Tropinin and Tropomyosin in the Cardiomyocyte Nucleus: What for?

Faizal Z. Asumda

Abstract

A number of recent reports have showed the nuclear expression of troponin and tropomyosin in cardiac myocytes. This challenges the long standing view that these sarcomere proteins are physiologically restricted to the cytoplasm. A synthesis of the available data suggests that troponin and tropomyosin in the cell nucleus is not the result of cytoplasmic contamination. The data is suggestive of physiological roles outside their well-recognized function in excitation-contraction coupling.

Keywords

Troponin; Tropomyosin; Actin; Mesenchymal stem cells; Cardiomyocytes; Nucleus; Calcium

Abbreviations

BM-MSCs: Bone marrow mesenchymal stem cells; cTn: Cardiac troponin; cTnC: Cardiac troponin C; cTnI: Cardiac troponin I; cTnT: Cardiac troponin T; CEF-1 CSCs: Cardiac stem cells; cTn: Cardiac troponin; cTn: Cardiac tropomyosin; ESCs: Embryonic stem cells; MScs: Mesenchymal stem cells; NLS: Nuclear localization sequence; NVCM: Native ventricular cardiomyocytes; S2: Drosophila Schneider 2 cells; S-K: BR-3: Human breast adenocarcinoma cells; SUMO: Small ubiquitin-like modifier; Tn: Troponin; Tn: Tropomyosin.

Introduction

Cell transformation from the progenitor state to a differentiated tissue specific phenotype is a tightly governed process. Precise changes in gene expression constitute the developmental regime for cardiac specification [1-6]. The fluid and dynamic nature of nucleocytoplasmic interactions influences specific phenotypic changes [5,6]. As a result, an alteration in nuclear protein organization is expected to have physiological consequences [7-9]. Data from a number of different investigators showing known myofilament lattice and cytoskeletal proteins in the cell nucleus have implications. The overarching question is where, and what do we look to, and start from; in our attempts to elucidate the mechanistic details that underlie these strange but interesting phenomena? Troponin (Tn), tropomyosin (Tm) and actin are critical components of the contractile apparatus. Tm and actin might have nuclear analogues that serve a physiological purpose in the cardiac nucleus distinct from their customary roles in the contractile apparatus.

There are three separate subunits of cardiac specific troponin (cTn) — troponin C (cTnC), the 18-kD Ca2+ binding subunit; troponin I (cTnI), the 23-kD inhibitory subunit that shuttles between binding actin and cTnC in response to intracellular calcium concentration [10-12]. Troponin T (cTnT) is the 35-kD subunit that attaches troponin to tropomyosin and to the myofilibrillar thin filament [10-12]. Actin-troponin interaction is inhibited by cTnI in the absence of cTnC-Ca2+ binding [10-12]. Tropomyosin (Tm) is a double stranded α-helical protein critical to the regulation of thin filament function [16]. The cardiac specific isoform of tropomyosin (cTm; αα-Tm homodimer) is an alternative splicing product of the TPM1 gene [16]. During excitation-contraction coupling, and following Ca2+ release from the sarcoplasmic reticulum, Tm moves on the surface of the actin filament to a position near the groove of the actin double helix [10-12,16]. Several sarcomere proteins are now known to be associated with the nucleus; most have been assigned nuclear functions. Notable examples are non-cardiac Tm [17], nuclear actin (Figure 1) and its functions in nuclear processes such as chromatin remodeling, transcriptional regulation, RNA processing, nuclear export [15,18,24], and most recently, transcriptional reprogramming and activation of embryonic genes [25,26]. Titin, the large sarcomeric protein responsible for the elasticity of striated muscle interacts with histones [27], is required for cardiac myocytes in conjunction with stem cells and their ability to differentiate into cardiomyogenic cells provide an excellent system within which to tackle this issue [3,13]. For the critical and objective eye, contamination and by extension, “spill over” from the cytoplasm [15] is a valid supposition. But such a conclusion is problematic; cardiac myocytes are highly specialized, and their expression of specific gene product subunits and isoforms is tightly orchestrated [1-4,10-13]. The specific transcriptional circuit that drives heart formation is integrated—multiple independent and interdependent regulatory factors come together to drive the expression of each cardiac gene [1,2,4]. It is therefore provocative to imagine that Tn, Tm and actin might have nuclear analogues that serve a physiological purpose in the cardiac nucleus distinct from their customary roles in the contractile apparatus.

