The discovery of the prokaryotic cytoskeleton: 25th anniversary

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ABSTRACT **The year 2017 marks the 25th anniversary of the discovery of homologues of tubulin and actin in prokaryotes. Before 1992, it was largely accepted that tubulin and actin were unique to eukaryotes. Then three laboratories independently discovered that FtsZ, a protein already known as a key player in bacterial cytokinesis, had the "tubulin signature sequence" present in all α-, β-, and γ-tubulins. That same year, three candidates for bacterial actins were discovered in silico. X-ray crystal structures have since confirmed multiple bacterial proteins to be homologues of eukaryotic tubulin and actin. Tubulin and actin were apparently derived from bacterial precursors that had already evolved a wide range of cytoskeletal functions.**

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INTRODUCTION

The 1970s and 1980s saw extensive research on microtubules and actin. During this period, the consensus developed that these cytoskeletal elements were unique to eukaryotes and that nothing related to tubulin or actin existed in bacteria or archaeans. This consensus was overthrown in the 1990s when a series of discoveries revealed that prokaryotes actually did have homologues of tubulin and actin and that these assembled into cytoskeletal filaments. It is now generally accepted that eukaryotic microtubules and actin filaments originated from these prokaryotic homologues. The key discoveries of bacterial tubulin and actin were published in 1992 and are reviewed here on their 25th anniversary.

PROKARYOTIC HOMOLOGUES OF TUBULIN

The discovery of FtsZ as a bacterial homologue of tubulin came first and was made independently by three groups (de Boer *et al.*, 1992; RayChaudhuri and Park, 1992; Mukherjee *et al.*, 1993). These independent studies each purified FtsZ protein from an *Escherichia coli* expression system and demonstrated that it bound and hydrolyzed GTP. They noted that bacterial FtsZs were missing the Walker sequence motifs characteristic of G proteins but that they had a conserved short sequence, GGGTGTG, that was almost identical to the G/AGGTGSG sequence conserved in all α-, β-, and γ-tubulins. That sequence, known as the tubulin signature sequence, was believed to be involved in the binding of GTP in the tubulins. The three groups all concluded that the GTP-binding site of FtsZ appeared to be related to that of tubulins.

A year earlier, before any link to tubulin was known, Bi and Lutkenhaus (1991) were the first to propose that FtsZ might be a cytoskeletal protein. They used immuno–electron microscopy to show that FtsZ localized to the invaginating septum in dividing *E. coli*: "In our model the role of FtsZ is to form a cytoskeletal element that is functionally analogous to the role of actin in cytokinesis in animal cells." The key discoveries in 1992–1993 advanced this suggestion. In particular, de Boer *et al.* (1992) noted that the GTPase activity showed a dependence on FtsZ concentration characteristic of the self-assembly of tubulin and actin.

A subsequent study by Mukherjee and Lutkenhaus (1994) provided two major advances. First, they demonstrated that purified FtsZ could assemble in vitro into filamentous polymers. This was strong support for the proposed role as cytoskeleton. Second, they extended the sequence alignment to identify >20 amino acids that were highly conserved in all tubulins and FtsZ. They did this by starting the alignment at the GGGTGTG sequence and inserting gaps in the FtsZ to maximize identity. Remarkably most of the gaps coincided with gaps already in the alignment of α-, β-, and γ-tubulins. I considered this alignment to be compelling evidence for full homology, but the editors of *Cell* were more cautious, insisting on adding a question mark to my 1995 minireview, "FtsZ, a prokaryotic homolog of tubulin?" (Erickson, 1995).

Our laboratory took up the question of in vitro assembly and showed that FtsZ assembled in vitro into sheets of protofilaments

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and mini-rings that were similar to tubulin polymers (Erickson *et al.*, 1996). Any question of homology was dramatically resolved when the structures of FtsZ (Löwe and Amos, 1998) and tubulin (Nogales *et al.*, 1998) were published simultaneously in 1998. They had an identical complex fold, which is the ultimate test of homology.

It turns out that FtsZ is not the only tubulin homologue in prokaryotes. Many archaeans have up to five FtsZ homologues, some with very divergent sequences that likely serve functions other than cell division. Even some plasmids and phage encode their own tubulin/FtsZ homologues. These TubZ proteins assemble into cytoskeletal filaments that function to partition DNA.

PROKARYOTIC HOMOLOGUES OF ACTIN

The discovery of bacterial actins was complicated by the homology of actin to other protein families. Protein homology means shared ancestry. Sometimes, this is indicated by amino acid sequence identity, but often this sequence identity is too weak to recognize. The most definitive demonstration of homology is from protein structure. When the x-ray structure of actin was determined (Kabsch *et al.*, 1990), it was seen to have a complex fold that was identical to that of hexokinase and also to Hsp70, a chaperonin. Because this fold is so complex yet was shared so precisely, it was concluded that the three shared a common ancestry. Actin, hexokinase, and Hsp70 are homologues and are considered to be members of an actin superfamily. Note that homology does not imply common function. Probably the original protein in this family was the sugar kinase, which underwent gene duplications that evolved into a chaperonin and separately into a protein that could assemble cytoskeletal filaments, actin.

The first strong suggestion for actin homologues in bacteria was a theoretical study by Bork *et al.* (1992). They used the recent x-ray structures of actin, hexokinase, and Hsp70 to do a structure-based sequence alignment, which identified four short signature sequences that were conserved across the superfamily. They then looked for these signatures in bacterial proteins and found them in three: FtsA, MreB, and ParM (StbA). These bacterial proteins were closest in sequence to Hsp70 and actin rather than the sugar kinases and were therefore candidates for bacterial actin.

Experimental confirmation that MreB was an actin homologue finally came 9 years later. Jones *et al.* (2001) studied the localization of MreB in bacteria by light microscopy, and found helical filaments running through the cell under the membrane. Van den Ent *et al.* (2001) isolated MreB protein and showed by electron microscopy that it assembled thin filaments. Their major discovery was x-ray crystallography, which showed that MreB had the actin fold and was assembled in the crystals into actin-like filaments. Later work has discovered multiple prokaryotic actins with a variety of cytoskeletal functions, although the functions of most are unknown.

It now seems clear that both tubulin and actin were invented in bacteria and/or archaeans and proliferated into diverse families of cytoskeletal filaments well before the emergence of eukaryotes; for various perspectives on the evolution, see Erickson (2007), Löwe and Amos (2009), and Wickstead and Gull (2011). An interesting irony is that roles of tubulin and actin have somewhat switched from bacteria to eukaryotes. FtsZ forms the cytokinetic ring in bacteria, whereas actin provides the major cytoskeletal framework in eukaryotes. Some bacterial (plasmid) actins function for nucleoid segregation, a role performed by microtubules in eukaryotes. However, some TubZ filaments also function for plasmid segregation. A global conclusion would be that once a protein has evolved the ability to assemble cytoskeletal filaments, these can be modified to perform a wide range of useful and sometimes overlapping cellular functions.

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