Effect of multiple copies of *rpoBC* on the rate of RNA synthesis in *Escherichia coli*

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Summary

The cloning of the *rpoB* and *rpoC* genes in a high copy number vector in *E. coli* increased the amount of the encoded gene products, the β and β' subunits of RNA polymerase. However, this unexpectedly caused a 30–50% decrease in RNA synthetic activity which alternatively induced a reduction of growth rate and enlargement of cell size, and decreased the DNA replication time. The results can be explained by autogenous regulation of the RNA polymerase genes by the $\beta\beta'$ subunits. A relation between the decrease in number of transcription units and the observed higher rate of movement of DNA replication forks is discussed.

1. Introduction

RNA polymerase is a central component in gene expression and is structurally one of the most complex enzymes in *E. coli*. It is a hetero-oligomer of four subunits, designated α (present in two molecules per polymerase enzyme), β and β'. The amount of β and β' subunits limits the amount of RNA polymerase since the cell contains an excess of the α subunit (Iwakura *et al.*, 1974).

The close metabolic ties which exist between transcription and translation are reflected in the organization of genes encoding the subunits of RNA polymerase. The α subunit is coded by gene *rpoA*, and is expressed in a transcription unit with four ribosomal protein genes (Yura & Ishihama, 1979) in a gene cluster of 30 genes for ribosomal proteins and stable RNA located at min 73 on the genetic map (Bachman, 1983). The β and β' subunits, the products of genes *rpoBC* located at min 90, are cotranscribed with ribosomal protein genes *rplJ* and *rplL*. The transcription of the β operon is complicated. Four promoters and a transcriptional attenuator have been identified. The major promoter of this operon is upstream of *rplJ* (p_{L10}) and by itself is sufficient for high level expression of these genes. There are also three internal promoters, one preceding *rplL*, another before *rpoB* and a third before *rpoC*. A translational attenuator is located between *rplL* and *rpoB* which terminates *ca.* 80% of the transcriptions initiated at the preceding promoters and accounts for the lower frequency of *rpoBC* transcription compared with that of *rplJL* (Barry *et al.*, 1979; Barry *et al.*, 1980; Blumenthal & Dennis, 1980;

