Development of the chick embryo mesoblast: pronephros, lateral plate, and early vasculature

By STEPHEN MEIER

From the Department of Anatomy,
University of Southern California School of Medicine,
Los Angeles, California

SUMMARY

The early development of the mesoblast in the intermediate and lateral regions of the chick embryo was examined with the scanning and transmission electron microscope. It was found that primary mesenchyme here becomes condensed into epithelial structures that emerge in a metameric pattern. Viewed in developmental sequence, the intermediate mesoblast condenses into a narrowing cord of axially oriented cells which divert medially at regular intervals into the intersegmental interfaces of somitomeres and somites. These cells give rise to the vascular channels of the posterior cardinal vein as well as to tubular elements of the pronephros. Intermediate mesenchyme cells become epithelial, forming zonular junctional complexes apically and depositing patchy basal lamina over their basal surfaces. The lateral plate mesenchyme organizes similarly into somatic and splanchnic epithelial sheets that utilize the body coelom as their lumenal surface. Cells of the lateral plate extend filopodia basally that interweave with adjacent cells, fibrillar extracellular matrix, as well as with interstitial bodies. The pattern in the lateral plate is subtly ribbed as bands of mesoblast undulate along the axis. The central region of each band is raised while there are grooves created along lines of band abutment, corresponding to intersegmental clefts in the paraxial region and reflecting an underlying metameric pattern. These grooves are usually demarked medially by the protrusion of short segments of adjacent intermediate mesoblast. Most of the remaining primary mesenchyme develops into a non-metameric vascular epithelium, which forms a prominent anastamosing plexus between splanchnic mesoderm and endoderm. It is proposed that the emergence of primary mesenchyme into patterned epithelial anlage facilitates the distribution of neural crest cells introduced subsequently.

INTRODUCTION

During the early course of development of the chick embryo mesoblast, cells comprising this layer interconvert between mesenchymal and epithelial morphologies. Primary mesenchyme cells originate from an epithelial epiblast layer at the primitive streak, separate from each other, and spread away from the midline (Bancroft & Bellairs, 1975; Wakely & England, 1977; Solursh & Revel, 1978). Mesenchymal cells sprout filopodia and contact one another focally (Trelstad, Hay & Revel, 1967). Freeze fracture has shown that these

1 Author's present address (for reprints): Department of Zoology, University of Texas at Austin, Austin, Texas 78712, U.S.A.
contact regions contain gap junctions and remnants of zonula occludens (Revel, Yip & Chang, 1973). Some of these cells reorganize into a thin, epithelial endoblast, the definitive embryonic endodermal germ layer (Wolk & Eyal-Giladi, 1977; Sanders, Bellairs & Portch, 1978). However, the majority of the cells gastrulating at the primitive streak become mesenchymal and contribute to the mesoblast layer. Meier (1979) has recently described the subsequent morphological conversion of primary mesenchyme into chordamesoderm. It was found that the primary mesenchyme cells reorganized into epithelial structures which exhibited regular pattern. Viewed dorsally, the embryonic axis is tripartite, being composed of a central axial segment flanked on either side in the paraxial region by circular domains (180 μm diameter) of mesoblast cells called somitomeres. The tandem addition of these segments generated by node regression down the primitive streak, establishes metameric pattern in the mesoblast layer. Cells of the axial segment eventually condense into an epithelial notochord ensheathed in basal lamina and extracellular matrix (Low, 1968; Ruggeri, 1972). Each paraxial somitomere originates as a slightly hollowed, squat cylinder composed of tapering mesenchymal cells whose long axes are directed toward the core center. Cells of somitomeres are obviously polarized (reform zonular junctions, deposit patchy basal lamina) while they continue to undergo morphogenesis into primitive, epithelial somites (Meier, 1979; Solursh, Fisher, Meier & Singley, 1979).

