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The Cardiac Sarcomere and Cell Cycle

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Abstract

Purpose of Review—The lack of adult human cardiomyocyte proliferative capacity impairs cardiac regeneration such as after myocardial injury. The sarcomere, a specialized actin cytoskeletal structure that is essential for twitch contraction in cardiomyocytes, has been considered a critical factor limiting adult human cardiomyocyte proliferation through incompletely understood mechanisms.

Recent Findings—This review summarizes known and emerging regulatory mechanisms connecting the human cardiomyocyte sarcomere to cell cycle regulation including structural and signaling mechanisms.

Summary—Cardiac regeneration could be augmented through targeting the inhibitory effects of the sarcomere on cardiomyocyte proliferation.

Keywords

Sarcomere; Cell cycle; Regeneration; Heart failure

Introduction

Heart failure (HF) is a rapidly growing cardiovascular condition with a prevalence of ~40 million individuals worldwide [1–3]. In the USA alone, HF affects over 6 million individuals and costs the healthcare system over $30 billion annually [4]. HF is a progressive condition that is frequently caused by cardiac injuries such as myocardial infarction, viral infection, and drug-related cardiotoxicity [5]. A shared consequence of many cardiac injuries is loss of cardiomyocyte number due to increased cell death, which promotes contractile dysfunction and maladaptive cardiac remodeling including cardiac chamber dilatation and fibrosis [6]. While in some contexts such as fetal cardiac development, it has been reported that cardiac

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regeneration can occur through cardiomyocyte proliferation (*as defined by an increase in cell division and growth*) [7], it is generally accepted to be insufficient in adult humans. The poor regenerative capacity of the adult human heart is also a consequence of the lack of a cardiac stem cell pool to replenish lost cardiomyocytes such that de novo cardiomyocyte generation must derive from proliferation of the existing cardiomyocyte pool [8]. While there are FDA-approved HF treatments such as adrenergic receptor blockers, neurohormonal antagonists, and implantable electrophysiological devices that have been shown to improve cardiac remodeling, HF symptoms, and even survival in human clinical studies [6], HF patients frequently develop advanced HF characterized by severe cardiac dysfunction that ultimately requires heart transplantation that is supply limited [5]. Therefore, the development of new HF therapeutics particularly those that target cardiac regeneration could be transformative.

Using development as a model to study cardiac regeneration (Figure 1), it has been observed that mammalian fetal cardiomyocytes utilize hyperplasia (*as defined as cardiac growth by cell proliferation*) for cardiac growth and in response to injury [8]; however, shortly after birth and distinct from other organisms such as the newt [9], cardiac growth and injury responses transition to cardiomyocyte hypertrophy (*as defined by cardiac growth by cell enlargement*) and fibrosis [10–12]. In parallel with the switch from hyperplasia to hypertrophy, adult mammalian cardiomyocytes also become progressively polyploid (*defined by greater than 2 paired sets of chromosomes per cell*) [13–17]. For example, the adult human cardiomyocyte is ~10% diploid, ~60% mononuclear polyploid, and ~30% multinuclear polyploid [12, 18]. While polyploidization can occur through several mechanisms [19], it is generally established that mammalian cardiomyocytes become polyploid through either endocycling (*defined as genomic replication without mitosis*) to generate mononuclear polyploid cells, or cytokinesis failure (*defined as genomic replication and mitosis without cell division*) to generate multinuclear cells [10]. Coincident with the shift from hyperplasia to hypertrophy and polyploidization, the mammalian adult heart also undergoes metabolic adaptations upregulating oxidative pathways [20] and biomechanical adaptations [21] including alterations in the expression of factors that sense and generate physical forces within the heart such as the force-producing sarcomere, a multiprotein machine evolved from the actin cytoskeleton that is responsible for cardiac pump function. Recent reviews of mammalian cardiac regeneration have focused on the therapeutic targeting of cardiac regeneration [22], biological relevance of polyploidy [10], and evolutionary perspectives [23], but the role of the sarcomere in cardiac regeneration has been less well summarized. Here, we address this gap in the literature by providing an overview of existing and emerging knowledge as well as potential future directions on the role of the cardiac sarcomere in mammalian cardiac regeneration focused on the cell cycle.

