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Phases and mechanisms of embryonic cardiomyocyte proliferation and ventricular wall morphogenesis

Yaacov Barak[1], **Myriam Hemberger**[2], **Henry M. Sucov**[3,4]

[1]Magee-Womens Research Institute, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA

[2]Depts. of Biochemistry & Molecular Biology and Medical Genetics, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

[3]Dept. of Regenerative Medicine and Cell Biology, and Div. of Cardiology, Dept. of Medicine, Medical University of South Carolina, Charleston, SC, USA

Abstract

If viewed as a movie, heart morphogenesis appears to unfold in a continuous and seamless manner. At the mechanistic level, however, a series of discreet and separable processes sequentially underlie heart development. This is evident in examining the expansion of the ventricular wall, which accounts for most of the contractile force of each heartbeat. Ventricular wall expansion is driven by cardiomyocyte proliferation coupled with a morphogenetic program that causes wall thickening rather than lengthening. Although most studies of these processes have focused on heart-intrinsic processes, it is increasingly clear that extracardiac events influence or even direct heart morphogenesis. In this review, we specifically consider mechanisms responsible for coordinating cardiomyocyte proliferation and ventricular wall expansion in mammalian development, relying primarily on studies from mouse development where a wealth of molecular and genetic data have been accumulated.

1. The timeline of ventricular wall expansion in mouse

In mice, with a total gestation time of 19 days, the heart first forms by the convergence of a prespecified domain of lateral mesoderm and begins to beat at embryonic day 7.0–7.5 (E7.0–7.5). This domain is now recognized as the first heart field (Kelly and Buckingham, 2002), to distinguish it from a subsequent progenitor territory that is described below. At this initial time, the heart consists of a single atrial and a single ventricular chamber, with a

[4]Correspondance author: sucov@musc.edu.

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constriction (the atrioventricular canal) that delimits the two. Throughout its length, the heart has an outer single cell layer of myocardium and an inner single cell layer of endocardium (the endothelium of the heart). During the E7.5–9.5 interval, the ventricle grows in length primarily by the addition of new cardiomyocytes at the ventricular outflow (anterior) pole from a splanchnic mesodermal progenitor territory called the second heart field (Kelly and Buckingham, 2002). Cardiomyocytes derived from the first heart field are destined to be in the left ventricle, whereas those from the second heart field are destined to be in the right ventricle, although there is no morphological distinction between the two sides at this time. Cardiomyocyte proliferation is robust during this period, in large part through the influence of the endocardium, which induces the formation of bundles of myocardium (called trabecular myocardium) that project into the ventricular lumen (Del Monte-Nieto et al., 2018). The non-trabecular portion of the ventricle is now morphologically identifiable as the ventricular wall, and through this period remains thin (1–2 cells across).

During the next few days, cardiomyocyte proliferation leads to progressive thickening of the ventricular wall, which reaches 10–15 cell layers by E14. In addition to clear contribution of cardiomyocyte proliferation to ventricular wall expansion at E10–14, this phase of heart development is likely to additionally involve a morphogenetic component that orients cell divisions in a direction perpendicular to the longitudinal axis of the heart, ensuring its thickening rather than lengthening. Some cellular and molecular components that underlie oriented cardiomyocyte division have been elucidated at earlier stages that account for formation of trabecular bundles (Wu, 2018); how such processes transition to ventricular wall thickening, or if new events account for ventricular wall growth, remains to be fully understood. More is known about the components that drive ventricular wall cardiomyocyte proliferation, as described below.

The pace of cardiomyocyte proliferation after E14 tapers down to a low level. This continues in late gestation and into the first few days after birth, then terminates (in rat, at postnatal day 3 (Li et al., 1996)) and remains negligible thereafter through postnatal life (Foglia and Poss, 2016). In mice, around the time of birth, a process known as compaction incorporates the majority of trabecular bundles from the ventricular lumen into the inner ventricular wall (Tian et al., 2014). Because there is essentially no further cardiomyocyte proliferation, the ventricular wall reaches its final cardiomyocyte cell count at this juncture. The ventricular wall and the heart overall further expand between birth and adulthood, but this occurs by the hypertrophic enlargement of preexisting cardiomyocytes and not by an increase in cardiomyocyte number.

