Proc. Nat. Acad. Sci. USA
Vol. 72, No. 12, pp. 5036–5040, December 1975
Genetics

Identification of a gene for the α-subunit of RNA polymerase at the *str-spc* region of the *Escherichia coli* chromosome*

(bacteriophage λ/ribosomal protein genes/protein identification/RNA nucleotidyltransferase)

S. RICHARD JASKUNAS†, RICHARD R. BURGESS‡, AND MASAYASU NOMURA†

† Institute for Enzyme Research, Departments of Biochemistry and Genetics, and ‡ McArdle Laboratory for Cancer Research, The University of Wisconsin, Madison, Wisc. 53706

Communicated by James F. Crow, September 8, 1975

ABSTRACT **A structural gene for the α-subunit of RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase; EC 2.7.7.6) has been identified and mapped between *spcA* and *trkA*, near 64 min on the *E. coli* chromosome. It appears to be coordinately expressed and possibly cotranscribed with the genes for ribosomal proteins S11, S4, and L17.**

Both ribosomes and RNA polymerase are made up of several macromolecular components. An understanding of the organization of the genes for the various components is essential for understanding the regulation of the biosynthesis of the complex cellular machinery responsible for transcription and translation of genetic information.

RNA polymerase (nucleosidetriphosphate:RNA nucleotidyltransferase; EC 2.7.7.6) from *Escherichia coli* is composed of four different proteins, α, β, β′, and σ (1). The *rif* gene, which is at 79 min on the genetic map, is the structural gene for β (2, 3). It is closely linked to the structural gene for β′. These two genes appear to be cotranscribed (4). The transducing phage λ*rif*^{d}18 (5), which carries bacterial DNA from the *rif* region of the chromosome, has been shown to carry the β and β′ genes (6). Recently it has been established that this phage also carries several genes for 50S ribosomal proteins (7, 8), a gene for elongation factor EF-Tu (*tufB*) (9), and a set of genes for rRNAs (7). Thus genes for several components of the transcriptional and translational machinery are clustered together near *rif*.

Another cluster of genes for components of the translational machinery is located near 64 min, the *str-spc* region of the chromosome. This cluster includes genes for approximately 30 ribosomal proteins (Jaskunas and Nomura, unpublished experiments; refs. 10 and 11), a gene for elongation factor EF-G (12, 13), and a second gene for elongation factor EF-Tu (*tufA*) (9). This cluster of genes is organized into four or more transcriptional units (see refs. 9 and 14).

We have now found that the cluster of genes at the *str-spc* region includes a gene for the α subunit of RNA polymerase. Furthermore, the α gene appears to be coordinately expressed and possibly contranscribed with genes for ribosomal proteins S11, S4, and L17.

METHODS

The isolations of λ*trkA*, λ*spc*1, λ*spc*2 (10), and λ*rif*^{d}18 (5) have been described. The isolation of λ*fus*2 (9, 11, 14) will be described elsewhere. λ*spc*2-Δ9 and λ*spc*2-Δ16 are deletion mutants of λ*spc*2 that were isolated by selecting citrate-resistant phages (see ref. 15) on which the *spc*^{s} gene of the original λ*spc*2 genome had been inactivated. The inactivation of the *spc*^{s} gene in these mutant phages is due to its deletion. However, both mutant phages still carry *trkA*$^{+}$ gene intact. They were purified and further characterized by the DNA fragments produced by digestion of phage DNA with *Eco*RI restriction endonuclease as described (16), by their ability to stimulate the synthesis of ribosomal proteins in UV-irradiated bacteria (10), and by heteroduplex analysis (17) to determine the size and position of the deletion. Their structure is given in Fig. 5.

UV-irradiated bacteria were infected with purified transducing phages in the presence of [^{35}S]methionine and the radioactive proteins were analyzed on sodium dodecyl sulfate ($NaDodSO_4$)-polyacrylamide gels (18) as described (9, 10).

Specific goat antisera against purified RNA polymerase and rabbit antisera against RNA polymerase subunit α were gifts from Dr. W. Zillig; rabbit antisera against purified EF-Tu and EF-G were gifts from Dr. H. Weissbach. Both goat anti-rabbit gamma globulin and rabbit anti-goat gamma globulin were purchased from Calbiochem, La Jolla.

