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Genetics

A mutant of *Escherichia coli* with an altered elongation factor Tu

(*tufB* gene product/two-dimensional polyacrylamide electrophoresis/peptide mapping)

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ABSTRACT **A previously isolated mutant of *E. coli* K12, HAK 88 [Kuwano, M., Endo, H. & Yamamoto, M. (1972) *J. Bacteriol.* 112, 1150–1156], contains a new protein that in two-dimensional gel electropherograms has the same molecular weight as normal elongation factor Tu, but whose isoelectric point is altered approximately 0.1 pH unit in the acidic direction. Peptide mapping, purification properties, and the ratio of leucyl plus isoleucyl residues to methionyl plus cysteinyl residues of the normal elongation factor Tu protein and the new protein show a close similarity between the two. The mutation causing the altered electrophoretic mobility is located between *argH* and *rif* (79 min on the *E. coli* genetic map). These biochemical and genetic data indicate that strain HAK 88 has a mutationally altered *tufB* gene.**

The development of two-dimensional polyacrylamide electrophoresis (1) has made it possible to identify and study individual components of a complex mixture of proteins, such as those in a crude cell extract. In a continuation of our physiological and genetic studies of elements of the transcription and translation apparatus in *Escherichia coli*, we have analyzed extracts from the previously isolated putative mutant of elongation factor Ts (EF-Ts), HAK 88 (2), on two-dimensional gels. Much to our surprise, extracts of this mutant evidently contained two spots for elongation factor Tu (EF-Tu) which differed from each other in isoelectric point by approximately 0.1 pH unit. We report here the evidence for this and show that the mutation leading to the alteration in one of the EF-Tu species in strain HAK 88 lies in the *tufB* gene.

MATERIALS AND METHODS

E. coli K12 strains HAK 88 and the parent strain HAK 8, isolated and characterized by Kuwano *et al.* (2), were obtained from the strain collection of A. A. Travers. HAK 88 from the strain collection of F. Young was indistinguishable from the above HAK 88. Strain JF 844 [*E. coli* B (AS 19), *leu, argH, rif*^{r}, *valS*], strain JF 376 (*E. coli* K12, *thi, thr, leu, argH, his, rif*^{r}), and strain JF 744 (*E. coli* K12, *trkA, kdp, str*) were used as recipients in P1 transduction experiments.

EF-Tu and elongation factor G (EF-G) were located on the two-dimensional gels¶. Strains HAK 8 and HAK 88 were grown in low-sulfate minimal medium with glucose as the sole carbon source and supplemented with tryptophan and thiamine. Cells were labeled with $^{35}SO_4^{-2}$ or [^{3}H]leucine and [^{3}H]isoleucine‖. Cell lysis, electrophoresis, staining of the gels with Coomassie blue, and determination of radioactivity in the gels were done as described elsewhere[11].

For partial purification of EF-Tu from HAK 88 the first three purification steps of the procedure of Arai *et al.* (3) were used. The procedure was scaled down 100-fold; after the 37–64% $(NH_4)_2SO_4$ precipitation, the precipitate was dissolved in buffer of Arai *et al.* (3) and diluted to a conductivity equivalent to that of 0.1 M $(NH_4)_2SO_4$. After application of the cell extract to the DEAE-Sephadex column the column was washed with 0.2 M KCl buffer. Elution was carried out in a stepwise manner with increasing concentrations of KCl, ranging in steps of 0.02 M, from 0.22 M to 0.30 M.

Preparation of Tryptic Peptides. Stained spots of each protein containing 150,000–300,000 cpm were cut from the two-dimensional gel of $^{35}SO_4^{-2}$-labeled HAK 88 extract. Seven hundred micrograms of salt-free unlabeled EF-Tu (*E. coli*), which had previously been denatured in 6 M guanidine·HCl, was added to each of the gel slices. The mixture was suspended in 150 μl of 0.1 M NH_4HCO_3, and 50 μg of (L-1-tosylamido-phenylethyl chloromethyl ketone)-treated trypsin was added (4). The mixture was kept at 37° for 4 hr, then centrifuged, and the supernatant was pipetted out. The gel was then treated as before with 50 μg more of trypsin at 25° for 12 hr. The combined supernatants were pooled and lyophilized. The residue was dissolved in 150 μl of 90% HCOOH and 10 μl of CH_3OH, and the solution was cooled to −5°, after which 800 μl of cold performic acid (5) was added to oxidize cysteine and methionine. The mixture was kept at about −5° for 2.5 hr, and then the solvent was removed under reduced pressure.

