Identification of a Putative Binding Site for a Sea Urchin 53-kD Myosin Binding Protein[a]

D. R. BURGESS,[b] R. YABKOWITZ,[c] AND G. R. WALKER[b]

[b]*Department of Cell Biology and Anatomy*
University of Miami School of Medicine
Miami, Florida 33101

[c]*Department of Pathology*
University of Michigan Medical School
Ann Arbor, Michigan 48109-0650

The actomyosin cytoskeleton provides the structural framework for an array of directed cellular movements such as cytokinesis and morphogenesis. Myosin is possibly the major mechanochemical transducer in such movements. The exact structural state of myosin in nonmuscle cells is unknown and may involve cycles of assembly and disassembly.[1] Therefore, the regulation of myosin structure and distribution in cells may have a fundamental physiologic role. In a number of cells the redistribution of myosin has been observed to correlate with changes in cell state.[1,2]

Myosin's catalytic activity resides in the head of the molecule, and phosphorylation of myosin light chains in this region mediates conformational changes necessary for the regulation of activity. The formation of bipolar filaments is a function of the rod portions of myosin heavy chain. The head-rod junction of myosin heavy chain contains the binding sites for the light chains and plays a role in conformational changes.[3] This head-rod junctional region retains a large degree of flexibility. Thus, the neck region determines myosin's shape and, consequently, filament assembly and enzymatic activity.[4,5]

We have identified a myosin-binding protein in sea urchin eggs that mediates myosin's low ionic strength solubility. This protein has the subunit molecular weight of 53 kD and binds to myosin in a nucleotide-dependent manner. In this study the 53K binding site on myosin was examined to determine the mechanism of interaction that gives rise to a change in myosin's properties. We used several methods to identify the 53K binding site on the myosin molecule (summarized in FIGURE 1).

Myosin and 53K were isolated from soluble sea urchin extracts as described by Yabkowitz and Burgess.[6] Papain and chymotrypsin digests were performed on myosin and the insoluble and soluble fragments were separated according to Barylko *et al.*[7] Papain gives rise to 165, 130, 107, 80, 62 and 24-kD fragments. The 80, 62, and 24-kD fragments are head fragments as defined by actin binding and ATPase activity

[a]This work was supported by NIH grant GM 40086.

and solubility at low ionic strength. The remaining fragments are from the rod as defined by lack of ATPase activity and insolubility at low ionic strength. Chymotrypsin generates a 165-kD rod fragment and two head fragments (69 and 52 kD).

Blot overlay experiments were carried out using the papain myosin fragments essentially as described by Glenney and Weber.[8] The bound 53K was detected with the use of a rabbit antibody against 53K. 53K was observed to bind strongly to the papain 107- and 80-KD fragments and weakly to the 130- and 165-kD fragments. The other head fragments did not bind 53K.

Chemical cross-linking 53K to myosin head and rod papain fragments was accomplished by preincubating fragments and 53K for 1 hour on ice. Cross-linking was initiated by addition of disuccinimidyl suberate or m-maleimibenzoyl-N-hydroxylsuccinimide and incubation at 25° C for 30 minutes or 5 minutes, respectively. The reactions were stopped by addition of 4× sample buffer. The 53K protein was cross-linkable to the 107-, 130-, and 165-kD rod fragments and also to the 80-kD head fragment.

To further identify the binding site for 53K, its ability to inhibit proteolyic cleavage at specific sites was determined. Whole myosin was incubated in the presence or absence of 53K in buffer containing ATP for 1.5 hours on ice. Digestion was carried out by addition of papain or chymotrypsin and incubation at 25° C for up to 1 hour. In both cases 53 kD inhibited proteolytic cleavage at the site that gives rise to the 165-kD rod fragments.

In summary, these experiments indicate that 53K binds at or very near the head-rod junction (FIG. 2), placing 53K in a region known to be important for myosin regulation. It is possible that 53K promotes or inhibits conformational changes in myosin, leading to altered solubility characteristics. The mechanism of this interaction is currently under investigation.

