Animal models to study cardiac regeneration

Michael Weinberger[1,2] *& Paul R. Riley*[1†]

[1]Institute of Developmental & Regenerative Medicine, University of Oxford, Oxford, UK.

[2]MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.

[†]e-mail: paul.riley@idrm.ox.ac.uk

Abstract | Permanent fibrosis and chronic deterioration of heart function in human patients following myocardial infarction present a major healthcare burden worldwide. In contrast to the restricted potential for cellular and functional regeneration of the adult mammalian heart, a robust capacity for cardiac regeneration is seen during the during the neonatal period in mammals as well as in the adults of many fish and amphibian species. However, we lack a complete understanding as to why cardiac regeneration takes place more efficiently in some species than in others. The capacity of the heart to regenerate following injury is controlled by a complex network of cellular and molecular mechanisms that form a regulatory landscape, either permitting or restricting regeneration. In this Review, we provide an overview of the diverse array of vertebrates that have been studied for their cardiac regenerative potential and discuss differential heart regeneration outcomes in closely related species. Additionally, we summarize current knowledge about the core mechanisms that regulate cardiac regeneration across vertebrate species.

[H1] Introduction

Cardiovascular disease continues to be one of the major causes of death worldwide[1]. Although the survival rate for myocardial infarction (MI) — the death of heart muscle tissue due to a lack of oxygen caused by the disruption of coronary blood flow[2] — has been steadily

increasing[3], muscle tissue lost to ischaemia is replaced by a collagen-rich scar[4]. The scar tissue is noncontractile and interferes with cardiac function, eventually leading to heart failure. Research on model organisms across various species has, however, shown that the outcome of heart injury is not always as detrimental as in humans. Indeed, the adult heart in several fish and amphibian species displays robust cardiac regeneration potential[5-7], illustrating a requirement for comparative studies across species to better understand the regulators of this phenomenon. Furthermore, even though adult cardiac regeneration capacity is much less pronounced in mammals, the neonatal mammalian heart has been found to possess substantial regenerative potential[8-10]. Considerable effort has, therefore, been invested into elucidating the mechanisms that determine heart regeneration and into finding ways to improve the regenerative outcome in the injured adult mammalian heart. In this Review, we provide an overview of heart regeneration research conducted across species, the mechanisms that have been found to control regenerative potential in various species as well as the latest advances towards enabling adult heart regeneration in mammals.

[H1] Cardiac regeneration in vertebrates

The capacity for heart regeneration seems to be unevenly distributed across the vertebrate subphylum (Fig. 1, key studies in Fig. 2, Table 1). Several fish species and amphibian species have substantial cardiac regenerative capacity that persists into adulthood, but mammals lose most of their potential for heart regeneration shortly after birth.

[H2] Fish and amphibians

The zebrafish (*Danio rerio*), a member of the cyprinid family of ray-finned fish (*Actinopterygii*), has been intensively studied for its remarkable cardiac regenerative capacity, after it was shown to completely regenerate the injured adult heart within 60 days of

ventricular resection[5,11]. Cardiac regeneration has also been demonstrated with cryoinjury[12-14], genetic ablation of cardiomyocytes[15], hypoxia–reoxygenation injury[16] and cauterization[17]. Cryoinjury and cauterization are used as injury models in non-mammalian species as they are thought to closely resemble mammalian MI due to the presence of large amounts of necrotic tissue in the wound and the destruction of a wide range of cardiac cell types. Interestingly, small scars persist in 75% of cryoinjured zebrafish hearts[18] and lasting alterations in ventricular wall motion have been reported[19]. These findings indicate that the regenerative capacity of the zebrafish heart is not complete in response to cryoinjury, contrasting with the response to ventricular resection. Furthermore, although the capacity for cardiac regeneration in the zebrafish persists into late adulthood[20], it declines in old age[21]. The giant danio (*Devario aequipinnatus*) and the goldfish (*Carassius auratus*), both cyprinids, also display robust heart regeneration potential in response to cauterization; however small areas of fibrotic tissue remain in the heart at 60 and 45 days post-injury, respectively [22,23]. Full morphological regeneration of the resected ventricle has been described in the African turquoise killifish (*Nothobranchius furzeri*; family: Nothobranchiidae)[24], but whether the heart regenerates in response to cryoinjury or cauterization is unknown. In the Senegal bichir (*Polypterus senegalus*), a member of the most basal family (Polypteridae) of ray-finned fish, cardiomyocyte proliferation is induced by resection or stab injury[25]. However, the regenerative potential of the *P. senegalus* heart remains unclear because the specific information on the resolution of fibrotic tissue and restoration of the myocardium following cardiac injury have not been reported.

In the Western clawed frog (*Xenopus tropicalis*), the adult resected ventricular myocardium can regenerate and become almost scar-free within 30–60 days[26,27]. The axolotl (*Ambystoma mexicanum*) is also capable of completely regenerating lost cardiac muscle tissue within 90 days of ventricular resection or cryoinjury[6,28-30]. Cardiac regenerative

potential has long been recognized in the Eastern newt (*Notophthalmus viridescens*)[31,32], and studies published in the 2000s have highlighted full regeneration of the newt heart within 70 days of ventricular resection and mechanical injury[33-35]. In the Iberian ribbed newt (*Pleurodeles waltl*), conflicting reports exist as to whether the post-metamorphic heart regenerates efficiently following cryoinjury. In one study, cardiac scarring that gradually reduced in extent, but persisted for at least 10 months after injury, was described, indicating the absence of an efficient regenerative response[36]. A second study, however, demonstrated complete resolution of cardiac fibrotic tissue within 210 days of cardiac injury[37]. These contradictory findings are likely due to injuries inflicted in the first study being more severe than those in the second, as the duration of cryo-cauterization was three times as long. Therefore, it is important to note that the severity of injury is a factor that influences cardiac regeneration outcome, illustrating the need for standardized injury procedures across studies.

[H2] Mammals

Generally, the consensus is that regenerative potential in the adult mammalian heart is very limited. This phenotype is likely to have evolved in parallel with the need to maintain highly specialized function against the increasing physiological demand of elevated pressure–volumes associated with a full pulmonary circulation[38]. In evolutionary terms, the mammalian heart needs to function until reproductive age; thereafter there is minimal selective pressure to ensure heart function in response to injury during later adult life. In humans, this is a new world problem, whereby extended lifespan has meant coronary artery disease resulting in MI is increasingly prominent and requires solutions to improve repair or enhance regenerative capacity.

Studies in multiple mammalian species have highlighted a brief postnatal window of increased regenerative capacity. In the house mouse (*Mus musculus*), for example, the heart

at postnatal day 1 (P1) is able to efficiently regenerate in response to resection injury[8], ischaemic MI[9,39] and nontransmural cryoinjury[40]. However, this regenerative potential is no longer present following resection injury or MI at P7[8,9,39] and might already be diminished by P2[41]. Although regeneration of the adult murine heart following cryoinjury has been reported in some studies[42,43], these findings have been disputed in other studies in which cryoinjury and MI models were combinined[44-48]. Neonatal capacity to restore lost cardiac muscle tissue has also been described in P1 brown rat (*Rattus norvegicus*) MI and resection injury models, but, as for the mouse, regeneration is absent from P7 onwards[49,50]. The adult guinea pig (*Cavia porcellus*) responds to cardiac cryoinjury with scarring and functional impairment[51] and, to date, no neonatal heart injury studies have been carried out in guinea pigs. In a study published in 2022, heart regeneration capacity was investigated in neonatal European rabbits (*Oryctolagus cuniculus*)[52]. Infarcted rabbit hearts displayed reduced scarring, as well as improved functional recovery, following MI at P1 when compared with MI at P7, mirroring the results obtained in the neonatal mouse heart. However, the researchers only assessed infarcted hearts until 3 weeks post-MI, by which time fibrotic tissue was still evident even in the hearts injured at P1. The neonatal human heart also seems to possess the capacity for regeneration, as rapid cardiac functional recovery has been reported in cases of newborn infants with MI due to congenital anomalies of coronary vessels or blood clotting factor mutations[53-55]. However, whether recovery in humans who survive a neonatal MI persists into adulthood has not yet been established. Adult rhesus macaque (*Macaca mulatta*) hearts show functional deterioration and fibrosis following MI[56,57]. In two investigations, cardiac regenerative potential was found in 1-day-old and 2-day-old neonatal piglets (*Sus domesticus*) following MI[58,59]. However, it should be noted that a subsequent study did not confirm morphological or functional recovery of the infarcted neonatal porcine heart[60]. In older piglets and 1 month old pigs, MI or ischemia–reperfusion injury leads to scarring and

permanently decreased cardiac function[58,59,61]. In sheep (*Ovis aries*), fetal hearts have been reported to fully repair following MI, whereas adult hearts form a scar and show a gradual decline in function[62]. However, quantitatively comparing these results with those obtained in other species is difficult, as the extent of injury post-MI was quantified via echocardiography and not on stained sections. In addition, whether fetal and larval heart repair truly represent regeneration processes rather than an enhancement of pre-existing developmental processes is debatable. Interestingly, the heart of the grey short-tailed opossum (*Monodelphis domestica*) shows reduced fibrosis following ventricular resection or MI at P14, when compared with injury at P29[10]. This finding represents the longest neonatal cardiac regenerative window in mammals reported to date. However, marsupials are born at an unusually early stage of development and comparing rodent and opossum heart developmental stages can be challenging. Collectively these studies indicate that heart regeneration is severely limited in adult mammals, but evidence exists across species for neonatal regenerative potential.

[H2] Exceptions to the trend

Although a trend exists for cardiac regenerative capacity to be more robust in fish and amphibians than in the mammalian species studied to date, it is important to note that there are several outlier species. In fish, the medaka, or Japanese rice fish, (*Oryzias latipes;* family: Adrianichthyidae) is a freshwater teleost like the zebrafish. Unexpectedly, studies of the injured adult medaka heart showed a markedly restricted regenerative capacity characterized by persistent fibrosis following ventricular resection and cryoinjury[63,64] (Fig. 3b). Furthermore, the resected medaka heart was reported to lack a myocardial proliferation response[63]. However, as the analysis included the entire ventricle, it is possible that a local response restricted to the wound border region might have been missed. Even within a single species, differences in cardiac regenerative potential have been found. Two forms of the

Mexican tetra (*Astyanax mexicanus;* family: Characidae) — surface dwelling and blind, Pachón cave-dwelling (blind cavefish) populations — have been evolving separately for approximately 1.5 million years. Whereas adult surface-dwelling cavefish are able to restore lost myocardium in a scar-free manner within 60 days of ventricular resection injury, their cave-dwelling counterparts develop a permanent scar, even in the presence of elevated cardiomyocyte proliferation[7] (Fig. 3c).

In amphibians, although the heart of the African clawed frog (*Xenopus laevis*) repairs within 90–180 days at the tadpole stage[65], fibrous scarring close to the site of injury persists post-resection in the juvenile and adult heart[65,66], contrasting with the reported adult cardiac regenerative capacity of the Western clawed frog (*X. tropicalis*)[26,27]. In mammals, adult spiny mice of the genus *Acomys* display more robust cardiac recovery following MI than the adult house mouse[67,68] (Fig. 3a).

[H1] Mechanisms

Functional restoration of the injured heart is a complex process and determined both by local events (such as the activation of resident fibroblasts) and by systemic responses (such as the influx of inflammatory immune cells into the heart). Although cardiomyocyte proliferation is at the core of restoring cardiac function, several other interconnected mechanisms are crucial to enable a robust regenerative response (Fig. 4). Among these are the formation of fibrotic tissue and its subsequent resolution, the regrowth of coronary blood vessels, inflammatory and reparative immune responses as well as altered energy metabolism.

[H2] Restoration of the myocardium

One of the challenges during cardiac regeneration is to replace the cardiomyocytes that have been lost due to injury and restore a fully functional myocardium. The two major strategies

through which restoration of cardiac muscle function can be achieved are the proliferation of surviving resident cardiomyocytes and the recruitment of cardiomyocytes from other cell sources.

[H3] Cardiomyocyte proliferation in fish and amphibians. In the injured zebrafish heart, the predominant mechanism of restoring lost cardiac muscle tissue is via the proliferation of pre-existing cardiomyocytes[69,70]. High levels of myocardial cell division are present close to the site of injury, where cardiomyocytes dedifferentiate towards a less mature phenotype and form a blastema-like structure[71]. However, increased mitotic activity is also present in the fully differentiated myocardium distal to the injury site, indicating global stimulation of cell division[71]. The pronounced proliferative capacity of adult zebrafish cardiomyocytes is linked to the fact that almost 99% of these cells are mononucleated and diploid[72]. These features seem to be pre-requisites for regenerative potential, as enforced polyploidization of zebrafish cardiomyocytes reduces their cell cycle re-entry and impairs heart regeneration[73].

Substantial proliferative capacity has also been found in the adult amphibian myocardium. In the adult Eastern newt heart, 29% of cardiomyocytes are able to divide[74,75]. In frogs, the discrepancy in regenerative capacity between the African clawed frog and the Western clawed frog might be partially due to differences in cardiomyocyte proliferation potential. The African clawed frog genome is pseudo-tetraploid, which might result in its cardiomyocytes being more prone to polyploidy and hypertrophy than in the Western clawed frog, which has a diploid genome[76]. However, other factors, such as the methods used to quantify cardiomyocyte proliferation and fibrosis, are also likely to have influenced the difference in *Xenopus* spp. cardiac regeneration potential reported[76].

[H3] Cardiomyocyte proliferation in mammals. Genetic lineage tracing indicates that, like zebrafish, the majority of the regenerated myocardium in the neonatal mouse heart is formed via proliferation of pre-existing cardiomyocytes[8]. Neonatal mouse cardiomyocytes are mononucleated, diploid and able to proliferate at birth. However 95% of these cells stop dividing and become binucleated by the end of the first week after birth[8,77]. Although MI increases cell cycle activity in the adult mouse heart, significant proliferation of cardiomyocytes has not been found[78]. Nevertheless, a population of mononucleated diploid cardiomyocytes remains present in the adult mouse heart and the size of this population, which is altered across genetic backgrounds, is correlated to functional outcome after MI, suggesting proliferative potential[79].

In humans, the majority of cardiomyocytes are mononucleated and diploid shortly after birth, and cardiomyocytes proliferate at a modest rate[80,81]. Whereas the proportion of mononucleated human cardiomyocytes remains unchanged throughout life, the DNA content in the majority of cardiomyocytes increases via endoreplication, and the annual cardiomyocyte turnover rate drops to around 1% by early adulthood[80-82].

Marsupials have a short gestation and are thus born in an immature state of development. Therefore, it is interesting that the rate of cardiomyocyte proliferation in 14-day old opossums is equivalent to that in P1 mice[10], indicating that cardiomyocyte proliferative potential in marsupials is not adversely affected by prolonged cardiogenesis outside the uterus.