Figure 1: Localization of nuclear actin in neonatal ventricular cardiomyocytes. Asumda et al. [13] and Schoenenberger et al. [21] observed the expression of nuclear actin with antibodies 1C7 and 2G2. The actin conformation identified by antibody 1C7 which is shown here, is predominantly nuclear and evenly distributed, with increasing intensity towards the periphery of the nucleus. Neonatal ventricular cardiomyocytes were isolated from 48 hours old Sprague Dawley rats.

40x Oil/225um 40x Oil/225um

1C7

DAPI
for chromosome integrity [28] and for control of gene expression via its kinase domains [29]. The nuclear equivalent of myosin I is distinct from its cytoplasmic counterpart; both in form and function [30-33]. Nuclear Myosin I serve evolutionarily conserved roles in RNA polymerase I and II transcription, and intranuclear transport process [30-33]. Zhang et al. [34,35] demonstrated the nuclear localization of fragmented and whole fast skeletal muscle TnT3. The presence of native TnI in non-muscle cell nuclei was demonstrated by Sahota et al. [36] in a set of experiments which showed that, the Tn-Tm complex is critical for the maintenance of nuclear integrity. Data from the Sahota et al. [36] report implicates the small ubiquitin-related modifier (SUMO) in the nuclear translocation of TnI. Fast skeletal muscle TnI (TNNI2) also localizes to the nucleus of SK-BR-3 cells and functions as a nuclear receptor coactivator [37]. This review makes a case, based on recent experimental findings, for a probable physiological role for the nuclear analogues of cTn and cTm.

Troponin and Tropomyosin in Cardiac Myocyte Nuclei: Fact or Artifact?

Differentiated cells, under normal in vivo developmental conditions do not revert back to the undifferentiated state. The transformation process once instigated, for the most part, is linear and monolithic. Dedifferentiation requires active and exogenous intervention. Stem cell specific data from Asumda and Chase [13] establishes that undifferentiated multipotent stem cells do not express cytoplasmic or nuclear cTn and cTm. Their cardiomyogenesis data [13] is suggestive of a link in terms of physiological function between nuclear cTn, cTm and actin in cardiac myocytes. In the absence of cardiac specific differentiation of stem cells, nuclear actin and its potential functions in stem cells can be discussed independent of the nuclear cTn and cTm issue. For the purposes of simplicity, the nuclear actin issue is mentioned here as it relates to cTn and cTm in the cardiac myocyte nucleus. There is evidence in support of the definitive expression of cTn and cTm in the cardiac myocyte nuclei. The bona fide presence of nuclear cTnI and cTnT was used by Bergmann et al. [38] and Kajstura et al. [39] to isolate cardiomyocyte nuclei and assess cardiomyogenesis in the adult human heart. From a mechanistic standpoint, the two important questions to address are how cTn and cTm enter the cardiac nucleus and what physiological purpose this serves? A synthesis of recent experimental evidence indicates that the nuclear translocation of key cardiac transcription factors is requisite for successful differentiation into functional cardiac myocytes. Nuclear pore complex reorganization [7-9], and subsequent GATA4, MeF2C and Nkx2.5 translocation into the nucleus of differentiating embryonic stem cells (ESCs) [8], and mesenchymal stem cells (MSCs) [6,40,41] yields sarcomere-containing myocytes with electro-mechanical functionality. It is known that cTnI and cTnT contain evolutionarily conserved nuclear localization signals [42] which explain their partial localization in the nuclei of cardiac myocytes; presumably, the same applies to cTnC. It is however not clear that the nuclear translocation of cTn and cTm is an absolute requirement for in vitro cardiomyogenesis or transdifferentiation during cardiomyoplasty. What is known is that, in the absence of GATA4 and GATA6, cardiac myocyte differentiation typified by the expression of structural proteins such as cTn and cTm is completely blocked with ensuing acardia [43]. Furthermore, GATA4 transactivates the cTnC promoter-enhancer in non-muscle cells [44] and has major binding activity on the CEF-1 site of the cTnC enhancer in cardiac nuclear extracts [44]. Intraembryonic expression of GATA4 precedes cTnC expression by .5 to 1 day [44] and precedes cTnT expression in differentiating ESCs by 3-4 days [11]. Data from Asumda and Chase [13] and that of Bergman et al. [38] suggests that cTn and cTm in the cell nucleus is not out of place—it is a normal biological process. Based on these data, a transplanted stem cell or an endogenous cardiac stem cell undergoing cardiomyogenesis in host tissue should in theory express cTn and cTm along with actin in its nucleus as a part of its natural progression into a fully functional cardiac myocyte. An intervention that blocks nuclear translocation of GATA4 and conceivably, inhibition of GATA4 SUMOylation [45-47] in differentiating stem cells should hypothetically disrupt myofilament lattice expression of cTn and cTm and their nuclear analogues. This presumption is also based in part on inferred circumstantial evidence from Asumda and Chase [13] and others [36-46]. It should be noted that the nuclear expression of proteins not necessarily associated with this cell compartment raises red flags—it might allude to a pathological state. Data from D’Angelo et al. [48] suggest an age-associated change in the nuclear envelope structure that allows the free flow of cytoplasmic proteins into the nucleus. Kajstura et al. [39] show for example that the nuclear expression of cTnI occurs predominantly in a subset of senescent cardiac myocytes with leaky nuclei. Such a blanket supposition [49] is however not consistent with the available data. The evidence points to nuclear expression as a normal part of transformation towards the cardiac lineage. The observation made in senescent or unhealthy cells with leaky nuclei [39,48], while credible, is only one of a number of different possible explanations. It does not fully elucidate the mechanistic details that underlie the observation of cTn and cTm in cardiac myocyte nuclei. Bergman et al. [50] found no direct relationship between advancing age and the expression of cTnI or cTnT in the cardiac nucleus. Their data [50] show that the highest level of cTnI and cTnT expression occurs in cells derived from younger donors. Asumda and Chase [13] did not directly test cells from aged rodents; experiments were carried out on neonatal ventricular cardiac myocytes. Interestingly neither their studies [13] nor that of Bergman et al. [50] identified cardiac myocytes without nuclear expression of cTnI or cTnT using immunocytochemistry under different staining conditions. These studies [13,38,50] suggest that the nuclear expression of cTn, cTnT, cTnC and cTm in cardiac myocytes occurs naturally and is uniform across a given cardiac myocyte population. The overall data [13,34-38,50] validate the conclusion that a fully functional cardiac myocyte should express each one of these proteins both in the nucleus and cytoplasm [51].

