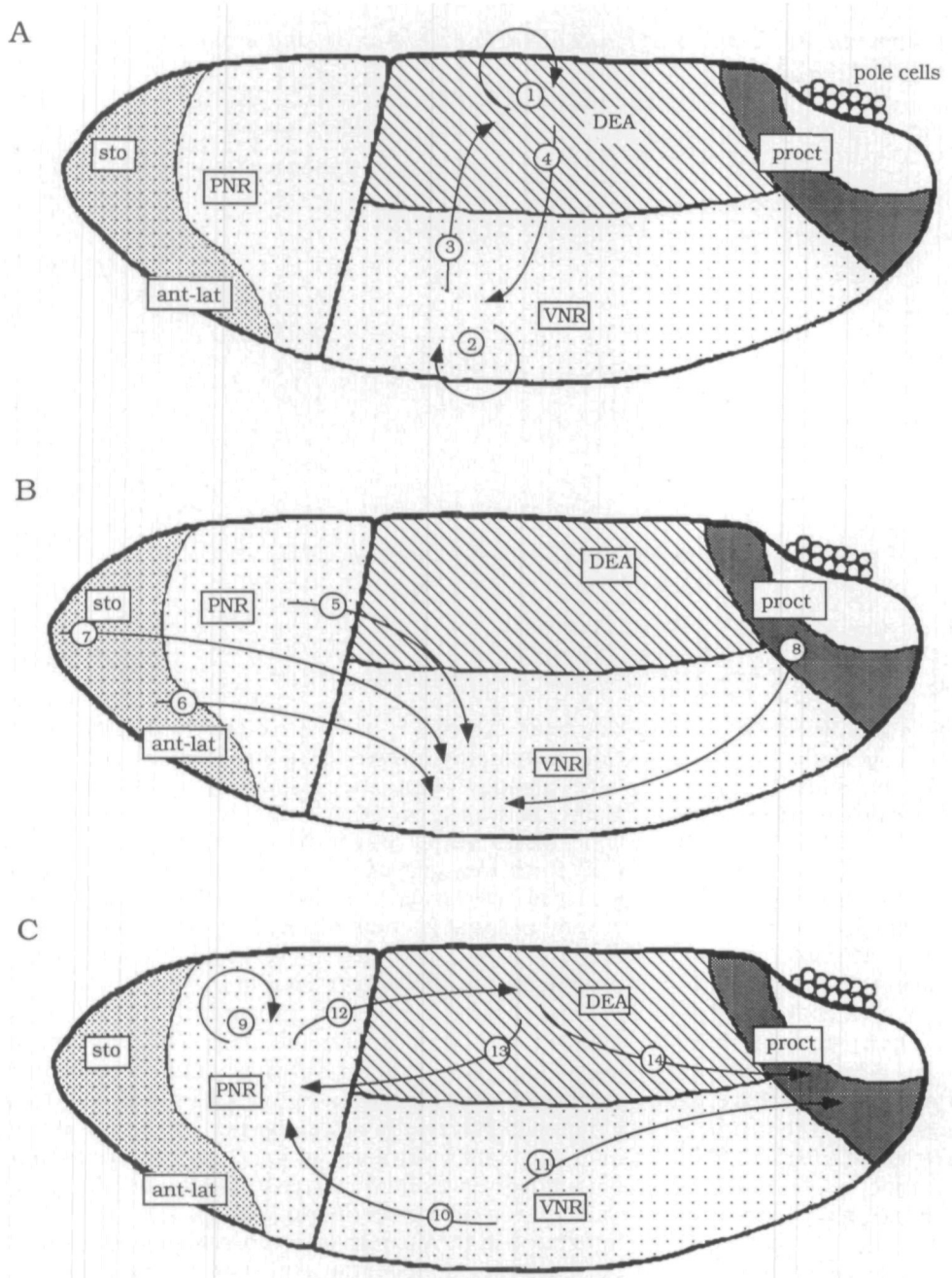

Fig. 1. A, B and C show a fate map of the gastrula stage, at which our experiments were done, and the various transplantation experiments (numbered 1–14). Refer to text. Abbreviations: ant-lat, anterolateral ectoderm; DEA, dorsal epidermal anlage; PNR, procephalic neuroectoderm; proct, proctodeal anlage; sto, stomodeal anlage; VNR, ventral neuroectoderm.

therefore, the latter hypothesis appears *prima facie* to be more plausible than the former.