[H3] Regulation of cardiomyocyte proliferation potential. Several cellular signalling factors, such as components of the Hippo pathway[83,84], have been implicated in the regulation of cardiomyocyte cell cycle withdrawal. These have been reviewed in detail elsewhere[85,86]. The perinatal withdrawal of mammalian cardiomyocytes from the cell cycle coincides with a

switch in cardiomyocyte energy metabolism from glycolysis to oxidative phosphorylation (reviewed by Lopaschuk and Jaswal[87]). Although increased reliance on mitochondrial metabolism boosts cellular energy production, cardiomyocytes are exposed to elevated levels of oxidative stress due to mitochondrial-derived reactive oxygen species (ROS)[88]. The resulting DNA damage response has been identified as a driver of cardiomyocyte cell cycle withdrawal in the postnatal murine heart[89]. Forcing cardiomyocytes to metabolically shift away from mitochondrial oxidative phosphorylation has been shown to prolong the postnatal window of cardiomyocyte proliferation and improve functional outcome following MI in adult mice[90]. Furthermore, transcriptomic profiling revealed that downregulation of genes involved in oxidative metabolism precedes the activation of cell cycle genes in neonatal mouse cardiomyocytes following MI, indicating that a shift away from mitochondrial metabolism is required to enable cell cycle entry[91]. Adaptation responses to elevated oxygen levels in the postnatal mammalian myocardium are, therefore, likely to contribute to the deterioration of cardiac regenerative capacity shortly after birth by promoting cardiomyocyte cell cycle withdrawal. Indeed, subjecting adult mice to chronic severe hypoxaemia results in reduced myocardial ROS levels, increased cardiomyocyte proliferation and improved outcome following MI[92]. In this context, it would be of interest to study the oxygenation status of the postnatal opossum heart, as opossum cardiomyocytes preserve their proliferation potential for at least 14 days after birth, despite being exposed to an extrauterine environment[10]. Adult zebrafish cardiomyocytes exist in a much more hypoxic environment than their mammalian counterparts, partly because the zebrafish heart is two-chambered[93], resulting in the mixing of oxygenated and deoxygenated blood. Even in this setting, ventricular resection has been shown to result in hypoxia close to the injury area, and induced systemic hyperoxia reduces zebrafish cardiomyocyte proliferation, blunting the regenerative response[94].

Another reason for the high cardiomyocyte proliferation potential in adult zebrafish and newt hearts might lie in the characteristics of their cellular structure. Centrosomes, which are required for the proliferation of vertebrate non-cancer cells[95], are functional in adult zebrafish and newt cardiomyocytes, but absent from adult mouse cardiomyocytes[96]. Furthermore, rat cardiomyocytes largely disassemble their centrosomes during the first few days after birth, possibly due to increased haemodynamic stress, promoting cell cycle arrest[96]. Given that there is a link between oxygen responsive factors and centrosome maturation[97], it would be interesting to study whether hypoxia is involved in maintaining centrosome integrity in cardiomyocytes.

The sarcomere is the structural element that enables contraction of cardiomyocytes, but also has a putative role in inhibiting cardiomyocyte proliferation (reviewed by Pettinato et al.[98]). During mitosis and cytokinesis of mammalian cardiomyocytes, the sarcomere complex is disassembled[8,99], and incomplete sarcomere disassembly has been implicated in endoreplication of postnatal rat cardiomyocytes[100]. Sarcomere disassembly thus seems to be an integral part of the cardiomyocyte cell cycle, but might be difficult to achieve during late developmental and adult life stages because several cardiomyocyte sarcomere genes switch splice isoforms during development, accompanied by sarcomere expansion and improved alignment[101]. This maturation of the sarcomere also increases the contractile force generated by cardiomyocytes, and increased ventricular wall tension might pose another hurdle to cardiomyocyte cytokinesis in the adult mammalian heart. That said, promoting sarcomere disassembly in the adult mammalian heart post-MI seems to be possible, via the administration of pluripotency factors (as discussed below).

[H3] Augmenting cardiomyocyte proliferation in the injured adult mammalian heart.

The low rate of cardiomyocyte turnover in the adult human and mouse heart[81,102] has

motivated researchers to focus on amplifying the pool of proliferative cardiomyocytes in the adult mammalian heart. Transient overexpression of the cell cycle regulators cyclin-dependent kinase 1 (CDK1), G2/mitotic-specific cyclin-B1 (CCNB1), cyclin-dependent kinase 4 (CDK4) and G1/S-specific cyclin-D1 (CCND1) achieves a cardiomyocyte proliferation rate of 15% in the adult mouse heart, as evidenced by a Cre-recombinase-based double labelling approach[103]. Overexpression of the four cell cycle factors, even when initiated 1 week after treatment, significantly reduced scar size and improved functional outcome in injured adult mouse, rat and pig hearts[103,104]. Partial reprogramming of cardiomyocytes towards a more proliferative phenotype has also been demonstrated using the Yamanaka (or OSKM) factors — Oct4, Sox2, Klf4 and c-Myc. Transient overexpression of these factors in the adult mouse heart before and during MI leads to a reduction in scar size and improvement of functional parameters[105]. Both the four cell cycle regulators and the Yamanaka factors induce a metabolic shift away from mitochondrial energy production in treated cardiomyocytes.

Cardiomyocyte proliferation in the injured adult mammalian heart is also increased upon overexpression of the neuregulin receptor tyrosine-protein kinase erbB-2[106] and following administration of microRNAs (reviewed by Ouyang and Wei[107]). A common challenge in all these approaches is the need for tight spatiotemporal control of cardiomyocyte reprogramming, because large-scale disassembly of sarcomere structures is likely to adversely affect cardiac function, and uncontrolled cell division risks the formation of cardiac tumours. Promisingly though, transient overexpression of cell cycle regulators elicits only a single round of division in transduced cardiomyocytes[103], and Yamanaka factor-mediated myocardial dedifferentiation was found to be reversible if treatment was temporally restricted[105].

[H3] Myocardial regeneration and general cell proliferation. As discussed above, robust heart regeneration in cyprinid fish, urodeles (newts and salamanders) and neonatal mammals is driven by the capacity of cardiomyocytes to proliferate following injury, linked to the level of cardiomyocyte proliferation in the intact heart. For example, cardiomyocytes in the neonatal mouse heart proliferate related to continued development whereas, at later life stages, the myocardium typically expands via cardiomyocyte polyploidization and hypertrophy[77]. Adult zebrafish show a general, continuous growth in size directed by environmental cues, such as food availability, water quality and population density[108]. A high capacity for cell proliferation in general, and a robust proliferative potential of cardiomyocytes in particular, therefore, seems to be indicative of the ability to regenerate the heart following injury.

The presence of cardiomyocyte proliferation alone, however, is not sufficient to enable robust cardiac regeneration. Notable precedents here include juvenile pigs, which lack cardiac regeneration in response to ischaemia–reperfusion injury even though their cardiomyocytes still show mitotic activity[61], and medaka which grow continuously, similar to zebrafish[108], but do not regenerate the heart following ventricular resection and cryoinjury[63,64].

[H3] Contributions from non-cardiomyocyte lineages. An alternate route towards restoring the damaged myocardium is the differentiation of non-cardiomyocyte cells into heart muscle cells. The presence and possible beneficial effects of cardiac stem cells in the injured adult mammalian heart have been extensively probed. However, their myogenic potential remains controversial, with the majority of publications in the past 10 years arguing against significant trans-differentiation into cardiomyocytes (reviewed by He et al.[109]). In the zebrafish, no apparent stem cell source of cardiomyocytes has been identified to-date, but

transgenically labelled atrial myosin heavy chain (*amhc)*$^{+}$ atrial cardiomyocytes have been reported to be reprogrammed into ventricular cardiomyocytes following ablation of the larval ventricular myocardium; a process that is dependent on Notch signalling[110]. However, in another study, *amhc* expression was found in a subset of larval ventricular cardiomyocytes[111], raising questions as to the ratio of atrial reprogramming versus ventricular proliferation following larval ventricular myocardial ablation.

The epicardium, an important signalling centre during heart regeneration (reviewed by Cao and Poss[112]) has been investigated as another potential source of cardiomyocytes following cardiac injury. The reliance of approaches to epicardial lineage tracing on non-epicardium-specific markers has posed a challenge to the characterization of epicardial contributions to other cardiac tissues following injury[113]. In the injured mouse heart, epicardial conversion to cardiomyocytes seems to be severely restricted[114]. However, priming with thymosin β4 modestly increases the rate of epicardial trans-differentiation into cardiomyocytes[115]. In the injured adult zebrafish heart, lineage tracing has been supplemented with cell transplantation, and both approaches seem to refute epicardial trans-differentiation into cardiomyocytes during regeneration[116,117]. However, a myogenic epicardial niche might still exist in the zebrafish heart, as a single cell RNA sequencing (scRNA-seq) study published in 2022 identified a cardiac cell cluster in which the epicardial marker gene *tcf21* was co-expressed with cardiomyocyte markers, such as *myl7*[118]. Based on lineage tracing, it has been reported that epicardium-derived cells contribute substantially to the regenerated myocardium in the injured Iberian ribbed newt heart[37]. However, lineage tracing was not based on an epicardial marker, but on the cellular uptake of Cre transposase injected into the pericardial cavity. Even though the authors ruled out the possibility of Cre uptake into cortical cardiomyocytes, based on fluorescence microscopy, this cannot exclude the

possibility that minimal amounts of Cre eventually did enter cardiomyocytes rendering them falsely lineage-traced.

Genomic *cis*-regulatory elements/enhancers that can drive reporter gene expression following cardiac injury have been identified in mouse and zebrafish epicardium[119,120], in zebrafish myocardium[121,122] and endocardium[123,124], as well as in the African turquoise killifish heart[24]. One intriguing question, therefore, is whether such enhancers could be used as a tool to promote trans-differentiation or in vivo reprogramming of both cardiomyocyte and non-cardiomyocyte cells following heart injury.

[H2] Resolution of fibrotic tissue

Formation of fibrotic tissue ('scar') or fibrosis in the acute phase after MI is crucial to the prevention of ventricular rupture in mammals, and is also a feature of several heart injury models in non-mammalian species. Fibrosis as an initial response to cardiac insult is, therefore, not exclusive to the regeneration-impaired heart. However, to achieve full cardiac regeneration, the initial fibrosis needs to be resolved to make way for newly formed functional heart tissue. In species with a low cardiac regenerative potential, such as adult mammals, this early fibrosis is not resolved and the permanent presence of noncontractile, nonconductive scar tissue leads to long-term deterioration of cardiac function. Consequently, the efficiency of resolving fibrotic tissue is an important factor in determining cardiac regenerative outcome.

[H3] Transient collagen deposition in zebrafish and newt hearts. In the highly regenerative zebrafish heart, fibrosis following ventricular resection is minimal and characterized by clot formation and abundant fibrin deposition[5,12,13]. By contrast, cryoinjury, as a model of tissue damage rather than tissue loss, induces substantial fibrosis, with

increased deposition of collagen[12,13]. Crucially, the initial fibrosis in the injured zebrafish heart is mostly resolved following a peak at 4–7 days post-injury, and fibrotic tissue is replaced with new functional tissue. The timing of fibrosis correlates with the presence of fibroblasts in the wound area[12,13]. Cardiac fibroblasts are a dynamic set of interstitial cells with several functions, including the production and remodelling of extracellular matrix (ECM) scaffolds (reviewed by Tallquist and Molkentin[125]). Upon injury, fibroblasts are activated and differentiate into myofibroblasts, which express high levels of ECM factors such as periostin and collagens. Most cardiac fibroblasts in the zebrafish are derived from the epicardium and from epicardium-derived cells — migratory cells that invade the wound area[117,126,127]. A scRNA-seq study published in 2022 demonstrated several fibroblast clusters in the cryoinjured zebrafish heart, and genetic scarring indicated that the majority of these fibroblast clusters were developmentally derived from the epicardial lineage[127]. Epicardium-derived fibroblasts have also been identified using scRNA-seq following ventricular resection[118]. Ablation of collagen-producing cells following cryoinjury impairs cardiomyocyte proliferation, suggesting a pro-regenerative role of cardiac fibroblasts[126,127]. However, whereas ablation of *col12a1a* expressing cells was found to impair the resolution of fibrotic tissue in one study[127], another study showed that ablation of *col1a2* expressing cells had no effect on fibrosis[126], which suggests the existence of subpopulations of fibroblasts and heterogeneity in function. In addition to epicardium-derived cardiac fibroblasts, the cryoinjured zebrafish heart harbours endocardium-derived fibroblasts[127], labelled by both *fli1a*[126] and *runx1*[128], which contribute to collagen production in the wound area post-cryoinjury. Another important contribution to fibrosis following zebrafish heart injury is made by collagen-depositing macrophages[129]. Collectively these studies reveal a diverse community of ECM-producing cell populations in the injured zebrafish heart.

Initial fibrosis and ECM production are also a hallmark of the regenerating Eastern newt heart, and the ECM factor tenascin-C promotes cell cycle re-entry of newt cardiomyocytes in vitro[130]. Additionally, ECM in the injured *N. viridescens* heart has been suggested to structurally guide the re-establishment of the myocardium[34].

[H3] Persistent scarring in the adult mammalian heart. Both neonatal and adult mouse hearts contain several fibroblast populations[131-134] that, in the adult, are derived from either the epicardium or the endocardium[133,134]. Tissue-resident fibroblasts give rise to activated, periostin-expressing fibroblasts that locate to the wound area following MI in adult animals[134]. These activated fibroblasts are required for fibrosis, and stabilize the wound area even before mature fibrotic tissue has formed[134,135]. Although the structural support by fibroblasts is beneficial in the acute phase of heart injury, these cells might also restrict the regenerative capacity of the adult mammalian heart. The finding that mouse cardiomyocyte proliferation is significantly higher when co-cultured with neonatal mouse cardiac fibroblasts than with adult mouse cardiac fibroblasts, supports this theory[136]. In the same study, co-culture of human stem cell-derived cardiomyocytes with adult human cardiac fibroblasts impaired their proliferation[136].

Further evidence underlining the influence that fibroblasts and fibrosis have on cardiac regenerative potential has emerged from reports on the composition of fibrotic tissue following MI. The extracellular proteoglycan agrin enhances cardiomyocyte proliferation through the receptor dystroglycan 1, whereas loss of agrin in mesodermal cells results in persistent scarring and reduced function in neonatal mouse hearts following MI[137]. Conversely, intramyocardial injection of agrin into infarcted adult mouse hearts promotes the resolution of fibrotic tissue and functional recovery[137]. Although a minor component of the adult mouse cardiac scar, collagen alpha-1(V) chain (COL5A1) has been found to play an

important role in regulating fibrotic tissue composition[138]. COL5A1 restricts scar size, with loss of COL5A1 resulting in over-activation of cardiac fibroblasts, increased fibrosis and worsened functional outcome. COL5A1 is proposed to mediate its influence on heart regeneration, at least in part, by altering the mechanical properties of fibrotic tissue[138]. Additionally, altering ECM stiffness has been shown to influence neonatal rat cardiomyocyte proliferation potential in vitro, with a less stiff 'compliant' matrix promoting cardiomyocyte cytokinesis[139]. Furthermore, pharmacologically reducing ECM stiffness improves regeneration in P3 mice following ventricular resection[41]. These findings suggest that loss of cardiomyocyte proliferation and regeneration potential in the postnatal mammalian heart might, in part, be due to changes in ECM properties, and that modulating fibrotic tissue composition post-MI could enhance cardiac regeneration capacity.

