Effect of Amino Acid Substitutions in the GerAA Protein on the Function of the Alanine-Responsive Germinant Receptor of *Bacillus subtilis* Spores

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Received 22 November 2010/ Accepted 22 February 2011

Spores of *Bacillus subtilis* require the GerAA, GerAB, and GerAC receptor proteins for L-alanine-induced germination. Mutations in *gerAA*, both random and site directed, result in phenotypes that identify amino acid residues important for receptor function in broad terms. They highlight the functional importance of two regions in the central, integral membrane domain of GerAA. A P324S substitution in the first residue of a conserved PFPP motif results in a 10-fold increase in a spore's sensitivity to alanine; a P326S change results in the release of phase-dark spores, in which the receptor may be in an "activated" or "quasigerminated" state. Substitutions in residues 398 to 400, in a short loop between the last two likely membrane-spanning helices of this central domain, all affect the germination response, with the G398S substitution causing a temperature-sensitive defect. In others, there are wider effects on the receptor: if alanine is substituted for conserved residue N146, H304, or E330, a severe defect in L-alanine germination results. This correlates with the absence of GerAC, suggesting that the assembly or stability of the entire receptor complex has been compromised by the defect in GerAA. In contrast, severely germination-defective mutants such as E129K, L373F, S400F, and M409N mutants retain GerAC at normal levels, suggesting more local and specific effects on the function of GerAA itself. Further interpretation will depend on progress in structural analysis of the receptor proteins.

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