Recent studies of the mesoblast with the electron microscope have been confined to the differentiation of the paraxial mesoblast because its development into somitomeres is strictly correlated with the development of epiblast into neural plate (Lipton & Jacobson, 1974a, b; Meier, 1979). However, to what extent the mesoblast is organized in regions not under jurisdiction of the neural plate has not been scrutinized with the electron microscope. Rosenquist (1966, 1970) has shown, utilizing autoradiographic techniques, that the intermediate and lateral plate mesoblast originate from a region of epiblast initially lateral to the primitive streak. During gastrulation these cells move medially and invaginate along the lower two thirds of the primitive streak, spreading away from the midline into the mesoblast layer. Recently, morphological analysis of Amblystoma embryos (Poole & Steinberg, 1979) suggests that directed extension of the pronephric duct is accomplished by rearrangement of intermediate mesenchymal cells. With stereo-scanning electron microscopy it is possible to also obtain a three-dimensional appreciation of cell-to-cell associations in these regions. By careful removal of epiblast or endoblast, large expanses of developmentally sequenced mesoblast can be observed in great detail. The purpose of this study was to trace the early morphological conversion of primary mesenchyme into epithelial embryonic anlage of the intermediate and lateral plate mesoblast. Particular attention was paid to the pattern of organization in these regions as it relates to metamerism of the chordamesoderm.
MATERIAL AND METHODS

Fertile Rhode Island Red chick eggs were obtained from the Red Wing Hatchery, Los Angeles, California, and incubated at 38 °C for up to 48 h. Eggs were cracked into Petri dishes, slits were made in the blastoderm, and a patch containing the embryo was removed and transferred to warmed Hanks balanced salt solution (GIBCo) for rinsing and trimming prior to fixation. The salt solution was withdrawn and replaced with half-strength Karnovsky's fixative (1965) supplemented with 2 % tannic acid in 0·1 M cacodylate buffer (pH 7·3) (Meier, 1978). Embryos were fixed for 30 min at room temperature, transferred to another vial and rinsed for 10 min in four to five changes of 0·1 M cacodylate buffer. Appropriately staged embryos were dissected with electrolytically sharpened needles in buffer under a dissecting microscope using transmitted light. Dissected embryos were thoroughly rinsed in buffer and transferred to 1 % osmium for 60 min. After several buffer rinses, samples were treated with 0·5 % thiocarbohydrazide for 15 min, rinsed in buffer, and transferred back to 1 % osmium for another 60 min. After this second osmium treatment, specimens were rinsed in buffer, dehydrated through a graded alcohol series and critical-point dried using CO₂ as the exchange fluid. Embryos were mounted on double-stick tape covering the top of aluminum studs and sputter-coated with 8–9 nm of gold–palladium alloy. Specimens were observed with a JEOL JSM-35 scanning electron microscope at 25 kV. Stereo pair photographs were obtained using a tilt angle of 12°.

In some cases, specimens were not dissected after aldehyde fixation, but were processed and mounted as described. These embryos were dissected with patches of double-stick tape (held with forceps). Tape was strategically placed on the specimen, and whole sections of epiblast or endoblast would be removed. By examining both the portions of embryoblast removed by the tape, as well as the embryo itself, it was possible to assess directly the complementary components of the dissection. For the regions reported here, the mesoblast layer is not adherent to either endoblast or epiblast layers removed by tape after critical point drying. In addition, specimens dissected immediately after aldehyde treatment were also free of mesenchyme. Therefore, most specimens were dissected immediately after aldehyde treatment were also free of mesenchyme. Therefore, most specimens were dissected with needles after prefixation, since subsequent osmium and thiocarbohydrazide treatment makes the embryos very dark, brittle and more difficult to dissect precisely with double-stick tape.

For transmission electron microscopy, samples were fixed as described except that the thiocarbohydrazide treatment was omitted. After postfixation in osmium, embryos were stained en bloc for 2 h with 0·5 % Mg uranyl acetate in 0·9 % NaCl, dehydrated with graded alcohols and embedded in Epon. Thin sections, collected on carbon-supported 200 mesh grids, were treated further with uranyl acetate and lead citrate (Reynolds, 1963), prior to examination
with a JEOL 100-C transmission electron microscope. Over 125 embryos were examined and nearly 750 micrographs were analyzed.

