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Epithelial-mesenchymal transitions in the developing heart of the dogfish (*Scyliorhinus canicula*). A scanning electron microscopic study

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Abstract

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An epithelial-mesenchymal transition is involved in two main morphogenetic events of cardiac morphogenesis, namely the differentiation of the valvuloseptal tissue from the endocardial endothelium, and the formation of subepicardial mesenchyme from the epicardial mesothelium. We have proposed that the dogfish (*Scyliorhinus canicula*) is a suitable model for the study of basic processes of cardiac morphogenesis in vertebrates, since the heart of this primitive fish probably outlines the original *bauplan* of the vertebrate heart. In order to study in this model the endocardial and epicardial epithelial-mesenchymal transition under scanning electron microscopy, we have used a technique of paraffin-embedding, partial sectioning, dewaxing and critical-point drying. Our results showed: 1) A centrifugal pattern of epicardial development from the atrioventricular groove to the sinus venosus and conus arteriosus; 2) A close spatial and temporal relationship between the endocardial and epicardial epithelial-mesenchymal transition, although the transformation of the endocardium starts earlier and ends later the epicardial transformation; 3) A complex arrangement of the fibrous extracellular matrix which is established prior to the migration of the mesenchymal cells. Subepicardial, but not subendothelial mesenchymal cells, coalesce in unicellular or pluricellular ring-like structures that probably are related to the origin of the cardiac vessels.

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Introduction

In previous reports we have shown that early cardiac morphogenesis in the dogfish *Scyliorhinus canicula* (Linneus, 1758) shares many similarities with that of higher vertebrates, including the development of the epicardium, the subepicardial mesenchyme and the cardiac vessels (Muñoz-Chápuli *et al.* 1996, 1997; Macías *et al.* 1998). We had already suggested that this species can provide a suitable model for the study of the basic processes of cardiac development (Muñoz-Chápuli *et al.* 1994). Dogfish eggs can be easily kept in aquaria, and they are translucent, allowing for a continuous monitoring of developmental stage. Dogfish embryos are large, and their development is very slow, lasting for about five months. The adult dogfish heart retains the basic primitive design of the vertebrate four-chambered heart, i.e. it consists of a sinus venosus, atrium, ventricle and conus arteriosus, with no septation nor chamber reduction, as it occurs in tetrapods and teleost fishes, respectively.

During cardiac morphogenesis, the endocardial endothelium transforms into mesenchyme in the atrioventricular canal and proximal outflow tract (the conus arteriosus of the dogfish). This epithelial-mesenchymal

transition supplies the mesenchymal tissue that fills the atrioventricular and conal/outflow tract valves. The process of endocardial transformation has been extensively studied (reviewed in Markwald *et al.* 1995; 1996)

The epicardium is the outermost layer of the vertebrate heart. Endocardium and myocardium derive from the mesoderm of the cardiogenic plate (García-Martínez and Schoenwolf 1993) but the epicardium has an extracardiac origin from the coelomic mesothelium. The proepicardium (i.e. the epicardial primordium) consists of mesothelial outgrowths whose characteristics are similar in all vertebrates that have been studied (Viragh and Challice 1981; Kuhn and Liebherr 1988; Hiruma and Hirakow 1989; Fransen and Lemanski 1990; Männer 1992, 1993). Proepicardial cells attach to the heart, spread on the myocardial surface and form the epicardial mesothelium. A space, the subepicardium, appears soon between the primitive epicardial cells and the myocardium. The subepicardium is populated by mesenchymal cells whose origin is unclear (Hiruma and Hirakow 1989; Tidball 1992). We have suggested that the epicardium is the main source of subepicardial mesenchymal cells in the dogfish heart through a process of epithelial-mesenchymal transition (Muñoz-Chápuli *et al.* 1996).

The aim of this study is to corroborate, through scanning electron microscopy (SEM), the spatial and temporal correlation between the endocardial and the epicardial epithelial-mesenchymal transition. Such a correlation could indicate the existence of a myocardial-derived, regional signalling system which induces the epithelial-mesenchymal transition (Muñoz-Chápuli *et al.* 1996). We have combined SEM with a special technique to obtain suitable sections of the embryonic heart, in order to show three dimensional evidence of transformation of the inner and outer cardiac epithelia and migration of subepicardial and subendothelial mesenchymal cells throughout the extracellular matrix. We have proposed elsewhere (Muñoz-Chápuli *et al.* 1997) that growth of the epicardium in the dogfish is centrifugal, i.e. from the atrioventricular groove to the inflow and outflow tracts of the heart. The sinus venosus and the distal part of the conus arteriosus would be the latest areas to be covered by the epicardium. We aimed to demonstrate, through SEM, this pattern of epicardial development, unique among vertebrate embryonic models studied to date. Finally, our purpose was to provide SEM images of the developing heart in an elasmobranch model, images which are uncommon in the literature.

