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Primary culture of single ectodermal precursors of *Drosophila* reveals a dorsoventral prepattern of intrinsic neurogenic and epidermogenic capabilities at the early gastrula stage

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Summary

We have analyzed the development in vitro of individual precursor cells from the presumptive truncal segmental ectoderm of the *Drosophila* embryo to study the intrinsic component in the determination of cell fate. For each cultured cell, the original position within as well as the developmental stage of the donor embryo were known. Cells removed from the ventral neurogenic region develop neural clones. Cells from the dorsal ectoderm and from the dorsalmost part of the ventral neurogenic ectoderm develop epidermal clones. These two classes of clones differ with respect to their division pattern, adhesiveness, cell morphologies and the expression of cell-specific markers. Mixed neural/epidermal clones were obtained from a fraction of precursors at almost all dorsoventral sites.

We conclude that, at the onset of gastrulation, precursor cells of the truncal segmental ectoderm already have the capability to develop as either neuroblasts or epidermoblasts in the absence of further cell interactions. At the same time, positional cues distributed along the dorsoventral axis equip precursors with intrinsic preferences towards the neural or epidermal fate, thus defining a prepattern of high neurogenic preferences ventrally, and high epidermogenic preferences dorsally. It is likely that this prepattern is involved in defining the extent of the ventral neurogenic and dorsal epidermogenic regions of the ectoderm. The roles of intrinsic capabilities versus extrinsic influences in the regulation of the characteristic pattern of segregation of the two lineages are discussed.

Key words: single cell culture, determination, ectoderm, *Drosophila.*

Introduction

Neural and epidermal cell lineages are the two major contributions of the ectoderm. In the ventral neurogenic region of *Drosophila*, which covers the ventral half of the truncal ectoderm, neural and epidermal precursor cells are intermingled. The dorsal half of the truncal ectoderm gives rise to the dorsal epidermis and its derivatives and does not contain presumptive neuroblasts (Hartenstein and Campos-Ortega, 1984, 1986; Technau and Campos-Ortega, 1985). However, dorsal ectodermal cells have neurogenic potencies as disclosed by their heterotopic transplantation into the ventral neurogenic ectoderm (Technau and Campos-Ortega, 1986). These and other experiments (Technau et al., 1988, Stüttem and Campos-Ortega, 1991; for grasshoppers see also Doe and Goodman, 1985) provide evidence that the decision of the cells to adopt the epidermal or the neural fate is mediated by a delicate balance between intrinsic factors and intercellular communication (for reviews see Campos-Ortega, 1992; Campos-Ortega and Haenlin, 1992). Two well-studied groups of genes play an important role in this process. The products of the proneural genes are required for the formation of CNS precursors (Cabrera et al., 1987; Jimenez and Campos-Ortega, 1990) and sensory organ mother cells (Ghysen and Dambly-Chaudiere, 1989; Bodmer et al., 1989; Caudy et al., 1988; Simpson, 1990) and there is some experimental evidence for neuralizing signals (Technau and Campos-Ortega, 1986; Stüttem and Campos-Ortega, 1991). The neurogenic genes, however, are thought to provide an intercellular signal that inhibits cells adopting their primary neural fate by allowing epidermal development (for reviews see Campos-Ortega, 1992; Campos-Ortega and Knust, 1990).

Thus, the regulation of the neural/epidermal dichotomy of ectodermal cells is complex as many different factors are involved. So far, the functional significance of these factors has been analyzed in situ by deleting or changing one or more of them genetically or experimentally. However, these approaches do not allow a complete separation of intrinsic factors from extrinsic factors such as cell communication. One possibility is to separate cells and grow them in vitro. In *Drosophila*, aspects of the differentiation of embryonic neuroblasts have been extensively studied in primary culture (e.g. Seecof et al., 1973; Furst and

Mahowald, 1985; Salvaterra et al., 1987; Huff et al., 1989; Wu et al., 1990) whereas, as yet, epidermogenic capabilities of cultured cells have been rarely considered (Chan and Gehring, 1971; Dübendorfer and Eichenberger-Glinz, 1980). Since, in most of these experiments, large numbers of cells from dissociated embryos or a neuroblast-enriched fraction of cells were cultured, the exact site of origin of the cells in the embryo was not known. Therefore, information is scarce about how far the developmental potencies exhibited by cells in vitro might be a function of the intrinsic manifestation of positional values.

Investigation of the intrinsic neurogenic and epidermogenic properties of ectodermal cells, required the following: (1) cells were cultured individually, (2) they were explanted from donors shortly after cell formation, i.e. before the segregation of the neural and epidermal lineages and (3) the site of origin of the cells with respect to the early gastrula fate map was known. We present evidence that ectodermal precursor cells at the beginning of gastrulation are already equipped with intrinsic neurogenic and epidermogenic properties, the realization of which does not depend on further cell interactions. These properties are differentially distributed along the dorsoventral axis of the presumptive truncal ectoderm, thus defining a prepattern that may serve to separate the ventral neurogenic from the dorsal epidermogenic region.

Materials and methods

Stocks

Cells were taken from the Canton S wild-type strain of *Drosophila* or from a transformant strain carrying a P-element vector with the P-*lacZ* fusion gene (O'Kane and Gehring, 1987) with specific expression of the reporter gene in epidermal cells (courtesy of J. A. Campos-Ortega; Stüttem and Campos-Ortega, 1991).