Peptide Mapping. The oxidized sample was disolved in 80 μl of water, and the solution was applied to a 20 × 20 cm precoated silica gel plate (E. Merck, no. 5763). The plate was developed in $CHCl_3$–CH_3OH–concentrated NH_4OH (2:2:1) and allowed to dry in air until the odor of ammonia had disappeared. The plate was then moistened with pH 1.8 buffer, H_2O–CH_3COOH–HCOOH (90:2:8), and subjected to electrophoresis at 1000 V for 1.75 hr, after which it was allowed to dry in air at room temperature. To locate the ^{35}S-labeled peptides, we exposed the plates to Kodak Blue Brand x-ray film for 3 days and then sprayed them with ninhydrin-collidine reagent.

RESULTS

Occurrence and Biochemical Characterization of the Altered EF-Tu. Cultures of strain HAK 8 and strain HAK 88

Abbreviations: EF-Tu, elongation factor Tu; EF-Ts, elongation factor Ts, EF-G, elongation factor G.

¶ S. Pedersen, J. Parker, S. Reeh, R. J. Watson, N. P. Fiil and J. D. Friesen (1976) "Analysis of the proteins made by ultraviolet light irradiated *E. coli* after infection with the bacteriophages λ*drif*^{d}*18* and λ*dfus*-3," *Mol. Gen. Genet.*, in press.

‖ R. M. Blumenthal, S. Reeh and S. Pedersen (1976) "Regulation of transcription factor ρ and the α subunit of RNA polymerase in *Escherichia coli* B/r," submitted for publication.

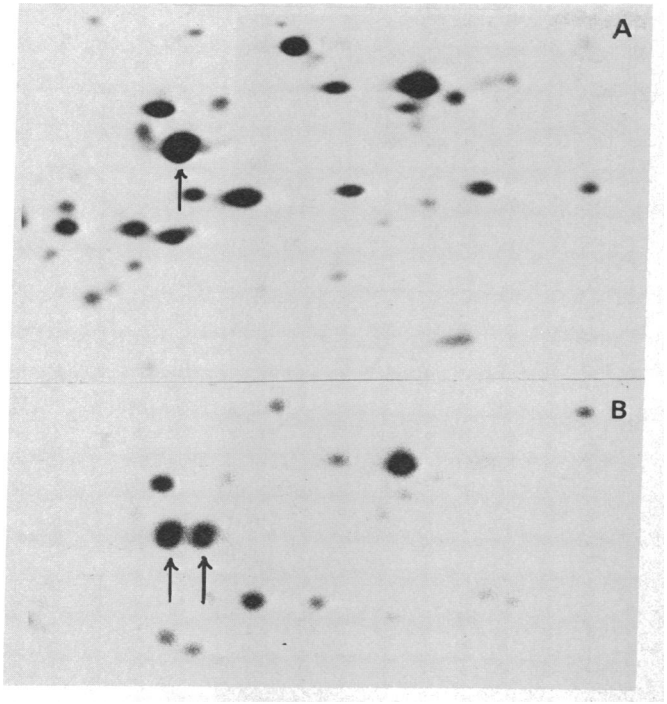

FIG. 1. Autoradiograms of extracts of strain HAK 8 (A) and strain HAK 88 (B) electrophoresed in the two-dimensional gel system (1). Only that portion of the autoradiogram showing proteins with isoelectric points in the range of 5.5–6.5 and having molecular weights in the range 55,000–35,000 is shown. The protein spots discussed in the *text* are marked with arrows; the left-hand arrow in part B points to normal EF-Tu.

were labeled with $^{35}SO_4^{-2}$, and extracts were electrophoresed in the two-dimensional polyacrylamide gel system of O'Farrell (1). Fig. 1 shows the portion of these gels on which EF-Tu is located. It is seen that in strain HAK 88 (Fig. 1B), in addition to the normal EF-Tu spot, a new protein spot is present whose molecular weight is identical with EF-Tu but whose isoelectric point is approximately 0.1 pH unit more acidic than the normal EF-Tu protein. Extracts of strain HAK 8, the parent of strain HAK 88 (2), show only one spot in the EF-Tu region of the electropherogram (Fig. 1A). These data suggest that the new electrophoretic spot appearing in extracts of strain HAK 88 might be an altered form of EF-Tu that arose in the selection of that mutant from its parent, strain HAK 8. Several experiments were undertaken to substantiate this inference.

