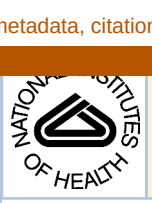

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Cardiac Progenitors and the Embryonic Cell Cycle

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Abstract

Despite the critical importance of proper cell cycle regulation in establishing the correct morphology of organs and tissues during development, relatively little is known about how cell proliferation is regulated in a tissue-specific manner. The control of cell proliferation within the developing heart is of considerable interest, given the high prevalence of congenital cardiac abnormalities among humans, and recent interest in the isolation of cardiac progenitor populations. We therefore review studies exploring the contribution of cell proliferation to overall cardiac morphology and the molecular mechanisms regulating this process. In addition, we also review recent studies that have identified progenitor cell populations within the adult myocardium, as well as those exploring the capability of differentiated myocardial cells to proliferate post-natally. Thus, the exploration of cardiomyoctye cell cycle regulation, both during development as well as in the adult heart, promises to yield many exciting and important discoveries over the coming years.

Keywords

cell cycle; cardiac; morphogenesis; development; chamber formation; mitogens; cardiac progenitors

During development, processes such as cell fate specification, proliferation, migration, and cell death, must occur in a coordinated fashion. The failure of any of these processes during embryogenesis can lead to developmental abnormalities, while the mis-regulation of many of the same pathways in adulthood is associated with disorders such as cancer. Thus, understanding the cellular and molecular mechanisms of development can provide crucial insight into human health. The mechanisms by which cell proliferation is regulated have been extensively studied in single-cell eukaryotes such as yeast, as well as in cultured mammalian cells. These studies have provided numerous valuable insights into how the different stages of the cell cycle are regulated, as well as the mechanisms by which cells exit the cell cycle and cease proliferating. Comparatively less is known about developmental regulation of cell proliferation, and especially about how this is achieved in a tissue-specific manner. In recent years, studies characterizing factors that regulate the cell cycle within the developing heart have advanced our understanding of how the morphology of the vertebrate heart is established and broadened our knowledge of cell cycle control in early development.

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CELL CYCLE REGULATION DURING DEVELOPMENT

Much of what is known about the regulation of the cell cycle and DNA synthesis has been studied primarily in yeast, mammalian cell culture, or in cell-free systems using Xenopus oocyte extracts.[1–6] During embryonic development, however, the cell cycle is modified by extracellular signaling events, with some cell types exiting the cell cycle early in development, and others continuing to proliferate extensively to adulthood. To address the in vivo requirements for important cell cycle regulators during development, initial studies have been carried out in Drosophila.[7,8] For example, cyclins A and E, which were shown to promote cell cycle progression in yeast and in Xenopus oocyte extract,[5,6,9,10] are both necessary and sufficient to initiate S-phase in Drosophila embryos, and are thereby essential for embryonic development.[8,11]

More recently, studies have been undertaken in the mouse to determine the requirements for various cell cycle regulators during vertebrate embryonic development (reviewed in ref. [12]). Surprisingly, despite the key role that cyclin/CDK complexes play during cell cycle progression by phosphorylating and inactivating the cell cycle inhibitory factor, retinoblastoma protein (Rb)[13,14] (Fig. 1), individual cyclins and CDKs largely do not appear to be required for normal embryonic development in mammals.[15–18] For example, deletion of the G_1 cyclins, cyclin E1 and E2, does not affect embryo viability or cell cycle progression.[15] This is in contrast to Drosophila, where cyclin E is required for progression to S-phase, and its absence results in embryonic lethality. Furthermore, knockout of all three mammalian D cyclins, cyclins D1, D2 and D3, failed to cause widespread proliferation defects in mouse embryos,[16] and mice lacking both Cdk4 and Cdk6, which partner with the D cyclins to promote the initial phosphorylation of Rb, are viable until late stages of gestation. In addition, deletion of Cdk2, the binding partner of the E and A cyclins, does not result in major cell cycle defects in vivo.[19] Only the combined knockout of Cdk2 and Cdk4 has been shown to result in defects in cell cycle progression and loss of Rb phosphorylation in vivo. These embryos only survive until embryonic day (E) 15 in the mouse, and die primarily due to cardiac defects.[20] Together, these data suggest that a high level of compensation can occur between the different cyclins and Cdks. The functional redundancy between the various cyclin/Cdk complexes is not total, however, as other complexes cannot fully compensate for the loss of both Cdk2 and Cdk4.[20]

The unexpected degree of functional compensation between the cyclin/Cdk complexes in mammalian embryos compared with what has been observed in yeast and in Drosophila embryos underscores the importance of understanding how the cell cycle is regulated in the context of a developing vertebrate embryo. Of particular interest is the observation that, while knockouts of individual or various combinations of cyclins and Cdks do not necessarily cause massive defects in cell proliferation throughout the embryo, deletion of each of these genes results in subtle and tissue-specific defects. For example, deletion of Cdk4 affects primarily pancreatic islet cells,[21] while the loss of cyclin E results in a failure of endoreplication in trophoblast giant cells,[15] and deletion of cyclins D1, D2 and D3 primarily affects heart and hematopoietic development.[16] In contrast, targeted deletion in mice of cyclin A2, which is required for both G_1/S and G_2/M transition, does result in early embryonic lethality at E5.5.[22] This suggests that despite the broad expression patterns of these cyclin/Cdk complexes, the ability of remaining complexes to compensate for the loss of one or more cyclin/Cdks may vary between cell and tissue types. Thus, further examination of tissue-specific cell cycle regulation during development is necessary and will provide important insights into organogenesis.

