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Heart development and regeneration in urodeles

ANTON W. NEFF[1]*, ARLENE E. DENT[1] and JOHN B. ARMSTRONG[2]

[1]*Medical Sciences Program, Indiana University, School of Medicine, Bloomington, Indiana, USA and* [2]*Department of Biology, University of Ottawa, Ottawa, Canada*

ABSTRACT Classical fate mapping and transplantation studies have yielded a rich embryological understanding of heart development in urodeles. Recent advances in understanding the molecular nature of many early developmental events can be applied to urodele heart development. In this review we examine urodele heart development from both morphological and molecular viewpoints. We focus primarily on cardiac induction, early cardiogenesis, and heart regeneration.

KEY WORDS: *heart development, heart induction, heart regeneration, urodele, heart field*

Introduction

In this review we will discuss cardiac induction, early cardiogenesis, and heart regeneration in urodeles. Amphibians such as urodeles have historically been one of the best animal models for studying heart development. Urodeles are an embryologically friendly model system that possesses many advantages: the urodele embryo is easily visualized as it is large in size, is available in large quantities, and has external development. More important, it is also a slow developer that is amenable to embryonic manipulations such as explantation and transplantation. In addition, the urodele has extensive regenerative capacity throughout its development and also in the adult. These advantages make the urodele a good model system for studying heart development and regeneration.

Classically, cardiogenesis in urodeles (and other vertebrates) was studied using embryonic fate mapping to determine the origin and movement of the cardiac mesoderm. Tissue explantation and transplantation were used to determine when cardiac mesoderm becomes specified to form heart. Heart morphology or heartbeat were used as assays for heart development. The sequences of developmental events leading to a beating heart have been well documented in urodeles (see Jacobson and Duncan, 1968 and Jacobson and Sater, 1988 for reviews). Fate and specification mapping has localized the two mesodermal heart primordia to the deep marginal zone at the lateral edge of the dorsal lip of the blastopore at the initiation of gastrulation. During gastrulation the paired mesodermal heart primordia migrate to lie at the dorsal lateral edges of the future neural plate and to lie over the anterior lateral endoderm. These heart primordia migrate to the mid-ventral site during the late neurula stage and early tail bud stages. In the Mexican axolotl *(Ambystoma mexicanum)* these bilateral primordia fuse in the ventral mid-line of the embryo at stage 29 (day 4). The fused primordia gives rise to a single linear heart tube. The heart tube then folds upon itself to form a three chambered heart.

With the advent of immunocytological and molecular techniques, specific proteins (e.g., sarcomeric proteins such as actin and myosin heavy chain) or their mRNAs have been identified and localized during cardiogenesis. For instance, upon forming the heart tube, organized myofibrils appear and rhythmic contractions ensue. In the axolotl, the contractile proteins troponin-T, myosin heavy chain, α-actinin and tropomyosin can be detected in the pre-heartbeat embryos (stage 32) just after the right and left pre-cardiac mesodermal sheets have fused in the ventral midline (stage 30) to form the heart tube (Fuldner *et al.*, 1984; Lemanski *et al.*, 1980). By stage 33, hearts show only amorphous non-sarcomeric localization of sarcomeric proteins such as tropomyosin (Lemanski *et al.*, 1980). Myofibrils can be detected by electron microscopy in ventricular cardiomyocytes at stage 34 (Lemanski, 1973). Rhythmic contractions begin shortly thereafter (stage 35).

Even though the traditional morphological approach has led to a rich embryologic understanding of cardiogenesis, the molecular analysis of cardiogenesis remains underdeveloped. Use of molecular methods has been limited to the study of later cardiogenic events due in part to a lack of early cardiac-specific molecular markers in urodeles. For further molecular analysis of cardiogenesis, early cardiogenic molecular markers must be developed. One approach may be to apply molecular data gleaned from other model systems to the embryologically friendly urodele system.

The heart is a multi-chambered complex organ that contains cardiomyocytes, connective tissue, nervous tissue, and endothelium. In this review we will emphasize myocardial development as more is known about this process. However, it must be realized that the contribution of neural crest, endothelium, and connective tissue (i.e., valves) components of the heart must be coordinated and integrated to form a complex functional heart.

