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The extracellular matrix during heart development

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Abstract. The embryonic extracellular matrix, which is comprised of glycosaminoglycans, glycoproteins, collagens, and proteoglycans, is believed to play multiple roles during heart morphogenesis. Some of these ECM components appear throughout development, however, certain molecules exhibit an interesting transient spatial and temporal distribution. Due to significant new data that have been gathered predominantly in the past 10 years, a comprehensive review of the literature is needed.* The intent of this review is to highlight work that addresses mechanisms by which extracellular matrix influences vertebrate heart development.
Key words. Heart; extracellular matrix; development; embryonic.

There is currently considerable interest in the extracellular matrix (ECM) within the biomedical community, however, this was not always the case. For many years, the importance of the ECM was appreciated by a relatively small number of developmental biologists[41,45,46]. The spurt of interest in recent years can be traced, in part, to the 42nd Annual Symposium of the Society for Developmental Biology; held at the University of California, Irvine in 1983. This meeting brought together a mix of ECM biologists who worked on a number of model systems and organs. One of the topics, presented by Markwald and colleagues, was embryonic heart ECM. This symposium, and subsequent work by this group of investigators, directly stimulated a dramatic increase in studies on embryonic heart extracellular matrix. Sufficient new information has now accumulated to justify an overview of pertinent information derived from the literature and current investigations on the role of the ECM during heart development.

The embryonic heart ECM is comprised of glycosaminoglycans (hyaluronic acid, chondroitin sulfate), glycoproteins (fibronectin, laminin, vitronectin, cytotactin, fibulin, fibrillin, thrombospondin), collagens (I, III, IV) and proteoglycans[4,34,54,55,59,63,68,73,77,83,84,105,121]. Investigators in the field believe that these ECM molecules play multiple roles during heart development, including mediating cell shape changes, migration, proliferation, and differentiation during heart morphogenesis. ECM molecules are involved in the binding/sequestering of growth factors and other molecules which further regulate cell behavior, and are believed to facilitate cell-cell interactions. Although it has not been demonstrated in the heart, models in related studies have demonstrated that the ECM can directly influence the developmental fate of cells. Indeed, the ECM plays a fundamental role in both the control of gene expression and the induction and maintenance of tissue-specific function[57]. At later periods of development, the structural properties of the early ECM allow it to transmit forces during myocardial contraction. During vascularization of embryonic heart muscle, the ECM influences the adhesive, migratory, and proliferative responses of vascular precursor cells. A discussion of blood vessel formation in the heart proper, however, is beyond the scope of this review. The interested reader is directed to several pertinent citations[10,27,47,96,103].

Although the authors have attempted to present a comprehensive picture of the role of the ECM during heart development, this review is by no means exhaustive. In addition, although many molecules and time points are included, large gaps in knowledge remain. Due to the predominance of work in avian models, in many cases, time points are indicated in Hamburger Hamilton (H&H) stages[42].

Vertebrate cardiac morphogenesis and the ECM

Pretubular heart (H&H stages up through 8)

Commitment to a cardiogenic fate is established during gastrulation, as presumptive heart-forming cells pass through the primitive steak. Fate-mapping studies in quail/chicken transplantation chimeras using vital fluorescent dyes and marker antibodies indicate that the heart originates from the primitive streak in a rostrocaudal sequence[36]. The gastrulating cells then establish the bilateral pattern of cardiogenic fields first described by Rawles[101], and later elaborated upon by Rosenquist and DeHaan[108].

On each side of the midline, the heart splanchnic mesoderm forms two columnar epithelia, the cardiac primordia (fig. 1). The majority of these cells are destined to

