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# The *Aspergillus niger* RmsA protein

## A node in a genetic network?

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**Many cells and organisms go through polarized growth phases during their life. Cell polarization is achieved by local accumulation of signaling molecules which guide the cytoskeleton and vesicular trafficking to specific parts of the cell and thus ensure polarity establishment and maintenance. Polarization of signaling molecules is also fundamental for the lifestyle of filamentous fungi such as *Aspergillus niger* and essential for their morphogenesis, development and survival under environmental stress conditions. Considerable advances in our understanding on the protagonists and processes mediating polarized growth in filamentous fungi have been made over the past years. However, how the interplay of different signaling pathways is coordinated has yet to be determined. We found that the *A. niger* RmsA protein is central for the polarization of actin at the hyphal tip but also of vital importance for the metabolism, viability and stress resistance of *A. niger*. This suggests that RmsA could occupy an important position in the global network of pathways that balance growth, morphogenesis and survival of *A. niger*.**

**Key words:** *Aspergillus niger*, morphogenesis, polar growth, TOR signaling, RmsA, Sin1, Avo1, environmental stress, metabolism

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*Aspergillus niger* is a eukaryotic microorganism belonging to the group of filamentous fungi, which are naturally capable of secreting large amounts of proteins and metabolites. Therefore, *A. niger* and other filamentous fungi are exploited in biotechnology as cell factories for the commercial production of chemicals, pharmaceuticals and enzymes.[1] Growth of filamentous fungi is in general characterised by the formation of highly polarised hyphae which send out branches from apical or lateral regions thereby forming a highly dense cellular meshwork, the mycelium. Long-distance transport of RNA, proteins, vesicles and organelles along cytoskeletal tracks ensures that new cell material is exclusively added to the growth zone at the hyphal tip. Thus, transport along microtubules and actin is essential to maintain the morphology of filamentous fungi and has also been shown to be a prerequisite for polarised protein secretion.[2-5]

Recently, we demonstrated that the RmsA protein of *A. niger* plays a key role in the maintenance of hyphal polarity.[6] We obtained evidence that RmsA is a functional equivalent of the Avo1p/Sin1 protein, which as a component of the highly conserved protein complex TORC2 is essential for actin polarisation and hence determination of cell polarity in *Saccharomyces cerevisiae*, Dictyostelium and mammals.[7] By using the strain *ramosa-1*, which carries a temperature-sensitive allele of RmsA ($RmsA^{Y447N}$), we could show that disturbed RmsA function causes actin depolarisation and thereby loss of hyphal polarity. *A. niger* cells, however, are very dynamic in counteracting this event—if the integrity of the polar axis is disturbed, two new polarity axis become established resulting in apically branched tips. The transcriptomic fingerprint of apically branched hyphae suggested that the stage for actin repolarisation and formation of two new branches is set by increased activity of different signaling pathways such as TORC2 signaling, phospholipid signaling, cell wall integrity signaling and calcium signaling.[6]

