Fate mapping the neural plate and the intraembryonic mesoblast in the upper layer of the chicken blastoderm with xenografting and time-lapse videography

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Summary

The disposition of the Anlage fields of the neural plate and the intraembryonic mesoblast in the upper layer of the chicken blastoderm was studied at the primitive streak stage prior to the regression of Hensen's node (stages 5V to 6V, L. Vakaet (1970) Arch. Biol. 81, 387-426). Chicken blastoderms were cultured by New's technique on a mixture of thin egg white and agar. The anterior half of the deep layer was reflected with a tungsten needle. A circular fragment of the upper layer was punched out with a pulled out Pasteur pipette and discarded. It was replaced with an isotopic and isopolar piece of quail upper layer that was punched out with the same pipette. The deep layer was replaced and the chimeras were reincubated for 24 hours. The xenografts were followed with time-lapse videography. After fixation, the quail cells were located using Le Douarin's quail nucleolar marker technique. Integrating the observations with time-lapse videography and the results of Feulgen stained sections, we have drawn a new fate map of the disposition of the Anlage fields in the upper layer of the chicken blastoderm at stages prior to the regression of Hensen's node (stages 5V to 6V). The disposition of the neural plate and of the notochord, somites, nephrotome and lateral plates was therefore determined before the Anlage fields are morphologically discernible. The pathway of the fields in the upper layer towards their disposition was documented with time-lapse videography in chimeric chicken blastoderms that developed normally.

Key words: fate map, time-lapse videography, xenografts, chicken blastoderm.

Introduction

The neural plate of avian embryos becomes visible at stage 7V (Vakaet, 1970) corresponding to stage 4 of Hamburger and Hamilton (1951). Pasteels (1937) and Malan (1953) used vital staining to construct fate maps in prestreak and primitive streak stages (stages 0V to 6V). Due to diffusion and fading of the marks, this technique has limited value for determining the final destination of the marked fields. The maps of Spratt (1952) are based on carbon marking at primitive streak and headfold stages (stages 7V to 9V). Carbon marks on the upper side of the upper layer are not reliable because the carbon particles can remain unattached or move randomly. At stages prior to the regression of Hensen's node (which starts at stage 7V corresponding to stage 4 of Hamburger and Hamilton), the disposition of the prospective neural plate and the intraembryonic mesoblast has not been exactly determined as appears from the differences between the maps of Rawles (1936), Rudnick (1944), Waddington (1952), Rosenquist (1966) and Vakaet (1984). We have studied the Anlage fields of both the neural plate and the intraembryonic mesoblast in the upper layer in stages prior to the regression of Hensen's node (5V to 6V). At stage 5V, upper layer cells start to ingress through the primitive streak. At the beginning of stage 6V, the primitive groove is fully developed. The ingression of the endoblast is completed by the end of stage 6V (Vakaet, 1962). We used time-lapse videography to follow the chick-quail xenografts. After 24 hours of culture, the grafts and their position within the embryo were determined by using the nucleolar marking technique (Le Douarin, 1973). By combining xenografting and registration of the morphogentic movements of the grafts with videography, we have drawn a new fate map of the neural plate and the intraembryonic mesoblast before they are morphologically discernable.

Materials and methods

Chicken eggs (White Rock, from the Rijksstation voor Pluimveeteelt, Merelbeke, B-9820) and quail eggs (from

laboratory stock) were incubated at 38°C for 15 hours to obtain stage 5V to 6V blastoderms. They were cultured by New's (1955) technique, except that the substratum used was a mixture of 25 ml thin egg white and a gel made of 150 mg Bacto-agar Difco (Detroit, Michigan) in 25 ml Ringer's solution, instead of pure thin egg white. This semi-solid medium allowed microsurgery and further culturing on the same substratum.

For microsurgery, we used a Pasteur pipette with a tip diameter of 0.20 mm to 0.25 mm. The experiments consisted in punching out and discarding a circular fragment of the upper layer. It was replaced with an isotopic and isopolar piece of quail upper layer that was punched out with the same pipette (Bortier and Vakaet, 1992). After the intervention a Polaroid photograph was taken using an M8 stereomicroscope (Wild, Heerbrugg, CH-9435) at magnification 25×. The culture vessels were covered with a glass lid, sealed with melted paraffin and further incubated at 38 ± 0.5°C.