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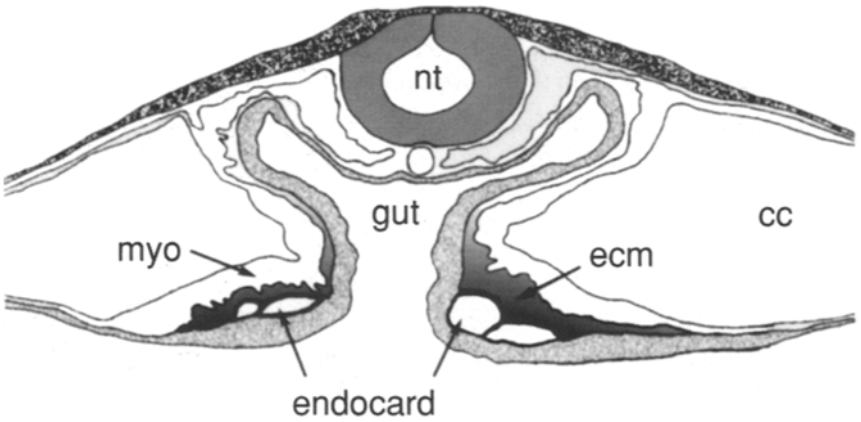

Figure 1. Diagram of a transverse section of a H&H stage 8 avian embryo just caudal to the anterior intestinal portal (gut), based on tracings of differential interference contrast micrographs. This drawing graphically demonstrates the bilateral nature of the heart prior to fusion at the midline. The bilateral heart-forming region is comprised of presumptive myocardium (myo), presumptive endocardium (endocard) and primitive heart extracellular matrix (ecm). The ECM is depicted as a gradient, since our interest is focused on the heart-forming regions. Note the endoderm and ectoderm are stippled on a light and dark background respectively.
cc = coelomic cavity; nt = neural tube; arrows denote lumens of endocardial tubes.

form the muscular, outer tube of the heart (myocardium). In addition, a small subset of these cells, termed 'angioblasts', are thought to form cords of endocardium[27]. The exact origins and movements of the 'heart angioblasts', however, are not precisely known[92,97]. Keep in mind that at these early stages, the heart is still a bilateral structure, comprised of a primitive myocardial sheet and endocardial cords on each side of the midline. Concomitant with formation of presumptive myocardium and endocardium, the anterior intestinal portal moves caudally and draws the bilateral cardiac primordia toward the midline where they will eventually fuse, forming a single tubular structure[28,117].

During this early period of development, a layer of ECM, probably of endodermal origin, lies between the basal surface of the endoderm and the basal surface of the heart splanchnic mesoderm/presumptive myocardium (fig. 1). This acellular material, which can operationally be referred to as the primitive-heart ECM[27] will presumably contribute to a thickened ECM (cardiac jelly) that exists later in heart development[59]. Several molecules have been localized to this primitive-heart ECM, including chondroitin sulfate[56], collagens I and IV, laminin and fibronectin[27], and more recently, fibrillin[33,128] and fibulin[114].

One of the earliest ECM molecules demonstrated in pretubular heart stages is fibronectin. This glycoprotein is present in the lateral heart-forming regions at H&H stage 5 in chicken embryos, and increases in abundance during the period of cell migration[65], in which it has been implicated to play a role[66,67]. Studies of mutant mice which lack the fibronectin gene have helped to elucidate the role of fibronectin in normal development. Homozygous mutant embryos implant and initiate gastrulation normally, however, at later stages of embryogenesis this defect is lethal. In these mutants, the heart and embryonic vessels are variable and deformed; abnormalities which can be interpreted as arising from fundamental deficits in mesodermal migration, adhesion, proliferation, or differentiation as a result of the absence of fibronectin[37]. Other studies suggest a role for fibronectin in early heart development. Specifically, the microinjection of peptides which contain the arginine-glycine-aspartic acid (RGD) cell attachment sequence of fibronectin into *Xenopus* embryos results in a randomization of the development of right/left asymmetry of the heart[130].

Recent studies have suggested that microfibrillar proteins such as fibrillin may play an important role in heart development. Fibrillin (350–390 kDa), which was originally identified by Sakai and colleagues[112], has been observed in the mesocardium of gastrulation stage avian embryos[33]. A fibrillin-like molecule, defined by the monoclonal antibody JB3, appears as early as stage 4 in the paired heart-forming regions in chicken embryos, and is seen in the mesocardium, distributed identically to fibrillin[128]. In addition, immunochemical analysis shows that the JB3 antibody recognizes a polypeptide that migrates near the molecular weight position of fibrillin[128]. Based on the molecular mass and similar immunostaining patterns in early embryos, it is believed that the JB3 antigen may be an avian fibrillin isotype.

