# Molecular evolution: **Actin's long lost relative found**

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**The bacterial protein MreB has been identified as a prokaryotic homolog of the eukaryotic cytoskeletal protein actin. While we still know little about MreB's function, the structural similarities and differences between MreB and actin provide more insight into the remarkable properties of actin.**

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Actin is one of the most abundant and highly conserved eukaryotic proteins. While actin was first identified in vertebrate skeletal muscle, where it forms the thin filaments, it was subsequently shown to be essential in the cytoskeleton of non-muscle cells for many aspects of the control of cell form and motility. The selective pressures that have prevented significant divergences in actin sequences across eukaryotic evolution are poorly understood. But these pressures must be enormous, as there are no amino-acid substitutions between human and chicken skeletal muscle actin. Looking at a much greater evolutionary separation, the yeast actin amino-acid sequence is 87% identical to that of skeletal muscle actin. Recent structural studies of the bacterial MreB protein [1] may not only tell us about the bacterial cytoskeleton, but also provide a wealth of information on the evolutionary origin of actin and the forces that have conserved it in both sequence and structure.

Several earlier observations suggested that MreB might be a bacterial actin-like protein. The first crystal structure of actin [2] led to the realization that actin is part of a large superfamily of structurally homologous proteins that included chaperones and sugar kinases. The simplest assumption is that these proteins are all related to a common ancestral protein, with a large divergence in both sequence and function having occurred at the same time that the structures remained largely conserved. This realization led to database searches, and the bacterial protein MreB was identified as a putative member of this superfamily [3]. It had been known that MreB was involved in the control of cell shape, as deletion of the *mreB* gene in *Escherichia coli* resulted in a switch of cell shape from rod-like to spherical [4]. A more recent study [5] was able to show with light microscopy that MreB assembles into filamentous bundles in *Bacillus subtilis*, forming an apparent cytoskeleton under the cell membrane.

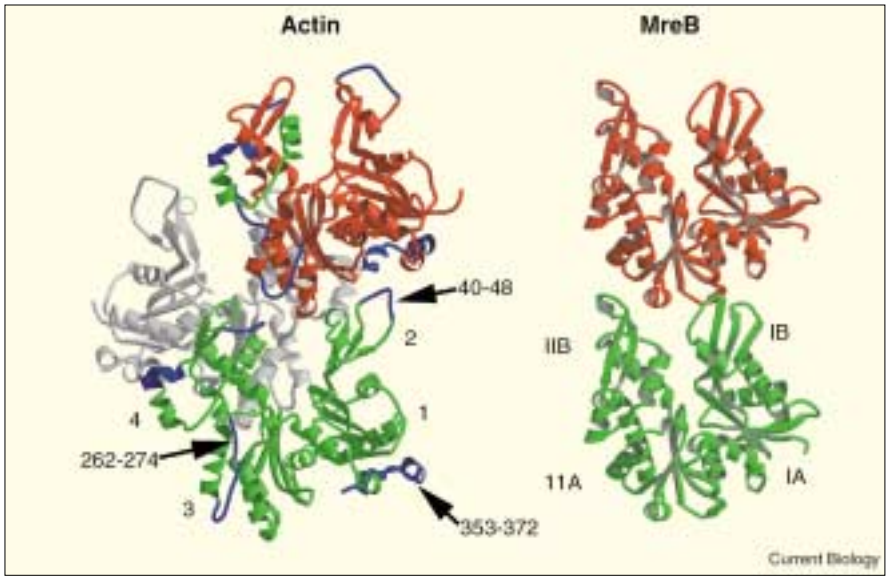
The breakthrough, however, has now come with the work of van den Ent *et al.* [1], who have used both electron microscopy and X-ray crystallography to examine MreB. They found that purified MreB protein assembles into protofilaments and bundles of protofilaments *in vitro*. They determined a crystal structure for MreB, and showed that it is strikingly similar to the structure of actin. Within both the crystals and the protofilaments observed by electron microscopy, the MreB subunits are arranged in a similar manner to that of the actin subunits in each of the long-pitch strands of the actin filament (Figure 1).

These findings may clarify the relationship of other bacterial proteins to actin. The bacterial protein FtsA was originally postulated to be a prokaryotic actin homolog [3]. Another protein product from the *fts* family of genes, FtsZ, has been shown, by structural homology, to be the bacterial relative of eukaryotic tubulin [6]. FtsA and FtsZ are both involved in cell division [7]. The crystal structure of FtsA [8] did, indeed, confirm that FtsA belongs in the family of actin-like proteins, as three of the four FtsA subdomains are in a position similar to that of the corresponding subdomains 1, 3 and 4 in actin. But the fourth subdomain of FtsA, called 1C, is located on the opposite side of subdomain 1A in FtsA from where subdomain 2 would be located in actin. Furthermore, no conditions have yet been found where FtsA polymerizes. The structure of MreB [1], on the other hand, contains four subdomains — IA, IB, IIA and IIB — which are also in the same positions as the corresponding subdomains 1, 2, 3 and 4 in actin (Figure 1). Thus, the presence or absence of a subdomain 2 homolog appears to distinguish MreB from FtsA, and makes MreB the true ortholog of actin. In the language of Platonic taxonomy, a protein is classified as an actin because it resembles an 'ideal' actin, and subdomain 2 appears to figure prominently in this ideal actin.