In summary, ventricular wall morphogenesis in the mouse embryo has three anatomical phases, each influenced by a different cellular mechanistic process: (1) Formation and lengthening while remaining thin-walled during the E7–9.5 period, which primarily involves recruitment of new cardiomyocytes from the second heart field; (2) Further elongation with thickening during the E10–14 period, which is primarily driven by ventricular wall cardiomyocyte proliferation presumably coupled with oriented cell division; and (3) Compaction around the time of birth, which involves aggregation of trabecular myocardium into the inner ventricular wall. The focus of this review is on the middle period of ventricular wall growth between E10–14.

2. Role of the epicardium in ventricular wall morphogenesis

From the time of its initial formation and through E9.5, the myocardium is on the outer side of the heart, facing the coelomic fluid of the pericardial cavity. The endocardium, as noted above, is on the inside of the heart. A third component – the epicardium – forms during the E9.5–10.5 period. It originates from a location adjacent to albeit external to the heart itself, and migrates over the myocardial surface, forming a single outer cell layer of non-contractile mesothelium that faces the pericardial cavity, with the myocardium now underneath (Manner, 1993; Sengbusch et al., 2002).

Mechanical perturbation studies in avian embryos (Gittenberger-de Groot et al., 2000; Manner, 1993) that either delayed or prevented the formation of the epicardium resulted in diminished proliferation of ventricular wall cardiomyocytes and a persistently thin-walled ventricle, without hampering normal trabecular development (which, as noted above, is dependent on the endocardium). Thus, formation of the epicardium around E10 is both coincident with and required for the onset of ventricular wall expansion. A potential role for the epicardium in directing oriented cardiomyocyte division (see also earlier comments on this subject related to trabecular formation) is reasonable but not demonstrated. Much more is known of epicardial control of ventricular wall cardiomyocyte proliferation.

A number of mouse genetic studies reported similar phenotypes of a persistently thin-walled ventricle around E14 (Rossant, 1996). In many cases, the ventricle is so severely impacted that heart functional output is too impaired to sustain further embryonic survival. Experimentally, E14 is a convenient time point for histological evaluation of ventricular wall morphology in the mouse embryo, as the wall is too thin at earlier time points to convincingly demonstrate a difference between mutants and normal controls. Moreover, even in cases that are lethal at E14, mutant embryos generally display normal size and normal overall developmental progression, such that a proper comparison of heart morphology to control embryos can be made. This hypoplastic phenotype has been clearly demonstrated to be the consequence of insufficient cardiomyocyte proliferation between E10 and E14, based on markers of cell cycle activity and because cardiomyocyte apoptosis does not appear to be a significant process in the developing heart even in mutant backgrounds. Although it may be somewhat of an oversimplification, genes that are involved in endocardium-myocardium trabecular growth generally have earlier phenotypes and do not have E14 ventricular wall phenotypes. Conceptually, then, genes whose mutations yield a hypoplastic ventricular wall phenotype can be grouped into three major categories: those that function autonomously in cardiomyocytes to control proliferation, those that function in the epicardium to directly or indirectly influence cardiomyocyte proliferation, and those that function extrinsic to the heart and control its development indirectly.

A number of cell cycle regulators, when mutated in mouse, result in ventricular wall hypoplasia by E14; notable examples include combined deficiency of the three cyclin D genes Ccnd1–3 (Kozar et al., 2004), dual deficiency of the two cyclin-dependent kinase genes Cdk2 and Cdk4 (Berthet et al., 2006), K-ras mutation (Koera et al., 1997), and N-myc knockout (Moens et al., 1993). These observations implicate canonical cell cycle regulators in normal embryonic cardiomyocyte proliferation. For most such genes, a conclusive genetic

analysis that would confirm a cardiomyocyte-autonomous function of the genes has not been performed, although in some cases (e.g., N-myc), the expression profile of the genes makes this the most plausible conclusion. Why these canonical cell cycle regulators do not appear to be required for earlier cardiomyocyte proliferation (during the E7–10 period) remains to be understood.

