IN VITRO TRANSCRIPTION OF THE BIPOLAR ARGININE *ECBH* OPERON OF *ESCHERICHIA COLI* K 12

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1. Introduction

The arginine (*arg*) regulon of *E. coli* K12 comprises 9 structural genes, four of which are clustered in the order *argECBH*. Genetic data [1,2] and *arg* messenger (m-) RNA determinations [3,4] have shown that the four genes constitute a bipolar operon, divergently transcribed from an internal control region which is situated at or near the *argE–argC* boundary and contains a repressor binding site common to the two wings of the cluster.

The present report describes a system for the in vitro synthesis of *arg* m-RNA, with a ratio of leftwards (*argE*) over rightwards (*argCBH*) transcription similar to that observed in vivo. As in the case of the in vitro transcription of another biosynthetic operon, the tryptophan operon [5], the specific synthesis of m-RNA requires only the presence of RNA polymerase holoenzyme.

2. Materials and methods

Transducing phages: ϕ80d *arg*, also carrying the neighbouring *ppc* gene, 95% cotransducible with *argE* and situated on the *argE* gene side of the *argECBH* cluster, has been isolated by B. Konrad. The λd *arg* phages (λ14, carrying *argECBH* and *ppc*, λ23, carrying only *argECBH*) have been isolated by the method of Shimada et al. [6], starting from λ199 (a gift of Dr Weisberg) which is thermoinducible (c I 857) and lysis defective (S7). They carry the *argECBH* genes in the reverse orientation with respect to ϕ80d *arg* and appear to differ only by the amount of bacterial DNA situated on the side of *argE*, i.e. the *ppc* region (Cunin, Boyen and Glansdorff, in preparation; Palchaudhuri, Mazaitis, Glansdorff and Maas, in preparation). Large amounts of the ϕ80d *arg* and the two λd *arg* phages have been prepared by inducing lysogens with mitomycin C (1 μg/ml) at 37°C or by raising the temperature of the cultures for 35 min from 32°C up to 41.5°C, respectively, under conditions of vigorous aeration. In both cases, incubation was continued for 3 hr at 37°C after induction was over. ϕ80 phages were collected after precipitation with polyethylene glycol [7] ; the λ-phages were liberated by chloroform treatment after concentrating the induced lysogen about 50 times. The transducing phages were purified and separated from the helper phage by differential centrifugation and 3 or 4 isopycnic centrifugations in CsCl. ϕ80 DNA was extracted with phenol and strand separation of the λd *arg* DNA was achieved in the presence of poly U, G (2:1) as described previously [3].

In vitro transcription system: RNA polymerase (Holo-enzyme) was purified from *E. coli* MRE 600 (RNase I$^-$) by the method of Burgess [8] up to the phosphocellulose step and was further purified by native DNA-cellulose chromatography [9]. Rho factor was purified from the same strain according to the procedure of Roberts [10] as modified by Darlix et al. [11]. Both proteins were shown to be free from detectable amounts of RNAase. In vitro RNA synthesis was carried out for 1 hr at 37°C. The reaction mixture was as follows : 25 mM Tris–HCl pH 7.9,

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Abbreviations: ppc: genetic determinant of phosphoenol pyruvate carboxylase.

8 mM $MgCl_2$, 0.13 M KCl, 0.1 mM dithiothreitol, 0.2 mM of ATP, GTP, CTP, 0.1 mM [^{3}H]UTP (specific radioactivity as indicated in the legends), 50 μg/ml φ80d *argECBH ppc* DNA, 25 μg/ml RNA polymerase (holo-enzyme) and 1 μg/ml Rho factor. The reaction was stopped by the addition of Pancreatic DNAase I at a final concentration of 20 μg/ml and the mixture was incubated for 10 min at 37°C. Then sodium dodecyl-sulfate was added up to a concentration of 0.2% and the mixture was chilled for 10 min at 0°C. The precipitate which was formed was removed by centrifugation and the supernatant solution was passed through a Sephadex G-50 column in order to remove unincorporated [^{3}H]UTP.

One step hybridizations and competition hybridization assays were performed as described before [12]. Competitor *E. coli* RNA was extracted from *E. coli* K 12 *argR* P4XB2 and from *E. coli* K12 deletion [*argECBH ppc*](MN42) by the procedure of Summers [13], followed by filtration on Schleicher and Schüll filters BA-85 (0.45 μ).

3. Results

To detect *arg* m-RNA we have used a one-step hybridization assay, taking advantage of the low cross-hybridization between φ80 RNA and λ-DNA [14], especially when φ80 RNA is synthesized in the presence of Rho (Pannekoek and Pouwels, in preparation). RNA was synthesized in vitro on φ80d *argECBH ppc* DNA as template and hybridized with the separated strands of λ23 (d *argECBH*) and λ14 (d *argECBH ppc*) and λ199 (helper). *argE* m-RNA hybridizes with the light strand, *argCBH* m-RNA with the heavy strand of both λd *arg* phages (Cunin et al., in preparation). The results are presented in table 1. Our data reveal an asymmetric transcription pattern with a substantially lower percentage of RNA hybridizing with the light strand of λ23 (lacking *ppc*) than that of λ14, while the values of the heavy strand are not significantly different between the two d *arg* phages. The ratio of leftward over rightward transcription falls in the range of values determined in vivo under conditions of maximal repression of the *ppc* gene. Indeed, in the case of λ23, leftward transcription represents 26% of the total *argECBH* m-RNA, while estimates of the fraction of *arg* m-RNA transcribed in vivo as *argE* m-RNA vary between 15 and 30% [3,15]. The difference observed between the hybridization percentage obtained with λ14 and λ23, plus the fact that the two phages seem to differ only by the *ppc* region, suggests that *ppc* DNA is transcribed in vitro from the same DNA strand as *argE* (see also below).

