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# In Vitro Transcription of the *Escherichia coli* K-12 *argA*, *argE*, and *argCBH* Operons

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Deoxyribonucleic acid isolated from *argA* and *argECBH* transducing phages was utilized to study the in vitro synthesis of *argA*, *argE*, and *argCBH* messenger ribonucleic acid. The specific regulation of these operons by the arginine holorepressor was demonstrated, providing evidence that the majority, if not all, of the control of these operons is exercised at the transcriptional level. Data are presented which indicate that the arginine holorepressor functions by binding to the operator region and concomitantly prevents the binding of ribonucleic acid polymerase to the corresponding promoter region.

The genes involved in arginine biosynthesis are scattered around the *Escherichia coli* chromosome, constituting a regulon with six or seven distinct operators (1, 16, 17) controlling nine genes (Fig. 1). The arginine genes *ECBH* are continuous, and studies have been presented (11, 20, 35, 38) that indicate that the *argECBH* gene cluster is a bipolar operon divergently transcribed from an internal control region situated between *argE* and *argCBH*. Further analysis (1, 5, 15) of this gene cluster suggested that one operator controls *argE* with transcription oriented counterclockwise and that another operator controls the *argCBH* genes with transcription oriented in the opposite direction. Extensive genetic studies by Bretscher and Baumberg (3), employing four-factor crosses and deletion mapping with mutants involving the *argE* and *argCBH* control regions, 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 operons.

The results of in vivo studies have demonstrated that the synthesis of enzymes in the arginine pathway is repressed when wild-type cells are grown in the presence of arginine and is derepressed in the absence of exogenous arginine (16, 17, 29). It has been demonstrated (21, 29) that the *argR* gene product is a protein, which, in concert with a corepressor, regulates the synthesis of all the arginine genes in a coordinate but nonparallel manner. The exact nature of the corepressor for the arginine pathway is unknown, but data have been presented (4) that suggest that arginyl-transfer ribonucleic acid (RNA) is not the corepressor, and the preliminary results of Cunin et al. (7) suggest that arginine may be the corepressor for the *argCBH* operon.

The question of whether the *argR* gene product functions at the transcriptional or translational stage has not been answered definitively for all the genes in this regulon; however, Sens and James reported (42) that a minimum of 95% of the regulation of the expression of the *argF* gene is mediated at the level of transcription. The studies of Sens, Natter, and James (submitted for publication) have confirmed the notion that the majority, if not all, of the regulation of the *argF* gene is effected at the transcriptional stage, and these workers have shown that the *argI* gene is also controlled at this level. Hybridization studies have been performed (6, 8, 23, 24, 25) in which the level of *argECBH* messenger RNA (mRNA), produced in vivo by the *argECBH* cluster, has been measured under conditions of repression and derepression by hybridization to deoxyribonucleic acid (DNA) isolated from a number of specialized transducing phages carrying the *argECBH* gene cluster, and it was demonstrated that the levels of *argECBH* mRNA and the corresponding enzymes do not correlate. Lavellé and Dehauwer (26) reported similar results for the tryptophan operon of *E. coli* and proposed two regulatory systems: one controlling transcription of DNA, and another controlling the translation of the resulting mRNA. McLellan and Vogel (31) proposed a similar mechanism for the regulation of the arginine regulon based on studies of the *argECBH* gene cluster. However, the recent results of Bertrand et al. (2), involving studies of the tryptophan operon, provide evidence for the existence of a second regulatory site, the "attenuator," functioning at the

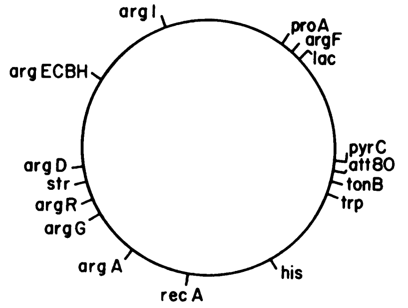

FIG. 1. *Simplified genetic map of E. coli K-12.*

transcriptional level, which accounts for the discrepancy in mRNA and enzyme level. Thus, the development of a thorough understanding of the precise mechanism for the regulation of the arginine regulon will depend upon the further development of both in vivo and in vitro systems for the analysis of the products of the various genes of the arginine regulon.

The gene coding for the first enzyme of the arginine biosynthetic pathway, *argA*, has received little attention in studies regarding the control of gene expression in the arginine regulon. One reason for this situation is the instability of this enzyme, *N*-acetylglutamate synthetase (EC 2.3.1.1), even in crude extracts of *E. coli*; however, the synthesis of this enzyme is repressible, and also the enzyme is subject to feedback inhibition (27).

In this work the in vitro transcription of the *argA*, *argE*, and *argCBH* operons was studied by using cytoplasmic extracts of *E. coli* and DNA isolated from specialized transducing phages carrying these arginine genes.

## MATERIALS AND METHODS

**Materials.** Trizma base and dithiothreitol (DTT) were purchased from Sigma Chemical Co., St. Louis, Mo. All radioisotopes were obtained from New England Nuclear Corp., Boston, Mass. Uridine, adenosine, guanosine, and cytidine 5'-triphosphates (UTP, ATP, GTP, and CTP, respectively) were supplied by P-L Biochemicals, Inc., Milwaukee, Wis. Polyuridylic acid-polyguanylic acid [poly-(U-G)] was purchased from Biopolymers, Inc., Cleveland, Ohio, and had a U-G ratio of 1.9:1. Electrophoretically purified deoxyribonuclease (DNase) and ribonuclease (RNase) were obtained from Worthington Biochemicals Corp., Freehold, N.J. Spectinomycin and streptolydigin were the generous gifts of The Upjohn Co., Kalamazoo, Mich. Selectron B-6 filters were supplied by Arthur Thomas Co., Philadelphia, Pa. Media were purchased from Difco Laboratories, Detroit, Mich. Cesium chloride was obtained from Columbia Organic Chemicals, Columbia, S.C.

**Media.** Bacteria used for the preparation of phage stocks and cell extracts were grown in L-medium (28). Bacteriophages were titered on TYE plates in H-top agar (18). F-top agar was used for plating cells on minimal medium (33). Selection plates contained medium A (9) and supplemental growth factors as required, 2% agar, and 0.5% glucose as the carbon source. Supplements were used at the concentrations previously described (13).

**Bacterial strains and bacteriophages.** The genotype and origin of bacterial strains and bacteriophages used in this work are listed in Table 1.

The specialized transducing bacteriophage λ*h*80d*argECBH*1, which is the parent of the hybrid phage used as DNA template in this work, was isolated by Press et al. (39) by the technique of episome fusion. From this φ80d*argECBH* phage, a hybrid phage, φ*h*80d*argECBH*1, was constructed (P. James, unpublished data). This was found to have a density similar to that of its helper phage which severely hindered separation of the transducing phage from the helper phage. When DNA isolated from λ*h*80d*argECBH*1 was used as hybridization probe for the determination of the derepressed/repressed ratio of *argE* and *argCBH* mRNA formed in vivo, a ratio of about 2 was determined. It was felt that this low ratio was a result of hybridization of mRNA from other bacterial genes, neighboring the *argECBH* operons, to the complementary DNA sequence carried on the specialized transducing phage (due to the relatively large amount of bacterial information carried on this phage). A light mutant (lower density) of the λ*h*80d*argECBH*1 transducing phage was isolated by the ethylenediaminetetraacetate (EDTA) procedure of Parkinson and Huskey (37). This selection resulted in the isolation of a phage, λ*h*80d*argECBH*2, that possessed a much decreased density and greatly facilitated the purification of the bacteriophage (M. Cleary, unpublished data).