Newman & Hayward, 1980; Ralling & Linn, 1984). Although the internal promoters contribute little to the expression of their downstream genes they may have a physiological importance in specific regulation of these genes. Synthesis of ribosomal proteins and RNA is subject to stringent control whereas expression of RNA polymerase genes is not under such control (Kajitani & Ishihama, 1984; Maher & Dennis, 1977; Yura & Ishihama, 1979). The rate of synthesis of ribosomal proteins, relative to the rate of synthesis of total protein, is known to increase with growth rate, but the relative rate of synthesis of the β and β' subunits remains constant with increasing growth rate (Ralling *et al.*, 1985). Restriction of RNA polymerase activity dissociates the coordinate transcription of the genes of increasing the recognition of the *rpoB* promoter or by readthrough of the *rplL-rpoB* attenuator (Blumenthal & Dennis, 1980; Little *et al.*, 1981). Under this restrictive condition an increased synthesis of β' has been attributed to the recognition of the *rpoC* promoter (Blumenthal & Dennis, 1980). Rifampicin induces synthesis of all polymerase subunits, but not ribosomal proteins (Hayward & Fyfe, 1978*b*). On the other hand, increased amounts of RNA polymerase inhibit the synthesis of β and β' at the transcriptional and post-transcriptional level (Lang-Yang & Zubay, 1981; Little *et al.*, 1981). The simplest mechanism to explain this non-coordinate synthesis of RNA polymerase subunits seems to be autogenous regulation (Kajitani *et al.*, 1980; Yura & Ishihama, 1979) in which one or more polymerase subunits have been proposed as regulatory molecules. Relatively little is known about the control of *rpoA* and its coordination
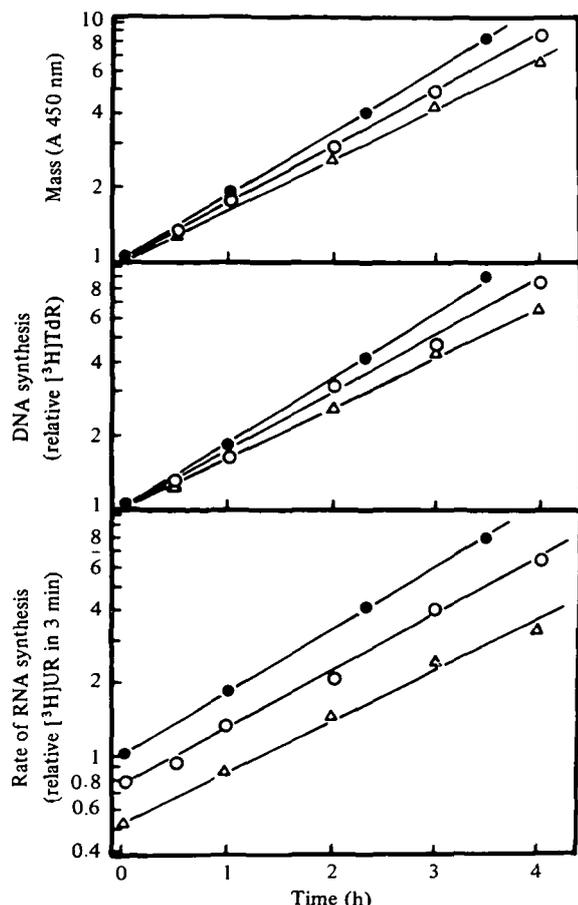
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Fig. 1. Mass increase of exponentially growing cells (*a*), DNA synthesis (*b*) and rate of RNA synthesis (*c*) in LE234 (●), LE234/*rpoB* (○) and LE234/*rpoBC* (△), relative to values of LE234 at time 0.

with the *rpoBC* operon, although they must in some way be linked since total enzyme is positively correlated with rate of growth.

2. Materials and Methods

Escherichia coli LE234 (*metB argH ilv thi*) and CO113 (*metB*) are from the Dept. of Genetics, University of Leicester (U.K.). TAU (*arg met pro trp ura thyA*) is a derivative of strain 15T-. Bacteria were incubated at 30 °C in M9 minimal medium supplemented with requirements, and growth rate was determined by mass doublings/hour. The rate of RNA synthesis was determined by acid precipitable [³H]uridine incorporated in 3 min in aliquots of 0·5 ml of culture. β and β' were measured by polyacrylamide gel electrophoresis of a ³⁵S-labelled exponential culture and gel fragments were counted by liquid scintillation. Average number of replication forks (*n*) and the time required to replicate the whole chromosome (*C*) were measured according to Pritchard & Zaritsky (1970), inhibiting new DNA initiation cycles by adding 100 μg/ml of rifampicin and measuring accumulated DNA relative to the amount present of the time of addition of the drug (Δ*G*).

pJLB1 and pNF1493 are pBR322 plasmids carrying the *Bgl*II fragment of the operon which includes genes *rplJ*, *rplL* and *rpoB*. pLBC1 and pNF1310 are pBR322 plasmids carrying the *Hind*III fragment which includes genes *rplL*, *rpoB* and *rpoC* (Fiil *et al*, 1979; Ishihama & Fukuda, 1980). Plasmid isolation and bacterial transformation were carried out as described (Maniatis *et al*., 1980).

