Development 112, 713-722 (1991)
Printed in Great Britain © The Company of Biologists Limited 1991

Specification of neurepithelium and surface epithelium in avian transplantation chimeras

GARY C. SCHOENWOLF* and IGNACIO S. ALVAREZ

Department of Anatomy, University of Utah, School of Medicine, 50 North Medical Drive, Salt Lake City, Utah, 84132, USA

* Author for correspondence

Summary

Previous studies of the avian blastoderm have revealed that extensive displacements occur within the epiblast during gastrulation and neurulation. The present study had two main purposes: (1) to map the origin and movement of prospective surface epithelial cells, and (2) to ask whether neurepithelial and surface epithelial cell fates are determined prior to cell movement, or whether they arise later as a result of the ultimate position attained by cells through their movement. Our results show that the rostral and lateral intraembryonic and extraembryonic surface epithelium originates as far laterally as at the area pellucida–area opaca interface of the early epiblast. Intraembryonic surface epithelial cells rearrange relative to one another, extending medially to contribute to the formation of the neural folds, whereas extraembryonic surface epithelial cells maintain their lateral positions, spreading uniformly as the epiblast expands. Our results further show that surface epithelial and neurepithelial cell fates are labile at the onset of neurulation, suggesting that cell fate is specified following cell movement.

Key words: ectoderm, epiblast, neural plate, neural tube, neurulation, notochord, mesoderm.

Introduction

A central question in developmental biology is: how do different populations of cells arise during embryogenesis? A second, associated question concerns the relationship between cell fate and cell movement; namely: do cells with a predetermination for a particular developmental fate move to a location in the embryo appropriate to express this fate, or do cells become determined for a particular fate as a result of the ultimate position they acquire through their movement? These two questions are examined in the present study of avian epiblast cells during neurulation.

Neurulation involves a number of morphogenetic cell behaviors, which occur both within the neural plate and surrounding tissues (reviewed by Schoenwolf and Smith, 1990). Such behaviors include changes in cell shape (i.e. cytomorphogenesis), position (i.e. cell rearrangement or intercalation) and number (i.e. cell proliferation and death). Cell rearrangement, coupled with cell division, results in the displacement of small perinodal groups of neurepithelial cells (i.e. MHP and L cells) down the length of the neuraxis during shaping and bending of the neural plate (Schoenwolf and Alvarez, 1989; Schoenwolf and Sheard, 1989, 1990; Schoenwolf *et al.* 1989; Alvarez and Schoenwolf, 1991). To what extent epiblast cells residing outside the neural plate (e.g. surface epithelial cells) display similar behaviors that cause them to be displaced down the length of the embryo during neurulation is unknown.

Heterotopic transplantation of plugs containing prospective MHP and L cells of the neural plate has confirmed previous studies (summarized by Placzek *et al.* 1990) that MHP cells are induced by the notochord and has further revealed that the fate of both MHP and L cells is determined by their ultimate position: prospective MHP cells isolated in L-cell territory display typical L-cell morphology (i.e. increased height with a spindle-like configuration), and prospective L cells isolated in MHP-cell territory display typical MHP-cell morphology (i.e. reduced height with a wedge-like configuration) (Alvarez and Schoenwolf, 1991). In contrast to cell fate, characteristic patterns of MHP- and L-cell rearrangement are established independently of notochordal inductive interactions: donor prospective MHP cells, transplanted heterotopically to host embryos prior to formation of the notochord, preferentially intercalate with host MHP cells, avoiding host L cells; similarly, heterotopic prospective L cells preferentially intercalate with host L cells, avoiding host MHP cells (Alvarez and Schoenwolf, 1991).

In the present study, we examined four new epiblast sites, expected to lie outside the neural plate, by homotopic grafting of quail epiblast plugs to chick epiblasts. The sites were chosen in an attempt to map the origin of the surface epithelium, as well as to assess

the morphogenetic behaviors of its cells (i.e. SE cells). Additionally, heterotopic grafting was performed to isolate prospective neural plate cells (both prospective MHP and L cells) in SE-cell territory and *vice versa*. Our results delineate the origin of SE cells in the early epiblast (i.e. at Hamburger and Hamilton, 1951, late stage 3), as well as characterize their morphogenetic behaviors, and suggest that neurepithelium and surface epithelium are specified following their displacement.

Materials and methods

Experimental procedures

Quail–chick transplantation chimeras were constructed as described previously (Schoenwolf and Alvarez, 1989; Schoenwolf *et al.* 1989; Alvarez and Schoenwolf, 1991). All grafting was done isochronically and either homotopically or heterotopically (Fig. 1). In previous studies, homotopic grafts were placed at three sites (Fig. 1A) designated as 'a', 'b', and 'b_L' (i.e. 'b' lateral). In the present study, homotopic grafts were placed at four additional sites (Fig. 1B) designated as 'd', 'e', 'e_c' (i.e. 'e' caudal) and 'e_r' (i.e. 'e' rostral). Grafts at site 'e' were placed 500–600 μm lateral to Hensen's node, those at site 'e_c' were placed 350–400 μm caudal to site 'e', those at site 'e_r' were placed 350–400 μm rostral to Hensen's node and grafts at site 'd' were placed at the midpoint of an imaginary line joining the centers of site 'e' and 'e_r'. Two kinds of heterotopic grafts also were done in the present study (Fig. 1C): (1) grafts obtained from site 'e' were placed at either site 'a' or site 'b' (that is, prospective SE cells were placed into the prospective MHP or L regions of the neural plate); (2) grafts obtained from either site 'a' or site 'b' were placed at site 'e' (that is, prospective MHP or L cells of the neural plate were placed into the prospective SE region). Cultures were collected at 24 h of incubation; in addition, plugs were fixed immediately and embedded in agar, exactly as done previously (Schoenwolf and Alvarez, 1989).

Differences existed in the size of blastoderms from one case to another at the same stage in the same species (i.e. two randomly selected chick blastoderms at stage 4 will likely have different sizes) and in different species (i.e. chick blastoderms are often somewhat larger than quail blastoderms at the same stage). Consequently, for grafts at sites 'e' and 'e_c' we used the area pellucida/area opaca border as a landmark for graft placement. Variation among blastoderms was likely responsible for the variation obtained in the results from these two sites (e.g. the variation between Figs 3A,B).