The specialized transducing phage λd*argECBH*26 was constructed from an *att* lambda deletion strain (KY3304) into which λcI857*sus xis*6Δ*b*515Δ*b*519 was inserted into the *bfe* gene by the technique of Shimada et al. (43). The *bfe* gene is located less than 1 min from the *argECBH* gene cluster, and the subsequent induction of this strain gave rise to the λd*argECBH*26 transducing phage (W. Natter, D. Sens, and E. James, unpublished data). Subsequent characterization of this phage revealed that it was easily purified from helper phage, that the light DNA strand of λd*argECBH*26 carried sense information for *argCBH*, and that the heavy strand carried sense information for *argE* (W. Natter, D. Sens, and E. James, submitted for publication).

The specialized transducing phage λcI857d*argA*2 was isolated by Sens and James (unpublished data) as a light mutant of λcI857d*argA* by the procedure of Parkinson and Huskey (37).

**Construction of RNase-negative strains.** An overnight culture of cells was subcultured and grown to the late log phase in L-medium with glucose. A 1-ml amount of this culture was centrifuged, washed with TM buffer, and resuspended in 1.0 ml of TM buffer. [TM buffer is (per liter): tris-(hydroxymethyl)aminomethane (Tris), 24.2 g; maleic acid, 23.2 g; $(NH_4)_2SO_4$, 1.0 g; $MgSO_4 \cdot 7H_2O$,

TABLE 1. *Bacterial strains and bacteriophages*

| Strain | Genotype | Source |
|---|---|---|
| *E. coli* | | |
| CA8000 | HfrH *thi* | J. Beckwith |
| DF634 | *thi leu his pyrB strA* | D. Fraenkel |
| GJ1 | thi (*proA/B argF lac*)Δ *argI* RNase | NTG mutagenesis of GL5 with low-RNase phenotype |
| GL5 | *thi* (*proA/B argF lac*)Δ *argI* | N. Glansdorff |
| JC12R15 | *thi met purC argR15 spc*$^r$ *lac xyl mel* | G. Jacoby |
| KY3304 | *thi bfe* (λcI857*xis*6Δ515Δ*b*519) | H. Yamagishi |
| MA4A4 | *thi argA* (λcI857) (λcI857d*argA*) | N. Kelker |
| MG427 | *thi* (*ppc argECBH*)Δ *str*$^r$ | G. Jacoby |
| EJ113 | *thi* (*proA/B argF lac*)Δ *argI argR*15 *spc*$^r$ | GL5 × JC12R15 |
| EJ113* | *thi* (*proA/B argF lac*)Δ *argI argR15* RNase *spc*$^r$ | NTG mutagenesis of EJ113 with low-RNase phenotype |
| EJ123 | *thi argA* (λcI857) (λcI857d*argA*2) | Our collection |
| EJ142 | *thi (ppc argECBH)*Δ *str*$^r$ (λ*h*80cI857) (λ*h*80d*argECBH*2) | M. Cleary |
| EJ217 | *thi* (*ppc argECBH*)Δ *str*$^r$ (λcI857*S*7) | Our collection |
| EJ218 | *thi* (*ppc argECBH*)Δ *str*$^r$ (λcI857*S*7) (λd*argECBH*26) | Our collection |
| Bacteriophage | | |
| *ϕ*80 | | L. Gorini |
| *ϕ*80d*argECBH* | | R. Press |
| λ*h*80cI857 | | L. Gorini |
| λ*h*80d*argECBH*2 | | M. Cleary |
| λcI857*S*7 | | N. Kelker |
| λd*argECBH*26 | | Our collection |
| λcI857d*argA* | | N. Kelker |
| λcI857d*argA*2 | | Our collection |

0.1 g; $Ca(NO_3)_2$, 5.0 mg; and $FeSO_4 \cdot 7H_2O$, 0.25 mg.] To the cell suspension was added 0.1 ml of a solution containing 1 mg of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (NTG) per ml, and the mixture was incubated at 37°C for 30 min without aeration. The mutagenized culture was then washed three times with TM buffer, resuspended in 1.0 ml of TM buffer, and diluted to give a concentration of 5,000 viable cells per ml; a 0.1-ml quantity was then spread on each of 100 TYE plates. The plates were incubated overnight at 30°C and then replica-plated onto fresh TYE plates. The master plates were stored at 4°C, and the replica plates were incubated at 30°C for 3.5 h. (GSA is 0.7% agar, 0.4% EDTA, and 3.0% yeast RNA [Sigma] adjusted to pH 7.0.) After 3.5 h of incubation, 1.0 ml of 1.0 N HCl was added to each plate, and any colony not surrounded by a clear halo was scored as RNase positive, located on the master plate, purified by streaking to single colonies, and retested for the RNase phenotype.

**Propagation and purification of bacteriophages.** The bacteriophages λ*h*80d*argECBH2*, λd*argECBH*26, and λcI857d*argA*2 were propogated from the appropriate lysogenic strains as described by Miller (32). Bacteriophages were purified as described previously (22, 41).

**Resolution of phage DNA strands by poly(U-G).** Strand resolution of bacteriophage DNA by complexing with poly(U-G) was performed as described by Hradecna and Szybalski (19) with the modification of Sens et al. (41).

**Isolation of DNA.** Bacteriophages were purified immediately prior to isolation of DNA by centrifugation to equilibrium in a cesium chloride gradient (density, 1.5 g/cm$^3$). Extraction of DNA for use as a template for in vitro transcription was performed as described by Miller (32) and for use in binding to nitrocellulose filters as described previously (41).

**Preparation of cell extracts.** Cell extracts were prepared from strains EJ113* and GJ1 by the method of Miller (32). The S-30 cell extracts were dialyzed twice against 50 volumes of buffer containing 20 mM Tris-hydrochloride (pH 7.9), 15 mM $MgCl_2$, 150 mM KCl, 1 mM DDT, and 2 mM L-arginine and were then stored at −70°C. Care was taken to select S-30 cell extracts prepared from strains carrying the *argR*$^-$ and *argR*$^+$ alleles, which were equally efficacious in directing the in vitro synthesis of β-galactosidase as described below.

**In vitro transcription.** Standard reaction mixtures for in vitro transcription contained (per milliliter): Tris-hydrochloride (pH 7.9), 23 mM; $MgCl_2$, 15 mM; KCl, 150 mM; DDT, 1 mM; L-arginine, 1 mM; ATP, UTP, and GTP, each at 0.15 mM; [$^3$H]CTP (specific activity, 23.2 Ci/mmol), 0.075 mM; DNA, 50 μg; and S-30 extract, 300 μl. The reaction mixture was first incubated without nucleoside 5′-triphosphates for 5 min at 37°C; the reaction was initiated by the addition of nucleoside 5′-triphosphates and terminated by the addition of 2.0 ml of a cold solution containing 100 mM Tris-hydrochloride (pH 7.0), 3 mM $MgCl_2$, 0.2 mg of carrier RNA per ml, and 25 μg of RNasefree DNase per ml. After 15 min at 4°C, 20-μl fractions were removed in triplicate; 50 μg of carrier RNA, 460 μl of 10 mM EDTA, and 500 μl of 20% trichloroacetic acid were added to each of the triplicate samples, and the mixture was allowed to stand at 4°C for 25 min. The precipitate was collected on a glass-fiber filter (Whatman GF/C) and washed with 100 ml of 2% trichloroacetic acid. The filter was dried, and $^{3}$H-labeled RNA was determined by using Redi Solv IV in a Beckman LS 230 scintillation spectrometer. The remaining [$^{3}$H]RNA was extracted with an equal volume of phenol (saturated with 50 mM sodium acetate [pH 5.2] and 30 mM $MgCl_2$), RNA was precipitated by the addition of 2 volumes of 95% ethanol (−20°C), and the mixture was allowed to stand at −20°C until a flocculent precipitate had formed. The precipitate was collected by centrifugation at 10,000 rpm for 20 min in a Beckman J-21B centrifuge. The precipitate was dried and then dissolved in 1 ml of 4× SSC (SSC is 0.15 M NaCl plus 0.015 M trisodium citrate, pH 6.8), and 20-μl fractions were removed in triplicate for the determination of [$^{3}$H]RNA as described.