Cardiac induction

The development of cardiac muscle during embryogenesis is a multi-step process which involves commitment of mesodermal

***Address for reprints:** Medical Sciences Program, Indiana University, School of Medicine, Bloomington, IN 47405, USA. FAX: 812.855-4436. e-mail: neff@indiana.edu

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progenitor cells to the cardiomyogenic lineage, differentiation of the cardiomyocytes, and enlargement of the number of committed cells by cell proliferation. Subsequently these cells mature and diversify to form functional cardiac muscle tissue in response to various influences. Each of these steps, beginning with commitment, involves induction.

Embryonic induction is a process whereby one group of cells controls the fate of its neighbors through interactions. In general, every tissue and organ formation involves some kind of induction. The best studied vertebrate inductions are mesodermal and neural, for which multiple recent reviews are available (examples are Slack, 1993; Fukui and Asashima, 1994; Kessler and Melton, 1994; Ruiz i Altaba, 1994). Amphibian embryos have been used extensively for induction studies. Historically, grafting experiments have been performed to find out when and where different inductions occur. Much is known about mesoderm induction including several good candidates for the inducer molecules, their receptors, and gene regulators. However, the series of cell interactions termed "cardiac induction" which begins with mesoderm induction is less well understood. Urodeles provide the most comprehensive understanding of cardiac induction. In these animals cardiac induction occurs when the anterior pharyngeal endoderm is in direct contact with the cardiogenic mesoderm. Several lines of evidence have shown that this endoderm induces heart formation (reviewed by Jacobson and Sater, 1988). However, the molecular aspects of cardiac induction and early cardiogenesis have not been worked out in any system.

What is the relationship between cardiac induction and the "heart field"?

One of the first questions to be asked is whether, from the beginning, "cardiac induction" leads to the specification of only those cells destined to form the heart, or of a larger region which is refined by subsequent events. The existence of morphogenetic fields that are larger than the organs they will form has been a concept in classical embryology for more than 70 years. Does one exist for the heart?

Traditionally, the extent of a morphogenetic field has been established by removing the tissue fated to form a particular organ or structure (heart, limb, lens, etc.) and determining whether the structure is still formed by the remaining tissue. If regulation occurs (and the structure forms), the field is larger than the excised region. Copenhaver (1926) first determined the extent of the heart field, in *Ambystoma punctatum (maculatum)* in this way. When Copenhaver excised only the heart-forming region, a heart formed, leading him to conclude that the heart field was larger than the heart primordium. However, a possible alternative explanation is that neighboring mesoderm moved into the heart region and was induced. Thus, regulation would occur due to a belated response from tissue that was not initially within the heart field. If true, Copenhaver's (1926) extirpation experiments would have been testing the competence of non-heart-field mesoderm to respond, and its ability to move in fast enough, rather than the extent of the heart field.

An alternative approach to assessing the extent of a morphogenetic field is to explant various regions of tissue to determine whether they have the capability of forming the structure in isolation (in other words, to see if they are "specified"). This approach was used by Rawles (1943) to map the chick heart field, which was clearly shown to be larger than the myocardial primordium fate-mapped by DeHaan and coworkers (Rosenquist and DeHaan, 1966; Stalsberg and DeHaan, 1969). Sater and Jacobson (1990a) also used specification mapping to study the heart field in *Xenopus laevis*.

In the axolotl, Easton *et al.* (1994) used specification mapping to test whether the heart field expands as more mesoderm comes in contact with inductive endoderm. Their results confirmed the hypothesis that the specified region grows larger between stage 20 (end of neurulation) and stage 28, when the migrating sheets of lateral plate mesoderm finally meet at the ventral mid-line of the

