Molec. gen. Genet. 144, 339–343 (1976)

MGG

Analysis of the Proteins Synthesized in Ultraviolet Light-Irradiated *Escherichia coli* Following Infection with the Bacteriophages λ*drif*^{d} 18 and λ*dfus*-3

Steen Pedersen* and Solveig V.Reeh*

Department of Microbiology, University of Michigan, Ann Arbor, Michigan 48104, U.S.A.

Jack Parker, Robert J.Watson, and James D. Friesen

Department of Biology, York University, 4700 Keele Street, Downsview, Ontario M3J 1P3, Canada

Niels P. Fiil

University Institute of Microbiology, Øster Farimagsgade 2A, Copenhagen K, DK-1353, Denmark

Summary. The presence of EF-Tu [1], RNA polymerase subunit α, and EF-G on the λ*dfus*-3 genome and EF-Tu, ribosomal proteins L7/L12, and RNA polymerase subunit β on the λ*drif*^{d} 18 genome has been confirmed using a two-dimensional gel electrophoresis technique sensitive to changes in isoelectric point and molecular weight. In this system two EF-Tu gene products could not be resolved. Following infection of ultraviolet light-irradiated *Escherichia coli* with either λ*dfus*-3 or λ*drif*^{d}18, the EF-Tu gene, *tufA*, near 65 minutes on the genetic map is expressed as 3–4 copies per EF-G molecule. The EF-Tu gene, *tufB*, near 79 minutes on the genetic map, is expressed at about one-third of this rate. α is expressed as 1 copy per EF-G molecule, β as 0.14 per EF-G molecule and L7/L12 as 2.5 per EF-G. These figures compare well with the relative amounts found in exponentially-growing cells, in which the ratio of EF-Tu to EF-G is approximately 5. Almost 90% of the total number of proteins (calculated on a molecular weight basis) which theoretically can be encoded on the λ*drif*^{d}18 have been identified on the two-dimensional gel.

Introduction

It has been shown that the *Escherichia coli* bacteriophage, λ*dfus*-3, carries the genes for α, EF-Tu, EF-G and several ribosomal proteins (Jaskanus et al., 1975a, 1975b). The λ*drif*^{d}18 bacteriophage was shown to have the β and β′ genes (Kirschbaum and Scaife, 1974), genes for 5S, 16S and 23S rRNA, genes for several ribosomal proteins (Watson et al., 1975; Lindahl et al., 1975), a second EF-Tu gene (Jaskunas et al., 1975b), and the *relC* gene which is most likely the structural gene for ribosomal protein L11 (Parker et al., 1976). Several questions concerning the two EF-Tu genes come to mind, among them whether the two EF-Tu genes are regulated in the same way and whether their gene products are identical. In this communication, we present evidence which bears on the relative expression of these two genes with respect to each other and to other host genes on these phages. For this purpose we have analyzed the proteins synthesized in UVL-irradiated bacteria following infection with these bacteriophages using a two-dimensional gel electrophoresis technique (O'Farrell, 1975).

Materials and Methods

Bacteriophage λcI857S7*drif*^{d}18 (Kirschbaum and Konrad, 1973) was obtained from J. Kirschbaum. Bacteriophage λcI857S7*dfus*-3 (Jaskunas et al., 1975a, 1975b) was obtained from M. Nomura. Purified proteins used as markers for identification of the proteins on the two-dimensional gels were obtained from J. Gordon, F. Engbaek, B. Glick and L. Lindahl. Infection of the UVL-irradiated cells (strain JF298, *uvrB Δatt*λ) was performed as described previously (Watson et al., 1975) except that the radioactive labelling was with a mixture of 14 [^{14}C] labelled amino acids (57 mCi/mAtom carbon, 3 μCi/ml medium) (Amersham) or 15 [^{3}H] labelled amino acids (average specific activity 24 Ci/mmole, 40 μCi/ml medium) (Amersham). The bacterium used for the labelling of exponentially growing cells was *E. coli* B/r strain NC3, which was grown and labelled as described (Blumenthal et al., 1976). Following radioactive labelling, the cells were collected by centrifugation, washed with one volume of 10 mM Tris, 5 mM $MgCl_2$, pH 7.4 and then resuspended in 0.08 ml of the same buffer. The cells were lysed by sonication and then treated with 4 μg of RNase A and DNase I for 15 minutes at 0° C. Following this 80 mg urea and 0.2 ml lysis buffer (O'Farrell, 1975) was added. The electrophoresis was carried out essentially as described by O'Farrell (1975) using 10% or 7.5% acrylamide in the second dimension. The gels were stained overnight in 50% ethanol, 7.5% acetic acid, 1% trichloroacetic acid and 50 mg/l of Coomassie brilliant blue. The follow-

