JOURNAL OF BACTERIOLOGY, Mar. 1982, p. 1175–1177
0021-9193/82/031175-03$02.00/0

Vol. 149, No. 3

The B66.0 Protein of *Escherichia coli* Is the Product of the *dnaK*$^+$ Gene

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Received 6 July 1981/Accepted 16 September 1981

B66.0 is one of the most abundant proteins of *Escherichia coli*. Its relative rate of synthesis is highly regulated depending on temperature and the growth rate of the culture. We identified the B66.0 protein to be the *dnaK*$^+$ structural gene product since *dnaK756* mutant bacteria synthesized a B66.0 protein with a more acidic isoelectric point.

Protein B66.0 (defined by the nomenclature in reference 8) is an abundant *Escherichia coli* protein whose rate of synthesis increases with increasing temperature and growth rate relative to most other proteins (8). In addition, the B66.0 protein belongs to the HTP (high-temperature production) group of *E. coli* proteins (6). The HTP proteins are a group of nine proteins whose members show a dramatic increase in the relative rate of protein synthesis upon a temperature shift to 42°C (6). A regulatory gene, *htpR*, has been identified which maps at 75 min and whose gene product is a positive regulator for the increased rate of synthesis of the HTP proteins (6). *htpR* mutant bacteria lyse at high temperature, suggesting that the increased rate of synthesis of the HTP proteins is important for *E. coli* viability at that temperature (6). Other members of the HTP group of proteins include the lysyl-tRNA synthetase (6), the *groEL* gene product (protein B56.5 [5]; unpublished data), and the *groES* gene product (unpublished data). The *groEL* and *groES* gene products are essential for bacteriophage morphogenesis and bacterial growth (8). The identity of the genes which code for the remaining HTP proteins is unknown.

Bacteria carrying the *dnaK756* mutation have previously been shown to block λ phage growth (3). The block was shown to be at the level of DNA replication, specifically at the stage at which the λ *P* gene product acts (2, 3). The bacterial *dnaK*$^+$ gene has been cloned into a phage λ cloning vehicle (2), and its gene product has been identified on one-dimensional sodium dodecyl sulfate-polyacrylamide gels (4).

E. coli B178 proteins were labeled with [^{35}S]methionine, as described in the legend to Fig. 1, at 30°C for 10 min and 10 to 20 min after a shift-up from 30 to 42°C. The B66.0 protein was labeled at 30 (Fig. 1A) and 42°C (Fig. 1B). Only a small portion of the gels, corresponding to a pH gradient of 5.0 to 5.8 (in the horizontal dimension) and an M_r of 55,000 to 80,000 (in the vertical dimension) is shown. Figure 1C shows the *dnaK*$^+$ gene product made in UV-irradiated bacteria which were subsequently infected with a λ *dnaK*$^+$ transducing phage and labeled as previously described (4). The identity of the B66.0 protein and the *dnaK*$^+$ gene product was shown by mixing the extracts shown in Fig. 1B and C and running them on two-dimensional gels. Figure 1D shows that only one protein spot corresponding to the B66.0 protein position can be seen.

Figure 1E shows the protein pattern of *dnaK756* bacteria labeled between 10 and 20 min after a temperature shift-up. It can be seen that a more acidic form of protein B66.0 was present. The magnitude of this shift is shown in Fig. 1F, which contains mixed extracts of *dnaK756* bacteria and the *dnaK*$^+$ protein of Fig. 1C. Instead of one spot, as in the case of Fig. 1D, two clearly distinguishable spots can be seen. This result is in complete agreement with our previous tryptic peptide maps of the *dnaK*$^+$ gene product and the *dnaK756* gene product extracted from one-dimensional sodium dodecyl sulfate-polyacrylamide gels (4).

Since bacteria carrying the *dnaK756* mutation were isolated after nitrosoguanidine mutagenesis, we wished to verify that the isoelectric point change was due to the *dnaK756* mutation and not to another unrelated one. To do this, we took advantage of the fact that the *dnaK756* mutation, in addition to interfering with λ *P* gene product action, inhibits bacterial growth at 42°C (2, 3). Temperature-resistant (Ts$^+$) revertants were isolated at 42°C at a frequency of 10^{-7}. Revertant Ts$^+$7 formed large wild-type-size colonies at 42°C, whereas revertant Ts$^+$26 formed small colonies at 42°C. However, both Ts$^+$7 and Ts$^+$26 revertants supported λ growth. Figure 1G