The leading model for epicardial influence on cardiomyocyte proliferation invokes secreted growth factors that cross the subepicardial space and directly activate proliferative signaling in the outer layer of cardiomyocytes (Chen et al., 2002). While it is clear that the epicardium secretes at least several such factors, insulin-like growth factor 2 (IGF2) may be the most influential during the E10–14 window. Thus, genetic absence of IGF2, or of its main receptor IGF1R, both result in a thin-walled ventricle at E14 (Li et al., 2011; Wang et al., 2019). The epicardial source of IGF2 in this context was confirmed by tissue-specific mutational analysis in each of the three primary lineages of the embryonic heart (epicardium, endocardium, and myocardium), with only the epicardial knockout resulting in a hypoplastic ventricular phenotype (Shen et al., 2015). IGF2 was estimated to account for 70–80% of the mitogenic activity secreted from epicardial cells in culture (Li et al., 2011), although this measurement does not necessarily extrapolate to the in vivo context. Adrenomedullin is another secreted factor that might also have a similar paracrine effect on the myocardium, based on its mutant phenotype (Caron and Smithies, 2001).

Detailed analyses (Brade et al., 2011; Shen et al., 2015) revealed that epicardial Igf2 expression is initiated by erythropoietin (Epo), signaling through its receptor EpoR, which is expressed in the epicardium (Wu et al., 1999). However, this mechanism is operant only at E10.0–11.5. Epo is not expressed in the heart or in most other embryonic tissues, but is expressed in the fetal liver (Wu et al., 1999), which is likely the source of the Epo protein that influences epicardial Igf2 expression. At this time of development, the pericardial and peritoneal cavities are connected via natural gaps (the pericardioperitoneal canals) in the still-incomplete diaphragm. Indeed, India ink microinjected into the peritoneal cavity of E10.5 embryos rapidly spread into the pericardial cavity to surround the heart (Sucov and colleagues, unpublished observations). Thus, by extension, it is conceivable that Epo from the liver passes via coelomic fluid into the pericardial cavity, where it binds to Epo receptors on the epicardial cell surface to induce Igf2 expression. This implied effect at a distance supports the notion that hepatic Epo acts on the heart as a bona fide endocrine signal, a mode of action not commonly observed in developmental processes.

Epo expression in the liver is activated by hypoxia, via an enhancer element that includes a binding site for the hypoxia-regulated transcription factor HIF1 (Makita et al., 2001). Prior to E12, the fetal liver is hypoxic, HIF1 activity is high, and Epo expression is robust. Hepatic Epo expression drops dramatically (>10-fold) around E12 (Makita et al., 2005). Because hepatic HIF1 drops at the same time, the decline in Epo expression is likely to be related to increased placental functionality and increased perfusion of the liver with oxygen-rich blood from the placenta. Around the same time, the pericardioperitoneal canals, which are the conduits for coelomic fluid carrying hepatic Epo to the heart, close with the completion of the diaphragm. Therefore, Epo ceases to directly influence epicardial Igf2 expression beyond E11.5. Indeed, EpoR mutation in all heart mesoderm or selectively in the epicardium

causes a transient absence of epicardial Igf2 expression only from E10 to 11.5, which recovers thereafter to normal levels (Shen et al., 2015) through an alternative, Epo-independent regulatory mechanism (see below). This, in turn, restores cardiomyocyte proliferation and allows attainment of a normal ventricular wall morphology by E14, and survival to full term.