**Hybridization procedures.** The quantity of RNA synthesized in vitro was determined by the hybridization procedure of Gillespie and Spiegelman (14). In experiments for the determination of *argA*-specific mRNA (where only a lambda phage is at present available), lambda mRNA was removed prior to the determination of *argA*-specific mRNA by prehybridization of a quantity of RNA ($4 \times 10^4$ cpm) for 24 h at 67°C to 10 μg of lambda DNA immobilized on a nitrocellulose filter in 400 μl of 4× SSC. Under these conditions, lambda mRNA was removed, and specific *argA* mRNA remained in solution. The amount of mRNA was determined by permitting 150 μl of the supernatant solution to hybridize for 16 h at 67°C to 1 μg of the individual, separated DNA strands of λ*c*I857d*argA*2 immobilized on a nitrocellulose filter. This general procedure also was used to determine *argE*- and *argCBH*-specific mRNA, directed by λ*h*80d*argECBH*2 template DNA, by hybridization to the separated DNA strands of λd*argECBH*26.

**In vitro protein synthesis.** Cell-free synthesis of β-galactosidase was performed essentially as described by Miller (32). All components (as listed in Table 2), except DNA and S-30 extract, were assembled at room temperature, DNA was added, and preincubation was allowed to proceed at 37°C for 5 min. Protein synthesis was initiated by the addition of the appropriate quantity of S-30. All reactions were performed in a total volume of 0.1 ml and were terminated by the addition of 100 μg of chloramphenicol per ml and by transfer to an ice-water bath.

## RESULTS

**DNA dependence.** The in vitro synthesis of mRNA described in this study was completely dependent upon the addition of DNA carrying the *argECBH* gene cluster and the *argA* operon for the production of *argE*-, *argCBH*-, and *argA*-specific mRNA. The production of *argE*- and *argCBH*-specific mRNA proceeded linearly over a range of λ*h*80d*argECBH*2 DNA concentrations from 0 to 10 μg of added DNA per 100-μl reaction volume (as judged by hybridization to the separated DNA strands of λd*argECBH*26, after removal of lambda mRNA transcripts by prehybridization to lambda DNA) (Fig. 2). The synthesis of *argE*- and *argCBH*-specific mRNA accounted for approximately 1.3 and 3.2%, respectively, of the mRNA produced by the in vitro system when 5 μg of DNA template was used. Hybridization background to lambda DNA, after prehybridization, accounted for approximately 0.3% of the total RNA synthesis, and the production of mRNA complementary to λ*h*80d*argECBH*2 accounted for 67% of the radioactivity incorporated into trichloroacetic acid-precipitable material.

The in vitro synthesis of *argA*-specific mRNA was also entirely dependent upon the addition of λ*c*I857d*argA*2 DNA (Fig. 3) and was a linear function of the λ*c*I857d*argA*2 DNA concentration from 0 to 10 μg of added DNA per 100-μl reaction volume (as judged by hybridization to the light DNA strand of λ*c*I857d*argA*2 after removal of lambda mRNA transcripts by prehybridization to lambda DNA). Synthesis of *argA*-specific mRNA accounted for approximately 6% of the total RNA produced in vitro, and the background hybridization to lambda DNA (after prehybridization) was 0.4% of the total

TABLE 2. *Composition of incubation mixture per milliliter*

| Component | Quantity | Component | Quantity |
|---|---|---|---|
| 2 M Tris acetate (pH 8.2) | 22 μl | 1 M ammonium acetate | 27.3 μl |
| 1 M DTT | 91 μl | 1 M cAMP | 10 μl |
| 2 M potassium acetate | 28.2 μl | 1 M IPTG | 5.5 μl |
| 5 mM amino acids | 45.5 μl | 0.27% folinic acid | 10 μl |
| 0.1 M CTP, GTP, UTP | 5.5 μl | 1 M magnesium acetate | 10 μl |
| 0.2 M ATP | 11 μl | 1 M calcium chloride | 7.3 μl |
| 0.1 M $Na_3$ PEP | 210 μl | 30% PEG 6000 | 20 μl |
| DNA | 50 μg | S-30 | 6,500 μg of protein |
|  |  | Water | 137.1 μl |

[a] PEP, Phosphoenolpyruvate; cAMP, cyclic adenosine 3′,5′-monophosphate; IPTG, isopropyl-β-D-thiogalactopyranoside; PEG 6000, polyethylene glycol 6000.

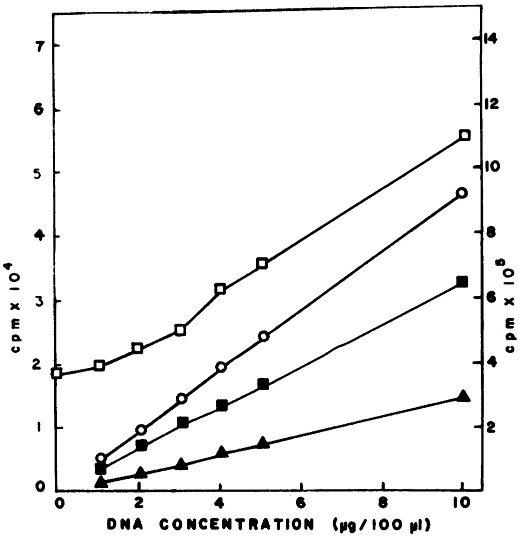

FIG. 2. *Synthesis of total RNA, template-specific mRNA, and argE- and argCBH-specific mRNA as a function of λh80dargECBH2 DNA concentration. The abscissa indicates DNA concentration expressed as micrograms per 100-µl reaction mixture. The left-hand ordinate indicates synthesis of argE- and argCBH-specific mRNA expressed as counts per minute per 100-µl reaction mixture after the removal of lambda mRNA transcripts by prehybridization to lambda DNA. The right-hand ordinate indicates total RNA synthesis (determined by trichloroacetic acid precipitation) and template-specific mRNA (determined by hybridization to λh80dargECBH2 DNA). An argR$^-$ S-30 cell extract was utilized, and synthesis was allowed to proceed for 2 min. The determination of argE-specific mRNA (▲) was monitored by hybridization to the heavy DNA strand of λdargECBH26, and argCBH-specific mRNA (■) was monitored by hybridization to the light DNA strand of λdargECBH26. In both cases, lambda transcripts were removed by prehybridization to lambda DNA; the background hybridization to lambda DNA after removal of lambda transcripts was less than 0.3% of the total RNA synthesized. Total RNA synthesis (□) was determined by trichloroacetic acid precipitation, and the quantity of template-specific mRNA (○) was determined by hybridization to 2 µg of λh80dargECBH2 DNA immobilized on a nitrocellulose filter. Each data point is the average of three determinations.*

RNA produced by the in vitro system when directed by 5 µg of template DNA. Synthesis of mRNA complementary to λcI857d*argA* template accounted for 95% of the radioactivity incorporated into acid-precipitable material.

