INVOLVEMENT OF ARGININE IN IN VITRO REPRESSION OF TRANSCRIPTION OF ARGININE GENES C, B AND H IN ESCHERICHIA COLI K 12

R. CUNIN[*], N. KELKER[**], A. BOYEN[*], HUEY-LANG YANG[***], G. ZUBAY[***], N. GLANSDORFF[*], and W.K. MAAS[**].

[*] Department of Microbiology, Vrije Universiteit Brussel, 1, E. Grysonlaan, B-1070 Brussels, Belgium.

[**] Department of Microbiology, N.Y.U. School of Medicine, New York, N.Y., USA.

[***] Department of Biological Sciences, Columbia University, New York, N.Y., USA.

Received January 27,1976

Summary : The combination of L-arginine and partially purified arginine repressor (the argR gene product) represses transcription of argCBH in vitro.

INTRODUCTION

The expression of the only cluster of genes found in the arginine regulon of E. coli - the bipolar argECBH operon (Fig. 1) - is regulated mainly at the level of DNA transcription into messenger (m-)RNA (1 - 4). Although there is ample evidence that the argR gene product is involved in this regulation as an aporepressor (1, 5 - 7) the identity of the corepressor is still uncertain. Indirect evidence suggests the participation of either arginine (8), arginyl-t-RNA (9) or, possibly, arginyl-t-RNA synthetase itself (9). We have approached the question by using an in vitro transcription system for argCBH (10) and partially purified arginine repressor (argR gene product) (11). This repressor is free of arginyl-t-RNA synthetase activity (11).

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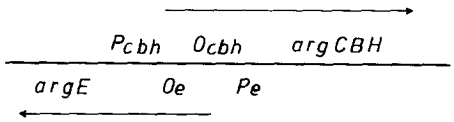

Fig. 1 : The argECBH bipolar operon (4) with the proposed organization (13) of the control region.

MATERIALS AND METHODS

Template DNA (16 µg/ml in the assay) was extracted (10) from a ø 80 d ppc$^+$ argECBH transducing phage or from a derivative harbouring the argEC1 deletion (12). Lambda 14 is a ppc argECBH transducing derivative (12) of λ 199 (14). Purified RNA polymerase (a gift from H. Pannekoek) was added at a ratio of about 20 molecules of enzyme per DNA molecule. The reaction mixture contains 0.2 mM GTP, ATP, CTP, 0.1 mM ^{3}H UTP, 25 mM Tris-HCl pH 7.9, 0.13 M KCl, 8 mM $MgCl_2$ and 0.1 mM dithiothreitol. The concentration of arginine when present, was 0.5 mM and that of partially purified repressor 40 - 100 µg protein per ml. Repressor has been purified as described by Kelker et al. from strain 514-H440-6 (11), the last step consisting in elution from a DNA cellulose column.

The reaction was started by the addition of DNA. Incubation was 15 or 20 min at 37°C. The reaction was stopped by a further 10 min incubation in the presence of DNase (20 µg/ml), followed by chilling in the presence of 0.2 % sodium dodecyl sulfate. ArgCBH m-RNA was measured by hybridization (4) with the r- strand of λ 14 DNA (3, 10). Hybridization to the homopolar strand of λ 199 was taken as blank.

RESULTS

a. Repressor and arginine repress argCBH transcription.

In the first set of experiments, only one concentration (100 µg/ml) of a particular repressor preparation was tested at a L-arginine concentration of 0.5 mM. The data in Table 1 clearly indicate that neither repressor nor arginine, taken separately, repress argCBH transcription, whereas repression (up to 60 %) is observed when both are present. An extract from a genetically derepressed strain (514-X5a44-10 argR), prepared identically through the last DNA cellulose step of the purification procedure, failed to repress.

The repressor preparation used in this set of experiments was contaminated with RNA polymerase : in the absence of purified polymerase, the repressor preparation alone gave an incorporation of 812 pmoles XMP/ml. This explains the increase in XMP incorporation whenever repressor is present (compare in Table 1, lines 1 and 2 with lines 3

Table 1

Repressor (DNA cellulose eluate) 100 µg/ml extracted from	L-arginine 0.5 mM	Total RNA synthesis (pmolesXMP / 100 µl)	argCBH RNA % of control value
(1) none	-	416	100
(2) none	+	544	105
(3) argR$^+$	-	2240	91
(4) argR$^+$	+	1840	39
(5) argR$^-$	+	520	104

RNA synthesis is estimated from ^{3}H-UMP incorporation into TCA precipitable material. Since the yield[1] of RNA synthesis is different in each case, the radioactivity input in the hybridization assay also varies (from 92,000 to 35,000 cpm). (For each assay hybridization with the r-strand of λ199 has been taken as blank.)

and 4). The repressor preparation used in the next experiments was almost free of contaminating RNA polymerase activity, although prepared by the same procedure.

b. Influence of repressor concentration.