Cardiac Sarcomere Structure

The sarcomere is the basic contractile unit of myocytes and is a unique specialization of the actin cytoskeleton found in higher organisms [24–26]. It is a cytosolic multiprotein complex that functions in mechanical twitch force production, as well as transcriptional regulation [27], cell signaling [28], and metabolic regulation [29]. The sarcomere is organized into parallel repeats of myosin-containing thick filaments, actin-containing thin filaments, and

stabilizing titin filaments, all of which is laterally bounded by actinin-containing Z-disks that are crosslinked to the cytoskeleton by actinin. The area immediately surrounding the Z-disk lacks thick filament structures and is known as the I band, which is adjacent to a region that comprises the entire length of the thick filament known as the A band. Within the A band is a portion devoid of thin filament overlap called the H zone, containing the myomesin-rich M-line that corresponds to the center of the sarcomere [30–32]. In response to intracellular calcium, the troponin regulatory complex on the thin filament undergoes a series of conformational changes that expose myosin binding sites on actin, allowing the myosin head to bind actin and promote twitch contraction through an ATP-consuming power stroke that brings the Z-disk closer to the M-line, shortening the sarcomere [33]. When the sarcomere is not properly assembled, cardiac contractile dysfunction and heart failure can occur such as in the setting of sarcomere gene mutations [34–36]. Mechanosensory and signaling factors are also localized to multiple sarcomere sub-compartments including the Z-disk [37], titin filaments [38], and the M band [39]. The sarcomere also functions as a protein-protein interaction hub integrating mechanosensing with cell signaling pathways previously implicated in cell cycle regulation, in addition to a putative role in cell cycle inhibition through structural hindrance.

The Actin Cytoskeleton and Cell Cycle

Because the sarcomere is a specialized version of the actin cytoskeleton, it is important to first consider how the actin cytoskeleton functions in the cell cycle. Cell division is a complex process [40], which is highly regulated and commonly organized into five sequential cell cycle phases—G0, G1, S, G2, and M. The G0 phase is a quiescent or resting state, which is followed by a gap/growth 1 (G1) phase in which RNA and protein synthesis occur in preparation for the subsequent phases. The synthesis (S) phase is when the genome is duplicated, which includes DNA replication, histone synthesis, and nucleosome formation. This is followed by a G2 phase in which rapid cell growth and protein synthesis occur in preparation for the mitotic (M) phase, in which the nucleus divides and the cell undergoes subsequent division or cytokinesis [40]. The final process of cytokinesis involves a contractile network composed of actin filaments, non-muscle myosin II, and other accessory factors that physically divide the parental cell into two daughter cells [41]. There are a number of key checkpoints that exist within and between phase transitions [42, 43]. Among these, the G1/S checkpoint is well-established to regulate the commitment to the cell cycle and has numerous stimuli that exert control, while the G2/M checkpoint regulates commitment to mitosis that is largely controlled by DNA damage or incomplete DNA replication, ensuring complete DNA replication in preparation for cytokinesis. While numerous factors control the cell cycle phases and checkpoints, key regulators are the cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors (CDKIs) [42]. It is known that cyclin-CDK complexes control specific steps of the cell cycle, and their respective protein levels and activities are tightly controlled through coordinated mechanisms such as by interactions with CDKIs [44–46]. For example, the cyclin D-CDK4/CDK6 complex plays a role in the progression through G1, while cyclin E-CDK2 is important for the G1/S transition. For S phase progression, the cyclin A-CDK2 complex is required, followed by

cyclin A-CDK1 and cyclin B-CDK1 as critical for the progression through G2 and M phases [47, 48].