Quantitative procedures

Plugs

Serial sections of plugs from site 'e' and 'e_c' were selected for quantitative analysis on the basis of histological quality. The following parameters were determined (Table 1): (1) nucleolar-marker diameter – obtained by averaging the diameters (measured with an eyepiece micrometer) of 30 randomly selected markers in each plug; (2) number of cells per plug – obtained by totaling counts from every section and correcting the value according to the calculated Abercrombie (1946) correction factor (i.e. the actual number of cells was estimated by counting the number of markers, multiplying this number by section thickness and dividing the product by the sum of section thickness and nucleolar-marker diameter); (3) plug height – obtained by averaging the measured apicobasal extent of each plug at its midpoint in the 10 centermost sections; (4) plug diameter – obtained by averaging the measured widths of the 5 centermost sections through each plug; (5) number of cells spanning plug diameter – obtained by totaling raw counts of the number of cells containing nucleolar markers in the 5 centermost sections, dividing the total by 5 and correcting the value according to the calculated Abercrombie correction factor; and (6) calculated cell diameter – obtained by dividing plug diameter by the number of cells spanning it. Values obtained from all plugs were averaged to yield a mean±standard error of the mean for each parameter. Mean values for plugs from site 'a' and 'b' were obtained from a previous study (Schoenwolf and Alvarez, 1989). Although these were collected and sectioned over 1 year earlier than those of the present study, they were processed in exactly the same way and cut on the identical microtome. Thus differences among values obtained from these plugs and from those of the present study are not likely to be artifactual.

Chimeras

Serial sections from chimeras constructed by homotopic and heterotopic grafting were selected for quantitative analysis on

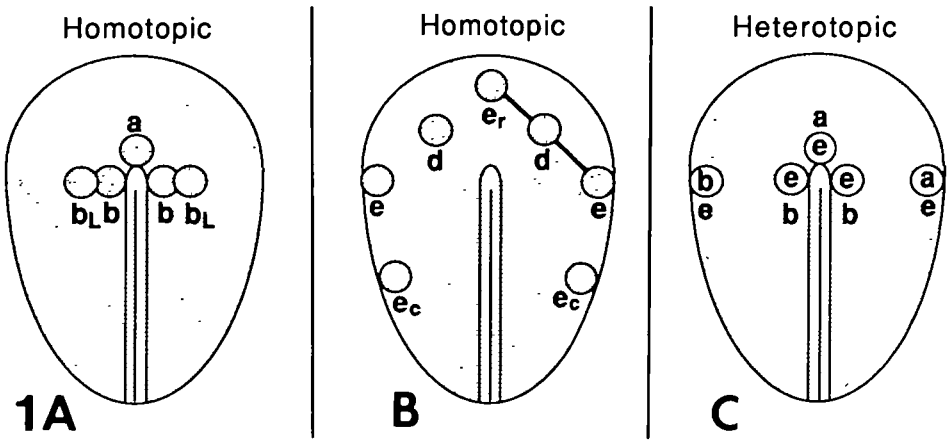

Fig. 1. Diagrams showing the sites of homotopic grafting (A, previous studies; B, present study) and heterotopic grafting (C, present study). Grafts at bilateral sites were done without restricting sidedness. Thus plugs obtained from the left side were placed on the right side and *vice versa*. Although it is not indicated by the figure, grafts obtained from site 'a' were placed at site 'e' on both the right and left sides; this was also true for grafts obtained from site 'b'. Site 'c' is not shown; it consists of Hensen's node and different levels of the primitive streak, and it is being mapped currently.

the basis of their histological quality. Homotopic grafts at site 'd' and 'e_r' were not analyzed quantitatively because the head fold of the body created a three-dimensional configuration that interfered with such an analysis. Heterotopic grafts of site 'e' to site 'a' also were not analyzed quantitatively because several graft cells moved rostrally into the head fold. The following parameters were determined in chimeras (Table 2): (1) number of cells per graft – obtained by counting cells containing nucleolar markers in every 6th section, multiplying the total by 6 and applying the calculated Abercrombie correction factor; (2) graft length (separate values were calculated for this parameter and the remaining parameters described below for portions contributing to extraembryonic surface epithelium, intraembryonic surface epithelium and neurepithelium) – obtained by counting the number of 5 μm serial sections through each graft and multiplying this number by 5 (section thickness in microns); (3) graft height – obtained by averaging the measured apicobasal extent of each graft at its midpoint in every 6th section; (4) graft width – obtained by averaging the measured transverse extent of each graft in every 6th section; (5) number of cells spanning graft width – obtained by dividing the corrected number of cells per graft by the number of sections through each graft; and (6) calculated cell diameter – obtained by dividing the graft width by the number of cells spanning it. Values obtained from all grafts were averaged to yield a mean±standard error of the mean for each parameter.

The following parameters were calculated from the values obtained from the quantitative analysis of the chimeras (Table 3): (1) rounds of cell division – obtained by comparing the number of cells per plug to the number of cells per graft (i.e. cell number doubles with each cell division); (2) rounds of graft rostrocaudal extension – obtained by comparing the plug diameter to graft length (i.e. each round consists of a doubling in graft length); (3) percentage increase or decrease in graft height – obtained by comparing the heights from plugs and grafts; (4) percentage increase or decrease in the calculated cell diameter – obtained by comparing the calculated cell diameters from the plugs and grafts.

Results

Histological analysis of plugs

In an example illustrating the effects of plug removal at site 'e', note the lateral position of the graft site with respect to the primitive streak and that the thickness of the epiblast tapers from medial to lateral (Fig. 2A). Further note that the endoderm and ingressed mesoderm have been reflected from the site. Nuclei and their contained nucleolar markers were readily identifiable in isolated quail plugs (Fig. 2B). Many plugs quickly curled upon isolation such that their basal side became concaved and their apical side (indicated by the presence of mitotic figures) became convexed (not illustrated).

Quantitative analysis of plugs

Plugs obtained from all sites had nucleolar markers of similar size, ranging from 2.1–2.4 μm (Table 1). Site 'e' and site 'e_c' plugs had somewhat fewer cells and smaller diameters than did site 'a' and site 'b' plugs. More importantly, the former plugs had shorter heights, fewer cells spanning their diameters and larger calculated cell diameters than did the latter plugs. This suggests that individual cells of site 'e' and site 'e_c' plugs are shorter and less densely packed than cells of site 'a' and site 'b' plugs.

