Localised mutagenesis of the \( fts \) \( YEX \) operon: conditionally lethal missense substitutions in the FtsE cell division protein of \( Escherichia \) \( coli \) are similar to those found in the cystic fibrosis transmembrane conductance regulator protein (CFTR) of human patients

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Summary. After localised mutagenesis of the 76 min region of the \( Escherichia \) \( coli \) chromosome, we isolated a number of conditionally lethal mutants. Some of these mutants had a filamentation temperature sensitive (\( ftS \)) phenotype and were assigned to the cell division genes \( ftsE \) of \( ftsX \), whereas others were defective in the heat shock regulator gene \( rpoH \). Both missense and amber mutant alleles of these genes were produced. The missense mutant \( ftsE \) alleles were cloned and sequenced to determine whether or not the respective mutations mapped to the region of the gene encoding the putative nucleotide binding site. Surprisingly, most of these mutant FtsE proteins had missense substitutions in a different domain of the protein. This region of the FtsE protein is highly conserved in a large family of proteins involved in diverse transport processes in all living cells, from bacteria to man. One of the proteins in this large family of homologues is the human cystic fibrosis transmembrane conductance regulator (CFTR), and the FtsE substitutions were found to be in very closely linked, or identical, amino acid residues to those which are frequently altered in the CFTR of human patients. These results confirm the structural importance of this highly conserved region of FtsE and CFTR and add weight to the current structural model for the human protein.

Key words: \( Escherichia \) \( coli \) – Cell division – FtsE – Cystic fibrosis transmembrane conductance regulator

Introduction

There are several genes involved in the process of cell division in \( Escherichia \) \( coli \) and some of these lie in cluster

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The products of these \( fts \) genes have been identified in expression systems and they are cytoplasmic membrane-associated (Gill and Salmond 1987). The FtsY protein is homologous with the SR\( \alpha \) protein of eukaryotes which is important for protein secretion (Bernstein et al. 1989; Römisch et al. 1989; Gill and Salmond 1990). Recently there has been considerable interest in testing the hypothesis that FtsY may have a role in a primitive prokaryotic signal recognition particle (Bernstein et al. 1989; Römisch et al. 1989; Poritz et al. 1990; Rapaport 1991; B. Dobberstein, personal communication). Interestingly, a homologue of FtsY has very recently been identified in an archaeabacterium (Ramirez and Matheson 1991) although the relevant gene is not flanked by \( ftsE \), \( ftsX \) or \( rpoH \) homologues. The FtsY protein has the putative nucleotide binding site consensus GXXGXS/T, as has the FtsE protein (Gill et al. 1986; Gill and Salmond 1990).

FtsE is the smallest member of a very large family of proteins which share a high degree of homology. The members of the so-called ABC (ATP-binding cassette) family (Higgins et al. 1990) include several prokaryotic nucleotide binding proteins (Ames 1986; Gill et al. 1986; Higgins et al. 1986, 1990) and eukaryotic proteins such as the P-glycoprotein of multidrug resistant tumour cells and the cystic fibrosis transmembrane conductance regulator (CFTR) protein of humans (Chen et al. 1986; Gros et al. 1986; Riordan et al. 1989; Gill and Salmond
The CFTR protein has been intensively investigated and two speculative structural models have been constructed based on an adenylate kinase "skeleton" (Hyde et al. 1990; Mimura et al. 1991). In these models, nucleotide binding is important for the energetics of the transport process, as has been shown for other homologues but in different transport processes (Hobson et al. 1984; Higgins et al. 1985; Hyde et al. 1990; Mimura et al. 1991).

We therefore presumed that, because of the strong conservation of the nucleotide binding site in all of these homologues, we might find that some thermosensitive \textit{ftsE} mutants are blocked in cell division because of disruption of the structure in or around the consensus sequence. Consequently we decided to use localised mutagenesis to isolate a bank of mutants with conditionally lethal \textit{ftsE} alleles, then sequence them to determine the molecular basis of the temperature-sensitive (Ts) phenotype. At the same time we expected to generate Ts-lethal \textit{ftsY} mutants with a cell division phenotype, because of our earlier indirect evidence that \textit{ftsY} was an essential gene (Gill and Salmond 1990). Our results show a pronounced clustering of the \textit{ftsE} mutations, reminiscent of those found in the CFTR protein of human cystic fibrosis patients.

