FUNCTIONAL CHARACTERIZATION OF AzoA, A NOVEL MOLECULAR DETERMINANT OF SENSITIVITY TO AZOLES IN *Aspergillus nidulans*

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Azoles constitute an important class of antifungal agents widely used in both agriculture and clinical medicine. Their efficacy is often limited by the overexpression of membrane-efflux pumps of the ABC (ATP-binding cassette) and MF (major facilitator) families, which lower intracellular drug concentration and results in multidrug resistance (MDR). Here we describe the cloning and characterization of *AzoA*, which is a novel molecular determinant of sensitivity to azoles in *A. nidulans*. This gene was identified from an azole-hypersensitive mutant of *A. nidulans,* obtained by insertion mutagenesis followed by plasmid rescue. Blast analysis of AzoA revealed similarity to hypothetical proteins with unknown function from yeasts and other fungi. Northern analysis revealed increased transcript levels of *azoA* upon treatment of fungal germlings with azoles. Gene-replacement mutants of *azoA*, also displayed increased sensitivity to azoles, confirming its functional role. Interestingly, transcript levels of the ABC transporter *atrG* , a previously characterized efflux pump involved in protection of *A. nidulans* against azoles is higher in Δ*azoA* mutants. Additionally, Δ*azoA* mutants displayed lower accumulation of [^{14}C]fenarimol, as compared to a control strain. This situation mimics MDR, *e.g* reduced intracellular accumulation due to overexpression of an efflux pump (AtrG). Nevertheless, the resulting phenotype observed for Δ*azoA* mutants is increased sensitivity to azoles. These results suggest a major role for *azoA* on fungal sensitivity to azoles.