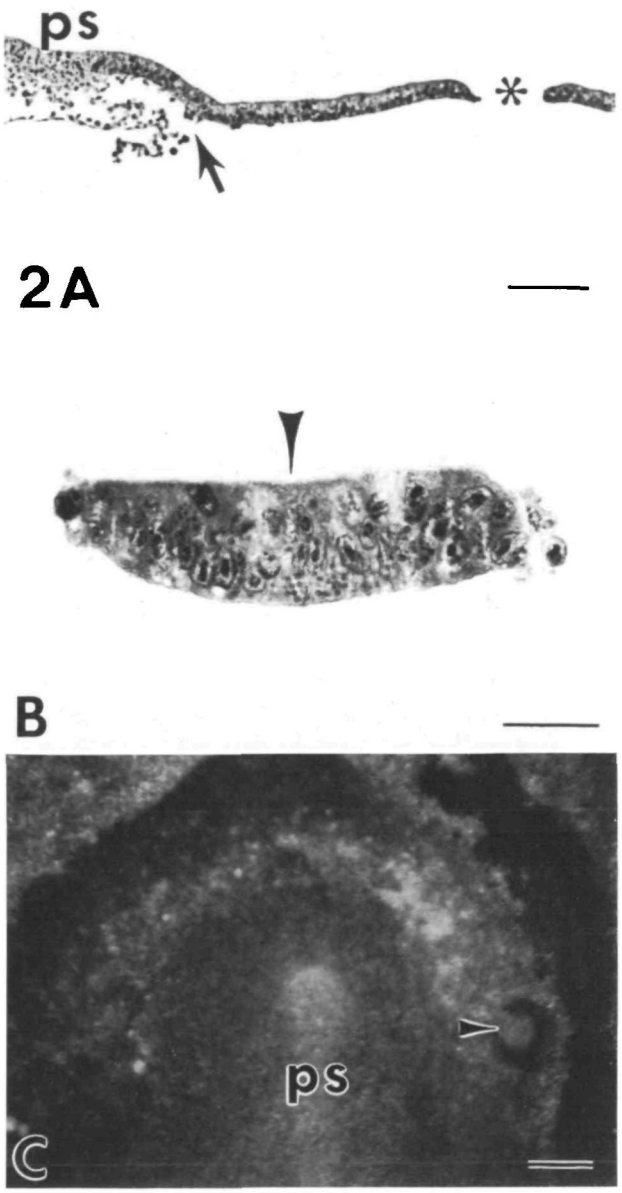

Fig. 2. Views of a donor blastoderm (A, transverse section), isolated plug (B, transverse section) and host blastoderm with grafted plug in place (C, whole embryo viewed ventrally) at 0 h postgrafting; site 'e' is illustrated in all cases. Asterisk (A), hole in the epiblast at site 'e'; arrow (A), area of mesoderm/endoderm reflection; ps (A, C), primitive streak; large arrowhead (B), apical (dorsal) side of plug; small arrowhead (C), graft *in situ* viewed through the replaced endoderm. Bars=200 μm (A,C); 20 μm (B).

Table 1. *Quantitative analysis of quail epiblast plugs*

Plug type	n[1]	Nucleolar-marker diameter (μm)	Number of cells per plug	Plug height (μm)	Plug diameter (μm)	Number of cells spanning plug diameter	Calculated cell diameter (μm)
'e'	6	2.3±0.1[2]	244±57	15±1	144±8	12±1	13
'e_c'	5	2.4±0.1	204±39	15±2	139±5	10±1	14
'a'	10	2.1±0.1	285±19	35±1	161±10	20±2	8
'b'	6	2.3±0.1	302±15	32±1	168±7	21±1	8

[1] Number.
[2] Mean±standard error of the mean.

Histological analysis of chimeras

Homotopic grafts

Fig. 2C illustrates an example of a homotopic site 'e' graft photographed immediately following replacement of the host's endoderm. Time-lapse microscopy revealed that such grafts typically healed into the epiblast very quickly (over the next 4–6 h) and became indistinguishable from host tissue.

An average of 72 % of homotopic grafts incorporated into the epiblast; examples are shown in Figs 3–5. Homotopic site 'e' grafts contributed quail cells to the lateral extraembryonic surface epithelium (i.e. the surface epithelium *lateral* to the lateral body folds), the lateral intraembryonic surface epithelium of the head and trunk, including the auditory placode, the migrating neural crest (the latter cells were defined as those lying adjacent to the dorsomost portion of the neural tube, beneath the surface epithelium; cells within the roof of the neural tube, which may have been neural crest precursors and which may have migrated out of the tube at a latter stage, were not scored as neural crest cells) and the lateromost (dorsomost) L region of the spinal cord (to the latter region in only 1 of 6 cases) (Figs 3A,B, 4A–C). Site 'e' thus contributed cells to virtually all levels of the lateral surface epithelium, but it typically contributed principally (that is, the majority of its cells in most embryos) to the *embryonic* surface epithelium. Grafts that contributed cells to the more medial surface epithelium underwent considerably more convergent-extension (i.e. transverse narrowing and longitudinal lengthening) than did grafts that contributed cells to the more lateral surface epithelium (cf. Fig. 3A,B), and elongated grafts usually extended along the interface between the two layers of the neural fold. Grafts contributed cells to the ectoderm as coherent patches having varying sizes, shapes and positions. Typically, the patch extended from lateral to medial, as it was followed from rostral to caudal, and it tapered rostrocaudally. The height of graft cells varied according to their position: extraembryonic surface epithelial cells were the shortest, L cells were the tallest and intraembryonic surface epithelial cells had intermediate heights (Table 2).

Homotopic site 'e_c' grafts contributed quail cells to both the lateral extraembryonic and intraembryonic surface epithelium, including an auditory placode in one case (Figs 3C, 5A, 5B), but such grafts typically contributed cells principally to the *extraembryonic*

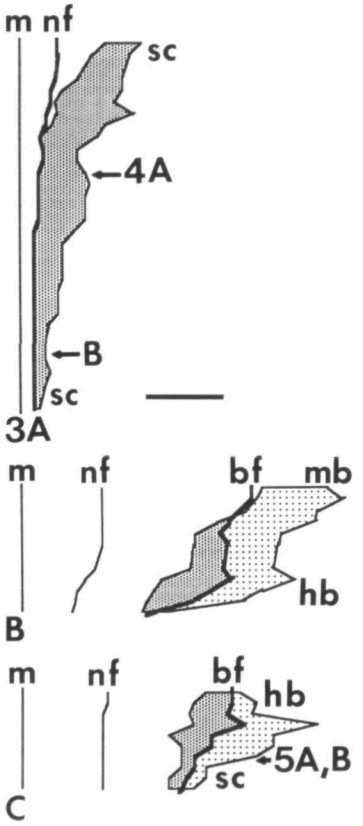

Fig. 3. Two-dimensional reconstructions of homotopic grafts, from chimeras obtained 24 h postgrafting, at site 'e' (A,B) and site 'e_c' (C). The levels of sections shown in Fig. 4 and 5 are indicated. The cranial end of each graft is at the top. bf, lateral body fold; m, midline; nf, neural fold; mb, hb and sc indicate, respectively, that the graft begins or ends in the midbrain, hindbrain or spinal cord levels of the body (this designation only indicates the rostrocaudal level of the body containing the graft, not the *type* of tissue containing graft cells). Bar=200 μm.

surface epithelium. The height of graft cells varied according to their position: extraembryonic surface epithelial cells were the shortest, auditory placode cells were the tallest and intraembryonic surface epithelial cells had intermediate heights (Table 2). As in the case of homotopic site 'e' grafts, cells were contributed to the ectoderm in coherent patches.