First, two identical cultures of strain HAK 88 were grown in glucose-minimal medium and labeled with either $^{35}SO_4^{-2}$ or [^{3}H]leucine and [^{3}H]isoleucine. Appropriate amounts of each culture were mixed and the extracts were coelectrophoresed. Portions of the EF-Tu spot and the new spot were cut from the dried gels and the isotope ratio ($^3H/^{35}S$) was determined by liquid scintillation counting¶. To provide a normalization base in this experiment we arbitrarily set the isotope ratio of the total cell protein applied to the gel at 1.0. The isotope ratio in both the normal and presumed altered EF-Tu protein was 1.0 ± 0.06 (mean ±SEM), which is compatible with the notion that the two proteins have similar amino acid composition. For comparison, the isotope ratio in other protein spots varies from 0.3 to 2.0 (Pedersen, Reeh, Bloch, and Neidhardt, manuscript in preparation).

Second, partially purified EF-Tu was prepared from ^{3}H-labeled extracts of strain HAK 88 as described in *Materials and Methods*. Two-dimensional gel electrophoresis was carried out on both the S-100 extract and on the proteins eluting from the DEAE-Sephadex column. On the basis of the radioactivity in the EF-Tu proteins and the amount of total protein, we estimate that the purification of EF-Tu after the DEAE-Sephadex step was about 10-fold. Fig. 2 shows the stained gels of the S-100 extract (A) and of the proteins eluting from the DEAE-Sephadex column at 0.28 M KCl (B). Three heavily stained proteins are seen, together with some impurities. The major spots are EF-Tu, the putative altered EF-Tu, and EF-G, which has been shown to copurify with EF-Tu in this method (3). Thus, the new protein and EF-Tu in strain HAK 88 have similar purification characteristics.

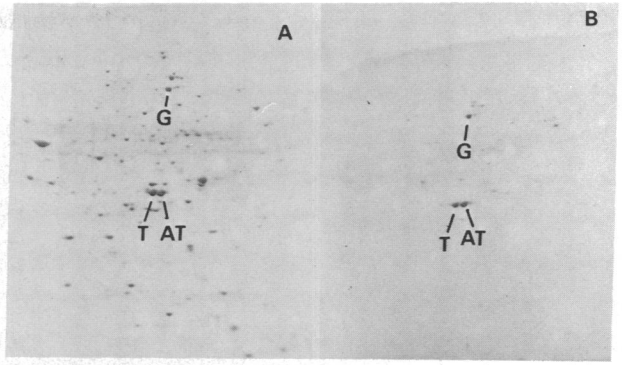

FIG. 2. Stained gels of S-100 extract (A) and of the 0.28 M KCl DEAE-Sephadex eluate (B). EF-G (G), EF-Tu (T), and the putative altered EF-Tu (AT) are indicated.

Third, a more direct identification of the new protein spot as altered EF-Tu was obtained from peptide mapping of proteins purified on two-dimensional gels as described in *Materials and Methods*. The peptide map obtained for the new protein (Fig. 3B) is nearly identical to that for normal

