# LOCALIZATION OF THE STRUCTURAL GENE FOR THE β' SUBUNIT OF RNA POLYMERASE IN ESCHERICHIA COLI

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## SUMMARY

A minicell-producing strain of E. coli carrying an F' factor, KLF10-1, forms minicells that contain plasmid but not chromosomal DNA. These minicells were found to synthesize two polypeptides corresponding precisely to the β and β' subunits of RNA polymerase in SDS-polyacrylamide gel electrophoresis. In contrast, minicells obtained from an isogenic strain carrying F13-1 do not synthesize these proteins under similar conditions. These results indicate that the structural genes for the β' as well as β subunits of the polymerase are located on the chromosomal segment (78 to 81 min on the standard genetic map of E. coli) carried by KLF10-1.

Genetic and enzymatic studies of mutants resistant to antibiotics that specifically bind to RNA polymerase have revealed that the structural gene for the β subunit of this enzyme is located between argH and purD on the genetic map of Escherichia coli (see reviews, 1 and 2). However, little is known about the location of the genes for other subunits, except for the indirect evidence that might be taken to suggest the close linkage between the structural genes for the β and β' subunits (3, 4). In an attempt to locate the structural gene for the β' subunit by more direct experiments, we have taken advantage of the facts that a minicell-producing strain (5) of E. coli carrying certain plasmids forms minicells containing plasmid but not chromosomal DNA (6-11), and that these minicells are capable of synthesizing proteins specifically coded by the plasmid DNA (10, 11). The exceptionally large molecular size of the β and β' subunits also permits the identification of those polypeptides among other proteins in crude extracts by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) (3). The results presented below strongly suggest that the structural genes for the β' as well as β subunits are located between 78 and 81 min on the standard genetic map of E. coli (12).

## MATERIALS AND METHODS

The minicell-producing $F^-$ strain used in this study (KY1381) carries the argH

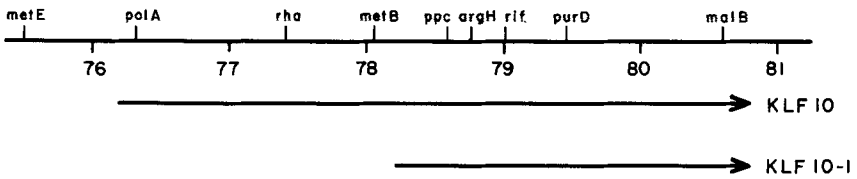



Fig. 1. Genetic map of the rif region covering the intervals, 76 to 81 min, of the E. coli chromosome (12). The lower lines show the extents of the region carried by the F' factors. rif stands for resistance to rifamycins and represents the structural gene for the $\beta$ subunit of RNA polymerase.