RESULTS

Intermediate mesoderm

At about the time of formation of the first somites (stage 8–9, Hamburger & Hamilton, 1951) the mesoderm immediately lateral to the paraxial region can be seen emerging as a narrow band of cells elongating parallel to the embryonic axis (Fig. 1). At the anteriormost end, this cellular organization first appears in the mesoderm adjacent to the fifth somite and can be seen in various stages of organization thereafter as far posteriorly as the segmental plate. Since much of the intermediate mesoderm organized in the region of the head process participates in branchiomeric and cardiovascular development, observations here were confined primarily to the posterior somitic regions of 10- to 20-somite embryos. Viewed dorsally, the intermediate mesoderm is seen as a condensing cord of somatic cells. Adjacent to fully formed somites, the band of intermediate mesoderm is approximately 45 μm wide, spreading gradually to a width of 80 μm posteriorly, adjacent to the segmental plate. Here the intermediate mesoderm becomes reduced to a series of condensations that are connected to each other by a few narrow cellular cords. The narrowing of the cellular band cranially and its shift toward the axis accompanies the condensation in the paraxial mesoderm, where somitomeres undergo morphogenesis into somites (Meier, 1979). Usually, at discreet intervals along the intermediate band, cells protrude at nearly right angles into adjacent paraxial and lateral regions. In the anterior region, the medial diversions are mainly tubular intrusions into deep intersomitic clefts. However, they occur as cord-like protrusions between somitomeres (incipient somites) in the anterior-most third of the segmental plate. It is the pattern of the developing paraxial mesoderm that is faithfully exploited by the adjacent intermediate mesoblast.

Figures 1 and 2

Fig. 1. A scanning electron micrograph of a dorsal view of the right half of a chick-embryo mesoblast in the region where somites emerge from the cranial end (right) of the segmental plate. Epiblast and neural tube have been removed. Outlining the lateral margin of the segmental plate, intermediate mesoderm can be seen condensing into a narrow cord. Tubular elements protrude into the paraxial region at regular intervals, suggesting the location of intersomitic regions, future sites of intersomitic clefts (arrowhead). x 320.

Fig. 2. A stereo scanning electron micrograph (tilt angle 12°) of a dorsal view of intermediate mesodermal elements coursing next to a somite of a stage-10 chick embryo. The epiblast has been removed. Branches of the posterior cardinal vein divert laterally, and medially into regions of intersomitic clefts (obliterated by ECM). Somitic cell processes reach into the covering blanket of ECM which is orientated primarily in a cranial (upper) caudal (lower) direction. The nephrotome courses axially, subjacent to the vascular element. Stereo viewer. x 625.
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Segmental plate

Intermediate mesoderm

Nephrotome

Posterior cardinal vein
The intermediate mesoderm develops principally into nephrotome and venous vasculature. Viewed in stereo, it is possible to distinguish between these elements. Figure 2 shows the posterior cardinal vasculature as a flattened tube wrapping around a subjacent cord of nephrotome. The main body of the vessel is flattened against the lateral surface of the somite, although some of the cells extend narrow channels deep into the intersomitic region as well as superficially into the lateral plate. The cells of the intermediate mesoderm come to develop in a groove created by the condensation of the somites towards the axis. In the space between intermediate mesoderm and overlying ectoderm, considerable extracellular matrix is deposited. The matrix is principally fibrillar with most of the strands studded with granules and coursing in an axial direction. Adjacent somites become so enshrouded with matrix that only the tips of somitic cell processes are visible beneath it. By carefully stripping away the vasculature and extracellular matrix, the deeper running nephrotome is revealed.