Homotopic site 'e_r' grafts contributed quail cells to the extraembryonic surface epithelium of the proam-

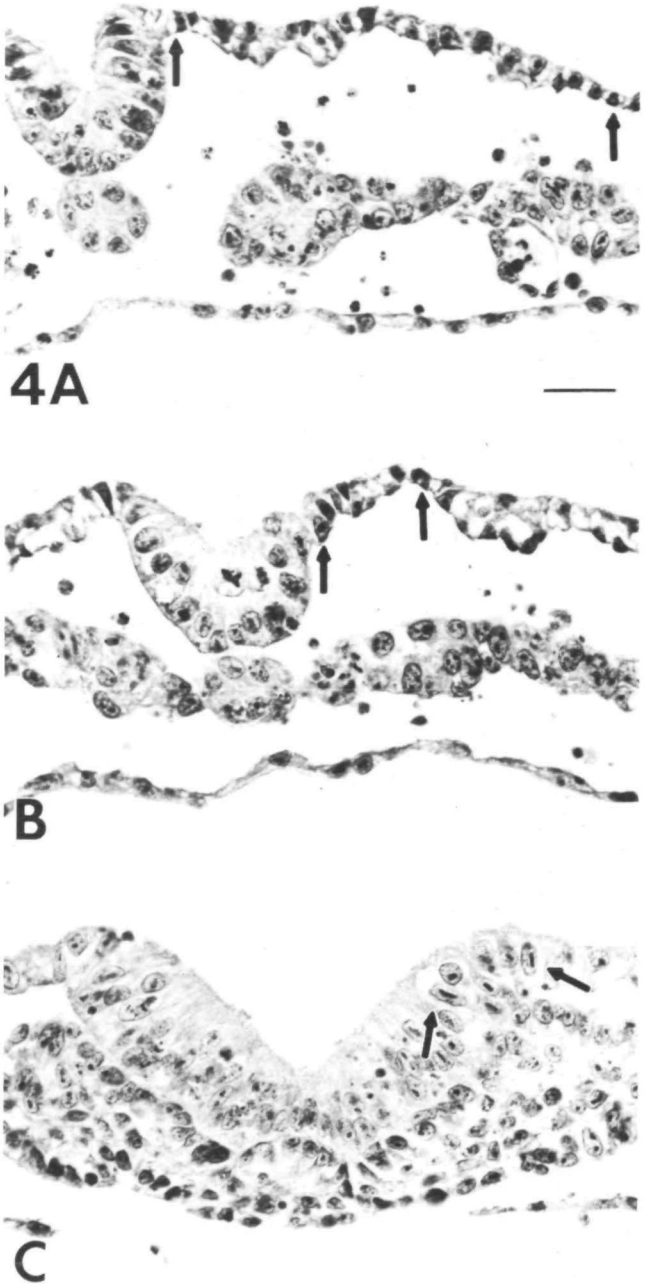

Fig. 4. Transverse sections through chimeras, obtained 24 h postgrafting, containing homotopic site 'e' grafts. Arrows indicate the mediolateral extents of the quail grafts in the ectoderm. A, B, sections through the spinal cord levels shown in Fig. 3A. C, section through the spinal cord level of a site 'e' graft (its reconstruction is not shown). Bar=20 μm.

nion and subcephalic pocket (i.e. the rostral surface epithelium cranial and ventral to the head), the intraembryonic surface epithelium of the cranial and ventral head, including the oral membrane, and the neurepithelium of the rostral neuropore. In virtually all embryos, graft cells were intermixed with host cells and were dispersed along the entire extent of the subcepha-

Table 2. *Quantitative analysis of quail–chick transplantation chimeras*

Chimera type	n[1]	Number of cells per graft	Graft length (μm)[2]			Graft height (μm)			Graft width (μm)			Number of cells spanning graft width			Calculated cell diameter (μm)		
			xe	se	ne	xe	se	ne	xe	se	ne	xe	se	ne	xe	se	ne
'e'/'e$_c$'	12	833±73[3]	291±46[4]	351±81[5]	–[7]	6±0.7	13±1	–	153±22	104±12	–	6±0.7	9±12	–	25±2	13±0.6	–
'a'/'b' to 'e'	10	1077±155	–	547±118[6]	712±245[8]	–	11±1	30±2	–	130±20	36±7	–	10±2	6±1	–	15±2	7±0.5
'b'	9	1404±169	–	–	1239±89[9]	–	–	32±2	–	–	32±3	–	–	5±0.4	–	–	6±0.5
'e' to 'b'	5	1029±16	–	–	792±77[10]	–	–	33±1	–	–	41±6	–	–	7±1	–	–	6±0.3

[1] Number; a minimum of 5 chimeras of each type was available for analysis (e.g., for 'a'/'b' to 'e', 5 'a' to 'e' and 5 'b' to 'e' chimeras were available).
[2] Determined separately for regions of the graft contributing to extraembryonic surface epithelium (xe), intraembryonic surface epithelium (se) and neurepithelium (ne).
[3] Mean±standard error of the mean.
[4] n=9.
[5] n=9.
[6] n=8.
[7] n=Value not included because only 1 case was available.
[8] n=4.
[9] n=9.
[10] n=5.

