The mvrA locus of Escherichia coli does not encode a ferredoxin–NADP+ reductase

Ferredoxin–NADP+ reductases (FRNs) are FAD-containing enzymes found in bacteria, plastids and mitochondria that catalyse the reversible electron transfer reaction between NADP+ and ferredoxin or flavodoxin (1). They are involved in a wide variety of redox metabolic pathways in different tissues and organisms, functioning as a universal class of one- and two-electron carriers (1). The isoform present in Escherichia coli, encoded by the fpr gene (2), is a member of the soxRS regulon, and is therefore involved in the concerted cell response towards superoxide intoxication (7).

In 1988, Morimyo (8) selected a number of mutants of E. coli strains that were abnormally sensitive to the superoxide-propagating compound methyl viologen (MV). One of these strains was used as a host in complementation experiments to clone a gene (named mvrA) that was able to rescue the mutant cells from killing by MV (8). Further research showed that, despite some framshitsu errors in the published sequence, the complementing gene was clearly related to the previous assignment.

However, several observations challenged the validity of the previous assignment. Bianchi et al. (2) showed that the mvrA and fpr loci were located at different positions in the E. coli chromosome. Also, the phenotypes displayed by the two mutant strains were markedly different (3, 6, 8), although this discrepancy could be attributed to the different genetic backgrounds and mutagenesis procedures employed to generate them (3, 8). In order to solve this question, we undertook a reassessment of the relationship between these loci, by determining the expression and activity levels of FRN in mvrA and fpr mutant cells under different growth regimes.

The genotypes and culture conditions of the fpr, mvrA and MC48 strains are described by Krapp et al. (6). To induce the soxRS response, bacterial cultures grown to the early exponential phase (OD600 ~0.3) were incubated for 1 h at 37 °C in Luria–Bertani broth containing 0.2 mM MV (7). Cells were then collected, washed and ruptured by sonication (6). The presence of FRN in the various E. coli strains was analysed by SDS-PAGE and immunoblotting (6). Bacterial lysates were separated on 12 % polyacrylamide gels, transferred to nitrocellulose membranes and immunodecorated with rabbit antisera raised against E. coli FRN (3). FRN activity in cleared lysates was determined by measuring the diaphorase reaction, namely, the ability to catalyse NADPH oxidation by artificial electron acceptors (1). In order to identify individual enzymes displaying diaphorase activity, E. coli extracts were resolved by electrophoresis in non-denaturing 10 % polyacrylamide gels, and stained for activity using nitro blue tetrazolium as electron acceptor (4).

The MC48 strain, from which the mvrA mutants were derived (8), was used as an FRN-proficient control. Lysates from these bacteria show a single protein species reacting toward FNR antisera (Fig. 1, lane 1), whose levels were increased ~20-fold upon a brief challenge with MV (Fig. 1, lane 2). FNR could not be detected in the soluble fractions obtained from fpr cells, even after MV treatment (Fig. 1, lanes 5 and 6), as expected from a bona fide FNR-deficient strain (3). At variance, the FRN patterns observed in mvrA mutant cells were similar to those displayed by the parental strain MC48 (Fig. 1, lanes 3 and 4), indicating that the enzyme was expressed in the mutant bacteria, and readily induced in the course of the soxRS response. These experiments cannot rule out the possibility that the FNR expressed in mvrA cells might be inactive. Indeed, the procedure employed to generate the mutants, random

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**Fig. 1.** Expression of ferredoxin–NADP+ reductase in different E. coli strains. FRN immunoblots were carried out on E. coli lysates as described in the text. Cells from strains MC48 (lanes 1 and 2), mvrA (lanes 3 and 4) and fpr (lanes 5 and 6) were challenged (lanes 2, 4 and 6) or not (lanes 1, 3 and 5) with 0.2 mM MV prior to rupture and analysis. Lane 7, 0·2 μg E. coli FNR.

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### References


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this possibility, soluble extracts from the role in the metabolic context of the stressed mutagenesis with ethyl methanesulfonate (8). The ability of oxidoreductase. Evidence for the formation of a left and right sides, respectively.

Fig. 2. Evaluation of the FNR-dependent diaphorase activity of E. coli lysates. Extracts were resolved by non-denaturing PAGE and analysed by activity staining (a) or FNR immunoblotting (b). E. coli cells from the MC48 (lanes 1 and 2), mvrA (lanes 3 and 4) and fpr strains (lane 5) had been treated (lanes 2, 4 and 5) or not (lanes 1 and 3) with MV as indicated in the text. The positions of the front dye and FNR are indicated by arrows on the left and right sides, respectively.

The lesions that cause the mvrA phenotype are therefore not related to FNR inactivation, but their effects can be bypassed by expression of an active reductase from either bacterial (8) or eukaryotic origin (5, 6). Participation of FNR in the antioxidant response of E. coli is well documented (3, 5–7), but its mechanism of action remains obscure. The ability of this enzyme to suppress the deleterious effects of an unrelated mutation suggests that it may be playing a general protective role in the metabolic context of the stressed cell.

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Four different molecular species displaying NADPH-dependent diaphorase activity could be resolved by this procedure (Fig. 2a, lane 1), presumably corresponding to the four diaphorases identified by Liochev and co-workers using ion-exchange chromatography (7). Comparison of the patterns exhibited by fpr and MC48 lysates suggests that FNR corresponds to the band with the higher electrophoretic mobility (Fig. 2a, lanes 1, 2 and 5), as confirmed by reaction with FNR antiserum (Fig. 2b, lane 2). A protein comigrating with FNR could be detected by activity staining in mvrA cells (Fig. 2a, lane 3). This protein was strongly expressed in response to MV exposition (Fig. 2a, lane 4), and was recognized by antibodies raised against E. coli FNR (Fig. 2b, lanes 3 and 4). Taken together, these results indicate that the mvrA mutant strain contains a fully active ferredoxin–NADP+ reductase.