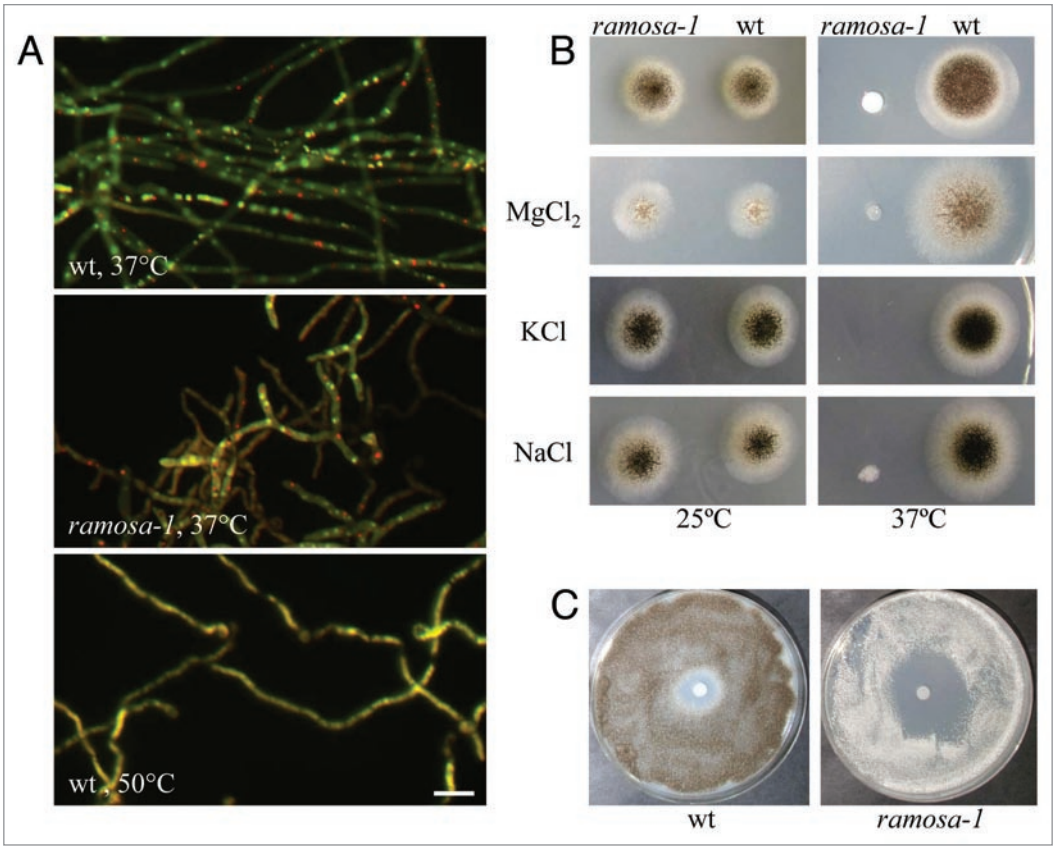


**Figure 1.** Phenotypic analyses of *ramosa-1* and the wild type strain T312. (A) Spores of both strains were allowed to germinate for 17 h at 25°C, after which the temperature was set to 37°C for 4 h. Hyphae were stained with 2 µM FUN-1 and subjected to microscopy using FITC and dsRed filters. FUN-1 stains nucleic acids yielding to a diffuse green cytoplasmic fluorescence. Only metabolic active cells transport FUN-1 to the vacuole and convert it to red fluorescent CIVS.[9] A control staining is shown in the lower panel where the wt strain was heat-inactivated for 4 h at 50°C. Bar, 10 µm. (B) $10^4$ spores of both strains were point-inoculated on minimal agar plates containing 500 mM of different salts. Control plates did not contain any additional supplements (upper). Plates were incubated at 25°C for 4 days or at 37°C for 3 days. (C) 5 x $10^5$ spores of both strains were evenly spread on minimal agar plates. A Whatman paper disc was wetted with 5 µl of a 30% $H_2O_2$ solution and the plates were incubated at 37°C for 3 days.

Surprisingly, the transcriptomic fingerprint obtained uncovered that inactivation of RmsA also results in considerable changes in the transcription of genes predicted to function in ion homeostasis, energy metabolism and protein fate,[6] which led us to speculate that the function of RmsA is not only important for actin polarisation but also to ensure redox balance and metabolic integrity of *A. niger*. Supportive for this assumption is the observation that prolonged cultivation of *ramosa-1* at restrictive temperature slows down its growth rate, prevents asexual development and that deletion of the *rmsA* gene is lethal for *A. niger*.[6,8] To further substantiate the hypothesis that RmsA is crucial for the metabolism of *A. niger*, we cultivated *ramosa-1* and the respective wild type strain T312 at restrictive temperature (37°C) and subjected it to staining with the vital dye FUN-1. Living and metabolically active cells metabolise FUN-1 to red fluorescent intravacuolar structures known as CIVS. CIVS formation is thus a reflection of the metabolic activity of a cell and depends on intact cellular ATP synthesis and on functional vesicle cycling between the prevacuolar compartment and the Golgi.[9,10] As shown in **Figure 1A**, loss of RmsA activity results in hyphae with reduced CIVS formation, suggesting that RmsA is of vital importance for the energy status of *A. niger* hyphae. As, moreover, exposure of *ramosa-1* to different salts (NaCl, KCl, $MgCl_2$, LiCl), oxidative agents ($H_2O_2$) and cell wall stress inducing compounds (Calcofluor white) results in a hypersensitive phenotype (**Fig. 1B and C** and data not shown), indicates that RmsA also safeguards *A. niger* against environmental stress.

In summary, these studies highlight multiple cellular roles of RmsA. Not only spatial growth and thereby morphogenesis of *A. niger* is dependent on RmsA, also temporal growth, viability and survival of *A. niger* require an intact RmsA protein. But how does RmsA achieve so many functions? In all organisms studied so far, the homologous Avo1p/Sin1 proteins have been identified as a stabilising component of the TORC2 complex. However, the function of TORC2 is not conserved, e.g., in the *S. cerevisiae*, TORC2 is crucial for actin polarisation but not involved in sexual reproduction; in the fission yeast *Schizzosaccharomyces pombe*, TORC2 is required for sexual development but dispensable for proper actin polarisation.[7] In mammalian cells, TORC2 seems to exert its most complex function—it is important for many processes including actin

polarisation, salt balance, metabolism and survival.[7] In addition to a role in TORC2 signaling, Avo1p/Sin1 proteins have been reported to interact with other proteins, e.g., with stress-activated protein kinases in *S. pombe* and humans,[11,12] with *S. cerevisiae* and human Ras GTPases,[13,14] with the apoptosis-related human transcription factor ATF-2,[11] and with the human anti-apoptotic RNA binding protein PCBP2,[15] suggesting that Avo1p/Sin1 proteins orchestrate organism-specifically many cellular processes. With our recent genetic, transcriptomic and phenotypic studies on *ramosa-1* and the RmsA protein, we got only a first glimpse of the role(s) of RmsA for *A. niger*. Future studies will disclose on how RmsA is embedded in different signaling pathways and participates in driving growth, morphology and development of *A. niger*.


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