What is the basis for cTn-Tm entry into the cardiac myocyte nucleus?

An answer to the question of how cTn and cTm enter the cardiac nucleus requires an acceptance that their nuclear presence is not artefactual or the result of cytoplasmic contamination. The in vitro cardiomyogenesis model of Asumda and Chase [13] strongly suggests a tightly controlled regime for the nuclear transcription and eventual expression of cTn and cTm. MSCs, like ESCs are known to display the cardiac phenotype following induction [3,52]. The ability of MSCs to display a phenotype completely synonymous with neonatal ventricular cardiac myocytes (Figure 2) takes away from arguments [39,48,49] in favor of a “condition dependent” cytoplasmic “spill over” [51]. Under normal physiological conditions, the cTnT subunits have low solubility [53-55]. It is therefore fascinating to envision some form of association with cTm and nuclear actin. Data from Zhang et al. [34,35] shows

that fast skeletal muscle TnT3 is expressed in the muscle nucleus as a full length or fragmented protein. Their data also demonstrates that full length TnT3 or its COOH-terminus contributes to muscle cell apoptosis. The identified nuclear export and localization signals [35] are located on the COOH terminus. It can be inferred from these data [34,35] that cTn or any one of the cTn subunits (cTnC, cTnI, or cTnT) might have greater nuclear expression or translocation under pathological conditions. The presumption here is that, they will be misfolded, damaged or fragmented [34,35] in the case of pathology. The exact mechanistic details of how cTn and cTm might enter the cell nucleus and subsequently induce cytotoxicity or any other related deleterious effect is an entirely separate issue. Immunocytochemical data from Asumda and Chase [13] depicts an even nuclear distribution of each protein. The highest staining intensity of cTn and cTm is observed towards the periphery of the nuclear compartment [13] which is consistent with their abundance in the myofilament lattice. The staining data [13,39,50] also suggests expression in the nucleus as intact and not fragmented proteins. Direct support for this line of thinking is premised on the fact that cTnI and cTm do in fact have evolutionarily conserved nuclear localization signals [11]. cTnC and cTm (Figure 3) are not known to have nuclear localization signals, so the explanation for those two is not straightforward. The association of cTnC with the cardiac nucleus has previously been explored [42,44]. Parmacek et al. [42] identified a cardiac muscle-specific transcriptional enhancer which acts along with the cTnC promoter to program high-level transcription in cardiac myocytes. Ip et al. [44] extended this finding by showing that the cTnC enhancer contains two nuclear protein DNA binding sites (CEF-1 and CEF-2), both of which bind and interact with cardiac muscle-restricted nuclear protein complexes.

