Origin of Cushion Tissue in the Developing Chick Heart: Cinematographic Recordings of in situ Formation

Abstract. *Differential interference microscopy and time-lapse cinematography were used to determine unequivocally the origin of cushion tissue cells migrating in situ in the atrioventricular region of the embryonic chick heart. These studies have verified the presumed endocardial origin of cushion tissue mesenchyme.*

A major developmental event in cardiac morphogenesis is the formation and migration of a population of cells traditionally referred to as endocardial cushion tissue (CT). Cushion tissue appears in specific regions of the cardiac jelly, an extracellular matrix interposed between the peripheral myocardium and the endothelium lining the heart lumen. Hypertrophy of the cardiac jelly in prospective regions of CT formation, primarily the atrioventricular (AV) canal and truncus arteriosus, results in the formation of endocardial pads, which represent the septal and valvular primordia of the heart (*1*).

Previous ultrastructural investigations by Markwald and his co-workers (*2, 3*) have suggested that the endocardium is the primary source of CT mesenchyme. In addition, both the myocardium (*4*) and the aortic arch mesenchyme (*2, 3, 5, 6*) have been implicated as possible CT progenitors. However, the nature of all of these studies precluded direct observation of the processes described, and none of their conclusions have been verified experimentally. No definitive study establishing the origin of CT mesenchyme has, to our knowledge, been reported. We now describe a method for preparing embryonic heart sections that allows the direct in situ observation of presumptive CT-forming regions during this critical developmental period. We have used this method to verify the purported endocardial origin of CT mesenchyme in the AV canal.

White Leghorn chicken eggs were incubated for 60 hours in a humidified atmosphere at 37.5°C. Embryos at stage 17 (*7*) were removed to a dish containing medium [Ham's F12M (Gibco) with 10 percent fetal calf serum and antibiotic-antimycotics (in each milliliter, 100 U of penicillin, 100 μg of streptomycin, and 0.25 μg of Fungizone); it was buffered with 10 m*M* Hepes]. Hearts were removed from the embryos and placed in dishes of 1.8 percent agar overlain with 10 to 15 percent gelatin, which had been dialyzed previously against medium and warmed to 37°C. After allowing the gelatin overlay to solidify, transverse sections of the AV canal region of the heart were made with sharpened tungsten needles (*8*). The gelatin overlay was melted, and AV canal sections (doughnuts) were removed and placed on the surface of a glass cover slip. The cover slip carrying the doughnut was inverted over a second cover slip set in an aluminum holder tooled to fit the microscope stage. The remainder of the space between the cover slips was filled with medium, and the edges of the preparation were sealed against evaporation with Valap (Vaseline, lanolin, and paraffin, in a ratio of 1 : 1 : 1). Time-lapse cinematography (four frames per minute) was performed with a photomicroscope (Zeiss II) outfitted with a 16-mm camera (Bolex). Preparations were maintained at 37°C with an incubator (Sage Air Curtain) while being photographed, and were protected by a heat filter and specimen shielding shutter between frames. Cinematographic recordings were made using differential interference optics with Plus-X negative film, which was developed commercially.

We have confined our observations to the initial stages of the process of CT formation in the AV canal. This process may be divided into two general categories: (i) extensive membrane activity by presumptive CT cells prior to delamination from the endocardium and (ii) seeding of endocardially derived mesenchymal cells into the cardiac jelly.

In a typical AV canal doughnut, sectioned from the heart before CT migration, two types of membrane activity by endocardial cells are evident. Cells associated with the endocardium exhibit elongated filopodial processes (Fig. 1). Cinematographic records demonstrate that filopodia of cells associated with the endocardium are extended (as far as 75 μm) and retracted, probing the matrix across the full breadth of the cardiac jelly. This activity is reminiscent of the membrane activity of invaginating primary mesenchyme cells in the sea urchin gastrula, reported in similar time-lapse studies (*9*). Our recordings of filopodial probing by endocardial cells and migrating CT cells also support the ultrastructural observation (*10*) that "pioneer" CT cells probe the matrix and modify their substratum before migration [see also (*11*)].

Another type of intense membrane activity limited to endocardial cells in our preparations is characterized by rapid blebbing and ruffling of both the luminal and matrical endocardial surfaces. Such activity often indicates intensive physiological activity and may be associated with lumen-matrix transport and the acquisition of secretory potential of "activated" endocardium in regions of presumptive CT formation (*2*). Both filopodial probing and localized membrane activity by endocardial cells (recorded as early as stage 12) precede the appearance of CT cells in the cardiac jelly.

The acquisition of cellular processes by endocardial cells may be related to subsequent CT cell migration. However, since we cannot establish whether all cells of the endocardium bearing processes eventually become CT mesenchyme and because process formation begins many hours before CT cell seeding, our observations do not necessarily support a simple cause-and-effect relationship between process formation and cell migration. Rather, we suggest that both phenomena are responses to an unspecified developmental signal that induces the formation of "activated" endocardium (*2*).