TABLE 1

EXAMPLES OF SIGNALING PATHWAY MOLECULES POTENTIALLY INVOLVED IN CARDIAC INDUCTION AND/OR DIFFERENTIATION

Molecule	Species	Comments	Reference
activin-A	newt	induced beating hearts (2/23) in presumptive ectoderm (animal caps)	Moriya and Asashima, 1992
activin-A	*Xenopus*	induced the expression of cardiac-specific myosin heavy chain-α (XMHC-α) in animal cap explants	Logan and Mohun, 1993
activin-A	chick	induced differentiation of explanted stage 6 cardiogenic mesoderm	Sugi and Lough, 1995
activin-A	axolotl	induced beating hearts in neurula stage precardiac mesoderm explants	Mangiacapra *et al.*, 1995
TGF-ß1	axolotl	induced beating hearts in neurula stage precardiac mesoderm explants	Muslin and Williams, 1991
TGF-ß2	mouse	expressed in cardiomyocyte progenitors and foregut endoderm	Dickson *et al.*, 1993
FGF-2	chick	induced differentiation of explanted stage 6 cardiogenic mesoderm	Sugi and Lough, 1995
FGF receptor-1 (FGFR-1)	chick	present in precardiac endoderm, precardiac mesoderm and myocardium at the heart tube stage. Anti-FGFR-1 retarded proliferation and multi layering of explanted cardiogenic cells	Sugi *et al.*, 1995
PDFG	axolotl	induced beating heart in neurula stage pre-cardiac mesoderm explants	Muslin and Williams, 1991
insulin	chick	induced differentiation of explanted stage 6 cardiogenic mesoderm.	Sugi and Lough, 1995
wnt-1	mouse	antisense oligo suppression of *wnt-1* expression resulted in cardiomegaly	Augustine *et al.*, 1993
msk	mouse	putative serine/threonine kinase showed restricted expression to myocardial cells and their progenitors	Ruiz *et al.*, 1994

embryo. The data strongly suggest that the field is, by the latter stage, considerably larger than the heart primordium.

The existence of a heart field that is larger than the organ it will form implies that the heart field must normally be partitioned into heart-forming and non-heart-forming areas. This led Armstrong to propose that the size of the heart must be determined by post-inductive dynamics, rather than by the inductive process itself (Armstrong, 1989; Smith and Armstrong, 1991, 1993; Holloway *et al.*, 1994). Extensive computer modeling showed that a two-morphogen "Turing" system could lead to gradients of the appropriate form (Holloway *et al.*, 1994). Based on their work on *Xenopus*, Sater and Jacobson (1990a) proposed a similar model invoking a single diffusible inhibitor produced within the heart field itself. However, models of this type are speculative, and will remain so until the postulated morphogens can be identified and their distribution measured.

When does cardiac induction occur?

Another fundamental question in the study of cardiogenesis is "when does cardiac induction occur?" In most vertebrate systems, cardiac induction appears to begin during gastrulation and continues through neurulation (see review by Jacobson and Sater, 1988). In *Xenopus*, cardiac induction occurs during gastrulation (Sater and Jacobson, 1989). Sater and Jacobson (1990b) found that the dorsal lip of the blastopore (organizer) dorsalizes the deep mesoderm adjacent to it. Because in *Xenopus* it is difficult to separate the deep dorsal anterior endoderm from the prospective heart mesoderm during gastrulation, this endoderm was thought to act as the inducer. This is supported by recent evidence that shows that the dorsal anterior endoderm is essential for heart formation during early gastrulation (Nascone and Mercola, 1995).

Molecular evidence to support the idea that cardiac induction occurs during gastrulation comes from the following studies: *Xenopus* cardiac myosin heavy chain message can be detected by RT-PCR as early as stage 10 and in the heart primordium by stage 13 (Cox and Neff, 1995). Nkx2.5, a homeodomain containing gene (with sequence homology to the *Drosophila* gene, *tinman*) expressed in the heart primordia and adjacent pharyngeal endoderm in the mouse during gastrulation, can be detected by RNase protection by stage 10 (gastrulation) in *Xenopus*, and can be localized to the left and right cardiogenic primordia at stage 15 (neurulation) (Tonissen *et al.*, 1994).

Cardiac induction is also thought to occur during gastrulation in the chick. Cardiac-specific myosin heavy chain expression can be detected in the cardiogenic mesoderm by stage 7 (Bisaha and Bader, 1991). Between stages 4 and 7, the cardiogenic region becomes terminally committed to the cardiomyogenic lineage (Gonzalez-Sanchez and Bader, 1990). Explanted stage 4 cardiogenic mesoderm expresses cardiac-specific genes in the absence of endoderm. However, endoderm is necessary for these explants to initiate contractions (Gannon and Bader, 1995).

Studying cardiac induction during gastrulation presents an experimental problem because other inductions, including mesoderm and neural inductions, occur simultaneously in the same dorsal anterior region of the embryo. Because the urodele develops slowly, the experimenter has the opportunity to view and dissect the multiple sequential induction events individually. This advantage can be fully exploited in the examination of early heart events, especially the cardiac induction processes.