Culture medium

We used the primary culture medium for *Drosophila* embryonic cells of Shields and Sang (1977). Non heat-inactivated fetal calf serum was added to 10%. After addition of the serum, the medium was held for 1-3 days at room temperature before use. To condition the medium, 3-6 hour embryos were chemically dechorionized, washed in H_2O and subsequently in medium, dissociated in a glass homogenizer under sterile conditions, washed in medium, spun 3-4 times at 620 ***g*** (2 minutes) and then transferred in fresh medium to a culture bottle with a final concentration of about 3.5×10^6 cells/ml (Dübendorfer and Eichenberger-Glinz, 1980). Primary culture was kept without CO_2 at 26°C. Once a week roughly half of the medium was removed and replaced by fresh medium. Conditioned medium can be harvested from 3 days to 6 weeks after starting the culture.

Primary culture of single ectodermal precursor cells

Embryos at the blastoderm stage were washed in 70% ethanol, dechorionated, mounted in an appropriate orientation on a coverslip coated with glue, desiccated and covered with fluorocarbon oil as described before (Technau, 1986). Embryos were selected as donors at about 10 minutes after the onset of gastrulation shortly after completion of cell formation (stage 7; stages according to Campos-Ortega and Hartenstein, 1985). With the aid of a micromanipulator, a capillary (provided with an approximately 45° bevel and an inner tip diameter of 15-18 μm) was introduced into the embryo at around 50% EL (egg length). 1-3 cells were gently removed from a given position along the ventrodorsal diameter (%VD). Positions were defined relative to the first morphogenetic landmarks (ventral furrow, cephalic furrow, position of pole cells) according to the early gastrula fate map (Fig. 1; Hartenstein et al., 1985; Technau and Campos-Ortega, 1985; Technau, 1987).

A drop (10-20 μl) of conditioned medium was passed through a sterile filter and placed in the center of a clean, sterile coverslip. Immediately after removal from the embryo, a single cell was released from the capillary and placed centrally onto the coverslip in the medium. In order to attach the cell to the coverslip, the ground end of the capillary must be obliquely oriented towards the coverslip. In cases when more than one cell was introduced into the medium (up to 4 cells), they were deposited individually with large distances between each other in order to prevent cell interactions. The preparation was then sealed to a culture vessel. The culture vessel was constructed as described by Dübendorfer and Eichenberger-Glinz (1980). It consists of two microscope slides cemented on top of each other with silicone rubber, the top one having a hole of 15 mm diameter in its center. The chamber was siliconized, washed and heat-sterilized. Before use, some vaseline was smeared around the whole of the slide. The vessel was then turned upside down, placed over the coverslip and gently pressed down so that the vaseline sealed the chamber tightly. The drop of medium adhered to both sides to form a column in the center of the chamber. The whole procedure was performed as rapidly as possible to avoid evaporation of the medium. For further development, cultures were kept for 16-20 hours in the dark in an incubator at 26°C.

Time-lapse recordings

In order to follow the dynamics of their development directly (cell division patterns, movements, differentiation), time-lapse recordings were made from some (n=32; ca. 15%) of the preparations. This was done on an inverted microscope (Zeiss Axiovert 35) equipped with Nomarski and phase-contrast optics and a videocamera (Hamamatsu, C2400-07). In most cases, we used a 63× oil immersion objective and an 1.6× optovar. Every three seconds (time-lapse: 160×), a frame was taken by the recorder (Panasonic AG 6720). Single pictures were analysed on the video screen and drawings were made directly from the screen.

Staining procedures

Clones derived from cells of the transformant strain were stained for β-galactosidase activity. The coverslip was gently removed from the culture vessel. Cells were fixed for 10 minutes in 1.25% glutaraldehyde in phosphate buffer (PB, 0.2 M, pH 7.2), washed (5-10 minutes) in PB and subjected to the X-Gal staining solution (one part of 20% X-Gal in DMSO and 99 parts of a warm (60°C) solution of 150 mM NaCl, 1 mM $MgCl_2$, 3.3 mM $K_3[Fe(CN)_6]$ in PB) for 2 hours at 30°C and overnight at room temperature. The X-Gal staining solution was then exchanged by PB. Labelled cells were documented under a photomicroscope (Zeiss Axiophot). Since epidermal cells showed almost no adhesion to the coverslip or to each other, most of these cells are lost during the staining procedure.

For immunohistochemical detection of neural antigens by anti-HRP (Jan and Jan, 1982), the antibody (Dako-Diagnostika) was dissolved and diluted 1:20 with 10% serum in PBT. Cells were prefixed (15 minutes) by adding 10 μl of 2% formaldehyde (in PEM buffer) to the drop of culture medium and subsequently fixed (10 minutes) in 2% formaldehyde in PEM. The preparation was rinsed in PB, blocked with 10% goat serum in PBT and incubated with primary antibody for 1 hour. After rinsing with PBT, HRP-coupled secondary antibody (goat anti-mouse; dilution 1:200 in serum/PBT) was added for 1 hour. The preparation was washed in PB, reacted in DAB solution (1 mg/ml PB + 2 μl H_2O_2), washed in PB, dehydrated in graded ethanol, cleared in xylene and mounted in Araldite.