Expression of Igf2 in the epicardium is constant in wild-type embryos throughout the E10-E14 period (and beyond), yet Epo only regulates this expression from E10-E11.5. The substitute activity that maintains epicardial Igf2 expression after E11.5 appears to be the influx of glucose and oxygen associated with placental transport function. In both cell and organ culture studies, oxygen and glucose alone are sufficient to induce epicardial Igf2 expression (Shen et al., 2015). It is intriguing that the very same circumstances (placental transport) that cause loss of Epo expression in the fetal liver also directly induce Igf2 expression in the epicardium, thereby maintaining this growth-promoting signal during this critical period of heart development.

In contrast to conditional EpoR mutation, global mutation of Epo or EpoR results in embryo death at E13.5. This is most likely due to the critical role of Epo signaling in fetal erythropoiesis (Wu et al., 1999). Interestingly, however, global mutants of both Epo and its receptor exhibit a hypoplastic ventricular wall at death (Wu et al., 1999). This is unlikely to be for lack of epicardial Igf2 expression, since heart-specific EpoR mutants are restored in epicardial Igf2 expression, cardiomyocyte proliferation, and heart morphology after E12 (Shen et al., 2015). Rather, a more likely explanation is that the thin myocardial phenotype of Epo and EpoR global mutants is an indirect consequence of red blood cell deficiency. Heart contraction is particularly energyintensive and oxygen-demanding, in the embryo as well as postnatally, and the developing heart might be particularly sensitive to the progressively worsening fetal anemia of global Epo and EpoR mutants. This illustrates how growth of the ventricular wall is dependent on proper external environmental conditions (i.e., oxygen level) in addition to intrinsic signaling pathways such as IGF2.

In addition to serving as a source of secreted factors, epicardial cells are also a multipotent progenitor cell type, giving rise to several mesenchymal lineages that populate the heart. These lineages arise from the epicardium by epithelial-mesenchymal transformation, migrate into the subepicardial space starting at E11, and into the myocardium thereafter. They include fibroblasts that supply the extracellular matrix surrounding myofibers, adipocytes, and pericytes and smooth muscle cells that surround nascent coronary vessels (Wessels and Perez-Pomares, 2004; Yamaguchi et al., 2015). Endothelial cells of the coronary vasculature originate from non-epicardial lineages (Cavallero et al., 2015). Regarding the model of the epicardium as a source of secreted growth-promoting factors for the myocardium at E10–14, such signals are generally thought to originate from the epithelial epicardium and not from its mesenchymal derivatives, although lineage-specific analyses of gene function would not be able to discriminate between these alternatives. One example of such a function of the fibroblast lineage is the secretion of a matrix component that supports cardiomyocyte proliferation during late gestation (i.e., after E14) (Ieda et al., 2009).However, evidence that epicardium-derived mesenchymal cells have a direct role in supporting cardiomyocyte proliferation prior to E14 is scant.

3. Cardiomyocyte proliferation and ventricular development after E14

By E14 in normal mouse heart development, under the influence of epicardial mitogens and supported by the placenta, the ventricle wall has thickened to 10–15 cell layers. At least for the major mitogen IGF2, it is likely that the source of this signal is the epithelial epicardium, rather than mesenchyme derived from the epicardium, in that Igf2 expression in the myocardium is not detectable during this period (Shen et al., 2015). This poses a logistical challenge: unlike at E10, when the small (8kD) IGF2 can easily diffuse across the 1–2 cells of the ventricle wall, diffusion through 15 cell layers at E14 would be unlikely, and therefore only the outer layers of myocardium would receive this signal. Indeed, there is evidence that cardiomyocyte proliferation is higher on the outside of the ventricle (Rumyantsev, 1977). Still, the relative impact of an external signal gradually diminishes as the thickness of the ventricle wall increases.