Differential distribution of neurogenic capabilities of ectodermal cells

To test the capability of other ectodermal cells to adopt neural fate under experimental conditions, as well as to further substantiate the hypothesis that neuralising signals operate within the VNR, we transplanted single cells from other ectodermal regions into the VNR. We found that cells from two major regions, i.e. the PNR and the anterior and lateral ectoderm (comprising the entire pharyngeal and stomodeal anlagen; Hartenstein *et al.* 1985), are capable of adopting neural fate when they develop in the VNR; in contrast, cells from the proctodeal anlage are apparently incapable of adopting neural fate upon transplantation into the VNR.

We removed cells from the PNR (from 65–75 % EL and 50 % VD, in front of the cephalic furrow; Table 2, experiment 5 in Fig. 1B), transplanted single cells into the VNR and found that they very frequently generated neural clones. Indeed, a significantly higher proportion of PNR cells than VNR cells produce neural clones after transplantation into the VNR (84 % *versus* 50 % of all clones; Tables 1 and 2), indicating that the PNR cells have a higher tendency to adopt neural fate. However, PNR cells also gave rise to a small number of epidermal and mixed clones, of epidermal and neural histotype, suggesting that some of the PNR cells are not firmly committed to neural fates and are still able to follow other developmental pathways, at least while develop-

Table 1. *Homotopic and heterotopic transplantations of single VNR and DEA cells, and groups of DEA cells*

Transplantation			Histotypes			
Type	No. of transpl.	No. of clones	neural	epidermal	epidermal/neural	others
VNR into VNR**†	189	97 (52)	37 (38)	43 (43)	11 (11)	6 (6) muscles
VNR into VNR	192	92 (47)	47 (51)	30 (33)	15 (16)	–
DEA into DEA**	185	52 (28)	–	40 (77)	–	11 (21) heart 1 (2) trachea
DEA into VNR**	249	89 (36)	19 (21)	55 (62)	15 (17)	–
DEA into VNR*	166	30 (18)	9 (30)	17 (57)	4 (13)	–
VNR into DEA**	240	62 (26)	25 (40)	37 (60)	–	–
DEA into VNR* (3–4 cells)	210	97 (47)	5 (5)	88 (91)‡	4 (4)	–
DEA into VNR* (10 cells)	160	109 (68)	1 (1)	101 (93)§	3 (3)	1 (1) muscle 3 (3) midgut

Figures in parenthesis are % of all clones.
* Transplantations carried out in part with HRP-labelled cells which specifically expressed β-galactosidase in epidermal cells.
** Data from Technau and Campos-Ortega (1986*a*), given for comparison.
† Data from transplantations from 0–50 % VD are pooled.
‡ includes three clones located subepidermally, whose cells expressed β-galactosidase.
§ includes eleven clones located subepidermally, whose cells expressed β-galactosidase.

ing within the VNR. But this result might also be explained by inaccurate removal of cells from the procephalic lobe of the donors, i.e. by taking cells from regions immediately in front of the PNR. Indeed, cells from anterior and lateral positions within the procephalic lobe (foregut anlage, experiment 6, Fig. 1B), flanking anteriorly the PNR, behaved like cells from the VNR after homotopic transplantation, that is to say, they gave rise to neural, epidermal and mixed clones in similar proportions as in experiment 1. In addition, a large number of clones with midgut epithelial histotypes was found incorporated into the midgut; these clones most probably originated from cells inadvertently removed from the neighbouring endodermal midgut anlage, which are known to be committed to endodermal fates (see Technau and Campos-Ortega, 1986*b*). Cells from the very anterior pole of the embryo (experiment 7, Fig. 1B), which normally invaginate with the stomodeum to give rise to the ectodermal portion of the anterior midgut (Technau and Campos-Ortega, 1985), adopted neural fate less frequently and epidermal fate more frequently than cells from the anterolateral regions. In addition, a larger number of midgut epithelial clones, as well as clones with vesicular character, which we believe to correspond to ectopically developing foregut structures, were found.

In analogy with the results of the heterotopic transplantations of DEA cells into the VNR, we conclude from the results of experiments 6 and 7 that cells from the anlagen of stomodeum–pharynx (foregut) and ectodermal anterior midgut have neurogenic capabilities that become manifest upon transplantation into the VNR, and that these capabilities are more pronounced in the former than in the latter cells.

