Cardiovascular regeneration in non-mammalian model systems: What are the differences between newts and man?

Thilo Borchardt, Thomas Braun
Department of Cardiac Development and Remodelling, Max-Planck-Institute for Heart and Lung Research, Bad Nauheim, Germany

Summary
The mammalian heart cannot regenerate substantial cardiac injuries, while certain non-mammalian vertebrates such as certain fish (Danio rerio) and amphibiae (Notophthalmus viridescens) are able to repair the heart without functional impairment. In mammals, the prevailing repair process is accompanied by fibrosis and scarring, while zebrafish and newts can replace lost contractile tissue by newly formed cardiac muscle with only little or no scar formation. A better understanding of cardiac regeneration in non-mammalian vertebrates might provide new insights for the manipulation of regenerative pathways in the human heart. Here, we summarize the current knowledge in cardiac regeneration of newts and the principal differences to repair processes in mammalian hearts.

Keywords
Newt, cardiac muscle, regeneration, dedifferentiation, proliferation

Introduction
The mammalian heart shows only little capability to regenerate. Instead, substantial injury of the myocardium results in loss of contractile tissue, fibrosis and scar formation. Although numerous approaches have been made to improve the regenerative capacity of mammalian hearts (1–5) clinical trials could not unequivocally demonstrate major improvements of cardiac function in human patients (6–8). Among vertebrate species certain organisms own exceptional regenerative potencies (9, 10). Zebrafish (Danio rerio, order: teleostei, family: cyprinidae) and newts (Notophthalmus viridescens, order: caudata, family: salamandridae) can rebuild functional myocardium after partial amputation or mechanical damage with only little or no scar formation. In Zebrafish, up to 20% of the ventricle can be regenerated after amputation within two months (11, 12). Newts can regrow large parts of the ventricular myocardium after amputation or mechanical damage (13–15). It seems reasonable to assume that a better understanding of the molecular processes of heart regeneration in zebrafish and newts will generate new perspectives to develop alternative approaches to stimulate regenerative pathways in mammalian hearts. Here, we focus on the current understanding of heart regeneration in newts and how it differs from the mammalian response to cardiac injury.

Proliferative potential of mammalian cardiomyocytes
Several classical studies have reported a restricted proliferative potential of cardiomyocytes in adult mammals (16–19). Hence, it seems very unlikely that cardiomyocyte proliferation contributes significantly to heart regeneration (17, 20–22). In fact, most cardiac injuries result in massive loss of contractile tissue, which is mainly replaced by scar tissue further demonstrating the limited regenerative ability of mammalian hearts. Despite the obvious restriction of the proliferative capacity of the myocardium, cardiomyocytes might be considered as ideal donor cells for replacement of lost contractile tissue, since cardiomyocytes already possess all necessary properties for efficient integration into resident myocardium. Functional coupling of individual transplanted cardiomyocytes has been demonstrated both by visualizing intercalated discs and gap junctions between donor and recipient cardiomyocytes (23, 24) and by measurements of calcium transients in neighbouring cardiomyocytes (25). Other approaches that utilize cardiomyocytes for therapeutic purposes are based on the forced re-entry of cardiomyocytes into the cell cycle by genetic manipulation or exogenously added factors (2, 21). These data provide evidence that cardiomyocyte cell cycle re-entry can be enhanced in combination with

Theme Issue Article

Received February 27, 2007
Accepted after revision May 28, 2007
Prepublished online July 6, 2007
doi:10.1160/TH07–02–0153

Correspondence to:
Prof. Dr. Thomas Braun
Department of Cardiac Development and Remodelling
Max-Planck-Institute for Heart and Lung Research
Parkstrasse 1, D-61231 Bad Nauheim, Germany
Tel.: +49 6032 705402, Fax: +49 6032 705419
E-mail: thomas.braun@mpi-bn.mpg.de
exogenously added factors in genetically modified animals. Some of these studies also suggested that it is possible to overcome functional defects that resulted from cardiac injuries (2, 26, 27). It cannot be excluded, however, that some of the beneficial effects that resulted in cardiomyocyte survival and positive ventricular remodelling were caused by indirect means possibly through paracrine effects, since none of the transplantation studies could demonstrate a comprehensive integration of transplanted cardiomyocytes in recipient myocardium (28, 29). One of the major drawbacks in all transplantation studies is the poor engraftment of cardiomyocytes into host tissue. It is therefore of major importance to develop methods that increase volume and stable integration of grafted cells along with an enhancement of donor cardiomyocyte survival.

