MESSENGER RNA FROM ARGININE AND PHOSPHOENOLPYRUVATE CARBOXYLASE GENES IN *arg* R^+ AND *arg* R^- STRAINS OF *E. COLI* K-12

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1. Introduction

The expression of the nine structural genes of the arginine (arg) regulon is submitted to repression by arginine through the mediation of at least one regulatory molecule: the protein produced by the *arg R* gene [1–4]. Recent indirect evidence [5–7] suggests that the expression of the *arg* genes is to some extent under translational control. Up to now, no direct information was available on a possible regulation of their transcription.

The use of ϕ 80 bacteriophages carrying either the whole *arg ECBH* cluster [8 and B. Konrad, unpublished] or an *arg E* deletion, allowed us to estimate the levels of messenger (m–) RNA complementary to the four genes or only to *arg E* in repressed and derepressed strains. The data point to the existence of a transcriptional control that does not operate in an *arg R* strain. While this report was being written, similar results, obtained by Rogers and coworkers [9], came to our knowledge. The two sets of data agree on the essential points and are complementary to each other: here, maximal m-RNA production is examined in genetically derepressed strains, while the other report studies physiological derepression. The present work provides also a direct estimate of the m-RNA originating from *arg E* alone and from the neighbouring *ppc* gene (structural determinant of PEP carboxylase). Additional interest arises from the unusual organization pattern of the *arg ECBH* cluster (see end of the discussion).

2. Materials and methods

All the bacterial strains used are derivatives of *E. coli* K-12: P678 $(\lambda)^-$, *thr, leu, thi, arg* R^+; P_4X $(\lambda)^+$, *met B, arg* R^+; MN42 $(\lambda)^-$, *met B,* deletion (*ppc*$^-$ *arg ECBH*); P_4XB_2 $(\lambda)^-$, *met B, arg R.*

The ϕ 80 d *arg (ECBH)*$^+$ phage used (B. Konrad, unpublished) is defective and very similar to the other ϕ 80 d *arg*$^+$ available [8] though not, like the latter ones, derived from a heat-inducible parent. A double lysogen, harbouring this phage and the heat inducible ϕ 80 h λc857 (thermosensitive λ type repressor), is nevertheless heat inducible (Glansdorff, unpublished) and has been used in the present experiments. This phage carries also the *ppc* gene. Phage suspensions were banded in CsCl gradients [8]; the heavier band, corresponding to ϕ 80 d *ppc*$^+$ *arg (ECBH)*$^+$, was isolated and used for DNA extraction.

A ϕ 80 d *ppc*$^+$ *arg (CBH)*$^+$ (carrying a deletion of *arg E* and of a very small fraction of *arg C*) has been prepared by selecting homozygote *arg*$^-$ strains from a double lysogen $(\phi 80)^+$ $(\phi$ 80 d *arg*$^+)^+$ harbouring the *arg EC-1* deletion [10].

Phage suspensions in CsCl were dialyzed overnight in TM buffer [8].

DNA was prepared according to [11].

Pulse labelled RNA: the cells were grown at 37° in aerated cultures on: (1) minimal mineral medium 132 [12] supplemented with 0.5% glucose, 200 μg/ml of L-arginine and other requirements (succinate used as carbon source for MN42). (2) AF medium (arginine-

Table 1
Percentage total RNA hybridized on ϕ 80 d arg DNA.

Growth medium (arginine always present: 200 μg/ml)	RNA source	DNA			*arg E* m-RNA
		ppc$^+$	*arg (ECBH)*$^+$	*ppc*$^+$ *arg (CBH)*$^+$	
Rich	P678(λ)$^-$ *arg R*$^+$	(a)	0.075	–	–
Minimal	P678(λ)$^-$ *arg R*$^+$	(b)	0.097	–	–
Rich	$P_4X(\lambda)^+$ *arg R*$^+$	(c)	0.089	–	–
Minimal	$P_4X(\lambda)^+$ *arg R*$^+$	(d)	0.140	0.138	0.002
Rich	$P_4XB_2(\lambda)^-$ *arg R*	(e)	0.450	–	–
Minimal	$P_4XB_2(\lambda)^-$ *arg R*	(f)	0.541	0.460	0.081
Minimal	MN42(λ)$^-$ *arg R*$^+$ (deletion *ppc-arg ECBH*)	(g)	0.057	–	–

free rich medium [13] supplemented with 0.5% glucose, 200 μg/ml of L-arginine and containing excess amounts of L-aspartate. When the absorbance reached 4×10^8 cells/ml, ^{3}H-uracil (263 μCi, 4 μg/100 ml) was added for 80 sec together with unlabelled uracil up to a final concentration of 20 μg/ml. The cultures were then poured on crushed ice 132 medium and the cells harvested by centrifugation.

