Cloning Strategies and Oligonucleotide Sequences

The gene encoding the wild type α-MyHC rat gene was amplified from the plasmid pMT21α with the forward primer 5’ -CCGGAATTCATGACGGATGCCC AGATGGCTG (carrying the EcoRI restriction site) and the reverse primer 5’ -GC TCTAGATTCATTCCTCATCGTGCATTTTCT (carrying a stop codon). The 5.8 kb PCR fragment was then treated with T4 Kinase, digested with EcoRI, and subcloned into vector pEGFP-C2 (Clontech) digested with EcoRI and XmaI. The resulting N-terminally tagged α-MyHC, is linked to EGFP through a 13 amino acid spacer (Ser-Gly-Arg-Thr-Gln-Ile-Ser-Ser-Ser-Ser-Phe-Glu-Phe) derived from the multi-cloning site of the plasmid. The N-terminal mCherry tagged α-MyHC vector was obtained by first removing the EGFP gene from the EGFP α-MyHC construct by inverse PCR using the forward primer 5’ -TCCGGCCGGACTCAGA TCTCG and the reverse primer 5’ -CATGGTGGCGACCGGTAGC, and then cloning the mCherry gene (amplified with the forward primer 5’ -GTGAGCAAGG GCGAGGAGGAT, and reverse primer 5’ -CTTGTACAGCTCGTCCATGCC) by blunt end ligation. The N-terminally tagged GFP/mCherry wild type and mutant MyHC rod constructs used in this paper were obtained by inverse PCR from the respective full length plasmids using the forward primer 5’ -CTGAAGAGCGC AGAGACAGA (starting at myosin residue 842) and the reverse primer 5’ -CTTGTACAGCTCGTCCATGCC annealing to the end of each fluorescence gene. Thus in these constructs the GFP/mCherry tags are directly fused to the myosin rod. The plasmids expressing the rod mutants R1500P and R1500W were obtained by inverse PCR from the EGFP α-MyHC rod construct using the common reverse primer 5’ -CTTGAAGGTCTCCAGGTGCTC and the forward

mutagenic primers 5’ -CCGGAGAACAAGAACCTCCAG and 5’ -TGGGAGAA CAAGAACCTCCAG respectively. The mutant L1706P construct was obtained with the same strategy using the forward mutagenic primer 5’ -CCGATCGAGA CCAGCGAGC, and the reverse primer 5’ -CTCCTGCTCTGCCAGCTTCC. The N-terminal EGFP-tagged *C. elegans* MHC B construct was obtained by amplifying the EGFP gene from the pEGFP-C2 plasmid with the forward primer 5’ -ATGGTGAGCAAGGGCGAG GAG, and the reverse primer 5’ -GGATCCC TTGTACAGCTCGTCCATG, which introduces two extra amino acids (Gly, Ser) at the end of EGFP. The PCR fragment was then treated with T4 Kinase and cloned into the inverse PCR product obtained from the pPD5.41 plasmid (gift from Andrew Fire) using the forward primer 5’ -ATGGAGCACGAGAAGGACCCA and the reverse primer 5’ -GATTTCTCGCTTCTTTCAAATGGTTTC. The human rod mutations were introduced in the corresponding C. *elegans* residues (R1500P/W=R1512P/W) by inverse PCR using the common reverse primer 5’ -GCGGAGTCCCTCAACAACCTC in combination with the following forward mutagenic primers: 5’ -CCAGAGAACAAGAGCTTGAGCCAA, for the R1512P mutation, and 5’ -TGGGAGAACAAGAGCTTGAGCCAA for the R1512W mutation. The L1706P mutation (A1718 in *C. elegans*) was introduced by inverse PCR using the forward mutagenic primer 5’ -CCAGCCGATGCTCGTGATCAA and the reverse primer 5’ -TTCATACTCGGCTTGCTTGCG. The reagents for the BiFC assay were developed in several steps. The R1500P-NGFP construct was obtained from the EGFP R1500P MyHC rod plasmid by inverse PCR using the forward primer 5’ -TCAGGAAGTTCCCTGAAGAGCGCAGAGACAGAGAAGGAG

and the reverse primer 5’ -TCCGCTTCCGCCCTGCTTGTCGGCCATGATATA GACGTTGT. The sequence in bold encodes for the eight amino acid linker Gly-Gly-Ser-Gly-Ser-Gly-Ser-Ser connecting the amino portion of EGFP (residue 1-158) with the N-terminus of the myosin rod at residue 842. The WT/R1500P-CGFP constructs were obtained from the EGFP WT/R1500P MyHC rod plasmids by two consecutive inverse PCR reactions. In the first reaction, the sequence encoding EGFP residues 1-158 was deleted using the forward primer 5’ -AAGAACGGCATCAAGGTGAACTTCAAG and the reverse primer 5’ -CATGGTGGCGACCGGTAGC which carries the EGFP ATG and Kozak sequence (underlined). From the constructs thus obtained, a second inverse PCR reaction was carried out with the forward primer 5’ -ACAAGTTCTAC CAGCTCAGGAATCCTGAAGAGCGCAGAGACAGAGAAGGAG and the reverse primer 5’ -GCCTGAACTGGTTCCGCTTGCTTCTGACTTGTACAGCTCGTCC ATGCCGAG. The sequence in bold encodes for the seventeen amino acid linker (Ser-Glu-Ala-Ser-Gly-Thr-Ser-Ser-Gly-Thr-Ser-Ser-Thr-Ser-Ser-Gly-Ile) connecting the carboxyl portion of EGFP (residue 159-239 preceded by a methionine) with the N-terminus of the myosin rod at residue 842.

Supplemental Figure Legends

Figure S1. Human β-myosin and *C. elegans* MHC B rod comparison.

(A) Percentage of amino acid conservation. (B) Plot of the Human and *C. elegans* rod periodicities calculated by Fourier transform analysis.[1] (C) Plot of the Human and *C. elegans* average charge profiles computed using a sliding window

of 14 amino acids.[2] x-axis: 0 corresponds to the beginning of the Human (AA 837) and *C. elegans* (AA 850) rods.

References

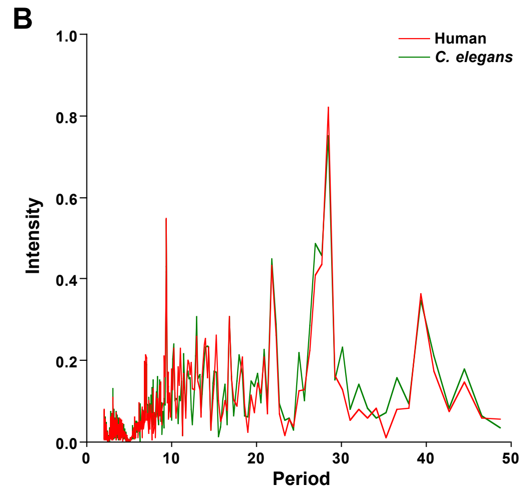
1. Parry, D. A. (1981). Structure of rabbit skeletal myosin. Analysis of the amino acid sequences of two fragments from the rod region. *J Mol Biol* **153**, 459-64.
2. Hostetter, D., Rice, S., Dean, S., Altman, D., McMahon, P. M., Sutton, S., Tripathy, A. & Spudich, J. A. (2004). Dictyostelium myosin bipolar thick filament formation: importance of charge and specific domains of the myosin rod. *PLoS Biol* **2**, e356.

A

Human vs *C.elegans*

Myosin Domains	AA identity (%)	AA similarity (%)
Head	54.4	68.7
Rod	44.9	68.1
Head & Rod	49.1	68.4

B



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