RESULTS

We have identified several of the genes present on the *E. coli* chromosome at the *spc-str* region and at the *rif* region by observing the stimulation of the synthesis of the gene products in UV-irradiated bacteria after infection with transducing phages carrying bacterial DNA from these regions of the genome (7, 9–11). Bacterial DNA from the *str-spc* region is carried by λ*spc*1, λ*spc*2, and λ*fus*2, whereas DNA from the *rif* region is carried by λ*rif*^{d}18. Of the phages carrying DNA from the *str-spc* region, λ*fus*2 carries the largest substitution of bacterial DNA; about 93% of the λ chromosome has been deleted and substituted by 96.5% λ units (2.9×10^{7} daltons) of bacterial DNA, including the genes *aroE*, *trkA*, *fus*, *tufA*, and genes for approximately 30 ribosomal proteins, including *spcA* and *strA* (see refs. 9–11). λ*spc*2, λ*spc*1, and λ*trkA* carry smaller amounts of the bacterial DNA present on λ*fus*2 (see Fig. 5).

Stimulation of the synthesis of several proteins in UV-irradiated bacteria after infection with λ*spc*1, λ*fus*2, and λ*rif*^{d}18 can be seen in Fig. 1. For these experiments, the proteins synthesized after phage infection were labeled with [^{35}S]methionine and analyzed on $NaDodSO_4$-polyacrylamide gels. λ*rif*^{d}18 stimulated the synthesis of β′, β, and EF-Tu. However, it did not stimulate the synthesis of any protein with the mobility of α. On the other hand, λ*fus*2 stimulated the synthesis of EF-G, EF-Tu, and a protein with the mobility of α. Similar results were found when the proteins were analyzed on urea-polyacrylamide gels (data not shown). Stimulation of the synthesis of the α-size protein was also observed with λ*spc*1 [Fig. 1, gel 2; Fig. 4, gels (a) 4 and (b) 4] and λ*spc*2 [Fig. 4, gels (a) 6 and (b) 6]. Some of the ri-

Abbreviations: EF, elongation factor; $NaDodSO_4$, sodium dodecyl sulfate; kb, kilobase.

* This is paper no. 1892 from the Laboratory of Genetics.

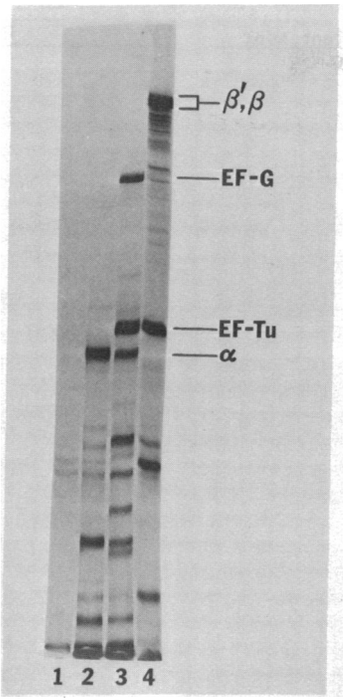

FIG. 1. Stimulation of the synthesis of proteins in UV-irradiated bacteria after infection with transducing phages. UV-irradiated bacteria, strain S159(λ), were infected with (*1*) λ*CI*857*S*7, (*2*) λ*spc*1, (*3*) λ*fus*2, and (*4*) λ*rif*^{d}18 in the presence of [^{35}S]methionine. The cells were lysed in NaDodSO$_4$ and analyzed by electrophoresis on 10% polyacrylamide gels containing NaDodSO$_4$ (9, 18). An autoradiogram of the dried gels is shown. The positions of β', β, EF-G, EF-Tu, and α on the gels, as determined from the staining of carrier reference proteins, are shown. In other experiments, the syntheses of both β' and β in λ*rif*^{d}18-infected cells were clearly demonstrated (unpublished experiments).