After a prolonged period of extensive endocardial cell membrane activity, individual CT cells have been observed to separate directly from the endocardium and migrate radially toward the myocardium (Figs. 2 and 3). Our observations indicate that most CT cell seeding into the cardiac jelly probably occurs by direct migration rather than by polarized mitotic activity within the endocardium, as has been previously proposed (*3*). Cells tended to stay within a given plane during migration, a fact confirmed by the shallow depth of focus in differential interference microscopy. Our observations disclosed no other source of CT mesenchyme in the AV canal during these initial stages of CT formation.

Although filopodia of presumptive CT cells approach the myocardium, CT cells begin migration immediately subjacent to the endocardium. The CT cells appear to use the cardiac jelly as a substratum and to move gradually, in contrast to studies of cell translocation in sea urchin gastrulae (*9*), in which cells moved across an intervening space in one step. Although we have observed no similar process, the presence of this mechanism of cell movement could explain both the appearance of CT mesenchyme adjacent to the myocardium (*4*) and the inference of a myocardial origin of these cells.

We have directly observed living heart tissue sections for evidence of an endocardial origin of AV canal CT mesenchyme. Although some CT cells are clearly derived from endocardium, our

0036-8075/80/0321-1359$00.50/0

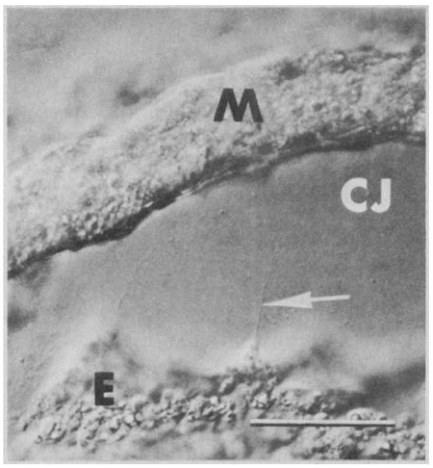

Fig. 1. Micrograph of a portion of a living AV canal doughnut from a stage 15 chick embryo. Differential interference optics reveal long filopodial processes (arrow) present on endocardial cells before CT cell migration. Abbreviations: *M*, myocardium; *CJ*, cardiac jelly; *E*, endocardium. Bar, 50 μm.

observations do not necessarily exclude other possible sources as CT progenitors. For example, recent descriptive studies (*6*) have suggested that aortic arch mesenchyme may migrate caudally and contribute to the formation of CT in the truncus, an anterior region of the heart not included in our observations. In addition, since AV canal doughnuts are isolated both distally and proximally from the heart tube continuum, mesenchymal migration along the axis of the heart could not contribute cells in this isolated system. Finally, our observations are limited to the initial stages of cushion tissue formation in the AV canal region. Hence, no attempt was made to discern future contributions, if any, of cells from other sources occurring at later stages of development.

Our heart preparations allow observations of CT migration in situ. However, CT formation is somewhat limited under our conditions with respect to the number of cells that appear in the cardiac jelly. The explants are viable for 12 to 24 hours, as determined by the formation of CT and the retention of contractile activity by the myocardium. In contrast, other studies involving the culture of whole explanted embryonic hearts have reported no development over the same time period (*12*). Since the increase of CT cell number has been suggested to be largely the result of interstitial growth (*3*, *6*), perhaps this process, rather than the process of cell seeding by the endocardium, was disrupted in our explants. Indeed, no cell divisions have been apparent among cushion tissue cells in our cinematographic records, although dividing cells should be evident over the time course of this study.