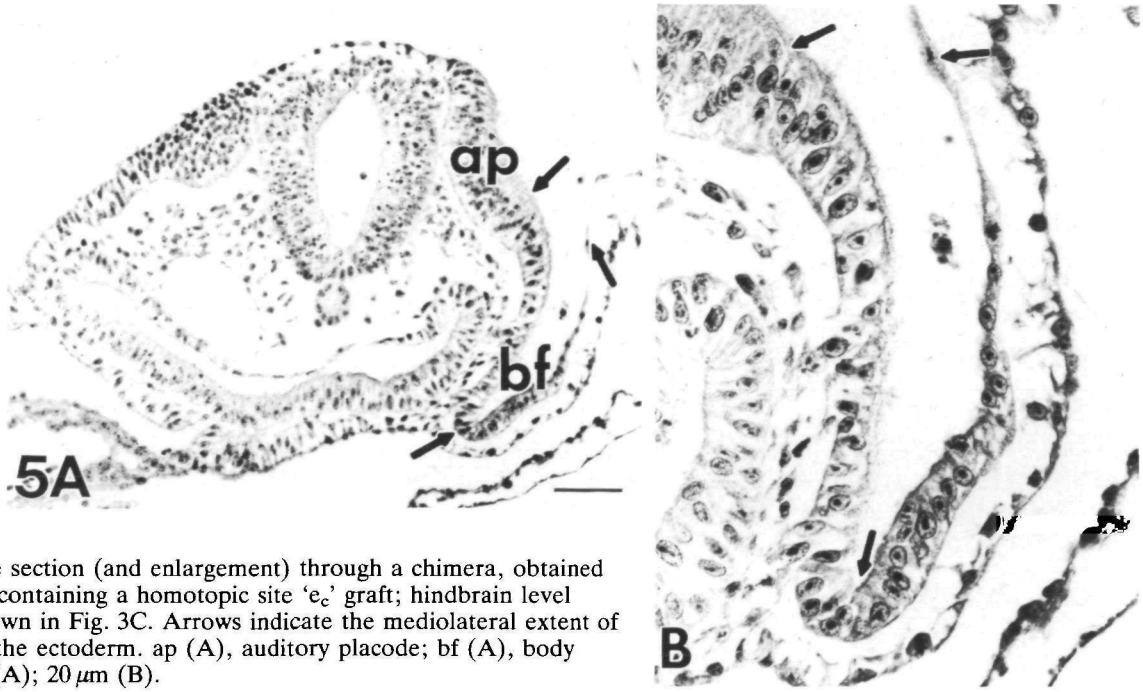

Fig. 5. Transverse section (and enlargement) through a chimera, obtained 24 h postgrafting, containing a homotopic site 'e_c' graft; hindbrain level similar to that shown in Fig. 3C. Arrows indicate the mediolateral extent of the quail graft in the ectoderm. ap (A), auditory placode; bf (A), body fold. Bar=60 μm (A); 20 μm (B).

lic pocket, contributing to both extraembryonic and intraembryonic ectoderm. Graft cells within the oral membrane were considerably taller than those in the surrounding surface epithelium; the height of cells in the neurepithelium of the rostral neuropore was not assessed, because the plane at which these cells were sectioned did not allow accurate measurements to be made.

Homotopic site 'd' grafts contributed quail cells to the intraembryonic surface epithelium of the dorsal and lateral head, the neurepithelium of the forebrain, including the optic vesicles, and the associated migrating neural crest. These grafts typically were very short (rostrocaudally) and wide (mediolaterally), and each contributed to all three of the ectodermal tissues in virtually every case. Graft cells in the forebrain and optic vesicles were substantially taller than graft cells in the surface epithelium.

Heterotopic grafts

An average of 64 % of heterotopic grafts incorporated into the epiblast (an average of 57 % for grafts placed at site 'e' and 72 % for grafts placed at sites 'a' and 'b'); examples are shown in Figs 6–9. Grafts of prospective MHP cells to SE-cell territory (site 'a' to site 'e') contributed quail cells to areas similar to those of homotopic site 'e' grafts. That is, these grafts contributed cells to the lateral intraembryonic surface epithelium, the lateromost L region of the spinal cord (only 1 of 5 cases) and the migrating neural crest (Figs 6A, 7A). They differed from homotopic grafts only in that they never contributed to *extraembryonic* surface epithelium, as the latter sometimes did. Behaviorally and morphologically, heterotopic grafts were indistinguishable from the comparable homotopic grafts: grafts contributing to the more medial surface

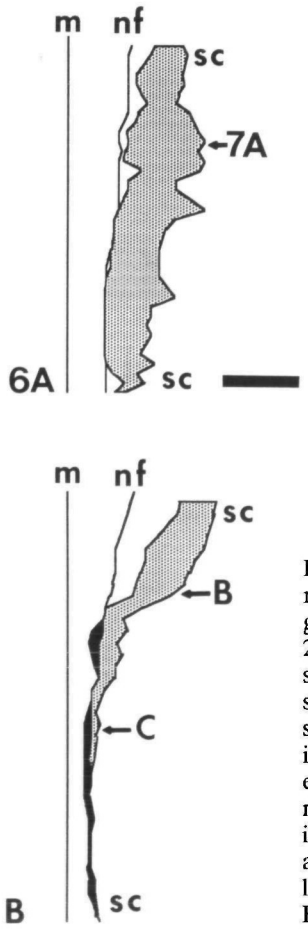

Fig. 6. Two-dimensional reconstructions of heterotopic grafts, from chimeras obtained 24 h postgrafting, of site 'a' to site 'e' (A) and of site 'b' to site 'e' (B). The levels of sections shown in Fig. 7 are indicated. The cranial end of each graft is at the top. m, midline; nf, neural fold; sc indicates that the graft begins and ends in the spinal cord level of the body. Bar=200 μm.

epithelium exhibited much more convergent-extension than did grafts contributing to the more lateral surface epithelium, and cells of the former were taller than cells