[H3] Fibrosis after MI in adult spiny mice. In the spiny mouse, injured tissues heal with surprisingly little scarring; a phenomenon that is likely to have co-evolved with strategies for predator avoidance and a regenerative skin response to injury (reviewed by Maden and Varholick[140]). In 2021, improved outcome of adult MI in the spiny mouse versus the house mouse was reported[67,68]. Qi et al. found the left ventricular ejection fraction (LV EF) of the spiny mouse heart to be substantially restored towards pre-injury levels within 4 weeks following MI[68], whereas Peng et al. did not report restoration but stabilization of the LV EF in the infarcted spiny mouse heart[67]. However, the initial reduction in the LV EF upon MI reported in Qi et al. is much larger than that reported in Peng et al., such that the reduction in the LV EF 4 weeks after MI is comparable (15%-20%) in both studies. Strikingly, scar size and collagen deposition within the scar are significantly reduced in the infarcted spiny mouse heart, accompanied by increased cardiomyocyte proliferation[68] and coronary vessel growth[67] (Fig. 3a, Table 2). Enhanced neovascularization was proposed as a driver of altered fibrosis

in the spiny mouse, allowing for fibrotic tissue with higher cellular content than in the house mouse[67]. As the anatomy and physiology of uninjured house mouse and spiny mouse hearts are comparable, these results indicate that the fibrotic response to MI can be modulated to limit scar size and improve functional outcome without risking ventricular rupture.

[H2] Neovascularization

Supplying the myocardium with oxygen and nutrients, as well as removing waste metabolites and excess fluid, is a crucial challenge for the heart. A specialized network of blood vessels, the coronary vasculature, has evolved in several vertebrate species to provide an efficient cardiac transport infrastructure that consists of arteries, capillaries, veins and lymphatic vessels. In these species, including humans, neovascularization of the injured area to re-establish blood flow is an important part of the cardiac wound-healing response. However, endogenous neovascularization is insufficient in the infarcted adult human heart, and no effective clinical treatment to induce coronary angiogenesis has been identified to date (reviewed by Lupu et al.[141]).

[H3] Coronary vessels across vertebrate species. The loss of cutaneous respiration in terrestrial vertebrates including reptiles, birds and mammals might have promoted a coronary vasculature in these species[142]. In fish, a variety of coronary vessel architectures have evolved that are correlated with the amount of compact myocardium present[143]. In zebrafish, coronary vessels start to form at 1–2 months of age, originating from a population of endocardial cells[144]. The cardiac lymphatic system in zebrafish arises from ventral facial lymphatics, with the ventricular lymphatic network becoming established from 3–4 months of age onwards and growing along the coronary arteries in a vascular endothelial growth factor (VEGF) C-

dependent manner[145,146]. In contrast to zebrafish, the medaka heart lacks coronary vessels and their function is fulfilled by a plexus of endocardial extensions[147].

Most amphibians, such as newts and frogs, lack a coronary vasculature[148]. In the mouse heart, coronary vessel formation starts at embryonic (E) day 11.5 and continues into the first few days after birth. During embryogenesis, most coronary endothelial cells originate from the sinus venosus, the inflow tract of the embryonic heart[149,150]. Additionally, endocardial cells contribute to the coronary vasculature, particularly during neonatal development[150-152]. The murine cardiac lymphatic vessels develop from E12.5 and continue to expand until around P15 (reviewed by Klaourakis et al.[153]). Mouse lymphatic cells originate from multiple sources, with venous endothelial[154] and non-venous contributions[155-157]. Both blood and lymphatic vascular beds contribute extensively to outcomes following cardiac injury and to regenerative potential.

[H3] Importance of neovascularization in heart regeneration. The potential for cardiomyocyte proliferation and neovascularization in the injured heart follow strikingly similar evolutionary trends. Efficient myocardial proliferation and vessel growth underpin regeneration in fish and amphibians and in the neonatal mammalian heart, compared with the greatly reduced efficiency of both processes in the less regenerative adult mammalian heart.

The injured zebrafish heart depends on rapid neovascularization to regenerate, and blocking coronary vessel growth greatly reduces cardiomyocyte proliferation at the injury border[158]. Newly formed coronary vessels in the regenerating zebrafish heart are derived from pre-existing vessels[159], their growth being guided by stromal cell-derived factor 1 (also known as C-X-C motif chemokine 12b; Cxcl12b)-chemokine (C-X-C motif) receptor 4a (Cxcr4a) and Vegfaa signalling[144,160]. These vessels are suggested to form a ‘scaffold’ along which de-differentiated cardiomyocytes repopulate the injury area[160]. The lymphatic system

in the injured zebrafish heart seems to be involved in the clearance of fluid, debris and immune cells[145,161]. Interestingly, lymphangiogenesis is more pronounced following cryoinjury than after ventricular resection, and a lack of lymphatic vessels increases the likelihood of defective regeneration only following cryoinjury[145,146,161]. The particular dependence on lymphatic clearance following cryoinjury is thought to be due to the presence of a substantial amount of necrotic tissue in the wound area, which is absent in the resection model. In the medaka heart, neovascularization of the injury area occurs more slowly than in zebrafish, and newly formed vessels seem to be unstable[63,64]. Lymphangiogenesis following injury has not yet been investigated in medaka.

Neovascularization is evident after MI in the adult mouse heart, with newly formed vessels being derived from pre-existing coronary vessels[162] and, potentially, from the endocardium[163]. However, several important pro-angiogenic pathways that promote coronary vessel growth following MI in the neonate, such as VEGF A–myocyte-specific enhancer factor 2D (MEF2) and bone morphogenetic protein (BMP)–Smad, are not efficiently activated in the adult infarct border zone[164]. Following MI, coronary vessel growth in the adult spiny mouse heart is more pronounced than that in the adult house mouse heart, contributing to stabilization of cardiac function[68]. Therefore, it would be interesting to investigate whether the pro-angiogenic pathways that are dysregulated in the infarcted house mouse heart are more efficiently activated in the spiny mouse. Collateral bridging of coronary arteries can re-route the flow of blood if an artery becomes blocked, and can prevent MI in humans[165]. Although collateral artery formation occurs in the infarcted P2 mouse heart, it is almost absent in adult hearts following MI[166]. The formation of new collateral vessels in the infarcted neonatal mouse heart involves the migration of single arterial endothelial cells along capillaries in a CXCL12-dependent manner. Endothelial deletion of *Cxcl12* reduces collateral

artery formation and cardiomyocyte proliferation in the infarcted neonatal heart, whereas CXCL12 treatment promotes adult mouse coronary collateral artery formation[166].

Together, these studies indicate that insufficient neovascularization is one of the factors restricting regeneration potential in the adult mammalian heart, but also that there are ways to exogenously stimulate coronary vessel growth. This finding is underlined by insights into the role of cardiac lymphatic vessels following MI in the adult mammalian heart. In adult infarcted mouse[155] and rat[167] hearts, cardiac lymphangiogenesis can be promoted via treatment with VEGF-C–Cys156Ser and VEGF-C–Cys152Ser, respectively, to improve injury outcome; partly due to lymphatic clearance of inflammatory immune cells from the infarcted heart[168]. Immune cell clearance is dependent on lymphatic vessel endothelial hyaluronic acid receptor 1 (LYVE1), and MI in *Lyve1* mutant mice leads to chronic inflammation and long-term deterioration of cardiac function[168]. Lymphatic neovascularization of the injured heart, therefore, modulates the immune response elicited by injury.

[H2] The immune response to heart injury

Massive cardiomyocyte death associated with heart injury results in a profound innate and adaptive immune response (reviewed by Simoes and Riley[169,170]), which seems to be evolutionary conserved. Shortly after the injury insult, innate immune cells, such as neutrophils and macrophages, initiate an inflammatory immune response, and large numbers of circulating immune cells are recruited to the heart. During the inflammatory phase, necrotic cells and cell debris are cleared via phagocytosis. This process is temporally restricted and succeeded by a reparative phase, during which inflammatory immune cells are cleared from the heart or adopt a pro-reparative phenotype. Reparative immune cells have a vital role in regulating fibrosis and neovascularization. Both inflammatory and reparative

phases of the immune response are crucial for cardiac regeneration following injury, however, the two phases need to be modulated and precisely balanced to enable robust regeneration.

[H3] Fish and amphibians. Cryoinjury of the zebrafish heart induces a biphasic immune response that involves the recruitment and expansion of innate and adaptive immune cell populations[64,129,171-173]. This response begins with an early influx of neutrophils and inflammatory macrophages[172]. Thereafter, neutrophils are cleared from the heart and macrophages change their molecular phenotype to enable the resolution of fibrotic tissue and regeneration[172,174]. Following ventricular resection, both inflammatory and reparative immune responses occur simultaneously, and macrophage gene expression in the resected heart differs from that in the cryoinjured heart, illustrating dynamic plasticity of the immune response to different injury conditions (e.g. the presence of large amounts of necrotic tissue)[129]. The immune system in the injured zebrafish heart fulfils various roles beyond the clearance of debris. Macrophages regulate the formation and resolution of fibrotic tissue[172], as well as directly contributing to fibrosis following cryoinjury[129]. The presence of macrophages in the injured adult zebrafish heart has also been correlated with cardiomyocyte proliferation potential[171,175], and macrophages actively promote the latter during repair of the larval zebrafish heart[176]. The adaptive immune system in the injured zebrafish heart has so far received less attention than the innate immune system. However, regulatory T cells expressing forkhead box protein 3a (*foxp3a*) have been found to infiltrate the cryoinjury wound area to promote cardiomyocyte proliferation, and their ablation results in the prolonged presence of fibrotic tissue in the wound[173].

The importance of a well-orchestrated immune response following cardiac injury has been illustrated by a study that compared immune responses in the zebrafish heart and the

medaka heart[64]. Recruitment of macrophages was found to be less efficient in the cryoinjured medaka heart than in the cryoinjured zebrafish heart (Fig. 3b, Table 2). In the same study, delaying macrophage recruitment in the cryoinjured zebrafish heart impaired neutrophil clearance, cardiomyocyte proliferation, neovascularization and resolution of fibrotic tissue[64].

The innate immune system also plays an important role during cardiac regeneration in the axolotl. Ablation of macrophages blocks the regenerative capacity of the cryoinjured axolotl heart, with increased fibroblast activation, collagen accumulation in the wound and reduced neovascularization[28]. However, unlike in zebrafish, depletion of macrophages in the injured axolotl heart does not reduce cardiomyocyte proliferation potential[28].

[H3] Mice. The immune response in the infarcted mouse heart involves several immune cell populations, both innate and adaptive. Neutrophils are recruited to the heart shortly after MI and persist only for a few days. Although their main function is to participate in the inflammatory phase of the immune response, neutrophils also assist in the reparative phase by promoting macrophages to adopt a reparative phenotype[177]. Macrophages in the infarcted mouse heart have historically been grouped into Ly6C^{high} monocyte-derived M1 and Ly6C^{low} monocyte-derived M2 populations, participating in the inflammatory (M1) and reparative (M2) phases[178]. However, the origin of inflammatory and reparative macrophages is more complex[179], and transcriptomic studies have revealed a broad spectrum of macrophage populations in infarcted mouse[180-183] and failing human heart[184]. Macrophages are required for regeneration of the injured neonatal mouse heart[185], which was found to be driven by tissue-resident C-C chemokine receptor type 2 (CCR2)$^{-}$ macrophages that promote cardiomyocyte proliferation and angiogenesis[186]. By contrast, in the injured adult mouse heart, the immune response includes the recruitment of CCR2^{+} macrophages that feature an inflammatory phenotype[186]. Furthermore, transfer of adult mouse splenic monocytes into the

infarcted neonatal heart inhibits fibrotic tissue resolution and promotes collagen deposition[129]. The adaptive immune system also exerts both positive and negative impacts on cardiac regenerative potential, which has been reviewed in detail elsewhere[169]. Additionally, a study published in 2022 showed that the numbers of $CD4^+$ and $CD8^+$ T cells increase postnatally in the mouse heart, and transfer of adult T cells into the infarcted neonatal mouse heart promotes macrophage recruitment, accompanied by increased fibrosis[187]. Collectively, these findings illustrate that altered immune cell functionality is a factor driving the loss of regeneration capacity in the postnatal mouse heart.

[H2] Metabolism and thyroid hormone signalling

The trend towards a decline in cardiac regeneration potential over the course of vertebrate evolution has prompted investigation into whether this decline correlates with general changes in metabolism, such as the thyroid hormone-driven acquisition of endothermy. Vertebrate cardiomyocyte diploidy has been linked with metabolic rate, body temperature and thyroid hormone levels[72]. The levels of thyroid hormones rise significantly in mice shortly after birth[188], and blocking thyroid hormone signalling in perinatal mouse cardiomyocytes decreases polyploidy and increases proliferation[72]. In the adult mouse heart, blocking thyroid hormone signalling increases cardiomyocyte proliferation following MI, accompanied by a reduction in fibrosis and improved functional outcome[72]. In the same study, reduced thyroid hormone signalling resulted in downregulation of molecular pathways, such as oxidative phosphorylation in P14 cardiomyocytes. This finding indicates that thyroid hormones might induce cardiomyocyte cell cycle withdrawal by promoting mitochondrial energy production in the neonatal mouse heart, similar to the effect described for increased cellular oxygen levels[89]. By contrast, a rise in thyroid hormone levels has been reported to correlate with a burst in cardiomyocyte proliferation in the P15 mouse heart[189]. However,

thyroid hormones were found to activate insulin-like growth factor I (IGF1) signalling in this setting, which might temporarily override the energy metabolism-related effects of earlier thyroid hormone signalling. Administration of a thyroid hormone (triiodothyronine; T_3) has been shown to restrict cardiomyocyte proliferation in the prenatal sheep heart[190], and T_3 treatment also reduces adult zebrafish cardiomyocyte proliferation[72], suggesting an evolutionarily conserved role for thyroid hormone signalling. Further comparative studies, possibly including endothermic fish, such as the opah (*Lampris guttatus*)[191], tuna (family: Scombridae) and lamnid sharks (family: Lamnidae), should be informative to gain deeper insight into the impact of thyroid hormones on heart regeneration across vertebrate species.

Changes in metabolism are also thought to drive the differential cardiac regeneration potential of surface dwelling and blind, Pachón cave-dwelling forms of Mexican tetra[7]. Although cardiomyocyte proliferation following ventricular resection is unchanged between the two populations, gene expression related to mitochondrial and glycolytic energy metabolism is downregulated in injured blind cavefish hearts (Fig. 3c, Table 2). This finding suggests that a lower metabolic rate, together with differences in fibrosis and immune response, might impair the cardiac regeneration potential of the blind, cave-dwelling form of Mexican tetra.

[H1] Link with general regenerative capacity

Robust regenerative potential of the heart is often correlated with a wider capacity for regeneration (Table 3). Several fish species[7,192,193] and amphibians, such as newts (reviewed by Tanaka et al.[194]) and the axolotl[195], are capable of regrowing fins or limbs, potentially as a response to cannibalism or fighting injury. The zebrafish, Eastern newt and axolotl also regenerate spinal cord injuries[196-198], and the axolotl has been shown to regenerate skin lesions[199]. Neonatal mice are able to regenerate injuries to the Achilles tendon[200], spinal

cord[201] and cochlear hair cells[202], whereas these tissues display impaired regeneration potential in the adult mouse[200,201,203]. The robust cardiac regenerative potential in spiny mice is accompanied by a high capacity for skin regeneration, whereby detachment of skin and subsequent restoration has evolved as a means to avoid predation[204].

