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Tarantula Myosin Free Head Regulatory Light Chain Phosphorylation Stiffens N-terminal Extension, Releasing it and Blocking its Docking Back

Lorenzo Alamo^a, Xiaochuan (Edward) Li^b, L. Michel Espinoza-Fonseca^c, Antonio Pinto^a, David D. 5 Thomas^c, William Lehman^b, and Raúl Padrón^{a*}

^aCentro de Biología Estructural, Instituto Venezolano de Investigaciones Científicas (IVIC), Apdo. 20632, Caracas 1020^a, Venezuela. ^bDepartment of Physiology and Biophysics, Boston University School, 72 East Concord Street, Boston, MA 02118, USA ^cDepartment of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, Minnesota 55455, USA.

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*Corresponding author: Raúl Padrón (raul.padron@gmail.com, fax: +58 212 504 1444, phone +58 212 504 1098)

SUPPORTING MATERIAL

SUPPLEMENTARY DISCUSSION

- 15 We discuss here the three prerequisites that need to be fulfilled for the proposed mechanism (Fig. 4) to operate in striated muscle: (1) the assembly of geometrically appropriate myosin interacting-heads motifs in thick filaments;¹⁻³ (2) the presence of RLCs with NTEs exhibiting a pair of phosphorylatable serines, associated with two specific kinase/phosphatase pairs; and (3) the formation of specific PKC- and MLCK-formed salt bridges.
- 20 (1) The myosin interacting-heads motif¹⁻³ is highly conserved, allowing relaxation in nonmuscle, smooth and striated muscles over a wide range of species of the animal kingdom (see introduction). The motif also allows simple steric/accessibility factors in the thick filament to control which of the two heads can become phosphorylated and thus is able to interact with actin to generate filament sliding⁴. When these motifs assemble on thick filaments with 4 helices, as in arthropod chelicerates like tarantula,^{2,3} *Limulus*⁵ or scorpion,⁶ and
- 25 platyhelminthes like Schistosome⁷ very specific inter-motif interactions along each helix are achieved. Very different interactions are achieved on the only other two thick filament geometries found to date: 3 helices (vertebrate cardiac muscle: mouse,⁸ human,⁹ zebra fish¹⁰) or 7 helices (molluscs striated muscle: scallop¹¹). Vertebrates –also with modulatory RLC phosphorylation- have a similar head organization to tarantula at two out of every three levels of heads, but with weaker inter-motif interactions along the helix.⁸⁻¹⁰ Molluscs with 30 direct Ca²⁺-binding regulation- exhibit heads protruding more from the backbone and with different inter-motif
- interactions than tarantula.¹¹

(2) RLC sequences from vertebrates or invertebrates have either short or long NTEs.^{3,4} Long NTEs on invertebrates,^{3,4} show pairs of non-contiguous phosphorylatable serines ("S...S") in sequences targeted for PKC and MLCK, homologous to Ser35/Ser45 in tarantula.⁴ As in tarantula, *Limulus* and scorpion have two

- 35 phosphorylatable serines.^{4,12} Short NTEs show either two contiguous serines "SS", a single serine "S" or contiguous "TS".⁴ In the species with long NTEs (arthropods, platyhelminthes and annelids), the proposed cooperative phosphorylation mechanism (Fig. 4) may be limited to arthropods (including insects) and platyhelminthes (like Clonorchis and Schistosoma), which possess constitutive/potentiating phosphorylatable serine pairs, while annelids have only one phosphorylatable serine (like Riftia) or none (like *Lumbricus*). While
- 40 there are no reports yet on head organization in annelids, arthropods^{2,3,13} and platyhelminthes⁷ have the same head organization and dual PKC/MLCK phosphorylation sites, consistent with this mechanism (Fig. 4). If these other species with long NTEs have the same head arrangement as in arthropods and platyhelminthes, they could have similar kinase accessibility constraints, suggesting a similar structural basis for their regulation. Even though constitutive and potentiating phosphorylation may not be the same in species with short NTEs, steric

constraints may still play a role in determining which heads are phosphorylated and in which order. Thus, the role of phosphorylation on both heads on these other species needs to be elucidated. The mechanism cannot be extended to NTEs with no serine pairs or duplets, as a pair of constitutive and potentiating serines is required. Therefore, the applicability of the proposed mechanism (Fig. 4) is limited to species with long NTEs like

