

Coiled coil cytoskeletons collaborate in polar growth of *Streptomyces*

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S*treptomyces* is a multicellular mycelial bacterium, which exhibits pronounced cell polarity and grows by extension of the hyphal tips. Similarly to other polarly growing walled cells, such as filamentous fungi or pollen tubes, *Streptomyces* hyphae face an intrinsic problem: addition of new cell wall material causes structural weakness of the elongating tip. Cellular strategies employed by walled cells to cope with this problem are not well understood. We have identified a coiled coil protein FilP, with properties similar to those of animal intermediate filament (IF) proteins, which somehow confers rigidity and elasticity to the *Streptomyces* hyphae. In a recent publication we showed that FilP forms extensive cis-interconnected networks, which likely explain its biological function in determining the mechanical properties of the cells. Surprisingly, the intrinsically non-dynamic cytoskeletal network of FilP exhibits a dynamic behavior in vivo and assembles into growth-dependent polar gradients. We show that apical accumulation of FilP is dependent on its interaction with the main component of the *Streptomyces* polarisome, DivIVA. Thus, the same polarisome complex that orchestrates cell elongation, also recruits an additional stress-bearing structure to the growing tips with an intrinsically weak cell wall. Similar strategy might be used by all polarly growing walled cells.

Mycobacterium tuberculosis or symbiotic, e.g., *Frankia spp*), physiology, and metabolism (e.g., production of most known antibiotics by *Streptomyces*), or colonization of various environmental niches. Besides having a large industrial and medical importance due to secondary metabolite production, *Streptomyces* species are also gaining importance as model organisms for cell biology research due to their intricate lifestyle involving multicellularity, differentiation and sporulation.¹ Vegetative hyphae grow by tip extension and branching to form a multicellular mycelium.² Such apical growth is one of the most extreme manifestations of cellular polarity, and has been extensively studied in filamentous fungi, yeasts, and pollen tubes and root hairs of plants. Polarized actin and tubulin cytoskeletons play key roles in these eukaryotic cells growing in apical manner. Polymerization of actin and tubulin subunits into filaments is coupled to hydrolysis of a nucleotide, and this enzymatic activity serves as an intrinsic regulatory mechanism of filament assembly, making actin- and tubulin-based cytoskeletons indispensable in dynamic cellular processes such as transport, cell locomotion, cell constrictions or establishment of polarity. The third main type of eukaryotic cytoskeleton, the intermediate filaments (IFs), consists of coiled coil proteins lacking any enzymatic activity, which spontaneously assemble into stable and intrinsically static filaments. IFs are important determinants of the mechanical properties, such as rigidity and elasticity of the cells and nuclei. Surprisingly, the intrinsically dynamic actin- and tubulin-like cytoskeletons are not involved in polar growth in *Streptomyces*, as mutants lacking FtsZ (tubulin) or MreB (actin) proteins

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Streptomyces are ubiquitous organisms in soil and belong to Actinobacteria, a large and diverse phylum of Gram-positive bacteria. Actinobacteria exhibit remarkable diversity regarding their morphology (from simple coccoid to complex branching filaments), lifestyle (pathogenic, e.g.,

are viable and grow via tip extension.^{3,4} Instead, three coiled coil proteins—DivIVA, Scy and FilP—play important roles in orchestrating tip growth. DivIVA is the key factor in generating cell polarity in *Streptomyces* and in many other Actinobacteria.^{1,2} The current model envisions that DivIVA assembles together with other proteins, such as Scy, into large complexes, termed polarisomes, at sites of de novo pole formation, which then serve as platforms to recruit factors needed for new cell envelope synthesis.⁵⁻⁷ Thus, the Achilles' heel of *Streptomyces*, and all other apically growing walled cells, is the growing tip where the nascent cell wall is being assembled and matured, making it by necessity more flexible and compliant than the more highly crosslinked pole-distal parts^{8,9} (Fig. 1). Despite extensive research, it is still not clear how cells of various kinds deal with this inherent problem. Below we discuss our recent publication in which we show that in actively growing hyphae an IF-like cytoskeleton consisting of the coiled coil protein FilP is recruited to the polarisome-proximal apical region by DivIVA, where it provides an additional stress-bearing structure.¹⁰ We also reveal an unexpectedly dynamic behavior of an intrinsically non-dynamic coiled coil cytoskeleton.