Results

Ectodermal precursors are capable of developing neural or epidermal clones in isolation

To test their intrinsic capabilities, we removed cells from individually selected, precisely staged embryos, shortly after the onset of gastrulation and grew them in isolation. Furthermore, the cells were removed from well-defined positions with respect to the early gastrula fate map (Hartenstein et al., 1985; Technau and Campos-Ortega, 1985; Technau, 1987).

In this series of experiments, we analyzed the development in vitro of cells from the presumptive truncal segmental ectoderm. More than 200 cells from a dorsoventral sector around 50% EL (egg length; Fig. 1) were individually cultured. In less than 10% of the cases, the cultured precursor did not divide. Although in some of these cases, the cells showed clear signs of differentiation they are not considered here (but see Discussion). In more than 90% of the cases, the cells divided to give rise to basically three types of ectodermal clones: neural, epidermal and mixed neural/epidermal clones. To distinguish between these clones, we used the following criteria: division pattern, structural phenotypes of progeny cells, adhesiveness (as qualitatively deduced from whether or not the cells tend to form clusters and attach to the substratum) and the expression of cell specific markers.

(1) Neural clones

Sizes of this type of clone are very variable. They comprise between 2 and about 30 cells (Fig. 2). Precursor cells generally divide asymmetrically, with each division budding off a smaller cell which in turn divides once symmetrically (Fig. 3A). This stem cell mode of division corresponds to that described for neuroblasts (NBs) in situ (Hartenstein et al., 1987; for grasshoppers, see Bate, 1976). The cell cycle of the daughter cells is considerably longer than that of their stem cell. This corresponds to the situation described for ganglion mother cells (GMCs) in situ (for embryonic GMCs see Hartenstein et al., 1987; for postembryonic GMCs see Truman and Bate, 1988; Ito and Hotta, 1992). In the case shown in Fig. 3A, the first GMC does not divide before the birth of the fifth GMC. Progeny cells form dense clusters (Figs 3A, 5A) as is the case for most of the neuronal clones in the embryo (Technau and Campos-Ortega, 1986; Prokop and Technau, 1991). In addition, they adhere to the slide. The cells differentiate processes and most of them stain positive with the anti-HRP antibody (Fig. 5A), which binds to neuronal-specific antigens (Jan and Jan, 1982). We made no attempt so far to distinguish within these clones between neuronal and glial cell types.

(2) Epidermal clones

Precursor cells of this type of clone divide almost symmetrically up to three times (Fig. 4) giving rise to maximally 8 progeny (2-8 cells; Fig. 2). This corresponds to the behaviour described for epidermoblasts in vivo (Technau and Campos-Ortega, 1986). In a few cases, during the proliferation period, clones bud off 1-2 small "sphericals" (Fig. 4B). This phenomenon has not been observed among neural clones. Because of their significantly smaller size and since we were not able to detect a nucleus (by inspecting them in vivo under the microscope), we do not believe that these sphericals represent cells. A clarification of what these derivatives might be, has to await a closer inspection on the EM-level and the application of specific staining procedures. Timing and number of cell-divisions varies between clones. In the case shown in Fig. 4A, both daughter cells divide once synchronously. In the case shown in Fig. 4B, daughter cells differ in their timing and number of further divisions: one daughter cell divides once, the other divides twice; division of the former precedes the first division of the latter. Cells attain a globular shape with smooth surfaces and almost no granulation of their cytoplasm. They resemble the type of cells in primary cultures of dissociated embryos described as "chitin-secreting epidermal cells" by Dübendorfer and Eichenberger-Glinz (1980). They do not form processes although, in one case (out of 15 cases monitored by time-lapse recordings), short processes were transiently formed before cells moved apart (Fig. 4A). On the surface of some of the progeny cells, we noticed a tiny, denticle-like specialization (Fig. 5B). Cells of this type of clone as well as small sphericals lose adhesion to the coverslip and separate from each other to float freely in the medium (Figs 4, 5B,C). We cultured single cells from early gastrula embryos of an enhancer-trap line

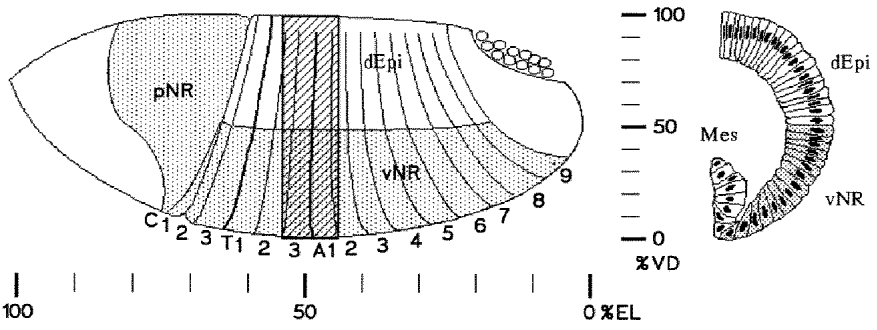

Fig. 1. Fate map of an early gastrula (stage 7) embryo (according to Technau and Campos-Ortega, 1985; Hartenstein et al., 1985). Cells to be cultured were explanted at this stage from different dorsoventral sites within the hatched region of the presumptive truncal ectoderm. Stippled area represents the neurogenic region. dEpi, dorsal epidermal anlage; Mes, mesoderm; pNR, procephalic neurogenic region; vNR, ventral neurogenic region; %El, % of egg length; %VD, % of the ventrodorsal dimension; C1-3, gnathal segments; T1-3, thoracic segments; A1-9, abdominal segments.

