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# A novel mechanism of intracellular protein localization in filamentous fungi

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## Research aims

Fungal cell walls are dynamic structures and crucial for fungal morphogenesis. Chitin is a homopolymer of *N*-acetylglucosamine and one of the major cell wall components in many filamentous fungi. To investigate the mechanism of fungal morphogenesis and hyphal tip growth, we have cloned six chitin synthase-encoding genes from the ascomycete filamentous fungi, *Aspergillus nidulans* and analyzed their functions. In the course of this study, we have found two genes, *csmA* and *csmB*, encoding chitin synthases that have very unique structures. CsmA and CsmB, the gene products of *csmA* and *csmB*, respectively, consist of a chitin synthase domain, CSD, at their C-termini and a myosin motor-like domain, MMD, at their N-termini (Fig. 1) (1, 2). Myosins are known to be motor proteins that move on actin filaments. There are no orthologs of *csmA* and *csmB* in the genomes of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Candida albicans*, but they are ubiquitous in the genomes of filamentous fungi and dimorphic yeasts of which cell wall chitin contents are relatively high.

*csmA* deletion (ΔA) mutant and *csmB* deletion (ΔB) mutant showed growth delay under low osmotic conditions and formed swollen hyphae, called balloons (2, 3). We showed that both the MMD and the CSD of CsmA were required for its function and that CsmA tagged with HA at its C-terminus localized primarily at hyphal tips and forming septa. We also showed that a direct interaction between the MMD and actin was essential for the proper localization of CsmA (4, 5). These results suggest that CsmA localizes at hyphal tips and forming septa by a novel protein sorting mechanism depending on its MMD. To investigate the mechanism in detail, we analyzed function of the MMD of CsmB and the interchangeability of the MMDs between CsmA and CsmB.

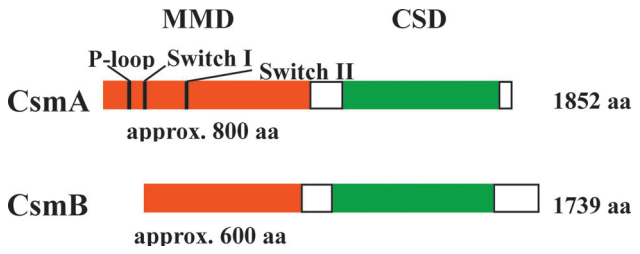


**Fig. 1.** Strucrures of CsmA and CsmB of *Aspergillus nidulans*.

## Methods

We constructed ΔB mutants that produced a truncated form of CsmB lacking its MMD under the control of the native *csmB* promoter and *alcA* promoter (*alcA*(p)), and designated them ΔMB strain and *alcA*(p)-ΔMB strain, respectively. The *alcA*(p) expressed strongly on the medium containing ethanol or threonine and fructose as carbon sources. We also constructed a strain that produced the truncated CsmB tagged with FLAG (ΔMB-FLAG protein) and determined its intrahyphal localization by indirect immunofluorescence. We made chimera genes encoding the MMD of CsmA and the CSD of CsmB (MACB) or the MMD of CsmB and the CSD of CsmA (MBCA). We constructed strains in which the gene encoding MACB fusion protein was expressed under the control of *csmA* promoter at the *argB* loci of the ΔA mutant and the ΔB mutant and designated them MACBΔA and MACBΔB strains, respectively. Similarly, we constructed MBCAΔA and MBCAΔB strains in which the gene encoding MBCA fusion protein was expressed under the control of *csmA* promoter at the *argB* loci of the ΔA mutant and the ΔB mutant, respectively. We also constructed CsmAΔA and CsmBΔB strains that expressed the wild-type *csmA* and *csmB* at the *argB* loci of the ΔA mutant and the ΔB mutant, respectively. We constructed a strain that produced FLAG-tagged MACB protein and determined its intrahyphal localization by indirect immunofluorescence.

## Results

*1. Function of the MMD of CsmB*

The MMD of CsmA contains the consensus sequences of myosins, such as P-loop, Switch I, and Switch II, which are essential for the function of myosins. In contrast, those consensus sequences are not found in that of CsmB. To evaluate the importance of the MMD in the function of CsmB, we constructed the ΔMB strain and the *alcA*(p)-ΔMB strain (see Methods section). The ΔMB strain grew slowly under low osmotic conditions and formed balloons, whereas the *alcA*(p)-ΔMB strain grew as fast as the wild-type strain and formed balloons less frequently than the ΔMB strain under the *alcA*(p)-inducing conditions. These results suggest that the function of CsmB partially depends on its MMD. This is in contrast to the case of CsmA in which the function of CsmA was completely dependent on its MMD (3, 5). To determine the localization of the N-terminally truncated

CsmB in the hyphae, we analyzed the intracellular distribution of ΔMB-FLAG protein. The wild-type CsmB tagged with FLAG localized primarily at hyphal tips and forming septa, whereas ΔMB-FLAG protein was distributed throughout hyphae. This indicates that the MMD of CsmB is important to its proper localization.

*2. Interchangeability of the MMDs between CsmA and CsmB*

Although deletion of *csmA* or *csmB* caused similar defects as described above, overexpression of *csmA* did not suppress the defects of the ΔB mutant and overexpression of *csmB* did not suppress those of the ΔA mutant (2, and data not shown). These results suggest that CsmA and CsmB have some different functions in growth and morphogenesis. The MMD of CsmB has only 21% amino acid identity with that of CsmA. To investigate the functional difference in the MMDs between CsmA and CsmB, we constructed the MACBΔA, MBCAΔA, MACBΔB, MBCAΔB, CsmΔA, and CsmBΔB strains (see Methods section). The CsmAΔA and CsmBΔB strains showed the same phenotype as the wild-type strain. The MACBΔA strain and the MBCAΔB strain exhibited the phenotypes similar to the ΔA mutant and the ΔB mutant, respectively, suggesting that their CSDs are not interchangeable. The MACBΔB strains grew as well as the wild-type strain and did not form balloons. Although the growth rate of the MBCAΔA strain was nearly the same as the wild-type strain, the frequency of balloon formation was not ameliorated to the level of the wild-type strain. We showed that MACB tagged with FLAG localized mainly at hyphal tips in the ΔB mutant.

## Conclusion

We demonstrated that the MMD of CsmB was important to both its proper localization and full function, and that the MMD functions of CsmA and CsmB were partially interchangeable. Since FLAG-tagged MACB localized at hyphal tips, the MMD of CsmA is suggested to have a function that is able to target proteins to hyphal tips.

## References


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