- 5 arthropods (including insects) and platyhelminthes (as Clonorchis and Schistosoma), but excluding the annelids, which have only one phosphorylatable serine (as Riftia) or none (as *Lumbricus*). This mechanism, however, could be applied –but with modifications- to some species with short NTEs with SS diphosphorylating duplets like vertebrates.
- (3) The mechanism depends on the ability of two kinases and phosphatases to establish and remove 10 specific salt bridges, as shown here for tarantula. In the tarantula NTE, the Ser35 forms salt bridges, either to Arg38 or to Arg39, located at 3 or 4 aa forward on the C-terminal direction from the Ser35. The residues located in these two positions in long NTEs are very conserved (positive), as they are either RR duplets, RK or KR³. The phosphorylatable serines could have evolved from simply negatively charged glutamate and aspartate residues which could also form salt bridges¹⁴ to keep ancient NTEs activated via a fold of straightened and
- 15 rigidified helices P and A. The phosphorylation of the novel serines could eventually replace these negatively charged residues. This could be advantageous as it would enable the formation of equivalent salt bridges for stabilizing the NTE fold when required for activation. The emerging of phosphatases to dephosphorylate these serines would break these salt bridges; de-activating the RLC and completing the regulation scheme shown in Fig. 4. Alternatively, another novel modification in which phosphorylatable serines are not involved, could
- 20 allow regulation by direct Ca²⁺-binding¹⁵ as in molluscs¹¹. Therefore these two myosin-linked regulation schemes could have evolved from a common ancestor, or the direct Ca²⁺-binding from the RLC phosphorylation scheme.¹¹ The third known scheme, actin-linked regulation, would have emerged later with the novel troponin added only to the earlier RLC phosphorylation scheme, now widely spread among the animal kingdom.
- We conclude that these requirements are only fulfilled by the arthropods (perhaps including insects) and 25 platyhelminthes as they have thick filaments with 4 helices of myosin interacting heads motifs, long NTEs and the required PKC and MLCK phosphorylatable serines. As no structural information has been reported yet for annelids, which also have long NTEs but only have one serine or none, it is currently unclear if the proposed mechanism would be used by them. Vertebrates, on the other hand, have 3 helices of motifs and short NTEs, so they may use a modification of this mechanism.

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SUPPLEMENTARY TABLES

Table S1 Bending angle between PKC and MLCK (θ_1), MLCK and helix A (θ_2), total angle (θ), and apparent ξ_a , dynamic ξ_d and static ξ_s persistence lengths of un-, mono- and Di-P tarantula RLC NTEs

| Phosphorylation status | Un-P | Mono-P | Mono-P | Di-P |
|---|---------------|---------------------|-------------------|------------------|
| | | Constitutive pSer35 | Activating pSer45 | pSer35and pSer45 |
| $<\theta_1 > \circ$ (rad) | 50.4 (0.8792) | 43.6 (0.7604) | 61.2 (1.0674) | 8.8 (0.1533) |
| $< \cos(\theta_1) >$ | 0.523 | 0.6023 | 0.4513 | 0.9844 |
| $<\!\theta_l^2\!>$ - $<\!\theta_l^2\!>^2$ | 0.3493 | 0.3575 | 0.1177 | 0.0079 |
| | | | | |
| $<\theta_2>^{\circ}$ (rad) | 53.2 (0.9291) | 40.6 (0.7079) | 33.8(0.5892) | 15.0 (0.2624) |
| $< \cos(\theta_2) >$ | 0.4934 | 0.6691 | 0.7566 | 0.9556 |
| $<\!\!\theta_2^2\!\!>$ - $<\!\!\theta_2^{>2}$ | 0.3358 | 0.2509 | 0.1955 | 0.0215 |
| | | | | |
| $<\theta>$ o (rad) | 51.8 (0.9042) | 42.4 (0.7341) | 47.5 (0.8283) | 11.9 (0.2079) |
| $< \cos(\theta) >$ | 0.5082 | 0.6357 | 0.60395 | 0.97 |
| $<\!\theta^2\!>$ - $<\!\theta\!>^2$ | 0.3493 | 0.3575 | 0.1955 | 0.0215 |
| | | | | |
| ξ_{a} (nm) | 6 | 8 | 8 | 126 |
| $\xi_{\rm d} (\rm nm)$ | 22 | 21 | 39 | 357 |
| $\xi_{s}(nm)$ | 8 | 13 | 10 | 195 |

 $<\theta>$ is the average value of θ_1 and θ_2 . $<|\cos(\theta)|>$ is the average value of $(<|\cos(\theta_1)|>, <|\cos(\theta_2)|>), <\theta^2> - <\theta>^2$ is the maximum value of (variance (θ_1) , 5 variance (θ_2)).