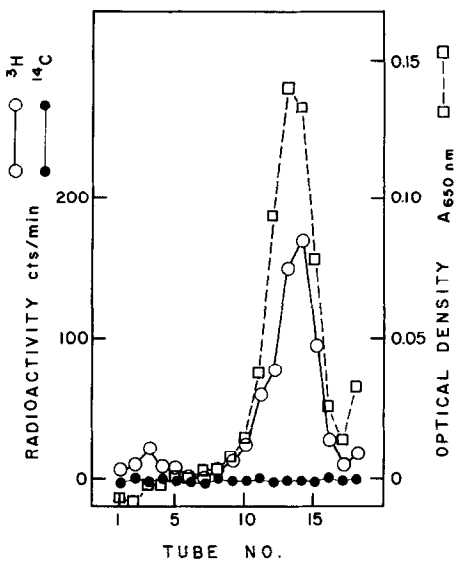



Fig. 2. Segregation of F' factor (KLF10-1) DNA into minicells. Optical density and radioactivity profiles of minicells after the fourth sucrose gradient centrifugation (20 min at 5,000 rev./min) are presented. A mixture of cultures (50ml each) of the F' (KY1385) and F$^-$ (KY1381) strains that had been grown with $^3$H- and $^{14}$C-thymidine, respectively, were used for purification of minicells. The medium contained $^3$H-thymidine (20$\mu$c/10$\mu$g/ml) or $^{14}$C-thymidine (0.5$\mu$c/10$\mu$g/ml) and 250$\mu$g/ml of deoxyadenosine. Treatment with pancreatic deoxyribonuclease (40$\mu$g/ml, 37$^o$C, 1 hr) was carried out after the first sucrose gradient centrifugation. Other procedures are as described in Methods. Total viable cells in this gradient were less than 50. The radioactivities represent those incorporated into 5% trichloroacetic acid-insoluble materials per 0.4ml aliquots. The $^3$H/$^{14}$C ratio for the starting material was 6.4.

and metB mutations that had been introduced into the original minicell producer (P678-54; ref. 5) by crossing to an appropriate Hfr strain. It also requires leucine for growth. Two F' strains (KY1385 and KY1386) were obtained by transferring the F' factor, KLF-10-1 or F13-1, respectively, into the F$^-$ strain by conjugation. KLF10-1 has been derived from KLF10 (13, 14) and was shown to carry the chromosomal genes extending from malB$^+$ to ppc$^+$, but not to metB$^+$ or rha$^+$ (Fig. 1). F13-1 was isolated by H. Ozeki

(person. commun.) from F13 (15); it carries the lac$^+$ genes but not phoA$^+$ or purE$^+$.

Cultures were grown overnight in 1 to 4 liters of medium ML (16) containing 0.5% Difco Casamino Acids, 2μg/ml of thiamine and 0.5% glucose with vigorous shaking at 37°C. Appropriate dilutions of all F' cultures used were plated on nutrient agar, and the colonies that appeared were checked for their ability to transfer the F' factor into an F$^-$ recA strain by the replica-plating method. In every case, more than 99% of the colonies tested proved to be F' by this criterion.

Minicells were separated from ordinary cells by two cycles of low and high speed centrifugations, followed by three or four cycles of sucrose gradient centrifugations employing 5 to 20% sucrose in 30ml of buffered saline with gelatin (BSG) in a Spinco SW25 rotor at 5,000 rev./min for 30 min, unless otherwise indicated. The purified minicell preparations contained less than 1 viable cell per $10^7$ minicells.

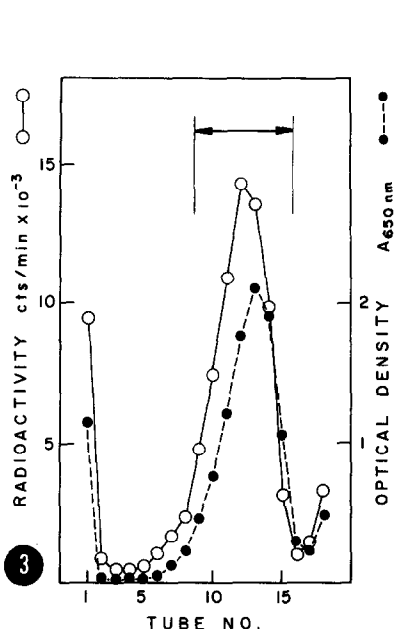
Thymidine-6-$^3$H, thymidine-2-$^{14}$C, and L-amino acid-$^3$H(G) mixture (NET250) used were the products of Radiochemical Centre (Amersham), Dai-ichi Pure Chemicals Co. (Tokyo), and New England Nuclear Corp. (Boston), respectively.

## RESULTS AND DISCUSSION

### Segregation of F' DNA into minicells

It was first determined whether the F' factor (KLF10-1) segregates into minicells, as has been found with strains carrying other plasmids. An F' strain harboring KLF-10-1 that carries the structural gene for the β subunit of RNA polymerase, was grown in the presence of $^3$H-thymidine to label the DNA. An isogenic F$^-$ strain was similarly grown with $^{14}$C-thymidine, under otherwise the same conditions. After confirming the minicell production under microscope (minicell/cell = about 1 for both strains), the two cultures were mixed, harvested, and then used for purification of minicells. Figure 2 shows the profiles of the fourth sucrose gradient centrifugation during this purification. The preferential enrichment of $^3$H-labeled DNA in the minicell fractions (the $^3$H/$^{14}$C ratio was more than 100 as compared to 6.4 for the initial mixture) suggests that minicells derived from the F' but not F$^-$ culture contain appreciable amounts of DNA.