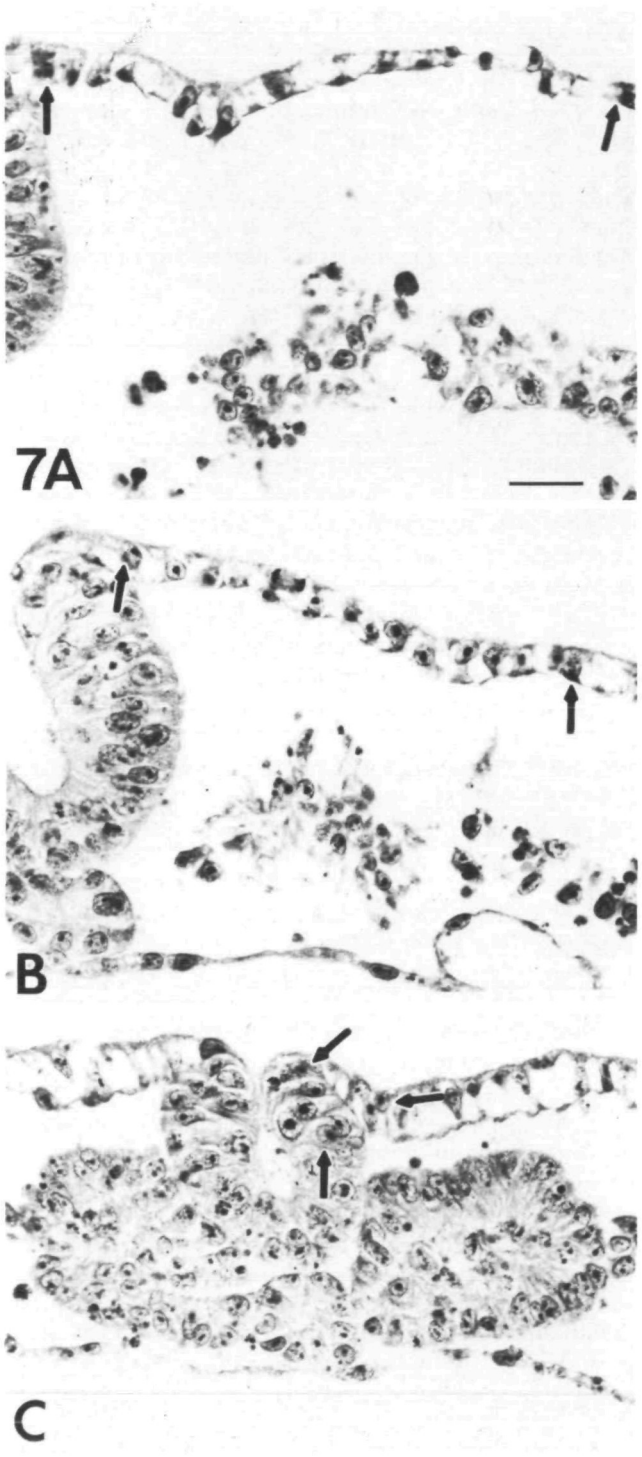

Fig. 7. Transverse sections through chimeras, obtained 24 postgrafting, containing grafts of site 'a' to site 'e' (A) and of site 'b' to site 'e' (B,C). Arrows indicate the mediolateral extents of the quail grafts in the ectoderm. A, section through the spinal cord level shown in Fig. 6A. B, C, sections through the two levels of the spinal cord shown in Fig. 6B. Bar=20 μm.

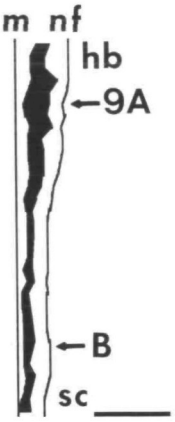

Fig. 8. Two-dimensional reconstruction of a heterotopic graft, from a chimera obtained 24 h postgrafting, of site 'e' to site 'b'. The levels of sections shown in Fig. 9 are indicated. The cranial end of the graft is at the top. m, midline; nf, neural fold. hb and sc, indicates, respectively, that the grafts begin in the hindbrain level of the body and ends in the spinal cord level. Bar=200 μm.

of the latter (also, L cells were taller than medial SE cells) (Table 2).

Grafts of prospective L cells to SE-cell territory (site 'b' to site 'e') gave very similar results to those just described with one exception (Figs 6B, 7B,C): graft cells typically entered the neural plate, contributing to the L cells along the neurepithelium-surface epithelium interface of the spinal cord neural folds (i.e. these cells distributed similarly to homotopic site b_L grafts of a previous study; Alvarez and Schoenwolf, 1991).

Grafts of prospective SE cells to MHP-cell territory (site 'e' to site 'a') contributed quail cells to the intraembryonic surface epithelium of the ventral head, including the oral membrane, the neurepithelium of the forebrain (especially in the region of the rostral neuropore) and to a few MHP cells of the cranial midbrain (only 1 of 5 cases). The height of graft cells in the surface epithelium was consistent with their position, and MHP cells (from the 1 case in which the MHP region of the cranial midbrain contained quail cells) were shorter than more lateral (L) midbrain (chick) cells and were typically wedgeshaped rather than spindleshaped.

Grafts of prospective SE cells to L-cell territory (site 'e' to site 'b') contributed quail cells principally to the L regions of the midbrain or hindbrain through spinal cord (Figs 8, 9). These grafts exhibited extensive convergent-extension and the height of the quail L cells was comparable to that of adjacent chick L cells (Table 2). In 2 of 5 cases, grafts contributed cells to the migrating neural crest and, in 3 of 5 cases, they contributed cells to the MHP region of the spinal cord (Fig. 9C); in the latter cases, the quail MHP cells had a morphology that was identical to that of adjacent chick MHP cells. Grafts typically extended from lateral to medial, as they were followed from rostral to caudal, and they tapered rostocaudally.

Quantitative analysis of chimeras

Table 2 shows the results of the quantitative analysis performed on serial transverse sections of chimeras, and Table 3 shows the parameters calculated from these results. Data obtained from similar groups were combined to facilitate comparison among groups. Data from site 'e' and site 'e_c' homotopic grafts were combined and compared to the combined data obtained from grafts of site 'a' or site 'b' to site 'e'. In addition,