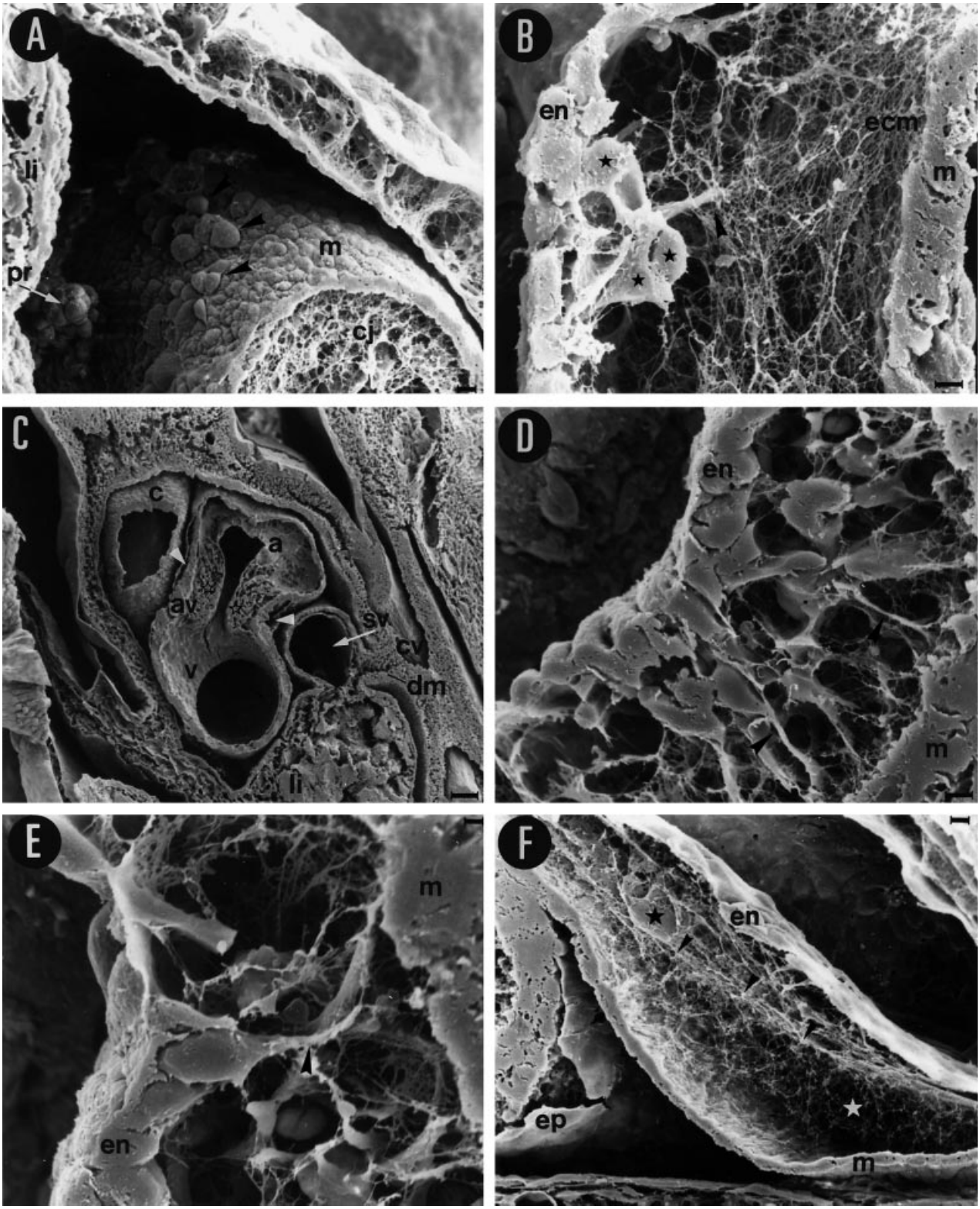
Material and Methods

Fertilized dogfish eggs were obtained from adult females collected in the bay of Málaga (Western Mediterranean) by commercial trawl vessels. The eggs were kept in indoor tanks of well-aerated sea water, which was monitored for nitrite, specific gravity and pH. Egg capsules were opened at intervals, and the embryos carefully removed, anaesthetized in 0.04% tricaine methanesulphonate (MS-222, Sigma, UK) in sea water and measured.

Seventeen dogfish embryos were used. Their sizes were 13 (2), 14, 18 (2), 20 (2), 24 (2), 27 (2), 28, 28.5, 31, 33 (2) and 35 mm in total length. The size of an embryo has proved to be well correlated with the successive developmental stages of the dogfish (De Andrés *et al.* 1993).

