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Metabolism of Arginine-Specific Messenger Ribonucleic Acid in *Escherichia coli* K-12

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Ribonucleic acid-deoxyribonucleic acid (RNA-DNA) hybridization was employed for the determination of the level of messenger RNA (mRNA) transcribed from seven of the nine genes of the arginine regulon of *Escherichia coli* K-12. The quantity of RNA complexing with each of the separated DNA strands of the *argA*, *argF*, *argE*, and *argCBH* operons carried on specialized transducing phages was measured. The derepressed:repressed ratio of mRNA formed in vivo was found to vary between about 3 and 4 when measured by hybridization to DNA isolated from specialized transducing phages carrying the *argA*, *argE*, *argCBH*, *argF*, and *argI* operons.

The nine genes specifying the enzymes of the arginine biosynthetic pathway in *Escherichia coli* K-12 are scattered around the chromosome, constituting a regulon with six or seven distinct operators (3, 16, 17) controlling nine genes. The arginine genes *ECBH* are contiguous, and studies have been presented (3, 8, 15) which indicate that one operator controls *argE*, with transcription oriented counterclockwise, and another operator regulates the *argCBH* genes, with transcription oriented in the opposite direction (12, 20). The remainder of the arginine genes are present as single entities with their own promoter and operator elements (21, 26). The extensive genetic studies of Bretscher and Baumberg (6) involving four-factor crosses and deletion mapping with mutants involving the complex control region of the arginine *E* and arginine *CBH* operons have not provided a definite answer regarding the structure of this important region controlling the divergent transcription of these two operons; however, the data of these workers seem to exclude the possibility of one operator controlling both the *argE* and *argCBH* operons in this gene cluster. Another interesting feature of the arginine pathway is the existence of two genes coding for ornithine transcarbamylase (EC 2.1.3.3.). One, *argF*, is located near *proA* at 6 min (15), and the other, *argI*, is between *pyrB* and *valS* at 95 min on the chromosome map of *E. coli* K-12 (2). These isoenzyme genes have been shown by Sens et al. (34) to have diverged by at least 25% since they arose from a presumptive ancestral gene.

The results of in vivo studies (15, 16, 26) have shown that the concentrations of enzymes in the arginine pathway are repressed when wild-type cells are grown in the presence of arginine and derepressed in the absence of exogeneous arginine. The derepressed:repressed ratio varies from a low value of 14 (8) for acetylornithinase synthetase (EC 2.3.1.1.) to about 400 for ornithine transcarbamylase (15). A number of mutations have been isolated (21) that map in the *argR* region and lead to constitutive expression of all enzymes in the pathway. An amber mutation in the *argR* gene has been isolated (21). These data demonstrate that the *argR* gene product is a protein that regulates, in concert with a corepressor, the synthesis of all the arginine biosynthetic enzymes in a coordinate but nonparallel manner. The nature of the corepressor and holorepressor for the arginine pathway is at present still at issue, but data have been presented (7) which suggest that arginyl transfer ribonucleic acid (RNA) is not the corepressor, and the preliminary results of Cunin et al. (10) suggest that arginine may be the corepressor for the *argCBH* operon.

The question of whether the *argR* gene product functions at the transcriptional level has been answered for the *argA*, *argECBH*, *argF*, and *argI* operons since the in vitro transcriptional studies of Sens et al. (35; D. Sens, W. Natter, R. T. Garvin, and E. James, Mol. Gen. Genet., in press) have demonstrated that between 80 and 95% of the regulation of the expression of these operons occurs at the transcriptional level, compared to the demonstration by Cleary, Garvin, and James (submitted to Mol. Gen. Genet.) of 95% regulation of in vitro ornithine transcarbamylase synthesis, using template deoxyribonucleic acid (DNA) isolated from the specialized transducing phages

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λ*argI*c I857 and φ80*argFi*λc I857.

The use of the RNA-DNA hybridization techniques of Gillespie and Spiegelman (14), whereby RNA is hybridized to DNA immobilized on nitrocellulose filters, provides a means for direct measurement of the transcriptional activity of a gene or a group of genes. The levels of messenger RNA (mRNA) have been determined for several bacterial operons (1, 9, 18, 19, 29, 31; Sens et al., in press). A number of studies have been reported (11, 31) concerning mRNA levels for the *argECBH* operons and of the corresponding enzyme activity for cells grown under conditions of physiological repression and derepression. It has been noted that the levels of mRNA and those of the corresponding enzymes do not correlate for the *argECBH* operons (11). In the case of the *trp* operon, noncorrelation of mRNA and enzyme levels has also been demonstrated, and this phenomenon has been shown to be the result of a post-transcription modulation of *trp* mRNA levels, which has been designated "attenuation" (5).

We describe the measurement of the levels of expression of the *argA*, *argE*, *argCBH*, *argF*, and *argI* operons under conditions of physiological repression and derepression and of genetic derepression. The quantity of mRNA complexing to each separated DNA strand of specialized transducing phages carrying the *argA*, *argE*, *argCBH*, and *argF* operons was determined, thus identifying the DNA strand that carries the sense information of each of these genes.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. Bacterial strains and bacteriophages used in this study are listed in Table 1. All strains were derived from *E. coli* K-12.

