A new multidrug ABC transporter from *Bacillus subtilis*: the heterodimer YheI/YheH

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YheI/YheH is a putative heterodimeric ABC transporter from *B. subtilis*, the model organism for Gram-positive bacteria, which contains more than 70 members of the ABC transporter family. YheI and YheH are encoded by two different genes, *yheI* and *yheH* and each monomer contains a membrane spanning domain fused to a nucleotide-binding domain. *yheI* and *yheH* have been overexpressed, either separately or in tandem under the same promoter, in the BL21(DE3) *Escherichia coli* strain and Inverted Membrane Vesicles (IMVs) enriched in the proteins have been prepared for each construction. Transport experiments showed that the presence of both subunits, YheH plus YheI, is required to measure a transport of different drugs, namely the Hoechst 33342, mitoxantrone and doxorubicin. The heterodimeric transporter YheI/YheH can thus be assigned as a new multidrug (MDR) ABC transporter. YheI/YheH does not seem to be capable to transport other fluorescent drugs such as daunorubicin or 7-aminoactinomycin D, typical substrates of ABC multidrug transporters related to the eukaryotic P-glycoprotein.

Mutational analysis of conserved residues in the nucleotide binding domains (NBD) revealed that the two subunits are not equivalent for ATP hydrolysis and drug transport: all mutations affecting the NBD of YheH fully abolished transport of the fluorescent compounds, while mutants in the NBD of YheI still conserved a certain degree of transport activity. These results suggest the existence of one canonical and one degenerate ATP binding site in the heterodimer.

Drug susceptibility assays performed with KAM32 *E.coli* cells expressing YheI and YheH wild-type and mutant proteins revealed no significant susceptibility differences to chloramphenicol, doxorubicine, ethidium bromide, kanamycin or spectinomycin. The same results were obtained with *B. subtilis* wild-type and YheI'/YheH' null strains. YheI/YheH might thus not be involved in multidrug resistance *in vivo*. Instead, we hypothesize that the natural substrate of YheI/YheH may be YheJ, a 53 amino acid protein similar to antimicrobial peptides (bacteriocins). The *yheJ* gene encoding for YheJ, is located 120 base pairs upstream of *yheI* in *B. subtilis* 168 chromosome and is probably co-transcribed with the transporter. Purification of YheJ will help to clarify its putative role as a bacteriocin and as a physiological substrate for YheI/YheH.