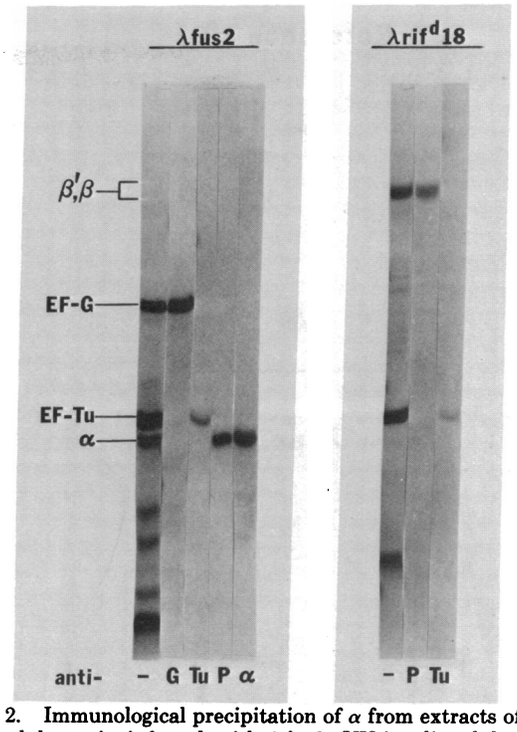

FIG. 2. Immunological precipitation of α from extracts of UV-irradiated bacteria infected with λ*fus*2. UV-irradiated bacteria were infected with λ*fus*2 and λ*rif*^{d}18 in the presence of [^{35}S]methionine. Proteins were precipitated from extracts of these cells using antisera to purified proteins as described (29). For each protein, antiserum was added to aliquots of the extract and the original antigen-antibody complex was precipitated with secondary precipitating antiserum (either goat anti-rabbit gamma globulin or rabbit anti-goat gamma globulin). The washed precipitates were electrophoresed with carrier reference proteins on NaDodSO$_4$-8.75% polyacrylamide gels in parallel with samples of the original untreated extracts. An autoradiogram of the dried gels is shown. Antisera to purified EF-G (G), EF-Tu (Tu), RNA polymerase holoenzyme (P), and α (α) were used as indicated. The positions of β' and β, EF-G, EF-Tu, and α, as determined from the stained gels and the mobility of the standards, are given on the left.

bosomal proteins whose synthesis was stimulated by λ*fus*2 can be seen as discrete bands below α in the gel shown in Fig. 1. Most of them migrated at the front and were not resolved by this gel system.

Evidence for the Identity of α. Several criteria have been used to prove that the radioactive protein with the mobility of α in Fig. 1 is α. The results of the experiment shown in Fig. 2 demonstrate that this protein had the antigenic determinants of α. In these experiments, extracts of the UV-irradiated cells infected with transducing phages in the presence of [^{35}S]methionine were incubated with various antisera prepared against purified proteins. The primary antigen-antibody complexes were precipitated with secondary precipitating antisera and electrophoresed on NaDodSO$_4$-polyacrylamide gels in parallel with samples of the original extracts. It can be seen from this figure that the α-sized protein was selectively precipitated with anti-α or anti-RNA polymerase while EF-G, EF-Tu, and β and β' were precipitated with anti-EF-G, anti-EF-Tu, and anti-RNA polymerase, respectively.

Results of a two-dimensional fingerprint of the tryptic digest of [^{35}S]methionine-labeled reference α and "α" synthesized in UV-irradiated bacteria after λ*spc*1 infection are shown in Fig. 3. The two fingerprints appear very similar. Lindahl and Nomura have also demonstrated the *in vitro* synthesis of α using λ*spc*1 DNA as template (unpublished experiments).

Location of the α Gene. The gene for α has been localized by comparing the ability of several transducing phages carrying only part of the bacterial DNA on λ*fus*2 to stimulate the synthesis of α in UV-irradiated bacteria. The structures of some of these phages are diagrammed in Fig. 5, and the results of the experiment are shown in Fig. 4. Both λ*spc*1 [gels (a) 4 and (b) 4] and λ*spc*2 [gels (a) 6 and (b) 6] stimulated the synthesis of α, as noted above, but λ*trkA* did not [gels (a) 3 and (b) 3]. A deletion mutant of λ*spc*2, λ*spc*2-Δ9, also stimulated the synthesis of α when the bacterial host for the experiment did not carry a λ prophage [gel (a) 7]. However, another deletion mutant, λ*spc*2-Δ16, did not [gels (a) 8 and (b) 8]. Thus we found that the α gene is present on λ*spc*1, λ*spc*2, and λ*spc*2-Δ9 in addition to λ*fus*2. The α gene must be on the bacterial DNA that is common to all four phages, which is the bacterial DNA to the left of the Δ9 deletion.

Phages λ*spc*1, λ*spc*2, and λ*fus*2 were isolated independently, and heteroduplex studies have indicated that they are homologous in the region of the α gene (unpublished experiments; cf. ref. 14). Thus the presence and location of the α gene on these phages almost certainly represents its position on the *E. coli* chromosome. From the relative position of the *trkA* and *spcA* genes on the *E. coli* chromosome (19) and the transducing phages, and knowing that Δ9 deletes the *spcA* gene, we conclude that the α gene is counterclockwise from *spcA*. Since λ*trkA* did not stimulate the synthesis of α, the gene for α is probably between *trkA* and *spc*.

