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 between NADP(H) 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 electron donors between transferases and obligate 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 et al. (8) selected a number of mutagenized 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 frame-shift errors in the published sequence, the FNR patterns observed in different E. coli strains were markedly different (3, 6, 8). In order to solve this question, we undertook a reassessment of the relationship (2), as expected from a bona fide FNR-deficient strain (3). At variance, the FNR 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...
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.

mutagenesis with ethyl methanesulfonate (8), is known to produce mostly point mutations that could inactivate the enzyme without affecting its expression. To assess this possibility, soluble extracts from the different *E. coli* strains were resolved in duplicate lanes by non-denaturing PAGE. Half of the gel was stained for 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 antisera (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.

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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