From classical embryological induction studies it was determined that urodele cardiac induction does not occur until after gastrulation, during neurulation (Jacobson and Sater, 1988). For example, one can explant the pre-cardiac tissue (and overlying ectoderm) from open neural plate (stage 14) axolotl embryos and place it in culture (Muslin and Williams, 1991). When cultured alone, it forms epidermis and mesenchyme. When cultured with the anterior pharyngeal endoderm, it forms rhythmically beating cardiac tissue. When pre-cardiac tissue from a later stage axolotl embryo (for example stage 15) is cultured alone, it can form beating cardiac tissue. This induction appears gradual. As the axolotl embryo develops, the percentage of pre-cardiac explants that form beating cardiac tissue gradually increases to almost 100% by stage 20 (Smith and Armstrong, 1990). Thus, urodeles appear to possess delayed cardiac induction in comparison to other vertebrates.

Although the evidence that cardiac induction in urodeles occurs during neurulation is extensive, there is some persistent evidence that indicates that in urodeles cardiac induction may also occur during gastrulation as in other vertebrates. It has been observed that a low but significant percentage of isolated "un-induced" pre-cardiac explants from stage 14 axolotls will form rhythmically beating cardiac tissue (Smith and Armstrong, 1990; Muslin and Williams, 1991). Also, using *Ambystoma punctatum (maculatum)*, Bacon (1945) found that some explants of the lateral blastopore lip developed heart tube formations that exhibited good pulsations (similar to the results seen with *Xenopus*). Further examination of this process awaits molecular analysis of the timing of the expression of cardiogenic mesoderm-specific transcription factors such as the homeodomain containing gene Nkx2.5 in urodeles.

What signal molecules might be involved in cardiac induction?

Understanding cardiogenesis requires identifying and studying the nature of the signaling molecules, the receptors, and the signal transduction pathways involved in cardiac induction and patterning. Because cardiac mesoderm is derived from dorsal mesoderm, potential molecules involved in mesoderm induction may also be involved in cardiac induction. Table 1 lists some of these candidates. Some of these molecules may even be able to form a gradient and therefore act as morphogens. Gurdon *et al.* (1994) recently showed that activin is capable of diffusing through 300 μm of non-responding tissue to affect responding tissues.

Cardiac induction signaling will probably be complex and may well involve multiple (Table 1) signaling molecules which are already know, as well as several as yet undiscovered molecules. This potential complexity is best illustrated in Table 2, which adds reality to this discussion by presenting conflicting evidence for the important role of some of the signaling molecules listed in Table 1.

It is evident from Table 1 that potential cardiac inducers have been identified in urodeles. Muslin and Williams (1991) sought to elucidate possible inducers of cardiac induction. In these studies, they cultured stage 14 axolotl explants with individual growth factors such as Transforming Growth Factor (TGF), Platelet Derived Growth Factor (PDGF), Epidermal Growth Factor (EGF), and Fibroblast Growth Factor (FGF) (see Table 1). They found that TGF>> PDGF> EGF individually increase the frequency of explants forming rhythmically beating cardiac tissue while FGF decreased the frequency of beating tissue formed (alone or in combination

with TGF). Also, activin A induced beatings hearts in explanted axolotl pre-cardiac mesoderm (Mangiacapra *et al.*, 1995). Thus, one signal molecule may not be responsible for this induction process. Alternatively, related TGF peptides could bind to the same receptor, or up-regulate another member of the family (e.g., van der Kruijssen *et al.*, 1993). The result that FGF may inhibit the induction event indicates that inhibitors may be involved.

The analysis of molecules involved in this induction pathway is complicated by explant studies involving co-culturing of pre-cardiac mesoderm and neural tissue. In these studies, culturing the urodele pre-cardiac mesoderm with tissue from the adjacent neural folds and plate results in a decrease in the frequency of cardiac tissue formation in the explants (reviewed by Jacobson and Duncan, 1988). It should be reiterated that during the cardiac induction process, the pre-cardiac mesoderm migrates away from the neural folds. Thus, several components may be involved in the cardiogenic commitment during the developmental window in axolotls of stage 15-20. Axolotls can be used to dissect the various elements of this process as each stage can be viewed and manipulated individually. Currently we are using Differential Display PCR (Liang and Pardee, 1992; McClelland *et al.*, 1995) to compare axolotl un-induced pre-cardiac tissue (stage 14) gene expression to induced pre-cardiac tissue (stage 18) gene expression. Several induced pre-cardiac tissue-specific cDNAs have been isolated and sequenced. None show nucleotide homology to known genes. Further characterization of the isolated cDNAs continues.

