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Energy landscapes reveal the myopathic effects of tropomyosin mutations

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ABSTRACT

Striated muscle contraction is regulated by an interaction network connecting the effects of troponin, Ca^{2+}, and myosin-heads to the azimuthal positioning of tropomyosin along thin filaments. Many missense mutations, located at the actin–tropomyosin interface, however, reset the regulatory switching mechanism either by weakening or strengthening residue-specific interactions, leading to hyper- or hypo-contractile pathologies. Here, we compute energy landscapes for the actin–tropomyosin interface and quantify contributions of single amino acid residues to actin–tropomyosin binding. The method is a useful tool to assess effects of actin and tropomyosin mutations, potentially relating initial stages of myopathy to alterations in thin filament stability and regulation. Landscapes for mutant filaments linked to hyper-contractility provide a simple picture that describes a decrease in actin–tropomyosin interaction energy. Destabilizing the blocked (relaxed)-state parallels previously noted enhanced Ca^{2+}-sensitivity conferred by these mutants. Energy landscapes also identify post-translational modifications that can rescue regulatory imbalances. For example, cardiomyopathy-associated E62Q tropomyosin mutation weakens actin–tropomyosin interaction, but phosphorylation of neighboring S61 rescues the binding-deficit, results confirmed experimentally by *in vitro* motility assays. Unlike results on hyper-contractility-related mutants, landscapes for tropomyosin mutants tied to hypo-contractility do not present a straightforward picture. These mutations may affect other components of the regulatory network, e.g., troponin–tropomyosin signaling.

Introduction

Muscle contractility depends critically on the role played by the coiled-coil regulatory protein tropomyosin to cooperatively activate and relax the myofibrillar thin filament. In turn, crossbridge cycling by the myosin motor on actin and thus muscle performance is also controlled cooperatively [1–3]. Tropomyosin, the lynchpin in this on–off switching process, relies on Ca^{2+} binding to troponin and myosin binding to actin to define its azimuthal position on successive actin subunits along the thin filament. In this way, tropomyosin sterically blocks or alternatively exposes myosin-binding sites, and thereby controls myosin motor activity and contractility [1–8]. End-to-end linked tropomyosin molecules form a continuous cable that hovers about 10 Å over the helical array of actin-subunits comprising the thin filament [8–12]. Since the cable is semi-rigid, local azimuthal movement at one site evoked by troponin or myosin is propagated along actin filaments, thus amplifying cooperative switching signals [13].

The axial position of tropomyosin along the actin filament is defined by residue-specific electrostatic interactions between amino acids within the "α-zones" of tropomyosin pseudo-repeating domains (seven each in cardiac and skeletal muscle) and complementary targets on successive actin subunits [3,10–12]. The large radial separation between actin and tropomyosin ensures that these interactions, while residue-specific, are relatively weak and thus compatible with tropomyosin's azimuthal movements on actin at low energy cost. Indeed, tropomyosin only binds to F-actin effectively by linking head-to-tail along F-actin to form a continuous cable with high collective binding strength [9,12]. Hence, locally tropomyosin affinity is low, while globally it is high.

The actin–tropomyosin assembly has been examined in atomic detail by Li et al. [10] who built a structural model of the troponin-free filament by selecting the position of tropomyosin on the F-actin surface that optimized electrostatic interactions. The near equivalence between the coordinates of this structure and the

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density profile of actin and tropomyosin in EM reconstructions validated the model [10]. Orzechowski et al. [11] then extended this work by mapping energy landscapes to determine interaction energy terms for tropomyosin located at different positions on F-actin that covered the entire surface of actin explored by tropomyosin. The lowest energy position of tropomyosin on actin was found to localize in a relatively shallow energy basin and, as in Li et al. [10], very close to tropomyosin's low-Ca^{2+} blocking-site on troponin-regulated thin filaments. This arrangement was corroborated by Spudich and colleagues using other models of the filament [14]. It follows that the role of Ca^{2+}-free troponin during muscle regulation is to decrease the azimuthal dynamics of tropomyosin and to fix tropomyosin at its energetic minimum on actin, blocking access of myosin to actin. In contrast, Ca^{2+}-saturated troponin and then myosin-binding itself function to distort the energy landscape and hence bring about tropomyosin movement to the "open" M-state position and thin filament activation [15,16]. Thus, mutations in thin filament proteins, particularly ones that strengthen or others that weaken interfacial electrostatic contacts between actin and tropomyosin, will deform the filament energy landscape and thus bias tropomyosin toward or away from the filament blocked-state. Indeed, actin mutants D292V and K326N have been shown to strongly alter the actin–tropomyosin landscape profile and interfere with thin filament regulation [17].