**In vitro transcription of λ*h*80d*argECBH*2 DNA.** RNA transcripts produced by λ*h*80d*arg-ECBH*2 template DNA, using an *argR*$^-$ S-30 cell extract (strain EJ113*), were analyzed by hybridization to the heavy and light DNA strands of λ*c*I857*S*7 (Fig. 4), φ80 (Fig. 4), and λd*argECBH*26 (Fig. 5). The quantity of mRNA that complexed specifically to the light DNA strand of lambda was negligible throughout the 10-min time course and comprised less than 1% of the mRNA complementary to λ*h*80d*argECBH*2 at times later than 1 min of synthesis (Fig. 4). The quantity of mRNA that complexed specifically to the heavy DNA strand of lambda rose rapidly during the first 3 min of synthesis, rose only slightly during the remainder of the time course, and accounted for approximately 29% of the total RNA synthesis (at 2 min) that was complementary to λ*h*80d*argECBH*2 template DNA. The quantity of synthesized mRNA complementary to the heavy DNA strand of φ80 (Fig. 4) rose slowly during the first 0.5 min of synthesis, increased more rapidly for a short time, and finally slowed with only a small net accumulation of mRNA occurring during the 3- to 10-min time period. Synthesis of mRNA

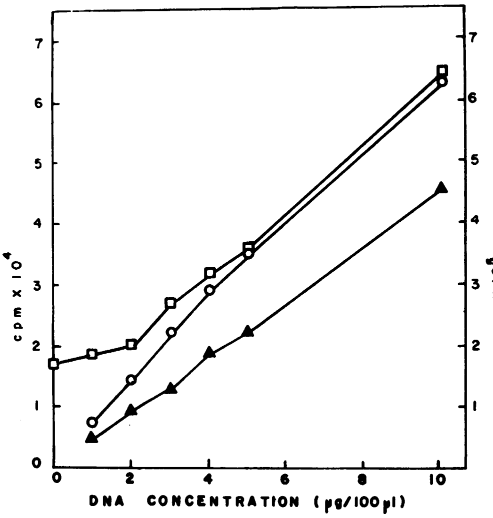

FIG. 3. *Synthesis of total RNA, template-specific mRNA, and argA-specific mRNA as a function of λcI857dargA2 DNA concentration. The abscissa indicates DNA concentration expressed as micrograms per 100-µl reaction mixture. The left-hand ordinate indicates synthesis of argA-specific mRNA (▲) expressed as counts per minute per 100-µl reaction (after removal of lambda mRNA transcripts by prehybridization to lambda DNA) and determined by monitoring hybridization to the light DNA strand of λcI857dargA2. Background hybridization to lambda DNA was approximately 0.4%. The right-hand ordinate represents total RNA synthesis (□) determined by trichloroacetic acid precipitation and synthesis of total template-specific RNA (○) determined by hybridization to λcI857dargA2 DNA. Each data point is the average of triplicate determinations.*

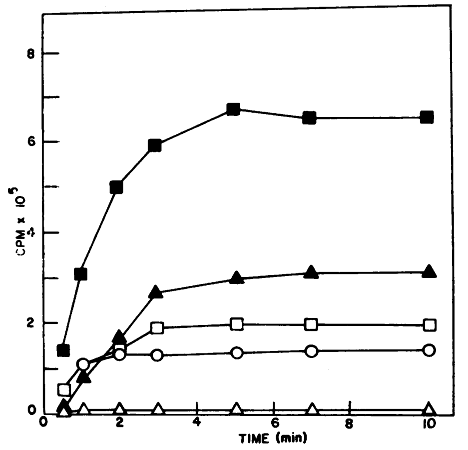

FIG. 4. *Time course of synthesis of mRNA complementary to λh80dargECBH2 DNA and the separated DNA strands of lambda and of φ80, with λh80dargECBH2 DNA template and an argR⁻ S-30 cell extract. Synthesis of mRNA complementary to λh80dargECBH2 DNA (■) was determined by hybridization to λh80dargECBH2 DNA. Synthesis of mRNA complementary to the light DNA strand of lambda (△) and the heavy DNA strand of lambda (□) was determined by hybridization to the appropriate separated DNA strands of lambda. Synthesis of mRNA complementary to the light DNA strand of φ80 (▲) and the heavy DNA strand of φ80 (○) was determined by hybridization to the separated DNA strands of φ80. Samples (50 μl) were removed at each time point, and a portion (2 × $10^4$ cpm) was used to determine the amount of each of the different mRNA transcripts present. Each time point represents that amount that would have been present in a 100-μl reaction mixture and is the average of three determinations.*

complementary to the light DNA strand of φ80 (Fig. 4) rose rapidly during min 1 of synthesis and then increased more slowly until at 3 min no further net accumulation of mRNA occurred. Synthesis of RNA complementary to the heavy DNA strand of φ80 accounted for approximately 34% of the total synthesis of RNA complementary to λ*h*80d*argECBH*2 template DNA, whereas synthesis of RNA complementary to the light DNA strand of φ80 accounted for approximately 27% of the total synthesis of RNA complementary to λ*h*80d*argECBH*2 templated DNA at the 2-min time point.

Analysis of mRNA produced from λ*h*80d*argECBH*2 template DNA by hybridization to the separated DNA strands of λd*argECBH*26, after the removal of lambda and φ80 mRNA transcripts by prehybridization, is shown in Fig. 5. The rate of specific *argE* mRNA synthesis was determined by hybridization to the heavy DNA strand of λd*argECBH*26 and exhibited three distinct phases. Synthesis of *argE*-specific mRNA occurred rapidly during the first 1 min of synthesis, much more slowly during the next 2 min, and then exhibited no net accumulation of *argE*-specific mRNA during the remainder of the time course (Fig. 5). The rate of *argCBH*-specific mRNA synthesis was monitored by hybridization to the light DNA strand of λd*argECBH*26 and exhibited two distinct phases of mRNA synthesis. During the first 1 min of synthesis, *argCBH*-specific mRNA rose rapidly, followed by a much reduced rate of synthesis for the remainder of the time course (Fig. 5). At a synthesis time of 2 min, approximately three times as much *argCBH*-specific mRNA was produced compared with *argE*-specific mRNA (*argE* mRNA accounted for 1.3% of the total RNA synthesized, and *argCBH*-specific mRNA accounted for 3.2% of the total RNA synthesized). Background hybridization to lambda DNA was ap-

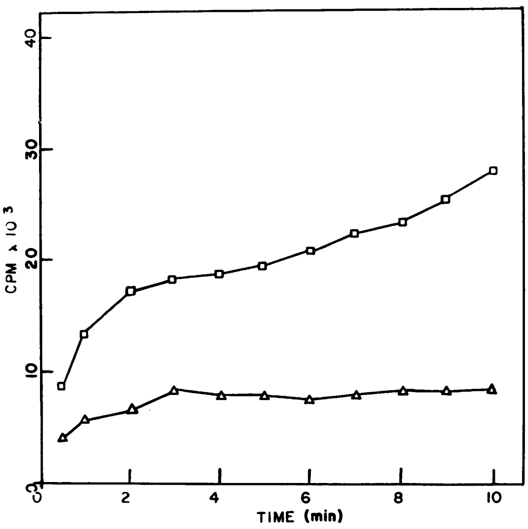

FIG. 5. *Time course of argE- and argCBH-specific mRNA synthesis directed by λh80dargECBH2 DNA with an argR⁻ S-30 cell extract. The quantity of argE- and argCBH-specific mRNA was determined by analysis of a portion of each 50-μl portion used in the experiment depicted in Fig. 4. The quantity of argE-specific mRNA was determined by hybridization to the heavy DNA strand of λdargECBH26 (△), and the quantity of argCBH-specific mRNA was measured by hybridization to the light DNA strand of λdargECBH26 (□) after removal of lambda mRNA transcripts by prehybridization to lambda DNA. The quantity of mRNA synthesized represents the amount that would have been present in a 100-μl reaction mixture. Each data point represents the average of three determinations.*

proximately 0.3% and has been subtracted from the values presented.