* *On leave from:* Department of Molecular Biology, University of Aarhus, Denmark

1 *Abbreviations:* α, RNA polymerase subunit α; β, RNA polymerase subunit β; β′, RNA polymerase subunit β′; EF-Tu, elongation factor Tu; EF-G, elongation factor G; L7/L12, ribosomal proteins L7/L12; UVL, ultraviolet light

ing morning the gels were soaked for 1 h in 7.5% acetic acid, 1% trichloroacetic acid, and 50 mg/l Coomassie brilliant blue, and then destained in 7.5% acetic acid. The gels were then dried under vacuum on filter paper and autoradiograms were made. Spots were cut out with a sharpened syringe needle and digested with 0.075 ml H_2O_2 for 2 h at 70° C. Since the H_2O_2 was found to reduce counting efficiency it was removed with 0.1 ml catalase, 0.25 mg/ml in 25 mM Tris pH 7.8. After incubation for 15 minutes at room temperature, 0.6 ml NCS (Amersham-Searle) and 5 ml of a standard toluene based scintillation mixture was added.

Results

Autoradiograms of the [^{14}C] labelled extract from the UVL-irradiated cells infected with λ*drif*^{d}18, λ*dfus*-3 and the helper phage λcI857S7b515b519*xis*6 are shown in Figures 1A, B, C, respectively. The indicated protein spots were identified by co-electrophoresis with purified proteins. The distinction between