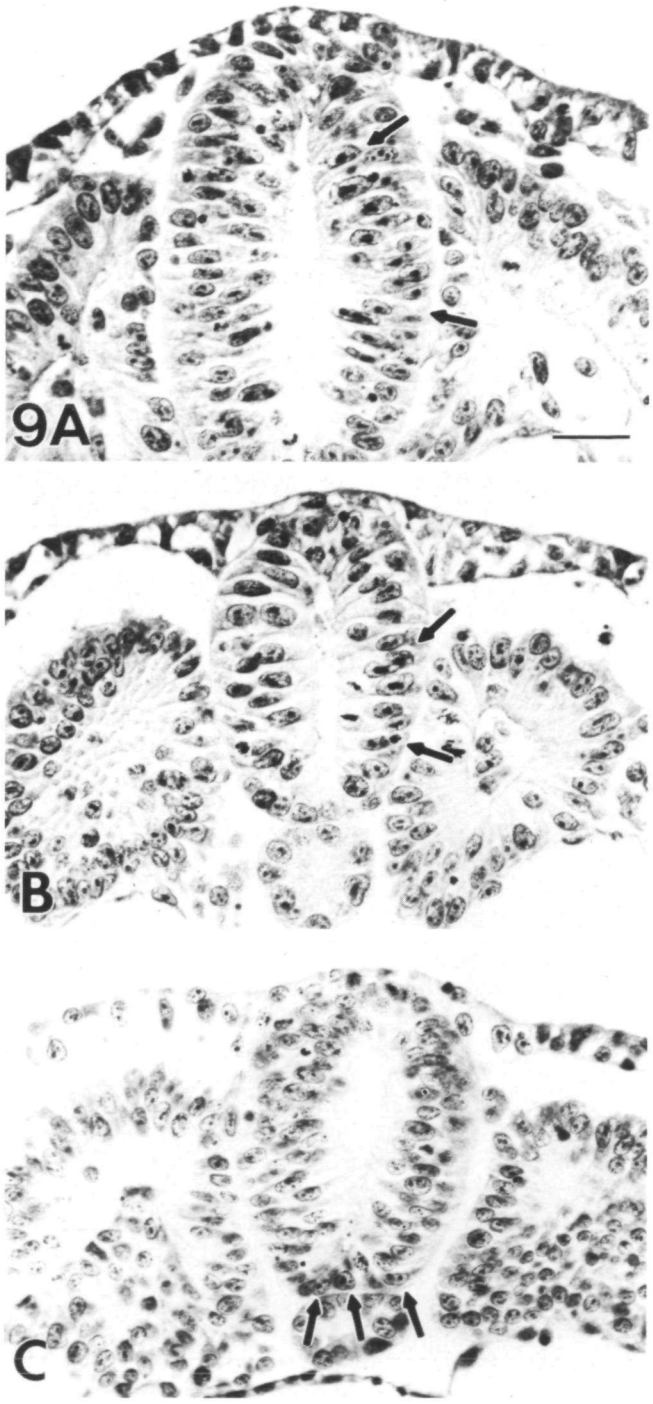

Fig. 9. Transverse sections through chimeras, obtained 24 h postgrafting, containing grafts of site 'e' to site 'b'. Arrows indicate the mediolateral extents of quail grafts in the neural tube. A, B, sections through the caudal hindbrain (A) and spinal cord (B) levels shown in Fig. 8. C, spinal cord level of a chimera, containing a graft of site 'e' to site 'b', whose reconstruction is not shown. Bar=20 μm.

data obtained from grafts of site 'e' to site 'b' were compared to data obtained from homotopic site 'b' grafts.

The number of cells per graft increased in each group during the 24 h period postgrafting (Table 2), suggesting that approximately 2 rounds of cell division occurred (Table 3). Graft length increased in all groups, as well as in all group subdivisions (i.e. extraembryonic surface epithelium, intraembryonic surface epithelium and neurepithelium) (Table 2), resulting in approximately 1 round of graft extension (i.e. rostrocaudal lengthening) for regions of the graft contributing to the extraembryonic surface epithelium, 1 or 2 rounds for regions contributing to the intraembryonic surface epithelium and 2 or 3 rounds for regions contributing to the neurepithelium (Table 3). Graft height (equivalent to cell height) either increased or decreased depending on the graft site (Tables 2, 3). The height of all plugs transplanted to site 'e' and site 'e_c' decreased their heights (by a factor ranging from 11 to 69 %; Table 3). In contrast, the height of homotopic site 'b' grafts essentially remained constant, and the height of site 'e' grafts transplanted to site 'b' increased substantially (by 118 %; Table 3). Graft width was determined separately for each group and its subdivisions (Table 2). These values were then used in two ways. First, for individual homotopic and heterotopic grafts contributing to *only* extraembryonic surface epithelium or *only* intraembryonic surface epithelium, length/width ratios were calculated. For those grafts contributing to extraembryonic surface epithelium, this ratio averaged 1.42 ± 0.16 (i.e. $\pm$the standard error of the mean; $n=2$), and for those contributing to intraembryonic surface epithelium, this ratio averaged 4.66 ± 1.62 ($n=7$). This suggests that the graft expanded more uniformly (i.e. isotropically in surface view) when it contributed to extraembryonic rather than intraembryonic surface epithelium, although in both cases, the length of the graft increased more than its width. Second, the widths of individual portions of the graft contributing to extraembryonic surface epithelium, intraembryonic surface epithelium and neurepithelium were each divided by the average number of cells spanning the individual widths (Table 2) to calculate the cell diameter in each area (Table 2). These data were then compared with data obtained from plugs to determine the percentage increase or decrease in the calculated cell diameter (Table 3). This value increased for site 'e' and site 'e_c' homotopic grafts contributing to extraembryonic surface epithelium (by 87 %), suggesting that this portion of these grafts spread, thereby increasing their surface area, but it remained essentially constant for those contributing to intraembryonic surface epithelium (i.e. decreased by only 6 %). Site 'a' and site 'b' grafts transplanted to site 'e' showed an increase in this value for regions of the graft contributing to intraembryonic surface epithelium (by 90 %), again suggesting that they spread, but showed a decrease when they contributed to neurepithelium (by 19 %), suggesting that they condensed. Both homotopic site 'b' grafts and grafts of site 'e' to site 'b' showed a decrease in this value (by 23 % and 56 %, respectively; Table 3), suggesting that they condensed.

Table 3. *Parameters calculated from the results of quantitative analysis of quail–chick tansplantation chimeras*

Chimera type	Rounds of cell division	Rounds of graft rostrocaudal extension[1]			Percentage increase (+) or decrease (−) in graft height			Percentage increase (+) or decrease (−) in the calculated cell diameter		
		xe	se	ne	xe	se	ne	xe	se	ne
'e'/'e_c'	2	1	1	–	−60	−16	–	+87	−6	–
'a'/'b' to 'e'	2	–	2	2	–	−69	−11	–	+90	−19
'b'	2	–	–	3	–	–	+0.6	–	–	−23
'e' to 'b'	2	–	–	2	–	–	+118	–	–	−56

[1] Determined separately for regions of the graft contributing to extraembryonic surface epithelium (xe), intraembryonic surface epithelium (se) and neurepithelium (ne).

Discussion

Origin and behavior of prospective surface epithelial cells

The results of the present study provide new information on the origin and behavior of prospective SE cells. First, all four new sites contributed cells to the surface epithelium. In addition, site 'd' routinely contributed cells to the neurepithelium of the forebrain, suggesting that this site straddled the prospective surface epithelium–neurepithelium interface at the cranial end of the neuraxis. It was surprising that grafts placed at or near the area pellucida–area opaca border contributed some cells to the intraembryonic surface epithelium of the neural folds; that is, to the most medial surface epithelium, as well as other cells to the extraembryonic surface epithelium (the epithelium lateral to the lateral body folds). This suggests that these sites straddled the extraembryonic–intraembryonic surface epithelium interface and that extensive displacement of the surface epithelium occurs during neurulation. Finally, site 'e_r' contributed cells to rostral extraembryonic and intraembryonic surface epithelium, as well as to the neurepithelium of the rostral neuropore. Thus cells within a small circumscribed area of the epiblast become widely dispersed with formation of the head fold of the body to contribute to the rostromost levels of the embryo.