The actin cytoskeleton undergoes dynamic structural and functional changes that are essential for normal cell cycle progression [49–51]. For example, disruption of actin assembly using the toxin dihydrocytochalasin B [52] activates a G1/S checkpoint through the retinoblastoma pathway involving inhibition of cycle E/CDK2 [53], while the actin polymerization inhibitor cytochalasin D delays mitosis [54, 55]. The actin cytoskeleton also plays a role in cellular rounding and cortical stiffening to prepare for spindle assembly [56], as well as centrosome separation that is essential for mitosis as demonstrated by elegant studies using the actin polymerization toxin latrunculin [57, 58]. Additionally, the actin-binding protein, cortactin, has been found to be a key anchor between actin and the centrosome and is important in actin-facilitated centrosome separation during mitosis [59]. Cortactin is also a substrate of CDK1, which directly links this kinase to cortactin-mediated centrosome separation during mitosis [60]. In cytokinesis, the actin cytoskeleton plays a well-established role in contractile ring assembly at the cleavage furrow. Through force-generating interactions with myosin II, the cleavage furrow ingresses until a midbody structure is formed between daughter cells, after which abscission results in midbody cleavage and separation of the two daughter cells. This process requires actin filament disassembly in the cleavage furrow through the PKCε-14-3-3 complex and RhoA inactivation [61]. Moreover, cell cycle factors can also directly regulate the actin cytoskeleton such as has been shown for CDK1 that can directly phosphorylate the actin crosslinking protein filamin A at serine residues 1084, 1459, and 1533, which is essential for postmitotic daughter cell separation and migration [62]. It is important to note that most of these studies implicating the role of the actin cytoskeleton in cell cycle regulation utilize transformed cell lines and yeast models, and while these are powerful models to study these processes, their generalizability to cardiomyocyte cell cycle regulation may be limited. For example, unlike classical cell cycle model systems such as transformed cell lines, mammalian cardiomyocytes become progressively polyploid through incomplete cell cycle progression by incompletely understood mechanisms [10]. In summary, actin cytoskeletal functions are critical for general cell cycle progression particularly for mitosis and cytokinesis, but have not been sufficiently studied in mammalian cardiomyocyte models that proceed through complex cell cycle patterns.

Sarcomere Structure and Cell Cycle

Relative to adults, fetal mammalian cardiomyocytes that proliferate contain less organized and less abundant sarcomeres that are composed of distinct sarcomere gene and splice isoforms including fetal-enriched myosin heavy chain (*MYH6* in humans), troponin I (*TNNI1*), and titin splice isoforms (*TTN* N2BA) [63]. With development, cardiomyocyte sarcomere content and longitudinal alignment both progressively increase (Figure 1) with a concomitant transition to adult-enriched sarcomere genes and splice isoforms including *MYH7, TNNI3*, and *TTN* N2B. It remains incompletely understood how the developmental transitions in sarcomere structure and gene expression contribute to the proliferative arrest observed in adult mammalian cardiomyocytes, which are considered essential to maintain normal cardiac contractile function in the adult mammalian heart.

During their proliferative phase, it has been observed that fetal mammalian cardiomyocytes in vivo undergo sarcomere disassembly prior to cytokinesis followed by reassembly in daughter cells [64]. This appears to be a coordinated process that involves marginalization of sarcomere structures to the cellular periphery during mitosis and cytokinesis [8, 65], which has been observed to occur by two sequential steps. The first step involves a collapse of the Z-disk and thin filament-associated proteins, followed by disassembly of titin, M band, and thick filament components including myosin heavy chain [64, 66, 67]. Hallmarks of this pattern of sarcomere disassembly have been observed in a number of studies investigating a broad scope of regulators of cardiomyocyte proliferation including overexpression of neuregulin1 (Nrg1) and its receptors (Erbb2 and Erbb4) [65, 68]; surgical resection of the left ventricular apex [8]; Meis1 gene knockout and Meis1-Hoxb13 double knockouts [69, 70•]; and treatment with the cyclin B1-CDK1 inhibitor Ro3306 [67]. In contrast, postnatal cardiomyocytes that express cell cycle markers but do not complete sarcomere disassembly have been observed to become multinucleated [71, 72]. Taken together, these studies provide evidence that sarcomere disassembly is a highly ordered process that is necessary for cell cycle completion, and the adult sarcomere may structurally impede cell cycle progression to promote cardiomyocyte polyploidization [73].

