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Transcription of Regions Within the Divergent *argECBH* Operon of *Escherichia coli*: Evidence for Lack of an Attenuation Mechanism

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Using in vitro and in vivo assays, we could detect no early termination of DNA transcription in the proximal part of the *argCBH* arm of the *argECBH* divergent operon. The discrepancy noted previously between the respective amplitudes of variation of messenger and enzyme synthesis must therefore be attributed to other causes than a difference in efficiency between attenuation and repression.

The bipolar *argECBH* operon is regulated primarily at the level of DNA transcription (4, 9) by a repressor protein (5, 12). However, assays of pulse-labeled *argECBH* RNA suggested that repression exerted at operator sites might not be the sole mechanism involved in regulation of arginine biosynthesis. For example, in the case of the *argCBH* arm of the operon, arginine was found to modulate the rate of synthesis of the *argH* gene product over an 80-fold range, whereas *argCBH* mRNA levels varied only 20-fold (4). A similar discrepancy was observed for *argE* (4) but not for the carbamoylphosphate operon, the expression of which is also regulated by the arginine repressor (11). Thus, even though arginine-mediated repression could largely be accounted for by control of transcription initiation, these and other (9) results were compatible with the notion that a certain proportion of *argECBH* transcripts were not contributing to enzyme synthesis, this proportion being higher under conditions of repression. This in turn raised the question (4, 9) of whether *arg* genes were not submitted to control by attenuation, like several other operons involved in amino acid biosynthesis (see reference 17). Indeed, the synthesis of an enzyme would appear more repressible than the synthesis of the cognate mRNA, were repression of DNA transcription less efficient than attenuation of messenger elongation.

To answer this question, we examined in vitro and in vivo the relative frequencies of transcription of different regions of the *argCBH* arm of the *arg* cluster under various conditions in order to detect putative transcripts that would correspond with the operator-proximal region of *argCBH*. A set of λ d *arg* transducing phages carrying different portions of the *arg* cluster provided the necessary probes.

MATERIALS AND METHODS

Bacterial strains. Hfr strain P4X6 λ^- and the *argR* (genetically derepressed) derivative P4XB2 have been described previously (4). Derivatives of strain MN42 λ^- (4) lysogenic for the helper phage λ 199 and all but one of the transducing λ d *arg* phages mentioned below (λ 14 *argEC1*) are from references 2 and 13; λ 14 *argEC1* was constructed by the method already used to obtain λ 13 *argEC1* (13). The pMC7 plasmid carrier strain is described in reference 2 (see note added in proof).

In vitro transcription assay. Template DNA from plasmid pMC7 was extracted (2) and used for in vitro transcription as described previously (15). The RNA polymerase (from Boehringer Mannheim)-to-DNA ratio was 30:1 (wt/wt). Both the yield and the specificity of transcription obtained with this enzyme were comparable with those observed previously with RNA polymerase purified in the laboratory (5). Partially purified repressor (8) was added to a final concentration of 30 μg/ml; the arginine concentration was 0.5 mM.

DNA-RNA hybridization assays. Hybridization assays for *argCBH* mRNA were performed with the "right" (r^-) DNA strand (4) of transducing phage λ 13. λ 13 *argEC1*, λ 13 *sup400*, λ 13 *sup102*, λ 14, and λ 14 *argEC1* as described previously (4, 5). Hybridization to the homopolar strand of the parental and helper phage λ 199 was taken as blank. In vivo pulse-labeling and extraction of RNA were as in reference 4.

RESULTS

In vitro experiments. As template DNA for the in vitro transcription of *arg* genes, we used the colicinogenic plasmid pMC7, which harbors a 9.5-kilobase fragment encompassing the whole

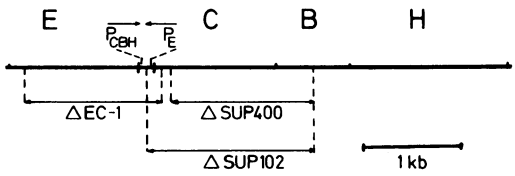

FIG. 1. Functional organization of the *argECBH* cluster and relevant features of template and probe DNAs. Estimates of physical distances are based on references 13 and 18.

arg cluster and part of the *ppc* gene (2). From previous transcription experiments including competition hybridization assays (5, 15), it was known that even chromosome fragments stretching much further to the left (Fig. 1) than *ppc* were predominantly transcribed in vitro into *arg* RNA, about 75% of the RNA hybridizing with the *argCBH* template strand being genuine *arg* mRNA. pMC7 supercoiled DNA was found to be three to four times as efficient a template (Table 1) as linear phage DNA (5, 15) in terms of RNA production per template molecule. In addition, superhelicity increased the transcription frequency of *argCBH* relative to that of *argE*; the ratio was about 10:1 (data not shown) with a 95% supercoiled plasmid template, versus 3:1 with linear phage DNA (15). The reason for this is presently unknown. The following data concern *argCBH* transcription only.

RNA synthesized from supercoiled pMC7 DNA in the presence or absence of repressor, with or without arginine, was hybridized with the template *argCBH* DNA strand from different probes. Figure 1 shows the relevant features of template and probe DNAs. The *argEC1* deletion enters the *argC* structural gene (6); it was recently shown by genetic analysis to eliminate all sites involved in initiation of *argE* transcription (1).

In Table 2, the values presented in Table 1 are given as percentage of the amount of RNA hybridizing with Arg^+ probe DNA (r-λ 13) for each set of conditions. There clearly was no preferential transcription of the region covered by the *argEC1* deletion. The results even suggested that transcription and repression of transcription took place relatively uniformly over the whole cluster, since the average percentages of Table 2 turned out to be in reasonable agreement with the relative sizes of the residual stretches of *argCBH* DNA remaining in each one of the three deletion mutants (bottom line of Table 2).