However, regeneration potential in the heart does not always correlate well with the capacity to regenerate injuries in other tissues. Robust fin regeneration potential in medaka and cave-dwelling Mexican tetra contrasts with their restricted cardiac regeneration capacity following ventricular resection and cryoinjury in medaka and ventricular resection in the Mexican tetra. This finding indicates that maintaining high regenerative potential in the heart is potentially more challenging than maintaining such capacity in other tissues or appendages, such as the fin. Moreover, in a panel of 32 genetically diverse mouse strains, cardiac regeneration potential varied and correlated poorly with the capacity to regenerate ear punch injuries[205]. In this study, the researchers carefully excluded some mouse strains because the left anterior descending artery was atypically positioned. However, subtle differences in coronary architecture between the remaining strains might still have introduced variability in initial injury size, thereby obscuring some of the variability in regenerative capacity.

[H1] Conclusions

Research across species suggests that heart regeneration is a multi-layered, complex process controlled both at an organ level and systemically. Of the approximately two dozen vertebrate species that have been examined to date, cardiac regeneration of the injured adult heart is frequently found in fish and amphibian species. In mammals, however, cardiac regenerative potential is mostly restricted to the early postnatal period. The fact that multiple species defy this evolutionary trend indicates that there are several independently evolving processes required for robust cardiac regeneration. Studying the differences in regenerative potential

between closely related species, therefore, promises to be particularly valuable to elucidate the mechanisms that drive heart regeneration.

The major regulators of cardiac regenerative potential — including cardiomyocyte proliferation, fibrosis, neovascularization, the immune response and metabolism — are highly interconnected. Evolutionary adaptations that have optimized cellular energy production, for example, have also promoted cardiomyocyte cell cycle withdrawal[72,89]. Furthermore, cardiomyocyte proliferation[173,186], fibrosis [28,129,172] and neovascularization[64,185,186] are all influenced by the interplay of inflammatory and reparative immune responses. The immune response to cardiac injury is, in turn, regulated via neovascularization of the coronary blood and lymphatic vascular systems[168]. Coronary neovascularization could also influence the composition of the fibrotic tissue formed in response to cardiac injury[67], whereas the properties of fibrotic tissue are important regulators of cardiomyocyte proliferation[130,137,139]. The landscape generated by the interplay of these processes seems to facilitate a regenerative response in species such as the zebrafish and axolotl, linked to a robust capacity for more general tissue regeneration and hyperplastic organ growth, that could have evolved as a response to extrinsic pressures, such as predation and variations in food availability. A less regenerative landscape has materialized in mammals, potentially due to increased complexity of heart function and higher physiological demand. However, even suboptimal conditions for cardiac regeneration can be modulated to achieve a better outcome, as is evident in the spiny mouse[67,68 204]. In the future, further insights into evolutionary drivers of heart regeneration arising from the study of other species will be important. In addition, promising developments, such as the partial reprogramming of mammalian cardiomyocytes, to improve heart regeneration offer the prospect of new therapies. These advances build on research in fish and amphibians, which have offered-up

potential cellular and molecular targets for translation towards clinical treatments following MI.

References

1 Tsao, C. W. *et al.* Heart Disease and Stroke Statistics-2023 Update: A Report From the American Heart Association. *Circulation* **147**, e93-e621, doi:10.1161/CIR.0000000000001123 (2023).

2 Frangogiannis, N. G. Pathophysiology of Myocardial Infarction. *Compr Physiol* **5**, 1841-1875, doi:10.1002/cphy.c150006 (2015).

3 Parikh, N. I. *et al.* Long-term trends in myocardial infarction incidence and case fatality in the National Heart, Lung, and Blood Institute's Framingham Heart study. *Circulation* **119**, 1203-1210, doi:10.1161/CIRCULATIONAHA.108.825364 (2009).

4 Kong, P., Christia, P. & Frangogiannis, N. G. The pathogenesis of cardiac fibrosis. *Cell Mol Life Sci* **71**, 549-574, doi:10.1007/s00018-013-1349-6 (2014).

5 Poss, K. D., Wilson, L. G. & Keating, M. T. Heart regeneration in zebrafish. *Science* **298**, 2188-2190, doi:10.1126/science.1077857 (2002).

6 Cano-Martinez, A. *et al.* Functional and structural regeneration in the axolotl heart (Ambystoma mexicanum) after partial ventricular amputation. *Arch Cardiol Mex* **80**, 79-86 (2010).

7 Stockdale, W. T. *et al.* Heart Regeneration in the Mexican Cavefish. *Cell Rep* **25**, 1997-2007 e1997, doi:10.1016/j.celrep.2018.10.072 (2018).

8 Porrello, E. R. *et al.* Transient regenerative potential of the neonatal mouse heart. *Science* **331**, 1078-1080, doi:10.1126/science.1200708 (2011).

9 Haubner, B. J. *et al.* Complete cardiac regeneration in a mouse model of myocardial infarction. *Aging (Albany NY)* **4**, 966-977, doi:10.18632/aging.100526 (2012).

10 Nishiyama, C. *et al.* Prolonged Myocardial Regenerative Capacity in Neonatal Opossum. *Circulation* **146**, 125-139, doi:10.1161/CIRCULATIONAHA.121.055269 (2022).

11 Raya, A. *et al.* Activation of Notch signaling pathway precedes heart regeneration in zebrafish. *Proc Natl Acad Sci U S A* **100 Suppl 1**, 11889-11895, doi:10.1073/pnas.1834204100 (2003).

12 Chablais, F., Veit, J., Rainer, G. & Jazwinska, A. The zebrafish heart regenerates after cryoinjury-induced myocardial infarction. *BMC Dev Biol* **11**, 21, doi:10.1186/1471-213X-11-21 (2011).

13 Gonzalez-Rosa, J. M., Martin, V., Peralta, M., Torres, M. & Mercader, N. Extensive scar formation and regression during heart regeneration after cryoinjury in zebrafish. *Development* **138**, 1663-1674, doi:10.1242/dev.060897 (2011).

14 Schnabel, K., Wu, C. C., Kurth, T. & Weidinger, G. Regeneration of cryoinjury induced necrotic heart lesions in zebrafish is associated with epicardial activation and cardiomyocyte proliferation. *PLoS One* **6**, e18503, doi:10.1371/journal.pone.0018503 (2011).

15 Wang, J. *et al.* The regenerative capacity of zebrafish reverses cardiac failure caused by genetic cardiomyocyte depletion. *Development* **138**, 3421-3430, doi:10.1242/dev.068601 (2011).

16 Parente, V. *et al.* Hypoxia/reoxygenation cardiac injury and regeneration in zebrafish adult heart. *PLoS One* **8**, e53748, doi:10.1371/journal.pone.0053748 (2013).

17 Dyck, P. K. V. *et al.* Cauterization as a Simple Method for Regeneration Studies in the Zebrafish Heart. *J Cardiovasc Dev Dis* **7**, doi:10.3390/jcdd7040041 (2020).

18 Bertozzi, A. *et al.* Is zebrafish heart regeneration "complete"? Lineage-restricted cardiomyocytes proliferate to pre-injury numbers but some fail to differentiate in fibrotic hearts. *Dev Biol* **471**, 106-118, doi:10.1016/j.ydbio.2020.12.004 (2021).

19 Gonzalez-Rosa, J. M. *et al.* Use of echocardiography reveals reestablishment of ventricular pumping efficiency and partial ventricular wall motion recovery upon ventricular cryoinjury in the zebrafish. *PLoS One* **9**, e115604, doi:10.1371/journal.pone.0115604 (2014).
20 Itou, J., Kawakami, H., Burgoyne, T. & Kawakami, Y. Life-long preservation of the regenerative capacity in the fin and heart in zebrafish. *Biol Open* **1**, 739-746, doi:10.1242/bio.20121057 (2012).
21 Reuter, H. *et al.* Aging Activates the Immune System and Alters the Regenerative Capacity in the Zebrafish Heart. *Cells* **11**, doi:10.3390/cells11030345 (2022).
22 Lafontant, P. J. *et al.* The giant danio (D. aequipinnatus) as a model of cardiac remodeling and regeneration. *Anat Rec (Hoboken)* **295**, 234-248, doi:10.1002/ar.21492 (2012).
23 Grivas, J. *et al.* Cardiac repair and regenerative potential in the goldfish (Carassius auratus) heart. *Comp Biochem Physiol C Toxicol Pharmacol* **163**, 14-23, doi:10.1016/j.cbpc.2014.02.002 (2014).
24 Wang, W. *et al.* Changes in regeneration-responsive enhancers shape regenerative capacities in vertebrates. *Science* **369**, doi:10.1126/science.aaz3090 (2020).
25 Kikuchi, K. *et al.* Retinoic acid production by endocardium and epicardium is an injury response essential for zebrafish heart regeneration. *Dev Cell* **20**, 397-404, doi:10.1016/j.devcel.2011.01.010 (2011).
26 Liao, S. *et al.* Heart regeneration in adult Xenopus tropicalis after apical resection. *Cell Biosci* **7**, 70, doi:10.1186/s13578-017-0199-6 (2017).
27 Wu, H. Y. *et al.* Fosl1 is vital to heart regeneration upon apex resection in adult Xenopus tropicalis. *NPJ Regen Med* **6**, 36, doi:10.1038/s41536-021-00146-y (2021).
28 Godwin, J. W., Debuque, R., Salimova, E. & Rosenthal, N. A. Heart regeneration in the salamander relies on macrophage-mediated control of fibroblast activation and the extracellular landscape. *NPJ Regen Med* **2**, doi:10.1038/s41536-017-0027-y (2017).
29 Flink, I. L. Cell cycle reentry of ventricular and atrial cardiomyocytes and cells within the epicardium following amputation of the ventricular apex in the axolotl, Amblystoma mexicanum: confocal microscopic immunofluorescent image analysis of bromodeoxyuridine-labeled nuclei. *Anat Embryol (Berl)* **205**, 235-244, doi:10.1007/s00429-002-0249-6 (2002).
30 Vargas-Gonzalez, A., Prado-Zayago, E., Leon-Olea, M., Guarner-Lans, V. & Cano-Martinez, A. [Myocardial regeneration in Ambystoma mexicanum after surgical injury]. *Arch Cardiol Mex* **75 Suppl 3**, S3-21-29 (2005).
31 Oberpriller, J. O. & Oberpriller, J. C. Response of the adult newt ventricle to injury. *J Exp Zool* **187**, 249-253, doi:10.1002/jez.1401870208 (1974).
32 Bader, D. & Oberpriller, J. O. Repair and reorganization of minced cardiac muscle in the adult newt (Notophthalmus viridescens). *J Morphol* **155**, 349-357, doi:10.1002/jmor.1051550307 (1978).
33 Witman, N., Murtuza, B., Davis, B., Arner, A. & Morrison, J. I. Recapitulation of developmental cardiogenesis governs the morphological and functional regeneration of adult newt hearts following injury. *Dev Biol* **354**, 67-76, doi:10.1016/j.ydbio.2011.03.021 (2011).
34 Piatkowski, T., Muhlfeld, C., Borchardt, T. & Braun, T. Reconstitution of the myocardium in regenerating newt hearts is preceded by transient deposition of extracellular matrix components. *Stem Cells Dev* **22**, 1921-1931, doi:10.1089/scd.2012.0575 (2013).
35 Laube, F., Heister, M., Scholz, C., Borchardt, T. & Braun, T. Re-programming of newt cardiomyocytes is induced by tissue regeneration. *J Cell Sci* **119**, 4719-4729, doi:10.1242/jcs.03252 (2006).
36 Uemasu, H. *et al.* Cryo-injury procedure-induced cardiac regeneration shows unique gene expression profiles in the newt Pleurodeles waltl. *Dev Dyn* **251**, 864-876, doi:10.1002/dvdy.450 (2022).
37 Eroglu, E. *et al.* Epicardium-derived cells organize through tight junctions to replenish cardiac muscle in salamanders. *Nat Cell Biol* **24**, 645-658, doi:10.1038/s41556-022-00902-2 (2022).

38 Laflamme, M. A. & Murry, C. E. Heart regeneration. *Nature* **473**, 326-335, doi:10.1038/nature10147 (2011).
39 Porrello, E. R. *et al.* Regulation of neonatal and adult mammalian heart regeneration by the miR-15 family. *Proc Natl Acad Sci U S A* **110**, 187-192, doi:10.1073/pnas.1208863110 (2013).
40 Darehzereshki, A. *et al.* Differential regenerative capacity of neonatal mouse hearts after cryoinjury. *Dev Biol* **399**, 91-99, doi:10.1016/j.ydbio.2014.12.018 (2015).
41 Notari, M. *et al.* The local microenvironment limits the regenerative potential of the mouse neonatal heart. *Sci Adv* **4**, eaao5553, doi:10.1126/sciadv.aao5553 (2018).
42 van Amerongen, M. J., Harmsen, M. C., Petersen, A. H., Popa, E. R. & van Luyn, M. J. Cryoinjury: a model of myocardial regeneration. *Cardiovasc Pathol* **17**, 23-31, doi:10.1016/j.carpath.2007.03.002 (2008).
43 Leferovich, J. M. *et al.* Heart regeneration in adult MRL mice. *Proc Natl Acad Sci U S A* **98**, 9830-9835, doi:10.1073/pnas.181329398 (2001).
44 Oh, Y. S. *et al.* Scar formation after ischemic myocardial injury in MRL mice. *Cardiovasc Pathol* **13**, 203-206, doi:10.1016/j.carpath.2004.03.610 (2004).
45 Abdullah, I., Lepore, J. J., Epstein, J. A., Parmacek, M. S. & Gruber, P. J. MRL mice fail to heal the heart in response to ischemia-reperfusion injury. *Wound Repair Regen* **13**, 205-208, doi:10.1111/j.1067-1927.2005.130212.x (2005).
46 Robey, T. E. & Murry, C. E. Absence of regeneration in the MRL/MpJ mouse heart following infarction or cryoinjury. *Cardiovasc Pathol* **17**, 6-13, doi:10.1016/j.carpath.2007.01.005 (2008).
47 Grisel, P. *et al.* The MRL mouse repairs both cryogenic and ischemic myocardial infarcts with scar. *Cardiovasc Pathol* **17**, 14-22, doi:10.1016/j.carpath.2007.01.007 (2008).
48 Cimini, M. *et al.* The MRL mouse heart does not recover ventricular function after a myocardial infarction. *Cardiovasc Pathol* **17**, 32-39, doi:10.1016/j.carpath.2007.06.006 (2008).
49 Wang, H. *et al.* Natural Heart Regeneration in a Neonatal Rat Myocardial Infarction Model. *Cells* **9**, doi:10.3390/cells9010229 (2020).
50 Zogbi, C. *et al.* Early postnatal rat ventricle resection leads to long-term preserved cardiac function despite tissue hypoperfusion. *Physiol Rep* **2**, doi:10.14814/phy2.12115 (2014).
51 Shiba, Y. *et al.* Human ES-cell-derived cardiomyocytes electrically couple and suppress arrhythmias in injured hearts. *Nature* **489**, 322-325, doi:10.1038/nature11317 (2012).
52 Wang, H. *et al.* A neonatal leporine model of age-dependent natural heart regeneration after myocardial infarction. *J Thorac Cardiovasc Surg* **164**, e389-e405, doi:10.1016/j.jtcvs.2021.08.013 (2022).
53 Haubner, B. J. *et al.* Functional Recovery of a Human Neonatal Heart After Severe Myocardial Infarction. *Circ Res* **118**, 216-221, doi:10.1161/CIRCRESAHA.115.307017 (2016).
54 Cesna, S., Eicken, A., Juenger, H. & Hess, J. Successful treatment of a newborn with acute myocardial infarction on the first day of life. *Pediatr Cardiol* **34**, 1868-1870, doi:10.1007/s00246-012-0417-2 (2013).
55 Deutsch, M. A. *et al.* Successful management of neonatal myocardial infarction with ECMO and intracoronary r-tPA lysis. *Congenit Heart Dis* **9**, E169-174, doi:10.1111/chd.12117 (2014).
56 Yang, P. *et al.* Electrocardiographic characterization of rhesus monkey model of ischemic myocardial infarction induced by left anterior descending artery ligation. *Cardiovasc Toxicol* **11**, 365-372, doi:10.1007/s12012-011-9129-8 (2011).
57 Wang, T., Xiao, Y., Zhang, J., Jing, F. & Zeng, G. Dynamic regulation of HIF-1 signaling in the rhesus monkey heart after ischemic injury. *BMC Cardiovasc Disord* **22**, 407, doi:10.1186/s12872-022-02841-0 (2022).
58 Ye, L. *et al.* Early Regenerative Capacity in the Porcine Heart. *Circulation* **138**, 2798-2808, doi:10.1161/CIRCULATIONAHA.117.031542 (2018).
59 Zhu, W. *et al.* Regenerative Potential of Neonatal Porcine Hearts. *Circulation* **138**, 2809-2816, doi:10.1161/CIRCULATIONAHA.118.034886 (2018).