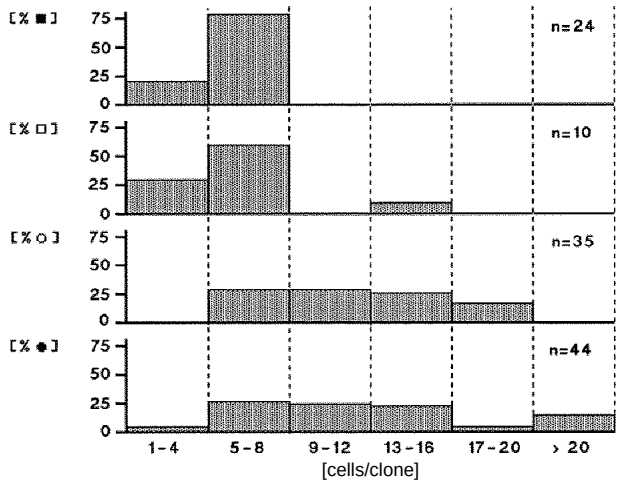

Fig. 2. Distribution of clone sizes in vitro given as % of epidermal, neural and mixed epidermal/neural clones, respectively. Epidermal clones (■) consist of up to eight cells. Sizes of neural clones (●) vary between 2 and up to approx. 30 cells. Among the mixed clones, comprising neural derivatives in addition to epidermal cells, we distinguish between two types, depending on whether the initial division of the precursor cells is symmetric (□) or non-symmetric (○). Whereas the former generally produce low numbers of progenies (□), the latter give rise to mixed clones comprising up to 20 cells (○).

which specifically expresses β-galactosidase in epidermal cells (Stüttem and Campos-Ortega, 1991) and stained the resulting clones for β-gal expression. Progeny cells of the type described here stained positive for β-gal (Fig. 5B,C). Neural clones did not express the marker.

(3) Mixed neural/epidermal clones

These clones consist of both types of cells described above. The initial division of precursors giving rise to mixed clones may be asymmetrical or almost symmetrical (Fig. 3B). From precursors dividing asymmetrically, we obtained mixed clones of up to 20 cells whereas mixed clones from precursors dividing symmetrically generally did not exceed 8 cells (Figs 2, 3B). Since we continuously monitored the development of clones (by time-lapse recordings) in only a limited number of cases, it is possible that we did not detect all of the epidermal cells that had left the neural cluster to float freely in the medium. In some cases, epidermal cells transiently formed short processes before attaining their final globular shape and leaving the cluster. Within the neural clusters we did not distinguish between neuronal and glial cells. As we did not apply glia-specific markers, we also cannot exclude the possibility that, among the globular cells which leave the cluster, are glial cells. But this seems to be unlikely, since glial cells in culture would be expected to differentiate processes and to adhere to the substratum (Fredieu and Mahowald, 1989). Mixed neural/epidermal clones are also obtained in the embryo following transplantation of single ectodermal precursors (Technau and Campos-Ortega, 1986; Becker and Technau, 1990).

Expression of these clonal types in vitro indicates that, briefly after cell formation and before the first postblastodermal division, ectodermal precursor cells are already endowed with intrinsic capabilities to develop as a neuroblast or as an epidermoblast (or both). They are able to execute the respective developmental program in isolation.

Neurogenic or epidermogenic fate of singly cultured precursors is a function of their original position in the early gastrula ectoderm

Early gastrula precursors are already endowed with intrinsic preferences towards the neural or the epidermal fate depending on their position along the dorsoventral axis of the ectoderm. Fig. 6 shows the distribution of the basic clonal types obtained in vitro as a function of the site of explantation of the precursors from the early gastrula ectoderm: the pure neural type of clone developed from cells taken from the neurogenic ectoderm, which in the truncal region spans between 0% and about 50% VD (n=54; 1 exception), the pure epidermal type of clone developed from cells taken from the dorsal epidermal anlage and the dorsalmost region of the ventral neurogenic ectoderm (between about 40% and 100% VD; n=89). Mixed types of clones were obtained from precursors distributed between 5% and 90% VD (n=56). Precursors from between 90% and 100% VD exclusively produced epidermal clones. Precursors from 0-5% VD exclusively gave rise to neural clones. Among mixed clones, those with symmetric initial divisions and generally comprising no more than 8 cells were derived from precursors distributed between 20% and 90% VD. The field of distribution of precursors dividing asymmetrically and giving rise to mixed clones of up to 20 cells reaches more ventral sites and spans between 5% and 80% VD.

These results indicate that the cells differ in their relative neurogenic and epidermogenic capacities depending on their position within the ectoderm. Positional cues along the dorsoventral axis provide precursors with intrinsic preferences towards the neural or epidermal fate: neurogenic preferences are strongest ventrally, epidermogenic preferences are strongest dorsally. Transition of preferences occurs at a position that roughly demarcates the dorsal border of the neurogenic ectoderm.