The nephrotomal cells are generally oriented parallel to the embryonic axis (Fig. 3). Individual cells are somewhat ovoid and make broad contacts with each other. More slender filopodial contacts are made between cells not directly opposed. In regions where cells are dense, there is considerable overlapping and intermingling. The net effect of these cell associations is a rather unevenly surfaced cord coursing through the interior of the intermediate mesoderm. Cross-fractures of this cord (Fig. 4) reveal that many of the intermediate cells have developed a continuous channel. Cells of the tubular wall are nearly as wide as they are tall, and are considerably flattened. They have scalloped lateral borders and reveal some modification at their luminal surfaces, such as elongate microvilli and cilia that extend into the main channel. Examination of thin sections of cells comprising the nephric duct (Fig. 5) bear out the impressions cast by the scanning electron micrographs: the duct cells are polarized in an epithelial manner. The basal cell surfaces slip by and overlap each other, reducing the chance for direct continuity of inter-epithelial and

Figures 3-5

Fig. 3. A scanning electron micrograph of the dorsal surface of the nephrogenic cord in a posterior region, adjacent to somites of a stage-10 chick embryo. The elliptical cells form broad contacts with one another, and most are orientated with their long axes parallel to the midline (lower left to upper right). × 1130.

Fig. 4. A scanning electron micrograph of cross fractured nephrotome in a region adjacent to somites of a stage-11 chick embryo. Some nephric cells have organized into the pronephric duct, the main channel of the nephrotome, extending cilia into the lumen (arrowhead). × 1925.

Fig. 5. A transmission electron micrograph of the luminal surface of the pronephric duct taken from a thin section through a region similar to that for Fig. 4. Cells are closely interdigitated apically, and basally (insert), cells spread over one another at intercellular margins. × 16100, inset × 12000.
extracellular space (Fig. 5 inset). Often times, patchy basal lamina is adherent to the outer basal surface of the duct. At the lumenal surface, the cells undergo extensive interdigitation (Fig. 5) and junctional complexes appear between the membranes of cells along regions of broad contact. The apical cytoplasm is also the preferential site of location of the Golgi complex.

**Lateral plate**

A cross section of the embryo through a somite-containing region at 36 h of development shows that the lateral plate mesoderm is organized into epithelial layers (Fig. 6). The splanchnic mesoderm is compact, being composed of elongate, bottle-shaped cells. These tall, columnar cells are arranged in such a way that the bulbous end of one cell is interdigitated with the tapered ends of adjacent cells. The lateral surfaces of these tight-fitting cells are relatively smooth, showing only occasional small microvillous projections. The basal surface, however, is covered with filopodia, which course over one another and mesh with extracellular matrix fibers. In addition to fibrils of various caliber, the extracellular matrix contains interstitial bodies (Fig. 7), first described by Low (1970) and recently reported to contain fibronectin (Mayer, Hay & Hynes, 1979). These matrix aggregates range in size from 220 to 600 nm in diameter, and are located preferentially along the endoderm-mesoderm interface and in the crevices between individual splanchnic mesenchymal cells. The somatic mesoderm is also epithelial in appearance, forming a sheet of low columnar cells. However, individual cells are wedge-shaped, being somewhat taller than they are wide. Cellular surfaces facing the coelom are smooth, the ends of individual cells bulging convexly into the cavity. Examination of thin sections taken through the coelomic border of the somato- or splanchnopleure reveals that the cells are tied together apically by junctional complexes (Fig. 8). While portions of these complexes appeared in some sections but not in others, much of this region is zonular as revealed by serial sectioning. Those membrane differentiations found in each section usually lacked well-developed supporting cytoplasmic filaments, but showed punctate membrane contacts reminiscent of profiles of sectioned tight junctions.

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**FIGURES 6-8**

Fig. 6. A scanning electron micrograph of cross-fractured lateral plate from the posterior region of a stage-10 chick embryo. ×1800.

Fig. 7. A transmission electron micrograph of the basal surface of the splanchnic mesoderm facing endoblast from a region similar to that shown in Fig. 6. While some fibrillar and granular elements are present in the ECM, the most common components are interstitial bodies (arrowhead) of various sizes. ×8500.

