Abstract

ABSTRACT

Chromosome segregation is a critical process in eukaryotic cell development, which is brought about by the multi-protein kinetochore complex along spindle microtubules (MTs). The outer kinetochore DASH complex proteins maintain spindle structure and chromosome-MT association to bring about high-fidelity chromosome segregation during anaphase. While the role and sub-cellular localisation of DASH complex proteins varies among yeasts, their role in filamentous fungi had not been investigated in detail thus far. Pathogenic filamentous fungi cycle through diverse developmental stages associated with changes in cell polarity. Pathogenic development in the rice blast fungus *Magnaporthe oryzae* requires an isotropic distension of the polarised germ tube to form the appressorium (infection structure). These developmental transitions are precisely regulated progression of nuclear segregation and migration. Vegetative growth, on the other hand, proceeds by hyphal extension and lateral branching. Here, I studied the dynamics of GFP-tagged middle (Mis12) and outer (Dam1 and Ask1) kinetochore proteins and their role in *M. oryzae* vegetative and pathogenic development. The Mis12 foci constitutively associated with the nucleus throughout the cell cycle, clustered into a single patch during interphase, and de-clustered during mitosis; however, Dam1 and Ask1 showed a mitosis-specific nuclear association. Absence of Dam1 function, (*dam1*Δ) led to atypical mitotic progression with sluggish nuclear movement and difficulty in segregation. Dam1 was involved in the three consecutive rounds of mitosis required for development of the three celled conidia such that loss of Dam1 led to defects in conidial development and impaired appressorium formation. The *dam1*Δ mutant also showed reduced hyphal growth and morphological defects in vegetative hyphae, indicating altered cell polarity during filamentous growth in *M. oryzae*. The *ask1*Δ strain showed all the defects seen in the *dam1*Δ mutant. Interestingly, both Dam1 and Ask1

localised to the hyphal tip during vegetative growth and to the germ tube tip during pathogenic development, and oscillated back and forth from the tip. Thus, the findings indicate that *Magnaporthe* Dam1 and Ask1, in addition to their function at mitotic spindle, likely perform a broader role in regulating microtubule dynamics and/or maintenance of cell polarity even during interphase. Aberrant appressorial (infection structure) development and impaired host penetration by the *dam1*Δ mutant suggest that the fungus-specific DASH complex proteins Dam1 and Ask1 could constitute attractive targets for development of novel antifungal strategies.