**Materials and methods**

**Bacterial strains, phages and plasmids.** The bacterial strains, phages and plasmids used are listed in Table 1. Some of these have been described before (Crickmore and Salmond 1986; Gill et al. 1986; Gill and Salmond 1990). All of the SG mutants were isolated in this study by localised mutagenesis. The pH3C deletion derivatives were provided by N. Crickmore (1987). The pH3C plasmid carries the 4.5 kb \textit{HindIII} fragment, containing ORF4, \textit{ftsY}, \textit{ftsE} and \textit{ftsX}, in pBR322 and is equivalent to pDB1 which was described previously (Gill et al. 1986; Crickmore 1987).

**Media.** Oxoid nutrient broth (NB) or agar (NA) was used throughout, except with a few strains which showed enhanced temperature sensitivity on Luria broth agar containing no salt (see Results). All other complex and minimal media and antibiotics were as described previously (Salmond and Plakidou 1984; Crickmore and Salmond 1986; Gill and Salmond 1990). Glp- mutants were assessed by their inability to grow on minimal agar (MA) containing 0.5% glycerol, in place of glucose, as a sole carbon source.

**Genetic methods.** P1 transductions were done as before (Salmond and Plakidou 1984). All mutations generated by localised mutagenesis were transduced out into OV2 via T4GT7 (Salmond and Plakidou 1984; Plakidou et al. 1984) prior to further analysis. Transformations and lysogen construction were as previously described (Salmond and Plakidou 1984; Gill et al. 1986; Crickmore and Salmond 1986). Genetic selection of transductants and transformants was on NATc (tetracycline at 10 \(\mu\)g/ml) and NAAp (ampicillin at 50 \(\mu\)g/ml), respectively.

**Localised mutagenesis.** The strategy used was adapted from Hong and Ames (1971). High titre lysates of P1 of > \(10^{11}\) pfu/ml (0.5 ml) were mixed with 1 ml of phosphate-EDTA buffer (1 M \(\text{K}_2\text{HPO}_4\) added to 1 M \(\text{KH}_2\text{PO}_4\) to give pH 6.0 followed by an equal volume of 10 mM EDTA), 1.5 ml sterile \(\text{H}_2\text{O}\) and 2 ml freshly prepared hydroxylamine solution (0.35 g NH\(_2\text{OH}\) plus 560 \(\mu\)l of 4 M NaOH made up to 5 ml with sterile \(\text{H}_2\text{O}\)). After incubation at 37°C, phage were precipitated at 36,000 \(\times\) g for 2.5 h at 4°C. Phage were recovered from the pellet by adding 0.5 ml LBSE (Luria broth plus 1 M NaCl and 1 mM EDTA) and allowing slow resuspension overnight at 4°C. The resultant lysate was titrated to quantify the effects on plaque formation as an indirect monitor of general mutagenesis of P1-packaged DNA. The mutagenised lysate was then used to transduce OV2 to tetracycline resistance (Tc') on NATc at 30°C (or 37°C) and transductants were replicated to 42°C (or 25°C) to assay for temperature sensitivity (or cold sensitivity).

**Ts mutation mapping, cloning and sequencing.** Linkage of each Ts mutation to the zhf::Tnl0 transposon was confirmed by transducing each mutation out (via T4GT7) into a "clean" OV2 genetic background, selecting Te' cells at 30°C then replicating to 42°C to score for thermosensitivity. The Ts mutations were then localised by complementation assays using \(\lambda\) derivatives and plasmids carrying DNA from the 76 min region of the \textit{E. coli} chromosome (ORF4, \textit{ftsY}, \textit{ftsE}, \textit{ftsX} and \textit{rpoH}). The respective mutations were cloned using the strategy described in the Results section. DNA sequencing in M13 derivatives was as described before (Gill et al. 1986).

**Results**

**Localised mutagenesis**

A P1 lysate was made on strain OV2 zhf::Tnl0 to a titre of \(2 \times 10^{11}\) pfu/ml. After hydroxylamine mutagenesis for 40 h, the survival rate was approximately \(10^{-3}\). Mutagenised lysates were used to transduce OV2 to Te' on NATc plates at 30°C. Transductants were replicated to 42°C and 30°C to screen for conditional lethals on NA plates and MA plates to screen for auxotrophs. Of 10,000 transductant screened, 28 Ts mutants were isolated, but no auxotrophs. Additionally, 2000 transductants were also screened for psychrosensitive mutants at temperatures between 37°C (permissive) and 25°C (restrictive), but none were found. In control experiments \(glpRD\) mutants were also screened for, because \(glpRD\) is located close to zhf::Tnl0. Of 800 Te' transductants tested, 5 were Glp-. The latter gave a localised mutagenesis frequency of 0.62%. The low recovery rate of Ts mutants (0.28%) compared with Glp- mutants is, presumably, a reflection of the higher degree of linkage of the latter (\(glpRD\)) genes to the transposon. We presumed that this low level of mutagenesis would make the isolation of double mutants unlikely.