After transplantation of proctodeal cells into the VNR (Table 2 and experiment 8, Fig. 1B), 85 % of the transplants gave rise to vesicular, hollow structures composed of 6–8 cells; these vesicles were situated either subepidermally or among the yolk grains in the midgut and may well correspond to ectopically developing hindgut epithelium. We also found one neural and four epidermal clones in this series, the latter ones integrated in the epidermis of thoracic segments. Since the frequency of appearance of such clones is very low indeed, we believe that they developed from cells removed from neighbouring abdominal segments of the donor embryos, rather than from proctodeal cells. Therefore, the proctodeal cells seem to be committed

Table 2. *Transplantations of single ectodermal cells from various positions into the ventral neuroectoderm*

Transplantation			Histotypes						
Type	No. of transpl.	No. of clones	neural	epidermal	vesicles	epidermal/neural	midgut	muscle cells	non-diff.*
PNR into VNR	180	79 (44)	66 (84)	7 (9)	–	5 (6)	–	–	1 (1)
AL into VNR	188	70 (37)	31 (44)	3 (4)	–	6 (9)	23 (33)	6 (9)	1 (1)
Sto into VNR	267	87 (33)	14 (16)	20 (23)	13 (15)	5 (6)	28 (32)	3 (3)	4 (5)
Proct into VNR	114	46 (60)	1 (2)	4 (9)	39 (85)	–	1 (2)	–	1 (2)

Figures in parentheses are % of all clones.
* Clones were classified as non-differentiated when several HRP-labelled cells did not show any recognisable histotype. AL, anterolateral ectoderm (foregut anlage); Sto, stomodeum anlage (anterior pole); Proct, proctodeal anlage.

Table 3. *Homotopic and heterotopic transplantations of single cells*

Transplantation			Histotypes						
Type	No. of transpl.	No. of clones	neural	epidermal	vesicles	epidermal/ neural	frontal sack	pharynx	non-diff.*
PNR into PNR	97	24 (25)†	23 (96)	–	–	1 (4)	–	–	–
VNR into PNR**	280	80 (22)†	74 (93)	–	–	–	–	–	6 (7)
VNR into Proct**	310	61 (20)†	49 (80)	1 (2)	–	–	–	–	11 (18)
PNR into DEA	243	18 (7)†	4 (22)	4 (22)	1 (4)	–	–	–	9 (45)
DEA into PNR	157	42 (27)†	9 (21)	2 (5)	10 (24)	–	16 (38)	3 (7)	2 (4)
DEA into Proct	252	37 (15)†	3 (8)	2 (5)	21 (58)				11 (30)

† Figures in parentheses are % of all transplants. All other figures in parentheses are percentage of all clones.
* Clones were classified as non-differentiated when several HRP-labelled cells did not show any recognisable histotype.
** Experiments carried out with HRP labelled cells which specifically expressed β-galactosidase in neural cells.
Epidermal clones are integrated within the epidermis.
Proct, proctodeal anlage.

to their proctodeal fate and are not prone to take on a neural fate following transplantation into the VNR.

The results of experiments 5–8 thus indicate that neurogenic capabilities are differentially distributed among the different territories of the ectoderm that we have tested under the present experimental conditions. These abilities are highest in the cells of the PNR, decrease progressively in the anterolateral direction, from the PNR to the anterior pole, and are apparently lacking from the proctodeal anlage cells. Cells from the foregut anlage may react to whatever conditions exist in the VNR that favour neural development, whereas proctodeal cells do not react to these conditions. The results also point to a relatively high degree of developmental commitment of the cells of the various anlagen prior to their transplantation. Thus, cells from the PNR that developed in the VNR produced almost exclusively neural clones, anterolateral cells frequently produced foregut or midgut clones, and proctodeal cells generated only hindgut clones.

Epidermalising signals do not operate in the PNR

The previous series of experiments has provided evidence for a high degree of neurogenic capabilities of the cells of the PNR. Laser ablations carried out during the blastoderm stage on cells from the PNR had previously failed to produce epidermal defects in the larvae (Jürgens *et al.* 1986), indicating that all the cells in the PNR normally enter the neural pathway of development and, in striking contrast to the situation in the VNR, are not intermingled with presumptive epidermoblasts. Indeed, cells from the PNR transplanted homotopically into host embryos (Table 2, and experiment 9 in Fig. 1C) were found to generate almost exclusively neural cell clones, which were located throughout the supraoesophageal ganglion. Only one clone was found containing both neural and epidermal cells, which were located in the supraoesophageal ganglion and in the frontal sack, respectively. Therefore, these results suggest that the PNR as a whole essentially lacks epidermalising influences; adoption of a neural fate by a PNR cell is thus a consequence of intrinsic properties, rather than being mediated by cellular interactions. Results of transplanting cells from the VNR into the PNR (Table 3, and experiment 10, Fig. 1C) confirm the lack of epidermalising influences in the PNR itself and further demonstrate that the VNR cells have a high degree of neurogenic capability: 93 % of the clones scorable in this experiment were neural; the remaining clones did not differentiate into any recognisable histotype.

