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Transcription of Ribosomal Genes during a Nutritional Shift-Up of *Escherichia coli*

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We measured the differential transcription rates of individual ribosomal operons after a nutritional shift-up. All operons showed a transient increase in transcription. However, the response of the S10 ribosomal protein operon was much stronger than that of any other operon. We propose that only the S10 operon is autogenously regulated by a transcription attenuation mechanism.

The synthesis of bacterial ribosomes is regulated in response to growth conditions (6, 8, 12, 14). Cells growing in rich medium devote a larger fraction of their RNA and protein synthesis capacity to the formation of new ribosomes than do cells growing in poor medium. One aspect of this growth medium-dependent regulation is the nutritional shift-up response. When cells are shifted from a poor growth medium to a richer growth medium, the rates of synthesis of rRNA and ribosomal proteins (r-proteins) are rapidly changed to the values typical of the postshift medium. However, before the final postshift rate is obtained, the synthesis rate of ribosomal components goes through a transition period with several oscillations (6).

We have previously analyzed the response of the 11-gene S10 r-protein operon after a nutritional shift-up (5, 16). We found that the shift-induced oscillations in the synthesis of proteins encoded by this ribosomal operon are due to changes in the differential rate of transcription of the structural genes (16). The mechanism mostly responsible for this response is the regulation of readthrough at an attenuator in the S10 leader (5, 16). This attenuator is the site for autogenous control of the S10 operon (L. Freedman, J. M. Zengel, and L. Lindahl, manuscript in preparation), mediated by r-protein L4, the product of the third gene in the S10 operon (19). When the free form of L4 (i.e., L4 not bound to rRNA) accumulates in the cell, transcription of the S10 operon is prematurely terminated at the attenuator (10). Conversely, increased synthesis of 23S rRNA would result in a sequestering of free, inhibitory L4 molecules, leading to increased readthrough at the attenuator. During steady-state growth, only one of every two to three transcripts is elongated past the attenuator (10). Thus, the attenuator mechanism has the capacity to both increase and decrease the expression of the S10 operon. The increased readthrough that we observed immediately after a nutritional shift-up presumably reflects a rapid sequestering of free L4 molecules (16).

Although increased readthrough at the attenuator accounts for much of the transcription regulation of the S10 operon, attenuation is not the only mechanism which contributes to the regulation of the S10 operon after a nutritional shift-up. Immediately after the shift-up there is a small, but reproducible, increase in the differential synthesis rate of the S10 leader, an effect presumably due to the regulation of the rate of transcription initiation at the S10 promoter (5, 16). These results suggest that the S10 operon is subject to at least two regulatory processes after a shift-up: an immediate and small increase in the differential rate of transcription initiation at the promoter, followed by a more dramatic increase in readthrough at the attenuator.

Transcription attenuators have not been found in other r-protein operons. In the cases of the L11-L1 operon (2) and the *spc* operon (L. Lindahl and J. M. Zengel, unpublished results), experiments indicate that autogenous control occurs exclusively at the level of translation regulation and does not involve the modulation of transcription. Since our experiments with the S10 operon had suggested that the operon-specific autogenous control mechanism makes an important contribution to the shift-up response of r-protein synthesis, we were interested in analyzing the transcription of other r-protein operons after a nutritional shift-up. Specifically, we predicted that the L11-L1 and *spc* operons, which do not have attenuator mechanisms, would not exhibit the same strong changes in the differential transcription rate as we previously had observed for the S10 operon.

To test this prediction, we measured the relative differential transcription rates of r-protein genes from the L11-L1, *spc*, and S10 operons following a nutritional shift-up. Transcription rates of several other r-protein operons and of rRNA were also monitored. The experiments were carried out with *Escherichia coli* K-12 LL308 (11), the strain used for the shift-up experiments previously described (5, 16). Some experiments were conducted with the same strain carrying a multicopy plasmid (pLL36 or pLF1) carrying the proximal part of the S10 operon (16). The presence of this plasmid had no effect on the shift-up response of the synthesis of ribosomal transcripts.

We induced the nutritional shift-up by adding glucose and 19 amino acids (no methionine) to a culture which had been growing exponentially at 37°C for at least two doublings in AB minimal medium (3) supplemented with glycerol and thiamine. To determine how the rates of transcription of rRNA and r-protein mRNA changed in response to the shift, we pulse-labeled cell samples with [^{3}H]uridine before and at various times after induction of the shift. RNA was extracted and hybridized to DNA probes specific for different ribosomal genes.