Since Hatshorne et al. [56] first showed that there are at least two different proteins in troponin, several studies have purified and reconstituted the individual troponin components and their activity. Troponin is not a single homogeneous protein [54-55,57]. The separation of troponin into its constituent components has shown that, the active complex can be assembled in vitro under the right conditions, and all three subunits are required for the activity of the complex [54-56]. The individual subunits are also significantly different in their amino acid sequence composition. They do not interconvert—anyone of the three components cannot be derived from another via proteolysis [55]. Troponin might therefore exert its presumed nuclear function (s) as a complex. Of importance is the observation of slow skeletal muscle TnC mRNA by Reddy et al. [57] in the perinuclear region of C, C, derived myocytes. This particular observation is interesting to consider in light of the model suggested by Gurdon [58] and Bonner [59-61]. They both posit that selected accumulation of proteins in the cell nucleus is mediated by free diffusion in the case of small proteins. On the other hand, selective retention of bona fide nuclear proteins is accomplished via binding of the specific nuclear protein to a component of the nucleus that is not diffusible. This is suggestive of a scenario where primarily cytoplasmic or non-nuclear proteins might be able to freely diffuse into the nuclear compartment by virtue of size (less than 60kDa) [62] as opposed to a nuclear localization sequence (NLS) [63]. Microinjection experiments [59-62] support this view—that the nuclear envelope does not restrict small proteins; instead, it blocks the access of large proteins. It should therefore come as no surprise that predominantly cytoplasmic proteins are observed in the cardiac myocyte nucleus. Each of the three cTn subunits and cTm has a molecular weight less than 60kDa. What this means is that, in the absence of an NLS, cTn and cTm can still diffuse into the cardiac myocyte nucleus under the right conditions.

The question that this model raises is whether their translocation into the nuclear compartment serves some physiological purpose or if it is simply a fluke of nature. For example, a large amount of TnC mRNA in the perinuclear region [57] is suggestive of polypeptide production in the same locale. Presumably, the endoplasmic reticulum along with translationally active ribosomes is located in this vicinity as well [57]. It is therefore reasonable to imagine that situating the site of active protein synthesis near the nucleus will in all probability ensure the rapid production and subsequent efficient entry of the polypeptide products into the nucleus, granted there is a need for such an occurrence [57]. The observation that selective retention in the nucleus of diffusible proteins is controlled in large part by binding and interactions within the nucleus [63-65] suggests that damage to the nuclear envelope does not fully account for the uptake of cTn and cTm as suggested elsewhere [39,48,49].

Modification by the small ubiquitin-like modifier (SUMO) potentiates nucleocytoplasmic translocalization. Nuclear retention of actin is SUMOylation dependent [45]. Nuclear translocation of GATA4 and its mediation of cardiac gene expression is potentiated by the SUMOylation process [46,47]. Tnf does in fact contain SUMOylation target sequences and SUMO modification is required for its nuclear translocation in S2 cells [36]. Of importance is the fact that the identified putative SUMOylation sites exist in all three
Ca²⁺ signals are key determiners of such development. Despite being the nuclear compartment and specificity in the spatial patterns of interaction is developmentally advantageous, spatial constraints in mechanistic work, but is not impossible to study. Whether such an approach could be considered—i.e., if all four proteins can be SUMOylated in cardiomyocytes and if so, what the consequence would be for their nuclear translocation?