Another experimental series demonstrates pronounced neurogenic capabilities of the VNR cells: upon transplantation of single VNR cells into the proctodeal anlage (Table 3, and experiment 11, Fig. 1C), 80 % of the clones were neural, one was epidermal and the remaining ones could not be assigned to histotypes. These results suggest that, during development *in situ*, the VNR cells must be forced into the epidermal fate by epidermalising signals.

To further verify the idea that the PNR cells are firmly committed to their neural fates, we transplanted PNR cells into the DEA (Table 3, and experiment 12, Fig. 1C). Surprisingly, however, the overwhelming majority of the cells transplanted in this experiment did not differentiate into recognisable structures. From a total of 243 transplants, only 18 clones differentiated; four of them were epidermal, another four neural, one formed a vesicle and nine did not differentiate into any recognisable histotype. In contrast, cells from the DEA transplanted into the PNR developed frequently and differentiated into four different classes (Table 3, experiment 13, Fig. 1C). In nine cases the cells took on neural fate and produced neural clones located within the larval supraoesophageal ganglion; in another two cases, the cells developed as epidermal progenitor cells, their progeny being incorporated in the epidermal derivatives of the maxillary segment. In addition, 11 clones of vesicular structure were found, several of which were located within the supraoesophageal ganglion. The spindle-like shape of their cells, the lack of neural processes and the fact that progeny of DEA cells in the VNR, which had formed vesicles, could be identified as epidermal in other experiments using specific markers (β-galactosidase, see next experiment), lead us to believe that these are epidermal

clones, which failed to integrate into an appropriate tissue and closed up to form spheres.

In experiment 14, we transplanted single cells from the DEA into the proctodeal anlage (Table 13, Fig. 1C) in an attempt to further qualify the results of transplanting DEA cells into the VNR and, thus, obtain additional evidence in support of neuralising influences within this latter region. The majority of clones (58%) were located within the abdomen and consisted of a vesicular array of cells, which we believe are epidermal (see previous paragraph); a high proportion of clones (30%) were located in the hindgut lumen and did not differentiate into any recognisable histotype; two clones were epidermal, incorporated into the epidermal sheath of the 8th abdominal segment, probably derived from having transplanted cells outside the proctodeal anlage; finally, a few clones (3) consisted of 2–3 neurones with long neural processes that had grown ectopically.

A community effect after transplantation of groups of DEA cells

The results above indicate that ectodermal cells are capable of interacting with each other. For example, the VNR provides single DEA or anterolateral ectodermal cells in conditions that make possible their development as neuroblasts, whereas the same cells will adopt epidermal fates when they develop *in situ*; epidermalising influences are required by the VNR cells to allow their development as epidermoblasts. Two additional experimental series were carried out to assess to what extent DEA cells maintain their original state and develop according to their origin, when they are transplanted into the VNR in groups rather than individually. In one series, we transplanted groups of 3–5 cells, in the other up to 10 or more cells; in both cases we tried to keep the transplanted cells together and avoid their separation, to permit them to maintain conditions as close as possible to those prevailing within the DEA. In both experimental series, most differentiated clones had epidermal histotype and were of large size (20–30 or more cells), and only very small numbers of neural or mixed, epidermal and neural, clones were found (Table 1). Neural clones were smaller (between 1 and 8 cells) than epidermal clones. In addition, several clones formed vesicular structures located subepidermally (Fig. 2D, E). The latter clones could, in this series, be unambiguously diagnosed as epidermal because a *lacZ* insertion line, which specifically expresses β-galactosidase in epidermal cells, was used as donor.