When DNA of the minicells as well as the cells was examined by alkaline sucrose gradient centrifugation, the fraction of radioactivity found in the region of covalently closed circular DNA was much higher for minicells than for cells, although the yield was not as high as expected. These results indicate that DNA of the F' factor, but not of the chromosome, segregates into minicells, as was reported by other workers for F8 (7) and other plasmids (6, 8-11). On the basis of several assumptions, the fraction of minicells containing F' DNA has been estimated to be 2 to 4%.



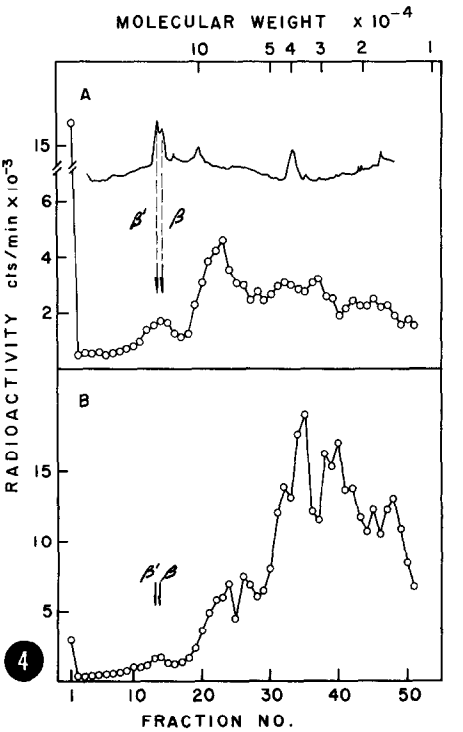




Fig. 3. Amino acid incorporation into minicells obtained from strain KY1385 carrying KLF10-1. Minicells (total $A_{650}$ = 25) obtained from 4 liters of culture after three cycles of sucrose gradient centrifugations (1 viable cell per 2 x $10^7$ minicells) were used. Incubation was carried out in 20ml of medium ML supplemented with 0.5% glucose, 0.1mc/ml of synthetic mixture of fifteen $^3$H-L-amino acids, and 10$\mu$g/ml each of unlabeled leucine, methionine, cystein and tryptophan. After shaking 90 min at 37°C, minicells were collected by centrifugation, washed in medium with 0.2% Casamino Acids, layered on a sucrose gradient and then centrifuged at 5,000 rev./min for 20 min. Aliquots (20$\mu$l) of each fraction were used to determine the radioactivity in 5% trichloroacetic acid-insoluble meterials. Arrows indicate the fractions that were pooled to be used for experiments shown in Figures 4 and 5.

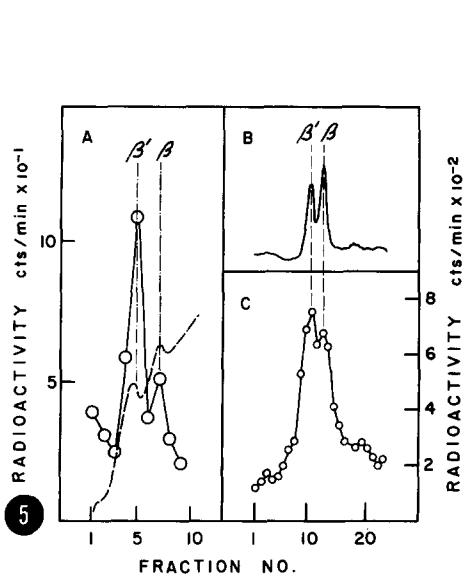
Fig. 4. SDS-polyacrylamide gel electrophoresis patterns of proteins synthesized by minicells (A) or cells (B) from strain KY1385 carrying KLF10-1 (obtained by the experiment of Fig. 3). Purified minicells and cells were disrupted by sonication in 0.5% SDS, centrifuged and the supernatants obtained were treated at 37°C for 20 min. Samples were then applied onto 5% polyacrylamide gel containing 0.1% SDS, according to the procedure of Shapiro et al. (18). Electrophoresis was run at 5mA for 8 hrs at room temperature. Gels were cut into 2mm-thick pieces, treated with 0.5ml of 30% $H_2O_2$ at 60°C for 3 hrs, and the radioactivities determined in Bray scintillation mixture. Purified RNA polymerase and 50 S ribosomes of E. coli were run simultaneously as references to calculate the molecular weight scale indicated. The densitometer tracing represents an optical density profile of purified RNA polymerase run simultaneously with other gels and stained by Coomassie Brilliant Blue. (A) Minicells (2 x $10^5$ cts./min, $A_{650}$ = 0.8) (B) Cells (1.1 x $10^6$ cts./min, $A_{650}$ = 0.48)