Contribution of extra-cardiac cells to heart repair in mammals

The claim that the mammalian heart has some limited regenerative potential is partially derived from transplantations in which hearts from gender mismatched individuals were found to harbour cells of recipient origin (30, 31). Several studies have reported a wide variety of the extent of myocyte chimerism (32–36) ranging from 0.04% to even 10% of total cardiomyocytes. Although it is accepted by most researchers that chimerism occurs only to a low extent, the presence of extracardial cells in the heart triggered several studies to mobilize non-resident stem cells or to transplant bone marrow derived cells to the site of injury.

Clinical trials to enhance homing of stem cells to sites of cardiac injuries reported different degrees of successful recruitment and improvement of heart function (37–41). It soon became obvious that this type of intervention does not give rise to efficient regenerative growth of the injured myocardium. Initial findings proposing that transplantation of haematopoietic stem cells leads to rapid and efficient regeneration of infarcted hearts (42) were not confirmed by other investigators (43–45). Nevertheless, a series of randomized controlled clinical trials where initiated based on Sca-1 expression. Sca-1 positive cells did not express some of the cardiac side population cells began to express the mature cardiac phenotype. Genetic fate mapping studies (65) demonstrated that isl1-positive cells from the secondary heart field might contribute to smooth muscle, endothelia and working myocardium. It should be kept in mind, however, that this type of intervention does not give rise to efficient regeneration of infarcted hearts. Moreover, the limited capacity of the human heart to give rise to new cardiomyocytes highlights the restricted differentiation capability of heart "progenitor" cells and emphasizes the importance of secreted factors to modulate repair and remodelling processes in the heart.

Resident heart progenitor cells

Several recent studies have identified resident heart stem cells with an apparent cardiomyogenic activity within the adult heart. It has been proposed that such cells might participate in the continuous replacement of apoptotic cardiomyocytes at a low basal level. Beltrami et al. (58) isolated cells from the adult rat heart that expressed c-kit. They reported that these cells differentiated into cardiomyocytes, smooth muscle cells, and vascular endothelium leading to a replacement of the majority of infarcted tissue although the morphology of newly formed cardiomyocytes was distinct from mature cardiomyocytes. The same authors also demonstrated a significant improvement of ventricular function after engraftment of c-kit positive cells into diseased hearts. More recently Dawn et al. (59) reported that, after intra-coronary administration, these cells traversed the vascular barrier and improved ventricular function in a rat infarct model.

Another putative progenitor cell population was described by Oh et al. (60, 61), who isolated cells from the adult mouse heart based on Sca-1 expression. Sca-1 positive cells did not express early cardiac markers, like Nkx 2.5, but could be induced in vitro to express cardiac marker genes after treatment with 5’aza-cytidine. Subsequent intravenous injection of Sca-1+ cells into infarcted hearts demonstrated that, two weeks later, engrafted donor cells expressed cardiac markers, such as sarcomeric actin and troponin I. Cre-Lox recombination studies indicated that approximately one half of the donor-derived cells had fused with host cardiomyocytes and the other half had differentiated without fusion.

A third progenitor population was described by Hierlihy et al. (62), who isolated Hoechst dye-effluxing side-population cells from the adult mouse heart, characterized by expression of the ATP-binding cassette transporter Abcg2 (63). They reported that some of the cardiac side population cells began to express the sarcomeric protein alpha-actinin when co-cultured with unfracticated cardiac cells.

The most recent addition to the group of potential progenitor cells are cells expressing the LIM-homeodomain transcription factor islet-1 (isl1). Laugwitz et al. (64) demonstrated that isl1-positive cells were present in the neonatal and – to a lesser extent – in adult hearts. Co-culture studies with neonatal myocytes induced differentiation of isl1-positive cells into cells displaying a mature cardiac phenotype. Genetic fate mapping studies (65) demonstrated that isl1-positive cells from the secondary heart field might contribute to smooth muscle, endothelia and working myocardium. It should be kept in mind, however,
that these studies were mostly performed in vitro using co-cultures of isl1-NlacZ-positive cells from embryoid bodies with cardiac mesenchymal cells. Clonal cell populations were isolated characterized by the expression of isl1/Nkx2.5/flk1 that might serve as common progenitor for all three heart lineages. The potential of such ES-cell-derived precursors for therapeutic approaches seems promising if these cells can be produced in sufficient amounts and if they are able to functionally replace damaged heart tissue. Careful in-vivo studies, however, which would prove this concept are still pending.