The size of the neural clones found in this series (compare Technau and Campos-Ortega, 1986*a*) suggests that they either derived from individual cells that had succeeded in differentiating, or from single cells that had separated from the mass of transplanted cells. In contrast, the epidermal clones suggested that their cells had developed from several transplanted cells that had differentiated together. Therefore, these experiments indicate that whereas single cells do react to influences from the environment, groups of DEA cells are not susceptible to these influences.

Discussion

Neural induction and/or neural inhibition?

Under normal conditions, the capability to produce neuroblasts is a characteristic of the VNR and the PNR, which other regions of the ectoderm do not possess; neuroblast development within the non-neurogenic regions of the ectoderm is only possible if cells from the VNR or the PNR are transplanted into these regions. Nonetheless, our results show that cells from most of the non-neurogenic ectodermal regions are capable of developing as neuroblasts within the VNR and the PNR, that is to say, the regions which are normally permissive for neurogenesis. Cells from the proctodeal anlage, on the other hand, did not respond to influences from the VNR cells and apparently assumed their normal fate as hindgut cells, forming vesicular structures ectopically. Hence, the proctodeal cells can be regarded as committed at the time of transplantation in the early gastrula stage. The question is, of course, why do cells from the different ectodermal regions behave in such different ways?

Although both regions are neurogenic, the VNR and the PNR differ from each other in that normally the cells of the former may develop either as neural or as epidermal progenitors, whereas those of the latter region develop exclusively as neural progenitor cells. In fact, the PNR cells showed a pronounced propensity to adopt neural fates, since, upon their heterotopic transplantation into the VNR most of them developed as neuroblasts and only a few reacted to the epidermalising signals seemingly available within the VNR. Moreover, these signals are essentially absent from the PNR, as shown by the homotopic transplantations of PNR cells and by the heterotopic transplantation of VNR cells into the PNR: in both cases, neuroblasts developed almost exclusively. These findings actually confirm results of previous experiments, in which laser ablations within the PNR failed to cause larval epidermal defects (Jürgens *et al.* 1986). In contrast, epidermalising signals are operative within the VNR and are apparently required to allow some of its cells to develop as epidermoblasts. In fact, the intrinsic neurogenic capabilities of the VNR cells seem to be as strong as those of the PNR cells, since almost all VNR cells when individually transplanted into the PNR or into the proctodeal anlage, that is to say, in the apparent absence of epidermalising signals, developed as neuroblasts. We, of course, assume that no neuralising influences exist in either region, since there is no indication of it.

Thus, it seems that neural development of the VNR cells has to be actively opposed to permit some of these cells to take on epidermal fate to form the ventral half of the epidermal sheath. After heterotopic transplantations of DEA cells (Technau and Campos-Ortega, 1986*a*, and present work) and of cells from the