OVERVIEW OF VERTEBRATE HEART FORMATION

In all vertebrate species, the heart is among the first organs to form, and the basic processes of cardiac development are largely conserved.[23] Two bilateral populations of cells on either side of the midline of the embryo are induced into the cardiac lineage during gastrulation by signals emanating from the anterior endoderm underlying the anterior lateral plate mesoderm and from the organizer.[24–27] Additionally, the poles of the heart tube gradually grow through the addition of cells from a region contiguous with the splanchnic and core pharyngeal mesoderm, and in mouse preferentially contribute cardiomyoctes in the case of the anterior heart field to the proximal outflow tract and right ventricle and in the case of the secondary heart field to the distal region of the outflow tract.[28–31]

Following induction, the two bilateral populations of cardiac precursors migrate to the midline of the embryo where they fuse and form the linear heart tube composed of both an outer myocardial layer, which will form the beating heart muscle, and an inner endocardial layer, which will form the endothelial layer of the heart contiguous with the vasculature (reviewed in refs. [23] and [32]). Once the bilaminar heart tube has formed, the heart begins the process of rightward looping, which will ultimately generate the distinct chambers of the heart.[33] Mammalian and avian hearts are comprised of two atria and two ventricles (Fig. 2), while amphibians have a three chambered heart with a single ventricle, and the heart of fish consists of a single atrium and ventricle.

During cardiac looping, the ventral portion of the heart tube rotates to form the outer curvature of the heart, with the dorsal side becoming the inner curvature.[34] The chambers of the myocardium form out of the outer curvature through a process of rapid cell proliferation and increased cell size,[35] while the cells of the inner curvature become the nonchamber myocardium of the atrioventricular canal (AVC) and outflow tract (OFT), which will play a role in the alignment and septation of the cardiac chambers.[34]

The early patterning of the myocardium is established, in part, by differential expression of cardiac transcription factors, such as the T-box transcription factors TBX2, 3, 5 and 20, the homeobox transcription factor NKX2.5, as well as GATA4, 5 and 6.[32,34,36] Other chamber-restricted cardiac transcription factors include HAND1 and HAND2 (also known as eHAND and dHAND, respectively). In mammals, *Hand1* and *Hand2* are restricted to the left and right ventricles, respectively, but are expressed ubiquitously in avian and amphibian hearts.[37,38] The *Iroquois* homeobox genes (*Irx*) are also expressed in specific cardiac chamber primoridia.[39]

The mechanisms by which these chamber-specific transcription factors promote cardiac morphogenesis are only beginning to be understood. TBX2, TBX5, TBX20, HAND1, HAND2 and IRX4 have all been shown to be required for proper heart development, and they act both individually and combinatorially to regulate the expression of other genes specific to distinct regions of the developing myocardium,[34,39–43] including *atrial naturitic factor* (*ANF*),[44] *Cited* (also called *Msg1*),[45] *Chisel*,[46] *connexin 40* and *connexin 43*.[47] However, relatively few targets of these chamber- restricted cardiac transcription factors have been identified, and the precise cellular and molecular mechanisms by which they function remain unknown. The polarity of the heart tube seen through the differential expression patterns of these and other cardiac genes is later reinforced by changes in cell proliferation, polarity, and size.[39] The roles of some of these transcription factors in cardiomyocyte proliferation will be further explored below.

REGULATION OF CELL PROLIFERATION DURING EARLY CARDIAC DEVELOPMENT

As mentioned above, cell proliferation plays a key role in heart formation. Following the initial induction of cardiac precursor cells in vertebrate embryos, the cells undergo a period of rapid proliferation beginning at about E8 in the mouse.[27,48,49] Proliferation within the heart field then transiently slows, followed by a second peak of proliferation at E11.[48] However, the molecular mechanisms regulating these waves of proliferation within the cardiac mesoderm are poorly understood.