Media. The medium used for the preparation of bacteriophage stocks and genetic procedures was described previously (22, 32). RNA was prepared from cells grown in S medium [0.1 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.4), 7 mM NaCl, 10 mM $(NH_4)_2SO_4$, 3 mM KH_2PO_4, 2 mM $MgSO_4$, 0.2 mM $CaCl_2$, 4 mg of vitamin B_1 per liter, 4 g of glucose per liter, and all amino acids, except arginine, at the concentrations previously described (13)]. Arginine, when used, was present at a concentration of 200 μg/ml. Guanosine and adenosine were added at a concentration of 30 μg/ml.

Preparation of φ80 and φ80*argECBH* bacteriophage. Strain LS527 (or EJ37) was grown at 37°C in L medium with glucose to a density of 2×10^8 cells per ml. At this time, the cells were centrifuged for 5 min at 5,000 × *g* and quickly suspended in one-half the volume of 0.1 M $MgSO_4$. The resuspended cells were irradiated with ultraviolet light for 50 s at a distance of approximately 50 cm, utilizing a General Electric 15-W germicidal lamp. The irradiated cells were added to an equal volume of L medium with glucose, and incubation continued at 37°C for 3 h, with vigorous aeration, in flasks protected from light. Chloroform was then added to the culture (1 ml/100 ml); agitation continued for 10 min at 37°C, and the lysed culture was clarified by centrifugation for 15 min at 7,500 × *g* and stored at 4°C.

Preparation of λc I857, λ*argF*2, λ*argF*23, λ*argA*c I857, φ80*argECBH*1*i*λc I857, φ80*argECBH*-2*i*λc I857, λ*argECBH*2, λ*argECBH*5, λ*argECBH*26, λ*argECBH*30, and λ*argI*c I857. Lysogenic strains (Table 1) carrying the above-mentioned bacteriophages were grown at 30°C with aeration to a density of approximately 2×10^8 cells per ml; aeration was increased, and the temperature was raised to 42°C for 20 min. The temperature was lowered to 37°C, and incubation with maximum aeration continued for 3 h. Phages were harvested as described previously (22, 34).

Purification of bacteriophage. Phage containing lysates was adjusted to 10% (wt/vol) in polyethylene glycol 6000 and 2.4% (wt/vol) NaCl and mixed until homogeneous, and the mixture was allowed to sediment for 16 to 24 h at 4°C. The sediment, containing the phage, was collected by centrifugation at 5,000 rpm for 5 min, and the resulting pellet was carefully suspended in 1/50 of the original lysate volume of T1 buffer (6×10^{-4} M $MgSO_4$, 5×10^{-4} M $CaCl_2$, 6×10^{-3} M Tris-hydrochloride [pH 7.3], 0.1% [wt/vol] gelatin). The concentrated phage was sedimented for 2 h at 30,000 rpm into a cushion comprised of 2 ml of 1.4-density (grams per milliliter) CsCl above 2 ml of 1.6-density CsCl, using a 50.2 Ti rotor in a Beckman ultracentrifuge. Phage was further purified by repeated centrifugation in a cesium chloride density gradient, using 1.4-, 1.6-block gradients for 12 to 16 h, or running to equilibrium in 1.5-density CsCl with the 50.2 Ti, the 50 Ti, or the 75 Ti angle rotor. After centrifugation, gradients were harvested by upward displacement with Fluorinert FC 40 (Minnesota Mining and Manufacturing Co.) with an ISCO gradient fractionator coupled to a Chromatronix dual-wavelength absorbance monitor equipped with a 0.5-mm-path length flow cell. Purified phages were stored at 4°C over a drop of chloroform.

Strand separation and isolation of DNA. (i) Resolution of phage DNA strands by poly(U)·poly(G). Strand resolution of phage DNA by complexing with polyuridylic acid·polyguanylic acid [poly(U)·poly(G)] was performed as described by Lozeron and Szybalski (27). Cesium chloride was added to high-purity water to give a saturated solution at 60°C, and the warm solution was rapidly filtered through a 0.45-μm membrane filter. Saturated CsCl solutions had an absorbance at 260 nm (A_{260}) of less than 0.010. Polyallomer centrifuge tubes (5/8 by 3 inches [ca. 1.6 by 7.6 cm]) were placed in 1 liter of 0.1 M ethylenediaminetetraacetate (pH 10.0) and heated to 100°C for 15 min. The tubes were then washed with glass-distilled water and stored at room temperature. A quantity of phage containing 350 to 450 μg of DNA, freshly purified in a CsCl gradient, with an A_{260} greater than 60, was placed into a dialysis bag; both ends were closed with hemostats, and the phage were dialyzed for 2 h against 1 liter of phage