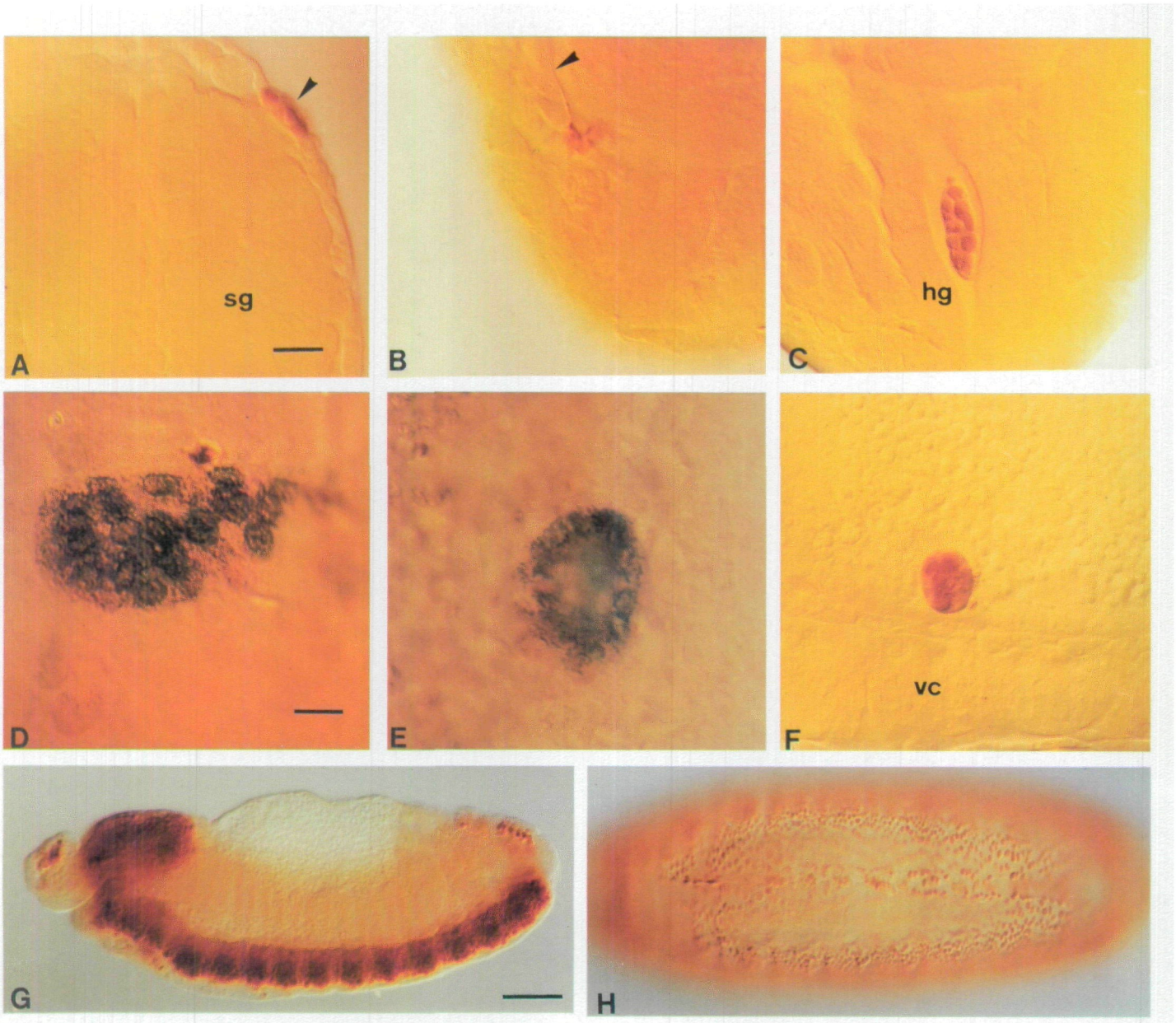

Fig. 2. Examples of cell clones. A shows an epidermal clone (arrowhead) located in the epidermis surrounding the supraoesophageal ganglion (sg) derived from a cell from the DEA transplanted into the PNR. B and C show two examples of neural clones, which developed from single cells from the VNR transplanted into the proctodeal anlage. The clone in B is located subepidermally; axons (arrowhead) grow ectopically from the clone. The clone in C consists of 14–16 cells; axons (not distinguishable here) can be seen intermingled between the cells. D and E show two examples of epidermal clones derived from groups of 3–4 cells transplanted into the VNR. Both clones are located subepidermally and their cells form closed vesicles; the cells in both clones show HRP activity and express β-galactosidase. F shows a vesicle located in the midgut cavity, which we believe is formed by hindgut cells derived from a cell from the proctodeal anlage transplanted into the VNR. vc, ventral nerve cord. G and H shows examples of embyros from the two lines in which β-galactosidase is specifically expressed in neural (G, midsagittal view) and in epidermal (H, dorsal view) cells, which were used as donors in some of the experiments. Magnification bar in A (applies to A–C and F) is 20 μm, in D (D and E) 10 μm, and in G (G and H) 50 μm.

anterolateral ectoderm into the VNR (present work), high numbers of transplanted cells took on neural fate. In another experiment, DEA cells were transplanted into the PNR and found to adopt neural fate in approximately the same proportion of cases as when transplanted into the VNR. Thus, both neurogenic regions appear to exert similar neuralising effects on the DEA cells. Were these cells actively induced to neurogenesis by the surrounding neuroectodermal cells? Or were they rather relieved from inhibitory influences which normally prevent them from developing as neuroblasts? The available evidence does not allow us to conclusively distinguish between neural induction within the VNR *versus* neural inhibition in the DEA and the stomodeal–pharyngeal anlagen. The data could actually be interpreted to support the occurrence of either one, or of both mechanisms, and do not exclude any. In fact, the occurrence of both neural induction within the VNR and neural inhibition within the DEA and the anterolateral territories of the ectoderm appears to us a very appealing possibility.