For time-lapse videography (Bortier and Vakaet, 1987) we used an M8 stereomicroscope (Wild, Heerbrugg, CH-9435), on top of which a WV-1850 camera (Panasonic, Osaka, Japan) was mounted in a plexiglass incubator at 38 ± 1°C (Fig.1). The camera was linked to a U-matic video-recorder VO-5850P (Sony, Tokyo, Japan) through an animation control unit EOS AC580 (EOS Electronics A.V., Barry, South Glamorgan). A time date generator WJ-810 (Panasonic, Osaka, Japan) displayed the chronological information on a WV-5340 monitor (Panasonic, Osaka, Japan). One video-image was recorded every 30 seconds, yielding an acceleration of 750× at normal projection speed (25 images/second). To reduce the temperature gradient due
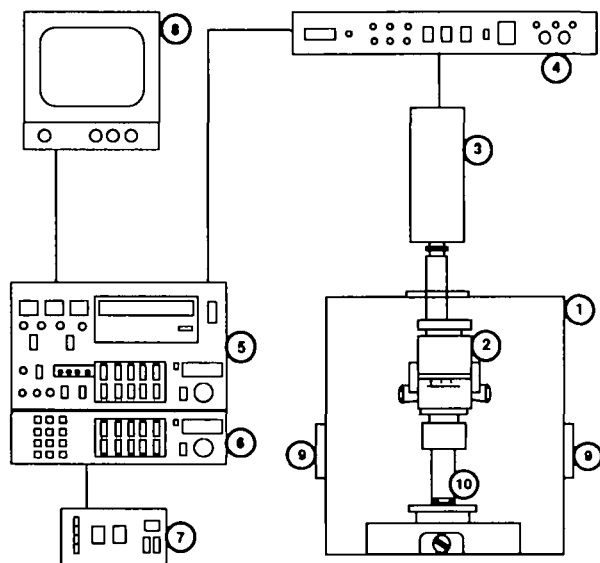

Fig. 1. Time-lapse videography installation. 1. Incubator; 2. Wild M8 stereomicroscope; 3. WV-1850 camera (Panasonic, Osaka, Japan); 4. Time date generator WJ-810 (Panasonic, Osaka, Japan); 5. U-matic video-recorder VO-5850P (Sony, Tokyo, Japan); 6. Animation control unit EOS AC580 (Electronics, Barry, South Glamorgan); 7. Interval timer (Vel, Leuven, B-3030); 8. WV-5340 monitor (Panasonic, Osaka, Japan); 9. Entrances; 10. Culture vessel.

to the light that is transmitted from below, an open transparent well with a planparallel bottom was filled with distilled water and placed under the culture vessel. The cooling of the bottom of the vessel thus provided was usually sufficient to prevent damping of the glass lid. If necessary, a thin layer of glycerin was spread on the inside of the glass lid.

All chimeras were reincubated for 24 hours. The culturing was interrupted by fixing the blastoderms in a mixture of absolute alcohol, formaldehyde 4%, acetic acid (75:20:5, v:v:v). After paraffin embedding and sectioning, they were stained after Feulgen and Rossenbeck (1924).

Results

The results are the integration of time-lapse videography and histology from 72 chimeras that developed normally by in ovo standards. The fate map is drawn for a chicken blastoderm at stage 6V, prior to the regression of Hensen's node.

Videography

Before healing, the quail grafts unroll at their edges and thereby enlarge. After healing they are distinguishable in the host as clearly delineated patches. This is due to the fact that the quail cells stay together and that they contain less yolk than the chicken cells.

Grafts anterior to a transverse line through Hensen's node can be followed up to stage 9V (headfold stage). Only grafts in a small area anterior to this line seem to move into the neural plate. To do so, they move as a field towards the midline. Concomitantly the more anterior grafts extend in an anterior direction, the more posterior grafts in a posterior direction. The neural plate therefore extends as a whole in an anterior as well as in a posterior direction (Fig. 2B). The plate becomes visible due to its convergence. After elevation of the neural lips, the whole neural plate is no longer in focus and the grafts can not be followed further. Grafts outside the neural plate form sharply delineated fields.

All grafts posterior to Hensen's node migrate towards the primitive streak in a rostrolateral to mediocaudal direction. They form patches that elongate in the direction of their migration. As they arrive at the primitive streak, the grafts extend in length. After ingression, mesoblast cells can no longer be followed. Sometimes a graft seen as a delineated patch, partly disappears and partly stays in the upper layer.

Histology

Histology of the grafts was performed at later stages than could be followed with videography. This enabled us to recognize the nature of the tissues formed from the grafts.