**In vitro transcription of λcI857d*argA*2 DNA.** RNA produced by λcI857d*argA*2 template DNA with an *argR*$^-$ S-30 extract was analyzed by hybridization directly to the separated DNA strands of lambda (Fig. 6) and to the separated DNA strands of λcI857d*argA*2 (Fig. 7) after removal of lambda RNA transcripts by prehybridization to lambda DNA as described in Material and Methods. The quantity of mRNA produced by λcI857d*argA*2 template DNA which specifically complexed to the light DNA strand of lambda increased steadily during the first 5 min of the time course, after which time net synthesis rapidly decreased to zero (Fig. 6). Synthesis of mRNA complementary to the heavy strand of lambda DNA proceeded linearly for 7 min, at which time net accumulation of mRNA approached zero (Fig. 6). Synthesis of mRNA complementary to λcI857d*argA*2 DNA template accumulated in a linear fashion for the first 7 min of synthesis, at which time net accumulation of template-specific mRNA ceased (Fig. 6). The synthesis of mRNA complementary to the light DNA strand of lambda accounted for approximately 34% of the template-specific mRNA, whereas synthesis of mRNA complementary to the heavy DNA strand of lambda accounted for approximately 50% of the template-specific mRNA (at 2 min). Synthesis of mRNA complementary to λcI857d*argA* template accounted for 95% of the radioactivity incorporated into trichloroacetic acid-precipitable material.

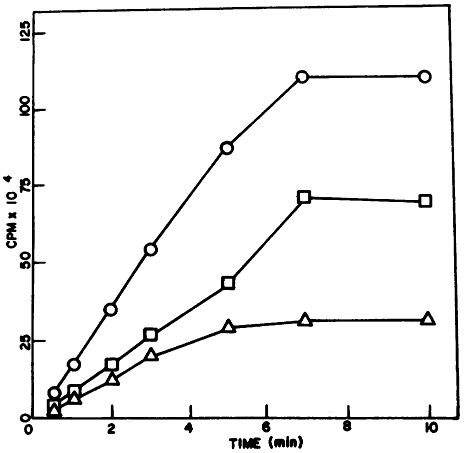

FIG. 6. *Time course of synthesis of mRNA complementary to λcI857dargA2 DNA and to the separated DNA strands of lambda with λcI857dargA2 DNA template and an argR*$^-$ *S-30 cell extract. Synthesis of mRNA complementary to λcI857dargA2 DNA (○) was determined by hybridization directly to 2 μg of λcI857dargA2 DNA immobilized on a nitrocellulose filter. Synthesis of mRNA complementary to the light DNA strand of lambda (△) and the heavy DNA strand of lambda (□) was measured by hybridization to the appropriate separated DNA strands of lambda (1 μg) immobilized on a nitrocellulose filter. Samples (50 μl) were removed at each time point, and a portion (2.0 × 10*$^4$ *cpm) was used to determine the amount of mRNA complementary to the template DNA and the separated strands of lambda DNA. Each time point represents the amount that would have been present in a 100-μl reaction mixture and is the average of three determinations.*

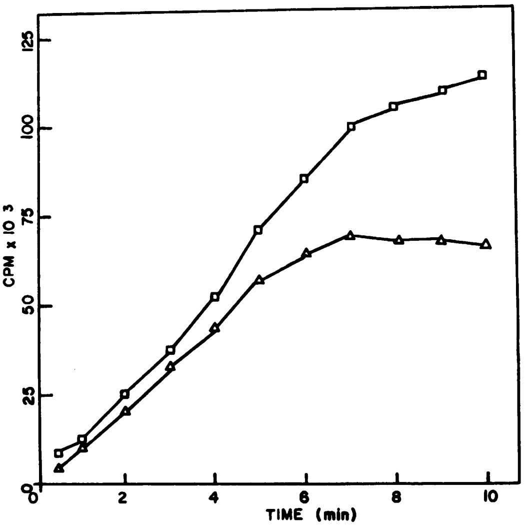

FIG. 7. *Time course of argA-specific mRNA synthesis directed by λcI857dargA2 template DNA with an argR*$^-$ *S-30 cell extract. The quantity of argA-specific mRNA was determined by analysis of a portion of each of the 50-μl portions used in the experiment described in the legend of Fig. 6. The quantity of argA-specific mRNA was determined by hybridization to the light DNA strand of λcI857dargA2 (△), whereas antisense argA mRNA was measured by hybridization to the heavy DNA strand of λcI857dargA2 (□) after (in both cases) the removal of lambda mRNA transcripts by prehybridization to lambda DNA. Background hybridization to lambda DNA (after removal of lambda transcripts by prehybridization) was 0.4% of the total template-specific mRNA produced. The quantity of mRNA synthesized represents the amount that would have been present in a 100-μl reaction mixture. Each data point represents the average of three determinations.*

Analysis of mRNA produced from λcI857d*argA*2 template DNA by hybridization to the separated DNA strands of λcI857d*argA*2 (after the removal of lambda mRNA transcripts by prehybridization) is shown in Fig. 7. The rate of *argA*-specific mRNA synthesis was determined by hybridization to the light DNA strand of λcI857d*argA*2 and was found to be a linear function of time during the first 7 min of synthesis, after which time the net accumulation of *argA*-specific mRNA rapidly became zero (Fig. 7). Synthesis complementary to the heavy DNA strand of λcI857*dargA*2 (antisense strand) also proceeded in a linear fashion during the first 7 min of synthesis, after which time the rate of formation of mRNA steadily decreased (Fig. 7). The synthesis of mRNA complementary to the heavy DNA strand of λcI857*dargA*2 was greater than that produced complementary to the light DNA strand of λcI857*dargA*2, especially after 5 min of synthesis (Fig. 7). At a synthesis time of 2 min, mRNA complementary to the light DNA strand (sense) of λcI857*dargA*2 (after lambda transcripts had been removed) accounted for approximately 6.5% of the total mRNA complementary to the λcI857*dargA*2 template (a similar amount was determined to complex to the heavy DNA strand of λcI857*dargA*2). The background hybridization to lambda DNA (after removal of lambda transcripts by prehybridization) was approximately 0.4% of the total template synthesis.