TABLE 1. *Bacterial strains and bacteriophage used in this work*

Strain	Genotype	Source
Bacterium		
AD1	HfrH *thi* (*proA/B argF lac*)Δ (*argI*)Δ	L. Gorini
CA8000	HfrH *thi*	J. Beckwith
GJ1	*thi* (*proA/B argF lac*)Δ *argI* RNase *strA*	Nitrosoguanidine mutagenesis of GL5 with low-RNase phenotype
DF634	*thi leu his pyrB strA*	D. Fraenkel
GL5	*thi* (*proA/B argF lac*)Δ *argI strA*	N. Glansdorff
JC12R15	*thi met purC argR15* spc^r *lac xyl mel*	G. Jacoby
KY3304	*thi bfe* (λcI857*xis*6Δ*b*515Δ*b*519)	Yamagishi
LS527	*thi trpR trpA his ilvA* (*arg ECBH*)Δ *pro* (*ϕ80*) (*ϕ80ppcargECBH*)	R. Press
MA4	*thi argA* (λ*argA*cI857)	N. Kelker
MA4A4	*thi argA* (λcI857) (λ*argA*cI857)	N. Kelker
MG427	*thi* (*ppc argECBH*)Δ str^r	G. Jacoby
EJ37	*thi thr leu proA/B argF argI lac strA* (*ϕ*80)	E. James
EJ113	*thi* (*proA/B argF lac*)Δ *argI argR15* spc^r	GL5 × JC12R15
EJ113*	*thi* (*proA/B argF lac*)Δ *argI argR15* RNase spc^r	Nitrosoguanidine mutagenesis of EJ113 with low-RNase phenotype
EJ114	*thi* (*ppc argECBH*)Δ *str* (*ϕ80i*$^\lambda$cI857) (*ϕ*80*ppcargECBH*1cI857)	P. James
EJ123	*thi argA* (λcI857*argA2*)	Our collection
EJ141	*thi* (*ppc argECBH*)Δ str^r (*ϕ*80*i*$^\lambda$cI857)	M. Cleary
EJ142	*thi* (*ppc argECBH*)Δ str^r (*ϕ*80*i*$^\lambda$cI857) (*ϕppcargECBH2i*$^\lambda$cI857)	M. Cleary
EJ149	HfrH *thi* (*proA/B argF lac*)Δ *argI*Δ *argR15* spc^r	AD1 × JC12R15, selected *spc* resistance
EJ199	*thi* (*proA/B argF lac*)Δ *pyrB argR15* RNase spc^r	P1 (DF634) transduction of EJ113*
EJ200	*thi* (*proA/B argF lac*)Δ *argR15* RNase spc^r	Spontaneous reversion of uracil requirement (EJ199)
EJ202	*thi* (*proA/B argF lac*)Δ (*argI*)Δ *argR15* RNase spc^r	P1 (EJ149) transduction of EJ200
EJ203	*thi* (*argI*)Δ *argR15* RNase spc^r	CA8000 × EJ202
EJ204	*thi* (*proA/B argF lac*)Δ (*pyrB*)Δ RNase *strA*	P1 (DF634) transduction of GJ1
EJ205	*thi* (*proA/B argF lac*)Δ (*argI*)Δ RNase *strA*	P1 (EJ149) transduction of EJ204
EJ206	*thi* (*argI*)Δ RNase *strA*	CA8000 × EJ205
EJ207	*thi* (*proA/B argF lac*)Δ RNase *strA*	Spontaneous reversion of EJ204 to $pyrB^+$
EJ213	*thi* (*proA/B argF lac*)Δ (*argI*)Δ RNase (λcI857) (λ*argF*2) *strA*	Lysogenization of EJ205
EJ214	*thi* (*proA/B argF lac*)Δ (*argI*)Δ RNase (λcI857) (λ*argI*cI857) *strA*	Lysogenization of EJ205
EJ215	*thi* (*proA/B argF lac*)Δ (*argI*)Δ RNase (λcI857) (λ*argF*23)	Lysogenization of EJ205
EJ217	*thi* (*ppc argECBH*)Δ *strA* (λcI857*S*7)	Lysogenization of MG427
EJ218	*thi* (*ppc argECBH*)Δ *strA* (λcI857*S*7) (λ*arg-ECBH*26)	Induction of KY3304 and transduction of EJ217 with LFT^a lysate
EJ219	*thi* (*ppc argECBH*)Δ *strA* (λcI857*S*7) (λ*arg-ECBH*2)	Induction of KY3304 and transduction of EJ217 with LFT lysate
EJ220	*thi* (*ppc argECBH*)Δ *strA* (λcI857*S*7) (λ*arg-ECBH5*)	Induction of KY3304 and transduction of EJ217 with LFT lysate
EJ221	*thi* (*ppc argECBH*)Δ *strA* (λcI857*S*7) (λ*arg-ECBH*30)	Induction of KY3304 and transduction of EJ217 with LFT lysate

TABLE 1—*Continued*

Strain	Genotype	Source
Bacteriophage		
	φ80argECBH	R. Press
	φ80i$^{\lambda}$cI857	L. Gorini
	φ80argECBH1i$^{\lambda}$cI857	P. James
	φ80argECBH2i$^{\lambda}$cI857	M. Cleary
	$^{\lambda}$cI857	M. Howe
	$^{\lambda}$cI857*S7*	N. Kelker
	$^{\lambda}$*argF*2 (YK611)	L. Gorini
	λ*argF*23(YK5512)	L. Gorini
	λ*argIvalSc*I857 (YK5)	L. Gorini
	λ*argECBH*2	Our collection
	λ*argECBH*5	Our collection
	λ*argECBH*26	Our collection
	λ*argECBH*30	Our collection
	λ*argA*cI857	N. Kelker
	λ*argA*2cI857	Our collection
	P1	J. Beckwith

[a] LFT, Low frequency of transduction.

dialysis buffer (0.1 mM ethylenediaminetetraacetate, 0.1 mM KH_2PO_4, pH 7.0) at 4°C. The phage were transferred to a test tube (16 by 125 mm), and the volume was adjusted to 1.8 ml with dialysis buffer; 0.50 ml of an aqueous solution of poly(U)·poly(G) (1 mg/ml) and 0.015 ml of 10% Sarkosyl NL 97 were added, and the mixture was heated for 2.5 min at 97°C in a hot-water bath with gentle swirling for the first 30 s. The mixture was rapidly cooled to 4°C, followed by the addition of 0.45 ml of 0.5 M Tris-hydrochloride (pH 7.6) and 10.9 ml of saturated CsCl. The density was adjusted to 1.725 g/ml (refractive index, 1.4022), and the mixture was centrifuged in a 75 Ti or 50 Ti rotor at 35,000 rpm for 67 to 72 h at 17°C. The contents of each tube were fractionated as described for the isolation of phage.

