Methionine Aminopeptidase Gene of *Escherichia coli* Is Essential for Cell Growth

SHENG-YUNG P. CHANG, ERIC C. McGARY, AND SHING CHANG*

Department of Microbial Genetics, Cetus Corporation, Emeryville, California 94608

Received 5 December 1988/Accepted 13 April 1989

We localized the methionine aminopeptidase (map) gene on the *Escherichia coli* chromosome next to the rpsB gene at min 4. Genetically modified strains with the chromosomal map gene under lac promoter control grew only in the presence of the lac operon inducer isopropyl-β-thiogalactoside. Thus, methionine aminopeptidase is essential for cell growth.

The map gene of *Escherichia coli* encodes the methionine aminopeptidase that preferentially removes the initiation methionine from certain intracellular proteins in vitro and in vivo (3). The substrate specificity of the methionine aminopeptidase is primarily determined by the residue adjacent to the methionine (3, 5, 11). The map location of the map gene and the biological significance of selective removal of the initiation methionine were not known. We report here the localization of the map gene on the *E. coli* genetic map and data indicating that methionine aminopeptidase performs an essential function in *E. coli*.

The coding and flanking nucleotide sequences of the map gene have been reported elsewhere (3). We searched the GenBank DNA data base for *E. coli* sequences that matched the upstream sequence of map and identified the first 143 base pairs of the reported 5' -flanking sequence of the rpsB-tsfs operon (ribosomal protein S2 and translation elongation factor EF-Ts genes) (1). Thus, map is located 357 base pairs from the rpsB-tsfs operon, and the two genes are transcribed divergently. The rpsB gene maps about 5 kilobases clockwise from the dapD gene at the 4-min position on the *E. coli* chromosome (2, 4, 8). The order of the four closely clustered genes is dapD-map-rpsB-tsfs (Fig. 1A). This conclusion is consistent with that deduced independently from the genomic restriction mapping data (9).

We attempted by gene replacement, but failed, to substitute the chromosomal map* gene with an inactivated copy of map (unpublished data). This led us to speculate that map is essential. Therefore, we substituted the chromosomal map* gene with an altered map gene fragment that allowed us to conveniently manipulate its expression. We deleted the lacZ promoter sequence between the PvuII site and the EcoRI site in the pUC18 (13) vector. We then placed between the EcoRI and the HindIII sites on this modified pUC18 plasmid the following genetic elements (in order): the linker-adapted 190-base-pair EcoRI-BamHI fragment located upstream from the map gene (nucleotide positions 1 to 190 [3]), the BamHI-EcoRI linker-adapted fragment containing the cat (chloramphenicol acetyltransferase) gene derived from a *HhaI* fragment from plasmid pACYC184 (6), the wild-type lac promoter and operator (lacZP0) sequence derived from pUC18 (the sequence between the PvuII and the polylinker sites), the promoterless map gene (between nucleotide positions 197 and 1032 as numbered in reference 3, adapted with CiaI at its 3' end), and the 1.4-kilobase CiaI-BstEII sequence 3' to the map gene (3) with the BstEII site converted to *HindIII* by a linker. This plasmid, designated pSYC1695 (Fig. 1B), contains the map gene controlled by the lac promoter and operator fragment (lacZP0-map), the cat gene as a selectable marker, and flanking homologous sequences to allow double recombination for gene replacement.

Plasmid pSYC1695 DNA was digested with Asp718 and SalI restriction enzymes, which cleave outside of the cat-lacZP0-map fragment. The DNA fragment was used to transform *E. coli* H205, an *hsdR* derivative from the recB recC sbeB strain JC7623 (12) that allows gene replacement through double homologous recombination between the linear transforming DNA and the chromosome (Fig. 1). Chloramphenicol-resistant (Cm*) transformants were selected on plates supplemented with the lac operon inducer isopropyl-β-thiogalactoside (IPTG) to induce and maintain the Map* phenotype in the transformants. One of these (designated EM1) was selected for further characterization. Southern genomic blotting (10) data for *BstHI*- and *ClaI*-digested chromosomal DNA of strains H205 and EM1 indicated that the map fragments from EM1 were longer than the corresponding ones from strain H205. The detected size differences were as expected for the intended gene substitution (data not shown).

Strain EM1 grew normally on plates containing IPTG but grew only to pinpoint colonies on plates without IPTG. This observation suggests that expression of the map gene is essential for cell growth. The cat-lacZP0-map gene cluster from strain EM1 was transduced into the lacP* strain DG101 (F− endA thi-1 *hsdR* supE44 lacI* lacZΔM15; from D. Gelfand) and into the (lac-pro) strain CM89 (7) by P1 transduction. CM89 cells do not produce lac repressor; therefore the Cm* CM89 transductants were expected to be Map*. Indeed, all Cm* transductants of CM89 grew equally well on plates with or without IPTG. On the other hand, all DG101 transductants depended on IPTG for growth. One of the DG101 transductants, designated strain EM9, was selected for further characterization.

The growth of the lacZP0-map lac* strains (strains EM1 and EM9) was IPTG dependent. The growth of the map* parental strains and the lacZP0-map lacI strain (CM89-derived transductants) was not IPTG dependent. The correlation of lac-controlled Map phenotype with cell growth dependency supports the hypothesis that map is an essential gene. Alternatively, the growth of the lacZP0-map cells may require the expression of another gene, located downstream from map, whose expression is also under lac repressor control. To rule out this possibility, we transformed EM9...
We thank David Gelfand and Hing C. Wong for valuable discussions.

LITERATURE CITED