Discussion

In the initial phase of neurogenesis at least two important determinative events have to operate before the first neuroblasts segregate from the neurogenic ectoderm, namely the definition of the neurogenic regions of the ectoderm and the subsequent decision of cells within these regions to develop as a neural or as an epidermal precursor. In order to analyse intrinsic properties of ectodermal precursor cells with respect to these early processes, cells must be isolated from exactly staged embryos, shortly after cell formation at the beginning of gastrulation. By dissociating embryos at the time gastrulation begins, it has been shown that a fraction of cells at this stage could differentiate in vitro to form neurons (Seecof et al., 1971; 1972). Furthermore, by dissociating blastoderm cells and culturing them in vivo in the hemolymph of adult and larval hosts, it has been shown that progenitor cells for the imaginal discs become deter-

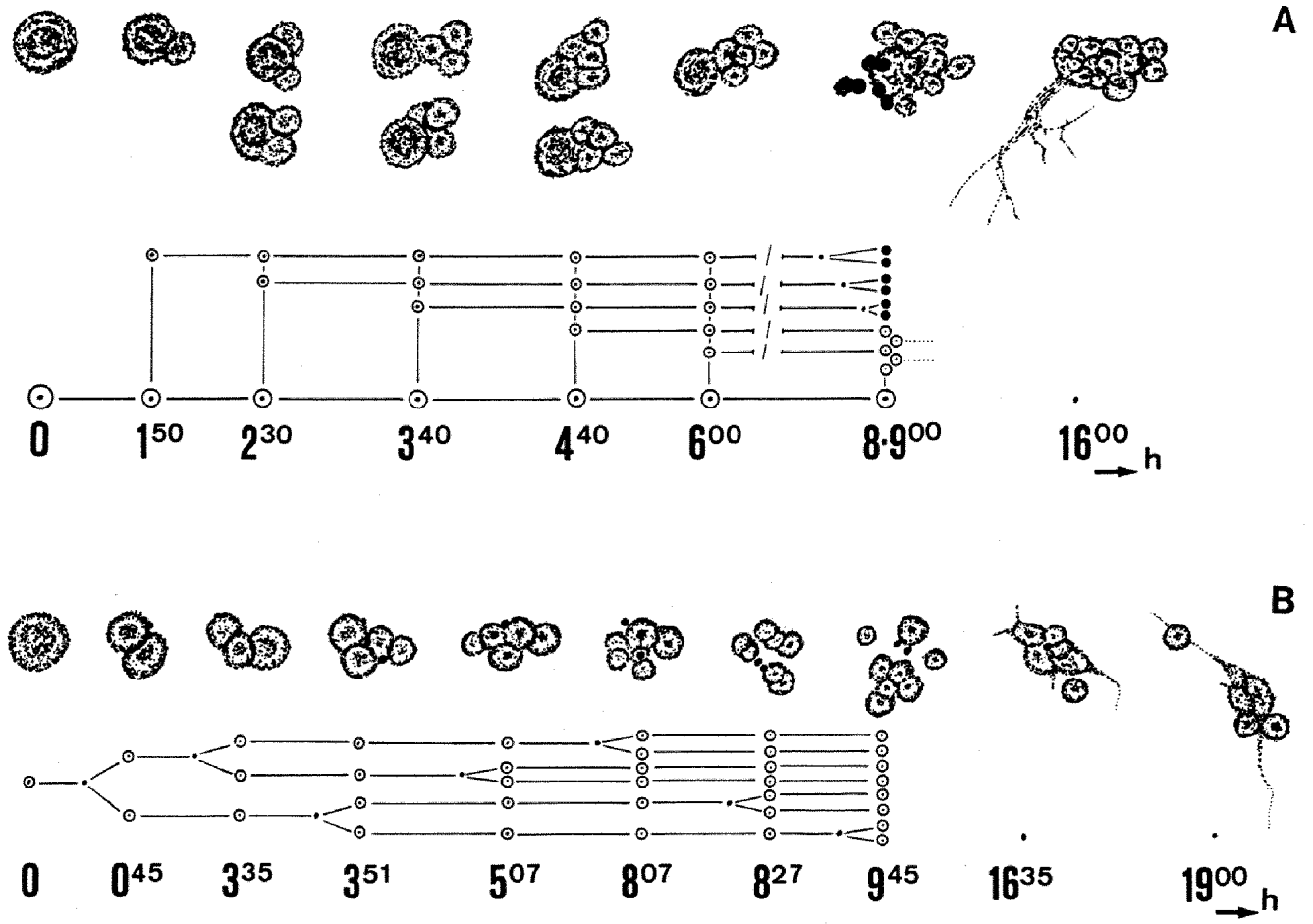

Fig. 3. Examples of a neural (A) and a mixed clone (B) developing from individual early gastrula cells in vitro. Drawings were made from time-lapse recordings on a video monitor. Schemes underneath illustrate mode and timing of divisions. (A) The neuroblast (large light cell) divides asymmetrically, budding off a chain of ganglion mother cells (smaller light cells). The size of the neuroblast decreases accordingly. Ganglion mother cells divide once symmetrically to give rise to two neurones (small black cells). Their cell cycle is much longer as compared to the neuroblast. In the case shown, the first ganglion mother cell divides after the fifth division of the neuroblast. At three stages (2^{30}, 3^{40} and 4^{40}) two drawings are shown to illustrate movements among cells that occurred within a few minutes. (B) The precursor divides almost symmetrically and gives rise to 8 progeny cells. At least two of them differentiate fibers, others become round and move apart. Two small "sphericals" are released by the cells (shown as black dots in upper part of B).

mined at the blastoderm stage and that they are already restricted in their potential to form anterior and posterior adult epidermal structures (Chan and Gehring, 1971). Although the cells were not cultured individually, these data suggest, that before the segregation of epidermoblasts and neuroblasts takes place in the embryo, a fraction of cells is already equipped with intrinsic properties to differentiate into neural and/or epidermal structures. The experimental conditions that we used were designed to test the developmental capacities of isolated precursors shortly after their formation (stage 7) and as a function of their original position along the dorsoventral axis of the presumptive truncal ectoderm.