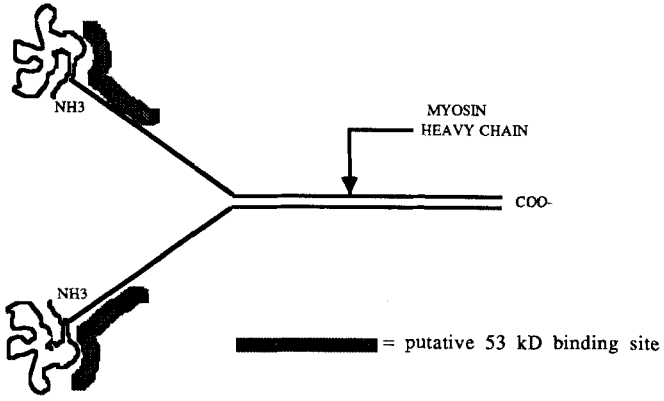

FIGURE 1. Myosin-53-kD complex.

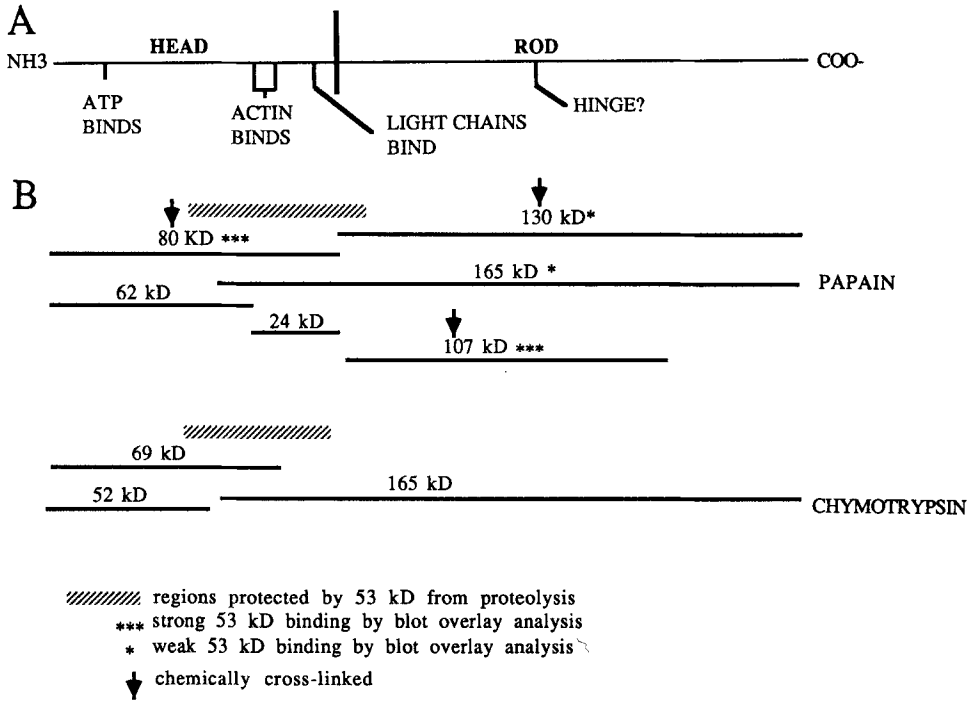

FIGURE 2. (**A**) Established map of smooth muscle myosin. (**B**) Summary of blot binding and protection experiments with egg myosin.

REFERENCES

1. YUMURA, S. & Y. FUKUI. 1985. Nature (Lond.) **314:** 194-196.
2. MITTAL, B., J. M. SANGER & J. W. SANGER. 1987. J. Cell. Biol. **105:** 1753-1760.
3. CRAIG, R., R. SMITH & J. KENDRICK-JONES. 1983. Nature (Lond.) **302:** 436-439.
4. SCHOLEY, J. M., K. A. TAYLOR & J. KENDRICK-JONES. 1981. Biochemie **63:** 255-271.
5. HARDWICHE, P. M. D., T. WALLIMANN & A. G. SZENT-GYORGYI. 1983. Nature (Lond.) **301:** 478-482.
6. YABKOWITZ, R. & D. R. BURGESS. 1987. J. Cell. Biol. **105:** 927-936.
7. BARYLKO, B., P. TOOTH & J. KENDRICK-JONES. 1986. Eur. J. Biochem. **158:** 271-282.
8. GLENNEY, J. R., JR. & K. WEBER. 1980. J. Biol. Chem. **255:** 10551-10554.