The early period of proliferation in the cardiac mesoderm corresponds with the close association between the endoderm and the cardiac progenitor population.[25] During this time, cardiac cells continue to be exposed to a variety of growth factors and mitogens, including fibroblast growth factors (FGFs) and bone morphogenic proteins (BMPs). In particular, studies carried out in avian embryos have demonstrated that *Fgf8*, expressed in the endoderm contacting the cardiac mesoderm, is required for the maintenance of *Nkx2.5* and *Mef2c* expression.[49] FGF signaling has also been implicated in cardiac cell proliferation specifically during this developmental window. Several studies have demonstrated that FGFs 1, 2 and 4, which are expressed beginning at gastrulation in the endoderm underlying the heart-forming field, are required for cardiac cell proliferation in explants of the avian cardiac mesoderm.[50–52] Additionally, a retro-viral vector encoding a dominant negative form of FGF receptor 1 (FGFR1) resulted in reduced cardiac cell proliferation when infected at day 3 and assessed at day 7 of avian development, demonstrating a requirement for FGF signaling in cardiac cell proliferation in vivo at stages corresponding to early cardiac morphogenesis. In contrast, no effect on cardiac cell proliferation was observed when hearts were infected at day 7 and observed at day 14.[53] Together, these data suggest a role for FGF signaling in regulating the high levels of cardiac cell proliferation observed during the early stages of cardiac migration, heart tube formation, and cardiac looping.[48] Moreover, the reduction in cardiac proliferation that occurs after stage 24 in chick (approx. E12 in mouse), corresponds closely with reduced expression of FGFs within the myocardium.[52]

BMPs are also present in the early heart field and myocardium during the same developmental window as FGFs. Together with FGF signaling, BMP signaling also plays a role in the induction and maintenance of early cardiac progenitor cells.[54] BMP2, expressed in the anterior endoderm, is required for the induction of cardiac progenitors in early heartfield explants,[55] and together with FGF4, is sufficient to induce noncardiac mesoderm to produce heart tissue.[56] BMP signaling downstream of *Gata6* also appears to be required after the initial induction of cardiac progenitors for the maintenance of early cardiac genes, including *Nkx2.5*.[57] Later in development, BMPs function to promote the proliferation of the myocardium, as mice lacking BMP receptor 1A exhibit a thin myocardial wall,[58] while mice lacking the BMP antagonist *Noggin* display hyperplastic growth of the ventricular myocardium.[59]

CELL PROLIFERATION AND CARDIAC CHAMBER FORMATION

In spite of the reduction in cell proliferation that occurs during cardiac chamber formation and remodeling, cell proliferation plays a critical role throughout heart morphogenesis. The high levels of DNA synthesis observed in the early cardiac mesoderm and myocardium are thought to be important both for the expansion and segmentation of the heart tube along its anterior-posterior axis, as well as for the formation of the cardiac chambers.[34,35]

Recent studies of the basic-helix-loop-helix transcription factor HAND1 underscore the importance of proper regulation of the early cardiomyocyte cell cycle in establishing the correct length of the early heart tube. Loss of function and overexpression studies support a role for

HAND1 in promoting the proliferation of cardiomyocyte progenitor cells prior to terminal differentiation.[60–62] Deletion of *Hand1* from the developing heart results in shortening of the outflow tract as well as hypoplasia of the right ventricle, resulting in the formation of an unlooped heart tube.[62] In contrast, overexpression of *Hand1* within the developing heart tube has the opposite effect, where the outflow tract is lengthened due to increased cell proliferation, resulting in abnormal cardiac looping, and overall abnormal cardiac morphology.[61] Thus, regulation of cardiomyocyte cell proliferation by HAND1 plays a necessary and sufficient role in establishing the correct length of the early heart tube, thereby contributing to the process of cardiac looping.

In addition to a requirement for the correct number of cells in order to generate a heart tube of the proper length, recent analysis suggests that the orientation of cell divisions plays a key role in cardiac morphogenesis. Retrospective clonal analysis of the developing mammalian heart has revealed two distinct phases of myocardial proliferation.[63] In the first phase, which corresponds to the formation and elongation of the heart tube during early stages of cardiac development (prior to E8.5), descendents of individual labeled cells are observed to disperse over fairly long ranges, and always along the arteriovenous axis. Thus, this phase is termed the dispersive phase.[63]

In the second phase, which begins after E8.5, clonal cells form larger clusters. This latter phase has been termed the coherent phase of growth, and further analysis has revealed that coherent clones have a distinct orientation of growth that corresponds with the chamber in which they reside.[63] For example, clones in the outflow tract take on a linear anterior-posterior orientation, while clones in the left ventricle tend to radiate out from the AVC.[63,64] This work provides evidence that the orientation of cell division, as well as the rate of proliferation, plays a critical role in generating the correct pattern and shape of the developing heart.

In further support of these findings, several zebrafish mutants have been identified that have severe cardiac abnormalities due to the failure of myocardial cells to divide along the proper axis. These include the mutants s*anta* (*san*), *valentine* (*vtn*) and *heart of glass* (*heg*). *San* and *vtn* encode zebrafish homologs of two genes associated with brain vasculature defects known cerebral cavernous malformations in humans, *CCM1* and *CCM2* respectively,[65] while *heg* encodes a novel, membrane-bound signaling protein expressed in the endocardium.[66] The phenotype of each of these mutants is very similar, wherein the hearts are enlarged and exhibit a thin ventricular wall due to the failure to add concentric layers to the myocardium. Normally, these concentric layers are added when cardiac cells shift their orientation of cell division to divide along the transmural axis (from the epicardium to the endocardium). Failure of this process results in expansion of the myocardium only along the initial axis, leading to severe morphological abnormalities and a failure of cardiac function.[65,66] Thus, generating both the correct number and orientation of cell divisions within the developing heart plays a critical role in generating proper the morphology and function of this organ.