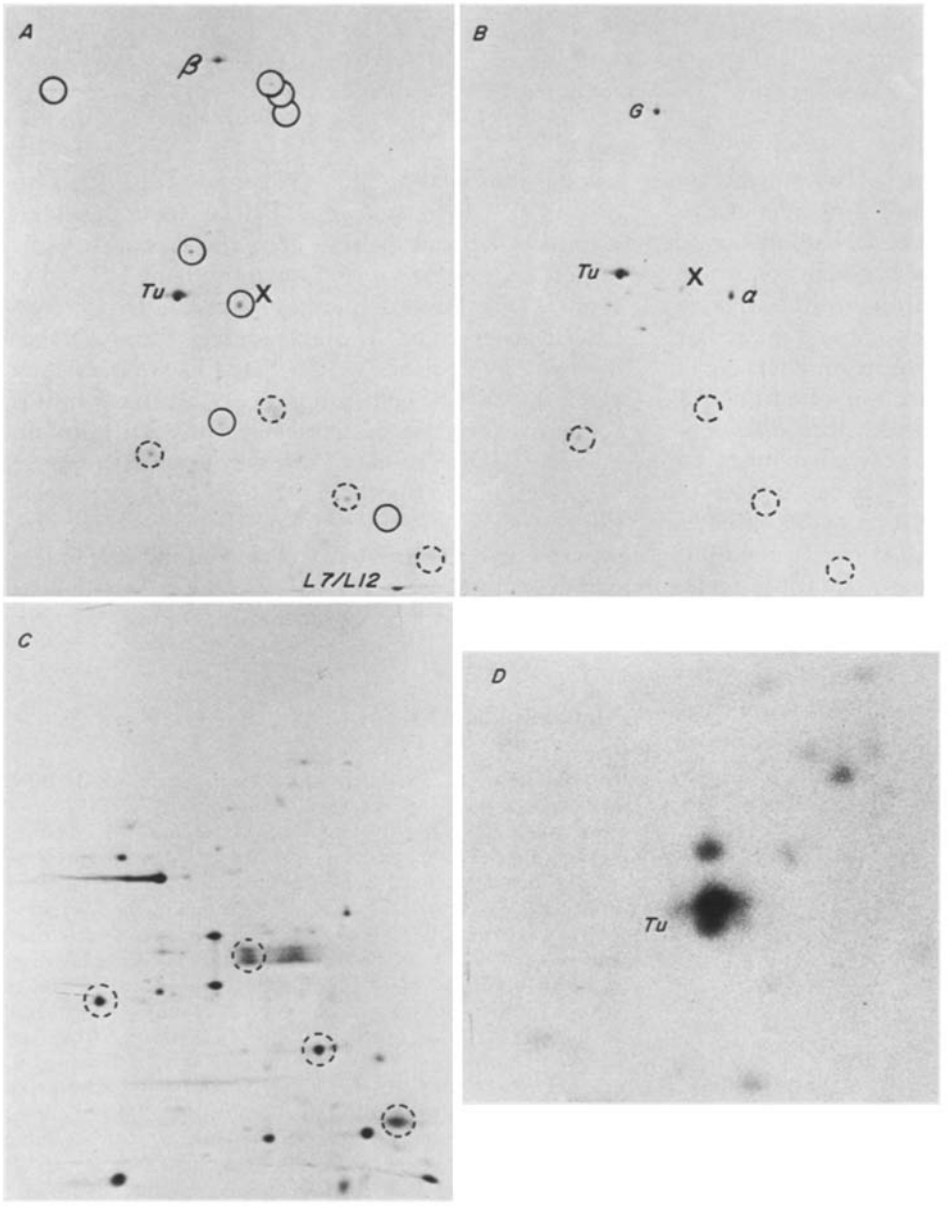

Fig. 1A–D. Two-dimensional electropherograms of extracts of UVL-treated, λ-infected *E. coli* labelled with [^{14}C] amino acids. (A) λ*drif*^{d}18. (B) λ*dfus*-3. (C) λcI857S7b515b519*xis*6. The extracts were prepared and analyzed as described in Materials and Methods. Spots in Figure 1A which are designated as *E. coli*-specific because they do not appear on the λ helper gel (Fig. 1C) are circled with a solid line. The *E. coli* proteins which are known are indicated (Tu, G, α, β, L7/L12). The four λ proteins which are common to both λ*dfus*-3 and λ*drif*^{d}18 and which were used for normalization (Table 1) are circled with a dashed line. (D) Enlargement of the Tu spot from exponentially growing cells showing the apparent double spot

Table 1. Quantitative determination of the radioactivity in the EF-Tu, EF-G, α, β, and L7/L12 proteins from extracts of UVL-irradiated cells infected with λ*dfus*-3 or λ*drif*^{d}18 and from extracts of *E. coli* grown exponentially in glucose

Radioactivity in	λ*dfus*-3		λ*drif*^{d}18		Exponential	
	[^{3}H] cts/min	Relative molar amount	[^{14}C] cts/min	Relative molar amount	cts/min	Relative molar amount
EF-Tu, *tufA* product	20,801	3.5	–	–	69,491	5.1
EF-Tu, *tufB* product	–	–	1,003	1.1		
EF-G, *fus* product	11,287	1.0	–	–	25,831	1.0
α, *rpoA* product	5,504	1.0	–	–	9,953	0.82
β, *rpoB* product	–	–	442	0.14	15,907	0.33
L7/L12, *rpsL* product	–	–	722	2.5	11,635	2.8
λ, four common proteins	444		69			

The experiments with extracts of infected, UVL-treated cells (columns 2, 3, 4 and 5) were carried out as described in Materials and Methods. The λ*dfus*-3-infected cells were labelled with [^{3}H] amino acids and the λ*drif*^{d}18-infected cells were labelled with [^{14}C] amino acids. Portions of these infected cells were mixed prior to extraction and electrophoresis. The [^{14}C] radioactivity cut out from the gels was standardized by multiplying by the [^{3}H]/[^{14}C] ratio in the four common lambda proteins coded for by both bacteriophages (see Fig. 1). These figures were then corrected to molar amount by dividing by the molecular weight of each protein. The relative molar amount of each protein was calculated, setting EF-G arbitrarily at one. The data from exponential cells (columns 6 and 7) were determined by quantitatively cutting the proteins from a gel on which [^{14}C] glucose labelled strain NC3 extract, kindly given to us by P. Bloch was electrophoresed Molecular weights of 83,000, 44,000, 39,000, 155,000, and 13,400 have been used for EF-G, EF-Tu, α, β, and L7/L12, respectively.