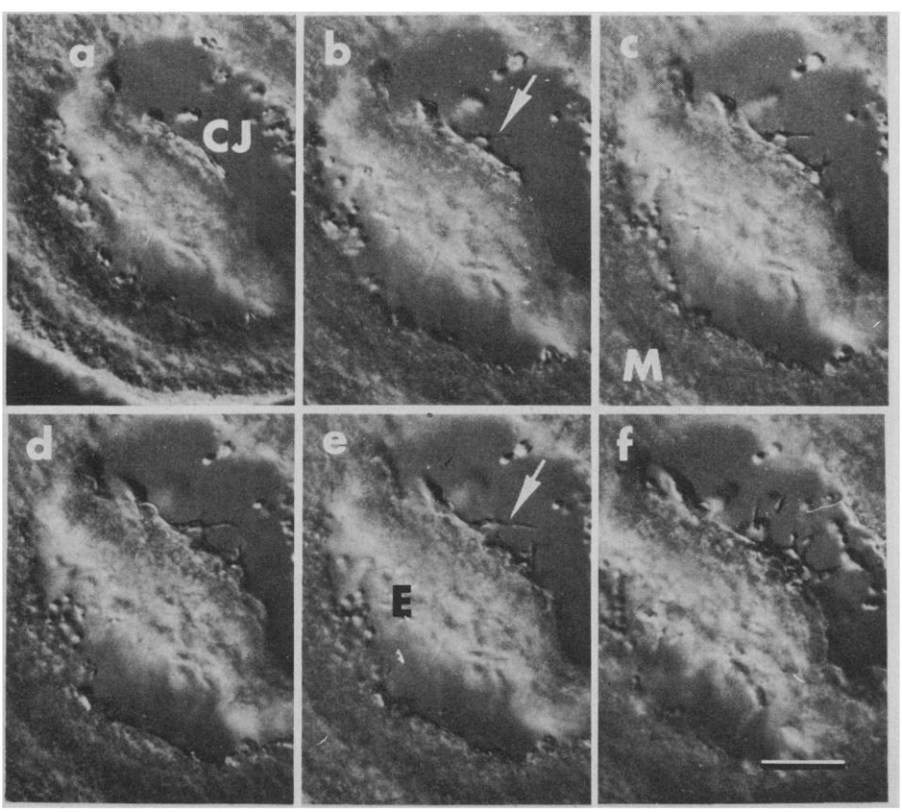

Fig. 2. Sequence from 16-mm film demonstrating the initiation of CT cell migration in a stage 17 chick heart AV canal doughnut. Bar, 50 μm. (a) 0.00 hours. Stage 17 AV canal prior to the initiation of cell migration. (b) 1.69 hours. Endocardial cell (arrow) can be seen as cushion tissue cell migration is initiated. Note long filopodium; (c) 2.67 hours; (d) 4.34 hours; (e) 5.99 hours. Endocardial cushion tissue cell (arrow) has nearly completed delamination from the endocardium; (f) 8.32 hours. Many cells have begun to emigrate from the endocardium.

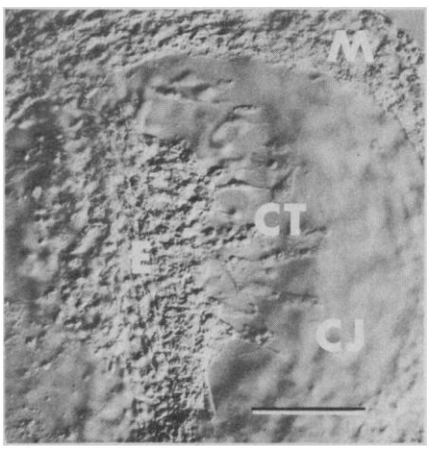

Fig. 3. Stage 18 chick heart AV canal doughnut. Bar, 100 μm. At a later stage of CT formation, cells moving in concert can be seen to have delaminated completely from the endocardium.

We have demonstrated that the endocardium is the origin of "pioneer" cells (*10*) initially seeded into the AV canal cardiac jelly. Also, the migration of CT cells from the endocardium is preceded by a prolonged period of extensive membrane activity by presumptive CT cells, characterized by extensive filopodial probing of the matrix. These observations provide proof for the contention (*2*, *3*, *10*) that CT cells have an endocardial origin.

MICHAEL G. KINSELLA
TIMOTHY P. FITZHARRIS
Department of Anatomy, Medical University of South Carolina, Charleston 29403

References and Notes

1. B. M. Patten, T. C. Kramer, A. Barry, *Anat. Rec.* **102**, 299 (1948); L. H. S. Van Mierop, R. D. Alley, H. W. Kausel, A. Stranahan, *Am. J. Cardiol.* **12**, 216 (1963).
2. R. R. Markwald, T. P. Fitzharris, W. N. Adams Smith, *Dev. Biol.* **42**, 160 (1975).
3. R. R. Markwald, T. P. Fitzharris, F. J. Manasek, *Am. J. Anat.* **148**, 85 (1977).
4. C. Chang, *Anat. Rec.* **51**, 253 (1932); W. N. Adams Smith, *ibid.* **147**, 507 (1963).
5. S. Virágh and C. E. Challice, *J. Ultrastruct. Res.* **42**, 1 (1973).
6. R. P. Thompson and T. P. Fitzharris, *Am. J. Anat.* **154**, 545 (1979).
7. V. Hamburger and H. L. Hamilton, *J. Morphol.* **88**, 49 (1951).
8. G. Dossel, *Lab. Invest.* **7**, 171 (1958).
9. T. Gustafson and L. Wolpert, *Int. Rev. Cytol.* **15**, 139 (1963); *Biol. Rev.* **42**, 442 (1967).
10. R. R. Markwald, T. P. Fitzharris, D. L. Bolender, D. H. Bernanke, *Dev. Biol.* **69**, 634 (1979).
11. R. W. Orkin and R. P. Toole, *ibid.* **66**, 308 (1978).
12. N. Sperelakis, M. S. Forbes, K. Shigenobu, S. Coburn, *J. Mol. Cell. Cardiol.* **6**, 473 (1974).
13. Supported by a grant in aid from the American Heart Association and with funds contributed in part by the South Carolina affiliate.

1 October 1979; revised 12 December 1979