Whole embryos were immersion-fixed for 2–3 h in freshly prepared 1% glutaraldehyde and 1% paraformalde-

Fig. 1—Dogfish embryos, 13–20 mm in total length. Early stages of the epithelial-mesenchymal transition of the endocardium. —**A,** 13 mm total length. Proepicardial cells are attached to the myocardial surface (m), at the level of the atrioventricular junction (arrowheads). These cells originate from the proepicardium (pr), an outgrowth of mesothelial cells located at the ventral and anterior part of the liver (li). The cardiac jelly (cj), a thick layer of extracellular matrix between the myocardium and the endocardium, shows a complex network of fibrillar material that will be colonized in later stages by endocardial-derived mesenchymal cells. Scale bar = 10 μm. —**B,** 13 mm total length. Earliest stage of the epithelial-mesenchymal transition of the endocardium in the atrioventricular canal. Endocardial cells (stars) are migrating into the fibrous underlying matrix (ecm) which is denser near the myocardium (m). Note the contact between the leading pseudopodium of a cell and the fibrous matrix of the cardiac jelly (arrowhead). en: endocardium. Scale bar = 4.5 μm. —**C,** 20 mm total length. General view of the heart, in sagital section, at the level of the atrioventricular junction (av). The areas where the subepicardium has developed (white arrowheads) coincide with the atrioventricular endocardial cushions (stars). However, although the endocardial cushions are filled with endocardial-derived mesenchymal cells, the subepicardium is still acellular. The sinus venosus (sv) and conus arteriosus (c) are devoid of epicardium. Note the small remain of the dorsal mesocardium (dm), which is attached at the posterior dorsal part of the sinus venosus and allows for the connection of the cardinal veins (cv) with this cardiac chamber. a: atrium; li: liver; v: ventricle. Scale bar = 50 μm. —**D-E,** 20 mm total length. Two details of the atrioventricular endocardial cushions, in the areas of active epithelial-mesenchymal transformation. Many endocardial cells display long pseudopodia (arrowheads), which attach to the extracellular network of fibres. en: endocardium; m: myocardium. Scale bars = 5 and 2 μm, respectively. —**F,** 20 mm total length. Conal endocardial cushion. There is a conspicuous boundary (arrowheads) between the proximal and distal areas. The proximal area is closer to the transforming endocardium (en), which is being populated by the mesenchymal cells (black star). The distal and outer area is closer to the conal myocardium (m), which is still acellular and contains thin fibres in its extracelullar matrix (white star). The epicardium (ep) is present in the atrioventricular junction, but not on the conus arteriosus. Scale bar = 10 μm.