We have previously reported that FilP has a protein architecture consisting of a segmented coiled coil rod domain, and forms stable non-dynamic filaments in vitro without additional cofactors or energy, and thus belongs to the growing group of bacterial IF-like proteins.¹¹ Interestingly, FilP also seems to have a similar biological function as the animal IFs in determining mechanical properties of the cells. We showed by atomic force microscopy that the FilP cytoskeleton contributes to the normal rigidity and elasticity of the *S. coelicolor* hyphae.¹² However, since the latter properties are usually determined by the peptidoglycan cell wall in bacteria, the role of FilP remained mysterious. Our recent finding that FilP can self-assemble into a *cis*-interconnected regular network without the involvement of additional crosslinking proteins in vitro, encouraged us to propose that this property constitutes the structural basis for the observed role

in cell rigidity and elasticity (Fig. 1). We also showed that the in vivo localization of FilP is consistent with a network structure. Numerous studies have established that in order to possess sufficient coherence to resist deformation and mechanical stress, the cellular cytoskeletal fibers need to be crosslinked into a network.¹³ For example, actin filaments in solution in the absence of crosslinks form a relatively soft material. However a sharp increase in viscoelasticity was observed upon addition of a crosslinking protein already at a low molar ratio of 1 per 800 polymerizing actin monomers.¹⁴ Similarly, modeling of the hypothetical IF networks of vimentin and neurofilaments using arbitrary values ranging from 0.3–0.7 μm for the average distance between crosslinks, has yielded elastic properties consistent with those of the cells.^{15,16} In contrast, the regular network formed by FilP in vitro is interconnected significantly more frequently at every 60 nm, which should render the FilP network a mechanically highly resilient material, and explain its ability to confer cellular rigidity. FilP appears as an attractive model system to study the mechanical properties of protein networks. Intriguingly, formation of extensive regular networks by FilP is strongly promoted by polymerization conditions in a complex buffer designed to mimic cytoplasmic conditions. In simple buffers, routinely used in studies of IF proteins, FilP forms compact branching filaments exhibiting a light and dark striation pattern, visually closely resembling those formed by IF proteins nuclear lamins.^{12,17} In vivo, however, lamins are similarly to FilP, known to form a filamentous network, the structure of which has remained elusive, partially because network formation has not been accomplished in vitro.¹⁸ Our results tempted us to speculate whether it might be possible to reconstitute networks similar to nuclear lamina in vitro by designing an experimental system to more closely resemble the complex physiological conditions of the cells. This is highly relevant, because several serious human diseases result from mutations in lamins impairing the mechanical properties of the laminal network of the nucleus.¹⁹

In vivo all actively tip-growing hyphae contain an apical gradient of FilP with

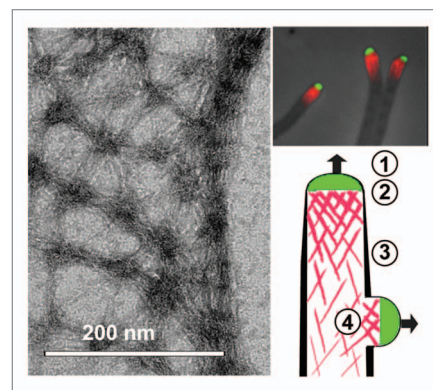


Figure 1. The intermediate filament-like protein FilP spontaneously assembles into a regular network in vitro, visualized by electron microscopy (left). A fluorescence micrograph of actively growing *Streptomyces* hyphae, in which FilP cytoskeleton forms an apical gradient (red) just behind the zone occupied by the determinant of polar growth, DivIVA (green) (top right). A model illustrating the concept that recruitment of an additional stress-bearing cytoskeleton to the area of intrinsic cell wall weakness is directly coupled to the mechanism driving polar growth. 1. Tip elongation and new cell wall insertion driven by DivIVA (green). Cell wall is compliant (thinner black outline). 2. Zone of DivIVA-FilP (red) interaction leading to recruitment of FilP to the growing tip. 3. Area of decreasing density of the FilP cytoskeleton and increasing resilience of the cell wall. 4. A new DivIVA focus recruits a new FilP gradient in branch formation.

the highest concentration of FilP present just next to the zone occupied by the DivIVA polarisome at the very tip (Fig. 1). Upon cessation of growth the apical gradients disappear and are remodeled into a FilP cytoskeleton that is spread evenly throughout the mycelium, indicating that FilP assembly and/or disassembly is a dynamic process and is controlled by specific cellular mechanisms. We demonstrate with in vitro, in vivo, and in situ methods that there is a spatially restricted interaction between the DivIVA polarisome at the very tip of the hyphae and the adjacent FilP cytoskeleton, which somehow stimulates accumulation of FilP at the interface of these two zones. However, for recruitment of FilP assembly and formation of apical gradients other *Streptomyces*-specific factors are needed, since we could not reconstitute these phenomena in a heterologous system by introducing FilP and DivIVA into *Escherichia coli* cells. The

role of the third coiled coil protein Scy in this process is so far unclear, because FilP exhibited normal localization in a *scy* null mutant strain. Why is the formation of spatial gradients of FilP induced during active polar growth? Our present data support the interpretation that a direct coupling of the DivIVA-driven process of tip extension to recruitment of the FilP cytoskeleton would ensure that each growing tip with a compliant cell wall receives an additional stress-bearing structure internally. However, the molecular mechanisms and the biological significance of the unexpectedly dynamic behavior of the intrinsically non-dynamic FilP assemblies need further research. Our results have shed some new light on principles how cell architecture is created by organized formation of large protein assemblies and illustrate the importance and complexity of this process also in bacterial cells.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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