During the developmental stages when these patterns of oriented cell divisions are being established within the myocardium, differential levels of cell proliferation are also observed in different regions of the myocardial tube. Increased levels of cell proliferation are observed in the presumptive chamber myocardium of the atria and ventricles, as compared with the non-chamber myocardium of the OFT and AVC.[34] The mechanisms by which cell proliferation is differentially regulated between the chamber myocardium and non-chamber myocardium are not well characterized, however some recent studies have begun to elucidate this process.

The early cardiac chambers of the vertebrate heart are initially formed by increased levels of proliferation in the presumptive chamber myocardium compared with non-chamber myocardium of the heart tube. This results in the “ballooning” out of the chamber myocardium.

[34,35] Prior to this expansion, chambers are specified in part by the activity of cardiac T-box transcription factors. *Tbx5* exhibits a graded expression pattern along the anterior-posterior axis of the heart tube, with the highest levels of expression seen in the future atria and left ventricle (common ventricle in amphibians),[34,42] while *Tbx20* is expressed throughout the developing myocardium.[67–69] In concert with NKX2.5, these two transcription factors promote the formation of the chamber myocardium by activating expression of chamber-specific genes. In addition, TBX5 and TBX20 have been shown to physically and functionally interact with one another,[40] suggesting that they may function together to regulate cardiomyocyte cell number as well as gene expression within the developing heart.

In the non-chamber myocardium, the activity of TBX5 and TBX20 is opposed by the transcriptional repressors TBX2 and TBX3,[43] which are thought to compete with TBX5 for binding to NKX2.5.[70] Furthermore, TBX20 has been shown to repress *Tbx2* expression within the chamber myocardium, as loss of TBX20 results in increased *Tbx2* expression throughout the heart tube.[71,72] Interestingly, TBX2 has been shown to repress transcription of *N-myc*, a protein required for G_1/S transition in cardiac and other cell types.[73] Deletion of *Tbx20* thereby results in decreased expression of *N-myc*, hypoplasia of the myocardium, and a resultant failure of cardiac morphogenesis.[71,72] Furthermore, TBX5 has been shown to be required for cardiac cell cycle progression, specifically at the G_1/S transition.[74] These data support a model of cardiac chamber formation whereby cell proliferation is maintained at low levels in the non-chamber myocardium by the transcriptional repressor activity of TBX2, while high levels of proliferation are promoted in the chamber myocardium by the activity of TBX5 and TBX20, either by repressing the activity of factors that inhibit cell cycle progression, or by directly promoting cell cycle progression (Fig. 3).

CARDIOMYOCYTE PROLIFERATION AND VENTRICULAR REMODELING

In addition to a role in chamber formation, cardiac cell proliferation is also important for the remodeling of the ventricles as the compact and trabecular layers of the myocardium are distinguished. Remodeling of the ventricles begins at the end of the cardiac looping stage[75,76] (E10.5 in mouse, HH stage 18 in chick, and Nieuwkoop and Faber stage 37 in Xenopus). The trabeculae of the ventricle are finger-like bundles of cardiomyoctyes that project into the chamber of the ventricle by about E10.5 in mouse. By contrast, the compact myocardium consists of cardiac myocytes that are more densely organized, forming the outer wall of the myocardium. The trabecular myocardium provides most of the contractile force during early embryonic heart function, while later in heart development this role shifts to the compact myocardium.[75] In addition to distinct cellular organization, the two myocardial layers also differ in their mitotic activity, with the compact myocardium being more highly proliferative overall, thereby driving the growth of the embryonic heart, and the trabecular myocardium proliferating at a much lower rate.[76] The timing of cell proliferation also differs between the compact and trabecular myocardium. The mitotic index (MI) of the trabecular layer reaches peak levels at E10.5 and declines rapidly, while in the compact layer, the MI peaks between E9.5 and E11.5 and declines more gradually.[77]

The fate of ventricular cardiomyocytes to become either compact or trabecular myocardium appears to be governed, at least in part, by signals emanating from the epicardium[78–80] and endocardium respectively.[79,81–83] Retinoic acid and other yet unidentified signals from the epicardium play a role in instructing myocardial cells to become compact myocardium.[79] The trabecular myocardium is patterned by neuregulin secreted from the endocardium and its downstream effectors, which induce these cell to form projections into the lumen of the heart tube.[81–83] Once this patterning is established, it is reinforced by the differential levels of proliferation observed between the compact and trabecular myocardium. The relatively higher

levels of proliferation in the compact myocardium are promoted primarily through FGF signaling downstream of retinoic acid secreted from the epicardium.[79,80]

Recently, several transcription factors have been identified that play important roles in cardiac morphogenesis by negatively regulating proliferation in the trabecular myocardium. The gene *jumonji* (*jmj*), which encodes a member of the AT-rich interaction domain (ARID) family of transcription factors, was found to negatively regulate the cell cycle, with over-proliferation observed in hearts of embryos lacking *jmj*.[84] The timing of *jmj* expression in trabecular and compact myocardium of the ventricle matches the time points at which each begins to reduce their respective MI.[77] JMJ has been shown to function in part by repressing cyclin D1 expression in the trabecular myocardium[77] and can also physically interacts both with NKX2.5 and GATA4. This results in repression of *ANF* in the AVC,[85] suggesting that JMJ may play a role in establishing chamber identity in addition to its function in regulating cell cycle progression. This finding further supports a link between molecules that establish cardiac chamber identity and those that are required for cell proliferation within the developing heart.