It is well known that point mutations found on practically all myofibrillar proteins profoundly affect cardiac and skeletal muscle contractility and lead to cardiomyopathies and skeletal muscle disease syndromes of varying severity. Over 30 of these mutations localize to residues present on the actin–tropomyosin interface and, as in the case of the actin mutants already mentioned, many may modify the actin–tropomyosin energy landscape, influence tropomyosin positioning on actin, and perturb cooperative interactions between actin, tropomyosin, troponin and myosin [17–23]. In order to investigate these possibilities further, the net energetic contributions made by each wild-type actin and tropomyosin residue to the interaction was first measured. Residues contributing most to actin–tropomyosin interaction were highlighted (Fig. 1) and then compared to the residues in missense mutants associated with myopathies reviewed in references [18–23]. Energy landscapes were then generated for each mutant tropomyosin on F-actin.

The information acquired in this study may prove to be useful as a diagnostic and/or predictive tool to assess effects of actin and tropomyosin mutations by relating the critical initial stages of disease development to alterations in thin filament stability and regulation. We find that the landscapes for mutant filaments associated with hyper-contractility, for example those linked to hypertrophic cardiomyopathy (HCM[1]), skeletal muscle arthrogryposis and congenital fiber-type disproportion (CFTD), provide a simple picture. In these cases, most of the mutations examined are associated with a decrease in actin–tropomyosin interaction energy that will tend to destabilize the blocked (relaxed)-state of the thin filament. Our measurements parallel previously noted enhanced Ca^{2+}-sensitivity conferred by these mutants [17–23]. We show, in addition, that energy landscape computation in combination with known actin–tropomyosin sequence and structural information can be used prospectively to identify potential effects of post-translational modifications to rescue regulatory imbalances. For instance, our *in silico* methodology shows that HCM-associated E62Q tropomyosin mutation weakens actin–tropomyosin interaction, but that phosphorylation of neighboring S61 rescues the binding deficit, a result that we then validate experimentally. In marked

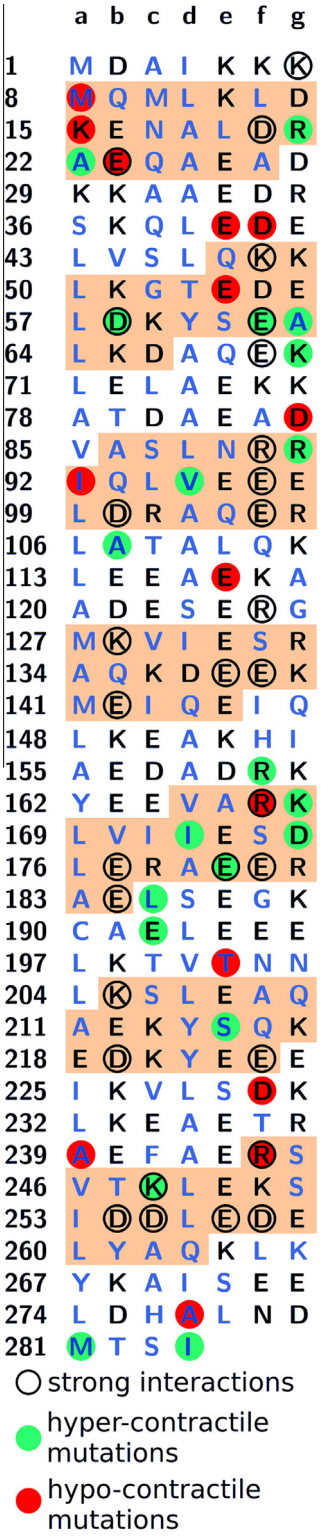

Fig. 1. Striated muscle α-tropomyosin sequence annotation. Figure adapted from Brown et al. [3] to emphasize tropomyosin heptad repeats (*a*, *b*, *c*, *d*, *e*, *f*, *g*) with the beginning residue of each repeat numbered. The so-called α-zone residues of tropomyosin [3] that locate close to actin sub-domain 1 [10] are shaded. Acidic and basic residues are indicated by black type, while others are in light blue. Residues noted in the present study that form strong electrostatic interactions with oppositely charge ones on actin are circled in black (cf. [10]), and ones associated with hyper- or hypo-contractile characteristics colored green or red respectively cf. [17,18]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

[1] *Abbreviations used:* HCM, hypertrophic cardiomyopathy; CFTD, congenital fiber-type disproportion; DCM, dilated cardiomyopathy.