**Repression of *argE*-specific mRNA synthesis by an *argR*$^+$ S-30 cell extract, using λ*h*80*dargECBH*2 template DNA.** The result of a set of experiments demonstrating in vitro regulation of *argE* mRNA synthesis directed by DNA isolated from the specialized transducing phage λ*h*80*dargECBH*2 is shown in Fig. 8. These experiments utilized varying proportions of S-30 cell extract, simultaneously added to the reaction mixture, isolated from strains carrying either *argR*$^+$ or *argR*$^-$ alleles and the heavy DNA strand of λ*dargECBH*26 as the hybridization probe. It was demonstrated that *argE*-specific mRNA was repressed significantly (Fig. 8) by increasing proportions of an S-30 extract obtained from a strain possessing the *argR*$^+$ allele when a synthesis time of 2 min was employed. When 40% of the total S-30 extract was prepared from a strain carrying the *argR*$^+$ allele, *argE*-specific mRNA synthesis was repressed approximately 30%, and this repression value increased as the proportion of *argR*$^+$ S-30 was increased, until a final repression value of 86% was obtained when the *argR*$^+$ extract comprised 100% of the S-30 cell extract. When synthesis was permitted to proceed for 15 min no significant difference in the extent of repression was noted (Fig. 8). When synthesis was allowed to continue for 30 min, a slight loss of repression was noted (66% of *argE*-specific mRNA synthesis was under the control of the arginine holorepressor, as shown in Fig. 8).

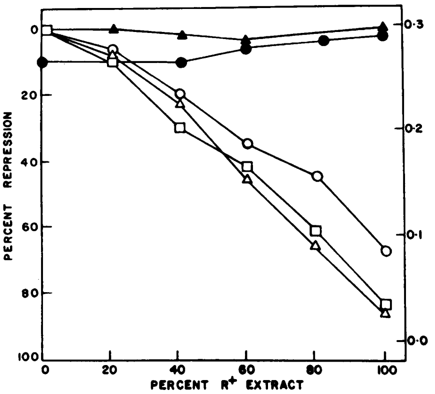

FIG. 8. *Repression of argE-specific mRNA as a function of S-30 composition. A series of experiments was performed in which S-30 cell extracts of different composition were used while λh80dargECBH2 template DNA was used to direct argE-specific mRNA synthesis. The cell extract used in each experiment was derived either from argR*$^-$ *S-30, argR*$^+$ *S-30, or from specific mixtures of the two S-30 cell extracts; the percentage of argR*$^+$ *extract is indicated on the abscissa. The ordinate represents the observed percentage of repression of argE mRNA synthesis. In this experiment, 60,000 cpm of total RNA were prehybridized (for each data point) to 30 μg of lambda DNA, and the resulting supernatant solution was analyzed for argE-specific mRNA by hybridization to the heavy DNA strand of λdargECBH26. When cell-free transcription was performed with S-30 entirely derived from an argR*$^-$ *strain, approximately 700 cpm of radioactive argE-specific mRNA were determined. Repression of argE-specific mRNA was measured by hybridization to the heavy DNA strand of λdargECBH26 after removal of lambda mRNA transcripts at a synthesis time of 2 min (□), 15 min (△), and 30 min (○). These values were corrected for background hybridization to lambda DNA, which was less than 0.25%. An identical experiment was performed with λcI857S7 DNA as the template (▲); the amount of lambda-specific mRNA was determined by hybridization to lambda DNA, and the value observed from synthesis directed by the S-30 extract prepared from a strain carrying the argR*$^+$ *allele was 2,000 cpm. In this experiment, the S-30 preparations were matched by selecting cell extracts with comparable activity in a coupled in vitro transcription-translation system as described by Cleary, Garvin, and James (submitted for publication). Synthesis of β-galactosidase is represented on the right-hand ordinate by the value of the absorbance at 420 nm determined per hour, per 100-μl reaction mixture, as a function of S-30 composition (●). The data presented are the average of duplicate determinations.*

Repression of *argE*-specific mRNA synthesis by an *argR*$^+$ S-30 cell extract was shown to be specific for the *argE* gene by employing two different controls. First, synthesis of β-galactosidase directed by 50 μg of φ80d*lac* DNA per ml was monitored in a coupled transcriptional-translational protein-synthesizing system using the respective S-30's used in the repression experiment presented herein, and it was demonstrated (Fig. 8) that the *argR*$^+$ S-30 was slightly superior in directing the synthesis of β-galactosidase. The second control involved the measurement of transcription of λ*c*I857*S*7 DNA by both the *argR*$^-$ and *argR*$^+$ S-30 cell extracts and combinations thereof. No difference in synthesis of lambda transcripts was observed (Fig. 8).

**Repression of *argCBH*-specific mRNA synthesis by an *argR*$^+$ S-30 cell extract, using λ*h*80d*argECBH*2 template DNA.** The ability of an *argR*$^+$ S-30 cell extract to repress *argCBH*-specific mRNA synthesis directed by DNA from the specialized transducing phage λ*h*80d*argECBH*2 was determined as a function of added *argR*$^+$ S-30 cell extract by using the light DNA strand of λd*argECBH*26 as the hybridization probe. At a synthesis time of 2 min, the addition of increasing amounts of *argR*$^+$ cell extract caused a concomitant decrease in the amount of *argCBH* mRNA produced, attaining a value of 90% repression when the S-30 cell extract was entirely derived from a strain carrying the *argR*$^+$ allele (Fig. 9). At a synthesis time of 15 min, increasing amounts of *argR*$^+$ S-30 cell extract decreased *argCBH*-specific mRNA synthesis to a slightly smaller extent, resulting in a maximum repression value of 81% when the S-30 cell extract was derived entirely from an *argR*$^+$ strain (Fig. 9). Increasing the synthesis time to 30 min resulted in a further loss of repression (66% when the S-30 cell extract was entirely derived from an *argR*$^+$ strain [Fig. 9]). It was also shown (Fig. 9) that synthesis of lambda mRNA directed by λ*c*I857*S*7 template DNA was unaffected by the use of S-30 cellular extract derived from strains carrying either the *argR* allele or mixtures thereof (Fig. 9). Synthesis of β-galactosidase was also shown to be independent of the composition of the S-30 utilized, as would be expected since S-30 extracts with matched protein synthesis characteristics were used.

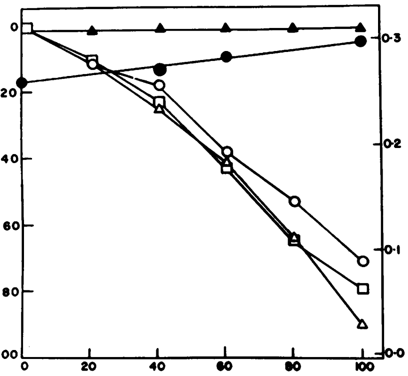

FIG. 9. *Repression of argCBH-specific mRNA synthesis as a function of S-30 composition. The experiment was performed as described in the legend of Fig. 8, except that the light DNA strand of λdargECBH26 was used to monitor argCBH-specific mRNA synthesis at times of 2 (△), 15 (□), and 30 (○) min of synthesis. As in Fig. 8, an input of 60,000 cpm of RNA was used, and 2,100 cpm of argCBH-specific mRNA were determined to be synthesized when the S-30 was entirely derived from a strain carrying the argR$^-$ allele. The controls were performed as described in the legend of Fig. 8. Each data point is the average of duplicate determinations.*

**Repression of *argA*-specific mRNA synthesis by an *argR*$^+$ S-30 cell extract, using λ*c*I857d*argA*2 template DNA.** The ability of an *argR*$^+$ S-30 cell extract to repress *argA*-specific mRNA synthesis directed by DNA isolated from the specialized tranducing phage λ*c*I857d*argA*2 was determined as a function of the quantity of *argR*$^+$ S-30 cell extract present in the incubation mixture. At a synthesis time of 2 min, the addition of increasing amounts of *argR*$^+$ extract caused a concomitant decrease in the amount of *argA*-specific mRNA produced complementary to the sense strand (light strand) of λ*c*I857d*argA*2 DNA, reaching a value of 90% repression when the S-30 was entirely derived from a strain carrying the *argR*$^+$ allele (Fig. 10). Increasing the time of synthesis to 15 or 30 min resulted in only a slight loss of repression (84% repression at 15 min and 78% repression at a synthesis time of 30 min [Fig. 10]). Synthesis of *argA* mRNA complementary to the heavy strand of λ*c*I857d*argA*2 DNA (the antisense strand) was also monitored as a function of the concentration of *argR*$^+$ cell extract at synthesis times of 2, 15, 30 min, and it was demonstrated (Fig. 10) that the arginine holorepressor had no effect on *argA*-specific mRNA produced complementary to this strand. Synthesis of β-galactosidase was shown to be independent of the *argR* allele present in the S-30 cell extract (Fig. 10), and synthesis of lambda mRNA directed by λ*c*I857*S*7 DNA was not affected by the *argR* allele present in the S-30 cell extract (data not presented).