The probe used to measure rRNA transcription was denatured DNA from the transducing phage λ *ilv5*, which carries a complete rRNA operon (9), or single-stranded DNA from an M13 phage carrying part of the 16S rRNA gene (Fig. 1). The probes for r-protein genes were single-stranded DNAs from M13 phages carrying r-protein genes from the S10, *spc*, alpha, *str*, L11-L1, or L10 operons (Fig. 1). In addition, we

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used an M13 phage carrying a fragment from the S10 leader. In all cases the single-stranded DNA was immobilized on nitrocellulose filters, and hybridization was carried out in a formamide-containing buffer at 41°C as previously described (10, 19). After hybridization the amount of radioactive RNA specifically binding to each probe was determined. The differential rates of transcription from each of the ribosomal operons were then determined by normalizing the amount of radioactive RNA found in hybrids to the total amount of trichloroacetic acid-precipitable radioactive RNA added to the hybridization reaction. Finally, these rates were normalized to their respective values measured prior to the shift-up.

The results shown in Fig. 2 demonstrate that the S10 operon is uniquely affected by the nutritional shift-up. While the differential rate of transcription of the structural genes of this operon increased about 2.5-fold within the first few minutes of the shift (Fig. 2a and b), the differential synthesis rates of structural gene mRNAs from other r-protein operons increased 50% or less (Fig. 2d to i). Similarly, the differential transcription of rRNA exhibited only a modest response (Fig. 2j) as compared with the structural genes of the S10 operon.

The differential synthesis of virtually all r-proteins increases about twofold during the first few minutes after a nutritional shift-up (4, 7). For the S10 operon, this increase is mainly due to increased transcription of the structural genes

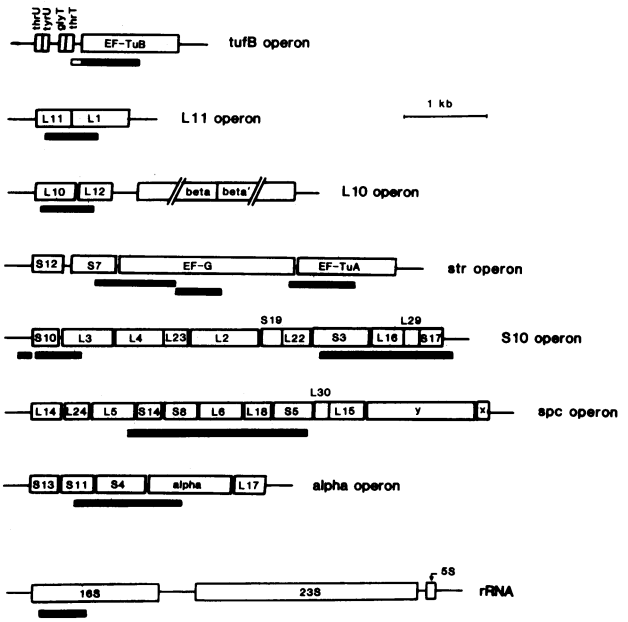

FIG. 1. Probes used for hybridization of rRNA and mRNA from different r-protein operons. The indicated fragments (solid bars) were cloned on M13 vectors (13). The constructions of the recombinant phages carrying the following fragments have been described previously (reference): L11-L1, S10 leader, S10-L3, the alpha operon, and the *spc* operon (10); 16S rRNA (18); S7 and EF-G (17). The probes indicated for the EF-TuA and EF-TuB genes both refer to the *Eco*RI-*Kpn*I fragment from the *str* operon. Since the sequences of the genes for EF-TuA and EF-TuB differ by only 13 bases (1, 15), our hybridization conditions do not discriminate between transcripts from the two genes. The probe for the L10 and L12 genes was a *Pst*I-*Eco*RI fragment cloned on M13mp8. This recombinant phage was a gift from S. Pedersen, University of Copenhagen. The synthesis of rRNA was also estimated by hybridization to DNA from λ *ilv*5 (9), which carries a full set of rRNA genes. kb, Kilobases.