Two repressor concentrations have been used in the experiments reported in Table 2. Repression increases in a roughly parallel fashion with repressor concentration (compare lines 2 and 3), going from 30 % of repression for a repressor concentration of 40 µg/protein/ml to 73 % for a 80 µg/ml concentration. The other results in Table 2 show that when the assay is repeated, the extent of repression is roughly the same although the actual amount of m-RNA synthesized (estimated by hybridization) may vary. In the experiments reported in the last four lines of Table 2, transcription factor rho (ρ) has been added and appears to stabilize the synthesis of arg m-RNA. This suggests that the variability of messenger levels observed between different sets of experiments are due to either a failure to terminate transcription properly at the end of the arg cluster, or to some readthrough transcription from promoters external to the arg region, or to both.

Table 2

Repressor concentration µg/ml	L-arginine 0.5 mM	RNA synthesis (pmoles XPM/100 µl)	Hybridization input (cpm)	Hybridized cpm [a]	Corrected % hybridization [(b)]	% repression
(1) 40	-	296	12.215	438	2.71 } 30	} 73
(2) 40	+	292	12.278	333	1.92	
(3) 80	+	292	13.146	225	0.73	
(4) 80	-	224	10.305	274	1.82	} 55
(5) 80	+	80	7.464	204	0.82	
(6) 80	-	488	25.905	726	2.13	} 58
(7) 80	+	480	25.968	408	0.91	
(8) 80	-	452	19.124	586	2.25	} 60
(9) 80	+	262	14.121	222	0.91	

(a) averaged from 2 values

[(b) hybridization with r- λ 199 DNA substracted as blank]

Lines 6 and 7 : termination factor rho present during transcription (0.1 µg/ml).
lines 8 and 9 : idem but rho concentration = 0.2 µg/ml.

It appears from an experiment in which RNA synthesized *in vitro* and RNA extracted from a genetically derepressed (*argR*) strain competed for hybridization with the r-strand of λ 14 DNA that 25 to 30 % of the RNA synthesized *in vitro* from the *argCBH*-sense DNA strand is extraneous to the cluster (10), so that the actual repression is higher than the observed 70 % . This is supported by the results of the following experiment. We have used as template the DNA from Ø80 *d arg* carrying the *argEC1* deletion. On this template, *argE* is absent, the control region of the cluster is missing, *argC* and *B* are silent (13) and *argH* is expressed *in vivo* at a low and constitutive rate (30 to 50 % of the wild type repressed level) owing to the presence of a secondary promoter at or near the *B-H* boundary (3, 13). Transcription from *argEC1* DNA was about 30 % of what can be measured on the r-strand of λ 14 when *argECBH*$^+$DNA is the template (Table 3). This value, very close to the 25 - 30 % given by the competition experiment reported

Table 3

Template DNA	Percentage[(a)] of transcripts hybridizable with	
	r- λ darg$^+$ppc$^+$DNA (argCBH RNA)	1- λ darg$^+$ppc$^+$ DNA (argE RNA)
ppc$^+$ argECBH$^+$	2.70	1.70
ppc$^+$ argEC1 deletion	0.84	0.48

((a) corrected for hybridization on homopolar λ199 DNA strands.)

Repressor concentration was 100 µg protein/ml. Values are averaged from 3 determinations.

above, reflects those classes of RNA whose transcription can be initiated outside of the control region (extraneous RNA, readthrough cluster RNA, argH RNA initiated at the secundary promoter). Both the competition experiment and the argEC1 transcription data concur in suggesting that the actual amplitude of *in vitro* repression is higher than the observed 60 - 70 % .

CONCLUSIONS

The results presented here show that L-arginine, together with partially purified arginine repressor, repress argCBH transcription *in vitro*. The efficiency of repression calculated from the data is 60 - 70 %, but, as discussed in the previous paragraphs, there are reasons to think that this is a minimal estimate. Our results thus strongly support the view that arginine itself is involved in the formation of active repressor. Since our repressor preparation is only partially purified, the present experiments do not provide critical information on the participation of other molecules besides the argR product in the formation of active repressor, but they exclude catalytically active arginyl-t-RNA synthetase as a necessary participant (see Introduction).

Involvement of the synthetase and/or arginyl-t-RNA in posttranscriptional control of *arg* genes expression is certainly not excluded.

Acknowledgements

This work has been supported by the Belgian Fund for Fundamental and Collective Reasearch (FKFO), U.S. P.H.S. Grant GM06048 to W.K.M., NATO Scientific Grant n°748 to W.K.M. and N.G., and A.C.S. Grant NP-12D to G. Zubay. W.K.M. is the recipient of Research Career Award K6GM-15.129.

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