Comparison of the Bacterial *HelA* Protein to the F508 Region of the Cystic Fibrosis Transmembrane Regulator

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The *HelA* protein of *Rhodobacter capsulatus* is the ATP-binding-cassette subunit of an exporter complex required for cytochrome *c* biogenesis. By primary sequence comparisons the F88 residue of *HelA* is similar to the F508 residue of the cystic fibrosis transmembrane regulator (CFTR) protein. Previous studies have established that CFTR F508Δ or F508R proteins are defective but F508C is functional. Our results demonstrate that the *HelA* F88 mutants functionally mimic the phenotypes of known CFTR F508 mutants. The phenotypes of additional *HelA* mutants and the in vivo steady-state levels of these proteins are also reported.

In *Rhodobacter capsulatus* cytochrome *c* biogenesis requires the four-subunit ABC (ATP-binding cassette) transporter encoded by the *helABCD* genes (3, 8, 11). This complex is of the export family (7, 8) and is proposed to deliver heme to the periplasm for cytochrome *c* assembly (e.g., see references 3 and 8). The *HelA* protein contains the nucleotide binding motifs (motifs A and B in Fig. 1) and a serine residue (S119) that are diagnostic for this family of transporters (1, 9). Many ABC transporters also appear to contain an intervening motif with the sequence E(D)NL(I,V) as a signature that separates motif A and the diagnostic serine residue (see box in Fig. 1). For select transporters this motif is followed by a phenylalanine that is equivalent to the F508 residue in the N terminus of the cystic fibrosis transmembrane regulator (CFTR). The CFTR F508 residue is important because 70% of the cases of cystic fibrosis (CF) are due to deletion of this residue (5, 10). All *HelA* proteins, from seven different bacterial genera (e.g., see references 3, 14, and 16), contain the ENL signature followed by an F88 residue (i.e., ENLXF), analogous to the CFTR F508 residue. Although a chimeric STE6:CFTR gene in *Saccharomyces cerevisiae* has been used as a microbial model for F508-equivalent defects in the CFTR protein (18), surprisingly, no bacterial models for the F508 defect have been established. This is in contrast to many other residues required for function which have been previously analyzed and compared (17; references cited in reference 1). A report on the maltose ABC transporter showed that the F508-equivalent residue, F98 in the MalK protein (Fig. 1), retained function when the F98 residue was deleted or changed to tyrosine or leucine (15), indicating that this residue is not essential for MalK activity. We describe here results of genetic experiments on the *helA* gene product, including the F88 residue, to derive clues to structure-function relationships in this region of the protein.

To mutagenize the *helA* gene, a pUC118 plasmid (19) containing the *helAB* and part of the *helC* genes on an *EcoRI* fragment was used as the template for site-directed mutagenesis by the method of Kunkel et al. (12) as previously described (13). The following oligonucleotides were used for mutagenesis: 5'-AAAACCTGCAGTGGGCGGCGATCCATGCGA-3' for F88Δ, 5'-GCGCGAAAACCTCCAGNNCTGGGCGGC

GAT-3' for F88X, and 5'-GCCTTANNGCGGGGCAAAAGCGTCGGCTTG-3' for S119X. The F88X oligonucleotide removes a *PstI* site (underlined) upstream of the F88 codon (without altering the leucine residue). The mutations were sequenced, and the *EcoRI* fragment was subcloned into the pUCA9 plasmid, a broad-host-range vector. These plasmids were then conjugated into either the *R. capsulatus* Δ*helAB* or Δ*helA* strain to test for function (see below). The mutants derived from these experiments and comparisons to analogous residues in the MalK and CFTR proteins are shown in Fig. 1.

The ability of plasmid-borne *helA* genes to complement an *R. capsulatus* chromosomal *helA* deletion mutant was used as the test for *HelA* function. Complementation is defined as the ability of cells to grow under anaerobic photosynthetic conditions (Fig. 2), for which *c*-type cytochromes are required (11). Growth of *R. capsulatus* was in RCV minimal medium (2) as previously described (11).