Intrinsic properties of newly formed ectodermal precursors

Cultured cells from the presumptive truncal ectoderm gave rise to ectodermal cell types exclusively. This is in accord with the fact that the lineages of the three germ layers are already separated at the early gastrula stage (Technau, 1987). Three basic types of clones were obtained, which, according to the criteria mentioned above, represent neural, epidermal and mixed neural/epidermal clones. Thus, in order to execute neural or epidermal differentiation, the precursors do not seem to depend on interactions among each other. Their capabilities include various developmental traits like frequencies and modes of division, adhesiveness, the differentiation of cell shapes and the expression of cell-specific markers. These developmental characteristics exhibited by singly cultured cells closely resemble the behaviour of epidermoblasts and neuroblasts in the embryo (Technau and Campos-Ortega, 1986; Hartenstein et al., 1987; for grasshoppers see also Bate, 1976). Thus, cells of the presumptive truncal ectoderm shortly after their formation are already endowed with the intrinsic property to develop as a neuroblast or as an epidermoblast including different consecutive steps of the neural or epidermal developmental program. Although our data suggest that the cells are able to execute this program autonomously, we cannot

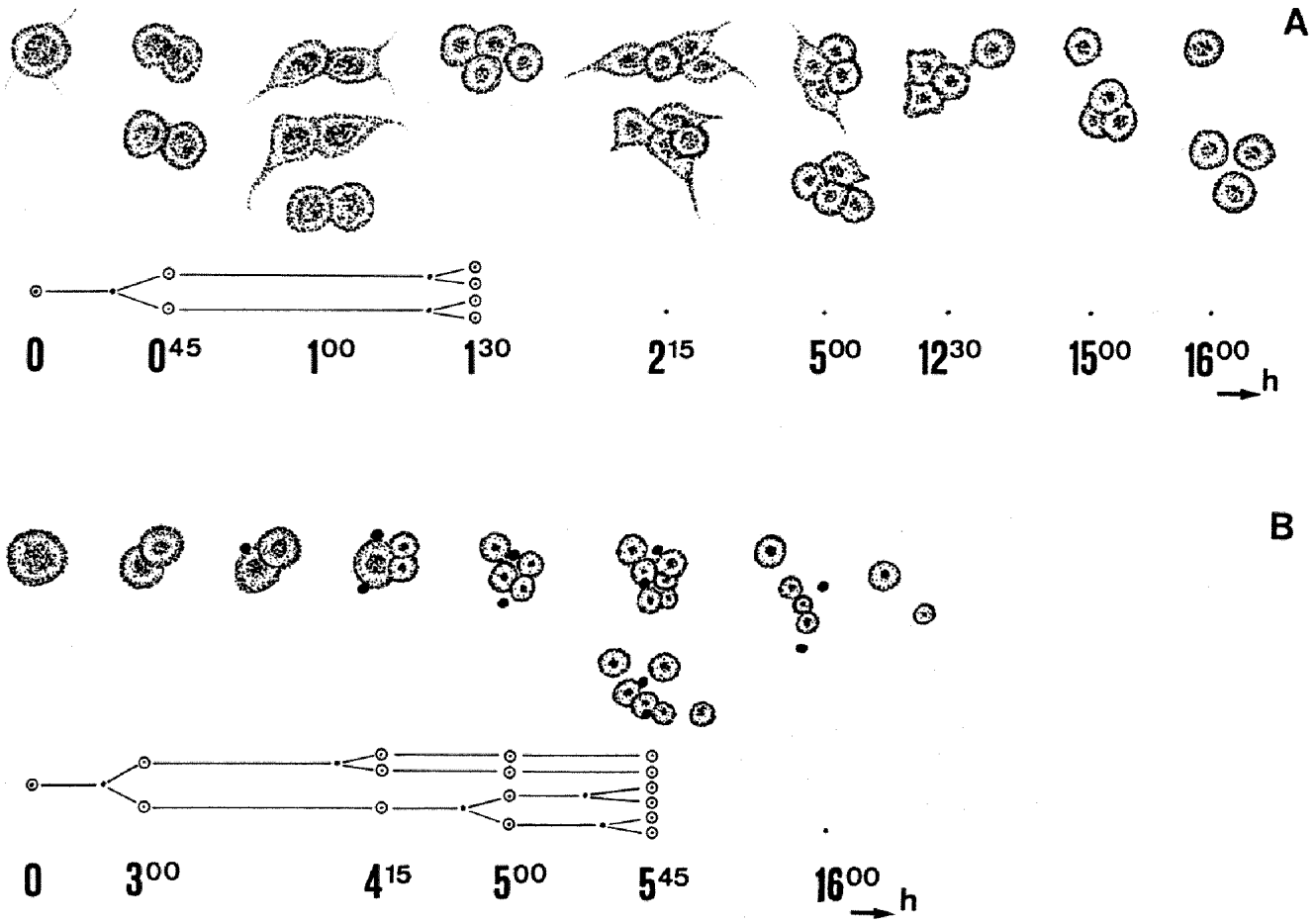

Fig. 4. Examples of epidermal clones developing form individual early gastrula cells in vitro. Drawings were made from time-lapse recordings on a video monitor. To illustrate changes in cell shape and movements occurring within a short period of time, two or three drawings are shown for certain stages. Schemes underneath illustrate mode and timing of divisions. In both cases, divisions are almost symmetrical. (A) Both daughter cells of the epidermoblast divide once more synchonously. Progeny cells transiently form processes before attaining their final sperical shape and separating from each other. (B) One daughter cell divides once, the other divides twice resulting in six globular, non-adhering cells; division of the former precedes the first division of the latter. Two small "sphericals" (black dots in upper part of B) are released by the cells.

exclude a possible role of components of the culture medium in initiating the program.

Mixed clones comprising both epidermal and neural types of progeny cells seem to be derived from precursors still lacking an intrinsic bias towards either fate. Following division of such a precursor, interactions among its daughter cells might then lead to differential behavior of these cells. Clones consisting of an epidermal and a neural subclone were also found in the embryo following transplantation of single ectodermal precursor cells. In