Regulators of sarcomere disassembly during cell cycle progression are not completely understood. This is in part due to the lack of robust mammalian cardiomyocyte model systems in which to study sarcomere disassembly through the cell cycle. In contrast, regulators of sarcomere assembly and maintenance have been better studied, such as members of the muscle chaperone [74] and E1-E3 ubiquitin proteasome systems [75]. For example, Hspb7 is a cardiac enriched heat shock protein that is essential for thin filament assembly in mice [76]. Disruption of the cardiomyocyte ubiquitin system also impairs sarcomere assembly as double knockout of E3 ubiquitin ligases MuRF1 and MuRF3 develops cardiac hypertrophy in vivo in association with accumulation of myosin heavy chains in the subsarcolemmal space [75]. While these studies have revealed factors regulating general sarcomere assembly and maintenance, how the sarcomere is regulated through the cell cycle remains largely unknown particularly the identity of the upstream regulatory factors as well as their sarcomere targets.

Sarcomere disassembly can be stimulated through activation of dedifferentiation programs that reprogram the sarcomere to its fetal-like structure, abundance, and gene expression pattern among other alterations in the state of the cardiomyocyte. For example, inhibition of miR-15 members or oncostatin M can induce dedifferentiation resembling a fetal-like cardiomyocyte state [77, 78], which results in enhanced capacity for proliferation and sarcomere disassembly. The dedifferentiated state also includes reduction in sarcomere content, re-expression of fetal-like sarcomere isoforms [78], upregulation of cell cycle promoting factors [79, 80], and downregulation of cell cycle checkpoint regulators including p21 and p53 [81]. Another method that has been demonstrated to activate a dedifferentiation program associated with enhanced cardiomyocyte proliferative capacity is transient expression of the Yamanaka reprogramming factors Oct3/4, Sox2, Klf4, and c-Myc [82], which has also been shown to improve cardiac stress responses following myocardial damage [83•]. Taken together, these dedifferentiation studies highlight the reversibility of

adult cardiomyocyte cell cycle exit and sarcomere disassembly deficits as well as their intimate relationship with cellular differentiation state.

In addition to dedifferentiation, adult cardiomyocyte proliferation can also be stimulated using enhanced expression of cell cycle activators, which suggests both that the sarcomere may not be an irreversible barrier to cardiomyocyte proliferation and that cell cycle activators may be regulators of sarcomere disassembly. Intriguingly, CDK1 has been show to phosphorylate the actin crosslinking protein filamin A at residues that are highly conserved to other filamins including filamin C that is a major sarcomere component [62]. Disruption of filamin A phosphorylation at CDK1 sites impairs post-mitotic daughter cell separation and cell migration in non-cardiomyocytes [84], but this process has not been studied in cardiomyocytes or filamins enriched in the sarcomere. Coincident with the loss of proliferative capacity of adult cardiomyocytes, cell cycle activators are downregulated including cyclins and CDKs [85–87•]. To test the functional impact of restoration of candidate cell cycle activators, rodent studies have evaluated the role of enhanced cyclin A2, cyclin B1, CDK1, cyclin D1, cyclin D2, and CDK4, among others [85, 87•–92]. With enhancement of a single activation factor, these studies have reported limited and variable proliferative rates including the promotion of polyploidization. For example, transgenic overexpression of cardiomyocyte G1/S-specific cyclins including cyclin D1 [91] and cyclin D2 [92] promotes polyploidization. In addition to enhancing a single cell cycle activator, recent studies have assessed the combinatorial overexpression of cyclin B1, CDK1, cyclin D1, and CDK4 that can induce adult cardiomyocyte cell cycle reactivation [85]. This combination of factors was observed to promote higher mitosis marker expression (histone H3 phosphorylation) relative to single factors in vitro. Interestingly, cyclin B1, CDK1, cyclin D1, and CDK4 overexpression did not promote cardiomyocyte multinucleation, though ploidy was not measured [85].

In association with the loss of cell cycle activator expression, adult mammalian cardiomyocytes also increase expression and activity for cell cycle inhibitors such as the CDKIs including p21 and p27 [93, 94] and tumor suppressors such as p53 and Rb [95]. While knockout of these factors such as p27 [94] and double knockout of p27 and p21 [96] can prolong the rodent cardiomyocyte proliferative window, targeting these repressive pathways exclusively in adult cardiomyocytes towards promoting cardiac regeneration has not been well studied, particularly how and whether these cell cycle inhibitory factors regulate sarcomere structure and function. In summary, while modulation of mammalian cardiomyocyte cell cycle regulators has had variable success in enhancing rodent cardiac regeneration, it remains incompletely known how these pathways converge on the regulation of the sarcomere particularly in the adult cardiomyocyte.