The locations of the borders of the prospective neural plate in the flat epiblast has been a controversial topic for over 50 years (discussed by Schoenwolf and Sheard, 1990). Spratt's (1952) fate mapping study supported the belief that the location and size of the *scheibe* of Wetzel (1929) coincided with that of the prospective neural plate, whereas the most recent fate mapping study suggested that the prospective neural plate is narrower and longer. The present study, in conjunction with previous studies from our laboratory (Schoenwolf and Alvarez, 1989; Schoenwolf and Sheard, 1989, 1990; Schoenwolf *et al.* 1989), provides an explanation for why the boundaries of the neural plate have been so difficult to delineate. Namely, that extensive cell displacements occur both within the neurepithelium and surface epithelium so that the juncture between these two layers becomes condensed and its position within the embryo changes. Both neurepithelial and intraembryonic SE cells move toward the midline as these two cells populations undergo convergent-extension; prospective neural crest cells do likewise, confirming the results of a previous study (Rosenquist, 1981). The present study shows that cell displacement within the surface epithelium involves at least three morphogenetic cell behaviors: (1) cell division (about 2 rounds in both the intraembryonic and extraembryonic surface epithelium, a value consistent with the previously reported cell-cycle length of about 10 h; Smith and Schoenwolf, 1987), (2) cell rearrangement (within the intraembryonic surface epithelium resulting in a narrowing and concomitant lengthening of the graft) and (3) change in cell shape from cuboidal to squamous (within the extraembryonic surface epithelium as indicated by a decrease in cell height and a concomitant increase in the calculated cell diameter).

Specification of surface epithelial and neurepithelial cell fate and behavior

The results of the present study provide strong evidence that the fates of SE and neurepithelial cells are determined as a result of the ultimate positions they attain in the epiblast. Prospective MHP or L cells placed into prospective SE-cell territory adopt the morphological and behavioral characteristics of SE cells. Depending on their exact position, these cells look and act like the surrounding intraembryonic or extraembryonic SE cells. For both homotopic site 'e' grafts and for heterotopic grafts of site 'a' to site 'e', contributions of cells to the neurepithelium were infrequent (homotopic, 1 of 6 cases; heterotopic, 1 of 5 cases). However, for heterotopic grafts of site 'b' to site 'e', contributions of cells to the neurepithelium were frequent (3 of 5 cases). This result is consistent with our previous finding that prospective L cells (originating from site 'b') intercalate with other L cells when given the opportunity to do so, but MHP cells (originating from site a) try to avoid prospective L cells when placed in heterotopic locations (Alvarez and Schoenwolf, 1991).

Prospective SE cells placed into prospective L-cell territory adopt the morphological and behavioral characteristics of L cells. Such grafts contributed cells not only to the L region of the neural tube, but in some cases they also contributed a few cells to the caudal

MHP region – a result identical to that obtained with homotopic site 'b' grafts. This provides evidence that paranodal cells normally contribute, albeit to a minor degree, to the caudal MHP region, because it seems impossible that heterotopic grafts of site 'e' to site 'b' could be contaminated with neighboring prospective MHP cells, a possibility that cannot be ruled out with homotopic site 'b' grafts.

Prospective SE cells placed into prospective MHP-cell territory gave less clear results. In most cases, these cells moved rostrally into the forebrain neurepithelium and adjacent surface epithelium, thereby avoiding intermixing with host MHP cells. In one case, a few graft cells contributed to the MHP region of the midbrain where they acquired typical MHP-cell morphology. However, the fact that these cells did not extend down the length of the midline neuraxis suggests that they failed to acquire the typical MHP-cell rearrangement behavior.

We gratefully acknowledge the assistance of Fahima Rahman and Jennifer Parsons. The research was supported by NIH grant no. NS 18112 and Fulbright Fellowship ref. FU89–8797464.

References

ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239–247.

ALVAREZ, I. S. AND SCHOENWOLF, G. C. (1991). Patterns of neurepithelial cell rearrangement during avian neurulation are established independently of notochordal inductive interactions. *Devl Biol.* **143**, 78–92.

HAMBURGER, V. AND HAMILTON, H. L. (1951). A series of normal stages in the development of the chick embryo. *J. Morph.* **88**, 49–92.

PLACZEK, M., TESSIER-LAVIGNE, M., YAMADA, T., JESSELL, T. AND DODD, J. (1990). Mesodermal control of neural cell identity: Induction of floor plate-specific properties by the notochord. *Science* **250**, 985–988.

ROSENQUIST, G. C. (1981). Epiblast origin and early migration of neural crest cells in the chick embryo. *Devl Biol.* **87**, 201–211.

SCHOENWOLF, G. C. AND ALVAREZ, I. S. (1989). Roles of neuroepithelial cell rearrangement and division in shaping of the avian neural plate. *Development* **106**, 427–439.

SCHOENWOLF, G. C., BORTIER, H. AND VAKAET, L. (1989). Fate mapping the avian neural plate with quail/chick chimeras: Origin of prospective median wedge cells. *J. exp. Zool.* **249**, 271–278.

SCHOENWOLF, G. C. AND SHEARD, P. (1989). Shaping and bending of the avian neural plate as analysed with a fluorescent-histochemical marker. *Development* **105**, 17–25.

SCHOENWOLF, G. C. AND SHEARD, P. (1990). Fate mapping the avian epiblast with focal injections of a fluorescent-histochemical marker: Ectodermal derivatives. *J. exp. Zool.* **225**, 323–339.

SCHOENWOLF, G. C. AND SMITH, J. L. (1990). Mechanisms of neurulation: Traditional viewpoint and recent advances. *Development* **109**, 243–270.

SMITH, J. L. AND SCHOENWOLF, G. C. (1987). Cell cycle and neuroepithelial cell shape during bending of the chick neural plate. *Anat. Rec.* **218**, 196–206.

SPRATT, N. T. JR (1952). Localization of the prospective neural plate in the early chick blastoderm. *J. exp. Zool.* **120**, 109–130.

WETZEL, R. (1929). Untersuchungen am hühnchen. Die entwicklung des keims während der erestn beiden bruttage. *Wilhelm Roux Arch. EntwMech. Org.* **119**, 188–321.

(*Accepted 4 April 1991*)