The Δ16 deletion overlaps the Δ9 deletion and deletes ap-

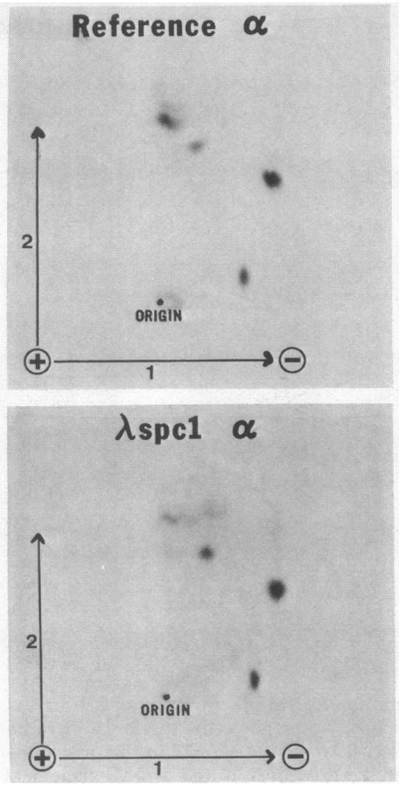

FIG. 3. Two-dimensional fingerprint of tryptic peptide from [^{35}S]methionine-labeled α. To obtain the reference α, RNA polymerase from strain S159(λ) grown in the presence of [^{35}S]methionine was isolated as described elsewhere (Gross, Engbaek, and Burgess, in preparation). The polymerase was electrophoresed on NaDodSO$_4$-polyacrylamide gels in parallel with samples of UV-irradiated cells infected with λ*spc*1 in the presence of [^{35}S]methionine. The α bands were cut out and digested with 1,1-tosylamido-2-phenylethylchloromethyl ketone-treated trypsin as previously described (7). The tryptic peptides were fingerprinted on silica gel thin-layer plates as described (30). The first dimension is at pH 4.7, 300 V for 3 hr. The second dimension is ascending chromatography with *n*-butanol/acetic acid/water (3:1:1). An autoradiogram is shown.

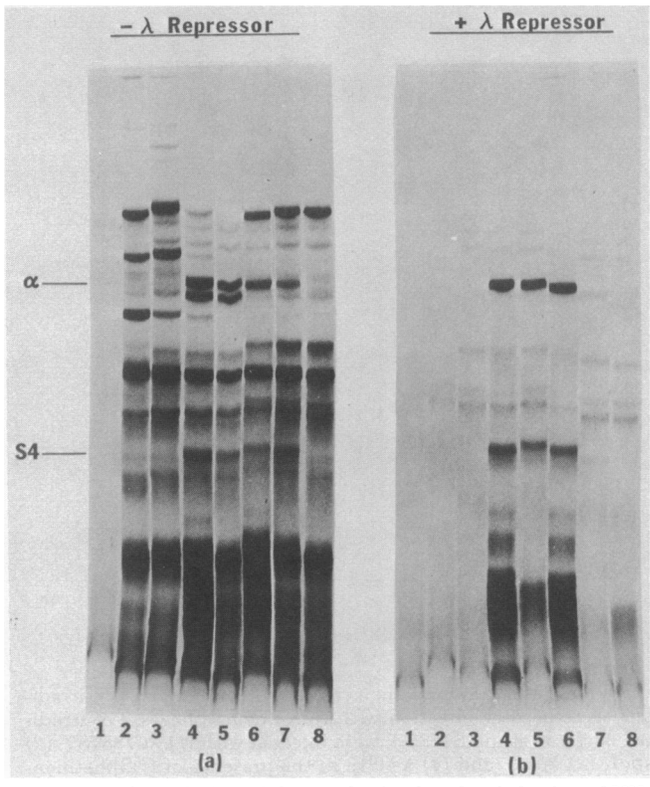

FIG. 4. Stimulation of the synthesis of α after infection of UV-irradiated bacteria with transducing phages. UV-irradiated bacteria were infected with (*1*) no phage, (*2*) λ*CI857S7*, (*3*) λ*trkA*, (*4*) λ*spc*1, (*5*) λ*spc*1-I16, (*6*) Δ*spc*2, (*7*) λ*spc*2-Δ9, and (*8*) λ*spc*2-Δ16 in the presence of [^{35}S]methionine and analyzed by NaDodSO$_4$-10% polyacrylamide gel electrophoresis. An autoradiogram of the dried gels is shown. The host for the "− λ Repressor" experiments (a) was S159 and the host for the "+ λ Repressor" experiments (b) was S159(λ). The bands for α and S4 are identified on the left. The identities of the α and S4 bands for the λ*spc*2 and λ*spc*2-Δ9 infected S159 cells were confirmed immunologically as described for Fig. 2.