Emerging Human Cardiomyocyte Models to Study the Sarcomere and Cell Cycle

To study how the sarcomere regulates the cell cycle, human cardiomyocyte models that lack sarcomeres have been recently developed [36]. While in vivo rodent models that lack cardiac sarcomeres are embryonic lethal, in vitro models are viable such as human

fetal-like cardiomyocytes (iCMs) differentiated from pluripotent stem cells using robust direct differentiation methods [97]. While iCMs may not fully recapitulate in vivo biology, iCMs have been observed to undergo differentiation time-dependent proliferative arrest and polyploidization resembling in vivo human cardiomyocyte patterns (Figure 2) [10]. After producing iCMs that lack cardiac sarcomeres using genetic knockout of cardiac troponin, enhanced cell cycle marker activation and reduced polyploidization rates could be observed [87•]. When sarcomere assembly-deficient iCMs were transplanted into myocardial infarction rodent models, graft size was increased ~4× relative to wildtype iCMs, which was related to enhanced iCM proliferation rates supporting an inhibitory role for the sarcomere. Moreover, sarcomere assembly-deficient iCMs did not result in a highly proliferative state 3-month post-engraftment as only 0.50% of iCMs demonstrated expression of the cell cycle marker Ki67 relative to 0.14% in wildtype iCMs. These proliferation levels were much lower than what was observed prior to transplantation. Also, no tumor-like intramyocardial growths were observed in grafts, confirming that additional mechanisms beyond sarcomere assembly may be responsible for promoting proliferative arrest and polyploidization in human cardiomyocytes. Recently, one such mechanism identified was cell-cell contact, which was shown in iCMs to promote inhibition of canonical Wnt signaling and promote early cell cycle exit [98]. Reducing cell-cell contact in 2D tissue culture via sparse seeding of iCMs suppressed maturation and stimulated cell cycle activation, promoting massive cellular proliferation without subsequent contractility deficits once assembled into 3D engineered heart tissues. Going forward, the iCM model system could be a powerful new tool to assist with the identification of additional mechanisms of human cardiomyocyte cell cycle control such as by the sarcomere and beyond.

ICM models have been recently engineered to harbor cell cycle reporters such as by fusing green fluorescent protein (GFP) to cyclin B1 within the endogenous genomic locus using CRISPR technology [99]. As cyclin B1 is expressed in S, G2, and M phases, GFP expression status can provide a live cell readout on iCM cell cycle status. Using this cyclin B1-GFP iCM model combined with a genome-wide CRISPR knockout library, genetic levers of iCM proliferation and polyploidization have been studied [87•]. Among hits, the well-studied tumor suppressor p53 was found to be a strong activator of polyploidization and inhibitor of iCM proliferation, which was found to also be dependent on sarcomere function through a p53-dependent DNA damage response [87•]. Intriguingly, sarcomere gene mutations that activate contractile function such as in hypertrophic cardiomyopathy individuals have also been shown to induce a DNA damage response [100]. Taken together, these emerging studies implicate sarcomere function as regulatory factor promoting proliferative arrest and polyploidization through a DNA damage response involving p53. Future studies utilizing iCM models could reveal additional mechanisms connecting the sarcomere to cardiomyocyte cell cycle regulation.

Conclusion

The sarcomere is an essential multiprotein machine that generates the pumping force necessary for the heart to deliver oxygen and nutrients to maintain organismal viability and functions. It is not surprising that this complex structure interfaces with nearly all functions

of the cardiomyocyte including the cell cycle. It has been postulated that adult mammalian cardiomyocytes do not proliferate because sarcomere disassembly would promote HF and death. Yet, emerging studies particularly in rodent models have demonstrated that reactivation of cardiomyocyte proliferative capacity can be protective against cardiac injuries such as myocardial infarctions [85]. Future studies particularly utilizing in vitro human iCM and in vivo murine models to delineate new levers to promote cardiac regeneration such as by targeting the sarcomere could be transformative for not only HF patients, but other conditions characterized by insufficient cardiomyocyte numbers such as congenital heart disorders like hypoplastic left heart syndrome [101].