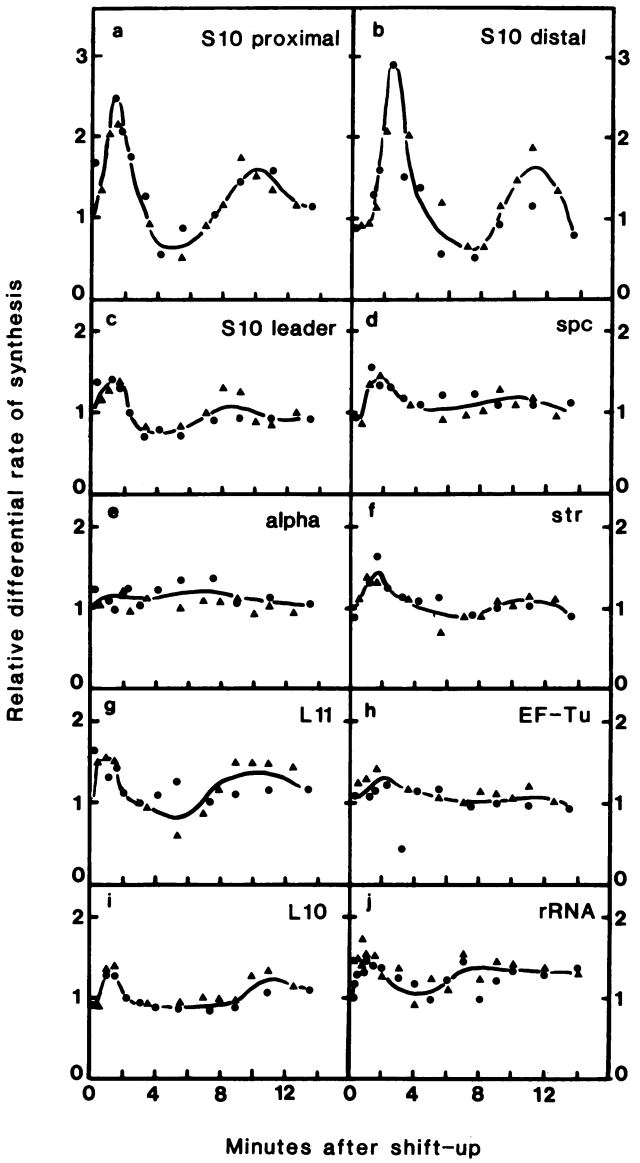

FIG. 2. Relative differential rates of synthesis of rRNA and mRNA from different r-protein operons after a nutritional shift-up. A nutritional shift-up was induced in a culture of *E. coli* K-12 as described in the text. For panels a to i, strain LL308 was used for experiment 1 (●), and the same strain carrying pLL36 was used for experiment 2 (▲). The experiment shown in panel j was done with LL308(pLF1). Immediately before and at the indicated times after the shift, 2-ml samples of the culture were labeled with 150 μCi of [^{3}H]uridine (40 to 50 Ci/mmol) and immediately lysed (19). Pulse lengths were 0.7 min (●) or 0.5 min (▲) for panels a to i and 0.4 min for panel j. RNA was extracted from the radioactive cell lysates and hybridized to probes from rRNA genes or different r-protein genes. The origins of these probes are indicated in Fig. 1. Hybridization conditions have been described previously (10, 19). The relative differential synthesis rates of specific RNA segments were calculated as described in the text. In experiment 1 in panels a to g the radioactive RNA was hybridized twice, and the average of the results is given. All other results are based on a single hybridization to each probe (each hybridization assay included two filters with each type of DNA). In panel f, experiment 1 was done with a probe which is entirely within the EF-G gene, and experiment 2 was done with a probe which carries sequences from both the S7 and EF-G genes (Fig. 1). In panel j, the circles represent hybridizations to λ *ilv*5 DNA, and the triangles represent hybridization to the probe from the 16S rRNA gene (Fig. 1).

mediated by the autogenous control mechanism (5, 16). However, the results shown in Fig. 2 suggest that for other r-protein operons (specifically, the *str*, *spc*, alpha, L11-L1, and L10 operons), a posttranscriptional mechanism is responsible for the shift-up response. A likely candidate for this regulation is autogenous control of translation initiation. It thus appears that autogenous (or feedback) regulation is accomplished mainly by the modulation of transcription attenuation in the S10 operon and by translation regulation in other r-protein operons. It is not obvious why different mechanisms have evolved for the autogenous control of the S10 operon and other r-protein operons.

Finally, it is interesting that the postshift transcription patterns for r-protein mRNAs from operons other than the S10 operon (Fig. 2d to i) were similar to the transcription pattern of the S10 leader transcript (Fig. 2c). With the possible exception of the alpha operon, transcription of these other r-protein operons showed a small, transient increase within the first 2 min of the shift-up. The differential transcription of rRNA also exhibited a similar pattern. These results suggest that there may be a general stimulation of transcription initiation at ribosomal promoters immediately after a nutritional shift-up.

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