What mutations affect cardiac development?

The "cardiac non-function" mutation (cardiac lethal gene c) results in incomplete differentiation and non-beating heart in homozygous recessive axolotls. Analysis of myofibrillar proteins in the cardiac mutant strongly suggests that some component necessary for assembly of functional sarcomeric myofibril is defective or missing (e g., tropomyosin), but does not yet firmly establish the nature of the defect (Lemanski *et al.*, 1980; Starr *et al.*, 1989; La France and Lemanski, 1994). This mutation appears to affect the induction of terminal cytodifferentiation of the heart but not organogenesis and therefore not early induction of the heart. Cardiac mutant hearts are phenotypically similar to normal hearts until the heartbeat stage (Lemanski, 1973). Wild-type pre-heartbeat stage, 30 anterior endoderm (Lemanski *et al.*, 1979) or RNA extracted from it (Davis and Lemanski, 1987) can rescue similar or later stage cardiac mutant hearts. Cardiac mutant endoderm can induce neurula stage wild-type cardiogenic mesoderm explants to form beating hearts (Smith and Armstrong, 1991). However, neurula stage 14 pre-cardiogenic cardiac mutant explants are not rescued by anterior endoderm (Smith and Armstrong, 1991), RNA-containing extracts similar to those of Lemanski (Smith and Armstrong, 1990), or growth factors such as activin A and TGF-ß (Mangiacapra *et al.*, 1995).

Aside from the work on the cardiac lethal gene c, little genetic analysis of cardiac development has been conducted in urodeles. Because molecular level discoveries made in one system can readily be incorporated into another, the heart development mutants being discovered in the zebrafish (Stainier and Fishman, 1994) and *Drosophila* (Bodmer, 1995) may provide a gateway into the molecular analysis of early cardiogenesis in urodeles. In *Drosophila* embryos lacking *twist* or *snail*, two genes involved in the establishment of the dorsal ventral axis, the presumptive mesoderm does not differentiate and the heart does not form. The gene *tinman* [Nkx homologs in *Xenopus* (Tonissen *et al.*, 1994) and in the mouse (Lints *et al.*, 1993)] may act in response to *twist*. In *tinman* mutants, no visceral or cardiac mesoderm forms, but the somatic mesoderm forms normally. Genes encoding signaling factors involved in regulating segment polarity may also play a role in heart formation. Loss of the function of the segmentation polarity gene *wingless* during the early phases of mesoderm formation in *Drosophila* affects the determination of the heart precursor cells (*tinman*-expressing cells) (Wu *et al.*, 1995). Thus genes involved in axis formation and consequential mesoderm patterning may directly affect cardiogenesis.

Does the amphibian heart regenerate?

Among vertebrates the urodele amphibians are the champions at wound healing and regeneration. They, like other vertebrates, are able to repair a wound by sealing the wound and by healing the damaged structure by fibrosis to prevent tissue fluid loss and infection. In addition, like other vertebrates, they are also able to repair selected tissue by limited regeneration using reserve stem cells such as osteoblasts during bone repair and satellite cells during skeletal muscle repair. However, it is the urodele's capacity for epimorphic regeneration, a process where a portion or a complete structure or organ, such as an appendage, is replaced through the formation of a transient blastema, that they stand out among all vertebrates. The high regeneration ability of urodeles provides a valuable model system to gain basic information on regeneration that may be transferable to human trauma and diseases that cause damage to such structures as the limbs and the nervous and cardiovascular systems. Because of the high incidence of heart disease that results in permanent damage to the cardiac tissue, there is currently considerable interest in the search for a method to repair damaged heart tissue. Urodeles, because of their high regenerative capacity, provide a potential model system to study heart regeneration.