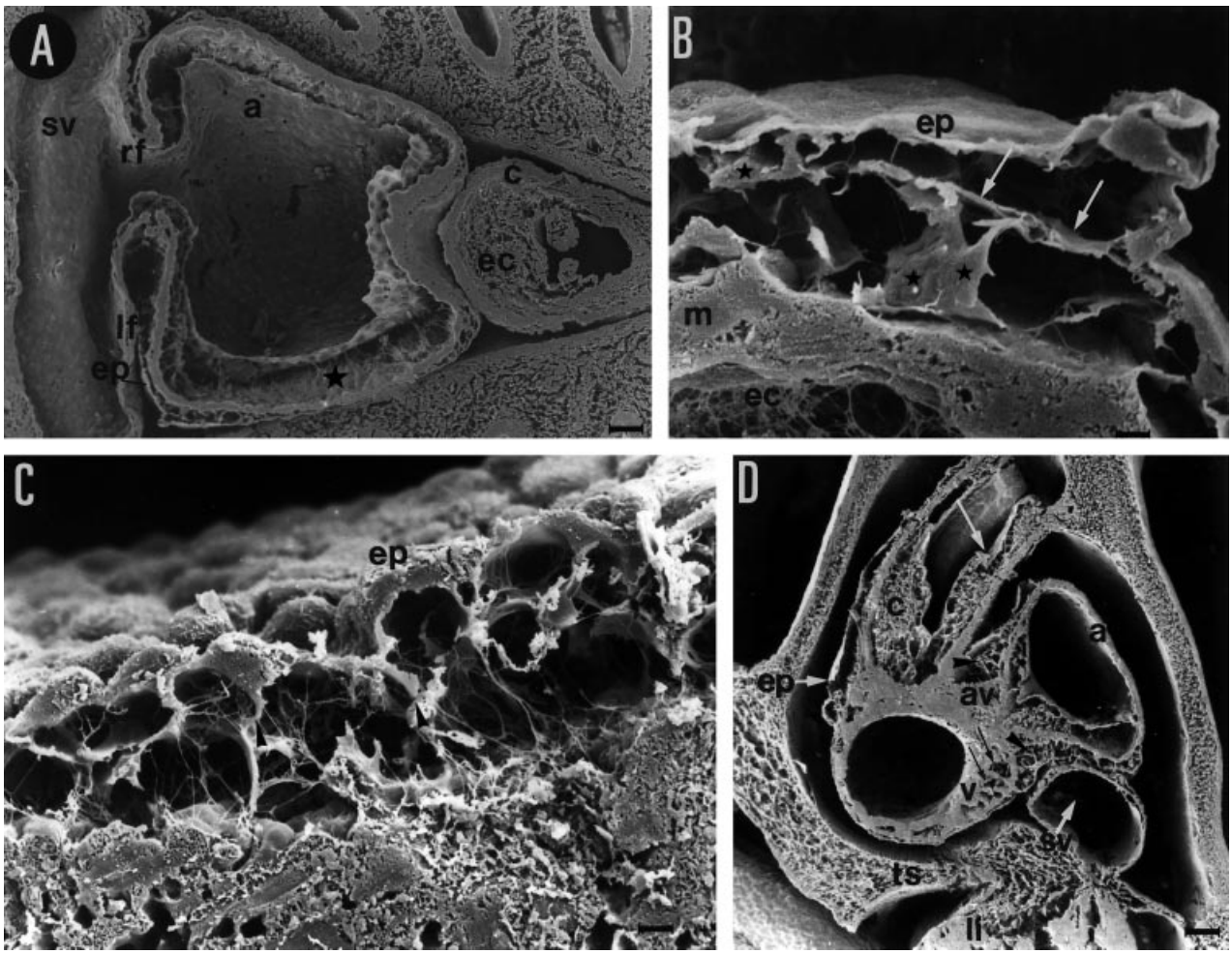


Fig. 2—Dogfish embryos, 24 and 27 mm in total length (total length). Development of the sinoatrial valve and epithelial-mesenchymal transition of the epicardium. —**A,** 24 mm total length. Frontal section showing the dorsal part of the sinus venosus (sv), atrium (a) and conus arteriosus (c). The lateral folds which give rise to the sinoatrial valve can be seen, as well as the absence of an epithelial-mesenchymal transition in this area. The left fold (lf) is deeper than the right one (rf), causing a shift of the sinoatrial junction to the right. The epicardium (ep) covers the sinoatrial groove, but it does not reach the posterior part of the sinus venosus. Note the larger amount of cardiac jelly at the left part of the atrium, the area closer to the atrioventricular junction (star). ec: conal endocardial cushion. Scale bar = 30 μm. —**B,** 24 mm total length. Detail of the epicardium (ep) of the dorsal part of the ventricle, close to the atrioventricular junction. The subepicardium is being populated by cells that migrate from the epicardium (stars). In some areas, the epicardium is apparently duplicated (white arrows), probably by the adhesion of proepicardial cells to the outer surface of the already established epicardium. The inner epicardial sheet probably transforms to mesenchyme. Note the smaller amount of fibrous extracellular matrix compared with the underlying endocardial cushion (ec) matrix. m: myocardium. Scale bar = 3 μm. —**C,** 24 mm total length. Epicardium (ep) of the dorsal part of the ventricle, showing the thin fibers of the subepicardial extracellular matrix and the pseudopodia of the premigratory epicardial cells (arrowheads). Scale bar = 4 μm. —**D,** 27 mm total length. General view of the heart in a sagittal plane. The epicardium (ep) covers the whole heart, except for the most posterior part of the sinus venosus (sv). The subepicardium is wide in the atrioventricular junction (av) and the conus arteriosus (c). Mesenchymal cells are abundant in the subepicardium of the atrioventricular junction (arrowheads). The division between the anterior and posterior segments of the conal endocardial cushions is already visible; it is indicated by an indentation of the endocardium and a lower density of mesenchymal cells (white arrow). These segments will give rise to the anterior and posterior rows of conal valves, respectively. In the ventricle (v), the early stages of the trabeculation are becoming apparent as myocardial clefts that will be invaded by the endocardium, forming the intertrabecular sinusoids (small arrows). Note the lacking of the dorsal mesocardium. a: atrium; li: liver; ts: transverse septum. Scale bar = 60 μm.

hyde diluted in elasmobranch buffer (16.38 g/L NaCl, 0.89 g/L KCl, 1.11 g/L $CaCl_2$, 0.38 g/L $NaHCO_3$, 0.06 g/L $NaHPO_4{\cdot}2H_2O$, 21.6 g/L urea, pH 7.2). The fixed embryos were washed in elasmobranch buffer, postfixed in 1% OsO_4 in the same buffer for two hours at 4°C, and then washed in distilled water. After washing, the embryos were dehydrated in an ethanolic series finishing in butanol, then paraffin-embedded and sectioned with a Leitz microtome. We monitored carefully the sections until we obtained the desired profile of the heart. Then, the paraffin blocks containing the embryos were dissolved in xylene and the embryos were washed several times in 100° ethanol. After drying from liquid CO_2 by the critical-point method, the samples were sputted with gold (about 450 Å) in a JEOL fine-coat ion sputter (JFC-1100) and observed and photographed in a JEOL JSM-840 scanning electron microscope operated between 10 and 20 kV.

