Filament assembly and structural studies of intermediate filament like protein, FilP, in *Streptomyces coelicolor*

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Akademisk avhandling

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Avhandlingen kommer att förvandas på engelska.
Fakultetsopponent: Professor, Martin Thanbichler, Faculty of Biology, Philipps University, Marburg, Germany.
Title
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Abstract
The cytoskeleton, known as intracellular connected filaments, has a prominent role in cellular behavior, motility, and stability. The following three major types of polymers have been characterized as cytoskeleton in eukaryotes: microtubules that are 25 nm in diameter, actin filaments that are 7 nm in diameter, and intermediate filaments (IF) that are 10 nm in diameter. IFs, unlike actin and microtubules, are not polarized and do not facilitate the directional movement of molecular motors. Further, IF assembly is different from that of actin and tubulin because they are independent of co-factors and they undergo instant self-assembly based on hydrophobic interactions.

Cytoskeleton proteins were initially thought to be unique to eukaryotic cells, but we now know that all three cytoskeleton types have bacterial counterparts. Bacterial cytoskeleton is a novel field and it is less characterized than the eukaryotic cytoskeleton. The IF subfamily in bacteria are called IF-like proteins because of a lack of conserved sequences. FilP is a bacterial IF-like protein that is localized to the sub-apical area of *Streptomyces coelicolor* hyphae tips. Moreover, FilP forms two distinct structures *in vitro*, as follows: 1) filaments in branching bundles with a repetitive striation pattern of 60 nm intervals between the repeats; and 2) an interconnected hexagonal meshwork, which has a three-dimensional morphology with the same 60-nm unit. There have been several studies on different IF-like proteins from different bacterial species; however, there are no studies that have investigated their assembly mechanism or atomic resolution of their structures before this study.

We present the first filament assembly model of an IF-like protein. The hierarchical stages of filament assembly were characterized and analyzed by utilizing physiological effects of different buffer systems. The basic building block was characterized by a single particle classification, revealing the length of primary coiled-coil unit. The following steps of protofilament assembly and filament bundling were revealed using negative-staining electron microscopy together with solubility assay and cryo-electron tomography. We demonstrated similarities and differences of FilP filamentation to eukaryotic IF lamin, because they both showed filaments with similar morphology *in vitro* conditions. In a cytoplasm-mimicking buffer (Polymix), FilP proteins form hexagonal meshworks. By subjecting FilP to the ion components of the Polymix buffer, we found that K⁺ and Na⁺ triggered FilP meshwork formation and increased its solubility.

Guided by the *in vitro* assembly studies of FilP we crystallized the 184–288 fragment, which is a tailless construct containing the C-terminal coiled-coil domain of the FilP rod domain to 2.3-Å-resolution. The crystal structure of the 184–288 fragment revealed that the C-terminus of FilP rod domain is composed of one single coiled-coil. Arrangement of the crystal indicated the formation of parallel homo-dimers and dissociation of the homo-dimers at the C-terminus, forming an open and fork-like structure. Further, the fork-like structure facilitates the end-to-end association of homodimers. These experiments were complemented by testing constructs containing different coiled-coil domains for *in vitro* filament assembly and their *in vivo* capability to restore the FilP phenotype AfilP *S. coelicolor*. Based on these findings, we showed a model for the *in vitro* FilP filament formation.

We have shown that FilP, like its orthologous in other *Streptomyces* species, has cellulose affinity. Investigation of cellulose affinity of other IF-like proteins and eukaryotic IF protein such as lamin showed that cellulose binding of certain coiled-coil domains is an intrinsic property of all the tested IF and IF-like proteins and thereby adds IF coiled-coil domains to the list of carbohydrate binding motifs. Building upon this, coiled-coil domains of FilP can be utilized to purify recombinant fusion proteins from *S. coelicolor* and *Escherichia coli* lysates. We used truncated constructs of FilP to find the coiled-coil domains with the highest affinity for cellulose, which can be used as a cellulose affinity tag.

Keywords
Cytoskeleton, Intermediate filament-like, FilP, *Streptomyces coelicolor*, filament assembly, cryo-electron tomography, single particle analysis, crystallography, cellulose affinity

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