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Structure and flexibility of the tropomyosin overlap junction

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To be effective as a gatekeeper regulating the access of binding proteins to the actin filament, adjacent tropomyosin molecules associate head-to-tail to form a continuous super-helical cable running along the filament surface. Chimeric head-to-tail structures have been solved by NMR and X-ray crystallography for N- and C-terminal segments of smooth and striated muscle tropomyosin spliced onto non-native coiled-coil forming peptides. The resulting 4-helix complexes have a tight coiled-coil N-terminus inserted into a separated pair of C-terminal helices, with some helical unfolding of the terminal chains in the striated muscle peptides. These overlap complexes are distinctly curved, much more so than elsewhere along the superhelical tropomyosin cable. To verify whether the non-native protein adducts (needed to stabilize the coiled-coil chimeras) perturb the overlap, we carried out molecular dynamics simulations of head-to-tail structures having only native tropomyosin sequences. We observe that the splayed chains all refold and become helical. Significantly, the curvature of both the smooth and the striated muscle overlap domain is reduced and becomes comparable to that of the rest of the tropomyosin cable. Moreover, the measured flexibility across the junction is small. This and the reduced curvature ensure that the super-helical cable matches the contours of F-actin without manifesting localized kinking and excessive flexibility, thus enabling the high degree of cooperativity in the regulation of myosin accessibility to actin filaments.

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1. Introduction

The actin-binding protein tropomyosin is a coiled-coil present on thin filaments in virtually all eukaryotic cells [1,2]. By associating head-to-tail it forms a continuous cable along the length of the actin-based filaments, thus conferring increased thin filament rigidity and protection against filament severing proteins [1–6]. In smooth and striated muscles, tropomyosin also cooperatively regulates myosin's association with the actin subunits of the thin filament and hence the myosin cross-bridge cycle and contraction [2–5,7]. In striated muscle, this regulation is controlled by interactions of tropomyosin with troponin and, in turn, by troponin binding Ca^{2+} [7]. In smooth muscles, tropomyosin interactions may be modulated similarly by caldesmon and calponin [8].

Despite the large number of tropomyosin isoforms, amino acid sequences of the coiled-coils are typically conserved [9,10]. Thus, tropomyosin variants are likely to bind to F-actin filaments by much the same well-characterized electrostatic mechanism [11]. While the specific positioning of tropomyosin on F-actin depends on the Coulombic interaction complementarity of residue pairs at the interface between actin and tropomyosin, the actual binding strength of a single tropomyosin molecule with F-actin is very low (reviewed in [4]). Its binding is augmented by head-to-tail tropomyosin polymerization with itself, forming a superhelical cable spiraling around the actin filament that prevents the detachment of tropomyosin from F-actin [2,3,5,6,10]. While the corresponding head-to-tail linkage is a universal attribute of all tropomyosin isoforms, the overlap structure appears to differ widely among the different family members [6,10,12,13]. In fact, the C-terminal tropomyosin sequences are not well conserved, and hence, head-to-tail complex formation is isoform-specific. For example, C-terminal sequence divergence is likely to be responsible for the different head-to-tail polymerization kinetics of smooth and striated muscle tropomyosin [6,9,10,12,13]. In fact, electron microscopy studies suggest that the smooth muscle junctional complex is more stable and less flexible than its skeletal muscle counterpart [14].

To date, no high resolution structures characterize the head-to-tail connection of full-length tropomyosin. However, the overlap structure for short C- and N-terminal sequences of tropomyosin have been determined after splicing them onto non-native peptides known to stabilize coiled-coils. These chimeric adducts

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86 form four-coiled complexes with an 8–15 residue overlap
87 [6,12,13]. In this nexus, the partially opened C-terminal coiled-coil
88 encloses the tight N-terminal coiled-coil (see Fig. 1A and B)
89 [6,12,13]. Both the smooth and the striated muscle adducts display
90 a near 90° rotation (i.e., twist around the longitudinal central axis)
91 of the C-terminal coiled-coil fragment relative to the N-terminal
92 coiled-coil fragment. In addition, the coiled-coil axis of the N-ter-
93 minal fragments is not aligned with that of the C-terminal frag-
94 ment, but instead they are angled relative to each other. This
95 bend (or curvature) angle ω is 12.4° in the smooth muscle nexus
96 and 19.6° in the striated muscle nexus. This is significantly more
97 curved than the curvature angle of canonical tropomyosin on
98 F-actin, which is 8.6° degrees (over a comparable length) [18].
99 Whether or not the presence of non-native protein adducts affects
100 the twist and the curvature of the synthetic overlap domains is
101 uncertain.

