Summary

Cytoskeletal proteins are essential for maintaining cell shape, facilitating DNA segregation, intracellular organization, and division across all three domains of life- Bacteria, Archaea, and Eukarya. A common feature of many of these proteins is their ability to polymerize in a nucleotide-dependent manner, where ATP or GTP binding and hydrolysis regulate filament assembly and dynamics. However, nucleotide hydrolysis plays diverse roles and serves distinct functions of the cytoskeleton, especially given the diverse repertoire in archaea and bacteria. In this study, we investigated the role of ATP hydrolysis in regulating the *in vivo* polymerization of four diverse cytoskeletal proteins: actin-related proteins (ARPs), Arp1 and Arp2 from *Lokiarchaeia* (Loki), MreB5 from *Spiroplasma citri* (Sc), ParM from *Clostridium perfringens* (Cp), and ParA from the F plasmid ($ParA_F$) of *E. coli* (Ec).

We show that Loki Arps from *Lokiarchaeota*, a member of the Asgard superphylum assemble into puncta or speckles in the eukaryotic cytoplasm, independent of host actin or tubulin, and remain unaffected by mutations in ATP binding region. We further show that Loki Arp1 filaments are sensitive and Loki Arp2 are resistant to Latrunculin A, but both of them are influenced by yeast actin regulators, indicating distinct modes of regulation.

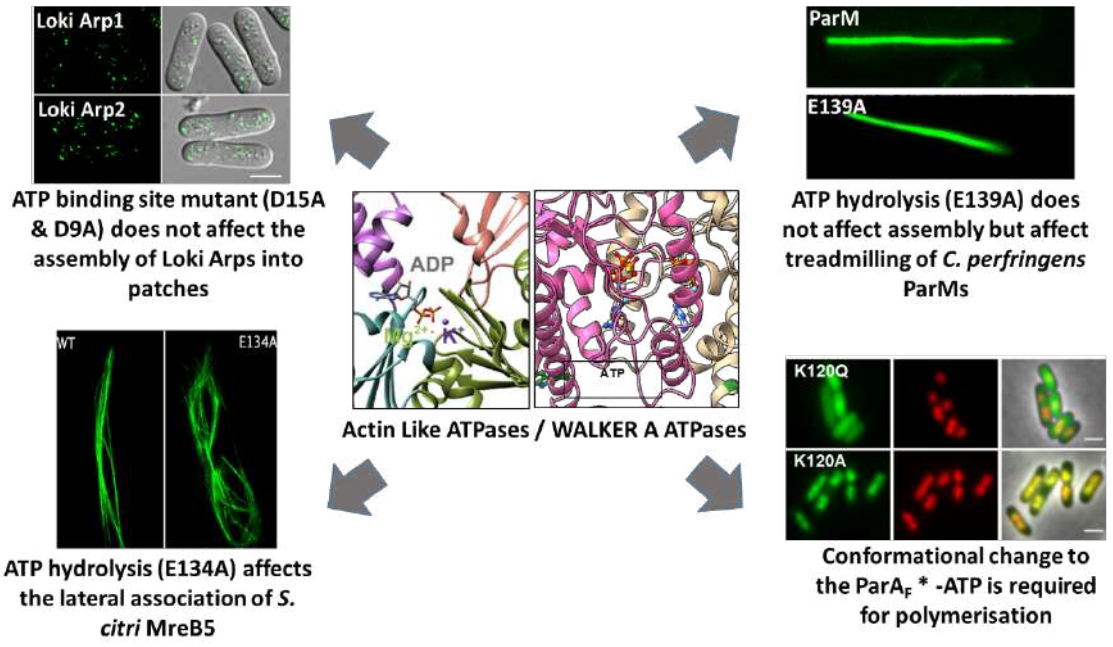
While cell shape maintenance by the actin homolog, MreB, and its nucleotide-dependent polymerization is well established in cell-walled bacteria, its dynamics in wall-less species remain less clear. In this study, we use the heterologous host fission yeast (*Schizosaccharomyces pombe*) and show that MreB5 from the wall-less bacterium *S. citri* (ScMreB) assembles as linear polymers that bundle into thick, long filaments oriented along the long-axis of the yeast cells. Further, imaging of the ATP hydrolysis defective mutant (E134A) suggests that the bundling of the filaments is strongly dependent upon ATP hydrolysis, highlighting the importance of nucleotide turnover in regulating filament architecture.

Actin homologs in bacteria not only play a role in cell shape, but actin-like proteins (Alps) such as ParM, play a pivotal role in plasmid partitioning. ParM from R1 plasmid of *E. coli* has been well studied and serves as a paradigm for actin mediated plasmid segregation in bacteria. Further, a study in 2009 by Pogliano and colleagues identified 40 actin-like proteins (Alps) from different species of bacteria, including *C. perfringens* where multiple plasmid-types coexist highlighting the diversity of Alps or ParM homologs. In this study, we analyze the polymerization dynamics of six *C. perfringens* ParM variants (*Cp*ParM (A), *Cp*ParM (B), *Cp*ParM (C), *Cp*ParM (D), *Cp*ParM (E), and *Cp*ParM (I). We find that four of these exhibit treadmilling behaviour, a hallmark of dynamic filament turnover. ATP hydrolysis mutants show impaired treadmilling, and co-expression studies further point to the importance of filament dynamics and its critical role in plasmid segregation.

Although actin-like proteins are involved in plasmid partitioning in many bacteria, a distinct family of proteins, known as the ParA family are most wide-spread in bacterial and archaeal genomes and play a role in a variety of functions including plasmid and genome segregation. Here in this work, we have characterized ParA from the F plasmid of *E. coli* ($ParA_F$) using two previously identified mutations, Q351H and W362E, that form cytoplasmic filaments independent of the $ParBS_F$ partitioning complex. Experiments combining the W362E mutation with the variants in the conserved K120 residue of the Walker A ATP binding motif, suggest that the polymerization of $ParA_F$ W362E requires the conformational switch to the $ParA_F$*-ATP state upon nucleotide binding. Further, we identify two residues, R320 in helix 12 and E375 in helix 14 at the interface of predicted $ParA_F$ filament structure, whose mutations abolish filament assembly of $ParA_F$ W362E and impair plasmid partitioning. Our results thus are suggestive of a role for the C-terminal helix of $ParA_F$ in assembly into higher-order structures and plasmid maintenance.

Together, these studies provide insights into diverse filament-forming mechanisms of actin-

like and Walker A cytoskeletal proteins, highlighting their roles across different domains.



Schematic overview summarizing the major findings presented in this thesis: This figure summarizes the role of ATP binding region mutations in regulating filament dynamics of actin-like proteins and Walker A-type ATPase cytoskeletal systems across diverse prokaryotic lineages. These studies highlight how alterations in ATP hydrolysis influence polymer assembly, stability, and function in different cytoskeletal ATPases.