Other transcription factors that act to restrict cardiac cell proliferation within the ventricular trabeculae include FOXP1, a member of the winged helix family of transcription factors,[86] and HOP1, an unusual homeobox-containing protein.[87,88] An increase in cell proliferation has been observed in the trabecular zone of *Foxp1* null embryos, however, *Foxp1* is expressed primarily in the compact myocardium and the mechanism by which it acts to restrict proliferation in the trabeculae has not been established.[86] The hearts of HOP1 null mice exhibit thickened ventricular walls in comparison with wild-type littermates, due primarily to an increase in cell number. HOP1 appears to be expressed primarily within the trabecular myocardium of the ventricle, and was shown to physically interact with Serum Response Factor (SRF). One molecular function of HOP1 is to antagonize the activity of SRF,[87,88] however, the exact molecular mechanism by which HOP1 acts to inhibit cardiac cell cycle progression is not yet known. Thus, further analysis of how tissue-specific transcription factors interact with, and regulate the cell cycle machinery during development is crucial to the understanding of how specific organs are patterned and how they achieve their required size and function.

THE RELATIONSHIP BETWEEN PROLIFERATION AND CARDIAC DIFFERENTIATION

In many tissues, such as muscle and the nervous system, the withdrawal of cells from the cell cycle is tightly associated with the onset of terminal differentiation.[89–92] In contrast, relatively little is known about the relationship between cell cycle progression and terminal differentiation in the heart. Myocardial cells continue to proliferate until just after birth in mammals, when nearly all exit the cell cycle.[48] As a result of this cell cycle exit, cardiomyocytes in the adult heart are thought to be very limited in their regenerative capacity. In skeletal muscle, by contrast, associated muscle progenitor cells, termed satellite cells, retain the ability to proliferate and differentiate into mature muscle upon injury.[93]

Mutations in a number of genes that are required for cell cycle progression in the heart also result in defects in cardiac differentiation. *Hand1* is required, primarily in the OFT and left ventricle, for cell cycle progression at the G_1/S transition by promoting the transcription of cyclin D2 and Cdk4.[61] Over-expression of *Hand1* in the developing heart results in increased cardiac cell proliferation as well as delayed and reduced expression of cardiac differentiation markers.[61] In addition, deletion of *jmj*, which promotes cell cycle exit by repressing cyclin D1 expression,[77] results in reduced expression of cardiac differentiation markers as well as increased proliferation within the heart.[84] These experiments lend support to a close association between cell cycle exit and terminal differentiation in myocardial cells, as has been demonstrated for other cell types. However, the consequences of disrupting the cell cycle upon

cardiomyocyte differentiation during development have never been directly examined, leaving open the possibility that cell cycle exit is not directly linked with terminal differentiation within the heart. Supporting this possibility is the observation that cell proliferation continues within the developing myocardium even after the fully differentiated, beating heart has formed, with myocardial cells ultimately exiting the cell cycle after birth.[48] In addition, work done in Xenopus has shown that in embryos at the tadpole stage, cells within the myocardium bearing structural hallmarks of differentiated cardiomyocytes stain positively for phosphohistone H3, a marker of M phase of the cell cycle.[74] Together, these data suggest that vertebrate cardiomyoctyes retain the ability to divide as they undergo terminal differentiation.

The potential for cells of the adult vertebrate myocardium to proliferate has generated considerable interest in recent years (reviewed in refs. [48, 94] and [95]). Several reports have indicated that rare cardiac progenitor cells exist within the mammalian myocardium. These progenitor populations are positive for distinct sets of markers, and were isolated from hearts at different stages, including embryonic, neonatal, and adult hearts.[96–100] One population of progenitor cells isolated from embryonic hearts and positive for the secondary heart field marker *islet1* is capable of forming fully differentiated cardiomyocytes in culture,[98] and is also capable of differentiating into endothelial and smooth muscle cells.[99] $cKit^+$ cells with the ability to differentiate into cardiomyoctes have been identified both in adult and embryonic hearts.[96,100] Finally, a $Sca1^+$ population of cells in the adult myocardium is also capable of differentiating into cardiomyocytes when isolated from the heart and cultured in vitro, as well as when transplanted into mice with ischemia/reperfusion injuries.[97] Thus, despite the fact that cardiomyocytes begin to exit the cell cycle during embryonic development following the onset of differentiation, and that most myocardial cells exit the cell cycle shortly after birth, one or more populations of undifferentiated cardiac progenitor cells exist in both the embryonic and adult myocardium. However, the molecular pathways by which these cells are specified and maintained within the heart during development are largely unknown.