Results

The results are organized clustering the embryos in groups which represent four developmental periods of the cardiac epithelial-mesenchymal transition. Figure 1 includes the embryos of the groups 13–14 and 18–20 mm in total length. Figures 2 and 3 show the embryos of the groups 24–28.5 and 31–35 mm, respectively.

13–14 mm total length

In the embryos of 13–14 mm, most of the myocardial surface was not covered by the epicardium. However, groups of rounded proepicardial cells were attached to the myocardiocytes in the atrioventricular groove and in the lateral ventricular walls (Fig. 1A). Some of them apparently were spreading over the myocardial surface. The progenitor tissue of these primitive epicardial cells, the proepicardium, was visible as a double mesothelial protrusion on the right and left ventrolateral parts of the liver primordium (Fig. 1A).

The prospective endocardial cushions of the conus arteriosus showed a few enlarged endothelial cells, although most endocardial cells were flattened and squamous, covering a subendothelial space devoid of cells but filled by an abundant extracellular matrix mainly constituted by fibrillar proteins. All these features also were seen in atrioventricular cushions, where some endocardial cells were already starting their migration into the subendocardial matrix (Fig. 1B). These cells were rounded, with long basal cytoplasmic projections that were tightly adhered to the matrix fibres.

18–20 mm total length

In the embryos of 18 and 20 mm, the conoventricular and atrioventricular grooves as well as most of the ventricle were covered by patches of epicardium (Fig. 1C). The conus arteriosus and the sinus venosus remained uncovered, except for the areas close to the conoventricular and sinoatrial grooves. Except for the atrioventricular groove, where a wide subepicardium develops very soon, most of the epicardium was directly attached to the heart surface (Fig. 1C). Subepicardial mesenchymal cells were not observed in the subepicardium of the atrioventricular groove. However, the primitive epicardium, at the atrioventricular groove, was starting to show cells with traits similar to those of the endocardial cells covering the endocardial cushions of early embryos (rounded cells bearing long basal cytoplasmic projections).

The atrioventricular subendocardial space was already filled by mesenchymal cells which, in some areas, had not reached the innermost part of the cushion (Fig. 1D-E). These endocardial-derived cells were embedded in the fibrillar matrix. The contact of the long leading pseudopodia with the fibrous extracellular material was especially tight. The conal subendocardium showed a much smaller cell population than the atrioventricular subendocardium (Fig. 1F). These cells were more abundant in the proximal part of the conus, at the level of the conoventricular groove. The distal areas of the developing conal cushions were virtually acellular. A clear difference was noted between the inner-proximal and the outer-distal cushion matrix, the latter being constituted of thinner fibres than the former (Fig. 1F).

In embryos of this size the myocardium was not yet trabeculated (Fig. 1C).

24–28.5 mm total length

The atrial and ventricular myocardium was virtually covered by the epicardium in 24 mm embryos. At this stage, only the distal part of the conus arteriosus and the caudal areas of the sinus venosus remained uncovered (Fig. 2A). The subepicardium of the atrioventricular and conoventricular grooves showed an increasing number of subepicardial mesenchymal cells that were apparently migrating from the epicardium of these regions (Fig. 2 A-C). In fact, several epicardial cells in these areas showed morphological features similar to those of the endocardial cushions. Rounded cells, probably proepicardial, were oçcasionally observed attached to the outer surface of the epicardium. This possibility was reinforced by some observations of epicardial duplication, probably due to the differentiation of a secondary epicardial layer from attached proepicardial cells (Fig. 2B).

Morphological differences between the structure of the sinoatrial valves and the cushion-derived valves (i.e. the atrioventricular and the conal ones) were evident. Sinoatrial valves originated from an asymmetrical, lateral infolding of the myocardium in the sinoatrial limit (Fig. 2A). The endocardium covering the myocardial folds did not generate mesenchymal cells as it occurred in the atrioventricular and conal cushions.

In the embryos of 27, 28 and 28.5 mm, the epicardium virtually covered the whole heart, although a subepicardium was not apparent in the posterior part of the sinus venosus (Fig. 2D). Epicardial mesothelial cells still showed some morphological signs of detachment and migration into the subepicardial space. Subepicardial mesenchymal cells were populating all of the atrioventricular subepicardium and, to a lesser extent, the conal subepicardium. Some of these subepicardial mesenchymal cells were connected, forming ring-like or capillary-like structures.

The subepicardial extracellular matrix was more complex in these embryos than in earlier stages. The matrix consisted of thick fibers joining the epicardial and the myocardial surfaces. These fibers were connected by an intricate mesh of thinner filaments.