At present, all resident cardiac progenitor cells seem to be distinct from each other, as exemplified by the differential expression of these markers in individual cell populations. It appears unlikely that an organ known for its lack of regenerative capacity harbours multiple non-overlapping sets of cardiomyocyte progenitors, raising doubts about the physiological function of such cells. Clearly, these cells do not function as robust progenitor cells after major tissue damage in vivo. If heart progenitor cells are indeed involved in the generation of cardiomyocytes in adult animals, it seems likely that they contribute to tissue maintenance at a rather slow turn-over rate rather than to regeneration processes.

In summary it is fair to conclude that mammalian organisms do not own the resources to effectively regenerate their hearts after cardiac injuries, although multiple types of cells exist in the heart that might be involved in the homeostasis of myocardial tissue and in remodelling processes. On the other hand, the finding that cardiac growth during prenatal (but not postnatal) development occurs mainly via division of embryonic cardiomyocytes (18, 22, 66) implies that a molecular program exists in mammals that enables cardiac cells to repeatedly re-enter the cell cycle.

Proliferative potential of newt and zebrafish cardiomyocytes

Newts can survive large excisions of the heart ventricle (13, 15, 67) or major mechanical damage (68). Cardiomyocytes of the uninjured heart are virtually non-proliferative (69) (Kostin, Borchardt, et al., unpublished observations), but respond to injury with a massive increase of DNA synthesis and mitosis (13, 15, 67, 69). This proliferative response has also been observed for atrial myocytes after ventricular damage (70) or direct damage of newt atria (71). Depending on the type of injury and regenerative stages, up to 30% of cardiomyocytes near the site of injury initiate DNA synthesis. About 10% of cardiomyocytes near the site of injury undergo mitosis. At later stages of regeneration more than 80% of all mitotonically active cells appear to be cardiomyocytes (13, 15, 67).

Cardiomyocytes in uninjured newt ventricles are mostly (98%) mononucleate and diploid. At later stages of regeneration approximately 50% of cardiomyocytes have undergone one round of DNA synthesis, and around 90% of them remain mononucleate and diploid (69), suggesting that most cardiomyocytes complete mitosis and cytokinesis after DNA synthesis. The response of atrial cardiomyocytes to injury is less pronounced although a considerable number of myocytes undergo DNA synthesis and subsequent mitosis. Only recently the regenerative capacity of zebrafish hearts has been discovered (11, 12). Zebrafish can regenerate up to 20% of ventricular mass after amputation. The number of cardiomyocytes, which start to synthesize DNA seems similar to newts, although mitotic cardiomyocytes have been observed only rarely in regenerating zebrafish hearts (11, 12).

Cultured cardiomyocytes from zebrafish can be stimulated to induce DNA synthesis upon treatment with recombinant PDGF-BB homodimers, although mitotic events were not observed in vitro experiments (72). Thus it seems that adult newts are the only adult vertebrate model system that allows growth of actively proliferating cardiomyocytes in vitro. In organ slice cultures from newt heart (73, 74) or dissociated primary cultures (75), 30% of ventricular cardiomyocytes responded with DNA synthesis and up to 9% of myocytes showed mitotic activity. Consecutive studies (76) demonstrated that 80% of cultured mononucleated cardiomyocytes produced mononucleated daughter cells and 20% binucleated progeny. With increasing culture time, the level of binucleated and multinucleated cells increased. Apparently, the age of the cultured cardiomyocytes leads to accumulation of bi- and multinucleated progeny, since non-mononucleate myocytes have already undergone at least one round of replication. DNA synthesis was stimulated more than two-fold when cultures were fed with conditioned media of older cardiomyocyte cultures (77). The same effect was observed when conditioned media of non-cardiomyocyte cultures, derived from the initial preplating steps, were used indicating an intense intercellular communication via soluble factors (77).

Several exogenously added factors have been tested for there ability to affect the proliferative response of cultured myocytes (77). TPA, retinoic acid, acidic FGF (FGF-1), ET-1, PDGF, bomsen and basic FGF (FGF-2) could stimulate DNA synthesis whilst thyroxin, TGF beta and heparin strongly inhibited DNA synthesis of cardiomyocytes. A more recent study has addressed the plasticity of newt cardiomyocytes in culture (78). Time lapse imaging of individual cells revealed that more than 75% of cardiomyocytes synthesizing DNA also underwent mitosis. About 29% of all imaged cells successfully completed one or more rounds of cell division giving rise to beating daughter cells. Although a substantial portion of all myocytes produced binucleated and multinucleated daughters almost one fifth of multinucleated progeny subsequently entered S-phase and completed mitosis and cytokinesis, which clearly demonstrated that formation of a multinucleated cell does not necessarily preclude further proliferation.