Later, in stage 7–8 chicken embryos, the primitive-heart ECM that lies between the endoderm and heart splanchnic mesoderm contains basement membrane components (laminin, collagen IV)[27], interstitial constituents (collagen I, fibronectin)[27], and a microfibrillar (fibrillin-like) protein[128]. At this time, embryos also contain fibulin-1, a member of the recently described ECM glycoprotein family of fibulins[2,3]. This protein appears to colocalize with fibronectin in the basement

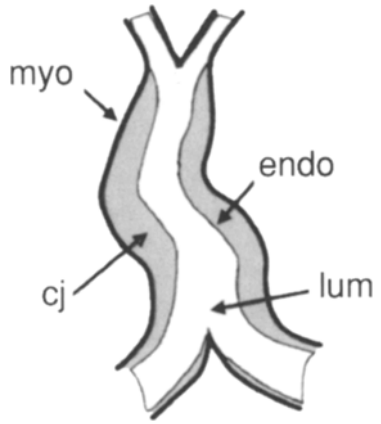

Figure 2. Diagram of the early tubular heart (in avians, ≈ H&H stage 9) during fusion of the two cardiac primordia. Note the cranial (top of figure) to caudal (bottom of figure) fusion of the two tubes. The myocardium (myo) and endocardium (endo) are separated by ECM/cardiac jelly (cj). (lum) denotes lumen of the heart tube.

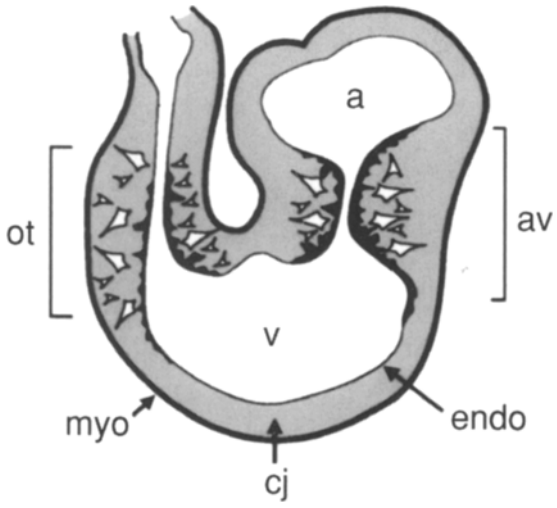

Figure 3. Diagram depicting endocardial cushion tissue formation (shown in dark grey) in the atrioventricular (av) and outflow tract (ot) regions at H&H stage 18 in avian embryos. The myocardium (myo) and endocardium (endo) are separated by ECM/cardiac jelly (cj). The transformed endocardial cells migrate into the cardiac jelly toward the myocardium.
a = atria, v = ventricle.

membrane of the heart splanchnic mesoderm[114]. At this point in development, the next major morphogenic change occurs, that is, formation of a tubular heart at the midline.

Tubular heart: H&H stages 9–13: (prior to cushion formation)