The conal endocardial cushions already showed the transverse division from which the anterior and posterior rows of valves will develop. This division consisted of an indentation of the endocardium and a lower density of mesenchymal cells The primordia of the anterior valves were already displaying their characteristic swallow-nest shape (Fig. 2D).

By 27 mm total length, myocardial trabeculation is apparent in the ventricle. The earliest sinusoidal spaces appeared as large myocardial clefts, which will be progressively lined by the endocardium (Fig. 2D).

31–35 mm total length

In the 31 and 33 mm embryos, the subepicardium was completely filled by subepicardial mesenchymal cells in the atrioventricular and conoventricular grooves (Fig. 3A). In these areas, most subepicardial mesenchymal cells showed no special arrangement, although some of them formed capillary-like structures constituted of isolated cells or rings of two, three or four cells connected between them (Fig. 3C,D). Some of these structures seemed to be more developed, showing aspect of short tubes. The innermost part of the subepicardium showed a lower density of cells (Fig. 3C).

Most morphological signs of delamination have already disappeared from the epicardium in the atrioventricular and conoventricular grooves, although a few cells were still large, rounded and bearing basal cytoplasmic processes. The epicardium covering the lateral areas of the ventricle was directly adhered to the myocardial surface. The subepicardium of these areas was reduced or virtually absent, and it contained only some extracellular matrix joining the epicardial cells with the underlying myocardium.

At this stage, the developing atrioventricular and conal valves included a great amount of subendocardial cells which formed the valvular tissue (Fig. 3A,B). Signs of epithelial-mesenchymal transition were restricted to the cephalic part of the conal valves and to the free edge of the atrioventricular valves (Fig. 3C). The conal valves were arranged in two rows (cephalic and caudal). The cephalic valves were more developed than the caudal, and they showed a very compact mesenchyme (Fig. 3A,B).

The myocardial trabeculation was well-developed in the ventricle. Large sinusoids were radially arranged around the U-shaped central lumen (Fig. 3A).

Fig. 3—Dogfish embryos, 33 and 35 mm total length. Late stages of the differentiation of the endocardial cushion mesenchyme and subepicardial mesenchyme. —**A,** 33 mm total length. General view, in a frontal plane, of the ventral part of the ventricle (v) and conus arteriosus (c). The epicardium (ep) covers the heart, and the subepicardial mesenchyme is abundant in the atrioventricular junction (av) and conus arteriosus. The atrioventricular and conal valves are well-developed and endowed with a compact mesenchyme. The anterior row of conal valves already shows the definitive swallow-nest shape (stars). Myocardial trabeculation is present throughout the ventricle, and the sinusoids (white arrows) are radially arranged from the main ventricular lumen, which is U-shaped. Scale bar = 50 μm. —**B,** Detail of A showing the anterior row of conal valves at higher magnification. Note the compact arrangement of the mesenchymal cells in the developing valves as well as the density of the extracellular matrix (arrowheads). Scale bar = 11 μm. —**C,** Detail of Fig.A showing the atrioventricular subepicardium at higher magnification. The epicardium (ep) has become squamous, and the signs of epithelial-mesenchymal transition are not conspicuous. However, in the developing atrioventricular valves the endocardial cells (en) still display a rounded shape, reduced intercellular adhesion (white arrows), cell overriding (black star) and basal appendages, suggesting that the epithelial-mesenchymal transition is still active. Another difference between the subepicardial and subendocardial mesenchyme is the differentiation, in the former, of ring-like structures, which are probably precursors of vessels (white star). m: myocardium. Scale bar = 4 μm. —**D,** 33 mm total length. A capillary-like structure (star) is formed by a single cell in the subepicardium of the conus arteriosus. Note the squamous appearance of the epicardium (ep). m: myocardium. Scale bar = 2.5 μm. —**E,** 35 mm total length. General view in a sagital plane. Although the whole heart is covered by the epicardial mesothelium, the subepicardium is wide and populated by mesenchymal cells only in those areas (labelled with white asterisks) where an epithelial-mesenchymal transition gave rise to the endocardial cushions, i.e. the atrioventricular junction (av) and the conus arteriosus (c). The sinus venosus (sv) lacks of a distinct subepicardium, and this probably precludes the migration of cells from the liver (li) and transverse septum (ts), as it has been suggested for other vertebrate embryos. On the other hand, the myocardial trabeculation is noticeable in the ventricle (v), but not in other cardiac chambers. a: atrium. Scale bar = 50 μm. —**F,** Detail of E showing the ventral part of the sinoatrial junction. A large vessel (star) is open to the lumen of the sinus venosus (sv). This will constitute the opening of the system of cardiac veins in the adult, because a true coronary sinus does not form in these vertebrates. ep: epicardium; m: ventricular myocardium; se: atrioventricular subepicardium. Scale bar = 5 μm.