The ability of newt cardiomyocytes to proliferate proficiently in culture but not in normal hearts is mainly mediated by mammalian serum components (78). The absence of fetal bovine serum (FBS) inhibited DNA synthesis of cardiomyocytes, whereas the administration of 10% FBS stimulated DNA synthesis in 30% of cardiomyocytes in culture. These results led to the hypothesis that a serum-activated pathway results in the phosphorylation of Rb-protein mediating cell cycle progression (79). In fact, inhibition of Rb phosphorylation through p16INK4 expression in cardiomyocytes caused a 13-fold inhibition of S-phase re-entry of cardiomyocytes compared to uninjected cells (78). A better understanding of the molecular mechanisms re-
sponding to these factors may help to understand the pathways leading to cardiomyocyte proliferation.

How do newt and zebrafish hearts regenerate?

The sequence of events following amputation of the newt ventricular apex was originally described by Oberpriller et al. (15). The cardiac ventricle of the newt consists of trabeculae, in which a single layer of epicardial cells envelopes myocytes, fibroblasts and nerve fibres. Upon amputation of the ventricular apex the initial wound is immediately sealed by a blood clot. After one week the clot is dissolved, and lymphocytes accumulate at day 10 at the margin of the clot. At this time point, first mitotic events in the trabeculae adjacent to the wound appear. At two weeks after amputation macrophage activity is observed within the blood clot, and a thin layer of epicardial cells seals the inner surface of the clot. At this time point, necrosis in adjacent trabeculae disappears. At the third week connective tissue fibres appear within the wound area and mitosis in trabeculae increases concomitant with the first appearance of myofibrils within trabecular structures near the wound. After four weeks, the wound area is mainly composed of connective tissue that extends into the wound, which contains only scattered myocytes, which contain smaller myofibrils than those found in uninjured animals. Oberpriller et al. therefore concluded that regeneration in the newt heart does not occur completely and results in a semi-functional tissue containing a mixture of contractile and residual connective tissue.

To increase the reactive area of the wound, the ventricular apex was minced in some experiments and placed onto the amputated ventricle (13, 67). By extending the observation period to up to 70 days the authors were able to observe a proliferative phase of partially dedifferentiated cardiomyocytes during the second and third week after injury. Finally, the grafts reorganized into functional miniventricles consisting primarily of cardiac muscle. Seventy-day-old grafts contained myocytes of uninjured ventricles carrying numerous myofibrillae, mitochondria, glycogen and intercellular junctions, demonstrating that newt hearts can effectively regrow organized cardiac muscle.

We reinvestigated heart regeneration in the newt by using a mode of injury that maintains the outer morphology of the newt ventricle (68). By mechanical disruption of the ventricular myocardium using repeated squeezing by fine forceps large parts of the inner ventricular mass were disorganized. We observed a complete functional reorganization of the trabeculated structure within 10–12 weeks after injury without major remnants of connective or scar tissue (Fig. 1). We concluded that the newt heart is able to fully regenerate its heart under conditions reminiscent of infarcted or inflammatory loss of contractile tissue. A similar sequence of events was also observed upon amputation of zebrafish ventricular tissue (11, 12). A dense fibrin clot is formed within one day after amputation, which is further compacted, sealing the initial wound area within one week. After one month the amputated part is completely replaced by a transiently hypertrophied compacted zone of regenerating tissue, which returns to normal size around two months after amputation when regeneration is completed (80).

Applying the cardiac injury model of mechanical damage, a large number of rapidly de-differentiating cardiomyocytes in the wounded area of newt hearts was observed. Within one day after injury, the expression of the cardiac markers alpha-myosin heavy chain and cardiac troponin T disappeared. These markers of mature cardiomyocytes were re-expressed within the regenerate only at later stages of regeneration. Similar changes were observed at the RNA level by semi-quantitative RT-PCR, demonstrating that the expression of several cardiomyocyte markers is strongly downregulated at early stages of regeneration (68). Whether dedifferentiation of cardiomyocytes is a necessary prerequisite for proliferation in vivo remains to be determined. Although cardiomyocytes maintain a limited proliferative potential in culture without the loss of sarcomeric proteins (68, 78, 81), de-differentiation of cardiomyocytes might enhance the proliferation potential of cardiomyocyte-derived cells dramatically and may thus enable the newt heart to repair even extensive damages.