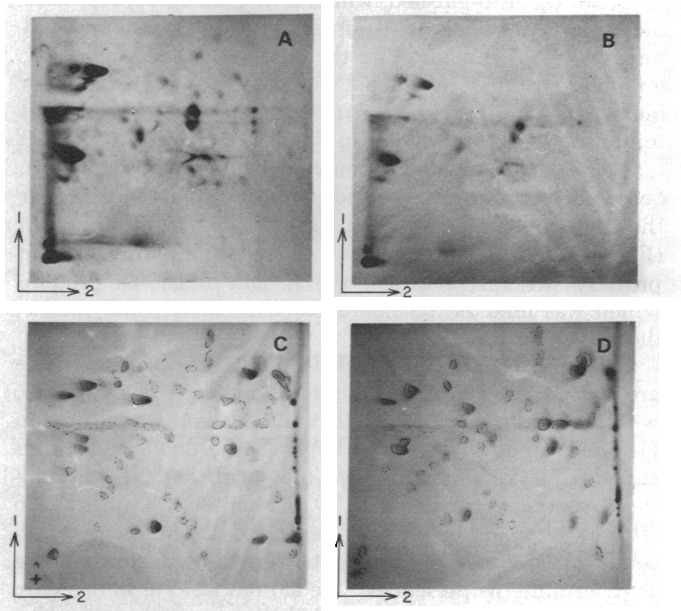

FIG. 3. Peptide maps of EF-Tu and the new protein spot (both from HAK 88) on silica-gel plates. The upper photographs show patterns obtained after autoradiography of peptide maps of normal EF-Tu (A) and of the putative altered EF-Tu (B). The lower photographs (C and D) show the same plates after they were stained with ninhydrin. The radioactive peptides are not present in sufficient quantity to be detected with ninhydrin, and thus only the unlabeled *E. coli* B EF-Tu carrier peptides are seen in (A) and (B). The origin is in the lower left-hand corner. The plates were subjected to chromatography in the first dimension and to electrophoresis in the second as described in *Materials and Methods*.

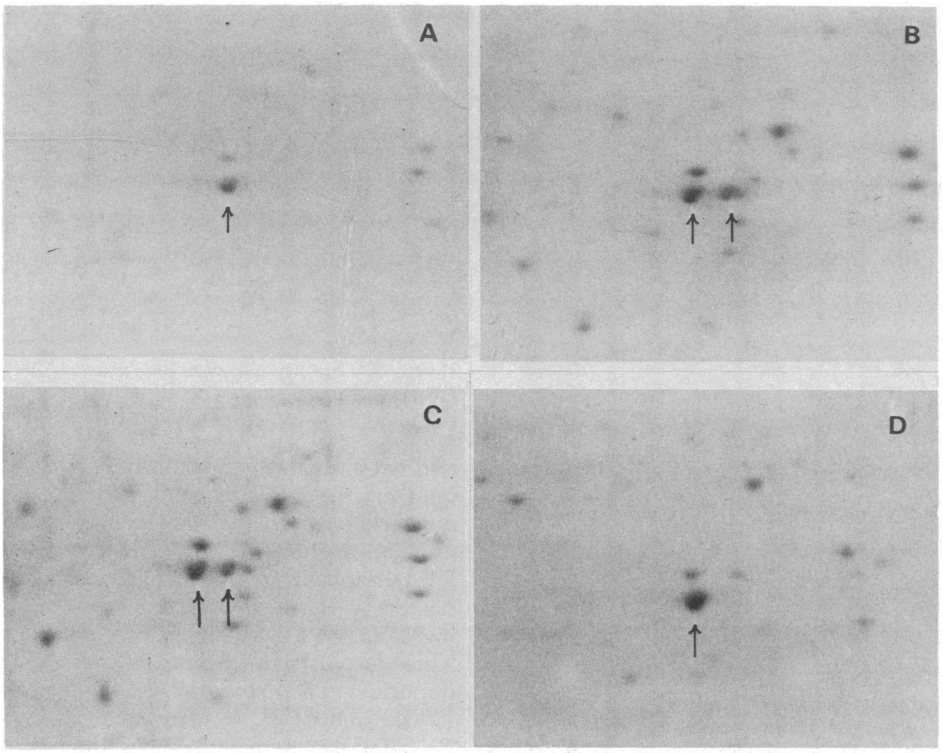

FIG. 4. Stained electropherograms of extracts of parent strain JF 376 (A), one of four recombinants of the cross P1 (HAK 88) × strain JF 376 (B), one of two recombinants of the cross P1 (HAK 88) × strain JF 844 (C), and one of five recombinants of the cross P1 (HAK 88) × strain JF 744 (D). The arrows point to the normal (left) and altered (right) EF-Tu proteins.

EF-Tu (Fig. 3A), indicating that the two proteins have very similar structures. Approximately nine major radioactive spots can be distinguished, which is in good agreement with the amino acid analysis (6) of EF-Tu (nine methionyl and three cysteinyl residues) and the observation (S. Nagarkatti, unpublished) that several of the tryptic peptides contain more than one methionyl or cysteinyl residue. The several minor spots that are present in Fig. 3A but absent in Fig. 3B may arise from a small amount of contaminating protein. Some of the radioactive peptides, for example, the pair near the centers of Fig. 3A and B, are not stained by ninhydrin (Fig. 3C and D). This may be a reflection of a difference in primary structure between the *E. coli* B EF-Tu protein, which was used as the nonradioactive carrier, and the radioactive K12 proteins.