proximately 1.5 kilobases (kb) of the DNA to the left of the Δ9 deletion, but not the *trkA* gene (Fig. 5). As mentioned above, the Δ16 deletion appears to eliminate the expression of the α gene. This could occur if Δ16 has deleted the α gene or some genetic element required for the expression of the α gene such as a promoter. But the bacterial promoter for the α gene appears to be deleted by Δ9 (see below). It appears that at least part of the α gene has been deleted by Δ16 and is located in the region that is covered by Δ16 but not by Δ9 deletion.

Although the precise locations of the left endpoints of Δ9 and Δ16 have not been determined, they are both within a 2.2 kb fragment called the "5%" fragment obtained after *Eco*RI digestion of λ*spc*2 DNA (data not shown; see Fig. 5). This fragment can be used as a template for the *in vitro* synthesis of S13, S11, and S4, and therefore it carries the genes for these three ribosomal proteins (Lindahl and Nomura, unpublished experiments; see ref. 20). The order of these genes is S13-S11-S4, in the direction of transcription, that is, from right to left (ref. 29, and the legend to Fig. 5). We have concluded that the α gene is to the left of the S4 gene since there is not room between the *Hin*dIII and the right *Eco*RI sites for the α gene in addition to the S13, S11, and S4 genes (see the legend to Fig. 5). This conclusion is consistent with the observation that besides eliminating the expression of α, the Δ16 deletion also abolishes the expression of S13, S11, and S4 genes (see Table 1).

We found that synthesis of L17 in UV-irradiated cells was stimulated by λ*spc*1, λ*spc*2, λ*fus*2, λ*spc*2-Δ9, and λ*spc*2-Δ16 but not by λ*trkA* (see Table 1 and ref. 10). The simplest interpretation of these results is that the L17 gene is to the left of the Δ16 deletion. Other ribosomal protein genes may also be left of the α gene. Therefore, the gene for the α-subunit of RNA polymerase appears to be intermixed with ribosomal protein genes.

Evidence for Cotranscription of the α Gene with Ribosomal Protein Genes. We found that the stimulation of the synthesis of α in UV-irradiated bacteria by λ*spc*2-Δ9 depended on whether the bacterial host contained a λ prophage [see Fig. 4, gels (a) 7 and (b) 7]. Synthesis was observed only when the host did not contain a λ prophage. λ*spc*2 stimulated the synthesis of α in both hosts. The presence of the λ prophage in the bacterial host conferred an immunity to these cells and few λ proteins were synthesized after infection with λ [compare gels (a) 2 and (b) 2 in Fig. 4]. These experiments indicate that Δ9 has deleted a genetic element required for the expression of the α gene and placed it under control of the λ repressor. Since the transcription of