The separated strands were dialyzed overnight against 140 mM phosphate buffer (pH 7.6), adjusted to 0.3 N KOH, and incubated for 12 h at 37°C to remove complexed poly(U)·poly(G). The reaction mixture was neutralized with 3 N HCl, adjusted to 0.14 M in potassium phosphate, annealed at 68°C for 3 h, and passed through a 5-ml hydroxylapatite column at 60°C to remove double-stranded DNA. Single-stranded DNA was dialyzed against 1 mM ethylenediaminetetraacetate (pH 8.0) and stored at 4°C over chloroform.

(ii) Isolation of DNA from phage for binding to nitrocelluose filters. Bacteriophage was purified immediately before isolation of DNA by centrifugation to equilibrium in 1.5-density cesium chloride, and purified phage was adjusted with 1.5-density cesium chloride to yield a concentration corresponding to an A_{260} of 13. The phage solution was dialyzed against 1× SSC (0.15 M NaCl plus 0.015 M sodium citrate, pH 7.4) for 3 h without stirring, and the phage was extracted three times with an equal volume of redistilled phenol saturated with 1× SSC. For the first extraction, 0.015 ml of a 25% solution of sodium dodecyl sulfate was added per ml. The third phenol extraction was followed by extraction with chloroform-isoamyl alcohol (24:1, vol/vol). The aqueous phase was then adjusted to 25 μg/ml in Pronase and incubated for 2 h at 37°C. Two additional phenol extractions were performed, followed by a final chloroform-isoamyl alcohol extraction. The aqueous layer was dialyzed exhaustively against 10 mM ethylenediaminetetraacetate (pH 8.0) and stored at 4°C over a drop of chloroform.

Preparation in vitro of [^{3}H]RNA. Strains genetically derepressed for enzymes of the arginine biosynthetic pathway and strains possessing a functional *argR* gene were grown in S1 medium supplemented with 200 μg of L-arginine per ml at 37°C to a concentration of approximately 3×10^8 cells per ml. The culture was pulsed-labeled with radioactive uridine for 50 s by the addition of 4 μCi of [^{3}H]uridine per ml. At the end of the pulse-labeling period, a freshly prepared solution of sodium azide was added rapidly (final concentration, 0.02 M), and the culture was immediately poured onto the same volume of frozen, crushed buffer containing 20 mM Tris-hydrochloride (pH 7.3) and 5 mM $MgCl_2$. The mixture was allowed to thaw, concentrated by centrifugation for 10 min at 8,000 rpm in a JA20 rotor, and suspended in a 0.1 volume of 0.1× SSC (pH 7.0). The cells were lysed by a single passage through a French press at 8,000 lb/in^2. Heparin was immediately added to the lysate to a final concentration of 0.02 mg/ml.

Deoxyribonuclease (ribonuclease [RNase]-free) was added to the supernatant solution (20 μg/ml), and the mixture was incubated at room temperature for 15 min. The solution of RNA was then extracted three times with phenol saturated with 0.1× SSC. The first extraction was performed in the presence of 0.4% sodium dodecyl sulfate. After each extraction, the mixture was centrifuged at 6,000 rpm for 5 min to facilitate phase separation. After the last phenol extraction, residual phenol was removed from the aqueous phase by extraction with chloroform-isoamyl alcohol (24:1, vol/vol). The aqueous phase was then adjusted to an overall concentration of 2× SSC by the addition of 20× SSC and passed through a

double layer of nitrocellulose filters. To the filtered solution, 2 volumes of 95% ethanol (−20°C) was added, and the mixture was left to stand at −20°C until a flocculent precipitate had formed. The precipitate was collected by centrifugation, suspended in 0.1× SSC, and passed through a Sephadex G-50 (fine) column equilibrated with 0.1× SSC. RNA eluted in the void volume. It was concentrated by ethanol precipitation and suspended in a small volume of 2× SSC, and the concentration of RNA was determined by measurement of A_{260} (1 A_{260} = 40 μg of RNA). The RNA solution was saturated with phenol and stored at 4°C.

RNA was prepared from strains grown under conditions of physiological derepression for the arginine biosynthetic pathway as described above but with the omission of arginine from the growth medium. The preparation of RNA without radioactive label, for use in competitive hybridization reactions, was performed exactly as for labeled RNA, except that radioactive uridine was omitted and replaced by an equal amount of uridine.

Binding of DNA to nitrocellulose filters. DNA was immobilized on 50-mm Schleicher & Schuell Co. Selectron B-6 filters that had been presoaked for at least 30 min in 6× SSC before binding of DNA. The DNA (in 4 ml of 1.0 mM ethylenediaminetetraacetate, pH 8.0) was denatured by heating at 97°C for 5 min followed by quick-chilling in an ice-water bath. The presoaked filter was placed on a 250-ml Millipore filtration unit, and 10 ml of 6× SSC was passed through the filter unit under the force of gravity. The DNA solution was then adjusted to an overall concentration of 6× SSC by the addition of the appropriate amount of 20× SSC, and the liquid was allowed to pass through the filter under the force of gravity. An additional 10 ml of 6× SSC was added to the filter and allowed to flow through under the force of gravity. Then 100 ml of 6× SSC was rapidly passed through the filter by the use of suction created by a water aspirator. The filter was then removed and placed on aluminum foil, and 13-mm filters were punched from the original 50-mm-diameter filter. These were allowed to dry overnight at room temperature. When dry, each filter was placed in a Mini-Vial (Beckman) and baked in a vacuum oven at 80°C for 2 h. After cooling, the vials containing the filters were capped tightly and stored at room temperature in the dark.