The resolution of this challenge is the formation of the coronary vasculature. Starting just after the formation of the epicardium, endothelial cells, mostly from the sinus venosus, migrate underneath the epicardium to the ventricle, and organize in the subepicardial space into nascent vascular elements (Red-Horse et al., 2010; Viragh and Challice, 1981). As the heart thickens over the next few days, these vessels either migrate or are passively incorporated into the ventricle wall, where they further mature. Coronary perfusion begins at E14. Gene mutations, for example in Cxcl12 or its receptor Cxcr4 (Cavallero et al., 2015), that specifically compromise coronary vasculogenesis have no morphological impact through E14, but then cause embryo lethality within a day or two later. In mutants deficient in IGF2 signaling, the E10.5-E13.5 impairment of cardiomyocyte proliferation is restored to normal at E14.5, and despite their earlier hypoplastic ventricular wall, these mutants recover to have a fully normal ventricular morphology by the time of birth (Li et al., 2011). Presumably, the onset of coronary perfusion distributes other growth-supporting factors to the myocardium, and at this point, the requirement of IGF2 for supporting cardiomyocyte proliferation is ended. At E14, therefore, the epicardium has completed several major tasks that are relevant to ventricular morphogenesis: it provided trophic factors during the E10–14 period to support cardiomyocyte proliferation, it provided a space for and instructively influenced the formation of the coronary vessels, and it provided mesenchymal cells that generate extracellular matrix as well as coronary vessel pericytes and smooth muscle. With the establishment of coronary perfusion, the primary developmental jobs of the epicardium are completed.

4. Role of the placenta in ventricular wall morphogenesis

The placenta and heart are the first organs to become functional during development and are linked by the feto-placental blood circulation. This temporal and functional association, as well as landmark genetic studies described below, have fueled a slowly-evolving appreciation of the potential importance of the placenta in heart development. This recognition of a putative placenta-heart connection prompted recent re-analyses of neonatal records for associations between human congenital heart and placental defects. Of these, three separate reports are noteworthy. The first found markedly increased prevalence of placental structural and vascular defects in neonates with hypoplastic left heart syndrome

(Jones et al., 2015). The second study reported strong association of tetralogy of Fallot, double-outlet right ventricle and major VSD with significantly reduced placental weight (Matthiesen et al., 2016). The latest report found independently that smaller placentas with vascular anomalies are significantly more prevalent in newborns with congenital heart disease (Rychik et al., 2018). Studies that corroborate, expand and refine these findings are currently underway by one of the authors and colleagues (Sharbaugh D, Weinberg J, Parks WT, Catov J, Barak Y, in preparation).

These clinical associations are buttressed by a recent large-scale, unbiased phenotyping screen of embryonic lethal and subviable mouse mutants, which found a strong co-association between heart and placental defects (Perez-Garcia et al., 2018). In this study, mutants that exhibited a placental defect at E14.5 - typically in the vascular interface of the labyrinth - were significantly more likely to also suffer from a cardiac abnormality (most commonly ventricular and septal defects) than mutants in which the placenta was normal. This systematic screen reaffirms nearly three decades of repeated, yet sporadic observations of concurring placental and cardiac defects in a variety of knockout mouse strains. For example, mutations of the genes Vcam1 or Itga4 (encoding alpha4 integrin) prevent fusion of the allantois to the chorion (a necessary early step in establishing feto-placental circulation), and result in lethality prior to E11 with impaired cardiomyocyte proliferation and an underdeveloped ventricular wall (Kwee et al., 1995; Yang et al., 1995). However, cause and effect interpretation of these and other mouse lines with germ-line mutations with concurrent placental and cardiac phenotypes is complicated by the potential expression of the genes in both extraembryonic and embryonic tissues.