60 Malliaras, K., Polychronopoulou, E., Poulakida, I., Sagris, D. & Makaritsis, K. Lack of macroscopically evident cardiac regeneration or spontaneous functional recovery in infarcted neonatal pigs. *Hellenic J Cardiol* **61**, 219-221, doi:10.1016/j.hjc.2019.10.012 (2020).
61 Agnew, E. J. *et al.* Scar Formation with Decreased Cardiac Function Following Ischemia/Reperfusion Injury in 1 Month Old Swine. *J Cardiovasc Dev Dis* **7**, doi:10.3390/jcdd7010001 (2019).
62 Herdrich, B. J. *et al.* Regenerative healing following foetal myocardial infarction. *Eur J Cardiothorac Surg* **38**, 691-698, doi:10.1016/j.ejcts.2010.03.049 (2010).
63 Ito, K. *et al.* Differential reparative phenotypes between zebrafish and medaka after cardiac injury. *Dev Dyn* **243**, 1106-1115, doi:10.1002/dvdy.24154 (2014).
64 Lai, S. L. *et al.* Reciprocal analyses in zebrafish and medaka reveal that harnessing the immune response promotes cardiac regeneration. *Elife* **6**, doi:10.7554/eLife.25605 (2017).
65 Marshall, L. N. *et al.* Stage-dependent cardiac regeneration in Xenopus is regulated by thyroid hormone availability. *Proc Natl Acad Sci U S A* **116**, 3614-3623, doi:10.1073/pnas.1803794116 (2019).
66 Marshall, L. *et al.* Persistent fibrosis, hypertrophy and sarcomere disorganisation after endoscopy-guided heart resection in adult Xenopus. *PLoS One* **12**, e0173418, doi:10.1371/journal.pone.0173418 (2017).
67 Peng, H. *et al.* Adult spiny mice (Acomys) exhibit endogenous cardiac recovery in response to myocardial infarction. *NPJ Regen Med* **6**, 74, doi:10.1038/s41536-021-00186-4 (2021).
68 Qi, Y. *et al.* Functional heart recovery in an adult mammal, the spiny mouse. *Int J Cardiol* **338**, 196-203, doi:10.1016/j.ijcard.2021.06.015 (2021).
69 Jopling, C. *et al.* Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. *Nature* **464**, 606-609, doi:10.1038/nature08899 (2010).
70 Kikuchi, K. *et al.* Primary contribution to zebrafish heart regeneration by gata4(+) cardiomyocytes. *Nature* **464**, 601-605, doi:10.1038/nature08804 (2010).
71 Sallin, P., de Preux Charles, A. S., Duruz, V., Pfefferli, C. & Jazwinska, A. A dual epimorphic and compensatory mode of heart regeneration in zebrafish. *Dev Biol* **399**, 27-40, doi:10.1016/j.ydbio.2014.12.002 (2015).
72 Hirose, K. *et al.* Evidence for hormonal control of heart regenerative capacity during endothermy acquisition. *Science* **364**, 184-188, doi:10.1126/science.aar2038 (2019).
73 Gonzalez-Rosa, J. M. *et al.* Myocardial Polyploidization Creates a Barrier to Heart Regeneration in Zebrafish. *Dev Cell* **44**, 433-446 e437, doi:10.1016/j.devcel.2018.01.021 (2018).
74 Bader, D. & Oberpriller, J. Autoradiographic and electron microscopic studies of minced cardiac muscle regeneration in the adult newt, notophthalmus viridescens. *J Exp Zool* **208**, 177-193, doi:10.1002/jez.1402080206 (1979).
75 Bettencourt-Dias, M., Mittnacht, S. & Brockes, J. P. Heterogeneous proliferative potential in regenerative adult newt cardiomyocytes. *J Cell Sci* **116**, 4001-4009, doi:10.1242/jcs.00698 (2003).
76 Marshall, L., Girardot, F., Demeneix, B. A. & Coen, L. Is adult cardiac regeneration absent in Xenopus laevis yet present in Xenopus tropicalis? *Cell Biosci* **8**, 31, doi:10.1186/s13578-018-0231-5 (2018).
77 Walsh, S., Ponten, A., Fleischmann, B. K. & Jovinge, S. Cardiomyocyte cell cycle control and growth estimation in vivo--an analysis based on cardiomyocyte nuclei. *Cardiovasc Res* **86**, 365-373, doi:10.1093/cvr/cvq005 (2010).
78 Kretzschmar, K. *et al.* Profiling proliferative cells and their progeny in damaged murine hearts. *Proc Natl Acad Sci U S A* **115**, E12245-E12254, doi:10.1073/pnas.1805829115 (2018).
79 Patterson, M. *et al.* Frequency of mononuclear diploid cardiomyocytes underlies natural variation in heart regeneration. *Nat Genet* **49**, 1346-1353, doi:10.1038/ng.3929 (2017).
80 Mollova, M. *et al.* Cardiomyocyte proliferation contributes to heart growth in young humans. *Proc Natl Acad Sci U S A* **110**, 1446-1451, doi:10.1073/pnas.1214608110 (2013).

81 Bergmann, O. *et al.* Dynamics of Cell Generation and Turnover in the Human Heart. *Cell* **161**, 1566-1575, doi:10.1016/j.cell.2015.05.026 (2015).
82 Bergmann, O. *et al.* Evidence for cardiomyocyte renewal in humans. *Science* **324**, 98-102, doi:10.1126/science.1164680 (2009).
83 Heallen, T. *et al.* Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size. *Science* **332**, 458-461, doi:10.1126/science.1199010 (2011).
84 Leach, J. P. *et al.* Hippo pathway deficiency reverses systolic heart failure after infarction. *Nature* **550**, 260-264, doi:10.1038/nature24045 (2017).
85 Foglia, M. J. & Poss, K. D. Building and re-building the heart by cardiomyocyte proliferation. *Development* **143**, 729-740, doi:10.1242/dev.132910 (2016).
86 Wang, J., An, M., Haubner, B. J. & Penninger, J. M. Cardiac regeneration: Options for repairing the injured heart. *Front Cardiovasc Med* **9**, 981982, doi:10.3389/fcvm.2022.981982 (2022).
87 Lopaschuk, G. D. & Jaswal, J. S. Energy metabolic phenotype of the cardiomyocyte during development, differentiation, and postnatal maturation. *J Cardiovasc Pharmacol* **56**, 130-140, doi:10.1097/FJC.0b013e3181e74a14 (2010).
88 Semenza, G. L. Life with oxygen. *Science* **318**, 62-64, doi:10.1126/science.1147949 (2007).
89 Puente, B. N. *et al.* The oxygen-rich postnatal environment induces cardiomyocyte cell-cycle arrest through DNA damage response. *Cell* **157**, 565-579, doi:10.1016/j.cell.2014.03.032 (2014).
90 Cardoso, A. C. *et al.* Mitochondrial Substrate Utilization Regulates Cardiomyocyte Cell Cycle Progression. *Nat Metab* **2**, 167-178 (2020).
91 Cui, M. *et al.* Dynamic Transcriptional Responses to Injury of Regenerative and Non-regenerative Cardiomyocytes Revealed by Single-Nucleus RNA Sequencing. *Dev Cell* **53**, 102-116 e108, doi:10.1016/j.devcel.2020.02.019 (2020).
92 Nakada, Y. *et al.* Hypoxia induces heart regeneration in adult mice. *Nature* **541**, 222-227, doi:10.1038/nature20173 (2017).
93 Staudt, D. & Stainier, D. Uncovering the molecular and cellular mechanisms of heart development using the zebrafish. *Annu Rev Genet* **46**, 397-418, doi:10.1146/annurev-genet-110711-155646 (2012).
94 Jopling, C., Sune, G., Faucherre, A., Fabregat, C. & Izpisua Belmonte, J. C. Hypoxia induces myocardial regeneration in zebrafish. *Circulation* **126**, 3017-3027, doi:10.1161/CIRCULATIONAHA.112.107888 (2012).
95 Wong, Y. L. *et al.* Cell biology. Reversible centriole depletion with an inhibitor of Polo-like kinase 4. *Science* **348**, 1155-1160, doi:10.1126/science.aaa5111 (2015).
96 Zebrowski, D. C. *et al.* Developmental alterations in centrosome integrity contribute to the post-mitotic state of mammalian cardiomyocytes. *Elife* **4**, doi:10.7554/eLife.05563 (2015).
97 Moser, S. C. *et al.* PHD1 links cell-cycle progression to oxygen sensing through hydroxylation of the centrosomal protein Cep192. *Dev Cell* **26**, 381-392, doi:10.1016/j.devcel.2013.06.014 (2013).
98 Pettinato, A. M., Ladha, F. A. & Hinson, J. T. The Cardiac Sarcomere and Cell Cycle. *Curr Cardiol Rep* **24**, 623-630, doi:10.1007/s11886-022-01682-9 (2022).
99 Ahuja, P., Perriard, E., Perriard, J. C. & Ehler, E. Sequential myofibrillar breakdown accompanies mitotic division of mammalian cardiomyocytes. *J Cell Sci* **117**, 3295-3306, doi:10.1242/jcs.01159 (2004).
100 Li, F., Wang, X. & Gerdes, A. M. Formation of binucleated cardiac myocytes in rat heart: II. Cytoskeletal organisation. *J Mol Cell Cardiol* **29**, 1553-1565, doi:10.1006/jmcc.1997.0403 (1997).
101 Guo, Y. & Pu, W. T. Cardiomyocyte Maturation: New Phase in Development. *Circ Res* **126**, 1086-1106, doi:10.1161/CIRCRESAHA.119.315862 (2020).
102 Ali, S. R. *et al.* Existing cardiomyocytes generate cardiomyocytes at a low rate after birth in mice. *Proc Natl Acad Sci U S A* **111**, 8850-8855, doi:10.1073/pnas.1408233111 (2014).

103 Mohamed, T. M. A. *et al.* Regulation of Cell Cycle to Stimulate Adult Cardiomyocyte Proliferation and Cardiac Regeneration. *Cell* **173**, 104-116 e112, doi:10.1016/j.cell.2018.02.014 (2018).

104 Abouleisa, R. R. E. *et al.* Transient Cell Cycle Induction in Cardiomyocytes to Treat Subacute Ischemic Heart Failure. *Circulation* **145**, 1339-1355, doi:10.1161/CIRCULATIONAHA.121.057641 (2022).

105 Chen, Y. *et al.* Reversible reprogramming of cardiomyocytes to a fetal state drives heart regeneration in mice. *Science* **373**, 1537-1540, doi:10.1126/science.abg5159 (2021).

106 Aharonov, A. *et al.* ERBB2 drives YAP activation and EMT-like processes during cardiac regeneration. *Nat Cell Biol* **22**, 1346-1356, doi:10.1038/s41556-020-00588-4 (2020).

107 Ouyang, Z. & Wei, K. miRNA in cardiac development and regeneration. *Cell Regen* **10**, 14, doi:10.1186/s13619-021-00077-5 (2021).

108 Farley, R. C. E. a. R. D. Growth and the Reduction of Depensation of Zebrafish, Brachydanio rerio, Reared in the Laboratory. *Copeia* **Vol. 1974, No. 1 (Mar. 28, 1974), pp. 204-209 (6 pages)**, doi:https://doi.org/10.2307/1443024 (1974).

109 He, L., Nguyen, N. B., Ardehali, R. & Zhou, B. Heart Regeneration by Endogenous Stem Cells and Cardiomyocyte Proliferation: Controversy, Fallacy, and Progress. *Circulation* **142**, 275-291, doi:10.1161/CIRCULATIONAHA.119.045566 (2020).

110 Zhang, R. *et al.* In vivo cardiac reprogramming contributes to zebrafish heart regeneration. *Nature* **498**, 497-501, doi:10.1038/nature12322 (2013).

111 Foglia, M. J., Cao, J., Tornini, V. A. & Poss, K. D. Multicolor mapping of the cardiomyocyte proliferation dynamics that construct the atrium. *Development* **143**, 1688-1696, doi:10.1242/dev.136606 (2016).

112 Cao, J. & Poss, K. D. The epicardium as a hub for heart regeneration. *Nat Rev Cardiol* **15**, 631-647, doi:10.1038/s41569-018-0046-4 (2018).

113 Simoes, F. C. & Riley, P. R. The ontogeny, activation and function of the epicardium during heart development and regeneration. *Development* **145**, doi:10.1242/dev.155994 (2018).

114 Cai, W. *et al.* Limited Regeneration Potential with Minimal Epicardial Progenitor Conversions in the Neonatal Mouse Heart after Injury. *Cell Rep* **28**, 190-201 e193, doi:10.1016/j.celrep.2019.06.003 (2019).

115 Smart, N. *et al.* De novo cardiomyocytes from within the activated adult heart after injury. *Nature* **474**, 640-644, doi:10.1038/nature10188 (2011).

116 Kikuchi, K. *et al.* tcf21+ epicardial cells adopt non-myocardial fates during zebrafish heart development and regeneration. *Development* **138**, 2895-2902, doi:10.1242/dev.067041 (2011).

117 Gonzalez-Rosa, J. M., Peralta, M. & Mercader, N. Pan-epicardial lineage tracing reveals that epicardium derived cells give rise to myofibroblasts and perivascular cells during zebrafish heart regeneration. *Dev Biol* **370**, 173-186, doi:10.1016/j.ydbio.2012.07.007 (2012).

118 Xia, Y. *et al.* Activation of a transient progenitor state in the epicardium is required for zebrafish heart regeneration. *Nat Commun* **13**, 7704, doi:10.1038/s41467-022-35433-9 (2022).

119 Huang, G. N. *et al.* C/EBP transcription factors mediate epicardial activation during heart development and injury. *Science* **338**, 1599-1603, doi:10.1126/science.1229765 (2012).

120 Cao, Y. *et al.* Identification of enhancer regulatory elements that direct epicardial gene expression during zebrafish heart regeneration. *Development* **149**, doi:10.1242/dev.200133 (2022).

121 Goldman, J. A. *et al.* Resolving Heart Regeneration by Replacement Histone Profiling. *Dev Cell* **40**, 392-404 e395, doi:10.1016/j.devcel.2017.01.013 (2017).