Table 1
Percentage of RNA synthesized in vitro on φ80 d*argECBH ppc* DNA hybridizing with the separated strands of λd *arg* DNA's

Separated DNA Strands of	% Hybridizable counts retained with	
	Light strand (*argE*)	Heavy strand (*argCBH*)
λ14 (d *arg ppc*)	1.9	3.4
λ23 (d *arg*)	1.3	3.7
λ199 (helper)	0.1	0.4

[^{3}H] φ80d *argECBH ppc* RNA was synthesized as described in Materials and methods. The specific radioactivity of [^{3}H]UTP was 2000 counts/min per μmol. The input of [^{3}H]RNA in the hybridization mixture was 3×10^4 counts/min of acid-precipitable material. A 10-fold excess of separated strands over RNA was used during hybridization.

The asymmetry of the transcription pattern observed is per se an indication that we are dealing with specific in vitro transcription of *argE* and *argCBH* m-RNA. The correctness of this assumption was tested by a competition hybridization experiment. The hybridization percentage of a fixed quantity of radioactive in vitro synthesized RNA with the separated strands of λ14 (d *arg ppc*) was determined in the presence of increasing amounts of cold competitor RNA extracted from an *arg ppc* deletion mutant (MN42) or from a genetically derepressed (*argR*), but otherwise isogenic strain (P4XB2). The results are presented in fig.1. When the heavy strand of λ14 DNA was used competition was not observed with RNA from the deletion mutant, but RNA from P4XB2 (*argR*) could efficiently compete the radioactive in vitro synthesized RNA. Approximately 25% of the hybridized radioactive material can not be competed by P4XB2 RNA. This residual hybridization is likely to be caused, at least in part, by hybridization between φ80 RNA and λ-DNA (see also table 1).

With the light strand of λ14 DNA competition was not observed with RNA from the deletion mutant (results not shown), while competition was observed

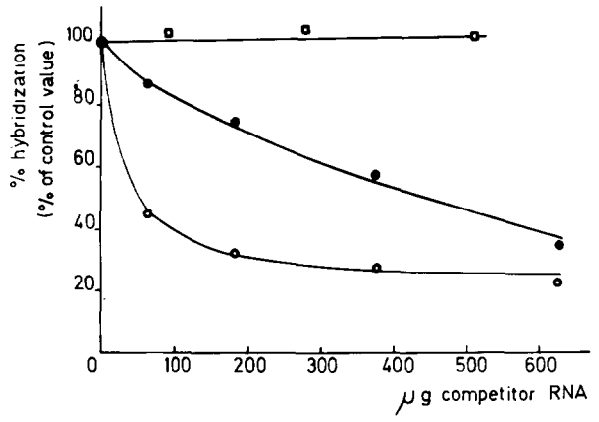

Fig.1. Competition hybridization between RNA synthesized in vitro on φ80d *argECBH ppc* DNA and RNA isolated from *E. coli* K 12 P4XB2 and *E. coli* K12 MN42. 0.08 μg of ^{3}H-labelled RNA synthesized in vitro (1.25×10^5 cpm) was hybridized with 0.11 μg of either light or heavy strands of λ14 DNA, in the presence of increasing amounts of unlabelled competitor RNA (circles = P4XB2 ; squares = MN42). Hybridization efficiency in the absence of competitor RNA, which represents the 100% value of the control is about 50% of that observed with a large excess of separated strands of λ14 DNA. Background, mock hybridizations performed at 0°C, were substracted from all values. (○–○–○) hybridization with the heavy strand of λ14 DNA; (●–●–●) hybridization with the light strand of λ14 DNA; (□–□–□) hybridization with heavy strand of λ14 DNA.

with RNA from P4XB2 although less efficient than for the heavy DNA strand : only 60% competition is achieved for 600 μg of competitor RNA and a plateau value has not been reached yet. Our results therefore clearly show that a large fraction of the radioactivity which hybridizes with the light and the heavy strand of λ14 represents *arg* m-RNA.

Our finding that the competing effect of P4XB2 RNA with the light DNA strand is less efficient than with the heavy strand may be explained by assuming that *ppc* and *argE* are transcribed from the same (= light) strand. Since P4XB2 has been grown under conditions of maximal repression of the *ppc* gene expression, RNA isolated from such a strain is expected to contain only small amounts of *ppc* m-RNA and therefore very large amounts of competitor RNA would be required to obtain complete competition of all the in vitro synthesized RNA.

4. Conclusions

Our results show that it is possible to achieve specific in vitro transcription of the bipolar, divergently transcribed *argECBH* operon. In its present state, the system may be used for the accurate determination of *argCBH* m-RNA levels which constitute the largest fraction (about 3/4) of the RNA transcribed from the whole cluster. No accessory factors appear to be required for the in vitro transcription of the *argECBH* cluster, a result which parallels the conclusions drawn from in vitro transcription of another biosynthetic operon, the tryptophan operon [5].

The exact nature of the corepressor of arginine biosynthesis is one of the pending questions that the system described here may contribute to resolve.

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