**Repression of *argE*-, *argCBH*-, and *argA*-specific mRNA synthesis as a function of the order of addition of *argR*$^+$ and *argR*$^-$ S-30 cell extract.** The effect of the order of addition of *argR*$^+$ and *argR*$^-$ cell extract on the regulation of *argE*-, *argCBH*-, and *argA*-specific mRNA synthesis was determined by performing cell-free synthesis as described previously, except that, in one case, the *argR*$^+$ cell extract was

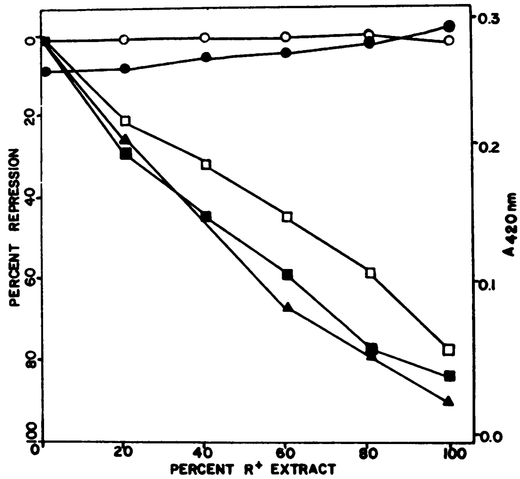

Fig. 10. *Repression of argA-specific mRNA synthesis as a function of S-30 composition. The experiment was performed as described in the legend of Fig. 8, except that in this case λcI857dargA2 DNA was used as template. Synthesis of argA-specific mRNA representing sense information was monitored at 2* (▲), *15* (■), and 30 (□) *min of synthesis by hybridization to the light DNA strand of λcI857dargA2. The input of total RNA in this experiment was 40,000 cpm, and, after removal of lambda transcripts by prehybridization to lambda DNA, approximately 2,400 cpm of mRNA were determined to be argA specific when the S-30 cellular extract was entirely derived from a strain carrying the argR*$^{-}$ *allele. Background hybridization to lambda DNA varied between 87 and 123 cpm. Synthesis of argA-specific mRNA representing antisense information was also monitored at 2, 15, and 30 min* (○) *by hybridization to the heavy DNA strand of λcI857dargA2 after the removal of lambda transcripts by prehybridization to lambda DNA. The input of RNA was 40,000 cpm, and, after prehybridization, approximately 2,700 cpm were argA antisense specific regardless of the argR allele present in the S-30. The β-galactosidase control* (●) *was performed as described in the legend of Fig. 8. Each data point represents the average of duplicate determinations.*

added first and then incubated for 1 min at 4°C, followed by the addition of the *argR*$^{-}$ S-30 cell extract. In the other case, the *argR*$^{-}$ cell extract was added first and allowed to incubate at 4°C for 1 min before the addition of the *argR*$^{+}$ S-30 cell extract. In both cases, the reaction mixture was allowed to preincubate at 37°C for 5 min, and the reaction was initiated by the addition of nucleoside 5'-triphosphates.

Experiments performed with the prior addition of cellular extract derived from a strain carrying the *argR*$^{-}$ allele followed by the addition of the appropriate quantity of S-30 derived from a strain carrying the *argR*$^{+}$ allele resulted in the synthesis of steadily decreasing amounts of *argE*-specific mRNA with increasing quantities of *argR*$^{+}$ S-30 (repression value of 15 and 38%, respectively, corresponding to an S-30 composition comprising 20 and 60% of the cellular extract derived from the *argR*$^{+}$ strain).

In sharp contrast, when the order of addition was reversed, with the prior addition of S-30 derived from a strain carrying the *argR*$^{+}$ allele followed by the addition of the appropriate quantity of *argR*$^{-}$ S-30, a dramatic increase in effectiveness of the arginine holorepressor was apparent. The synthesis of *argE*-specific mRNA was repressed 54% by the prior presence of arginine holorepressor present in only 20% of the S-30 utilized in the experiment. Increasing the quantity of S-30 derived from the strain carrying the *argR*$^{+}$ allele to 40% of the total resulted in the repression of the specific *argE* mRNA synthesis by 81% (Fig. 11). Similar results were obtained when the effect of the order of addition of *argR*$^{+}$ and *argR*$^{-}$ S-30 cellular extracts was determined for the *argCBH* operon (data not presented). When DNA isolated from λcI857d*argA*2 was used in this order of experiments, it was found that the repression of *argA*-specific mRNA followed a pattern qualitatively similar to that described for the *argE* and *argCBH* operons (data not presented). Synthesis of mRNA directed by DNA template isolated from λcI857 and cell-free synthesis of β-galactosidase directed by DNA isolated from φ80d*lac* was unaffected by the order of addition of S-30 cellular extracts (Fig. 11). In the case of experiments performed with the *argA* operon, synthesis of antisense *argA*-specific mRNA was also determined to be unaffected by the order of addition of S-30 cellular extracts.

## DISCUSSION

We have described the in vitro transcription of the *argECBH* gene cluster and the *argA* operon by using DNA template isolated from the specialized transducing phages λ*h*80d*arg-ECBH*2 and λcI857d*argA*2 and cytoplasmic (S-30) extracts prepared from strains of *E. coli* K-12 carrying the *argR*$^{-}$ and *argR*$^{+}$ alleles. The system used for in vitro transcription is identical to that used by McGeoch et al. (30) for the study of the tryptophan operon, except that S-30 extracts, which are capable of coupled transcription-translation, were used instead of S-180 cell extracts.

The system was shown to be entirely dependent on the addition of DNA containing the *argECBH* gene cluster and the *argA* operon for the synthesis of mRNA specific for the *argE*,

