MILESTONE 18

Actin structure enters the stage

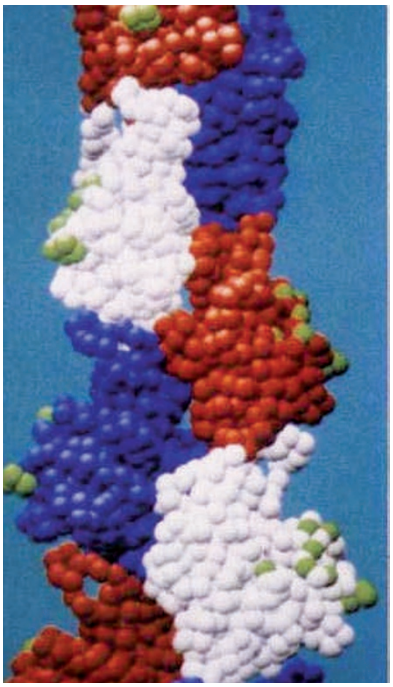
> “Holmes and colleagues managed what was thought to be impossible, namely obtaining extremely high level resolution maps of F-actin.” *William Bement*

By 1990, fundamental models underlying actin-based muscle motility were in place; however, despite a decade-long pursuit, high-resolution structures of the key components remained elusive. Motility assays indicated that myosin subfragment-1 (S1), which is a proteolytic fragment containing the actin and nucleotide binding sites, is the motor domain. S1 was tadpole-shaped and clearly resembled a series of oars rowing along a sea of actin filaments; however, the underlying conformational changes, and hence the motility mechanism, remained unclear.

The tendency of actin to polymerize initially thwarted crystal production. Holmes and colleagues overcame this by complexing rabbit skeletal actin with DNase I, thereby preventing polymerization and helping to solve the structure. Kabsch *et al.* saw that actin consists of a small and a large domain separated by a nucleotide-binding cleft. In an accompanying paper, this atomic structure of the actin monomer was used to achieve the unimaginable: a probable structure for the filament. Holmes and colleagues refined this model by comparing experimental diffraction patterns from orientated actin fibres with calculated diffraction patterns based on the atomic actin structure. The most likely model was consistent with the overall dimensions and inter-residue distances derived from functional studies. Actin-molecule orientation relative to the filament axis pointed to interfaces with myosin, as well as between actin monomers along the helix, and indicated where the fast-polymerizing barbed end of the filament lay.

Three years later, the long sought-after myosin S1 structure gave insight into the cross-bridges of the sarcomere and provided the first close glimpse of a molecular motor. The structure overcame obstacles, including the large size and heterogeneity of chicken myosin S1. Myosin S1 contains heavy and light chains, and is highly asymmetric, comprising a globular catalytic domain and a long projecting α-helix from the heavy chain around which the regulatory and essential light chains are wrapped. The latter forms what has become known as the lever arm, which is the key to the amplification of small conformational changes into large movements; the lever arm model for translocation forms the basis of our current understanding of myosin-based motility. The actin and nucleotide binding sites are linked by a narrow cleft, and conformational changes around this cleft were immediately proposed to mediate communication between the two binding sites. Rayment *et al.* combined this structural information with electron-microscopy data to generate a model for the actin–myosin complex. Having suggested a model for one state of the interaction, the authors used clashes in the model as well as previous data to suggest a unified model for the structural basis of a contractile cycle.

The muscle myosin structure provided a structural lynchpin for the then emerging family of myosin motors, and would shortly be shown to have unexpected structural relatives in what had been considered an unrelated class of motor proteins (see milestone 24).



Model of actin filament. © 1990 Nature Publishing Group.

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ORIGINAL RESEARCH PAPERS Holmes, K. C. *et al.* Atomic model of the actin filament. *Nature* **347**, 44–49 (1990) | Kabsch, W., Mannherz, H. G., Suck, D., Pai, E. F. & Holmes, K. C. Atomic structure of the actin:DNase I complex. *Nature* **347**, 37–44 (1990) | Rayment, I. *et al.* Three dimensional structure of myosin subfragment-1: a molecular motor. *Science* **261**, 50–58 (1993) | Rayment, I. *et al.* Structure of the actin–myosin complex and its implications for muscle contraction. *Science* **261**, 58–65 (1993)