Separated strands (resolved by ribopolymer binding) of phage DNA were immobilized in an identical manner except that the denaturation step was omitted.

Hybridization procedures for mRNA formed in vivo. The quantity of RNA synthesized in vivo was determined by the hybridization procedures of Gillespie and Spiegleman (14). The amount of mRNA produced was determined directly by hybridization to the appropriate DNA immobilized on nitrocellulose filters. Specifically, for each determination, 32 μg of RNA was added to a vial containing 2 μg of DNA immobilized on a nitrocellulose filter. Then, 2× SSC containing 0.4% sodium dodecyl sulfate was added to give a final volume of 0.3 ml, and the vial was incubated at 67°C for 16 h. Filters containing DNA-RNA hybrids were rinsed in 2× SSC and treated with 40 U of RNase A and 20 U of T1 RNase in 1 ml of 2× SSC for 1 h at 25°C. The filters were then washed on both sides with 50 ml of 2× SSC and dried, and the radioactivity was determined in Redi-Solv IV in a Beckman LS230 scintillation spectrometer.

Competitive hybridization procedures. Competitive hybridization reactions employing radioactive RNA formed in vivo were performed by first complexing unlabeled RNA (produced in vivo) to the appropriate immobilized DNA by hybridization at 67°C for 16 h in a volume of 0.30 ml of 2× SSC-0.4% sodium dodecyl sulfate. The hybridization vial was then chilled, and the filter was removed and washed on each side with 100 ml of 2× SSC. The filter was then placed in a new vial; 64 μg of the appropriate radioactive RNA (formed in vivo) was added, and hybridization performed as before, except that at the end of the hybridization reaction the filter was removed and treated with 20 μg of RNase A and 10 U of T1 RNase in 1 ml of SSC for 1 h at room temperature. The filter was dried, and the bound radioactivity was determined by using Redi-Solv IV in a Beckman LS230 scintillation spectrometer.

RESULTS

Standardization of hybridization procedures. The optimum volume for hybridization reactions was determined by hybridizing a constant amount of RNA (32 μg) extracted from an *argR*⁻ strain to 2 μg of λ*argF*2 DNA immobilized on a nitrocellulose filter in various volumes of 2× SSC (ranging from 0.1 to 1.0 ml). A volume of 300 μl was found to be convenient and efficient; therefore, this volume was used for all other hybridization reactions performed in this study.

The effect of hybridizing various quantities of labeled RNA isolated from an *argR*⁻ strain to a constant amount (2 μg) of DNA isolated from a specialized transducing bacteriophage carrying the appropriate arginine gene was determined. Increasing the quantity of RNA above 35 μg resulted in only a small increase in the quantity of RNA that complexed to DNA immobilized on the nitrocellulose filter (data not shown). The quantity of radioactive RNA used in hybridization reactions was standardized at 32 μg of RNA with 2 μg of denatured DNA or 1 μg of isolated single-stranded DNA bound to nitrocellulose filters.

Determination of the DNA strand carrying sense information for arginine genes. The procedure of Lozeron and Szybalski (27) using ribopolymer binding was used to resolve the DNA strands of all specialized transducing phages used in this work, except for DNA isolated from λ*argI*cI857 [the DNA strands of this bacteriophage are not amenable to strand resolution by the poly(U)·poly(G) binding technique since

the major guanine-plus-cytosine-rich region of the left arm has been replaced by DNA of bacterial origin] as described by Kikuchi and Gorini (23).

The separated DNA strands of the specialized bacteriophages listed in Table 2 were hybridized with RNA produced from strains possessing an ArgR$^-$ phenotype as described. A low background of hybridization to the separated DNA strands of both lambda and ϕ80 was measured (Table 2), as would be expected since the strains used are not lysogenic for lambda or ϕ80. The majority of RNA produced in an *argR*$^-$ strain that hybridized to DNA isolated from the specialized transducing phages λ*argF*2 and λ*argF*23 complexed specifically to the heavy DNA strand of both λ*argF* specialized phages, thus indicating that *argF* sense information is carried on the heavy DNA strand of λ*argF*2 and λ*argF*23. Both separated strands of λ*argA*2*c*I857 were found to complex approximately the same amount of RNA isolated from a strain producing constitutive levels of *argA* mRNA. Experiments were performed with mRNA isolated from a strain carrying the *argR*$^+$ allele which was grown under conditions of physiological repression and derepression for the arginine biosynthetic regulon. These studies demonstrated that the heavy DNA strand of λ*argA*2*c*I857 complexed approximately the same amount of RNA whether the RNA was isolated from a strain grown under conditions of physiological repression or derepression; in contrast, the light DNA strand of λ*argA*2*c*I857 complexed four times as much RNA when mRNA was isolated from a strain grown under conditions of physiological derepression compared with the quantity of RNA that hybridized when RNA was isolated from the strain grown in the presence of exogeneous arginine (Table 3).