122 Pfefferli, C. & Jazwinska, A. The careg element reveals a common regulation of regeneration in the zebrafish myocardium and fin. *Nat Commun* **8**, 15151, doi:10.1038/ncomms15151 (2017).

123 Kang, J. *et al.* Modulation of tissue repair by regeneration enhancer elements. *Nature* **532**, 201-206, doi:10.1038/nature17644 (2016).
124 Begeman, I. J. *et al.* Decoding an organ regeneration switch by dissecting cardiac regeneration enhancers. *Development* **147**, doi:10.1242/dev.194019 (2020).
125 Tallquist, M. D. & Molkentin, J. D. Redefining the identity of cardiac fibroblasts. *Nat Rev Cardiol* **14**, 484-491, doi:10.1038/nrcardio.2017.57 (2017).
126 Sanchez-Iranzo, H. *et al.* Transient fibrosis resolves via fibroblast inactivation in the regenerating zebrafish heart. *Proc Natl Acad Sci U S A* **115**, 4188-4193, doi:10.1073/pnas.1716713115 (2018).
127 Hu, B. *et al.* Origin and function of activated fibroblast states during zebrafish heart regeneration. *Nat Genet* **54**, 1227-1237, doi:10.1038/s41588-022-01129-5 (2022).
128 Koth, J. *et al.* Runx1 promotes scar deposition and inhibits myocardial proliferation and survival during zebrafish heart regeneration. *Development* **147**, doi:10.1242/dev.186569 (2020).
129 Simoes, F. C. *et al.* Macrophages directly contribute collagen to scar formation during zebrafish heart regeneration and mouse heart repair. *Nature communications* **11**, 600, doi:10.1038/s41467-019-14263-2 (2020).
130 Mercer, S. E., Odelberg, S. J. & Simon, H. G. A dynamic spatiotemporal extracellular matrix facilitates epicardial-mediated vertebrate heart regeneration. *Dev Biol* **382**, 457-469, doi:10.1016/j.ydbio.2013.08.002 (2013).
131 Wang, Z. *et al.* Cell-Type-Specific Gene Regulatory Networks Underlying Murine Neonatal Heart Regeneration at Single-Cell Resolution. *Cell Rep* **33**, 108472, doi:10.1016/j.celrep.2020.108472 (2020).
132 Hesse, J. *et al.* Single-cell transcriptomics defines heterogeneity of epicardial cells and fibroblasts within the infarcted murine heart. *Elife* **10**, doi:10.7554/eLife.65921 (2021).
133 Forte, E. *et al.* Dynamic Interstitial Cell Response during Myocardial Infarction Predicts Resilience to Rupture in Genetically Diverse Mice. *Cell Rep* **30**, 3149-3163 e3146, doi:10.1016/j.celrep.2020.02.008 (2020).
134 Kanisicak, O. *et al.* Genetic lineage tracing defines myofibroblast origin and function in the injured heart. *Nat Commun* **7**, 12260, doi:10.1038/ncomms12260 (2016).
135 Fu, X. *et al.* Specialized fibroblast differentiated states underlie scar formation in the infarcted mouse heart. *J Clin Invest* **128**, 2127-2143, doi:10.1172/JCI98215 (2018).
136 Wang, Y. *et al.* Single-cell analysis of murine fibroblasts identifies neonatal to adult switching that regulates cardiomyocyte maturation. *Nat Commun* **11**, 2585, doi:10.1038/s41467-020-16204-w (2020).
137 Bassat, E. *et al.* The extracellular matrix protein agrin promotes heart regeneration in mice. *Nature* **547**, 179-184, doi:10.1038/nature22978 (2017).
138 Yokota, T. *et al.* Type V Collagen in Scar Tissue Regulates the Size of Scar after Heart Injury. *Cell* **182**, 545-562 e523, doi:10.1016/j.cell.2020.06.030 (2020).
139 Yahalom-Ronen, Y., Rajchman, D., Sarig, R., Geiger, B. & Tzahor, E. Reduced matrix rigidity promotes neonatal cardiomyocyte dedifferentiation, proliferation and clonal expansion. *Elife* **4**, doi:10.7554/eLife.07455 (2015).
140 Maden, M. & Varholick, J. A. Model systems for regeneration: the spiny mouse, Acomys cahirinus. *Development* **147**, doi:10.1242/dev.167718 (2020).
141 Lupu, I. E., De Val, S. & Smart, N. Coronary vessel formation in development and disease: mechanisms and insights for therapy. *Nat Rev Cardiol* **17**, 790-806, doi:10.1038/s41569-020-0400-1 (2020).
142 Reese, D. E., Mikawa, T. & Bader, D. M. Development of the coronary vessel system. *Circ Res* **91**, 761-768, doi:10.1161/01.res.0000038961.53759.3c (2002).

143 Tota, B., Cimini, V., Salvatore, G. & Zummo, G. Comparative study of the arterial and lacunary systems of the ventricular myocardium of elasmobranch and teleost fishes. *Am J Anat* **167**, 15-32, doi:10.1002/aja.1001670103 (1983).
144 Harrison, M. R. *et al.* Chemokine-guided angiogenesis directs coronary vasculature formation in zebrafish. *Dev Cell* **33**, 442-454, doi:10.1016/j.devcel.2015.04.001 (2015).
145 Harrison, M. R. *et al.* Late developing cardiac lymphatic vasculature supports adult zebrafish heart function and regeneration. *Elife* **8**, doi:10.7554/eLife.42762 (2019).
146 Gancz, D. *et al.* Distinct origins and molecular mechanisms contribute to lymphatic formation during cardiac growth and regeneration. *Elife* **8**, doi:10.7554/eLife.44153 (2019).
147 Lemanski, L. F., Fitts, E. P. & Marx, B. S. Fine structure of the heart in the Japanese Medaka, Oryzias latipes. *J Ultrastruct Res* **53**, 37-65, doi:10.1016/s0022-5320(75)80085-2 (1975).
148 Kapuria, S., Yoshida, T. & Lien, C. L. Coronary Vasculature in Cardiac Development and Regeneration. *J Cardiovasc Dev Dis* **5**, doi:10.3390/jcdd5040059 (2018).
149 Red-Horse, K., Ueno, H., Weissman, I. L. & Krasnow, M. A. Coronary arteries form by developmental reprogramming of venous cells. *Nature* **464**, 549-553, doi:10.1038/nature08873 (2010).
150 Zhang, H. *et al.* Endocardium Minimally Contributes to Coronary Endothelium in the Embryonic Ventricular Free Walls. *Circ Res* **118**, 1880-1893, doi:10.1161/CIRCRESAHA.116.308749 (2016).
151 Wu, B. *et al.* Endocardial cells form the coronary arteries by angiogenesis through myocardial-endocardial VEGF signaling. *Cell* **151**, 1083-1096, doi:10.1016/j.cell.2012.10.023 (2012).
152 Tian, X. *et al.* Vessel formation. De novo formation of a distinct coronary vascular population in neonatal heart. *Science* **345**, 90-94, doi:10.1126/science.1251487 (2014).
153 Klaourakis, K., Vieira, J. M. & Riley, P. R. The evolving cardiac lymphatic vasculature in development, repair and regeneration. *Nat Rev Cardiol* **18**, 368-379, doi:10.1038/s41569-020-00489-x (2021).
154 Stone, O. A. & Stainier, D. Y. R. Paraxial Mesoderm Is the Major Source of Lymphatic Endothelium. *Dev Cell* **50**, 247-255 e243, doi:10.1016/j.devcel.2019.04.034 (2019).
155 Klotz, L. *et al.* Cardiac lymphatics are heterogeneous in origin and respond to injury. *Nature* **522**, 62-67, doi:10.1038/nature14483 (2015).
156 Lioux, G. *et al.* A Second Heart Field-Derived Vasculogenic Niche Contributes to Cardiac Lymphatics. *Dev Cell* **52**, 350-363 e356, doi:10.1016/j.devcel.2019.12.006 (2020).
157 Maruyama, K., Miyagawa-Tomita, S., Mizukami, K., Matsuzaki, F. & Kurihara, H. Isl1-expressing non-venous cell lineage contributes to cardiac lymphatic vessel development. *Dev Biol* **452**, 134-143, doi:10.1016/j.ydbio.2019.05.002 (2019).
158 Marin-Juez, R. *et al.* Fast revascularization of the injured area is essential to support zebrafish heart regeneration. *Proc Natl Acad Sci U S A* **113**, 11237-11242, doi:10.1073/pnas.1605431113 (2016).
159 Zhao, L. *et al.* Notch signaling regulates cardiomyocyte proliferation during zebrafish heart regeneration. *Proc Natl Acad Sci U S A* **111**, 1403-1408, doi:10.1073/pnas.1311705111 (2014).
160 Marin-Juez, R. *et al.* Coronary Revascularization During Heart Regeneration Is Regulated by Epicardial and Endocardial Cues and Forms a Scaffold for Cardiomyocyte Repopulation. *Dev Cell* **51**, 503-515 e504, doi:10.1016/j.devcel.2019.10.019 (2019).
161 Vivien, C. J. *et al.* Vegfc/d-dependent regulation of the lymphatic vasculature during cardiac regeneration is influenced by injury context. *NPJ Regen Med* **4**, 18, doi:10.1038/s41536-019-0079-2 (2019).
162 He, L. *et al.* Preexisting endothelial cells mediate cardiac neovascularization after injury. *J Clin Invest* **127**, 2968-2981, doi:10.1172/JCI93868 (2017).
163 Dube, K. N. *et al.* Recapitulation of developmental mechanisms to revascularize the ischemic heart. *JCI Insight* **2**, doi:10.1172/jci.insight.96800 (2017).

164 Payne, S. *et al.* Regulatory pathways governing murine coronary vessel formation are dysregulated in the injured adult heart. *Nat Commun* **10**, 3276, doi:10.1038/s41467-019-10710-2 (2019).
165 Seiler, C., Stoller, M., Pitt, B. & Meier, P. The human coronary collateral circulation: development and clinical importance. *Eur Heart J* **34**, 2674-2682, doi:10.1093/eurheartj/eht195 (2013).
166 Das, S. *et al.* A Unique Collateral Artery Development Program Promotes Neonatal Heart Regeneration. *Cell* **176**, 1128-1142 e1118, doi:10.1016/j.cell.2018.12.023 (2019).
167 Henri, O. *et al.* Selective Stimulation of Cardiac Lymphangiogenesis Reduces Myocardial Edema and Fibrosis Leading to Improved Cardiac Function Following Myocardial Infarction. *Circulation* **133**, 1484-1497; discussion 1497, doi:10.1161/CIRCULATIONAHA.115.020143 (2016).
168 Vieira, J. M. *et al.* The cardiac lymphatic system stimulates resolution of inflammation following myocardial infarction. *J Clin Invest* **128**, 3402-3412, doi:10.1172/JCI97192 (2018).
169 Simoes, F. C. & Riley, P. R. Immune cells in cardiac repair and regeneration. *Development* **149**, doi:10.1242/dev.199906 (2022).
170 Swirski, F. K. & Nahrendorf, M. Cardioimmunology: the immune system in cardiac homeostasis and disease. *Nat Rev Immunol* **18**, 733-744, doi:10.1038/s41577-018-0065-8 (2018).
171 de Preux Charles, A. S., Bise, T., Baier, F., Marro, J. & Jazwinska, A. Distinct effects of inflammation on preconditioning and regeneration of the adult zebrafish heart. *Open Biol* **6**, doi:10.1098/rsob.160102 (2016).
172 Bevan, L. *et al.* Specific macrophage populations promote both cardiac scar deposition and subsequent resolution in adult zebrafish. *Cardiovasc Res* **116**, 1357-1371, doi:10.1093/cvr/cvz221 (2020).
173 Hui, S. P. *et al.* Zebrafish Regulatory T Cells Mediate Organ-Specific Regenerative Programs. *Dev Cell* **43**, 659-672 e655, doi:10.1016/j.devcel.2017.11.010 (2017).
174 Xu, S. *et al.* Prolonged neutrophil retention in the wound impairs zebrafish heart regeneration after cryoinjury. *Fish Shellfish Immunol* **94**, 447-454, doi:10.1016/j.fsi.2019.09.030 (2019).
175 Sanz-Morejon, A. *et al.* Wilms Tumor 1b Expression Defines a Pro-regenerative Macrophage Subtype and Is Required for Organ Regeneration in the Zebrafish. *Cell Rep* **28**, 1296-1306 e1296, doi:10.1016/j.celrep.2019.06.091 (2019).
176 Bruton, F. A. *et al.* Macrophages trigger cardiomyocyte proliferation by increasing epicardial vegfaa expression during larval zebrafish heart regeneration. *Dev Cell* **57**, 1512-1528 e1515, doi:10.1016/j.devcel.2022.05.014 (2022).
177 Horckmans, M. *et al.* Neutrophils orchestrate post-myocardial infarction healing by polarizing macrophages towards a reparative phenotype. *Eur Heart J* **38**, 187-197, doi:10.1093/eurheartj/ehw002 (2017).
178 Nahrendorf, M. *et al.* The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. *J Exp Med* **204**, 3037-3047, doi:10.1084/jem.20070885 (2007).
179 Hilgendorf, I. *et al.* Ly-6Chigh monocytes depend on Nr4a1 to balance both inflammatory and reparative phases in the infarcted myocardium. *Circ Res* **114**, 1611-1622, doi:10.1161/CIRCRESAHA.114.303204 (2014).
180 Bajpai, G. *et al.* Tissue Resident CCR2- and CCR2+ Cardiac Macrophages Differentially Orchestrate Monocyte Recruitment and Fate Specification Following Myocardial Injury. *Circ Res* **124**, 263-278, doi:10.1161/CIRCRESAHA.118.314028 (2019).
181 Dick, S. A. *et al.* Self-renewing resident cardiac macrophages limit adverse remodeling following myocardial infarction. *Nat Immunol* **20**, 29-39, doi:10.1038/s41590-018-0272-2 (2019).