3. Results and Discussion

When bacteria were transformed with plasmids carrying *rpoBC*, a threefold increase in the amount of β and β' polypeptides was observed (data not shown). In contrast, the presence of *rpoBC*, and to a much lesser extent *rpoB* alone, on a multicopy plasmid, had a negative effect on the activity of RNA synthesis as shown by the decrease in its rate (Fig. 1*c*, Table 1). This reduction in RNA polymerase activity seemed to bring about a decrease in growth rate (Fig. 1*a*, Table 1) and to cause an enlargement of cell size (Fig. 2). In spite of these effects, strains carrying *rpoBC* plasmids were capable of steady growth (Fig. 1).

The rate of DNA synthesis decreased to the same degree as the growth rate but the average number of replication forks per chromosome (*n* in Table 1) diminished to 30% which gives, for the relationship $C = n\tau$ (Pritchard & Zaritsky, 1970), a replication time around 13% shorter than in parental strains (Table 1).

These results show that an increase in β and β' subunit synthesis brought about by an increase of their gene dosage unexpectedly reduced the total RNA synthetic activity, notwithstanding the excess of α subunit in normal growing cells. This result suggests that these subunits might inhibit the activity of RNA polymerase or, which is more likely, the excess of β and β' subunits reduced the expression of RNA polymerase genes. Plasmids pJLB1 and pNF1493, which carry the strong promoter p_{L10}, have a much higher expression of the *rpoB* gene than pLBC1 and pNF1310 (Fiil *et al*., 1979) but exhibit little or no restriction of RNA synthesis. This suggests that β', or the aggregate of this subunit with β, is an autogenous repressor of *rpo* genes. This rise in repressor activity will limit the amount of the *rpoA* gene product and accordingly the number of RNA polymerase enzymes will be diminished.

Hayward & Fyfe (1978*a*) observed that a clone diploid for *rpoBC* degrades part of the excess of β and β' subunits and synthesizes α at the same rate as the haploid. The high dosage used in our work apparently produced an amount of β and β' subunits that is sufficient to 'escape' total degradation, giving rise to the repressor activity. Inhibition in the total RNA polymerase activity could also be accounted for by reduction in σ subunit through degradation with β or β', but this suggestion is less plausible because higher amounts of β subunit are synthesized in the *rpoB*
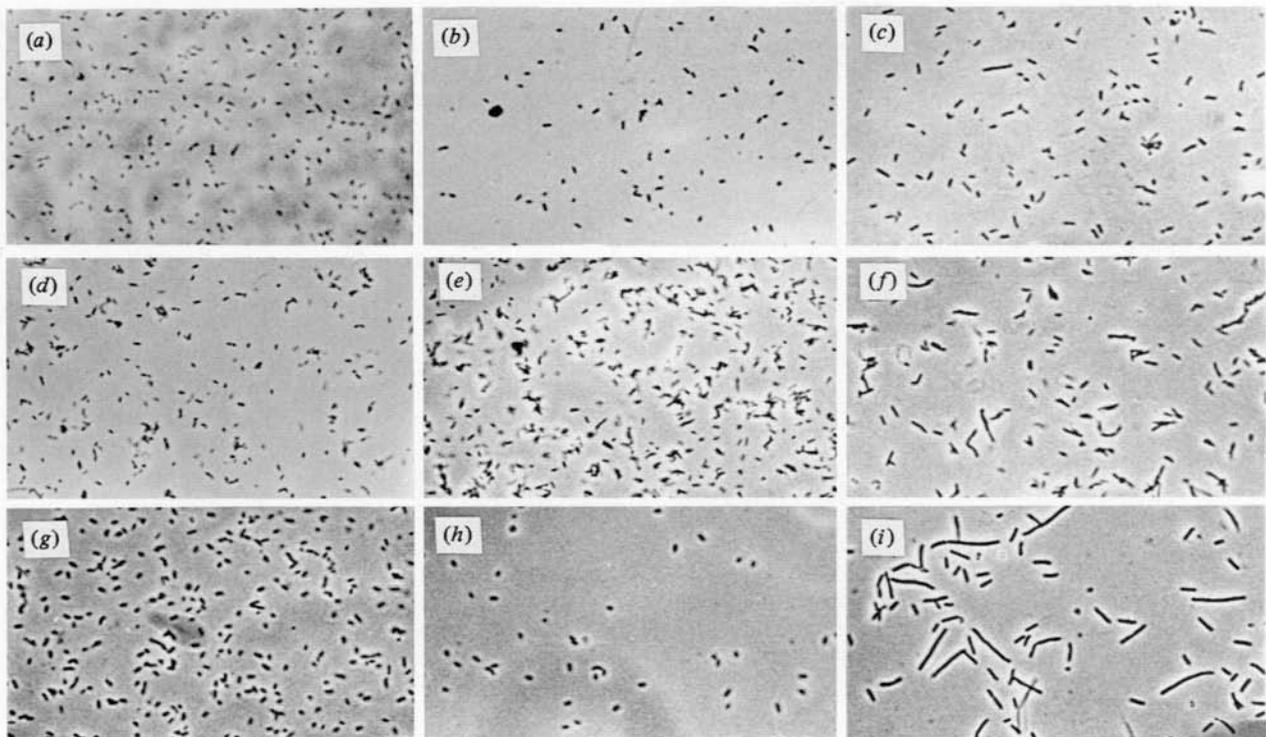
Fig. 2. Light micrographs of mid log-phase *E. coli* cells growing in M9 at 30 °C. (*a*), LE234; (*b*), LE234/*rpoB*; (*c*), LE234/*rpoBC*; (*d*), CO113; (*e*), CO113/*rpoB*; (*f*), CO113/*rpoBC*; (*g*), TAU; (*h*), TAU/*rpoB*; (*i*), TAU/*rpoBC*.