We found that the transverse line through Hensen's node in stage 6V constitutes a border between the neural plate and the intraembryonic mesoblast. Grafts anterior to this line only formed neural tissue. Grafts caudal to this line only yielded intraembryonic mesoblast. Grafts that partly became neural tissue and partly mesoblast had been inserted on the border between both fields, as could be seen with videography.
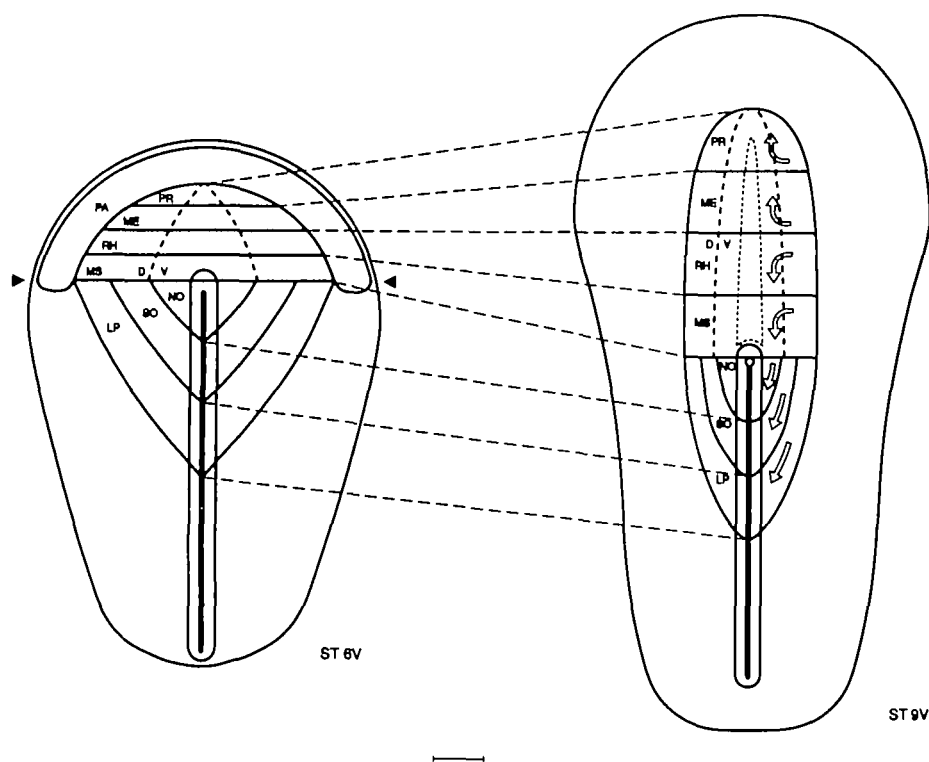
Fig. 2. Neural plate and intraembryonic mesoblast in stages 6V and 9V on dorsal views. (A) Disposition of the Anlage fields in stage 6V. A transverse line (between the arrowheads) through Hensen's node forms the border between neural tissue and intraembryonic mesoblast. PA, proamnion; PR, prosencephalon; ME, mesencephalon; RH, rhombencephalon; MS, spinal cord. A dotted line marks the border between dorsal and ventral neural structures; D, dorsal; V, ventral; NO, notochord; SO, somites; LP, lateral plate. (B) Disposition of the Anlage fields in Stage 9V. In the neural plate, the arrows indicate the convergence-extension movements within these fields. In the mesoblast area, the arrows show the convergence-regression movements of the Anlage fields. Scale bar : 200 µm.

Anterior to the transverse line through Hensen's node lies the neurectoblast area. Its peripheral border is situated at the inner arc of the endophyllic crescent, which marks the proamnion posteriorly.

(1) Grafts inside the inner arc of the proamnion were found in the nervous system. The most anterior grafts are found in prosencephalon, more posterior grafts successively in mesencephalon and rhombencephalon, while the most posterior grafts that formed neural tissue were found in the spinal cord (Fig. 2A). As observed with videography, each graft in the neural plate forms a field that extends length-wise. From stage 9V on, the posterior extension of the grafts was longer in the rhombencephalon and especially in the spinal cord. Grafts just rostral to Hensen's node eventually extend throughout the whole spinal cord (Rosenquist, 1966; Schoenwolf et al., 1989). Lateral grafts in the neural plate mark the dorsal parts of the neural tube, medial grafts mark ventral parts. The limit between dorsal and ventral lateral plates is indicated by a dotted line on Fig. 2.

(2) Grafts on the inner arc of the endophyllic crescent could be traced back in the rim of the neural plate, in neural crest and in head epiblast.

(3) Grafts outside the inner arc of the endophyllic crescent were found in epiblast-derived structures of the head (epiblast of the pharyngeal membrane, adenohypophysis, primordium of the lens placode, auditory placode) and extraembryonic epiblast of head and trunk (amnion).

The part of the upper layer caudal to the transverse line through Hensen's node at stage 6V contains presumptive intraembryonic mesoblast. In this area, the prospective notochord, somites and lateral plates occupy three fields (Fig. 2A). In a mediorostral triangle, we found future noto-chord. Bilaterally flanking the notochord, we found a strip

of future somitic tissue. A third strip flanked the somite area on both sides. It yielded nephrotome and lateral plate. The ingression of these fields at stage 5V has been described (Bortier and Vakaet, 1992).

