Proximity of Two Heads in Smooth Muscle Myosin

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Introduction

It is already established that the interaction between the two heads are involved in the activation process of Smooth Muscle Myosin. Nevertheless, which parts of the interacting heads are involved in the phosphorylation dependent activation is still controversial. Two principal models, also referred to as parallel (MD-MD interaction) [1] and antiparallel (RLC-RLC interaction) [2], are contradictory. The goal of this study is to identify the domains involved in the activation and distinguish which model is correct.

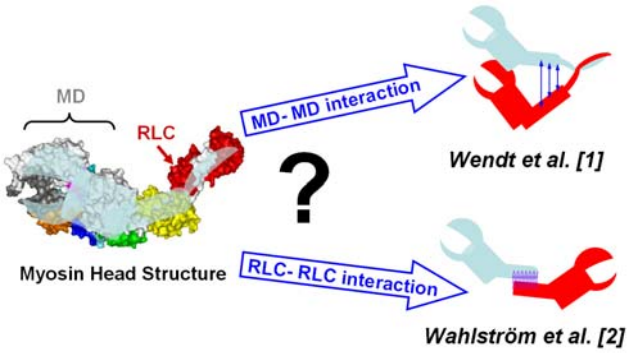

Figure 1: Structural models for the unphosphorylated Smooth Muscle Myosin: Interaction between two motor domains (MD-MD) [1] and model that interaction between RLCs brings.

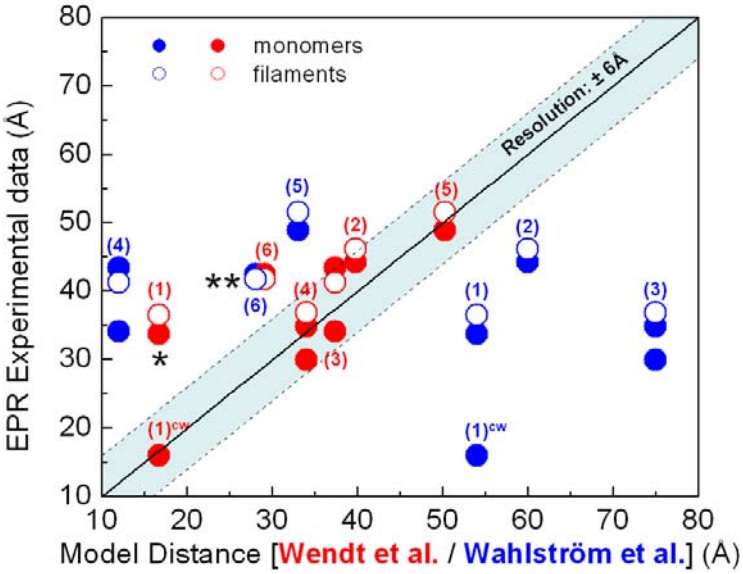

Figure 2: EPR experimental data vs. spin-spin distances from parallel (red) and antiparallel model (blue) and both monomers (full circle) and filaments state (open circle). The number 1,2,3,4,5 and 6 represents the spin-spin distances for the N38C, I48C, S59C, T96C, 108C and G165C mutants respectively. ***** N38C: Less than 20% of coupled spin [cf (1)cw]. ****** G165C: Wendt et al. model for RLC only have 165 amino acid (167 for Wahlström et al.) *vs.* 172 aa in reality.

Experimental

We measured six inter RLC residues distances in reconstituted unsphosphorylated smooth muscle myosin. For that purpose, site directed spin-label (SDSL) was used with both continuous wave electron paramagnetic resonance (cw-EPR) and double electron electron resonance (DEER).

Results and Discussion

To account for finite size of the SDSL and its mobility we performed a Metropolis Monte Carlo Minimization (MMCM) to find the lowest energy i.e. most probable position of the label. Spin-spin distance comparison from both models, and EPR experimental data obtained for unphosphorylated monomers as well as smooth myosin filament, support the parallel "MD-MD" model.

Conclusions

The use of EPR as an appropriate tool for validating structural models in solution is highlighted.

References

[1] Wendt, T., *et al*., Proc. Natl. Acad. Sci. U. S. A. **98**, 4361 (2001).
[2] Wahlström, J.L. J. Biol.Chem. **278**, 5123 (2003).