A large number of other observations have highlighted the key role of subdomain 2 in actin. There are only 39 amino acid substitutions, out of 375 residues, between yeast actin with the bovine cytoplasmic β actin isoform, and most of these are conservative changes — such as replacement of a hydrophobic residue by another hydrophobic residue. Within the approximately 36 residue subdomain 2, however, only one substitution has been found. Remarkably, this most conserved region of the molecule is also the most variable part structurally. Both electron microscopic and biochemical observations have shown that the position and conformation of subdomain 2 can be a function of the bound nucleotide, of whether $Ca^{2+}$ or $Mg^{2+}$ is bound at the high-affinity metal binding site, and of whether the carboxyl

**Figure 1**

Two subunits of bacterial MreB [1] (right) are compared with three subunits of eukaryotic actin [18] (left). MreB has been observed by both electron microscopy and X-ray crystallography to form protofilaments, and the subunits of MreB are shown as they are organized within the crystal protofilament. The crystal structure of G-actin has been modeled into a filament. Within the MreB protofilament, the red subunit is 5.1 nm above the green subunit, with no rotation. Within the actin filament, the red subunit is 5.5 nm above the green subunit and rotated by ~26º. A third actin subunit, shown in grey, is located behind the red and green subunits, and is part of a second long-pitch helical strand. The actin subdomains are labeled 1, 2, 3 and 4, and the corresponding MreB subdomains are labeled IA, IB, IIA and IIB, respectively. The inserts in the actin sequence that are not present in MreB [1] are shown in blue. Three of these inserts are indicated by arrows.




terminus of actin is modified [9–15]. The recent determination of a crystal structure for uncomplexed G actin has revealed that the DNase I-binding loop within subdomain 2, which exists as a β strand in several previous crystal structures, is now folded as an α helix [16]. This same loop was sufficiently disordered in another crystal that it was not visualized [17]. The entire subdomain 2 was seen to undergo a large rotation in yet another crystal structure [18].

The identification of subdomain 2 as the most structurally labile region of actin is in seeming contradiction with the fact that this region is also the most conserved in sequence. In general, one expects that a region which is highly mobile may also have the least selective pressure against mutations. A reconciliation of these two observations can be made by assuming that subdomain 2 is a molecular switch within actin, similar to the switches that have been observed within viral capsids [19]. The problem faced within spherical viral capsids is that subunits must make non-identical interactions with chemically identical neighboring subunits, because of the symmetry of the capsid. High-resolution structures have revealed that they do this by adopting discretely different conformations, which is an ability to switch their structure [20]. Within F actin, subunits need to make multiple types of interaction with their chemically identical neighbors, due to the fact that the subunits can exist in multiple states of twist [21] and tilt [22].

The crystal structure for MreB has allowed van den Ent *et al.* [1] to make a structure-based sequence alignment of MreB and actin. As the polypeptide chain of actin, with 375 residues, is longer than that of MreB, with 336 residues, there are a number of insertions that occur within the actin sequence in this comparison. These insertions are labeled in blue in Figure 1. One of these insertions, residues 40–48 in actin, has been called the DNase I-binding loop, as this is the region of actin that binds the nuclease DNase I [2]. It is this insertion that has been seen to fold as either an α helix [16] or a β sheet [2]. Several of the other insertions appear to be equally interesting. The insertion containing residues 262–274 in actin forms a loop with hydrophobic residues at the tip, and it was proposed that this loop must refold to make an important inter-strand interaction that holds the actin filament together [23]. This prediction has been experimentally tested and found to be valid [24]. The role of this loop may be more complicated, however, as it has been observed that modifications to this loop allosterically affect the DNase I-binding loop insert within subdomain 2 of actin [25]!

If allosteric interactions between these two actin inserts were not remarkable enough, it has also been extensively shown that the carboxy-terminal insert — residues 353–375 — is allosterically coupled to the DNase I loop insert [15,26]. Proteolytic cleavage of a few residues in the carboxy-terminal region of actin greatly decreases filament stability [27], and it has been shown that this occurs by a weakening of the inter-strand connectivity [28], most likely by affecting the 262–274 hydrophobic loop insert. Thus, the structure-based alignment of MreB and actin appears to highlight the extraordinary properties of at least three of the inserts — 40–48, 262–274 and 353–375 — that have appeared in actin since it diverged from MreB many years ago. These inserts not only play important roles in the actin filament, but they are all allosterically coupled despite being located far apart in the actin structure.

One of the most striking things about actin is the number of other proteins that specifically bind to it. Within skeletal muscle alone we know that myosin, tropomyosin, troponin, nebulin, dystrophin and α-actinin, among other proteins, bind to F-actin. There are probably more than 50 proteins whose actin-binding activity has been characterized in some detail. A very interesting question raised by the MreB structure is whether many prokaryotic proteins bind MreB, or whether this property of actin is mainly a eukaryotic development. Either answer will be interesting, and will provide additional insight into both the evolution of actin and the role of MreB in bacterial cells.

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