Discussion

Part of the discrepancies between earlier presumptive fate maps is due to the vagueness of the stage of the blasto-derms. Incubation hours can not be relied on for staging. For this reason, Vakaet (1970) defined ten stages between the unincubated and the headfold chicken blastoderm. He added three stages between stages 3 and 4 of Hamburger and Hamilton (1951). In stage 4V, the streak is rod-like and elongating; in stage 5V, it is elongating and grooving and, in stage 6V, it ends its elongation and is fully grooved. In stage 7V (stage 4 of Hamburger and Hamilton), regression of the anterior half of the streak takes place.

Another reason for the discrepancies between earlier presumptive fate maps is the use of different markers. Vital dyes (Pasteels, 1937 and Malan, 1953) fade away. Carbon marks (Spratt, 1952) are not reliable. In tritiated thymidine-labeled grafts (Rosenquist, 1966), only 50-70% of the graft nuclei were marked. Xenografting after Le Douarin (1973) is reliable cell per cell. Moreover, the grafts can be followed with time-lapse videography during their movements in the upper layer. Therefore, the movements need not be reconstructed afterwards, as they are observed.

The neural plate (Fig. 2A) does not extend caudally beyond a transverse line through Hensen's node. At stage 6V, it is remarkably small and occupies only a segment of a disk. It is limited anteriorly by the inner arc of the proam-
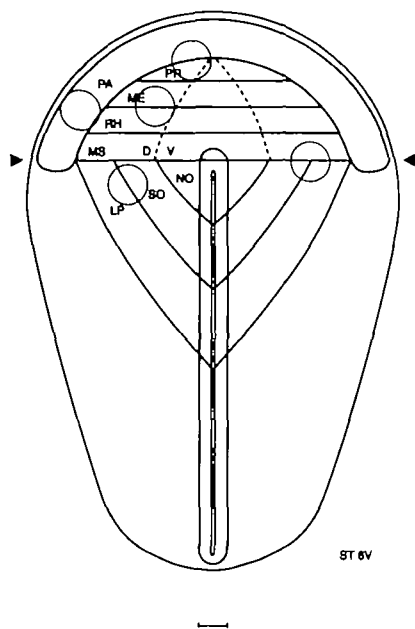
Fig. 3. Some examples of grafts to illustrate that one graft can mark several structures. Scale bar : 100 µm. Legends as in Fig. 2.

nion. Its posterior border is a transverse line with the intraembryonic mesoblast. This is in contradiction with the fate maps by Rawles (1936), Rudnick (1944), Spratt (1952), Rosenquist (1966) and Vakaet (1984). All these maps show a prospective neural plate with caudally directed wing-like extensions.

As demonstrated with videography, these fields converge. This reduces the width of the neural plate (compare Fig. 2A and 2B). Concomitantly the fields elongate in an anterior and posterior direction from the time Hensen's node starts to regress (convergence-extension as coined by Pasteels, 1937). In Fig. 2A, we have ascribed tentatively the same anteroposterior widths to the prosencephalon, mesencephalon, rhombencephalon and to the spinal cord. Moreover, we assumed in Fig. 2B that the elongation of these fields is similar up to stage 9V, the last presomitic stage. After stage 9V, the length over which grafts extend posteriorly is longer in the rhombencephalon and even more in the spinal cord.

In the region behind Hensen's node, the mesoblast cells ingress through the regressing anterior half of the primitive streak (Fig. 2B). Some grafts partly ingress and partly stay in the upper layer as described with videography. This illustrates the advantage of making grafts with a diameter of about 200 µm. Due to their size, they usually mark more than one Anlage field (Fig. 3). This marking of at least two Anlage fields shows that some fields have a border in common. Small grafts that only mark one Anlage field do not show its topographical disposition.

Our fate map complements the results of Couly and Le Douarin (1985, 1987, 1988) who studied with xenografts the neural plate and its periphery in presomitic and early somitic stages. At these stages the neural tube is already closing. Our xenografts were made in stage 5-6V blasto-

derms in which the topographical situation of the Anlage fields was not yet predictable. The resulting fate map shows the disposition of the Anlage fields prior to their final morphogenetic movements in the upper layer. The most extensive of these movements are: (1) the convergence-extension of the neural plate that is situated at stage 6V in a small segment of the area pellucida anterior to Hensen's node, (2) the backward movement of the border between the neural plate and the intraembryonic mesoblast that corresponds to the regression of Hensen's node and (3) the convergence-regression of the intraembryonic mesoblast fields and the shrinking of their surface by ingression of cells through the primitive streak.

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