Given the thereputic implications of cardiomyocyte regeneration for the treatment of pathological conditions such as myocardial infarction, the ability of differentiated adult myocardial cells to enter the cell cycle has been the subject of considerable research and controversy. It has been demonstrated, for example, that differentiated cardiomyoctyes continue to undergo DNA replication, as shown by incorporation of BrDU.[101] However, this may be indicative of the round of endoreplication that is known to occur in post-mitotic cardiomyocytes.[48,101] Very low percentages of adult human cardiomyoctyes have been observed to express markers associated with M-phase- approximately 14 myocytes per million.[102] Several lines of evidence also suggest that cardiomyocytes may have some capacity to reenter the cell cycle upon injury. Increased numbers of proliferating cardiomyocytes are observed in the hearts of patients who died of heart failure,[102] and in another study, even higher percentages of M-phase cardiomyocytes were observed adjacent to myocardial infarction sites.[103] More recent work has also suggested that over-expression of cyclin D2 in a mouse model of myocardial infarction promotes increased DNA synthesis in the injured hearts and leads to regression of the infarction.[104] Thus, the capacity of cells within the adult myocardium to proliferate, as well as factors that might promote cardiomyocyte cell cycle in response to injury are in need of further exploration.

CONCLUSIONS

Establishing the mechanisms by which cell proliferation is regulated in the developing heart is of critical importance, both for our understanding of the cellular basis of cardiac morphogenesis during embryonic development, as well as in establishing the origin and nature of cardiac progenitor cell populations existing in the adult myocardium. Cell proliferation levels within the developing myocardium appear to be highly specific to a given region and

developmental stage of the embryonic heart, thus playing an important role in the shaping and pattering of the heart.[35] Furthermore, not only are the levels of cell proliferation important, but the plane of cell division within the myocardium also appears to have a key function in morphogenesis.[63–66] The factors required to establish these regionalized patterns of cell proliferation within the heart, as well has how these patterns tie in with the identity of cardiac chambers, are only now beginning to be characterized.

In addition, the discovery that several populations of cardiac progenitor cells with unique properties exist within the postnatal and adult heart has lead to an important shift from the view that the mammalian adult myocardium is entirely post-mitotic with little to no regenerative capacity.[95] Thus, understanding how these populations are set aside during development and characterizing the signals that regulate their proliferation and differentiation is of crucial importance in regenerative medicine.

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ABBREVIATIONS

ANF
: atrial naturietic factor

AVC
: atrioventricular canal

BMP
: bone morphogenic protein

CDK
: cyclin-dependent kinase

FGF
: fibroblast growth factor

MI
: mitotic index

OFT
: outflow tract

RB
: retinoblastoma protein

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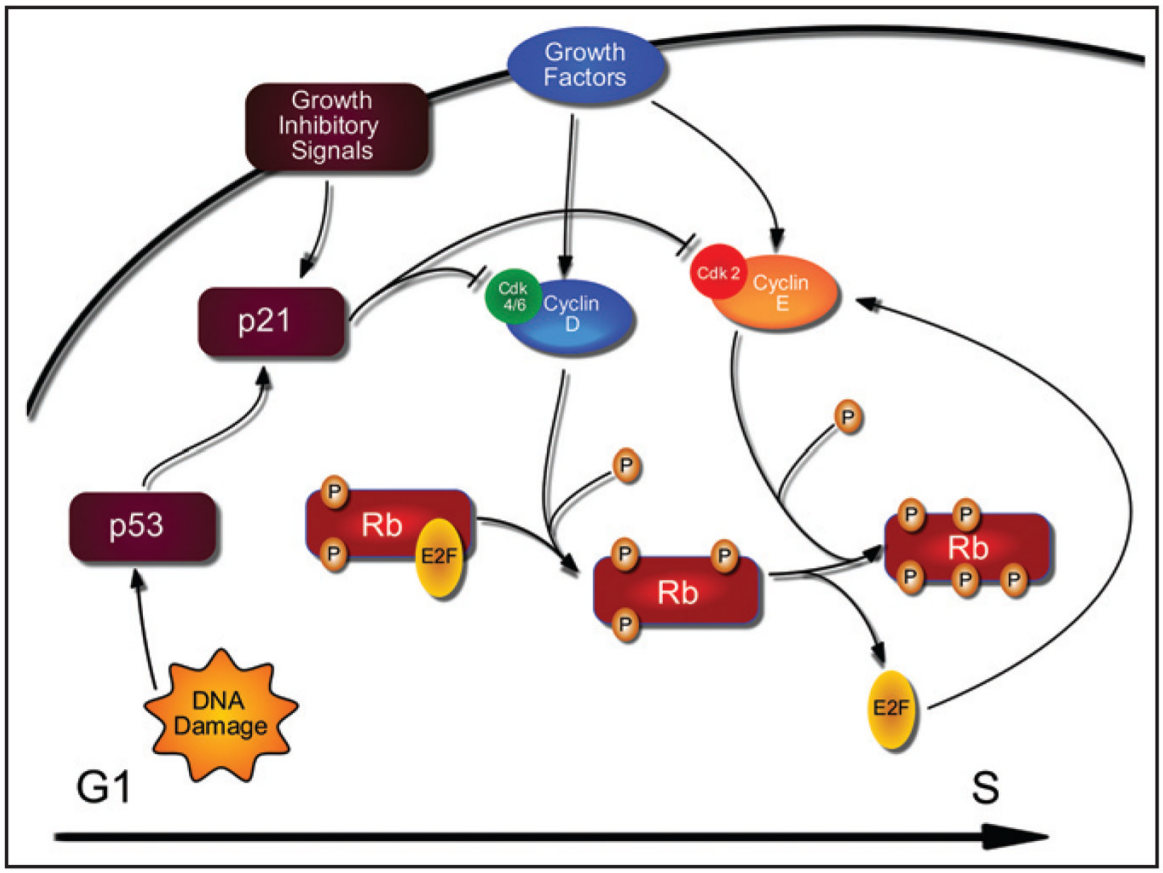