Why do newt and zebrafish hearts regenerate and mammalian hearts do not?

At present there is no evidence that adult stem cells within the heart may also contribute markedly to regeneration in the newt. In zebrafish a cardiac progenitor-mediated regenerative mechanism has been proposed. Lepilina et al. (82) provided evidence that newly formed myocardial cells arise from undifferentiated progenitor cells. Lepilina et al. utilized double-transgenic zebrafish, in which two fluorescent proteins with different folding properties and stability were expressed under the control of the cardiac myosin light chain 2 promoter (cmic2) (83). EGFP ex-
pression was detected at an early stage due to its fast folding properties, while RFP, which has a longer half-life due to its higher stability and slower folding kinetics, appeared only later. At seven days after amputation the authors detected a front of EGFP+/RFP+ cells at the apical edge of the regenerate and hence concluded that newly formed cardiomyocytes arise from undifferentiated cells. The up-regulation of early myocardial markers like nkx2.5, hand2 and tbx20 in cells at the apical edge further supported the conclusion that these cells constitute the source of newly formed cardiomyocytes. In another study (12) early cardiac markers like nkx2.5, tbx5 and CARP were not found in the regenerating myocardium, suggesting that new cardiomyocytes are not derived from undifferentiated precursor cells but from de-differentiating cardiomyocytes. The absence of RFP+/EGFP- cells, however, which would indicate inactivation of cmlc2 expression, implied that the proposed progenitor pool is not derived from dedifferentiating cardiomyocytes (82). At present it is not completely clear whether heart regeneration in zebrafish occurs solely via activation of a progenitor cell population or via dedifferentiation of cardiomyocytes. The use of a dual-transgenic EGFP/RFP system does not allow to monitor rapid de-differentiation of cardiomyocytes, which has been clearly demonstrated for newt cardiomyocytes transplanted into regenerating newt hearts and limbs (68). The conflicting findings for regenerating zebrafish and newt hearts clearly ask for accurate cell tracing experiments and a careful expression analysis of early cardiac markers, which might be found both in differentiating progenitor cells or in de-differentiating cardiomyocytes.

Interestingly, extensive DNA synthesis was observed in the entire epicardium of zebrafish hearts within 1–2 days after partial ventricular amputation accompanied by re-expression of embryonic epicardial markers like raldh2 and tbx18 (82). At later stages of regeneration epicardial cells invaded the myocardium, leading to the speculation that invading epicardial cells might play a role in establishing new blood vessels in regenerating zebrafish hearts. This process seems reminiscent of the epicardial to mesenchymal transition during mammalian heart development (84, 85). Surprisingly, such a type of epicardial invasion has not been observed in regenerating newt hearts, since newts lack an intracardial capillary system. It remains to be determined whether zebrafish and newts follow different strategies for myocardial regeneration or whether observed differences have different causes. Further studies will help to determine similarities and differences of heart regeneration in various non-mammalian vertebrates.

Numerous studies and observations in different regenerating organ systems of the newt including appendages and lens (10, 86) favour the view that regeneration in the newt is mostly accomplished by the generation of new progenitors through dedifferentiation of mature cells. These proliferating progenitor cells later redifferentiate to form all cell types necessary to rebuild lost or damaged organs. Further evidence for this hypothesis comes from studies of fluorescently labelled cardiomyocytes that were heterotopically transplanted into intact and regenerating limb buds of the newt (68). Transplanted cardiomyocytes retain their original morphology and expression pattern, when implanted into intact newt limbs. However, cardiomyocytes rapidly downregulated expression of cardiac maker genes and activated the expression of genes characteristic for undifferentiated blastema cells when implanted into a limb blastema five days after amputation. At later stages the expression of blastema markers disappeared, and cardiomyocyte-derived cells started to express markers of mature skeletal muscle. Moreover, the transplanted cells formed small muscle fibres that were associated with endogenous muscle tissue. In addition, Dil-labelled cardiomyocyte-derived chondrocytes were found at different planes in regenerating limbs. Transplanted cells did not only transdifferentiate robustly into cell types of the recipient organ, but also expressed phosphorylated Histon-H3, indicating that transplanted cells actively contributed to limb regeneration by proliferation. These findings demonstrated a remarkable plasticity of cardiomyocytes and favour the model that cardiac regeneration in newts is based on the capability of mature cardiomyocytes to de-differentiate and to form cardiomyocyte progenitor cells. Furthermore, cardiomyocytes seem to depend on the presence of factors in damaged tissues that provide the necessary cues for dedifferentiation and re-programming. It remains to be seen which types of cells other than cardiomyocytes in the newt contribute to an efficient regeneration of the heart. This degree of cellular plasticity is not observed in adult mammals and might explain some differences in regenerative abilities between salamanders, zebrafish and mammals.