The first confirmed demonstration of a placental influence on heart development came from studies of the gene Pparg; mouse embryos lacking this gene in the germline suffer from a hypoplastic ventricular wall, presumptively due to insufficient cardiomyocyte proliferation, and embryo lethality at E10 (Barak et al., 1999). Importantly, at that stage, Pparg is expressed exclusively in the placenta, and these mutants also exhibit severe placental defects consisting of impaired trophoblast differentiation and failed vascularization of the labyrinth. Tetraploid aggregation chimeric embryos, in which the entire embryo remained Pparg-deficient but the placenta was wild-type, survived to term and had no heart defects (Barak et al., 1999). Thus, in this model, placental defects are unequivocally the underlying cause of the heart defects. A later study of the gene Mapk14 (encoding the p38a MAP kinase) similarly used tetraploid aggregation to demonstrate that the hypoplastic heart phenotype of this mutant was also the downstream consequence of impaired placental development (Adams et al., 2000). However, in this case, the rescue was short-lived and the embryos died two days later of non-placenta-related causes.

Repeated genetic demonstrations of placental origin of heart defects continued to trickle over the intervening years. These included knockouts of ERK2 (Mapk1) (Hatano et al., 2003); c-myc (Dubois et al., 2008); the SUMO-specific protease Senp2 (Maruyama et al., 2016); and the syncytin receptor Ly6e (Langford et al., 2018). The latter three studies employed a higher-throughput, definitive method for generating completely knockout embryos attached to an entirely normal placenta by performing epiblast-specific excision of loxP-flanked (floxed) conditional alleles with Sox2-Cre (Hayashi et al., 2002). In all three,

germ-line deletion of the gene caused placental and heart defects and embryonic lethality, whereas embryo-restricted excision of the gene caused no cardiac defects or fetal growth deficits and allowed embryonic survival to term.

Mutation of the gene Rxra, which encodes a retinoid receptor that functions as an obligate heterodimeric partner for 19 different nuclear receptors, results in insufficient cardiomyocyte proliferation, a poorly formed ventricular wall, and embryo lethality at E14 (Sucov et al., 1994). Genetic analysis demonstrated that this is a cardiomyocyte non-autonomous phenotype (Tran and Sucov, 1998), and furthermore, likely arises from primary dysfunction of a non-cardiac lineage (Shen et al., 2015). Of the 19 RXR-interacting nuclear receptors, the only two that are indispensable for early embryonic survival are PPARγ and PPARγ, both of which are essential strictly in the placenta (Barak et al., 2002; Barak et al., 1999). As detailed above, PPARγ has already been implicated in placenta-driven heart defects, albeit of an earlier and more severe nature. Perhaps most importantly, Rxra is abundantly expressed in the placenta and its deficiency causes severe histological and ultrastructural defects in this organ (Sapin et al., 1997). Moreover, *Rxra*/*Rxrb* compound null embryos die at E9.5 in conjunction with placental defects that are harsher than those of *Rxra*-null placentas (Wendling et al., 1999). The likely interpretation is that Rxrb partially contributes to survival and development from E9.5 to E14 in embryos lacking only Rxra before the increasing demands of the growing embryo for unmitigated placental function become rate limiting. Taken together, the precedents of Pparg, Mapk14, Mapk1, c-myc, Senp2, Ly6e, and Rxra demonstrate that a hypoplastic ventricular wall phenotype, at least between E10 and E14, may stem from an underlying defect in placental morphology or function.

In these and many other models that exhibit both cardiac and placental defects, how the placenta influences heart development at a distance remains an open question. One relatively mundane explanation could be the indispensable role of the placenta in provision of oxygen and nutrients to the fetus in general and to the heart in particular. Indeed, placental embolization was shown to disrupt cardiomyocyte proliferation and maturation (Thornburg et al., 2008). In addition, hypoxia causes premature differentiation of the second heart field, associated with ventricular thinning and septal defects (Yuan et al., 1995). Moreover, the erythropoietic and heart defects of Epo and EpoR global mutants described above illustrate the acute need of the developing heart for liver-derived red blood cells (and by proxy, for ample oxygen supply).