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•• Of major importance

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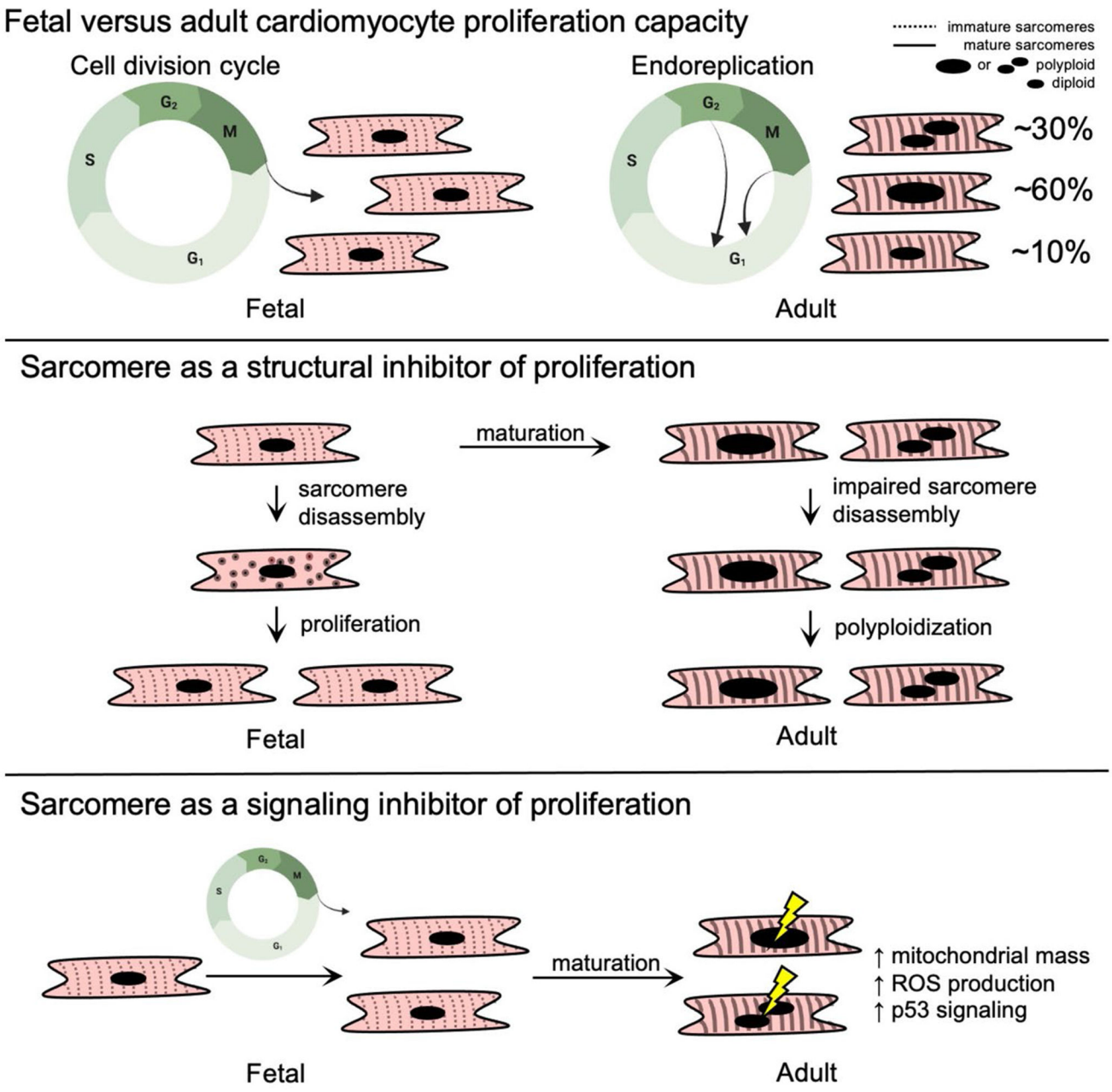

Fig. 1.

