Investigating the N-terminal Deletion in Mitochondrial Voltage-Dependent Anion Channels in *Neurospora crassa*

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Abstract

The mitochondrial outer membrane contains a class of porin – voltage dependent anion channels (VDAC) – that facilitate diffusion of small hydrophilic molecules across the outer membrane. VDAC proteins share a prominent property in which they display voltage-dependent conformational changes involved in gating when reconstituted into planar lipid bilayers. The N-terminus of VDAC is the focus of this study, due to conflicting structural and electrophysiological results presented by Geula et al. 2012 (Biochem J. 444: 475) and Teijido et al. 2012 (J. Biol. Chem. 287: 11437) with respect to the nature of the N-terminus' role in the gating mechanism. To verify the N-terminus' function and mobility in the VDAC gating mechanism, two *Neurospora crassa* strains (ΔN2a, WS125.5) were generated to contain identical VDAC N-terminal deletions of amino acids 2-12. However, the two strains exhibited incongruent phenotypes. Thus, the objective of this study was to determine the basis for the phenotypic differences seen in ΔN2a and WS125.5. Qualitative and quantitative phenotypic analyses were conducted using race tubes. In comparing growth rates of both strains to each other and to the growth rate of the wild-type, ΔN2a displayed a growth rate at a midpoint between wild-type and WS125.5 growth rates, while WS125.5 displayed significantly reduced growth rates compared to the wild-type and ΔN2a. Genomic DNA was extracted from both variants and sequenced using BigDye Termination 3.1, revealing the correct N-terminal deletion in ΔN2a. Sequences from WS125.5 failed to provide information regarding the N-terminal deletion in the strain due to incorrect annealing of primers. However, in amplifying the VDAC porin gene from the genomic DNA of WS125.5, the result was a double banding pattern – a band that is characteristic for wild-type and a band characteristic for the strain. This indicates that WS125.5 is a heterokaryon; however, it does not exhibit the wild type growth phenotype, suggesting that the N-terminal porin deletion is a dominant mutation. Further sequencing is currently being conducted to confirm the N-terminal deletion in WS125.5 and to examine the promoter and 3' UTR regions of both strains.

Keywords: VDAC; N-terminus; *Neurospora crassa*