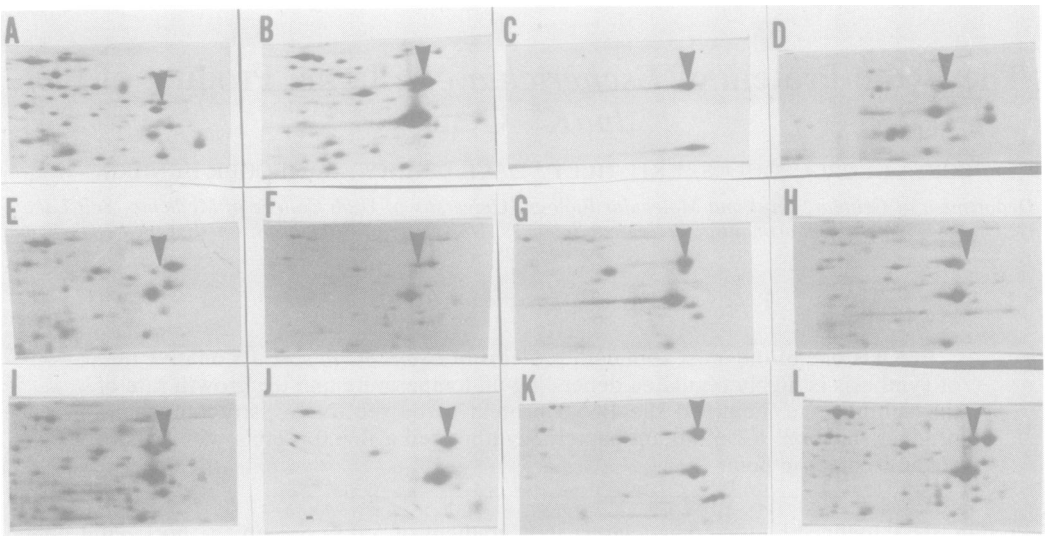

FIG. 1. Two-dimensional gel electrophoresis of [^{35}S]methionine-labeled extracts was done as previously described (9). The first dimension consisted of isoelectric focusing to equilibrium (6,400 V/h) in 1.6% (wt/vol) and 0.4% ampholine mixtures (pH 5 to 7 and 3 to 10, respectively) in a 4% (wt/vol) polyacrylamide gel. The second dimension was run in sodium dodecyl sulfate-12.5% polyacrylamide gels. The extracts in the various panels (A through L) are described in the text. Only the regions of the gels corresponding to a pH gradient of 5.0 to 5.8 (in the horizontal dimension) and an M_r of approximately 55,000 to 80,000 (in the vertical dimension) are shown. The arrow points to the position of the B66.0 protein (the *dnaK*$^+$ gene product). Its molecular weight is estimated to be M_r 75,000.

and H show the proteins made from 10 to 20 min after a temperature shift-up of Ts$^+$7 and Ts$^+$26 bacteria, respectively. If can be seen that Ts$^+$7 bacteria induced the synthesis of a normal B66.0 protein (Fig. 1G). Surprisingly, Ts$^+$26 bacteria induced the synthesis of a B66.0 protein with an isoelectric point more basic than that of wild-type bacteria (Fig. 1H). This is best seen in mixed extracts of *dnaK*$^+$ and *dnaK756* Ts$^+$26 cells (Fig. 1I). These results clearly demonstrate that the different isoelectric point of the B66.0 protein seen in *dnaK756* bacteria is due to *dnaK756*, and not to another, unrelated mutation.

We have previously described the Tro phenotype of phage λ (1). This phenotype is exhibited by lysogens of λ *c*I857 *cro*27 phage at 42°C, at which temperature both the *c*I857 and *cro*27 gene products are inactive (1). Under these conditions only λ early protein synthesis takes place, whereas most host protein synthesis is arrested. A few bacterial proteins are made under these conditions, however, even at 60 min after the shift to 42°C (1; C.G. and K.T., unpublished data). One of the proteins made appears to be B66.0 (Fig. 1J). That this protein is indeed B66.0 was shown in the behavior of mixed extracts with that of Fig. 1C (labeling after infection with λ *dnaK*$^+$), which showed the presence of a unique spot at the B66.0 position (Fig. 1K). Mixed extracts with that of Fig. 1E (that of *dnaK756* bacteria) showed the presence of two spots as expected (Fig. 1L).

The evidence for the identity of the *dnaK*$^+$ gene product and the B66.0 protein of *E. coli* can be summarized as follows. (i) The B66.0 protein and the *dnaK*$^+$ protein induced by the λ *dnaK*$^+$ transducing phage comigrated on two-dimensional gels. (ii) The *dnaK756* mutation resulted in the production of a more acidic form of B66.0. One of the *dnaK*$^+$ revertants of *dnaK756* examined, Ts$^+$7, induced the synthesis of a normal B66.0 protein, whereas another, Ts$^+$26, induced the synthesis of a more basic B66.0 protein. The Ts$^+$7 revertant formed large, wild-type-size colonies at 42°C, which is consistent with it being a true *dnaK*$^+$ revertant. The Ts$^+$26 revertant may be an intragenic suppressor of the *dnaK756* mutation, since it formed small colonies at 42°C, yet simultaneously becomes permissive for λ growth. (iii) The tryptic peptide maps of the *dnaK*$^+$ protein and the *dnaK756* protein are very similar (4).

Although the B66.0 protein is a member of the HTP group required for bacterial growth at 42°C (3, 6), the exact role that it plays in bacterial growth at all temperatures and its specific role in λ DNA replication and in preventing bacterial

lysis at 42°C are not understood at this time. The identification of the B66.0 protein as the product of the *dnaK*$^+$ gene should facilitate the elucidation of its role in phage and bacterial physiology.

This work was supported by grants GM23907 and AI12227 from the National Institutes of Health.

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