

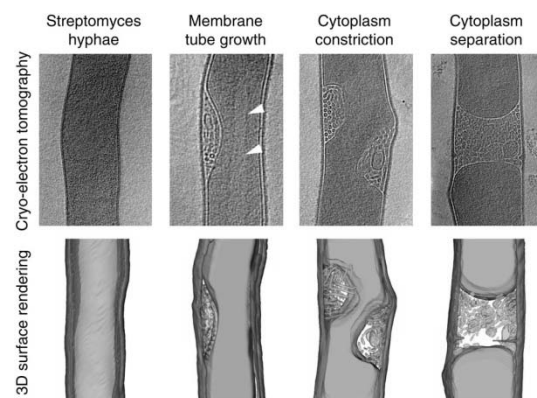
Newly identified cross-membranes orchestrate compartmentalization in substrate hyphae of *Streptomyces*

Joost Willemse^{1*}, K. Celler¹, R. I. Koning², A.J. Koster², Gilles P. van Wezel¹
¹Department of Molecular Biotechnology, IBL, Leiden University, PO Box 9505, 2300 AB Leiden, The Netherlands
²Department of MCB, LUMC, PO Box 9600, 2300 RC Leiden, The Netherlands.
jwillemse@biology.leidenuniv.nl

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Introduction. *Streptomyces* are filamentous soil-dwelling saprophytes, well-known for their complex multicellular life style and their ability to produce a plethora of natural products, including antibiotics, anticancer agents and immunosuppressants. After spore germination, hyphae grow by tip extension and branching to form a syncytial vegetative mycelium, occasionally separated by peptidoglycan-based septa or cross-walls. Cell division is not essential for growth in *Streptomyces* as shown by the fact that the gene for the cell-division scaffold protein FtsZ can be deleted. Surprisingly, the *ftsZ*-deletion mutant can be propagated from fragments obtained by mechanical maceration of mycelia, which suggests the presence of additional compartmentalization of the hyphae, besides that by peptidoglycan-based cross-walls. We have now demonstrated the presence of a membrane system in the substrate hyphae of *Streptomyces*, whereby so-called cross-membranes form protein-impermeable barriers, independent of cross-walls or the cell division machinery. Similar cross-membranes were discovered in very young vegetative hyphae.

Results. Membrane phospholipids were studied with fluorescence light microscopy. This revealed that 75% of membrane structures are independent of peptidoglycan cross-walls. These assemblies can be up to several micrometres in length, are capable of spanning the width of the vegetative hyphae. We then applied cryo-correlative light and electron microscopy, combining cryo-light microscopy with cryo-electron tomography, to study the fine structural detail of the membrane assemblies. These assemblies within hyphae revealed the formation of bundles of tube-like structures varying from small assemblies along



the cell wall to large structures completely delimiting the hyphae. Tomograms show that the membrane assemblies form in the space between the cytoplasmic membrane and cell wall. Large hyphae-spanning membrane assemblies were devoid of DNA, and served as a starting point for septum synthesis (Figure, middle and bottom images). To confirm that membranes formed independent of septa, we also studied the *S. coelicolor ftsZ* deletion strain, which grows without forming septa. Membrane formations in this strain were indistinguishable and as frequent as wild type membrane formation.

To assess if the large cross-membrane structures can form cytosol-impermeable barriers, we applied fluorescence recovery after photobleaching (FRAP), whereby GFP molecules were bleached in mycelial compartments, to see if GFP could cross the membrane barriers to restore fluorescence. These experiments demonstrated that some 30% of the cross-membranes are cytosol-impermeable. Importantly, the cross-membranes form DNA-free zones in the hyphae, offering a solution to the long-standing problem of how cell-wall remodelling and cell division can take place without DNA damage in the multinucleoid hyphae.

References.

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