Since no preferential transcription of the proximal region covered by the *argEC1* deletion could be detected, hybridization conditions were modified so as to increase the chances of detecting short RNA molecules: the amount of probe *arg* DNA was increased to 30 times in excess of the estimated amount of *arg* RNA, and incubation was prolonged up to 42 h and performed at 55°C as well as 66°C. Under none of these conditions was there evidence for large-scale synthesis of a short RNA species corresponding to the *argC*-proximal region. Indeed, the difference between hybridization percentages on λ 13 and λ 13 *argEC1* was never higher than 8% of total *arg* RNA, in keeping with the portion of *argCBH* deleted in mutant *argEC1*: 100 to 200 nucleotides from a total of 3,850 (13, 18).

In vivo assays. Transcription of the *argC*-proximal region was also estimated in vivo by comparing assays of pulse-labeled *argCBH* messenger with λ d *arg* 14 and λ 14 harboring the *argEC1* deletion as probes. We used λ 14 instead of λ 13 because, contrary to this latter phage, λ 14 lacks a DNA segment near the *bfe* gene which is usually expressed in the bacteria and therefore contributes a high background to the hybridization assays (4). The data (Table 3) showed no conspicuous relative increase of transcription in the *argC*-proximal region. This was particularly clear for cells grown under repression, where a bias in favor of premature transcription termination in that region would have explained the discrepancy observed between the modulations of messenger and enzyme synthesis (4, 9).

TABLE 1. Hybridization assays of RNA transcribed in vitro from pMC7 on the right DNA strand of phages carrying different portions of the *argCBH* operon (see Fig. 1)[a]

Template DNA	Repressor	0.5 mM L-arginine	% RNA[b] hybridizing with:							
			r-λ 13	% Repression[c]	r-λ 13 *argEC1*	% Repression	r-λ 13 *sup400*	% Repression	r-λ 13 *sup102*	% Repression
pMC7	−	−	26.90		22.10		16.10		14.50	
pMC7	+	−	19.80	57	21.60	65	12.91	51	9.92	42
pMC7	+	+	8.55		7.50		6.30		5.80	

[a] Values are average of two to six independent determinations; input ranged from 12,000 to 35,000 cpm.
[b] Percentage of trichloroacetic acid-precipitable counts.
[c] The addition of the repressor preparation alone resulted in uniformly lower hybridization values; therefore, values were calculated as previously (5) in relation to the other conditions in which repressor was present.

TABLE 2. Hybridization assays of Table 1 expressed in percentage of the value obtained with the r-λ 13 strand as probe

Template	Addition to system	% Hybridizing to: r-λ 13 *argECI*	r-λ 13 *sup400*	r-λ 13 *sup102*
pMC7	None	82	60	54
pMC7	Repressor	109 (93)[a]	65 (65)	50 (57)
pMC7	Repressor + 0.5 mM arginine	88	74	68
% of *argCBH* remaining on probe[b]		94	62	55

[a] Numbers in parentheses are averages.
[b] Based on heteroduplex mapping values (13, 18).

DISCUSSION

No evidence was found in vitro for the production of a short RNA molecule that would correspond to the most proximal region of the *argCBH* cluster. This contrasts wih the observations made in the case of the tryptophan and histidine operons (7, 10, 14) where, under conditions equivalent to ours, most of the transcripts were "leader" RNA molecules.

One could argue that, in vivo, the arginine repressor itself is involved in such a secondary control of arginine biosynthesis. However, addition of repressor and arginine to the in vitro system did not cause premature transcription arrest in the region covered by the *argECI* deletion (Table 1); more critically, no preferential transcription of this region could be detected in vivo under conditions of repression (Table 3). Therefore, early transcription termination cannot be invoked to explain the discrepancy observed between m RNA and enzyme levels. Our data are, however, not incompatible with the hypothesis that, in repression, transcripts of a distal portion of the cluster would be relatively less abundant or stable than those of more proximal segments. If this is true, the present results locate this portion well beyond the *argC*-proximal part of the cluster.

We have established the DNA sequence of the *argECBH* control region (3; 16; J. Piette, A. Boyen, R. Cunin, D. Charlier, M. Crabeel, F. Van Vliet, N. Glansdorff, C. Squires, and C. L. Squires, manuscript in preparation). The data display a promoter site 116 nucleotides upstream from the putative *argC* translational start. In keeping with the present results, this region does not display the features (extensive secondary structure, leader peptide) which are typical of leader sequences involved in attenuation control (17).

TABLE 3. Transcription of the *argC*-proximal region: in vivo assays

RNA source[a]		DNA probe	
Strain	Supplement to medium AUF[b] (100 μg/ml)	λ d *arg*[c]	λ d *argECI*[b]
P4XB2[d]	Arginine	0.514	0.415
P4X6	None	0.160	0.133
		0.157 (0.144)[e]	0.107 (0.127)
		0.114	0.140
P4X6	Arginine	0.031	0.034
		0.023 (0.034)	0.031 (0.035)
		0.057	0.040

[a] Pulse-labeled RNA of high specific radioactivity as described in reference 4; 0.1 represents about 3,000 cpm above the blank.
[b] Arginine- and uracil-free medium enriched with amino acids (4).
[c] Right strand of λ 14 or λ 14 harboring the *argECI* deletion.
[d] Genetically derepressed.
[e] Numbers in parentheses are averages.

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