SUPPLEMENTARY MOVIES LEGENDS



Movie S1. Movie of un-P tarantula myosin RLC NTE plus helix A peptide secondary structure 200 ns trajectories. The helix A is shown static at the bottom. The peptide is initially straight, with the α-helix A (purple) followed by helix P (Ser35/45 as green sticks, basic residues as balls and sticks), linker 5 (magenta) and α-helix L (top, with its 9 white positive Lys and 7 red acidic residues as balls and sticks). From the beginning helix L is highly movable through the flexible linker (white/magenta coil/turn), moving eventually closer to helix A. The helix P bends over helix A (white coil). No salt-bridges are established (shown in zoom on Movie S2). The final stabilized peptide is folded with a bend between helix P and A, with helix L close to helix A but without forming salt-bridges (See Fig. 2A).



10 Movie S2 Zoom of helix P of movie S1 showing that no salt-bridges are established showing loss of α-helix structure at Pro31-Lys32 that extends the linker and a helix-coil-helix (HCH) folding structure at helix P Ser42-Gly44 (See Fig. 2A)..



Movie S3. Movie of Ser45 mono-P tarantula myosin RLC NTE plus helix A peptide secondary structure 600 ns trajectories. This peptide corresponds to the Ser45 mono-P NTE of the blocked head. The evolution is similar to the described for the un-P peptide (Movies S1-2) except that a pSer45/Arg39 salt-15 bridge is established (shown in zoom on Movie S4) (See Fig. 2B). For details on displayed peptide structure see movie S1 legend.

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Movie S4. Zoom of helix P on movie S3 showing that a pSer45/Arg39 salt-bridge (dotted blue lines represent H-bonds) is established between the two pSer45 phosphates negative charges and the two Arg39 positive charges. No helix to turn/coil occurs but evolves to HCH folding structure at Ser43-GLy44 (See Fig.2B).



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Movie S5. Movie of Ser35 mono-P tarantula myosin RLC NTE plus helix A peptide secondary structure 350 ns trajectories. This peptide correspond to Ser35 constitutively mono-P NTE of the free head (swaying head) RLC. The evolution is similar to the one described for the un-P peptide (Movie S1) except that a pSer35/Arg38,Arg39 salt-bridge is established (see zoom on Movie S6) (See Fig. 2C). For details on displayed peptide structure see movie S1 legend.



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Movie S6. Zoom of helix P on movie S5 showing that pSer35/Arg38,Arg39 salt-bridges are established (dotted blue lines represent H-bonds) between the two pSer35 phosphates negative charges and the four Arg38 and Arg39 positive charges. The linker extended the turn up to Lys32 and the helix P folding into a HCH structure at Gly44 (See Fig.2C).

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Movie S7. Movie of di-P Ser35 and Ser45 tarantula myosin RLC NTE plus helix A peptide secondary structure 200 ns trajectories. The evolution is very different to the ones described for the un- and mono-P peptides (Movies S1-S6). From the beginning, helix L is highly movable through the flexible linker, eventually moving closer to helix A. Salt-bridges are established at pSer35/Arg38,Arg39 and pSer45/Arg42 (shown in zoom on Movie S8). The 5 final stabilized peptide is straight with no bends between helix P and A, and with helix L close to helix A forming a pSer45/Lys15salt-bridge (See Fig. 2D). For details on displayed peptide structure see movie S1 legend.



Movie S8. Zoom of helix P on movie S7 showing the early establishment of salt-bridges (pSer35/Arg38,Arg39 and pSer45/Arg42). A salt-bridge pSer45-Lys15 is also formed between helix L and helix P at the end of the simulation (See Fig. 2D).

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