Fusion of the bilateral cardiac primordia occurs at the embryonic midline, in a progressive, cephalo-caudal direction. The resulting tubular structure is formed of two concentric epithelial layers, the myocardium and endocardium (fig. 2). At H&H stage 9, the straight heart tube is constituted by two well-defined anatomical regions: the primordium of the trabeculated portion of the right ventricle (cranial) and the trabeculated portion of the left ventricle (caudal), reviewed in de la Cruz et al.[24]. This tubular heart begins to beat at H&H stage 10. It is not until the loop stage (H&H stage 12) that the primordium of the infundibulum of the right ventricle and the primitive atria are present[24]. At this time, the tubular heart undergoes a series of elongation and looping events in which the heart is transformed from a linear to a multi-chambered organ. The ECM has been demonstrated to play a role in this process[76]. The primordium of the arterial pole of the heart then appears in the early post-loop stage[24]. The myocardium and endocardium are now separated by a thick cell-free expanse of ECM termed the cardiac jelly by an early investigator[22], and more recently described as a myocardial basement membrane[59,81] (fig. 2). While no data exist, it is reasonable to assume that the primitive heart ECM (described above) persists as part of the myocardial basement membrane/cardiac jelly in the tubular heart, since only 10 h separate these stages[42]. Also, many of the molecules which are found in the primitive-heart ECM persist at later stages. For example, at the tubular heart stage, fibulin-1[114] and hyaluronic acid[8,38,77,84] are present in the myocardial basement membrane/cardiac jelly. Fibrillin has also been detected at these stages in the chicken[33]. Moreover, others have seen the fibrillin-like JB3 antigen distributed in all stages associated with the formation of the avian tubular heart in cardiogenic regions of the splanchnic mesoderm[128]. In addition to these molecules, expression of thrombospondin is observed in developing myocytes in the mouse (8.5 days of gestation), but not in the adjacent endocardium[19]. Thrombospondin is also present in late tailbud stage heart tissue in *Xenopus* (per. commun., C. Whittaker, Univeristy of Virginia).

Tubular heart: H&H stages 14–36 (endocardial cushion/valvuloseptal primordia formation)

Partitioning of the heart into a multi-chambered structure is accomplished by a complex sequence of events leading to the formation of definitive valves. Initially (H&H stages 14–17), there is a regionally specific differentiation of the endocardium in the regions of the atrioventricular canal and proximal outflow tract (fig. 3). In the endocardium, a subset of endothelial cells are 'transformed'. This process is characterized by cell hypertrophy, loss of cell-cell adhesion molecules, and increased expression of substrate adhesion molecules[20,32,79,80,89,110]. Thus, these endothelial cells acquire a mesenchymal morphology and invade the myocardial basement membrane/cardiac jelly separating the endocardium and myocardium (stage 17 in avian embryos/ ≈ day 9 in mouse)[80] (see fig. 3). The myocardial basement membrane/cardiac jelly at this time is thought to be a composite of two unequally sized basement

membranes; the thicker derived from the myocardium, while the thinner is endothelial-associated[59]. Prior to the invasion of mesenchymal cells, there is an expansion of the ECM to form local acellular tissue swellings[79]. These swellings may result from an interaction between collagen and hyaluronate, which produces fully hydrated gels[31]. Thus, mesenchymal cells 'seed' these tissue swellings to form the definitive cushion tissue[80].

Some investigators believe the cushions are transitory structures, important in patterning where the valves and septa form, but which do not directly contribute to valvuloseptal structures[125]. However, other data suggest that these endocardial cushions fuse to contribute to the connective tissue of septa and primordial atrioventricular valves in the maturing heart[16,72,75,79,82,83]. One recent study has indicated that the mitral valve leaflets contain solely endocardial cushion tissue, whereas the tricuspid band includes both myocardial cells and endocardial cushion tissue[16]. The myocardium of the ventricle forms trabeculae, the precursors of the interventricular and papillary muscles[44], which arise through localized clonal expansion[86].

The formation of endocardial cushion tissue and the concomitant epithelial-mesenchymal transition has been modeled in vitro using hydrated collagen gels as the scaffold for atrioventricular explants[9,83]. These experiments have demonstrated that a regionally specific interaction of the myocardium with the endothelium is required to initiate the formation of prevalvular mesenchyme[59,63,89]. An EDTA-soluble extract of myocardial basement membrane particles, or alternatively, conditioned medium from cultured embryonic cardiocytes, can also induce mesenchyme formation from endocardium[62,63]. According to Markwald and colleagues, these data suggest that an ECM signaling substance is produced by the embryonic myocardium which stimulates cardiac atrioventricular endothelial cytodifferentiation into valvular mesenchyme[62]. Antisera against the EDTA soluble (ES) antigens, block epithelial-mesenchymal transformation in culture, indicating their possible involvement in initiating the formation of the atrioventricular mesenchyme[88].