β and β′ was on the basis of the reported acidity of these subunits (Fujiki and Zurek, 1975); a protein with the isoelectric point of β′ (8.6) would run out of the gel in the ampholyte mixture used in our experiments and thus the indicated spot must be β. Ribosomal proteins L7/L12 are identified as the most acidic of the 50S ribosomal proteins (Kaltschmidt and Wittmann, 1970). It is clear from our data (Figs. 1A, B) that there is an EF-Tu gene on each of the two transducing bacteriophages, in confirmation of the findings of Jaskunas et al. (1975b).

The four spots outlined with a dashed circle on the gels shown in Figures 1A and 1B are λ gene products common to both transducing phages (albeit unidentified). (Note that contamination of transducing phages with helper must be very small because the major λ protein seen in Figure 1C is not present in Figures 1A and 1B.) We have used these four λ spots as a means of normalization in calculating the relative translation activities of the *tufA* and *tufB* genes, as well as other genes carried on λ*dfus*-3 and λ*drif*^{d}18. The underlying assumption in this calculation is that these λ-specific spots are under λ promoter control and that the *E. coli* genes are under their own promoter control. In this experiment (Table 1) UVL-irradiated cells were radioactively labelled with [^{14}C] amino acids following infection with λ*drif*^{d}18 or with [^{3}H] amino acids following infection with λ*dfus*-3. Cells were mixed in the appropriate ratio, and the extract electrophoresed. The appropriate spots were cut out for determination of radioactivity. [^{14}C] counts in the *E. coli* spots were multiplied by the [^{3}H]/[^{14}C] ratio in the four common λ spots and the relative molar amount of the different protein species was determined by dividing the normalized radioactivity in any given spot by the molecular weight of that protein. The amount of EF-G was arbitrarily set at one. It can be seen (Table 1) that according to these data *tufA* is expressed 3.2 times more than *tufB*; *tufA* is expressed 3.5 as much as *fus*, the gene for EF-G and *tufB* is expressed 1.1 as much as *fus*. Table 1 also shows data pertaining to the relative amount of translation of *rplL*, the gene for r protein L7/L12 (2.5 relative to *fus*), *rpoA*, the gene for RNA polymerase subunit α (1.0 relative to *fus*) and *rpoB*, the gene for RNA polymerase subunit β (0.14 relative to *fus*). These data are in remarkably close agreement with the relative translation of these genes as determined from experiments using exponentially growing cells (Table 1, columns 6 and 7). The dearth of β in the λ*drif*^{d}18 extract compared to the exponential culture might be due to the instability of β overproduced with respect to α (Kirschbaum and Scaife, 1974).

It can be seen in Figures 1A and 1B that two spots (marked with an x) with molecular weights of 41,000 and almost identical isoelectric points are coded for by both phages. However in a double-label experiment in which [^{3}H] labelled λ*dfus*-3 and [^{14}C] labelled λ*drif*^{d}18 extracts were mixed and co-electrophoresed, the [^{3}H]/[^{14}C] ratio within the spot(s) varied considerably. Thus we believe that these spots are not identical proteins coded on the two bacteriophages. Several other *E. coli* specific proteins can be seen on either of the two bacteriophages. In the case of λ*drif*^{d}18 in particular (Fig. 1A) several high-molecular weight proteins are observed. These are found

in exponentially growing cells as much less than one copy per ribosome and are regulated differently from ribosomal proteins (S. Pedersen and S. Reeh, unpublished observations). If one sums the molecular weights of the *E. coli*-specific proteins and λ-specific proteins on λ*drif*^{d}18 (939,700 and 503,000, respectively) and adds the DNA molecular weight corresponding to the rRNA genes (Jaskunas et al., 1975a) one can account for a DNA molecular weight of 28×10^6. Thus almost 90% of all the coding capacity of λ*drif*^{d}18 has been accounted for. The temperature-sensitive mutations covered by the λ*drif*^{d}18 phage (Watson et al., 1975; Kirschbaum et al., 1975) might well be due to mutations in the unidentified *E. coli* proteins detected on the gels (solid circles, Fig. 1A).

