The Periplasmic Protein MppA Is Not Involved in Regulation of *marA* in *Escherichia coli*[▽]

The *marRAB* operon of *Escherichia coli* is self-repressed by MarR, while the MarA protein activates a number of promoters, including those for the AcrAB/TolC multidrug efflux pump (1, 4, 5). Inactivation of *marR* leads to a “Mar” multiple-antibiotic resistance phenotype.

In 1999, the *mppA* gene was reported to be a novel factor involved in the regulation of the *marRAB* operon (3). Strain TP985 had been created by introducing *mppA*::Tn10Cm into *E. coli* strain AT980 by P1 transduction (6). AT980 was multidrug susceptible, but TP985 was multidrug resistant in a *marA*-dependent manner. It was initially proposed that *mppA*, the gene for a periplasmic murein tripeptide binding protein involved in transport, helped repress *marRAB* in AT980 (3).

However, the multidrug resistance of TP985 was later explained by the inadvertent acquisition of an inactivating *marRΔc18* mutation by that strain (2). Complementation with plasmid pTrc99A bearing the *mppA* gene (pMLD1285) was reported to cause loss of the resistance (3). This effect of pMLD1285 did not occur in a strain reconstructed to be like TP985, namely, strain AT980 *marRΔc18 mppA*::Tn10Cm (2). Therefore, a second (unidentified) locus, “X,” altered only in TP985, appeared to help *mppA* repress *marRAB* (2).

In the present work, all strains were cultured at 37°C in LB medium (10 g tryptone, 5 g yeast extract, and 10 g NaCl per liter) supplemented with 50 μg diaminopimelic acid per ml (for *dapD2*). The genotypes and phenotypes of the strains and the plasmid used in this study are shown in Table 1.

We found that the multidrug resistance of TP985 freshly transformed with pMLD1285 (carrying the *mppA* gene) was not suppressed, even when *mppA* transcription was induced with 300 μM isopropyl-β-D-thiogalactopyranoside (IPTG). This was contrary to the previous work, where pMLD1285 was reported to cause a decrease in resistance in TP985 (3). Moreover, clones of *mppA* in pJP105 (7) or in pACYC177 also failed to suppress resistance (Xiaowen Bina, unpublished data).

Therefore, we sequenced the *marR* locus of strain TP985 and the original TP985/pMLD1285 transformant construct obtained from our laboratory collection, as well as from the original stocks of J. T. Park. All of the strains had been stored at −70°C. We found that TP985 indeed had the expected

inactivating *marRΔc18* mutation. However, surprisingly, the original TP985/pMLD1285 strain no longer had the *marRΔc18* mutation but rather was wild type for *marR* (and *marA*) (Table 1). That the chromosomal *marR* gene of the original TP985/pMLD1285 strain had reverted to the wild type explains completely the loss of multidrug resistance in that strain. No role for the plasmid-borne *mppA* gene need be presumed.

We do not know how this restoration of *marR* to the wild type occurred. Strain TP985/pMLD1285 was indeed derived from TP985 since, like TP985, it required diaminopimelic acid, had *mppA*::Tn10Cm, and was lysogenic for bacteriophage phi80 (as was TP985 but not AT980; unpublished data).

In conclusion, the proposal that *mppA* contributes to repression of the *marRAB* operon (3) is no longer plausible.

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TABLE 1. Properties of the strains and plasmid used in this study

Strain or plasmid	Relevant chromosomal genotype (reference)	Multidrug phenotype
AT980	<i>dapD2</i> (3)	Susceptible
TP985	AT980 <i>mppA</i> ::mini-Tn10Cm (6) <i>marRΔc18</i> (2)	Resistant
TP985/pMLD1285	AT980 <i>mppA</i> ::mini-Tn10Cm (6) <i>marR</i> ⁺ (this work)	Susceptible
pMLD1285	pTrc99A bearing <i>mppA</i> regulated by the IPTG-inducible <i>trc</i> promoter; Amp ^r	

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