Fetal and adult human cardiomyocyte cell cycle patterns and sarcomere structure. While fetal human cardiomyocytes maintain their proliferative capacity as they progress through the stages of the cell cycle, adult cardiomyocytes lose their proliferative capacity and exit the cell cycle prior to completion of mitosis or cytokinesis (top panel). It has been proposed that the sarcomere inhibits proliferation because sarcomeres in the adult heart cannot be disassembled (middle panel). Alternative mechanisms may connect the sarcomere to cell cycle regulation such as contraction-induced metabolic and signaling alterations that promote cell cycle arrest (bottom panel)

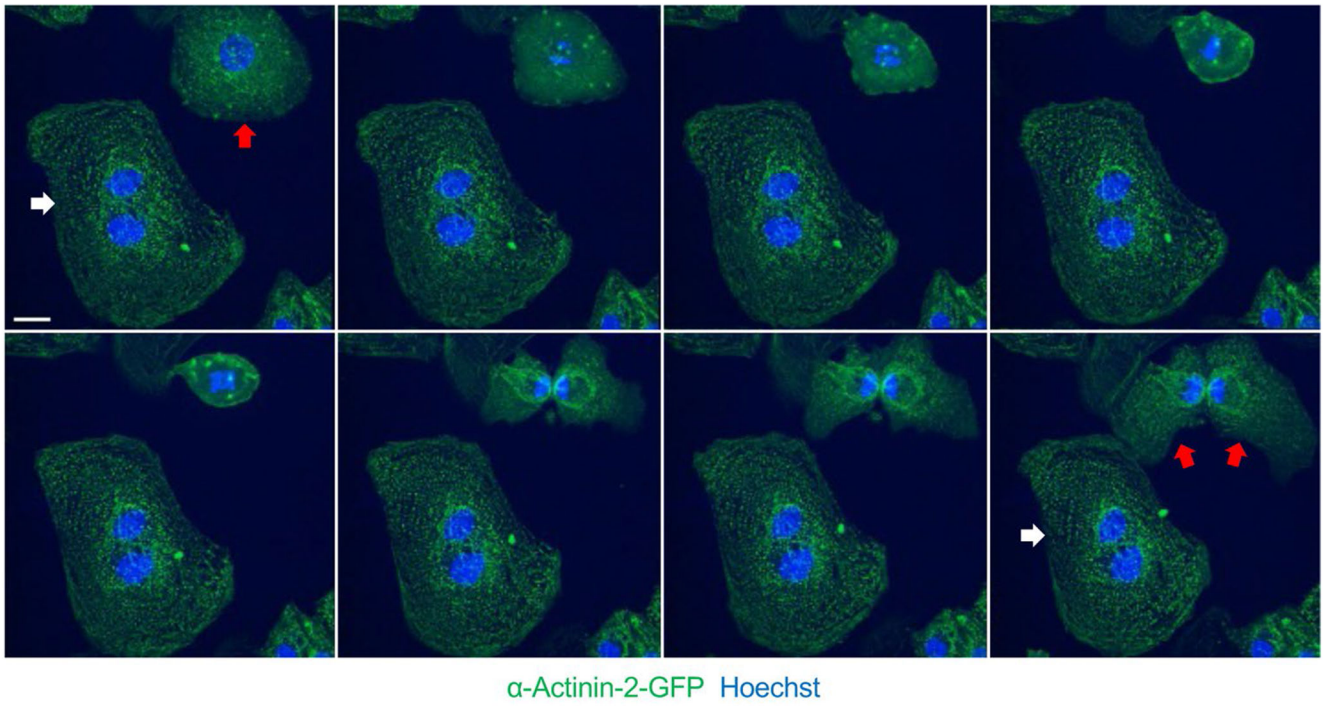

Fig. 2.

Human iPS–derived cardiomyocyte (iCM) mitosis and cytokinesis visualized using time-lapse live cell confocal imaging (micrographs at ~15-min intervals). Sarcomere Z-disk structures as demonstrated by alpha actinin 2-GFP signal disassemble during early stages of mitosis and reassemble after mitosis. *White arrow* denotes multinuclear iCM, while *red arrow* denotes a mononuclear iCM (top left image) that successfully completes cytokinesis (bottom right image). Scale bar = 10 μ