The general observation that heart muscle has no reserve satellite cells (Mauro, 1979) and that adult vertebrate cardiomyocytes are terminally differentiated has historically been utilized to account for its response to stress (e.g., increased chronic hemodynamic load) by cell enlargement (hypertrophy) rather than cell proliferation (hyperplasia) and therefore for the inability of heart tissue to regenerate damaged muscle. The idea that adult cardiomyocytes are terminally differentiated cells is, however, currently being questioned (e.g., Nag and Cheng, 1981; Rumyantsev, 1981; Quaini *et al.*, 1994). Several relevant considerations follow: First, it should be noted that in contrast to developing skeletal muscle, differentiating embryonic, fetal, and neonatal cardiomyocytes can proliferate (Manasek, 1968; Rakusan, 1984). This may be because cardiac muscle develops earlier than skeletal muscle and therefore becomes functional earlier. Thus, the heart must grow and function simultaneously. In addition, differentiating embryonic cardiomyocytes are mononucleated, while differentiating skeletal muscle cells are multinucleated. This implies that the differentiation/proliferation control differs between skeletal and cardiac myocytes. Even though heart and skeletal muscle express many similar sarcomeric proteins, these proteins appear to be regulated by common as well as by different transcription factors. This idea is supported by the observation that the basic helix-loop-

TABLE 2

EXAMPLES THAT QUESTION THE ROLE OF SOME OF THE SIGNALING MOLECULES LISTED IN TABLE 1 IN CARDIOGENESIS

Molecule	Species	Comments	Reference
activin,activin receptor follistatin	mouse	not essential for mesoderm and also heart formation in the mouse (transgenic knock-out mutants)	Matzuk *et al.*, 1995a,b,c
activin-A	axolotl	did not induce beating heart in explanted precardiac mesoderm	Muslin, 1992
FGF-2	axolotl	inhibited TGF-ß1 induction of beating hearts in neurula stage precardiac mesoderm explants	Muslin and Williams, 1991
FGF-2	*Xenopus*	induced the expression of cardiac a actin but not XMHC-α in animal pole explants	Logan and Mohun, 1993
insulin	axolotl	did not induce beating hearts in explanted precardiac mesoderm explants	Muslin, 1992

helix (bHLH) myogenic regulatory factors (MRF) (e.g. MyoD and Myf5) that are involved in skeletal muscle determination and differentiation have not been found in cardiac muscle. The detailed discussion of the similarities and differences in gene regulation between cardiac and skeletal muscle is beyond the scope of this review; the reader is referred to several recent reviews of this subject (e.g., Olson, 1993; Duprey and Lesens, 1994; Skerjanc and McBurney, 1994; Kern *et al.*, 1995). The key point is that cardiomyocytes have a developmental history of simultaneous differentiation and proliferation. Therefore, they may have an intrinsic potential for reentering the cell cycle in order to repair a damaged heart.

Second, it is generally accepted that urodele cardiac muscle can repair and/or regenerate. Mammalian cardiac muscle does not regenerate but responds to damage to the myocardium by necrosis and repair of the remaining myocardium by non-contractile fibrosis (scar tissue) and compensatory hypertrophy of the remaining myocardium. In contrast, cardiac muscle of urodeles is able to repair/regenerate considerable heart damage. Newts can survive the excision of 30% to 50% of the heart ventricle (Oberpriller and Oberpriller, 1974). This repair/regeneration process involves, adjacent to the damaged area, dedifferentiation of remaining cardiac muscle, loss of intercalated discs, followed by DNA synthesis and mitosis of cardiomyocytes and connective tissue cells (Oberpriller and Oberpriller, 1974). In addition, there is a limited proliferative response to the ventricular damage in the atria (McDonnell and Oberpriller, 1983). However, the regenerative response in the injured ventricle is limited; the defect is filled in with a fibrous scar that includes a limited number of differentiated cardiomyocytes (Oberpriller and Oberpriller, 1974). Although this repair and/or regeneration process results in a repaired functioning heart, it does not involve a typical regeneration blastema or the complete regeneration of the missing ventricle. It therefore is not a result of epimorphic regeneration. Rather, it represents an efficient repair mechanism with the involvement of proliferating cardiomyocytes in the repair response. Consistent with the *in vivo* data, *in vitro* cultured, differentiated ventricular cardiomyocytes from adult newts are able to synthesize DNA and undergo mitosis and cytokinesis (Nag *et al.*, 1979; Tate and Oberpriller, 1989; Soonpaa *et al.*, 1994; Matz *et al.*, 1995; mitotic differentiated newt cardiomyocytes divided to give rise to mononucleated daughters 80% of the time). These observations are consistent with the idea that adult urodele cardiomyocytes are not terminally differentiated (can never reenter the cell cycle) in that they can still proliferate under appropriate circumstances.