With the transplantation of VNR cells into the DEA, we failed to demonstrate influences which would completely inhibit neural development within this latter region (Technau and Campos-Ortega, 1986*a*, and present observations). In fact this result actually supports neural inhibition dorsally, since the transplantation of VNR cells into either the PNR or the proctodeal anlage (experiments 10 and 11) results in a much higher proportion of neural clones than after transplanting VNR cells into the DEA. Similar heterotopic transplantations were carried out with PNR cells into the DEA (experiment 12). But, surprisingly enough, in spite of a large number of transplants, only a few cells succeeded in developing. We do not understand why the frequency with which PNR cells developed within the DEA was so low; apparently, the DEA does not provide appropriate conditions for the development of the cells from the PNR. However, among the few clones obtained in this experiment, epidermal histotypes were present as frequently as neural ones, although, as discussed in the previous paragraph, the PNR cells showed a high propensity to adopt neural fates in all the other experiments in which they were involved. Consequently, this result could also be interpreted as support for signals inhibiting neural development within the DEA.

The transplantation of single cells from the DEA into the proctodeal anlage (experiment 14) supports neural induction. The transplantation of single VNR cells into this region (experiment 11) indicates that the proctodeal anlage lacks epidermalising influences. Thus, if neural inhibition would normally hinder the appearance of neuroblasts within the DEA, a large number of DEA cells should adopt neural fate upon their transplantation into the proctodeal anlage. However, clones with neural histotype differentiated in this experiment, but only very few of them, whereas most of the cell clones formed vesicles (probably of epidermal cells) or did not differentiate recognisable histotypes. Therefore, the intrinsic neurogenic capabilities of the DEA cells cannot be high, and this experiment points to neural induction within the VNR as the main mechanism that allows cells from non-neurogenic regions of the ectoderm to take on a neural fate while developing within the neuroectoderm.

A community effect after transplantation of several cells

Following transplantation of single DEA cells into the VNR, between one fifth and one third of them adopted neural fate. After transplanting groups of DEA cells, however, a much higher proportion of transplants (more than 90 % after transplanting about 10 cells) took on epidermal fate than in the case of single cell transplantations. It seems, therefore, that groups of cells can maintain the same conditions that prevail during development *in situ*, whereas individual cells are subjected to the conditions imposed by the environment with which they interact. Possible neuralising signals would act upon such a patch of cells only at its borders; thus, one would expect the effect of such signals on the subsequent developmental behavior of the cells to be considerably weakened. Indeed, only a few clones were found which consisted of neural cells; they had apparently originated from single cells that had succeeded in developing, whereas the other transplanted cells died. In addition, another few clones were mixed, with epidermal and neural cells. The neural components of these mixed clones – as deduced from their size and locations – seemed to have originated from single cells that had detached from the main mass of transplanted cells.

The interpretation of these results, with respect to the mechanism that led to a neural development of a few cells, poses in principle the same difficulties as discussed above for the single cell transplants. Yet, the results clearly show that interactions between ectodermal cells are an important factor in the process of cell fate acquisition and maintenance: neural induction and/or neural inhibition presupposes the exchange of signals between the cells, which is likely to involve direct cell-to-cell contact.

Cell interactions and the phenotype of neurogenic mutants

It is worth emphasizing the similarity between the regions whose cells have been found to be capable of developing as neuroblasts after transplantation into the VNR, and those contributing to the embryonic phenotype of neurogenic mutants (Lehmann *et al.* 1983). Inspection of the neurogenic phenotype reveals that the extent of the neuralized region in the most extreme neurogenic mutants is actually larger than the neuroectoderm, that is to say, than the VNR and the PNR together; the region that becomes neuralized in the mutants comprises a substantial fraction of the DEA and of the stomodeal–pharyngeal anlagen as well (Jiménez and Campos-Ortega, 1982), whereas the proctodeal anlage develops largely normally in these mutants.

We hypothesize that there is a causal relationship

between the extent of the region of neuralization in the mutants and the ectodermal cells' ability to take on neural fate in the transplantations, which may relate our present experiments to the genetic circuitry responsible for the neuroepidermal lineage dichotomy in *Drosophila* (Campos-Ortega, 1991) and thus help us in understanding the molecular basis of developmental commitment of the ectodermal cells under discussion. We are presently testing whether the content of so-called proneural gene products in a given cell (Ghysen and Dambly-Chaudière, 1989) is related to the cell's ability to take on neural fate upon transplantation. We believe this to be a plausible hypothesis, in view of the fact that these gene products have been shown to be involved in the commitment of the neuroblasts (Jiménez and Campos-Ortega, 1990).

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