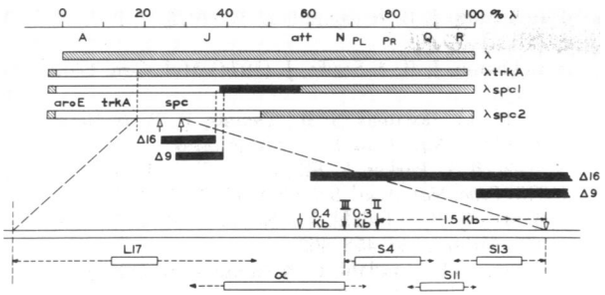

FIG. 5. The structure of various transducing phages and the location of the α gene on the λ*spc*2 chromosome. The location of the *Eco*RI fragment ("5% fragment") is shown by the two *Eco*RI sensitive sites (open arrows) that produce it. The regions representing λ DNA are hatched. The filled region in λ*spc*1 corresponds to the DNA present in λ*spc*2 but absent in λ*spc*1. The locations and the probable sizes of genes for α, S13, S11, S4, and L17 are indicated. The gene sizes were estimated from the molecular weights of the proteins (1, 31). As noted in the text, the 5% fragment carries the genes for S13, S11, and S4 in this order from right to left. The evidence for this order can be summarized as follows. λ*spc*2 stimulates the synthesis of S13, S11, and S4 in UV-irradiated bacteria, but λ*spc*2-Δ9 only stimulates the synthesis of S11 and S4 (see Table 1). Thus Δ9 appears to delete S13 but not S11 or S4, which indicates that S13 is to the right of S11 and S4. The positions of the *Hin*cII and *Hin*dIII restriction endonuclease sensitive sites in the 5% fragment were determined as indicated in the figure (arrows II and III, respectively). By examining the ability of several purified "restriction fragments" to code for S13, S11, and S4, it was found that the S11 gene is between the *Hin*cII site and the right *Eco*RI site, while the S4 gene is inactivated by the *Hin*cII, but not by the *Hin*dIII, cut (Lindahl and Nomura, unpublished experiments; see ref. 20). Therefore, the S4 gene is to the left of the S11 gene and to the right of the *Hin*dIII site. Since the distance between the *Hin*dIII and the right *Eco*RI sites is 1.8 kilobases (kb) and the sum of the sizes of the genes for S4, S11, and S13 is 1.4 kb, there is no room for α, whose size is 1.1 kb, to be located right of the *Hin*dIII site. For other arguments for the mapping, see the *text*.

the genes for α and the ribosomal proteins on λ*spc*2 is leftward (14), the simplest model to explain these results is that Δ9 has deleted the bacterial promoter for the α gene that is present on λ*spc*2 and "fused" the α gene with the lambda P_L promoter. In this model, the "normal" bacterial promoter for the α gene would be to the right of the genes for S4 and S11.

The Δ9 deletion has a similar effect on the genes for S11, S4, and L17, placing them under control of the λ repressor. As with α, we found that stimulation of the synthesis of these proteins by λ*spc*2-Δ9 only occurred in the absence of the λ repressor (Table 1). However, each could be synthesized by λ*spc*2 in the presence or absence of the λ repressor. Thus the α gene appears to be coordinately expressed and possibly cotranscribed with the genes for S11, S4, and L17. The gene for S13 may also be part of this unit.

We have previously isolated and characterized an IS2 insertion mutant of λ*spc*1, called λ*spc*1-I16 (14). This insertion is located 0.27 kb to the left of the bacterial-λ DNA junction in λ*spc*1. It inactivates most of the ribosomal protein genes on λ*spc*1 but not the genes for S13, S11, S4, and L17. The inactivated genes are located to the right of the S13 gene. We concluded that the inactivated genes probably belong to a single transcriptional unit, while the genes for S13, S11, S4, and L17 belong to another one or more transcriptional units (14). We have now found that the I16 insertion also appears to have little or no effect on the synthesis of α [compare gels (a) 4 and (a) 5, and gels (b) 4 and (b) 5 in Fig. 4]. This is consistent with the model that the α gene is in the same transcriptional unit as the genes for S11, S4, and L17, and the promoter for these genes is left of the gene cluster inactivated by the I16 insertion.

λ*spc*2-Δ16 stimulates the synthesis of L17 in the presence or absence of the λ repressor (Table 1). Thus the L17 gene appears to have a bacterial promoter in this phage mutant. From heteroduplex studies, we know that the right end of the Δ16 deletion is at a position corresponding to 0.42 kb to the left of the bacterial-λ DNA junction in λ*spc*1 (data not shown). This suggests that the bacterial promoter for the L17 gene in λ*spc*2-Δ16 is the promoter for the gene cluster inactivated by the I16 insertion in λ*spc*1.

DISCUSSION

We have identified a structural gene for the α-subunit of RNA polymerase in the *str-spc* region at 64 min on the *E. coli* chromosome. On the other hand, we failed to find a gene for α in λ*rif*^{d}18, which carries many bacterial genes in-

Table 1. Stimulation of synthesis of ribosomal proteins after infection of UV-irradiated bacteria with transducing phages