In recent years, investigators have tried to determine if specific ECM molecules are involved in mediating the events which lead to cushion and valve formation. Known components of the myocardial basement membrane/cardiac jelly at the time of transformation, invasion, and cushion formation include: fibronectin, collagens (I, III, IV), laminin, tenascin/cytotactin, vitronectin, thrombospondin, fibulins-1 and -2, a fibrillin-like molecule (JB3 antigen), chondroitin sulfate, and hyaluronic acid[8,20,35,49,53,59,68,70,74,78,89,93,114,118,119,128,132,133] and also data by D. Bouchey, University of Virginia (manuscript in preparation).

Although fibronectin is ubiquitously expressed during development, a unique, particulate form of fibronectin is seen within the myocardial basement membrane/cardiac jelly in the regions of transformation[58,59,89,90], but is absent in the ventricular region[59]. Furthermore, the signaling molecules (ES1 antigens) mention previously associate with fibronectin in vivo[88,89]. Thus, epithelial-mesenchymal transition may be mediated by a multicomponent complex involving fibronectin and other proteins[88,113], which appear as regionally distinct particulates only in areas of endothelial differentiation[89]. The temporal and spatial distribution of other ECM molecules indicates a possible involvement in various stages of valve formation. These include the traditional basement membrane components, collagen IV and laminin. Collagen IV staining is present on the endothelial and myocardial pericellular surfaces and within the myocardial basement membrane/cardiac jelly[59,68]. Laminin is detected in the distal portion of avian outflow tracts at stage 14, where a narrow connection between the myocardium and endoderm persists as the dorsal mesocardium[59], and is more widely distributed in the myocardial basement membrane/cardiac jelly at stage 15 in avian embryos[68]. In the mouse, however, others have shown that very little laminin is found in the endocardial cushions[133]. Thrombospondin is strongly present in atrial and ventricular myocytes, and bulbous arteriosus, however, one group reports that cushion cells do not show expression of this protein at day 10.5 in the mouse[19]. Furthermore, thrombospondin expression is not seen by this group in structures derived from the endocardial cushions[19]. In contrast, recent work by another group has indicated that a family of structurally related thrombospondin genes exist, and that thrombospondin 1 expression predominates in cardiac cushion tissue at days 10–13 in the mouse, but diminishes markedly after these stages[54]. Thrombospondin 2 expression is seen at low levels in cardiac cushions, and is observed in cardiomyocytes[54].

At the time of migration/invasion (H&H stage 17), laminin is present in the myocardial basement membrane/cardiac jelly surfaces of both the endothelium and myocardium, and in vitro studies show that motile endocardial-derived mesenchymal cells can migrate into hydrated, 3-dimensional laminin gels[23,69]. Another traditional basement membrane component present is type IV collagen[68]. Functional studies have demonstrated that hyaluronic acid is not required for looping of the tubular heart; however, its absence is associated with substantial hemodynamic alterations in the heart following looping[8].

More than any other known molecule, it is perhaps the fibulins that most distinctly localize to endocardial cushion tissue, although these glycoproteins are found in numerous other tissues[94,95,104,114,132,133]. Presently, two members of the fibulin family are known. Fibulin-1, an approximately 100 kDa calcium binding glycoprotein, exists as four alternatively spliced variants in

humans[2,3], and has been detected in avians using human fibulin antibodies[114]. Other investigators independently identified mouse fibulin-1, originally referred to as BM-90[60,94,95], and most recently mouse fibulin-2[95,133], which displays a higher apparent molecular mass, $M_r = 195{,}000$ (see ref. 95). Both fibulins share characteristic EGF-like domain repeats, and 43% sequence identity (see references above).

Avian and mouse fibulin-1, as well as recently-described fibulin-2, are markedly enriched in the areas of the developing heart that undergo the epithelial-mesenchymal conversion[114,132,133]. Fibulin-1 antibodies label the entire thickness of the cardiac jelly in stage 15 embryos, however by stages 17–19 ($\approx$ 9–9.5 day mouse embryo), fibulin-1 is highly enriched at the surfaces of motile endocardial mesenchyme cells. Indeed, laser scanning confocal microscopy shows that both leading and trailing cell protrusions are intimately associated with fibulin-1[114]. Subsequently, prominent fibulin-1 and -2 expression are observed in endocardial cushion tissue in both avian and mouse embryos[114,132,133], where fibulin-2 colocalizes with fibronectin[132].