182 Jung, S. H. *et al.* Spatiotemporal dynamics of macrophage heterogeneity and a potential function of Trem2(hi) macrophages in infarcted hearts. *Nat Commun* **13**, 4580, doi:10.1038/s41467-022-32284-2 (2022).
183 Rizzo, G. *et al.* Dynamics of monocyte-derived macrophage diversity in experimental myocardial infarction. *Cardiovasc Res*, doi:10.1093/cvr/cvac113 (2022).
184 Bajpai, G. *et al.* The human heart contains distinct macrophage subsets with divergent origins and functions. *Nat Med* **24**, 1234-1245, doi:10.1038/s41591-018-0059-x (2018).
185 Aurora, A. B. *et al.* Macrophages are required for neonatal heart regeneration. *J Clin Invest* **124**, 1382-1392, doi:10.1172/JCI72181 (2014).
186 Lavine, K. J. *et al.* Distinct macrophage lineages contribute to disparate patterns of cardiac recovery and remodeling in the neonatal and adult heart. *Proc Natl Acad Sci U S A* **111**, 16029-16034, doi:10.1073/pnas.1406508111 (2014).
187 Dolejsi, T. *et al.* Adult T-cells impair neonatal cardiac regeneration. *Eur Heart J* **43**, 2698-2709, doi:10.1093/eurheartj/ehac153 (2022).
188 Friedrichsen, S. *et al.* Regulation of iodothyronine deiodinases in the Pax8-/- mouse model of congenital hypothyroidism. *Endocrinology* **144**, 777-784, doi:10.1210/en.2002-220715 (2003).
189 Naqvi, N. *et al.* A proliferative burst during preadolescence establishes the final cardiomyocyte number. *Cell* **157**, 795-807, doi:10.1016/j.cell.2014.03.035 (2014).
190 Chattergoon, N. N. *et al.* Thyroid hormone drives fetal cardiomyocyte maturation. *FASEB J* **26**, 397-408, doi:10.1096/fj.10-179895 (2012).
191 Wegner, N. C., Snodgrass, O. E., Dewar, H. & Hyde, J. R. Animal physiology. Whole-body endothermy in a mesopelagic fish, the opah, Lampris guttatus. *Science* **348**, 786-789, doi:10.1126/science.aaa8902 (2015).
192 Johnson, S. L. & Weston, J. A. Temperature-sensitive mutations that cause stage-specific defects in Zebrafish fin regeneration. *Genetics* **141**, 1583-1595, doi:10.1093/genetics/141.4.1583 (1995).
193 Katogi, R. *et al.* Large-scale analysis of the genes involved in fin regeneration and blastema formation in the medaka, Oryzias latipes. *Mech Dev* **121**, 861-872, doi:10.1016/j.mod.2004.03.015 (2004).
194 Tanaka, E. M. The Molecular and Cellular Choreography of Appendage Regeneration. *Cell* **165**, 1598-1608, doi:10.1016/j.cell.2016.05.038 (2016).
195 Kragl, M. *et al.* Cells keep a memory of their tissue origin during axolotl limb regeneration. *Nature* **460**, 60-65, doi:10.1038/nature08152 (2009).
196 Mokalled, M. H. *et al.* Injury-induced ctgfa directs glial bridging and spinal cord regeneration in zebrafish. *Science* **354**, 630-634, doi:10.1126/science.aaf2679 (2016).
197 Zukor, K. A., Kent, D. T. & Odelberg, S. J. Meningeal cells and glia establish a permissive environment for axon regeneration after spinal cord injury in newts. *Neural Dev* **6**, 1, doi:10.1186/1749-8104-6-1 (2011).
198 Rost, F. *et al.* Accelerated cell divisions drive the outgrowth of the regenerating spinal cord in axolotls. *Elife* **5**, doi:10.7554/eLife.20357 (2016).
199 Seifert, A. W., Monaghan, J. R., Voss, S. R. & Maden, M. Skin regeneration in adult axolotls: a blueprint for scar-free healing in vertebrates. *PLoS One* **7**, e32875, doi:10.1371/journal.pone.0032875 (2012).
200 Howell, K. *et al.* Novel Model of Tendon Regeneration Reveals Distinct Cell Mechanisms Underlying Regenerative and Fibrotic Tendon Healing. *Sci Rep* **7**, 45238, doi:10.1038/srep45238 (2017).
201 Li, Y. *et al.* Microglia-organized scar-free spinal cord repair in neonatal mice. *Nature* **587**, 613-618, doi:10.1038/s41586-020-2795-6 (2020).
202 Cox, B. C. *et al.* Spontaneous hair cell regeneration in the neonatal mouse cochlea in vivo. *Development* **141**, 816-829, doi:10.1242/dev.103036 (2014).

203 Brigande, J. V. & Heller, S. Quo vadis, hair cell regeneration? *Nat Neurosci* **12**, 679-685, doi:10.1038/nn.2311 (2009).
204 Seifert, A. W. *et al.* Skin shedding and tissue regeneration in African spiny mice (Acomys). *Nature* **489**, 561-565, doi:10.1038/nature11499 (2012).
205 Salimova, E. *et al.* Variable outcomes of human heart attack recapitulated in genetically diverse mice. *NPJ Regen Med* **4**, 5, doi:10.1038/s41536-019-0067-6 (2019).
206 Near, T. J. *et al.* Resolution of ray-finned fish phylogeny and timing of diversification. *Proc Natl Acad Sci U S A* **109**, 13698-13703, doi:10.1073/pnas.1206625109 (2012).
207 Roelants, K. *et al.* Global patterns of diversification in the history of modern amphibians. *Proc Natl Acad Sci U S A* **104**, 887-892, doi:10.1073/pnas.0608378104 (2007).
208 Alvarez-Carretero, S. *et al.* A species-level timeline of mammal evolution integrating phylogenomic data. *Nature* **602**, 263-267, doi:10.1038/s41586-021-04341-1 (2022).

Author contributions

The authors contributed substantially to all aspects of the article.

Competing interests

The authors declare no competing interests.

Table 1 | Heart injury models and regeneration potential across vertebrate species

Species	Class	Order	Age	Cardiac regenerative potential	Injury	Refs
Fish						
Zebrafish (*Danio rerio*)	Actinopterygii (ray-finned fish)	Cypriniformes	Adult	High	Resection, cryoinjury, genetic ablation, cauterisation, hypoxia–reoxygenation	5,11-17
Giant danio (*Devario aequipinnatus*)	Actinopterygii	Cypriniformes	Adult	High	Cauterisation	22
Goldfish (*Carassius auratus*)	Actinopterygii	Cypriniformes	Adult	High	Cauterisation	23
Mexican tetra (*Astyanax mexicanus*)	Actinopterygii	Characiformes	Adult	High in surface-dwelling forms, low in cave-dwelling forms	Resection	7
African turquoise killifish (*Nothobranchius furzeri*)	Actinopterygii	Cyprinodontiformes	Adult	High	Resection	24
Medaka/Japanese rice fish (*Oryzias latipes*)	Actinopterygii	Beloniformes	Adult	Low	Resection, cryoinjury	63,64
Amphibians						
Western clawed frog (*Xenopus tropicalis*)	Amphibia	Anura	6 months, 1 year	High	Resection	26,27
African clawed frog (*Xenopus laevis*)	Amphibia	Anura	6 months, >5 years	Low	Resection	65,66
Eastern newt (*Notophthalmus viridescens*)	Amphibia	Urodela	Adult	High	Resection, mechanical injury	31-35
Iberian ribbed newt (*Pleurodeles waltl*)	Amphibia	Urodela	Adult	Low	Cryoinjury	36
				High	Cryoinjury	37
Axolotl (*Ambystoma mexicanum*)	Amphibia	Urodela	Adult	High	Resection, cryoinjury	6,28-30
Mammals						
Human (*Homo sapiens*)	Mammalia	Primates	Newborn	High	Myocardial infarction	53-55
Rhesus macaque (*Macaca mulatta*)	Mammalia	Primates	Adult	Low	LAD ligation	56,57
European rabbit (*Oryctolagus cuniculus*)	Mammalia	Lagomorpha	1 day	High	LAD ligation	52
			7 days	Low	LAD ligation	52
House mouse (*Mus musculus*)	Mammalia	Rodentia	1 day	High	Resection, LAD ligation, non-transmural cryoinjury	8,9,39-41
			7 days	Low	Resection	8,9,39
				High	Cryoinjury	42

			Adult C57BL6	Low	Cryoinjury, LAD ligation	[45-48]
			Adult MRL/MpJ$^{+/+}$	High	Cryoinjury	[43]
				Low	Cryoinjury, LAD ligation	[44-48]
Brown rat (*Rattus norvegicus*)	Mammalia	Rodentia	1 day	High	Resection, LAD ligation	[49,50]
			7 days	Low	Resection	[50]
			Adult	Low	LAD ligation	[49]
Cairo spiny mouse (*Acomys cahirinus*)	Mammalia	Rodentia	Adult	High	LAD ligation	[67,68]
Guinea pig (*Cavia porcellus*)	Mammalia	Rodentia	Adult	Low	Cryoinjury	[51]
Pig (*Sus domesticus*)	Mammalia	Artiodactyla	1-2 days	High	LAD ligation	[58,59]
				Low	LAD ligation	[60]
			>2 days	Low	LAD ligation, ischemia–reperfusion	[58-61]
Sheep (*Ovis aries*)	Mammalia	Artiodactyla	Embryonic (65-76 days)	High	LAD ligation	[62]
			Adult	Low	LAD ligation	[62]
Grey short-tailed opossum (*Monodelphis domestica*)	Mammalia	Marsupialia	14 days	High	Resection, LAD ligation	[10]
			29 days	Low	Resection, LAD ligation	[10]

LAD, left anterior descending coronary artery.

Table 2 | Cardiac phenotypes associated with differential cardiac regeneration potential in closely related species

Species	Cardiac regeneration potential	Adult cardiac phenotype	Refs.
Fish			
Zebrafish (*Danio rerio)*	Robust	Temporally restricted presence of neutrophils and early macrophage recruitment following injury	[64]
Medaka/Japanese rice fish (*Oryzias latipes)*	Restricted	Prolonged presence of neutrophils and delayed macrophage recruitment following injury	[64]
Mexican tetra (*Astyanax mexicanus;* cave-dwelling form)	Restricted	Low energy metabolism, strong immune response and high fibrosis following injury	[7]
Mexican tetra (*Astyanax mexicanus;* surface-dwelling form)	Robust	Increased energy metabolism, reduced immune response and reduced fibrosis following injury	[7]
Mammals			
House mouse (*Mus musculus)*	Restricted	Restricted neovascularization following MI	[164,166]
		Fibrotic tissue with high compression following MI	[67]
		Low frequency of diploid cardiomyocytes	[8,72,77]
Spiny mouse (*Acomys spp.*)	Robust	Robust neovascularization following MI	[67]
		Fibrotic tissue with lower compression and higher cellular density following MI	[67,68]
		Increased frequency of diploid cardiomyocytes	[67,72]

Table 3 | Vertebrate regeneration potential across organs and tissues

Species	Organ	Regeneration potential	Refs.
Fish			
Zebrafish *(Danio rerio)*	Heart	Robust	5,11-17
	Caudal fin	Robust	192
	Spinal cord	Robust	196
Medaka/Japanese rice fish *(Oryzias latipes)*	Heart	Restricted	63,64
	Caudal fin	Robust	193
Mexican tetra *(Astyanax mexicanus;* surface-dwelling form)	Heart	Robust	7
	Caudal fin	Robust	7
Mexican tetra *(Astyanax mexicanus;* cave-dwelling form)	Heart	Restricted	7
	Caudal fin	Robust	7
Amphibians			
Eastern newt *(Notophthalmus viridescens)*	Heart	Robust	31-35
	Limb	Robust	194
	Spinal cord	Robust	197
Axolotl *(Ambystoma mexicanum*)	Heart	Robust	6,28-30
	Limb	Robust	195
	Skin	Robust	199
	Spinal cord	Robust	198
Mammals			
House mouse (*Mus musculus;* adult)	Heart	Restricted	45-48
	Achilles tendon	Restricted	200
	Spinal cord	Restricted	201
	Cochlear hair cells	Restricted	203
House mouse (*Mus musculus*; neonatal)	Heart	Robust	8,39-41
	Achilles tendon	Robust	200
	Spinal cord	Robust	201
	Cochlear hair cells	Robust	202
Spiny mouse (*Acomys spp*.)	Heart	Robust	67,68
	Skin	Robust	204

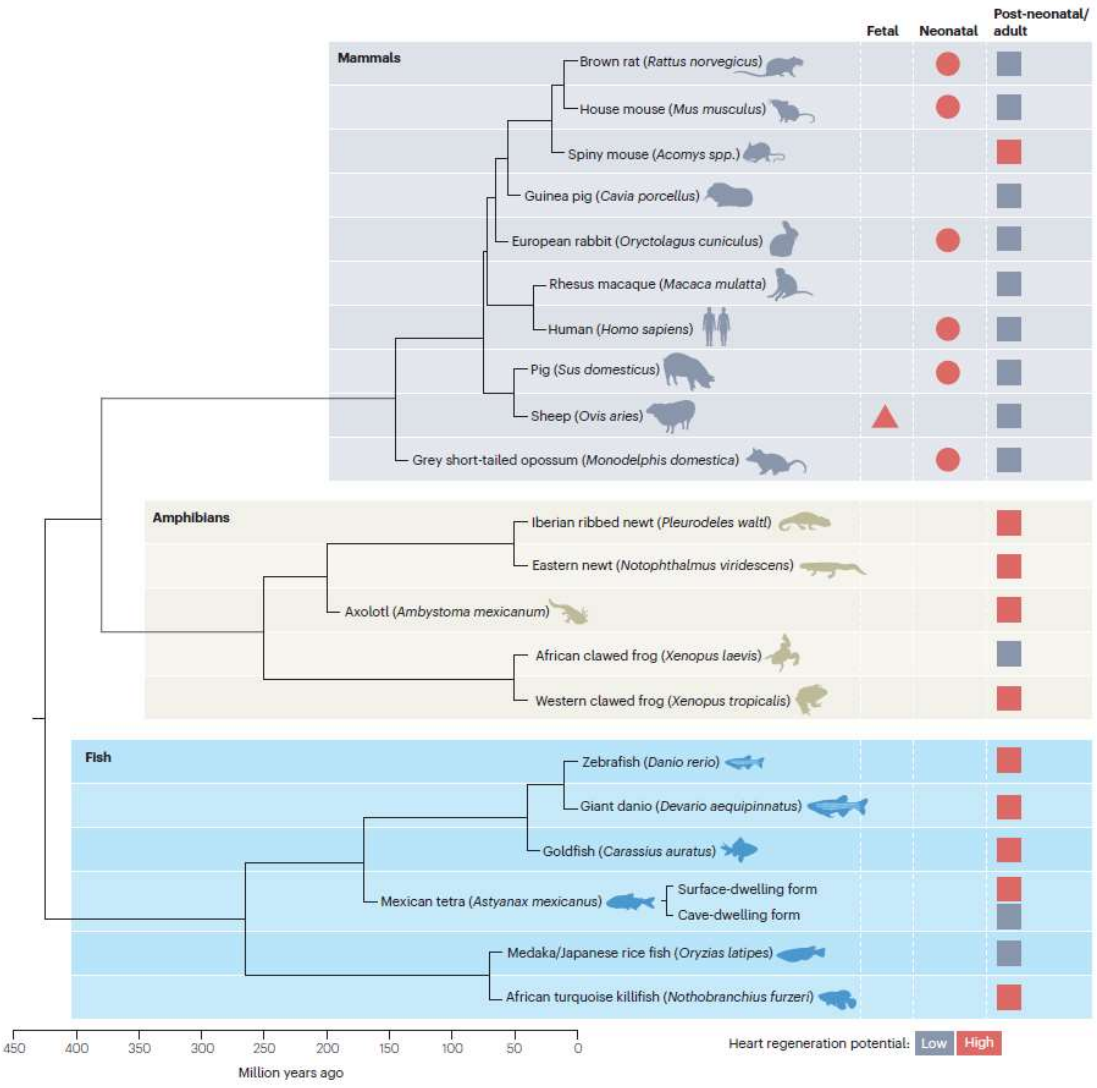
Fetal
Neonatal
Post-neonatal/ adult
Mammals
Brown rat (Rattus norvegicus)
House mouse (Mus musculus)
Spiny mouse (Acomys spp.)
Guinea pig (Cavia porcellus)
European rabbit (Oryctolagus cuniculus)
Rhesus macaque (Macaca mulatta)
Human (Homo sapiens)
Pig (Sus domesticus)
Sheep (Ovis aries)
Grey short-tailed opossum (Monodelphis domestica)
Amphibians
Iberian ribbed newt (Pleurodeles waltl)
Eastern newt (Notophthalmus viridescens)
Axolotl (Ambystoma mexicanum)
African clawed frog (Xenopus laevis)
Western clawed frog (Xenopus tropicalis)
Fish
Zebrafish (Danio rerio)
Giant danio (Devario aequipinnatus)
Goldfish (Carassius auratus)
Mexican tetra (Astyanax mexicanus)
Surface-dwelling form
Cave-dwelling form
Medaka/Japanese rice fish (Oryzias latipes)
African turquoise killifish (Nothobranchius furzeri)
450
400
350
300
250
200
150
100
50
0
Million years ago
Heart regeneration potential:
Low
High