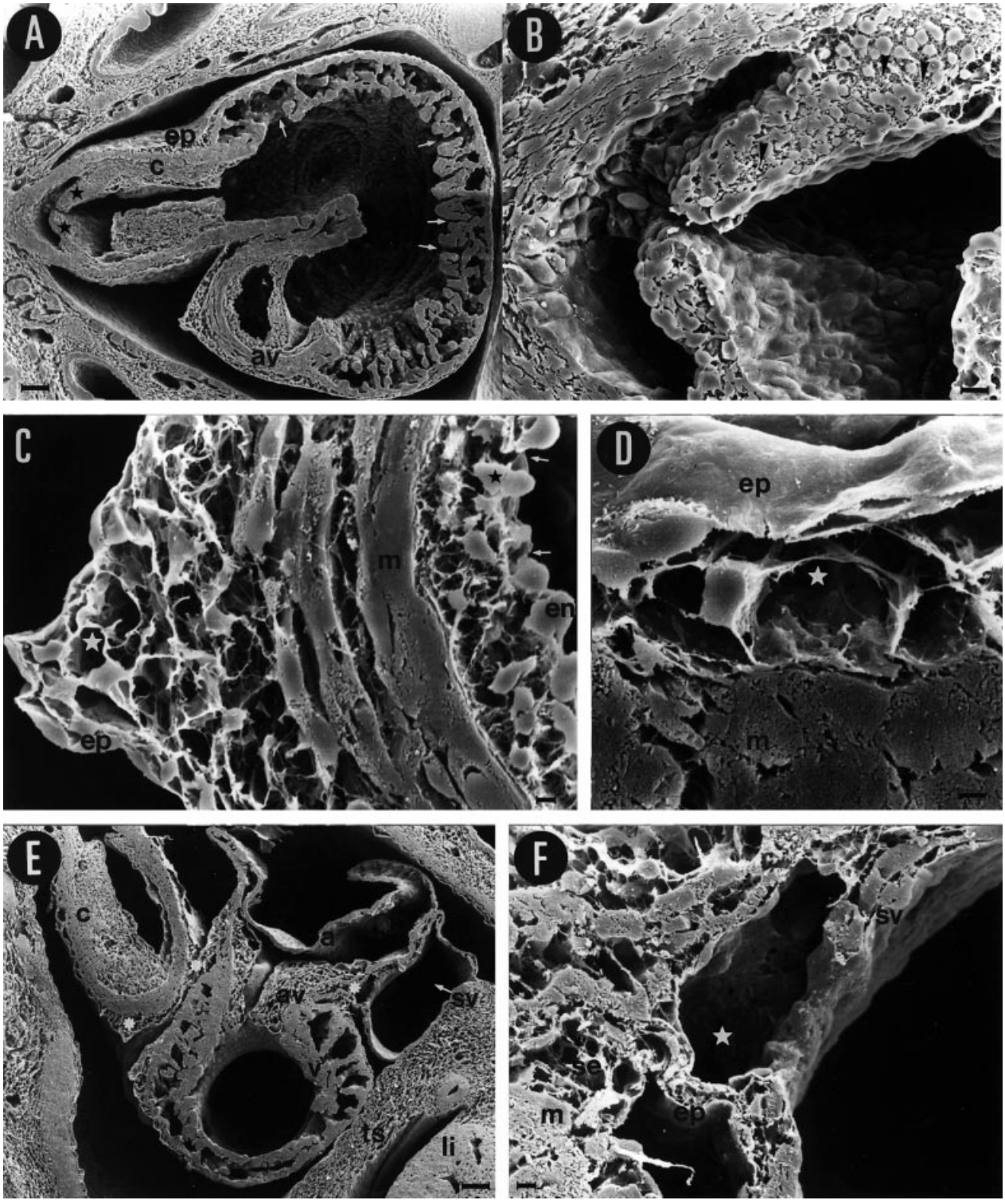


Fig. 3.

The 35 mm total length embryo represents the latest developmental stage included in our study (Fig. 3E,F). The epicardium of the lateral areas of the ventricle was flat, with small, elongated nuclei, no cytoplasmic processes and covering a narrow subepicardial space. Large vascular structures were present in the subepicardium of the atrioventricular and conoventricular grooves, some of them containing blood cells. Vessels were deeply located in the subepicardial space, usually in contact with the myocardial layer. A large vessel, located in the posterior part of the atrioventricular groove, was open to the sinus venosus (Fig. 3F). In adults, this opening constitutes the point of drainage of the cardiac veins to the systemic circulation. Some subepicardial mesenchymal cells were contacting the outer surface of the vessels. Probably, they are precursors of pericytes or smooth muscle cells.

Discussion

Epithelial-mesenchymal transitions are embryonic cellular processes that involve a radical morphological and molecular phenotypical shift in epithelial cells, leading to their transformation into migratory mesenchymal cells (Hay 1990, 1996). Such processes have been described in two main types of embryonic events. The first one includes the controlled disappearing of an epithelial sheet, such as happens during the fusion of the palatal shelves (Hay 1990). The second type is related to the generation of new mesenchyme mantaining the integrity of the original epithelium, as it occurs during the formation of the cardiac valvuloseptal mesenchyme (Markwald *et al.* 1977; Bolender and Markwald 1979).