these cases, the epidermal cells are either associated with CNS cells (Technau and Campos-Ortega, 1986, 1987; Becker and Technau, 1990) or with cells of the PNS that form a sensory organ (Bodmer and Technau, unpublished). In vivo tracings suggest that these lineages bifurcate after the first division of the precursor (Bossing and Technau, unpublished observation). The mixed clones we obtained in vitro may also be subdivided into two groups according to the mode of division and size distribution (Figs 2,6). Those of them assuming a non-symmetric mode of initial division and predominantly consisting of more than 8 cells resemble the CNS/epidermal type in the embryo, whereas mixed clones with an almost symmetric mode of division and generally no more than 8 cells resemble the PNS/epidermal type.

So far, we made no attempt to distinguish, within cultured clones, between neuronal and glial cell types.

Intrinsic properties, positional effects and cell communication

Singly cultured early gastrula cells systematically differed in their capabilities to develop neural or epidermal types of clones. These capabilities were clearly correlated with the positions the cells originally occupied along the dorsoventral axis of the presumptive ectoderm. The cells expressed intrinsic preferences towards the neurogenic pathway when located ventrally and epidermogenic preferences when located dorsally and no obvious preferences in a small lateral region. The border between the ventral neurogenic region and the dorsal epidermal anlage at the early gastrula

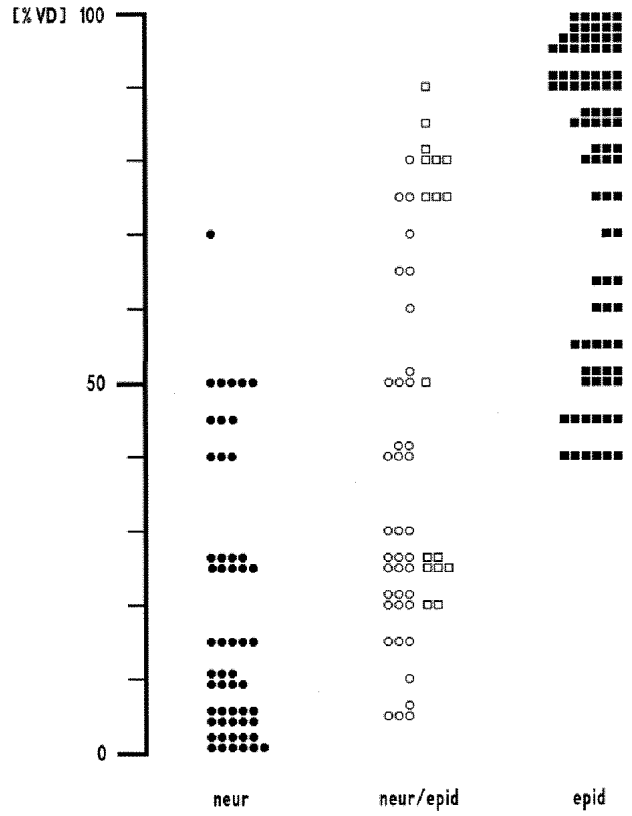

Fig. 6. Distribution of neural, epidermal and mixed neural/epidermal types of clones in vitro as a function of the site of removal of precursors at the early gastrula stage. Cells were explanted from different sites along the dorsoventral axis (given in %VD, 0%VD = ventral midline) of the presumptive truncal segmental ectoderm (at about 50% egg length; see Fig. 1). Neural clones (●) develop from cells taken from the ventral neurogenic region (approx. 0-50%VD; *n*=54; 1 exception). Epidermal clones (■) derive from cells explanted from the dorsal epidermal anlage (approx. 55-100%VD; *n*=69) or the dorsalmost region of the ventral neurogenic region (approx. 40-50%VD; *n*=20). Mixed clones are obtained from precursors distributed along the entire dorsoventral axis except the extreme dorsal and ventral ectodermal regions. The figure distinguishes between two types of mixed clones: □ represent mixed clones derived from precursors the initial division of which is almost symmetric and which generally give rise to no more than 8 cells (*n*=17); ○ represent mixed clones of up to 20 cells and asymmetric divisions of precursors (*n*=39). The field of distribution of ○ is shifted towards more ventral sites as compared to □.