Fig. 1

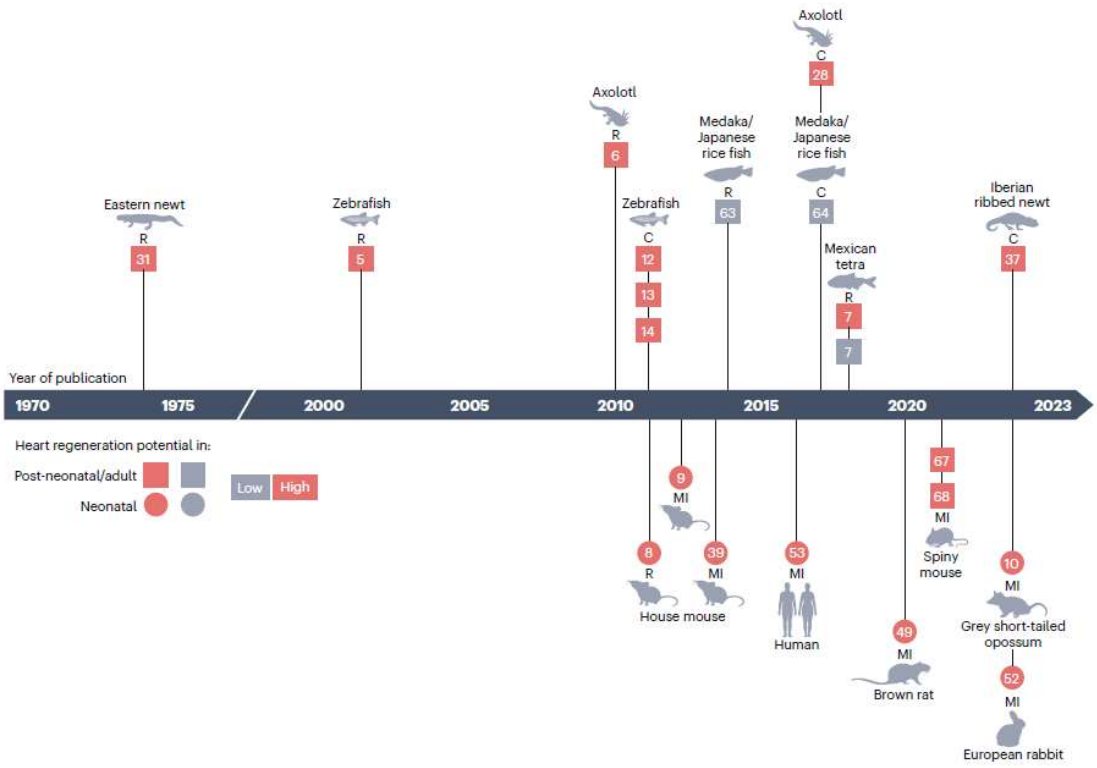
Axolotl
C
28
Axolotl
R
6
Medaka/
Japanese
rice fish
R
63
Medaka/
Japanese
rice fish
C
64
Iberian
ribbed newt
C
37
Eastern newt
R
31
Zebrafish
R
5
Zebrafish
C
12
13
14
Mexican
tetra
R
7
7
Year of publication
1970
1975
2000
2005
2010
2015
2020
2023
Heart regeneration potential in:
Post-neonatal/adult
Neonatal
Low
High
9
MI
8
R
39
MI
House mouse
53
MI
Human
67
68
MI
Spiny
mouse
10
MI
Grey short-tailed
opossum
49
MI
Brown rat
52
MI
European rabbit

Fig. 2

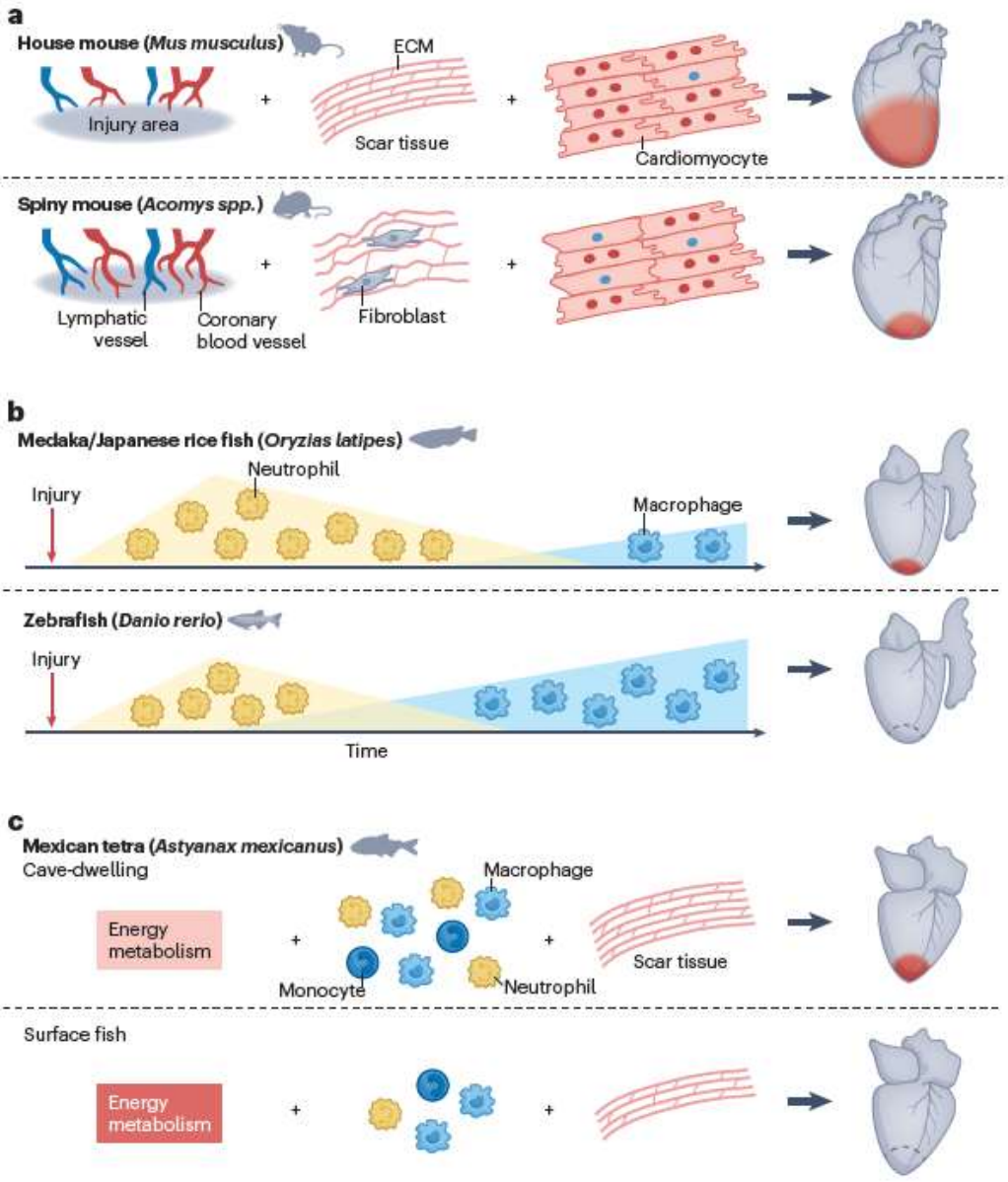
a
House mouse (Mus musculus)
Injury area
ECM
Scar tissue
Cardiomyocyte
Spiny mouse (Acomys spp.)
Lymphatic vessel
Coronary blood vessel
Fibroblast
b
Medaka/Japanese rice fish (Oryzias latipes)
Injury
Neutrophil
Macrophage
Zebrafish (Danio rerio)
Injury
Time
c
Mexican tetra (Astyanax mexicanus)
Cave-dwelling
Energy metabolism
Macrophage
Monocyte
Neutrophil
Scar tissue
Surface fish
Energy metabolism

Fig. 3

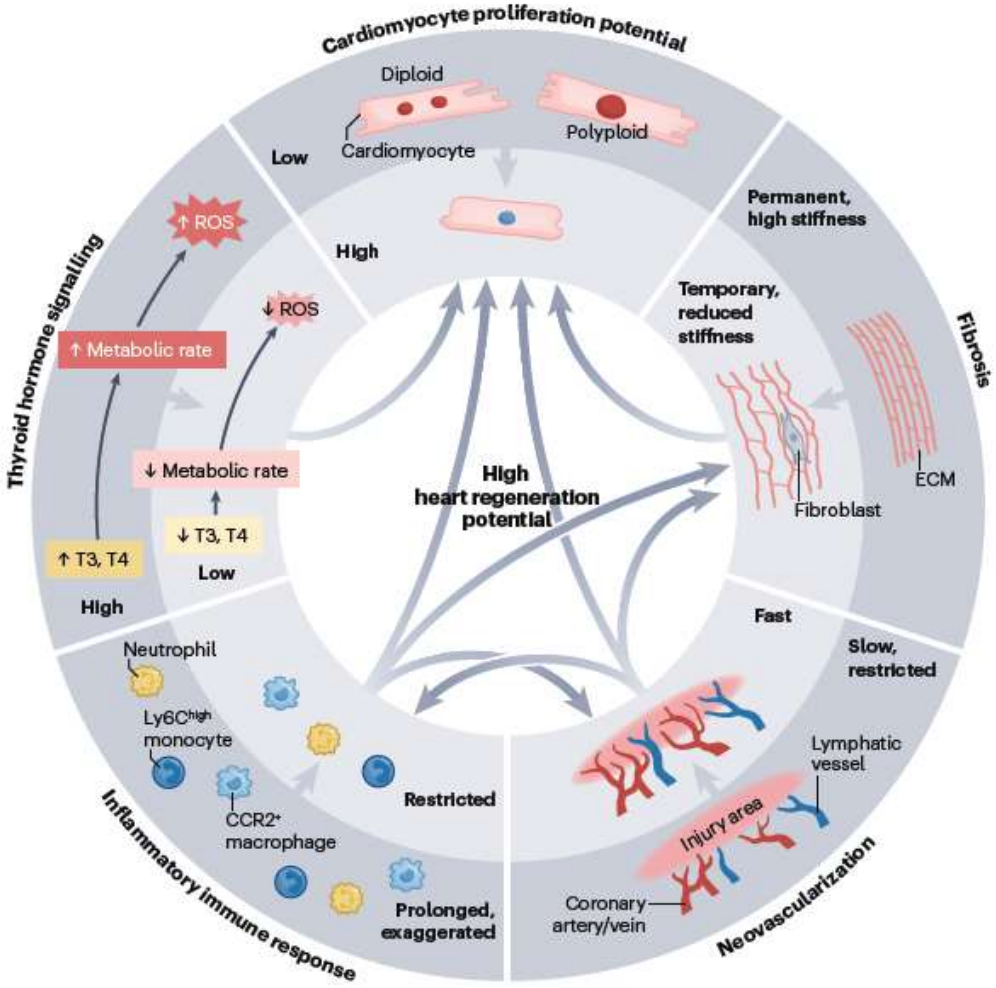
Cardiomyocyte proliferation potential
Diploid
Polyploid
Cardiomyocyte
Low
High
Permanent, high stiffness
Temporary, reduced stiffness
Fibrosis
ECM
Fibroblast
High heart regeneration potential
Thyroid hormone signalling
↑ ROS
↓ ROS
↑ Metabolic rate
↓ Metabolic rate
↑ T3, T4
↓ T3, T4
High
Low
Fast
Slow, restricted
Neutrophil
Ly6C^high monocyte
CCR2+ macrophage
Restricted
Prolonged, exaggerated
Inflammatory immune response
Lymphatic vessel
Injury area
Coronary artery/vein
Neovascularization

Fig. 4

Fig. 1 | Heart regeneration potential across vertebrate species. The phylogenetic tree was created using divergence time estimates for fish[206] , amphibians[207] and mammals[208]. Cardiac regeneration capacity at the post-neonatal or adult stage (square), at the neonatal stage (circle) and at the fetal stage (triangle) is indicated as low (grey) or high (red). See also Table 1.

Fig. 2 | Key studies on animal models of heart regeneration. Reported cardiac regeneration capacity at the post-neonatal or adult stage (square) and at the neonatal stage (circle) is indicated as low (grey) or high (red). The numbers indicate the study references, as stated in the main text and reference list. Pictograms indicate the model organism studied. See also Table 1. C, cryoinjury; MI, myocardial infarction; R, resection injury.

Fig. 3 | Cardiac characteristics associated with differential heart regeneration potential in closely related species. a | House mouse (*Mus musculus*) versus spiny mouse (*Acomys spp.*). Increased neovascularization, higher numbers of cells in the fibrotic wound tissue and elevated numbers of diploid, mononucleated cardiomyocytes are associated with the increased cardiac regeneration potential in the spiny mouse[67,68]. **b |** Medaka/Japanese rice fish (*Oryzias latipes*) versus zebrafish (*Danio rerio*). Restricted presence of neutrophils and early recruitment of macrophages are associated with the high cardiac regeneration potential in the zebrafish[64]. **c |** Cave-dwelling versus surface-dwelling forms of Mexican tetra (*Astyanax mexicanus*). Elevated energy metabolism, restricted immune response and reduced fibrosis are associated with the high cardiac regeneration potential in the surface-dwelling Mexican tetra[7]. See also Table 2.

Fig. 4 | Mechanisms that control heart regeneration potential. Grey arrows in the centre section indicate instances where pro-regenerative changes in one mechanism have been found

to result in pro-regenerative changes in another mechanism. Processes occurring in the light grey, central area of the circle are associated with high cardiac regeneration potential and those in the outer ring are associated with low cardiac regeneration potential. ROS, reactive oxygen species; T3, triiodothyronine; T4, thyroxine.

Key points

- Cardiac regeneration potential tends to be robust in fish, amphibians and neonatal mammals, whilst being restricted in adult mammals; in several model organisms however cardiac regeneration potential defies this trend.

- Cardiac regeneration potential is determined by multiple highly interconnected processes, including cardiomyocyte proliferation, fibrosis, neovascularization, immune response and energy metabolism.

- Mammalian cardiomyocytes exit the cell cycle postnatally due to changes in structure and energy metabolism; partial *in vivo* reprogramming of adult mammalian cardiomyocytes can however increase their proliferation capacity.

- Fibrosis in the injured heart is both beneficial and detrimental; altering fibrotic tissue composition and mechanical properties might improve adult mammalian heart regeneration.

- Fast neovascularization of the wound area is a hallmark of heart regeneration and absent in the adult mammalian heart; lymphatic coronary vessels modify the immune response post-MI via immune cell clearance.

- The immune response to cardiac injury consists of multiple phases; restricting the initial inflammatory phase and promoting the subsequent reparative phase poses a strategy to improve heart regeneration.