From day 12 forward in the mouse, protein and mRNA expression of fibulin-1 decreases in the myocardial ECM and becomes progressively confined to areas where growth and fusion of endocardial cushion tissues occur. Specifically, fibulin-1 is associated with the spiral aorticopulmonary septum, atrioventricular valves and semilunar valves of the pulmonary and aortic arteries[132,133]. Fibulin-1 also appears concentrated at the boundary between the mesenchyme of the inferior endocardial cushion and the myocardium of the muscular portion of the interventricular septum during the onset of septal fusion (unpublished data, D. Bouchey, University of Virginia). Fibulin-1 and -2 are expressed well into adult stages in the mouse heart valves[132], and a coexpression of fibulins -1 and -2 are found in the adult mouse heart[95]. Fibulin-1 has also been detected in the adult human heart muscle and valve tissue[104].

Tenascin/cytotactin is another glycoprotein which has been implicated in development[17]. (Please refer to Chiquet-Ehrismann's tenascin review in this volume, pp. 853–862.) In avians, tenascin-c is present in the myocardial basement membrane/cardiac jelly into which the endocardial cushion tissue cells invade in patterns that correlate with cell migration[20]. Interestingly, after cushion mesenchymal cell migration ceases, the expression of tenascin decreases, remaining high only in the peripheral portion of the aorticopulmonary septum[20]. Others have shown that in the cushions, tenascin-c shows considerable co-distribution with fibulin-1, but tenoscin-c expression is more focal and does not mark all areas of the cushion tissue[133]. In addition, tenascin-c is known to bind to fibronectin and this interaction may influence cell binding to this protein[48]. Thus, the presence of tenascin-c and fibronectin at the front of cushion cell migration may modulate the substrate's adhesive properties and allow not only for the attachment necessary for cell movement, but also for the detachment required to continue forward migration.

Vitronectin, a multifunctional protein with domains for cell binding[99], may also be involved in the regulation of cushion mesenchymal cell migration in vivo[119]. Vitronectin is present in myocardial cells in some mesenchymal cells (equivalent to H&H stage 19–20) of the truncus arteriosus and atrioventricular canal[119], and is also present in the endocardial cushions of embryonic chicken hearts at stages 23–29[118]. Furthermore, in vitro studies in the rat indicate that vitronectin is synthesized by myocardial cells[118] and some cushion mesenchymal cells, and that vitronectin inhibits cell movement on fibronectin[119]. Later (H&H stage 36), vitronectin is apparent in the connective tissue of quail epicardium[26].

Human fibrillin is now believed to be a member of a family of related gene products[131]. Furthermore, mouse fibrillin-1 has been recently cloned[129]. This data show expression of fibrillin-1 mRNA in mouse endocardial tissue at the time of cushion formation[129]. In addition, the fibrillin-like JB3 antigen shows a widespread distribution in nascent valves up until stage 28 in avian embryos[128]. After stage 28, the epicardium becomes intensely labeled with antibodies to the JB3 antigen, and staining persists in the valve leaflets.

Postseptated heart (H&H stage 37 to birth)

The ECM proteins which influence the heart during later stages of fetal development are qualitatively, but not quantitatively, similar to those in the adult ECM. The collagens are primarily the interstitial types I and III, however type IV collagen is present in the basement membranes surrounding individual myocytes[11]. Several glycoproteins (fibronectin, laminin, entactin)[71], proteoglycans, and glycosaminoglycans (dermatan sulfate, chondroitin sulfate, hyaluronic acid)[62] have also been demonstrated to be present.

Immunoelectron microscopic studies in rat cardiac myocytes indicate that laminin is localized to punctate patches on the surface plasma membranes with large gaps between areas of staining at 11.5 days[100]. The basement membrane is not evident as a distinct, contiguous layer at this time. Heavier concentrations of staining are associated with cell projections and in areas of close cell apposition. By 15 days, there is an increase in the distribution of laminin associated with the sarcolemma. Gaps between areas of localization are smaller[100]. Thrombospondin staining remains strong in the myocardium, but is negative in the valve leaflets[19].