	− λ Repressor					+ λ Repressor				
Proteins	− λ	λ	λ*spc*2	λ*spc*2-Δ9	λ*spc*2-Δ16	− λ	λ	λ*spc*2	λ*spc*2-Δ9	λ*spc*2-Δ16
α	−	−	+	+	−	−	−	+	−	−
S4	0.02	0.11	10.3	10.5	0.27	0.02	0.02	9.1	0.64	0.02
S11	0.07	0.10	1.9	1.7	0.41	0.06	0.04	1.2	0.17	0.08
S13	0.13	0.91	16.6	1.3	0.85	0.09	0.09	11.7	0.10	0.13
L17	0.06	0.16	16.1	9.6	5.1	0.05	0.04	16.2	0.70	5.8

The bacterial hosts were prelabeled with [^{14}C]leucine, UV-irradiated, infected with phage, and labeled with [^{3}H]leucine between 30 and 40 min after phage infection. Ribosomal proteins were extracted and separated by two-dimensional polyacrylamide gel electrophoresis in the presence of carrier ribosomal proteins. The spots for the ribosomal proteins were located by staining and cut out; the ^{3}H/^{14}C ratio for each spot was determined. The experimental procedures were similar to those described (7, 10). The results for S13, S11, S4, and L17 are shown in the table. Those cases where there appeared to be significant stimulation of synthesis by infection with a transducing phage compared to infection with λ are underlined. The spot for S11 on the gel is a mixture of S11 and S9 (31). The observed increase in the ^{3}H/^{14}C ratios for this spot is presumably only a reflection of the stimulation of the synthesis of S11 since there does not appear to be a gene for S9 on any of the phages used in this experiment (refs. 10, 20, and unpublished experiments of Lindahl and Nomura). Therefore, the true ^{3}H/^{14}C ratios for S11 after stimulation of its synthesis is probably greater than the values given in the table. The host for the "− λ Repressor" experiments was S159; the host for the "+ λ Repressor" experiments was S159(λ). Results are shown for uninfected cells and cells infected with λ*CI857S7* ("λ"), λ*spc*2, λ*spc*2-Δ9, and λ*spc*2-Δ16. Results of the experiment shown in Fig. 4 for the synthesis of α are also given; + indicates stimulation of synthesis; − indicates no observable stimulation of synthesis.

cluding the genes for β' and β at 79 min. The α gene in the *str-spc* region appears to be coordinately expressed and possibly cotranscribed with the genes for ribosomal proteins S11, S4, and L17, and possibly S13. There are several possible ramifications of these results.

There are some mutations that apparently affect transcription of certain genes and map at the *str-spc* region. For example, Sunshine and Sauer isolated a mutant of *E. coli* C called *gro*$^-$ which blocks growth of phage P2 (21). The *gro* gene was mapped close to *spcA*. It is possible that such a mutation is in the α gene.

Dennis and Nomura have found that ribosomal protein genes, including genes for S4, S11 and L17, are under stringent control (22) and that, in general, this control is exercised at the level of transcription (23). Since the expression of the α gene appears to be coordinated with the expression of the genes for S11, S4, and L17, it is likely that the α gene is also under stringent control.

In general, our results raise the possibility that the amount of RNA polymerase and ribosomes in *E. coli* are coordinately regulated. However, while both increase with an increase in growth rate, ribosome content appears to be more sharply dependent on growth rate than core RNA polymerase content (24–26) and there appear to be more ribosomes in the cell than core RNA polymerase molecules (24–28). Recent evidence (Engbaek, Gross, and Burgess, manuscript in preparation) indicates that the molar ratio of α to β' is 5–7 and not 2 as would be expected from the stoichiometry of purified core RNA polymerase. Thus, it is conceivable that the synthesis of α could be regulated independently of the synthesis of β and β'. However, Iwakura *et al.* found that the relative ratios of the core subunits were independent over the growth rate range examined (26). They concluded that the α, β, and β' subunits were synthesized coordinately. Further experiments are needed to determine whether the syntheses of ribosomes and RNA polymerase subunits are coordinately regulated.

We thank Drs. H. Weissbach and W. Zillig for providing us with antisera; Dr. J. B. Kirschbaum for λ*rif*^{d}18; and H. Ahrens, K. Ryan, L. Sadowski, and G. Strycharz for technical assistance. This work was supported in part by the College of Agriculture and Life Sciences, University of Wisconsin, and by grants from the United States National Science Foundation (GB-31086) and the National Institutes of Health (GM-20427) (administered by M.N.) and the National Institutes of Health (CA-07175) (administered by R.R.B.).