RNA prepared from a strain carrying the *argR*$^-$ allele was permitted to complex to the separated DNA strands of a series of specialized transducing bacteriophages, each carrying the *argE* and *argCBH* operons. Approximately 67% of the hybridizable RNA was found to complex to the light DNA strand of λ*argECBH*2, λ*argECBH*5, λ*argECBH*26, and λ*argECBH*30, while the remaining 33% hybridized to the heavy DNA strand of these specialized transducing phages (Table 2). These data are consistent with *argCBH* sense information being carried on the light DNA strand of these phages and *argE* sense information being carried on the heavy DNA strand. In contrast, the reverse pattern of hybridization was observed when experiments were performed with separated DNA strands isolated from ϕ80*argECBH*1*i*$^\lambda$*c*I857 and ϕ80*argECBH*2*i*$^\lambda$*c*I857, with the major proportion of mRNA complexing with the heavy DNA strand of these phages (Table 2).

TABLE 2. *Sense strand determination of arginine-transducing DNAs*[a]

Bacteriophage	DNA strand[b]	% of input bound	cpm bound
λ*c*I857*S*7	L	0.006	48
	H	0.004	32
ϕ80*i*$^\lambda$*c*I857	L	0.005	40
	H	0.003	24
λ*argF*2	L	0.030	240
	H	0.220	1,760
λ*argF*23	L	0.020	160
	H	0.210	1,680
λ*argA*2*c*I857	L	0.120	960
	H	0.070	560
λ*ppcargECBH*2	L	0.520	4,160
	H	0.220	1,760
λ*ppcargECBH*5	L	0.650	5,200
	H	0.210	1,680
λ*ppcargECBH*26	L	0.420	3,360
	H	0.220	1,760
λ*ppcargECBH*30	L	0.710	5,680
	H	0.290	2,320
ϕ80*ppcdargECBH* 1*i*$^\lambda$*c*I857	L	0.150	1,200
	H	0.270	2,160
ϕ80*ppcargECBH* 2*i*$^\lambda$*c*I857	L	0.060	480
	H	0.180	1,440

[a] RNA of specific activity 25,000 cpm/μg was prepared from strain EJ203 as described in the text. The DNA strands of the various specialized bacteriophages were separated by the use of ribopolymer binding, and 1 μg of each strand was bound to nitrocellulose filters. The input of RNA into the hybridization reaction was 32 μg, and hybridization was performed for 16 h at 67°C in 300 μl of 2× SSC-0.4% sodium dodecyl sulfate. At the conclusion of hybridization, the filters were rinsed with 2× SSC, treated with RNase as described in the text, and washed on both sides with 50 ml of 2× SSC. The filters were dried, and bound [^{3}H]RNA was determined with BioSolv IV (Beckman Instruments) in an LS230 scintillation spectrometer. Hybridization values were corrected for background hybridization to the separated strands of λ or ϕ80*i*$^\lambda$*c*I857 DNA, depending on the origin of the transducing phage. The results are the average of three determinations that deviated less than 5%.

[b] L, Light; H, heavy.

arg-specific mRNA levels of strains carry-

ing the *argR*$^+$ or *argR*$^-$ allele. The levels of arginine-specific mRNA were determined for a number of *E. coli* strains during growth in S medium in the presence of exogeneous arginine. RNA prepared from a strain (EJ200) possessing the *argR15* allele (thus genetically derepressed for arginine biosynthesis) and a deletion in the *argF* gene but having all other arginine genes functional was hybridized to *argA*, *argI*, and *argF* transducing DNA. The results shown in Table 4 for RNA isolated from strain EJ200 demonstrate the levels of expression of these various arginine genes under conditions of constitutive expression of the arginine regulon. It is apparent (Table 4) that a negligible quantity of mRNA complexed to the *argF* gene, implying that considerable genetic drift occurred between the *argF* and *argI* genes since they diverged from a common presumptive ancestral gene, as reported by Sens et al. (34). When similar experiments were performed with RNA isolated from strain EJ202, which is isogenic with strain EJ200 except for the introduction of a nonreverting mutation in *argI* (presumed to be a deletion [34]), it was noted that there was no significant difference in the quantity of mRNA that hybridized to the same arginine-transducing DNA. The levels of arginine-specific mRNA present in the strain EJ203 were also comparable to those measured for strains EJ200 and EJ202 except for the presence of mRNA that complexed specifically with the *argF* gene.

The level of expression of the *argF* and *argA* genes was also determined under conditions of physiological derepression and repression with strains EJ206 and EJ207 by measuring the quantity of mRNA that complexed with the resolved DNA strand carrying sense information for the genes involved (Table 4). Similar experiments were performed to measure the level of expression of the *argI* gene; however, in this case, resolved DNA strands were not utilized. The derepressed:repressed ratio of expression of these operons was found to be between 4 and 6. This is a value similar to that found for the *argF* and *argCBH* operons (11; unpublished data).

Competitive hybridization of arginine-specific mRNA. The relative amount of arginine-specific mRNA in a number of *E. coli* K-12 strains was determined by sequential hybridization competition experiments with mRNA isolated from a variety of strains grown under conditions of genetic derepression and physiological derepression and repression. The data presented in Fig. 1 show the effective competition of mRNA isolated from strains grown under conditions of genetic derepression and physiological derepression in contrast to limited competition in the case where RNA had been isolated from strains grown under conditions of physiological repression for the arginine regulon (by growth in the presence of 200 μg of arginine per ml). No competition with *argF* mRNA was observed when cold RNA was prepared from a strain carrying a deletion covering the entire *argF* gene (a functional *argI* gene was present).