stage has been mapped laterally near 50% VD (Technau and Campos-Ortega, 1985). Thus, the distribution of intrinsic preferences along the dorsoventral axis as disclosed by the single cell cultures roughly corresponds to the extension of the ventral neurogenic region and the dorsal epidermal anlage on the early gastrula fate map. We interpret this to mean that positional cues gradually distributed along the dorsoventral axis and roughly specifying the extent of the ventral neurogenic and the dorsal epidermogenic region of the ectoderm affect the intrinsic developmental properties of cells in these regions from the moment of their formation.

In situ the dorsalmost cells located between 95 and 100% VD have been shown to form the amnioserosa (Technau and Campos-Ortega, 1985) and to perform no postblastodermal divisions (Technau and Campos-Ortega, 1986; Foe, 1989). Among cultured cells explanted from the dorsalmost region (attempted sites of explantation between 90% and 100% VD; *n*=40), there were three cases of non-dividing cells which differentiated sheath-like extensions. It is possible that these cells represent amnioserosa types of cells. Alternatively, or in addition, cells of the amnioserosa anlage in vitro might behave like epidermoblasts.

In the embryo, only about 25% of the cells of the ventral neurogenic region develop as neuroblasts whereas about 75% develop as epidermoblasts (Hartenstein and Campos-Ortega, 1984). It has been previously hypothesized from histological observations (Hartenstein and Campos-Ortega, 1984) as well as experimental data (Stüttem and Campos-Ortega, 1991) that the cells of the neurogenic ectoderm have a primary neural fate. The fact that cells taken from the neurogenic ectoderm almost exclusively gave rise to neural clones when individually grown in culture clearly supports this hypothesis. It is possible that the products of proneural genes are among the factors that confer intrinsic neurogenic preferences upon cells, as transcripts of the *achaete-scute* gene complex (AS-C) (Cabrera et al., 1987; Romani et al., 1987), and even protein at least in the case of *lethal of scute* (Martin-Bermudo et al., 1991) are already found at the blastoderm stage, i.e. before we explanted the cells to grow them in culture. In situ other influences must be superimposed on the primary neural fate forcing about 75% of the neuroectodermal cells onto the epidermogenic pathway. Epidermalizing intercellular signals, which are mediated by the products of the neurogenic genes play an important role in this process (for reviews see Campos-Ortega and Knust, 1990; Campos-Ortega, 1992). Accordingly, in the absence of these signals in neurogenic mutants (Lehmann et al., 1983) or in single cell cultures, neuroectodermal cells follow their primary neural fate.

Heterotopic transplantations have shown that ectodermal cells from outside the neurogenic regions have neurogenic capabilities (Technau and Campos-Ortega, 1986; Stüttem and Campos-Ortega, 1991) except those of the proctodeal anlage (Stüttem and Campos-Ortega, 1991). From this, it could be deduced, that intercellular signals inhibit neuroblast development also outside the neuroectoderm (Campos-Ortega and Haenlin, 1992). Our cell culture experiments show that this is not the case for cells of the dorsal epidermal anlage, since these cells do not develop neural clones when deprived of cell interactions. As they develop into epidermal clones under these conditions, they would seem to have a primary epidermal fate.

The existence of neuralizing signals in the ventral neurogenic ectoderm has also been inferred from the above mentioned heterotopic transplantations. This hypothesis is strongly supported by our data: since dorsal ectodermal cells turned out to be intrinsically biased towards epidermogenesis, their neural development following heterotopic transplantation into the neurogenic ectoderm is likely to result from inductive forces.

The approach that we used represents a means of depriving precursor cells of interactions among each other and of disclosing their intrinsic developmental capabilities as a

function of their position in the embryo at a precisely known developmental stage. The data that we obtained in this way stress the important role of intrinsic factors in the process of determination of neural and epidermal fate of ectodermal precursor cells. Whereas the relevance of intrinsic factors in specifying neurogenic capabilities has been already suggested by previous experiments (for review see Campos-Ortega and Haenlin, 1992), our data provide the first evidence for the existence of intrinsic epidermogenic preferences of cells in the presumptive dorsal segmental ectoderm at the early gastrula stage. The distribution of these intrinsic preferences reflects the manifestation of positional cues which may be involved in delimiting the neurogenic and epidermogenic regions of the ectoderm. The decision of each single cell, which then leads to the characteristic spatial and temporal pattern of segregation of the two types of precursors, is likely to depend on the relative influences of their intrinsic preferences versus epidermalizing, neuralizing and probably other intercellular signals.

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Fig. 5. (A) Neural clone labelled with anti-HRP antibody. (B,C) Epidermal cells stained for β-gal expression. Cells developed from precursors taken from an enhancer-trap line that specifically expresses β-gal in the epidermis (Stüttem and Campos-Ortega, 1991). One of the cells in B is dividing symmetrically. The cell in C has differentiated a tiny, denticle-like specialization on its surface.