102 In the present study, we use Molecular Dynamics to assess the
103 effect of the non-native adjuncts on the overlap structure. In the
104 X-ray crystal and NMR structures of the smooth and striated mus-
105 cle overlap domains, the non-native residues were replaced by na-
106 tive tropomyosin sequences. Our study shows that the opening of
107 the C-terminal coiled-coil and the orthogonal twist angle is main-
108 tained in the fully native overlap domain, although the helical
109 content of unfolded chains is restored. In contrast, the curvature
110 angle of both the smooth and the striated muscle overlap domain
111 is reduced in the native structures compared to that in the chime-
112 ric structures. The average curvature (~9.4°, see Table 1) is similar
113 to the curvature found along the rest of actin-bound tropomyosin.
114 The curvature variance (i.e., the flexibility) is modestly greater for
115 the striated tropomyosin overlap domain than for the correspond-
116 ing smooth tropomyosin overlap, which is consistent with data
117 from electron microscopy also showing that the overlap domains
118 of striated muscle have a greater variance in curvature angle [14].

2. Methods 119

2.1. Reference models for MD simulations 120

121 Initial reference models for the striated and smooth muscle
122 α-tropomyosin isoform head-to-tail overlap domains were based
123 on NMR and crystal structures. In PDB ID code 2g9j (conformer
124 model-1) [6], residues 1–14 from the N-terminus of rat striated
125 muscle tropomyosin had been spliced onto last 18 residues of
126 the leucine zipper coiled-coil forming sequence of GCN4, while res-
127 idues 251–284 from the C-terminus of rat striated muscle tropo-
128 myosin were spliced onto GCG residues to stabilize the latter. In

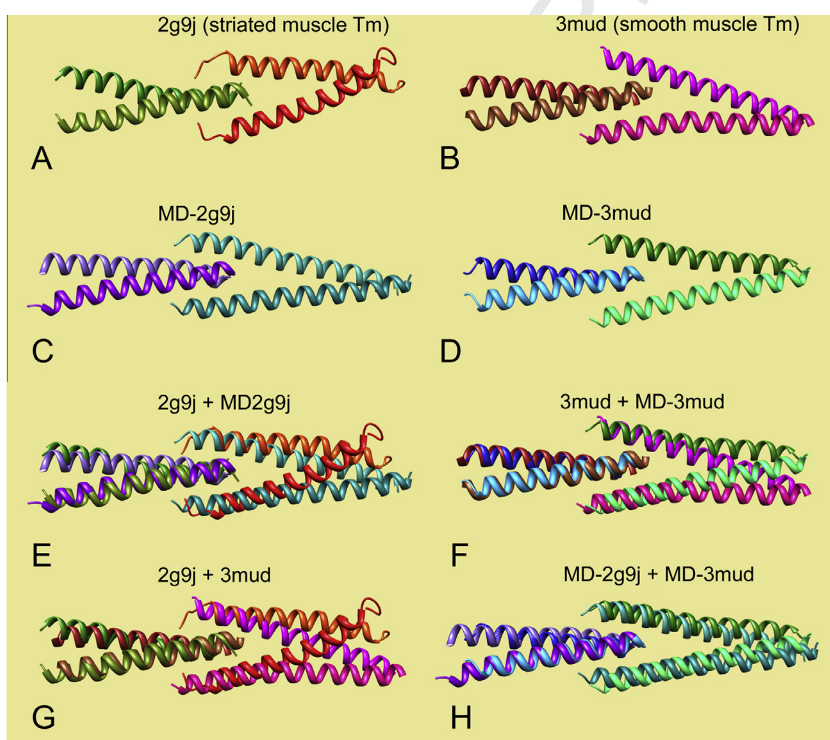

Fig. 1. Tropomyosin head-to-tail overlap domains. (A, C) striated muscle structures, (A) chimera from NMR, (C) native domain, averaged from MD. (B, D) smooth muscle structures, (B) crystal structure of chimera, (D) native domain, averaged from MD. In each panel, N-terminal residues are on the left, C-terminal residues are on the right. (E–H) superimposition of structures, after aligning the left pair of chains, superimposing: (E) A + C, (F) B + D, (G) A + B, (H) B + D. Graphics and alignment done with Chimera [19].

Table 1
Geometry of the overlap domain during Molecular Dynamics.

Tropomyosin source	Number of residue pairs in overlap	Distance covered by overlapping residues	Twist angle of C- *vs.* N-terminal fragments (θ)[a]	Curvature C- *vs.* N-terminal fragments (ω)[a,b]	Dynamic persistence length of overlap[c]
Striated muscle	10	13.5 Å	90.6 ± 2.5°	9.3 ± 4.8°	342 nm
Smooth muscle	9	12.0 Å	85.7 ± 2.4°	9.6 ± 3.8°	546 nm

[a] Values for average ± standard deviation.
[b] An *F*-test shows that the two standard deviations are significantly different from each other ($P < 0.001$).
[c] Measured over a 12 Å distance.

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