From $\approx$ stages 33–38 in chicken embryos, the subepicardial region is a site of rapid accumulation of ECM proteins[122]. These subepicardial components may play a role in determining cardiac mechanics during development. Fibronectin appears first in the subepicardium,

followed by collagen type III, and then collagen type I[122]. It is interesting to note that collagen type III is not seen in the embryonic chicken heart prior to ≈ stage 33[121], thus the subepicardium is the first site of its deposition in the heart.

Associations of ECM proteins with other molecules and receptors during cardiac development

Most ECM components are multifunctional molecules that can interact with other matrix constituents, growth factors or cell surface receptors to affect tissue properties and structure. Associations exist between collagens and other molecules, including proteoglycans, glycoproteins and glycosaminoglycans, which may have functional significance during development. For example, it is suggested that associations between collagens and hyaluronan play an important role in the initiation and maintenance of angiogenesis[106]. Mouse and human fibulin-1 bind to other fibulin molecules, fibronectin, laminin and entactin[6,94], while mouse fibulin-2 demonstrates binding to fibronectin and entactin, but not laminin and fibulin-1[133]. Laminin also interacts with entactin[85]. The significance of these differences in binding specificity are as of yet unknown.

ECM molecules can also bind and sequester growth factors, releasing them upon proteolytic degradation and thus mediating their availability and bioactivity. In return, growth factors may stimulate the synthesis of individual matrix components, or block matrix degradation by decreasing the synthesis of proteases and/or increasing the synthesis of protease inhibitors. In addition, growth factors can increase the synthesis of matrix receptors and alter their relative proportion on the surface of cells in a manner that could facilitate adhesion to matrix[91].

Surface receptors for ECM molecules allow cells to receive positional information from the environment during migration, as well as promote the recognition of and attachment to extracellular matrices and other cells. Furthermore, these receptors allow the cell to respond to signals that initiate proliferative and differentiation events. The integrins, a large family of α/β heterodimeric cell surface adhesion molecules[52,111], represent transmembrane receptors that have been proposed to integrate information from the ECM to the internal cytoplasm of individual cells[51,120]. During cardiac development, a regulated pattern of integrin expression is seen[7,11,14,19,69]. Periods of transient expression of specific receptors are observed, which may coincide with cell movement, differentiation and maturation of cells[40]. Integrin-ligand interactions also trigger specific organizational and physiological events within a cell, such as the production of metalloproteinases required for cell invasion and matrix reorganization[126]. In addition to the integrins, other cell adhesion molecules, such as members of the cadherin family and immunoglobulin superfamily (e.g. neural cell adhesion molecule[12,20,90], platelet endothelial cell adhesion molecule[7]) are present on embryonic heart cells. Other, non-integrin, receptors known to interact with ECM molecules during development include the hyaluronan receptor, CD44[87], which is expressed at high levels in the heart at critical stages of morphogenesis[127]. The interaction of hyaluronan with matrix hyaluronan-binding proteins and cell-surface hyaluronan receptors regulates many aspects of cell behavior such as cell migration[124], cell-cell adhesion[5], and cell differentiation[61]. During vertebrate development, multiple cases of hyaluronan involvement in cell proliferation[1] and histogenesis[123] have also been documented.

Future directions of research

Despite considerable work, much remains to be learned with respect to the role of the ECM during embryonic heart development. Recent studies have demonstrated the efficacy of using cold-blooded vertebrate embryos, such as *Xenopus* and zebra fish, to study heart development. Indeed, this may prove to be a productive and innovative avenue of future research. Amphibians and zebra fish produce abundant highly synchronous pregastrulation embryos, which thrive in aqueous solution and are easy to manipulate[43]. The zebra fish offer several additional advantages, including their amenability to genetic manipulation, a transparent embryo, and a prominent peripheral multichambered heart which develops precociously[98]. Thus, formation and proper functioning of the heart can be readily assayed by visual inspection[116]. Furthermore, the zebra fish is easily manipulated to induce heart defects in which embryos lack major regions of the heart primordia. For example, truncation of the arterial and venous regions of the heart can be induced by treatment of the embryos with retinoic acid[115] or lithium chloride (unpubl. data, R. A. McCarthy, College of Charleston) respectively. For these reasons, studies on the role of ECM proteins in the formation of the normal/mutant embryonic zebra fish heart should prove very fruitful, but will require antibody and hybridization probes specific for zebra fish ECM.