DISCUSSION

The results presented in this report demonstrate the detection and determination of *arg*-specific mRNA levels synthesized under conditions of genetic derepression, as well as physiological derepression and repression. The use of RNA prepared from a strain (EJ203) genetically depressed for the arginine regulon due to the *argR15* allele allowed the unambiguous determination of the sense strand for the *argF*, *argE*, and *argCBH* operons (Table 2). In the two λ*argF* transducing phages tested in this work, the heavy strand carried sense information. The light strand carried sense information for *argE* on the φ80*argECBHi*$^{\lambda}$cI857 transducing phages, and the heavy strand carried sense information for *argE* on the various λ*argECBH* transducing phages. Conversely, the heavy

TABLE 3. *Hybridization*[a]

RNA source	Input (cpm)	cpm hybridized to λ*argA*2cI857 LS[b] DNA	% input to λ*argA*2cI857 LS DNA	cpm hybridized to λ*argA*2cI857 HS[b] DNA	% input to λ*argA*2cI857 HS DNA
EJ203	9.2×10^5	1,140	0.124	645	0.070
EJ206 (−Arg)	8.9×10^5	908	0.102	625	0.071
EJ206 (+Arg)	1.2×10^6	260	0.024	807	0.064

[a] RNA was prepared from strains EJ203 and EJ206 as described in the text. Hybridization reactions were performed as described in footnote *a* of Table 2, and the hybridization values reported were corrected for hybridization to the separated DNA strands of lambda. Hybridization reactions were performed with 1 μg of DNA (resolved strand) isolated from the specialized transducing bacterophage λd*argA*2cI857 and with 32 μg of RNA. The results are the average of three determinations that varied less than 7%.

[b] LS, Light strand; HS, heavy strand.

TABLE 4. *arg-specific mRNA levels of wild-type and arg regulatory strains of E. coli K-12*[a]

Strain	Relevant genotype	Growth condition	% of input RNA bound to DNA isolated from:			
			λ*argF*2cI857 (HS)[b]	λ*argI*cI857	λ*argA*2cI857 (LS)[b]	λ
EJ200	(*argF*)Δ *argR15*	+Arg	0.03	0.22	0.13	0.01
EJ202	(*argF*)Δ (*argI*)Δ *argR15*	+Arg	0.02	0.23	0.12	0.01
EJ203	(*argI*)Δ *argR15*	+Arg	0.25	0.23	0.12	0.01
EJ206	(*argF*)Δ	−Arg	0.03	0.19	0.10	0.01
EJ206	(*argF*)Δ	+Arg	0.02	0.05	0.03	0.01
EJ207	(*argI*)Δ	−Arg	0.22	0.20	0.10	0.01
EJ207	(*argI*)Δ	+Arg	0.05	0.04	0.02	0.01

[a] Hybridization reactions were performed and the RNA was extracted as described in the text. For the preparation of RNA from cells growing under conditions of physiological repression, arginine (Arg) was added to the growth medium at a concentration of 200 μg/ml. The results presented are the average of three determinations that varied less than 6%.

[b] HS, Heavy strand; LS, light strand.