Fig. 8. A transmission electron micrograph of a thin section taken through the apical surface of the somatic mesoderm, facing the body coelom, of a stage-10 chick embryo. ×38000.
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The obvious segmental pattern of the axial mesoblast is extended subtly into the lateral plate region. By sectioning tangential to the midline, it is possible to see that the lateral plate is ribbed (Fig. 9) with tandem straps of mesoblast oriented perpendicular to the axis. Each band is raised and hollow within, but tapers along lines of band abutment (corresponding to intersegmental clefts in the paraxial region). Internally, the intersegmental regions are usually demarked by the close approximation of somatic and splanchnic mesoblast layers, pinching together. While there may still be some further organization of cells within each elongate segment, a consistent pattern was not found. Twisting and shrinkage of the plate, while occasionally accentuating parts of the pattern, usually disturbed the uniformity in size of the segments.

Early vasculature

As mentioned, there is early development of the posterior cardinal vessels in the intermediate mesoderm, but by far the more impressive vascular development occurs in the splanchnic layer. By carefully stripping away the endoblast, it is possible to assess the extent of vessel formation in the extracellular compartment subjacent to the mesoblast. As seen in Fig. 10, the vessels in the posterior region appear as an anastamosing network of mesenchymal cells. They are distributed alongside the newly formed somites and begin to permeate the crevices between them. At the opposite end of the segmental plate, near Hensen’s node, the vascular pattern is restricted to an area lateral to the midline. The line of vascular encroachment medially, exactly parallels the lateral limit of the paraxial mesoderm as it condenses toward the midline in the morphogenesis of somitomeres into somites (Meier, 1979). Those cells nearest the midline under the somite, begin to form larger tubes which are closely adherent to the endoderm. When these larger caliber tubes could be
observed in stereo, as in Fig. 11, it is apparent that the cells of the vessel wall have sealed along lines of cell contact. The apical or lumenal surface of the endothelium is moderately dotted with short microvilli and strings of granular material. Cell membranes often taper sharply at lines of contact with fellow endothelial cells, creating intercellular crevices. The accompanying bulges into the vessel lumen caused by the relief of the cell membrane passing over a nucleus creates a somewhat tortuous channel. The basal surface of the vessels are considerably smoother than the apical surface. There are relatively few microvilli, although most are found on flat cell processes that mark regions of adjacent cell contact. The borders of contacts are nearly obscured by close cell overlapping and by the buildup of extracellular matrix, principally fibrillar in form.
Fig. 11. A stereo scanning electron micrograph (tilt angle 12°) of a maturing blood vessel (cellular margins, arrow) coursing over the basal surface of the endoblast of a stage-12 chick embryo. A blood cell lies in the vascular channel. Stereo viewer. ×1080.

Fig. 12. A scanning electron micrograph of an elongating vascular element forming along the basal surface of the endoblast of a stage-10 chick embryo. Most cells are relatively smooth and flat surfaced, contacting each other along cell margins, but elongated cells usually extend broad filopodia at their tapered ends (arrow). ×2200.
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Fig. 13. A stereo scanning electron micrograph (tilt angle 12°) of mesenchymal cell recruitment into vascular development in a stage-11 chick embryo. A ventral view (endoblast removed) of the main channel of the dorsal aorta shows that it receives cellular contributions from the adjacent splanchnic layer (arrows). Stereo viewer. × 1100.

In general, vascular development appears to proceed along three general lines. First, as cells contact one another, they extend and flatten. An example of this phase of vessel development can be seen in Fig. 12, where individual mesenchymal cells overlap at cell margins, as they protrude along the endodermal surface near the midline. Cells which are more cylindrical and elongate usually bear both broad and slender filopodia, while those cells which are flatter in shape have only a few short microvilli. Second, cells from the adjacent splanchnic mesoblast seem to be recruited into vessel formation. As seen in stereo in Fig. 13, cells which have joined to form a portion of the dorsal aorta also appear to be receiving contributions from the surrounding mesenchyme. Oftentimes, mesenchyme cells can be seen joining to already well organized channels. Finally, endothelial cells are mitotically active (not shown) and at least some of the vascular extension and expansion in the splanchnic mesoderm can be attributed to an increase in endothelial cell number.