Table 1. *RNA synthetic activity, growth rate and DNA replication data in strains with or without* rpoBC *plasmids.*

Strain	RNA synthesis		Growth rate (doublings/h)	DNA replication		
	kcpm/ absorbance	Relative to parental		ΔG (%)	n	C (min)
LE234	187	1	0·85	55	1·37	96
LE234/rpoB	146	0·78	0·83	—	—	—
LE234/rpoBC	102	0·54	0·68	35	0·91	79
CO113	181	1	0·92	72	1·73	112
CO113/rpoB	166	0·92	0·89	—	—	—
CO113/rpoBC	124	0·69	0·75	50	1·26	101
TAU	189	1	0·92	—	—	—
TAU/rpoB	187	0·99	0·92	—	—	—
TAU/rpoBC	139	0·74	0·67	—	—	—

clones (Fiil *et al.*, 1979) without concomitant polymerase inhibition.

It is worth noting that rifampicin, which inhibits transcription by binding to the β subunit, increases the rate of synthesis of all *rpo* genes (Hayward & Fyfe, 1978*b*), and a mutation in *rpoC* which synthesizes a thermosensitive β' subunit gives rise to a rapid increase in the transcription of all *rpo* genes when the strain is shifted from 30° to 39 °C (Little & Dennis, 1979; Little *et al.*, 1981). Although these observations have been interpreted in terms of autogenous regulation by the core polymerase, they fit in with our suggestion that β' or β and β' are directly involved in modulating expression of all *rpo* genes. These conclu-

sions do not exclude the proposed autogenous regulation of the *rpo* genes by the core enzyme (Kajitani *et al.*, 1980; Yura & Ishihama, 1979).

As was also shown, the average number of replication forks in the strains which carry multiple copies of *rpoBC* was diminished more than expected by the increase in mass doubling time which means a shortening in the replication time of the chromosome. This shortening of the replication cycle suggests an increase in the rate of movement of DNA replication forks, due most likely to the lack of obstruction of fork movement in a chromosome with few transcription complexes. On the other hand, transcription complexes transiently immobilized on the chromosome present

a temporary obstacle to the replication advance, resulting in a decrease in the rate of movement of the replication machinery (Pato, 1975).

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