**Physiological significance of troponin and tropomyosin in cardiac myocyte nuclei**

With regard to function, it has to be assumed that the nuclear analogues of CTn and CTm serve a purpose. They are in all likelihood, there for a reason. The specific physiological function (s) of these proteins in the nucleus will depend to a large extent on their interactions with other compounds. Stem cell data from Asumda and Chase [13] raise one important question—what this means for cardiac specific differentiation and cell based approaches to cardiomyoplasty. Is this functionality (the synonymous nuclei expression of CTn and CTm), requisite for the attainment of the full cardiac myocyte repertoire? An approach based in part on speculation and imagination, in terms of what is known and what is possible should provide direction. A key metabolic component generated by the nucleus which interacts with the thin filament regulatory apparatus is calcium [66-68]. As a ubiquitous second messenger, Ca²⁺ mediates a vast array of processes. The regulation of nuclear Ca²⁺ is autonomous, and separate from the cytoplasm [68]. Nuclear Ca²⁺ has unique physiological effects that differ from cytosolic Ca²⁺ signals [69-71]. The presence of CTnC, which is a known calcium sink [12] in the cardiac myocyte nucleus, is physiologically significant for Ca²⁺ dependent processes such as gene transcription, metabolism, and differentiation [69-71]. With all components present in the nucleus, it is provocative but reasonable to imagine that CTn, CTnT, CTnC and CTm, along with nuclear actin interact [14]. An imaginary illustration of such an interaction might look like is shown by Asumda [14]. Exactly what physiological conditions might give rise to such a manifestation requires extensive mechanistic work, but is not impossible to study. Whether such an interaction is developmentally advantageous, spatial constraints in the nuclear compartment and specificity in the spatial patterns of Ca²⁺ signals are key determiners of such a development. Despite being borne purely out of imagination, the idea is not entirely anecdotal. The stability and specificity of such an interaction might be sufficient to generate force within the nucleus in theory [72]. Mechanotransduction in the form of force-induced changes in signaling, changes in higher order chromatin structure, transcription factor activation, gene transcription, cell cycle progression [73], and nuclear dependent sensitivity of mechanical stress resulting from cardiac pathology are possible downstream indicators and consequences. A synthesis of evidence from a number of different reports supports this line of thinking. In the myofilament lattice, the components of tropoion are arranged in a 1:1:1 stoichiometric ratio and are distributed along the thin filament with 1 tropoion complex bound to every 7 actin monomers [10-12] This model—the sliding filament concept is critical for force production. In the nucleus, actin forms filaments only under specific conditions; there is no evidence for classic filamentous actin [15,72]. The available data overwhelmingly supports the monomeric G-actin form [15,17-24,74,75] which does in fact exceed the critical concentration required for polymerization and forms oligomers [74,75]. Assuming actin formed condition dependent short filaments that escape detection by phalloidin, traditional anti-actin antibodies and current visualization techniques [15,72] such an interaction [13] might be possible. High level resolution microscopy along with the increasing capabilities of three-dimensional imagery of the nucleus should enable molecular level probing of such an interaction.

**Conclusion**

Decades of work conducted on the thin filament contractile apparatus and its constituent proteins—Tm, Tn and actin has resulted in the belief that these proteins are restricted to the cytoplasm. The observation made by a number of investigators, that CTn, CTnT, CTnC, CTm and actin are expressed in the cardiac myocyte nucleus [13,15,17-26,38,39,50] seriously challenges this long standing view. Nuclear actin is not known to form classic filaments, so it is not clear how CTn and CTm might be physiologically relevant in the cell nucleus. This does not discount the postulation of a probable novel role outside their more recognized functions in thin filament excitation-contraction coupling. Cardiomyogenic differentiation of adult stem cells is murky at best; we still do not have a firm grasp on the exact molecular programing mechanism responsible for cardiac specification in adult stem cells [13]. Thus, it is not clear if nuclear expression of CTn and CTm is required for generation of fully functional cardiac myocytes. Future directions should focus on predicting and verifying nuclear localization signals for all three CTn subunits and CTm. Identifying regulatory peptide sequences required for CTn and CTm nuclear targeting will enable more detailed studies on probable physiological function. It will also be important to determine if CTn and CTm freely diffuse into the cardiac nucleus by virtue of their size or under pathological conditions which might indicate therapeutic targeting.

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Author Affiliation

*Saint James School of Medicine, Chicago, USA

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