DISCUSSION

As the mesoblast layer becomes overtly patterned, primary mesenchyme cells undergo morphogenesis into epithelia. The expression of epithelial characters by virtually all regions of the mesoblast is timed to immediately coincide with the general condensation of the mesoblast layer accompanying neurulation (Fig. 14). The axial segment differentiates into a near lumen-less epithelial notochord surrounded by basal lamina (Low, 1968; Ruggeri, 1972) while cells of the adjacent somitomeres develop zonular junctions about a central myocoele and show patchy basal lamina deposition over their external
Fig. 14. Diagrammatic representation of the epithelial morphogenesis of primary mesenchyme in the region of the segmental plate of a stage-11 chick embryo. The bold arrowhead points cranially and the right side of the embryo has been cross-sectioned in steps. Individual condensed somites (two shown), having matured from somitomeres in the segmental plate (two and one-half shown), emerge in the paraxial region next to notochord and neural tube. Intersomitic clefts have been exploited by extensions of the adjacent posterior cardinal vasculature and pronephros. The intersomitic clefts are carried across to the lateral plate where they appear as a series of regular grooves, reiterating metamerism in this region. Most of the remaining primary mesenchyme, not participating in the morphogenesis of the metameric structures, differentiates into an extensive vascular endothelium.

faces (Meier, 1979). Anterior somitomeres participate in branchiometric development, while posterior ones condense into somites (Solursh et al. 1979). The intermediate mesoblast emerges mostly as an epithelial network of pronephric duct, consisting of lumen-containing tubules. Mesenchyme in the lateral plate shows even more rigorous epithelialization, forming sheets of compacted cells. Nearly all the rest of the mesenchyme not committed to the morphogenesis of these embryonic anlage participates in the development of vascular channels. These include cells in the crevices between somites and the axis, those from the dorsal region of the intermediate mesoblast, as well as those found at the
interface between the endoderm and splanchnic mesoblast, particularly in the lateral plate. By the time neurulation is completed, nearly all of the embryonic mesoblast, once composed of mesenchymal cells, has undergone morphogenesis into a variety of epithelial structures.

The epithelial structures of the intermediate and lateral plate mesoblast emerge in a repeating, segmental pattern. The intermediate mesenchyme sends out regularly spaced branches that mark the site of repeating grooves or crevices in the lateral plate. The metamerism in this region of the mesoblast correlates strictly to that occurring in the embryonic axis, since intersomitic and intersomitomeric clefts in the paraxial region, when extended laterally, emerge as the regular grooves seen in the lateral plate. It has been previously reported that the metameric pattern extends to the midline where it is realized as regular (although abbreviated) expansions of notochord and neural tube into intersegmental sites (Meier, 1979). Similar to the notochord, the general extension of aggregates of intermediate mesoblast is in an axial direction, but medially and laterally adjacent intersegmental seams are exploited by short extensions of nephric and vascular elements. Since the main nephric mass in chicks is trapped between the metameric domains of the somite and lateral plate, intermediate mesoblast extension may result from exploitation of pattern-restricted pathways. Indeed, Poole & Steinberg (1979) found that in Amblystoma embryos, pronephric duct is selective in choosing a path of elongation, but that it will accept other substrata if forced by blockages. Perhaps the brief digression of intermediate elements into intersomitic clefts in the paraxial region and into intersegmental grooves in the lateral plate is evidence that chick nephric elements have similarly probed alternate available spaces for extension.

Finally, it is tempting to speculate that the conversion of primary mesenchyme into patterned epithelial anlage facilitates the distribution of neural crest cells. At the conclusion of neurulation, neural crest cells are introduced at a strategic point (Tosney, 1978) into a chamber filled with extracellular matrix and patterned epithelial structures, stuccoed with basal lamina. The eventual distribution of neural crest cell populations in this layer may depend on their ability to exploit mesoblast topography and pattern. However, to what extent the distribution of neural crest cells is dependent on directional constraints and routing imposed by the metameric pattern of the mesoblast, remains to be examined.

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