Furthermore, though, it is evident that placental transport is used by the embryo not only for energetic input but also as a signal to initiate subsequent development. For example, as noted above, the influx of oxygen and glucose starting around E11.5 maintains ventricular wall cardiomyocyte proliferation by maintaining Igf2 expression in the epicardium. Thus, in this case, the developing heart is actively instructed rather than only passively supported by the metabolic environment that is established by the placenta.

In addition to nutrient transport, the placenta secretes active factors and hormones that may influence heart as well as embryo development at large (Costa, 2016). The list of placental expressed growth factors includes FGFs, IGFs, WNTs, BMPs, TGFb/NODAL and activins, SHH, as well as pro-angiogenic factors such as VEGF and prolactins. Intriguingly, the

signaling cascades activated by these ligands have pivotal functions in cardiogenesis, including in the differentiation of the second heart field (Parikh et al., 2015; Rochais et al., 2009). In most cases, the extent to which these cardiac signaling pathways are activated by factors that originate from the placenta (as opposed to those that originate from within the heart or embryo) remains to be clarified.

5. Concluding remarks

As summarized here, major events that account for cardiomyocyte proliferation and ventricular wall morphogenesis have been elucidated. In particular, the power of mouse reverse genetics has revealed the E10–14 period of ventricular development to be comprised of a number of sequential phases and mechanisms that together merge into a seamless process of growth and morphogenesis. We note the following three directions in which we feel that further insights (and further research) would be of particular importance.

The placenta-heart developmental axis represents a fertile area for future investigation. The placenta might have a disproportionate influence on heart development (more so than in most other embryonic tissues) because of the high energetic demands of the heart, because of the functional relevance of fetal-placental circulation, or for additional reasons. Most past and current studies have focused on local events that occur in or adjacent to the heart, as most developmental processes are regulated by local signaling or cell-cell interactions. Although still under-appreciated, the many unambiguous examples of placental influence on heart development described above imply that a placental defect may underlie or contribute to heart phenotypes much more frequently than currently recognized.

Despite the assembly of a sophisticated understanding of the many genes that have cardiac developmental phenotypes when mutated in mice, extension of these insights to human congenital heart defects has generally lagged behind. That is, mouse genetics as a technology has driven a detailed mechanistic awareness of the molecular and cell-cell signaling pathways that underlie heart development. Undoubtedly, the same genes participate in human heart development, yet the extent to which human congenital heart defects can be explained by mutation or dysregulation of these same genes is unclear. This is particularly so for ventricular wall defects; as noted above, compromised ventricular wall morphogenesis in mouse can cause embryo lethality around E14, but the equivalent developmental stage in human development is E56, i.e., very early in gestation, when fetal demise would manifest as a spontaneous miscarriage rather than a ventricular wall deficiency that was evident at birth. Furthermore, in some instances, embryos recover from a transient developmental perturbation in cardiomyocyte proliferation due to the fact that this is controlled in sequential phases (recovery in mouse after E14 from a deficiency in IGF2 signaling was described above), such that no obvious malformation may be present at birth. Whether some postnatal ventricular pathologies (i.e., various forms of cardiomyopathy) might have a yet-unrecognized developmental basis is another interesting and relevant topic.

Finally, the mechanisms of cardiomyocyte proliferation in heart development might be applicable to postnatal heart regeneration. As noted above, natural cardiomyocyte proliferation effectively terminates shortly after birth. This process can be reactivated

following early neonatal heart injury, allowing full regeneration of lost myocardium, as observed in several mammalian species (Porrello et al., 2011; Robledo, 1956; Ye et al., 2018; Zhu et al., 2018), and likely also true in humans (Haubner et al., 2016; Westaby et al., 2008). However, shortly following birth (in mouse, during the first postnatal week (Porrello et al., 2011)), this ability is mostly lost, and injured myocardium (e.g., resulting from infarction or ischemia) is not regenerated but rather replaced by scar, with consequent loss of contractile function and potential progression to heart failure. A significant degree of interest in studies of embryonic cardiomyocyte proliferation lies in the potential application of these insights to promote more effective regeneration in the injured adult heart.

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