Epithelial-mesenchymal transitions have been demonstrated to play an essential role in the development of the heart (reviewed in Markwald *et al.* 1996). In this paper we have performed a morphological study of the last epithelial-mesenchymal transitions that are involved in the cardiac morphogenesis, using the heart of the dogfish (*Scyliorhinus canicula*) as a model. We aimed to test the degree of colocalization of both types of epithelial-mesenchymal transitions, looking for evidence of a common inductive mechanism (Pérez-Pomares *et al.* 1997).

Dogfish endocardial and epicardial epithelia transform in a well-defined spatiotemporal pattern. Endocardial epithelial-mesenchymal transition is precisely restricted to the atrioventricular and conal endocardial cushions, while epicardial delamination mainly occurs in the atrioventricular and conoventricular grooves, extending to some adjacent areas of the ventricle and conus arteriosus. In the endocardial cushions, the epithelial-mesenchymal transition starts as early as 13 mm embryos and probably continues in embryos larger than 35 mm. The epicardial epithelial-mesenchymal transition starts later (around 18 mm) and finishes before 35 mm. These data agree with our previous histological results (Muñoz-Chápuli *et al.* 1996). Thus, colocalization between the endocardial and epicardial epithelial-mesenchymal transition seems clear. The delay shown by the epicardial epithelial-mesenchymal transition might be explained by the relatively late differentiation of the epicardial covering. The endocardium might be receiving an activating signal, thus acquiring a responsive status, much before the primitive epicardium.

Atrioventricular and conoventricular grooves were the first places in which primitive epicardium appeared. They were also the first areas where the epicardium delaminated and where the subepicardial mesenchymal cells formed the earliest cardiac vessels. These features resemble those reported in other embryonic vertebrate models such as chick (Hiruma and Hirakow 1989), mouse (Komiyama *et al.* 1987), tupaia (Kuhn and Liebherr, 1988) and hamster (Pérez-Pomares *et al.* 1997).

A common inductive process has been proposed to explain the similarities shared by the endocardial and epicardial epithelial-mesenchymal transition (Muñoz-Chápuli *et al.* 1996; Pérez-Pomares *et al.* 1997). Segmental myocardial signals are candidates to explain the localized transformation of the endocardial endothelium (Barton *et al.* 1995). The recently discovered ES/130 protein, which seems to be part of the signalling mechanism for the transformation of the endocardial cushions (Rezaee *et al.* 1993; Markwald *et al.* 1995), has been found in the epicardium and subepicardial mesenchymal cells of the avian embryo (Pérez-Pomares *et al.* 1997).

The subepithelial extracellular matrix seems to play a fundamental role in the epithelial-mesenchymal transition, not only providing a scaffold for the migration of the mesenchymal cells, but also serving as a source of growth factors and signalling molecules. The complexity of the subendocardial extracellular matrix (the so-called 'cardiac jelly') and the subepicardial extracellular matrix has been shown mainly by immunohistochemical techniques (Icardo and Manasek 1984; Tidball 1992). We have herein shown the abundance and diversity of the protein fibers which are probably used as a guide by cells migrating through substrate-adhesion molecules. Collagen, and fibronectin seem to be main components of these fibres (Tidball 1992). We have shown elsewhere that fibronectin immunoreactivity increases in the subepicardium of the dogfish embryo as it is being populated by subepicardial mesenchymal cells, as well as a strong fibrillin-2 immunoreactivity in the subepicardium and endocardial cushions of the dogfish embryo (Macías *et al.* 1998). The fibrillin-2 antigen recognized by the monoclonal JB3 antibody is involved in processes of epithelial-mesenchymal transition, as it has been demonstrated in chick embryos (Wunsch *et al.*1994). In the subepicardium, fibrillin-2 seems to organize the assembly of elastin (Bouchey *et al.* 1996).

Data shown in this work are consistent with our previous suggestions about an epicardial origin for most subepicardial mesenchymal cells in the dogfish embryo

and on the presence of vascular progenitors before the epicardium covers the caudal part of the sinus venosus. The connection of the epicardial-derived cells into capillary-like structures is a main difference between the two main populations of cardiac mesenchyme. Furthermore, while subendocardial mesenchyme is rapidly compacted to serve its mechanical function as valvular tissue, the subepicardial mesenchymal cells maintain their loose arrangement.

These results, together with our previous immunohistochemical and ultrastructural studies, suggest that both endocardial and epicardial epithelial-mesenchymal transition are closely related through a common, segment-specific, signalling system. This conclusion could be extended to other vertebrate models.

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