Figure 1.

Molecular regulation of the G_1/S transition. A schematic of the major molecular pathways both promoting and inhibiting the progression from G_1 to S-phase of the cell cycle. Growth factors promote entry into S phase by inducing expression and activity of cyclin D, cyclin E and their associated Cdks. These complexes in turn phosphorylate and inactivate the retinoblastoma protein (Rb), which then releases E2F, a transcription factor upstream of many other cell cycle-promoting factors, as well as DNA replication factors. Cell cycle progression can be inhibited by growth inhibitory signals, or by DNA damage, which results in p53 expression. Both of these pathways result in increased expression of Cdk inhibitors such as p21 and p27, which antagonize activity of the Cyclin/Cdk complexes, inhibiting entry into S-phase.

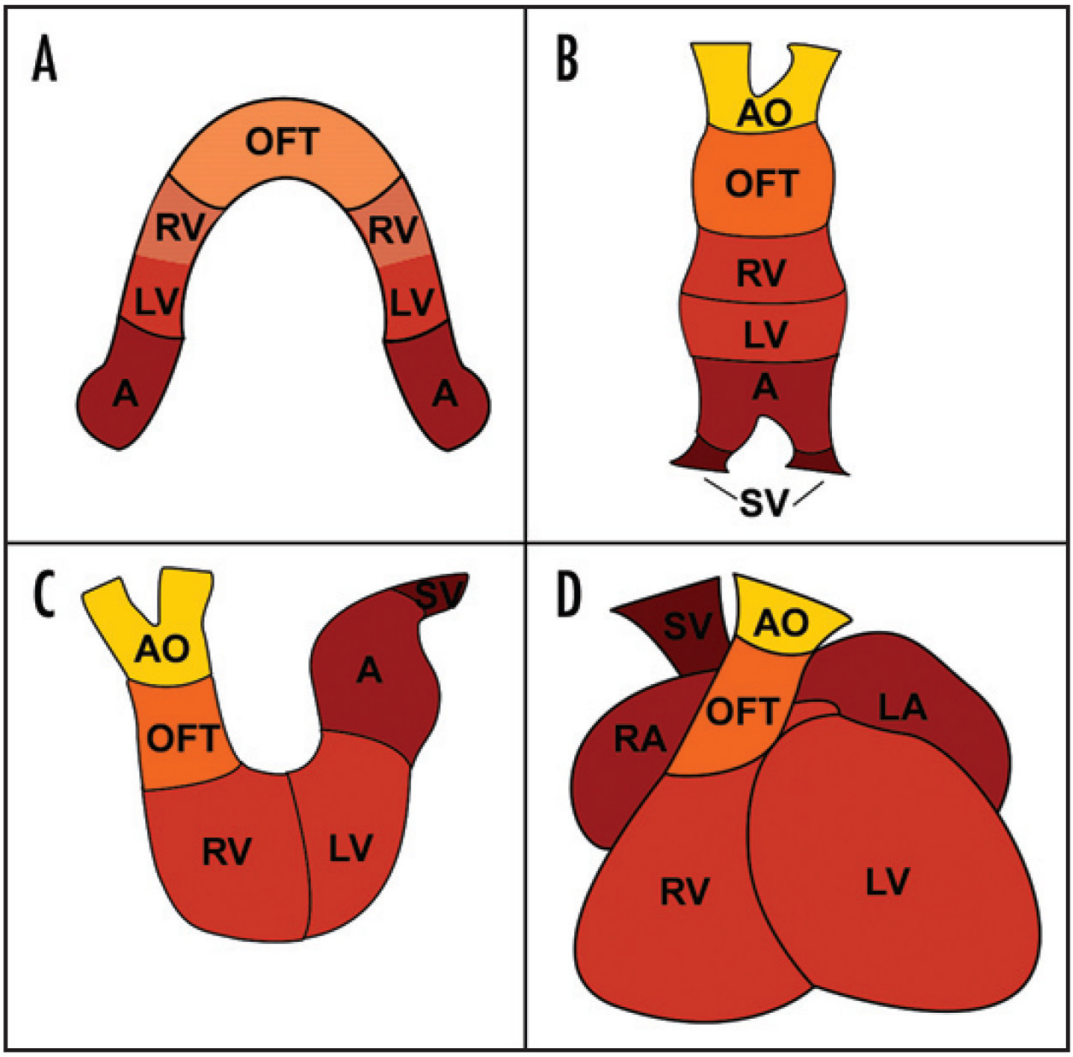

Figure 2.
Development of the vertebrate heart. A schematic depiction of vertebrate heart morphogenesis (based on the mouse) at the equivalent of mouse E7.5 (A), E8.5 (B), E9.5 (C), and E11.5 (D). OFT, outflow tract; RV, right ventricle; LV, left ventricle; A, atria; AO, aorta; SV, sinus venosus; RA, right atrium; LA, left atrium.