We have attempted to shed some light on whether the gene product from the two EF-Tu genes (*tufA* and *tufB*, Jaskunas et al., 1975a, b) are identical. Firstly, observations of the EF-Tu extracted from whole cell lysates of an exponentially growing culture showed a distinct double spot in the electropherogram (Fig. 1D). To investigate further the significance of the double spot a mixture of extracts of [^{3}H]-λ*dfus*-3-infected cells and [^{14}C]-λ*drif*^{d}18-infected cells were co-electrophoresed and the [^{3}H]/[^{14}C] ratio at various locations within the EF-Tu spot was determined. Within experimental error no trend in the [^{3}H]/[^{14}C] ratio within the EF-Tu spot was observed (unpublished observations). Therefore we believe that the double spot seen in extracts of exponential cells indicates either some form of precursor-product relationship, the existence of modified forms of Tu or a gel artifact possibly due to limited proteolytic cleavage. It should be noted that if the observed double EF-Tu spot is an artifact it is observed only for the EF-Tu protein and very few others. We estimate that the *tufA* and *tufB* gene products are identical to within 300 Daltons or less than 1 charge difference (O'Farrell, 1975).

Discussion

The close agreement in the relative translation amounts of the *E. coli* genes *fus*, *tufA*, *tufB* and *rpoA* when present on λ*drif*^{d}18 and λ*dfus*-3 as compared to in the exponential cell (Table 1) encourages us to assume that these genes on the transducing phages are under their normal promoter control. The remainder of this discussion is based on this assumption.

Characterization of polar effects of insertions or deletions in the *str-fus* region have revealed that *fus* and *tufA* are under the control of a common promoter and that *fus* is promoter-proximal compared to *tufA* (Jaskunas et al., 1975b). Our observation that the molar amounts of EF-Tu is 3.5-fold greater than EF-G thus leads to the suggestion that some type of translational control acts in this operon, since it is difficult to imagine a polar transcriptional control to suit this situation. A situation in which different genes on one RNA are expressed in different degrees is not without precedent, as for example in bacteriophage $Q\beta$ where the coat protein is expressed in excess over the replicase and indeed exerts a control over replicase translation (Weber and Konigsberg, 1975). No analogous mechanism has yet been demonstrated in *E. coli*, although the reverse has (Zabin and Fowler, 1970). In any case it is clear that the *tufA* and *tufB* genes are regulated in different ways since the former is three times more active than the latter. Whether the two genes are coordinately controlled is not yet known.

The *tufA* and the *tufB* gene products were found to be identical by this gel electrophoresis technique, but this does not rule out differences involving less than one charge in isoelectric point or 300 Daltons molecular weight. Two-dimensional fingerprinting of *tufA* and *tufB* gene products from normal cells, as well as from a mutant in which the *tufB* gene product is altered, are currently underway in an attempt to determine differences in the two gene products.

In exponentially growing cells the α subunit of RNA polymerase is made in 2.5 molar excess compared to the β subunit (Table 1). An excess of α has also been observed by F. Engbaek (personal communication). Our ratio of α to β is more close to that required to maintain the $\alpha 2\beta\beta'$ structure of RNA polymerase (Burgess, 1969). The amount of L7/L12 relative to the number of ribosomes both in λ*drif*^{d}18-infected cells and exponential cells is in agreement with the values found by Weber (1972) and Subramanian (1975), if one assumes one copy of EF-G per ribosome (Gordon, 1970).

Acknowledgements. This work was supported by a grant from the National Research Council (Grant No. 5734) and the National Cancer Institute of Canada to J.D.F. S.R. was supported by grants from the National Science Foundation (BMS 75-01135) and from the National Institute of General Medical Sciences (GM 17892) to Dr. F.C. Neidhardt in whose laboratory part of this work was done. S.P. received a fellowship from NATO. N.P.F. and S.P. were supported by Statens Naturvidenskabelige Forskningsraad, Denmark, R.J.W. was supported by the National Research Council of Canada. S.R. and S.P. are grateful to Dr. Neidhardt for encouragement.