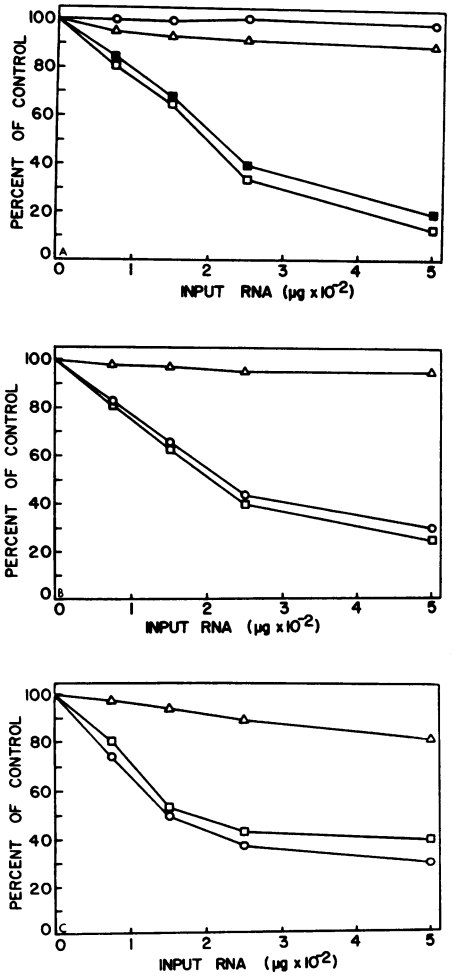

FIG. 1. *(A) Competition between unlabeled RNA formed in vivo and* ^{3}*H-labeled RNA formed in vivo from strain EJ203. Hybridization reactions were performed by first permitting the quantity of unlabeled RNA indicated on the abscissa to complex with 1 μg of the heavy DNA strand isolated from λargF2 for 16 h at 67°C. The filter was washed and placed into a new reaction vial;* ^{3}*H-labeled mRNA (64 μg, formed in vivo) was added, and hybridization was permitted to occur for an additional 16 h at 67°C. Prehybridization with unlabeled RNA formed from strains: EJ200, EJ202, and EJ207 (grown with arginine at 200 μg/ml) (○); EJ203 (□); EJ206 grown in the presence of arginine (△); and EJ206 grown in the absence of arginine (■). The data are the averages of duplicate determinations with a variance of less than 3%. (B) Competitive hybridization of* ^{3}*H-labeled argI mRNA, produced in vivo from strain EJ200, by unlabeled in vivo RNA. The experiment was performed as described, except that λargIcI857 DNA served as the hybridization probe. Competing in vivo RNA was prepared from strains: EJ200 and EJ203 (□), EJ207 grown without arginine (○), and EJ207 grown in the presence of 200 μg of arginine per ml (△). The data are the results of duplicate determinations with a variance of less than 4%. (C) Competitive hybridization of* ^{3}*H-labeled argA mRNA, produced in vivo from strain EJ203, by unlabeled in vivo RNA. The experiment was performed as described, except that λargA2cI857 light-strand DNA served as the hybridization probe. Competing in vivo RNA was prepared from strains: EJ203 (○), EJ206 grown in the presence of exogenous arginine (△), and EJ206 grown in the absence of arginine (□). The data are the results of duplicate determinations with a variance of less than 3%.*

strand of the φ80*argECBHi*$^{\lambda}$cI857 transducing phages was found to carry sense information for the *argCBH* gene, whereas the light strand of the λ*argECBH* transducing phages encodes sense information for the *argCBH* operon. This difference in sense strand configuration between φ80*argECBH* and λ*argECBH* phages is the consequence of the *argECBH* operons being oriented in opposite directions in these transducing phages.

Identification of the sense strand for the *argA*

gene could not be determined solely by the use of RNA formed in vivo from a strain constitutively expressing the arginine regulon since both strands of λd*argA*2*c*I857 bacteriophage DNA demonstrated hybridization values well above the background (Table 2); however, as shown in Table 3, the use of RNA formed in vivo under both physiologically derepressed and repressed conditions allowed the assignment of the light strand as the sense strand for the *argA* gene. Only the light strand demonstrated a significant loss of hybridization potential when hybridized with RNA isolated from a strain grown under conditions of physiological repression for the *argA* gene when compared with the amount hybridized when RNA was isolated from the same strain grown under conditions of derepression for the *argA* operon. The heavy DNA strand of the *argA* transducing phage did not vary in its hybridization potential regardless of whether RNA was formed under constitutive or repressed conditions.

The level of arginine gene expression under conditions of physiological derepression and repression was measured for the strains listed in Table 4. All of the arginine genes tested responded to the repression signal generated by excess arginine by forming lower *arg* mRNA levels in the case of cells carrying the *argR*$^+$ allele, whereas mRNA levels were unaffected in strains possessing a mutation in the *argR* gene. The presence of arginine in the growth medium resulted in an approximate fivefold repression of expression of the *argF*, *argI*, and *argA* operons (Table 4) when compared with levels of *arg* mRNA formed when arginine was absent from the growth medium, thus showing that some control of gene expression is mediated at the level of transcription as previously reported by Sens and James (33) for the *argF* operon, by Sens et al. (in press) for the *argF* and *argI* operons, and by Sens et al. (35) for the *argE*, *argCBH*, and *argA* operons. The use of competitive hybridization studies with the *argA*, *argF*, and *argI* operons gave rise to slightly higher repression values as judged by the difference in slopes of the lines between repressed and derepressed competing RNA species. An approximate 10-fold difference in the derepressed:repressed levels was determined for the *argF*, *argA*, and *argI* operons. This latter figure is in accord with the results of Cunin et al. (11), who reported work concerning the level of *argECBH*-specific mRNA and of the enzymes formed by the *argE* and *argH* genes. We have also determined the levels of expression of *argECBH* mRNA, with similar results (unpublished data).

Pouwels et al. (29) reported derepressed:repressed ratios for *argE* mRNA of between 3 and 15 depending on the strains chosen for comparison, with a corresponding ratio of acetylornithinease activity of between 10 and 17. These workers also reported derepressed:repressed ratios for *argCBH* operon mRNA of between 15 to 27 compared with a ratio of arginosuccinase activity of between 20 and 50. The noncorrespondence of arginine mRNA levels under derepressed and repressed conditions compared with the ratio of the levels of the corresponding enzyme activity may be the result of difficulties in the experimental protocol. For example, a significant portion of the mRNA measured as *arg* specific may be mRNA that is complexing to other unidentified *E. coli* genes carried by the arginine transducing phages. This would mask much of the true changes in *arg* mRNA levels. It is clear that different independently isolated specialized transducing phages carrying a particular operon may also have vastly different amounts of neighboring bacterial genes. These problems are demonstrated by an examination of the quantity of mRNA specifically complexing with DNA isolated from the various *argECBH* transducing phages listed in Table 2. The λ transducing phages all exhibit high levels of hybridization (0.710% to the light strand of λ*ppcargECBH*30) compared with a value of 0.180% for the heavy strand of φ80*ppcargECBH*2*i*$^\lambda$c1857 (although this is the other strand, it carries the same sense information as the light strand of the λ phage due to the opposite orientation of the bacterial genes in these phages).

Although the major proportion of the control of the expression of the arginine operons presented in this study has been shown by the in vitro transcriptional studies of Sens et al. (35; in press) to be at the transcriptional level, some other control mechanism operating beyond that of holorepressor-operator interaction may still exist. The recent studies on the regulation of the tryptophan operon (5) and the corresponding elucidation of a second regulatory site, termed the attenuator, could explain some of the discrepency that exists between in vivo and in vitro repression values and the corresponding higher enzyme values as was recently espoused by Krzyzek and Rogers (24).

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