The *HelA* S119 residue, when changed to lysine, methionine, or arginine, resulted in a defective *HelA* protein. Since this residue is conserved in all ABC transporters this result is not surprising. The *HelA* F88Δ mutant, analogous to the F508Δ mutant of CFTR, also exhibited a null phenotype (Fig. 2). To test the effect of substitutions, the *HelA* F88 residue was converted to 11 of 19 possible amino acids (Fig. 1). The *HelA* proteins with these changes were each functional, with two exceptions. When the amino acid residue histidine or arginine replaces *HelA* F88, complete loss of function was observed. This result suggests that a positive charge in this region is detrimental to function or folding of the *HelA* protein. We reasoned that a simple explanation might link the effect of deletion and positive charge substitution of the F88 residue. That is, a deletion could divert an adjacent basic residue into a misfolding pathway by ionic interaction with a negatively charged residue elsewhere in the protein. A candidate for such an adjacent basic residue is located in both the CFTR (K503) and *HelA* (R83) proteins but not the MalK protein (Fig. 1). To test this idea, we converted the *HelA* R83 residue to glycine in the *HelA* F88Δ background. However, this double mutant was still defective for function, while a single *HelA* R83G mutant was functional. Still, we cannot rule out this explanation completely because of the presence of other basic residues that are closely linked (e.g., *HelA* K77).

It has been demonstrated that the CFTR F508Δ and a site-directed mutant, CFTR F508R, are defective in intracellular transport and processing, and thus folding (4). To begin to understand the biochemical basis for the *HelA* defects de-

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FIG. 1. Amino acid sequences of the CFTR, MalK, and HeLa proteins and phenotypes associated with CF mutations at the F508 and S519 (and analogous HeLa) residues. For HeLa studies, single site-directed mutations were generated on a plasmid containing the *heLa* gene and tested for the ability to complement a Δ *heLa* strain (see Fig. 2 for complementation screen). Motif A and motif B represent the nucleotide binding folds. The CFTR mutation F508R (marked with an asterisk) was found to be defective in COS-7 cells (4) and was not associated with a CF patient. The CFTR F508Δ was from many CF patients and in vitro analyses, and F508C was found in a benign case (20).

scribed here, we measured the steady-state levels of the HeLa wild-type and mutant proteins in the cell (Fig. 3). Extracts of *R. capsulatus* Δ *heLa* strains carrying the various *heLa* alleles were immunoblotted, using HeLa antisera as previously described (8). The functional HeLa F88C mutant (Fig. 3, lane 8) was at steady-state levels equal to those of the wild-type (lane 9). The S119 (lanes 3 to 5) and the F88R and -H (lanes 6 and 7, respectively) substitutions resulted in approximately 50% of the wild-type levels of the HeLa antigen. In contrast, the HeLa F88Δ mutant (lane 2) contained barely detectable antigen, at least 10-fold-lower levels than each of the other mutant HeLa proteins.

We conclude, from primary structural and now functional analyses, that the HeLa F88 residue shares similarities with the

CFTR F508 residue. It is particularly intriguing that the HeLa F88R and the CFTR F508R mutants are both defective, whereas with other substitutions (e.g., cysteine) function is retained. Nevertheless, the basis for the HeLa F88Δ and F88R defects may be different, since the deletion results in an unstable protein, possibly a result of misfolding. We previously demonstrated that when either of the membrane subunits of the complex, HelB or HelC, are absent, HeLa is unstable and is rapidly degraded (8). Absence of the HeLa polypeptide would most likely indicate that it is either naturally unfolded and degraded or that it no longer interacts with the membrane components and is then degraded. Consistent with the aberrant folding for CFTR F508Δ, CFTR F508Δ proteins are temperature sensitive, with more functional protein reaching the

FIG. 2. Growth of *R. capsulatus* wild-type and *heLa* mutant strains on RCV medium. Plates were incubated for 72 h under aerobic and anaerobic, photosynthetic (PS) conditions; growth under anaerobic conditions requires *c*-type cytochromes and thus a functional HeLa protein.

FIG. 3. Western blot analysis of membrane fractions containing mutant and wild-type HelA proteins. *R. capsulatus* Δ helA strains containing plasmids carrying either the wild-type or mutated Δ helA gene. Lane 1, Δ helA alone; lane 2, F88 Δ ; lane 3, S119K; lane 4, S119M; lane 5, S119R; lane 6, F88H; lane 7, F88R; lane 8, F88C; lane 9, F88F (wild type). The same protein concentration (25 μ g) was loaded in each lane.

cytoplasmic membrane at lower temperatures in 3T3 fibroblasts (6). The HelA F88 Δ , -R, and -H proteins are not temperature sensitive, although the folding milieu and temperature effects within a bacterium are probably different than in the fibroblasts. Nevertheless, the results reported here indicate that HelA F88 Δ or F88R (or both) is a potential candidate for a bacterial model of CFTR F508 types of CF. The genetic screening and selection potential of the bacterial HelABCD system (Fig. 2) could be used to further understand the molecular basis for these defects.

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