Protein synthesis in minicells containing KLF10-1

Purified minicells from the same F' strain were then tested for their capacity to synthesize proteins. After incubating them in the medium with $^{3}$H-amino acids for 90 min, minicells were collected, washed, and then examined by sucrose gradient centrifugation. The results shown in Fig. 3 indicate that minicells containing KLF10-1 indeed synthesize fair amounts of protein under these conditions. As a control, cells obtained from the pellets of the first sucrose gradient centrifugation were also incubated under similar conditions, except that the amount of $^{3}$H-amino acids used was one tenth of that for minicells. The ratio of acid-insoluble radioactivity incorporated into a minicell and a cell was calculated to be about 0.3%, on the basis of several experiments. This value seems to be not inconsistent with the amounts of F' DNA present in the minicell preparation used. In contrast, minicells obtained from the $F^{-}$ strain did not show significant levels of incorporation. Protein synthesis with the F' minicell preparation was also inhibited by rifampicin, suggesting that it depends on messenger RNA synthesized in the minicells rather than that segregated into minicells when they are produced.

To see whether the $\beta$ and $\beta'$ subunits of RNA polymerase are found among the proteins synthesized, extracts of the radioactive minicells and cells were examined by polyacrylamide gel electrophoresis in the presence of 0.1% SDS. Purified RNA polymerase was always run simultaneously as a reference to locate the two subunits. As can be seen in Fig. 4B, about 0.7% (or less, in view of the results reported in ref. 17) of the labeled proteins synthesized by cells seems to represent the $\beta$ and $\beta'$ subunits of RNA polymerase. In contrast, much higher fraction (about 7%) of the radioactivity was found in this region among proteins synthesized by minicells (Fig. 4A). The overall protein profile for minicells is also quite different from that for cells. The comparison between the two profiles in the $\beta$-$\beta'$ region, together with distribution of the reference protein peaks suggests that both the $\beta$ and $\beta'$ subunits are synthesized in minicells as well as in cells, and this has been confirmed by closer analysis of this region after prolonged electrophoresis.

In these experiments, purified RNA polymerase was added to each extract as an internal reference before running gel electrophoresis. The gels were first stained with Coomassie Brilliant Blue to locate the $\beta$ and $\beta'$ subunits, and were then cut into pieces for radioactivity determination. Figure 5 shows the results of one such experiment. It can be seen that two radioactivity peaks corresponding precisely to the two polymerase subunits were found with the extracts of minicells as well as of cells. The rather low recovery of radioactivity in this experiment (Fig. 5A) is partly due to