RNA was extracted according to [14] but without using bentonite. The preparations had specific radioactivities ranging from 4×10^6 to 6×10^6 cpm/μg RNA.

Alkali-denatured DNA [15] was fixed to Schleicher and Schuell (type B-6 course) membrane filters, at 25 μg per filter, in the presence of 4 × SSC. Hybridization assays were performed as in [15]. For each sample, two to four RNA concentrations were used, each one in duplicate. The hybridization percentage was constant up to at least 200 μg RNA per assay. Blank disks and "mock hybridizations" [16] were performed and gave similar results.

3. Results and discussion

RNA from a genetically derepressed (*arg R*) strain and two *arg R*$^+$ strains, all grown in the presence of arginine, has been hybridized with *ppc*$^+$ *arg (ECBH)*$^+$ DNA (table 1, first column of data) and, in two cases, with the *ppc*$^+$ *arg E* deletion DNA (second column). The values have been corrected for the counts obtained when hybridizing RNA on ϕ 80 h λc857 DNA; this background is, as expected, somewhat higher (0.049% to 0.054%) when a lysogenic $(\lambda)^+$ strain is tested rather than a non lysogenic $(\lambda)^-$ one (0.038% to 0.041%). The absolute background (0.057%) is provided by the (*ppc*$^-$ *arg ECBH*) deletion strain (table 1, g).

Two different growth media (minimal and rich) have been used. The counts are always higher when RNA originates from a culture on minimal medium: the difference amounts to 0.022% for P678 (b–a) and to an average of 0.071% – between 0.051% (d–c) and 0.091% (f–e) – for P_4X and P_4XB_2. Since they all are *ppc*$^+$ strains, we may assume that these values reflect different activities of the *ppc* gene in minimal and rich medium: the latter contains an excess of aspartate and thus provides a physiological situation where the *ppc* enzyme is dispensable; moreover, aspartate is a potent inhibitor of the enzyme [17] and might also repress its synthesis. We indeed found that in P_4X the specific activity of PEP carboxylase amounts respectively to 0.6, 0.4 or 2.7 μM/hr/mg protein when the strain is grown on minimal medium plus 0.5% L-aspartate as carbon source, rich medium plus glucose or minimal medium plus glucose. The 5- to 6-fold repression of *ppc* expression encountered when the strain is grown in the presence of aspartate shows that our hybridization assay actually detects the m-RNA produced by the *ppc* gene.

Regarding the main object of this paper (*arg* m-RNA), the following information may be gathered from table 1.

The difference between the hybridization percentages obtained with genetically derepressed and repressed strains comes close to 0.4% (0.379 to 0.444%), a value very similar to that reported by Rogers et al. [9] with physiologically derepressed strains (0.3 to 0.5%). We have previously obtained nearly identical values (0.4 to 0.5%) with other DNA preparations than those used in this set of experiments. A maximal estimate of *arg ECBH* m-RNA in *arg R* cells (including the very low but unkown background due to the *ppc* gene in conditions of 6-fold repression of its expression) amounts to 0.393% (table 1, e–g). If this is compared with the message level in repression (a–g), which is 0.018%, a tentative repression coefficient of 22 may be computed.

The amount of *arg E* messenger alone may be estimated free of background by subtracting the counts obtained with the *arg E* deletion DNA (table 1, 2nd column) from the ones retained on *arg (ECBH)*$^+$ DNA; it comes close to a fifth (0.081%) of the total estimate for *arg ECBH* (0.393%). In conditions of repression, it falls to 0.002%, a value likely to be affected by a considerable relative error.

In conclusion, the difference found between the amounts of *arg E* or *arg ECBH* m-RNA synthesized in genetically derepressed cells and in repressed ones shows that the transcription of the cluster is submitted to a control mechanism involving the *arg R* gene product. Our experimental system also allows estimation of the variations in the amount of m-RNA produced by the neighbouring *ppc* gene. However, the RNA background resulting from the expression of this gene – even if it is very low in rich medium (about 0.010%) – prevents us from making accurate comparisons between repressibility coefficients expressed in terms of *arg* m-RNA levels and in terms of enzyme specific activities. When available, *arg* deletions leaving *ppc* intact will allow background-free estimates of *arg ECBH* m-RNA in repressed conditions and allow such comparisons; they would provide direct information on the possible translational control referred to in the Introduction. The enzyme synthesis repressibility coefficient amounts to 18 for *arg E* and to 50 to 70 for *arg C, B* and *H* [10]; the latter three constitute a clockwise polarized operon with control elements located at the E–C boundary [10]. Recent results from this and other laboratories [7, 18] suggest that the control elements of *arg E* also lie between E and C and raise the possibility that *arg E* and *arg CBH* share a complex common operator–promotor region. Further m-RNA measurements, this time performed on separated (light and heavy) DNA chains of the transducing phage will help in solving the functional organization of the *arg* cluster.

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