Several factors have been demonstrated to promote cell cycle re-entry and dedifferentiation of newt myotubes. A thrombin-activated factor that is present in the serum of several species has been proposed to serve as an effector for urodele myotubes to re-enter the cell cycle by phosphorylation of the Rb-protein, thereby providing a link between the reversal of differentiation and acute events of wound healing (79, 87, 88). Furthermore, Mxs1 (89), matrix metalloproteinases (90), hepatocyte growth factor (91), and the complement factors C3 and C5 (92) have been implicated in regeneration in amphibians. Interestingly, McGann et al. (93) described an activity derived from tissue extracts of the blastema that was able to induce dedifferentiation of newt and mouse myotubes. Unfortunately, other groups have not confirmed these results, and the nature of the molecules that might induce dedifferentiation of mammalian cells is still enigmatic.

Zebrafish with mutations in the Mps1 mitotic checkpoint kinase, a critical cell cycle regulator, failed to regenerate hearts and formed scars (11). In addition, evidence exists that PDGF signalling is required for heart regeneration, since treatment of cultured zebrafish cardiomyocytes with recombinant PDGF-B induced DNA synthesis, whereas treatment with a chemical inhibitor of PDGF signalling decreased DNA synthesis in cardiomyocytes in vivo by around 16% (72). In animals, in which Fgf receptor mediated signalling was inhibited after ventricular resection, myocardial regeneration was arrested 14 days after amputation. This defect was associated with a failure of epithelial to mesenchymal transition of epicardial cells and neovascularization of the regenerate (82), generating further evidence that the epicardial response and formation of coronary vessels are required for regeneration of zebrafish hearts.

Finally, the question remains why reprogramming of mammalian cells is rarely observed in physiological settings. It is tempting to speculate that evolution has selected against de-dif-
ferentiation and trans-differentiation for several reasons. First, de-differentiation within damaged tissue might cause an additional, transient decrease in the number of functional cells. Second, de-programming of cells is an additional step that will yield dysfunctional cells that might interfere with the function of certain organs. Third, de-differentiation of working cells will lead to the mobilization of cells that have already encountered many potentially hazardous conditions. Unlike stem cells, which are usually kept aside in well-protected niches, “working” cells might have collected mutations both in the nuclear and mitochondrial DNA, and carry otherwise dangerous cargo. Any future approach to utilize dedifferentiation for therapeutic purposes must take this into account and aim for a careful selection of appropriate cells.

Conclusions

Urodole amphibians and zebrafish provide powerful model systems to gain basic information on pathways that direct heart regeneration. A better understanding of the molecular signals that control heart regeneration in newts and zebrafish might also give way for heart regeneration in mammals. The zebrafish system already contains a large toolbox for forward and reverse genetic screens as reviewed in (94–96). Further technical advances that allow conditional expression of genes (97) or ablation of specific cell populations in zebrafish (98) will further increase the versatility of this model system and will help to gain further knowledge about regenerative pathways in vertebrate organisms. With the advent of transgenic newts and axolotls (99, 100) and the ability to knockdown (101) and overexpress target genes in urodele amphibians (102–104), new promising ways to study the function of selected genes in the newt system have become available. Recent sequencing approaches on regenerating salamander limb and tails (105, 106) and ongoing studies to access gene expression during cardiac regeneration in the newt will help to identify factors and signalling pathways that direct regeneration of damaged newt hearts. We propose that the highly efficient strategy employed by zebrafish and newts might eventually be utilized for human therapies. Mammalian organisms use a similar strategy to form myocardial tissue during heart development. Hence, it seems reasonable to assume that these pathways might be re-activated later in life helping to repair damaged hearts in adults.

References


102. Echeverri K, Clarke JD, Tanaka EM. In vivo imaging indicates muscle fiber dedifferentiation is a major contributor to the regenerating tail blastema. Dev Biol 2001; 236: 151–164.