Genetic Mapping of the EF-Tu Alteration. Since there are at least two genes coding for EF-Tu, *tufA* and *tufB* (4), we examined the possibility that one of them was altered in HAK 88. To do this we used HAK 88 as a donor and strains carrying markers flanking *tufB* (i.e., *argH* and *rif*) or flanking *tufA* (i.e., *trkA* and *str*) (4) as recipients. Strain JF 376 (*thi, thr, leu, argH, his, rif*^{r}) was used as one recipient, in which *argH*$^+$, *rif*^{s} recombinants were chosen for screening to determine the presence of the altered EF-Tu. In this cross four of four recombinants showed the electrophoretic alteration in EF-Tu. Gel electropherograms of extracts of strain JF 376 and one recombinant are shown in Fig. 4A and B, respectively. This result was confirmed in a second cross in which strain JF 844 (*E. coli* B, *leu, argH, rif*^{r}, *valS*) was used as a recipient; two of two *argH*$^+$, *rif*^{s} recombinants showed the altered EF-Tu. (One electropherogram of a recombinant extract is shown in Fig. 4C.) In a third cross the recipient was strain JF 744 (*trkA, kdp, str*). Five *trkA*$^+$, *str*^{s} recombinants were screened for the presence of the altered EF-Tu protein and *none* contained it (Fig. 4D); in all cases the recipients showed only the wild-type EF-Tu spot on the gels. From these data we conclude that HAK 88 is mutated in the *tufB* gene.

In one of the recombinants from the cross HAK 88 × JF 376 (Fig. 4B) the relative amount of the two EF-Tu proteins was determined by direct measurement of the radioactivity in the two spots. The *tufA* protein contained 134,433 cpm and the *tufB* protein contained 54,192 cpm, giving a ratio of 2.5 for the relative expression of the two genes in glucose minimal medium. This is in excellent agreement with the relative promoter efficiencies for *tufA* and *tufB* gene expression in ultraviolet light-irradiated cells after infection with bacteriophages carrying either EF-Tu gene[¶]; the *tufA* gene was found to be approximately 3-fold more active than the *tufB* gene[¶].

DISCUSSION

Our evidence indicates that the mutant strain HAK 88 (2) contains an electrophoretically altered EF-Tu. On the basis of the mapping data we have localized the mutation to the *tufB* gene (4). One cannot rule out the possibility that the EF-Tu change in strain HAK 88 is due to an altered EF-Tu modifying enzyme, although such a hypothetical enzyme would have to be located very close to the *argH-rif* region. Moreover, one would have to imagine an altered modifying enzyme that lacks the capacity to process only a portion of the EF-Tu molecules. The observation that in strain HAK 88, as well as in all the recombinants carrying the altered EF-Tu, less than half of the EF-Tu molecules are altered agrees qualitatively with the relative activity of *tufB* compared to *tufA*[¶] and supports the conclusion that the mutation is in the *tufB* gene itself.

We have found altered EF-Tu proteins in two independently maintained stocks of HAK 88. This makes it likely that the mutation arose in the isolation of strain HAK 88 from its parent, HAK 8, and is not, as could be imagined, an EF-Tu mutation arising during storage as a response in some way to the putative EF-Ts mutation in that strain (7).

The question arises whether any of the reported properties of strain HAK 88 could be accounted for by EF-Tu mutation rather than an EF-Ts mutation. Many of the properties of strain HAK 88, in particular tRNA growth-dependence (2) and the altered GTP binding site in Qβ replicase prepared from strain HAK 88 (8), might be explained by an EF-Tu mutation, since EF-Tu and not EF-Ts is known to have binding sites for tRNA and GTP (9). However, in the face of the evidence (7) that EF-Ts isolated from strain HAK 88 is temperature-sensitive *in vitro*, we must conclude that this strain has alterations in both EF-Tu and EF-Ts. We are unable to detect any mobility change in the EF-Ts spot in HAK 88, but not all mutations would be expected to give rise to such. Failure to demonstrate an enzymatic alteration in EF-Tu (7) might be because the *tufB* mutation is innocuous. Mutant HAK 88 itself is temperature-sensitive for growth (2), but none of the recombinants that have the electrophoretically altered EF-Tu show growth temperature-sensitivity (data not shown).

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