In avian and murine embryos, a large number of ECM molecules have been localized to the developing heart, however, in no case has a specific function been conclusively demonstrated. Recent evidence suggests that microfibrillar proteins may play a prominent role in valve formation. Microfibrils are 10–20 nm, extracellular tissue filaments. Some fibrils contain elastin, while others do not[18,107]. Fibulin-1 is present in the core of elastin-containing fibrils[104], while fibrillin is a constituent of the outer coat of the microfibrils[112]. It is important to note

that these microfibrillar glycoproteins are seen prior to tropoelastin in the early heart. Thus, fibulins and fibrillins may serve as an organizing scaffold in the formation of a tropoelastin-free microfibrillar network[18]. The earliest that tropoelastin expression is seen in the avian embryo is at H&H stage 21–22 in the wall of outflow tract vessels. However, tropoelastin is not detected in the heart at these stages[50,109].

Mutations in the fibrillin gene, located on human chromosome 15, have been strongly implicated as the cause of the Marfan Syndrome[25,64], which includes cardiovascular valvular defects. Molecular genetic studies of the Marfan Syndrome also point to the importance of elastic fibers in the development and function of the aorta[25,112]. In addition, supravalvular aortic stenosis (an inherited congenital heart defect that causes hemodynamically significant narrowing of the origin of the aorta)[29], maps to the same chromosomal subunit as elastin, which is a candidate for the defective gene[21,30]. In some instances, stenosis of the ascending aorta, similar to supraventricular aortic stenosis, is seen transiently in the Marfan Syndrome[13]. Thus, the relationships between fibrillin, fibulin, and elastin need to be further elucidated.

Another interesting microfibrillar protein, which may play a role in heart development, is the 31 kDa microfibrillar associated glycoprotein, MAGP[39], or the mouse homologue, Magp. *Magp* transcript is synthesized by the mesenchyme as early as day 8.5–9.0 in mouse embryos, and at day 13.5, *Magp* expression is observed in the atrioventricular bulbar cushion tissue[15].

Other recent work involves extrinsic signal-like ES antigens, such as ES/130. ES/130, a 130-kDa protein, is present both in EDTA extracts of embryonic hearts and the conditioned media of cardiocyte cultures which elicit mesenchyme formation[102]. Furthermore, antibodies to ES/130 inhibit the epithelial-mesenchymal transformation of cardiac endothelium in culture. It is important to note that these proteins are secreted only at regions of endothelial-mesenchymal transformation; furthermore, only a subset of endothelial cells respond at these sites. Thus, current work is focusing on the determination of cell fate based on the expression of ECM antigens such as JB3. For instance, work on the JB3 (fibrillin-like) antigen suggests that this molecule may distinguish between the cellular progenitors of atrioventricular/outflow tract and ventricular endothelium[128].

Conclusions

Cardiac defects occur at the rate of nearly 1% of live births, the greatest of any clinically relevant birth malformation, and many of these defects involve valvuloseptal abnormalities. Unfortunately, there are future implications with consequences even more grave. These derive from current data indicating that individuals with repaired lesions have a 100–250 fold higher increase in the frequency of congenital cardiovascular malformations than the general population. This will ultimately translate into a further increase in the incidence of valvuloseptal malformations within the general population. Thus, for reasons of human health and for understanding the molecular mechanisms of cardiac morphogenesis, it is imperative that developmental biologists continue the impressive progress made in recent years. We predict that another review of the role of ECM in heart development will be requred within a very few years. Much remains to be learned.

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