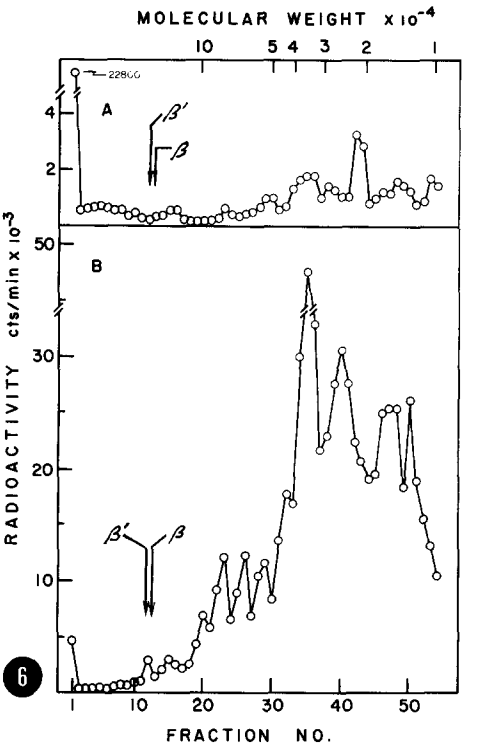




Fig. 5. SDS-polyacrylamide gel electrophoresis patterns of the $\beta$-$\beta'$ region of proteins synthesized by minicells (A) or cells (C) from strain KY1385 carrying KLF10-1. Procedures and conditions are as in Fig. 4, except that purified RNA polymerase (7.5$\mu$g) was added to each sample as an internal reference, and electrophoresis was run at 4mA for 27 hrs. Gels were stained to locate the $\beta$ and $\beta'$ bands, cut into pieces, and were treated either by a Teflon homogenizer with 0.1% SDS (A) or by $H_2O_2$ (C). The densitometer tracing (broken line) has been superimposed on the radioactivity profile (A). (A) Minicells ($5 \times 10^5$ cts./min, $A_{650} = 1.7$) (B) The densitometer tracing of purified RNA polymerase (2$\mu$g) run in a separate gel. (C) Cells ($1.1 \times 10^6$ cts./min, $A_{650} = 0.48$)

Fig. 6. SDS-polyacrylamide gel electrophoresis patterns of proteins synthesized by minicells (A) or cells (B) from strain KY1386 carrying F13-1. See legends to Fig. 4 for procedures and conditions. (A) Minicells ($2 \times 10^5$ cts./min, $A_{650} = 0.2$) (B) Cells ($1.1 \times 10^6$ cts./min, $A_{650} = 0.42$)

the low efficiency of protein elution employed. Similar results were obtained in several independent experiments using different preparations of minicells, but the relative amounts of radioactivity associated with the two peaks varied in different experiments. These results strongly suggest that the structural genes for the $\beta$ and $\beta'$ subunits are located on the F' factor, KLF10-1, and that they are active in making specific protein products in the minicells, although the products may not be as stable as those synthesized by ordinary cells.

Protein synthesis in minicells containing F13-1

To further substantiate the above conclusions, a series of control experiments was carried out with other F' strains harboring an F' factor that carries a different segment of the E. coli chromosome. The results obtained with a strain carrying F13-1 are shown in Fig. 6. It is apparent that no significant amounts of $\beta$ and $\beta'$ subunits are produced in minicells obtained from this F' strain, despite the fact that the total amount of protein made is not very different from that in minicells containing KLF10-1. The marked difference in protein profiles between minicells from the two F' strains (Fig. 4A and 6A) presumably reflects the difference in the chromosomal segments carried by the two F' factors. The parallel experiment with cells of the same strain revealed the production of the two polymerase subunits as expected (0.4% of total proteins) (Fig. 6B).

All the results presented above lead us to tentatively conclude that the structural genes for the $\beta'$ as well as the $\beta$ subunit of RNA polymerase are located on the chromosomal segment carried by KLF10-1. The possibility that the protein that we have tentatively identified as the $\beta'$ subunit represents the "$\beta'$-like protein" unrelated to the polymerase (17) cannot be excluded at present, but seems to be unlikely in view of the relatively short chromosomal segment carried by the F' factor employed. Obviously, further experiments are required to map the $\beta'$ gene more precisely, and studies along this line are in progress in our laboratory. The present system may also provide the useful means for locating the structural genes for other subunits of RNA polymerase, particularly when combined with immunochemical or other methods for analysis of specific proteins.

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