References

Blumenthal, R., Reeh, S., Pedersen, S.: Regulation of transcription-termination factor *rho* and the α subunit of RNA polymerase in *Escherichia coli* B/r. Proc. nat. Acad. Sci. (Wash.) (1976, submitted)

Burgess, R.R.: Separation and characterization and the subunits of RNA polymerase. J. biol. Chem. **244**, 6168–6172 (1969)

Fujiki, H., Zurek, G.: The subunits of DNA-dependent RNA polymerase from *E. coli*: Amino acid analysis and primary structure of the N-terminal regions. FEBS Letters **55**, 242–244 (1975)

Gordon, J.: Regulation of the *in vivo* synthesis of the polypeptide chain elongation factors in *Escherichia coli*. Biochemistry **9**, 912–917 (1970)

Jaskunas, S.R., Lindahl, L., Nomura, M.: Specialized transducing phages for ribosomal protein genes of *Escherichia coli*. Proc. nat.Acad. Sci. (Wash.) **72**, 6–10 (1975a)

Jaskunas, S.R., Lindahl, L., Nomura, M., Burgess, R.R.: Identification of two copies of the gene for the elongation factor EF-Tu in *E. coli*. Nature (Lond.) **257**, 458–462 (1975b)

Kaltschmidt, E., Wittmann, H.G.: Ribosomal proteins. VII. Two-dimensional polyacrylamide gel electrophoresis for fingerprinting of ribosomal proteins. Analyt. Biochem. **36**, 401–412 (1970)

Kirschbaum, J.B., Claeys, I.V., Nasi, S., Molholdt, B., Miller, J.H.: Temperature-sensitive RNA polymerase mutants with altered subunit synthesis and degradation. Proc. nat. Acad. Sci. (Wash.) **72**, 2375–2379 (1975)

Kirschbaum, J.B., Konrad, E.B.: Isolation of a specialized lambda transducing bacteriophage carrying the beta subunit gene for *Escherichia coli* ribonucleic acid polymerase. J. Bact. **116**, 517–526 (1973)

Kirschbaum, J.B., Scaife, J.: Evidence for a λ transducing phage carrying the genes for the β and β′ subunits of *Escherichia coli* RNA polymerase. Molec. gen. Genet. **132**, 193–201 (1974)

Lindahl, L., Jaskunas, S.R., Dennis, P.P., Nomura, M.: Cluster of genes in *Escherichia coli* for ribosomal proteins, ribosomal RNA, and RNA polymerase subunits. Proc. nat. Acad. Sci. (Wash.) **72**, 2743–2747 (1975)

O'Farrell, P.H.: High resolution two-dimensional electrophoresis of proteins. J. biol. Chem. **250**, 4007–4021 (1975)

Parker, J., Watson, R.J., Friesen, J.D., Fiil, N.P.: A relaxed mutant with an altered ribosomal protein L11. Molec. gen. Genet. (1976, in press)

Subramanian, A.R.: Copies of proteins L7 and L12 and heterogeneity of the large subunit of *Escherichia coli* ribosome. J. molec. Biol. **95**, 1–8 (1975)

Watson, R.J., Parker, J., Fiil, N.P., Flaks, J.G., Friesen, J.D.: New chromosomal location for structural genes of ribosomal proteins. Proc. nat. Acad. Sci. (Wash.) **72**, 2765–2769 (1975)

Weber, H.J.: Stoichiometric measurements of 30S and 50S ribosomal proteins from *Escherichia coli*. Molec. gen. Genet. **119**, 233–248 (1972)

Weber, K., Königsberg, W.: Proteins of the RNA phages. In: RNA phages (N.D. Zinder, ed.), p. 51–84. Cold Spring Harbor: Cold Spring Harbor Laboratory 1975

Zabin, I., Fowler, A.V.: β-galactosidase and thiogalactoside permease. In: The lactose operon (J.R. Beckwith and D. Zipser, eds.), p. 27–47. Cold Spring Harbor: Cold Spring Harbor Laboratory 1970

Communicated by H.G. Wittmann

Received December 27, 1975