Third, recent evidence has emerged that mammalian cardiomyocytes are also not terminally differentiated, and cardiomyocyte hyperplasia may constitute a reserve mechanism for adaptation to extreme overload stress or disease. Quaini *et al.* (1994) observed diffuse proliferating-cell-nuclear-antigen (PCNA) expression in approximately 50% of left ventricular cardiomyocytes, and incidence of mitotic divisions in patients with end-state cardiac failure. Hyperplasia of cardiomyocytes has also been documented in the rat heart subjected to coronary artery narrowing-induced overload stress (Kajstura *et al.*, 1994). These recent data support the sparse but persistent evidence that adult mammalian cardiomyocytes are also not terminally differentiated, and under certain conditions can be coaxed to re-enter the cell cycle and divide.

These observations call for an intensive investigation into the regulatory mechanisms that keep cardiomyocytes quiescent in adult hearts and into signaling pathways that are involved in their proliferative response to damage or overload. Undoubtedly some of these experiments will be done *in vitro* with explanted cardiomyocytes and in cardiomyocytes altered transgenically to proliferate *in vitro* (e.g., Daud *et al.*, 1993) and *in vivo* (e.g., Jackson *et al.*, 1990; Gruver *et al.*, 1993). However, repair/regeneration of heart involves more cells then cardiomyocytes (e.g., fibroblasts, etc.). Mammalian systems, because of their high metabolic rates and their intolerance for heart damage, are limited in their usefulness for *in vivo* studies. Urodeles, because of their lower metabolic rates (depending on temperature), their less complex heart structure (e.g., no arteries in the ventricle of the newt (Bader and Oberpriller, 1979), their tolerance for experimental manipulations such as mincing the myocardium (Oberpriller *et al.*, 1988), and their efficient heart injury repair, provide a valuable model system to study heart repair/regeneration *in vivo*. In addition, the analysis of hyperplasia in regenerating heart experiments is easier to interpret in the urodele because in contrast to mammals where a large percentage of the adult cardiomyocytes are binucleated (Katzberg *et al.*, 1977) or tetraploid (Zak, 1974), most urodele adult cardiomyocytes are mononucleated (e.g., in the newt 98.3% – Oberpriller *et al.*, 1988).

Viable areas of research are dedifferentiation of cardiomyocytes, remodeling of the heart extracellular matrix, growth factor regula-

tion of both cardiomyocyte and fibroblast cell proliferation, and differentiation. Interestingly, the cytokine TGF-ß which appears to be a key player in heart induction may also be a key player in heart regeneration. Considerable evidence shows that TGF-ß is involved in the initiation and termination of the tissue repair that results in tissue fibrosis (reviewed by Border and Noble, 1994). These important areas for research in the urodele system have the potential to provide basic information that could be used to induce and control heart repair in mammals favoring hyperplasia of cardiomyocytes over fibroblasts and resulting in replacement of the damaged area with functional heart muscle rather than with a fibrous scar.

Conclusion

We may reasonably expect that the regulation of the formation of a functional heart is going to be similar in complexity as the regulation of the formation of other tissues and organs such as skeletal muscle and brain. Many of the events, from cardiac induction through cardiogenesis, will probably involve repeated use of known signaling molecules and receptors. In addition, these events may be recapitulated during heart regeneration. Because of the apparent similarity of heart formation among vertebrates, embryological discoveries made in one system can be extrapolated to another. At the molecular level, the boundary between experimental systems is becoming transparent. Discoveries made in one system can be readily adapted to another. This is fortunate, for no single system is ideal for working out all of the biochemical pathways involved in cardiac induction and cardiogenesis. With respect to doing functional studies, such as gene knockouts, it is advantageous to utilize systems that are "genetically friendly." However, researchers have been humbled by the fact that some knockouts of presumably important regulatory genes have, disappointingly, yielded no phenotypes. These results imply that these regulatory genes frequently function in complex, often redundant, biochemical pathways. In light of the above, we feel that the "embryologically" friendly urodele system can make a substantial contribution to our understanding of cardiac induction, cardiogenesis, and heart regeneration, especially at the molecular level.

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