1. Burgess, R. (1971) *Annu. Rev. Biochem.* **40,** 711–740.
2. diMauro, E., Synder, L., Marino, P., Lamberti, A., Coppo, A. & Tocchini-Valentini, G. P. (1969) *Nature* **222,** 533–537.
3. Heil, A. & Zillig, W. (1970) *FEBS Lett.* **11,** 165–168.
4. Errington, L., Glass, R. E., Hayward, R. S. & Scaife, J. G. (1974) *Nature* **249,** 519–522.
5. Kirschbaum, J. B. & Konrad, E. B. (1973) *J. Bacteriol.* **116,** 517–526.
6. Kirschbaum, J. B. & Scaife, J. (1974) *Mol. Gen. Genet.* **132,** 193–201.
7. Lindahl, L., Jaskunas, S. R., Dennis, P. P. & Nomura, M. (1975) *Proc. Nat. Acad. Sci. USA*, in press.
8. Watson, R. J., Parker, J., Fiil, N. P., Flaks, J. G. & Friesen, J. (1975) *Proc. Nat. Acad. Sci. USA*, **72,** 2765–2769.
9. Jaskunas, S. R., Lindahl, L., Nomura, M. & Burgess, R. R. (1975) *Nature*, **257,** 458–462.
10. Jaskunas, S. R., Lindahl, L. & Nomura, M. (1975) *Proc. Nat. Acad. Sci. USA* **72,** 6–10.
11. Nomura, M. & Jaskunas, S. R. (1975) in *Control of Ribosome Synthesis, Proc. for Alfred Benzon Symp. IX*, eds. Kjeldgaard, N. O. & Maaløe, O. (Munksgaard, Copenhagen, Denmark), in press.
12. Tocchini-Valentini, G. P. & Mattoccia, E. (1968) *Proc. Nat. Acad. Sci. USA* **61,** 146–151.
13. Kuwano, M., Schlessinger, D., Rinaldi, G., Felicetti, L. & Tocchini-Valentini, G. P. (1971) *Biochem. Biophys. Res. Commun.* **42,** 441–444.
14. Jaskunas, S. R., Lindahl, L. & Nomura, M. (1975) *Nature* **256,** 183–187.
15. Smith, G. R. (1971) *Virology* **45,** 208–223.
16. Tanaka, T. & Weisblum, B. (1975) *J. Bacteriol.* **121,** 354–362.
17. Davis, R. W., Simon, M. & Davidson, N. (1971) in *Methods in Enzymology*, eds. Grossman, L. & Moldave, K. (Academic Press, New York), Vol. 21, pp. 413–428.
18. Laemmli, U. K. (1970) *Nature* **227,** 680–685.
19. Taylor, A. L. & Trotter, C. D. (1972) *Bacteriol. Rev.* **36,** 504–524.
20. Lindahl, L. & Nomura, M. (1975) in *Control of Ribosome Synthesis, Proc. for Alfred Benzon Symp. IX*, eds. Kjeldgaard, N. O. & Maaløe, O. (Munksgaard, Copenhagen, Denmark), in press.
21. Sunshine, M. G. & Sauer, B. (1975) *Proc. Nat. Acad. Sci. USA* **72,** 2770–2774.
22. Dennis, P. P. & Nomura, M. (1974) *Proc. Nat. Acad. Sci. USA* **71,** 3819–3823.
23. Dennis, P. P. & Nomura, M. (1975) *Nature* **255,** 460–465.
24. Matzura, H., Hansen, B. S. & Zeuthen, J. (1973) *J. Mol. Biol.* **74,** 9–20.
25. Dalbow, D. G. (1973) *J. Mol. Biol.* **75,** 181–184.
26. Iwakura, Y., Ito, K. & Ishihama, A. (1974) *Mol. Gen. Genet.* **133,** 1–23.
27. Skjold, A. C., Juarez, H. & Hedgcoth, C. (1973) *J. Bacteriol.* **115,** 177–187.
28. Kjeldgaard, N. O. & Gausing, K. (1974) in *Ribosomes*, eds. Nomura, M., Tissières, A. & Lengyel, P. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), pp. 369–392.
29. Roberts, J. W. & Roberts, C. W. (1975) *Proc. Nat. Acad. Sci. USA* **72,** 147–151.
30. Kahan, L., Zengel, J., Nomura, M., Bollen, A. & Herzog, A. (1973) *J. Mol. Biol.* **76,** 473–483.
31. Wittmann, H. G. (1974) in *Ribosomes*, eds. Nomura, M., Tissières, A. & Lengyel, P. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.), pp. 93–114.