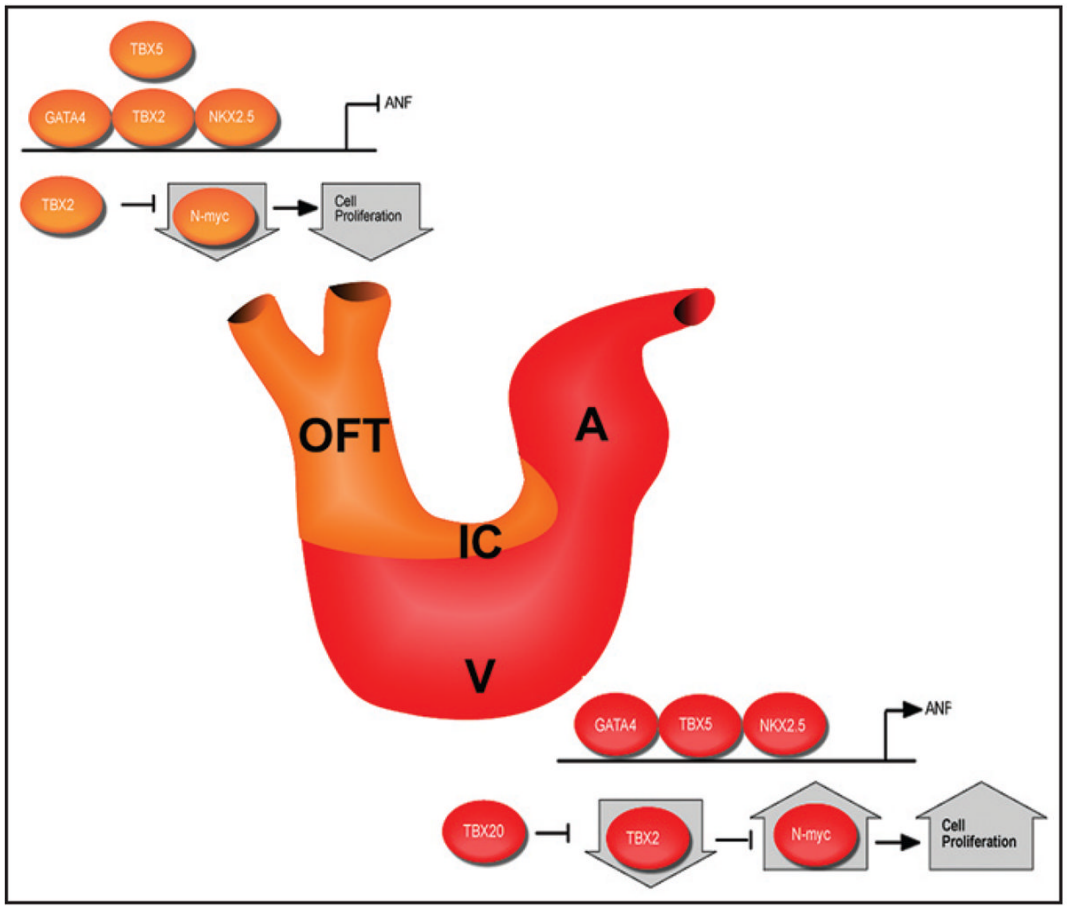

Figure 3.

T-box transcription factors and the development of the chamber and non-chamber myocardium. In the non-chamber myocardium (orange), TBX2 represses transcription of ANF and other genes specific to the chamber myocardium, and out-competes TBX5 for binding to NKX2.5 within the inner curvature. TBX2 also represses *N-myc*, a transcription factor that promotes cell proliferation, keeping proliferation levels low in the non-chamber myocardium. In the chamber myocardium (red), TBX5 binds to the *ANF* promoter and promotes its transcription. In addition, expression of TBX2 is repressed by TBX20, thus relieving repression of *N-myc*. Together with higher levels of TBX5 expression, which promotes cell cycle progression, this leads to higher levels of cell proliferation within the chamber myocardium. OFT, outflow tract; IC, inner curvature; V, ventricles; A, atria.