Project 10: Phosphate-nutrient control of the fungal cell cycle

(Supervising Investigator: apl. Prof. Dr. Ursula Fleig)

Infections by human fungal pathogens are a serious albeit often underestimated danger to human health. An estimated one million people die of fungal infections ever year, with global warming likely to significantly increase the number of pathogenic fungal species. For successful infection and propagation, pathogenic fungi need to sense, acquire and store micro/macronutrients such as phosphate. Virulence of the medically important fungal pathogens *Candida albicans* and *Cryptococcus neoformans* depends on balanced phosphate homeostasis. A pathway named PHO which is responsible for the sensing, the acquisition of phosphate and the synthesis of inorganic polyphosphate (polyP) which comprises a linear chain of up to hundreds of inorganic phosphate residues, is conserved to various degrees in all fungi.

The cellular functions of polyP have been severely understudied, as it was assumed for many years that polyP is simply a phosphate storage molecule. However, polyP is present from bacteria to man and is involved in numerous essential cellular processes ranging from virulence in bacteria and protozoan parasites to blood clotting and inflammation in human cells. In fungi, polyP regulates metal homeostasis, progression through the cell cycle and morphogenesis which is a virulence trait for pathogenic fungi. Furthermore, cell wall-associated polyP structures have been identified in *C. neoformans* which are required for capsule architecture and play a role in blood clotting of the infected host. Intriguingly polyP is involved in the post-translational modification of proteins as it can associate with specific lysine residues in a non-enzymatic manner. PolyP-mediated protein modification modulates biological processes in a manner dependent on the abundance of polyP. But how is polyP generation in the cell regulated? We have found recently that generation of polyP in the fission yeast is regulated by a unique subgroup of inositol-based signaling molecules: the highly phosphorylated inositol pyrophosphates (PP-InsPs). These are omnipresent in eukaryotes and control numerous biological processes. Specific PP-InsPs are synthesized by the highly conserved Vip1/PPIP5K enzyme family and we have shown that in the fission yeast intracellular polyP levels can be up- and down-regulated by Vip1-made PP-InsPs. How this regulation is achieved on a molecular level is unknown. Thus, the main objectives of this MOI IV project are to understand (i) how Vip1-made PP-InsPs regulate polyP-generation using mutational and genetic tools; and (ii) if this type of regulation is conserved in other fungi including pathogenic ones such as *C. albicans* and *U. maydis*; (iii) how are biological processes affected by the up and down-regulation of polyP? As we have recently found that mitotic chromosome segregation is controlled by both polyP and PP-InsPs, we will use extensive genetic and microscopic analysis to determine how chromosome segregation fidelity is affected by polyP. In particular, the occurrence of aneuploidy (loss/gain of entire chromosomes) will be monitored carefully via flow cytometry. Genome instability resulting in aneuploidy is a hallmark of pathogenic fungi to survive unfavorable conditions such as the presence of anti-fungal drugs. Thus, our analysis will uncover a possible connection between phosphate homeostasis, genome stability and resistance to anti-fungal drugs.