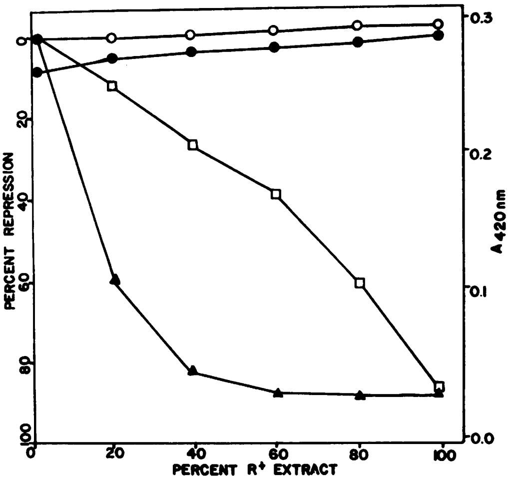

FIG. 11. *Determination of the effect of the order of addition of S-30 extracts (prepared from strains carrying the argR$^-$ and argR$^+$ alleles) on the repression of argE-specific mRNA synthesis as a function of S-30 composition. Synthesis of RNA was performed as described in the legend of Fig. 8, except the effect of the order of addition of the argR$^+$ and the argR$^-$ S-30 cell extract was determined. Template DNA isolated from λh80dargECBH2 was used to direct synthesis of argE-specific mRNA with a synthesis time of 1 min. The amount of argE-specific mRNA produced in the individual reactions was determined by hybridization to the heavy DNA strand of λdargECBH26. In one case, the quantity of argR$^-$ S-30 used in each experiment was added to the reaction mixture at 4°C and permitted to incubate for 1 min before the quantity of argR$^+$ S-30 cell extract was added (□). The reciprocal experiment was performed by adding the argR$^+$ S-30 cell extract first, incubating the mixture at 4°C for 1 min, and adding the appropriate quantity of argR$^-$ S-30 cell extract (▲). Polymerization was initiated by the addition of nucleoside 5'-triphosphates and terminated as described in Materials and Methods. The same quantity of RNA was used in the hybridization reaction as described in the legend of Fig. 8. Synthesis of lambda mRNA was also monitored by using λcI857S7 template DNA when the argR$^+$ was added first (O) and when the argR$^-$ was added first (O); the data points shown represent approximately 2,000 cpm of lambda-specific mRNA. Synthesis of β-galactosidase was also monitored when the argR$^+$ was added first (●) and when the argR$^-$ was added first (●). The data represent the average of duplicate determinations.*

*argCBH*, and *argA* operons (Fig. 2 and 3). The system was not completely dependent on added DNA for total RNA synthesis; this was not unexpected since McGeoch et al. (30) and Rogers et al. (40) reported similar data. The majority (90%) of radioactivity incorporated into trichloroacetic acid-precipitable material was found to complex specifically to DNA probe isolated from λcI857d*argA* when this same DNA was used as template; however, only 67% of the radioactivity incorporated into acid-precipitable material was determined to hybridize to the DNA probe isolated from λd*argECBH2* when DNA template was isolated from λ*h*80d*argECBH2*. It appears that the large amount of endogenous synthesis exhibited when λ*h*80d*argECBH2* DNA template was used to direct mRNA synthesis was a function of the particular template and not a deleterious aspect of the transcriptional system.

Synthesis of *argE*- and *argCBH*-specific mRNA was found to exhibit uncomplicated kinetics, with the rate of accumulation of mRNA steadily decreasing at extended periods of synthesis, indicating that the rate of formation of RNA was becoming balanced by degradation due to the action of RNase present in the S-30 extracts. Synthesis of *argCBH*-specific mRNA accounted for approximately 70% of the *argECBH*-specific mRNA produced. This agrees well with the ratio of *argE* mRNA to *argCBH* mRNA reported to be formed in vivo (8), as well as the ratio of the mRNA species formed in vitro (36). Initiation at the *argCBH* promoter appears to be the principal source of *argCBH*-specific mRNA synthesis, since 90% of the *argCBH*-specific mRNA synthesis was under control of the arginine holorepressor (Fig. 9) and since the time course of *argCBH* synthesis exhibited no plateau followed by a subsequent increase in synthesis (Fig. 5), as was observed for the *argF* operon carried on ϕ80d*argF* DNA, where readthrough from one or more upstream bacterial promoters was observed (Sens et al., submitted for publication).

Since experiments performed on the regulation of the *argI* and *argF* operons (Sens et al., submitted for publication) demonstrated poor repression values at extended times of mRNA synthesis due to readthrough of mRNA initiated at upstream bacterial promoters, these experiments were repeated for the *argECBH* gene cluster. Increasing the time of synthesis had little effect on the extent of repression of *argE*- and *argCBH*-specific mRNA synthesis, with substantial repression values being attained even at 30 min of synthesis (Fig. 8 and 9). These data support the notion that the DNA sequence carried on the specialized transducing phage λ*h*80d*argECBH2* in the region of the *argECBH* gene cluster probably has effective terminator sequences associated with any neighboring bacterial genes that have promoters functioning under the conditions utilized in this work, thus preventing readthrough of mRNA transcripts initiated at other bacterial promoters. The demonstration of regulation to the extent of 90% by the arginine holorepressor indicates that relatively little transcription is occurring from other bacterial operons. Furthermore, it shows that the majority, if not all, of the regulation of the *argECBH* operons is mediated at the level of transcription. It is necessary to reconcile this notion that regulation of gene expression for the *argE* and *argCBH* operons is mediated entirely at the level of transcription with the observaton of Cunin et al. (8) and Krzyzek and Rogers (24) that the derepressed/repressed ratios of the enzyme activities for the corresponding enzymes do not correlate. One factor contributing to this noncorrespondence is the difficulty in measuring accurately the quantity of mRNA formed in vivo, particularly under conditions of physiological repression. Another potential explanation is to invoke the possibility of post-transcriptional control operating in a manner analogous to that observed for the *trp* operon (2). This possibility has been espoused by Krzyzek and Rogers (24), who have reported preliminary results concerning the measurement of levels for two species of mRNA formed in vivo for the *argECBH* operons; however, these workers did not utilize separated DNA strands for monitoring these species.

To obtain meaningful data when performing repression experiments with two sets of cell extracts—one isolated from an *argR*$^-$ strain and the other prepared from an *argR*$^+$ strain—it is necessary to insure that the S-30 cell extracts are of equal competence in directing template-specific mRNA synthesis. This was accomplished in the present work by performing three different controls. First, in all cases, cellular extracts were utilized that exhibited comparable activity in an in vitro transcription-translation system producing $\beta$-galactosidase directed by $\phi$80d*lac* DNA (M. Clearly, R. T. Garvin, and E. James, submitted for publication). Second, the S-30 cell extracts were matched in their ability to synthesize *argA* antisense (heavy-strand-specific) RNA directed by $\lambda$*c*I857d*argA*2 DNA and mRNA specific to $\lambda$*c*I857*S*7. It is therefore clearly evident that repression of arginine-specific mRNA was due to the presence of functional arginine holorepressor.

Synthesis of *argA*-specific mRNA was monitored by hybridization using the separated DNA strands of $\lambda$*c*I857d*argA*2, and the rate of synthesis during a 10-min time period was found to exhibit uncomplicated kinetics (Fig. 7). Previous characterization of the bacteriophage (Natter et al., submitted for publication) has demonstrated that the light DNA strand of $\lambda$*c*I857d*argA*2 carries sense information for the *argA* gene. Since synthesis of mRNA complementary to both DNA strands of $\lambda$*c*I857d*argA*2 exhibited similar kinetics, it was of interest to ascertain what effect the presence of the promoter on the heavy DNA strand of $\lambda$*c*I857d*argA*2 would have opposite the *argA* operon and its interaction with the arginine holorepressor. The results (Fig. 10) show that substantial repression of *argA*-specific mRNA was obtained at all synthesis times and that no repression was noted for mRNA complementary to the heavy DNA strand of $\lambda$*c*I857d*argA*2. Thus, it appears that the promoter(s) located on the heavy DNA strand of $\lambda$*c*I857d*argA*2 has no adverse effect on mRNA produced that is complementary to *argA* sense gene DNA. This may be due to the *argA* operon lying downstream from the promoter on the heavy strand, thus giving rise to a situation in which RNA polymerase initiated at one promoter does not transcribe the other promoter region.

Since both $\lambda$*h*80d*argECBH*2 and $\lambda$*c*I857d*argA*2 DNA template provide an excellent source of DNA for regulation studies involving the *argECBH* gene cluster and the *argA* operon, it was of interest to ascertain if information regarding the control regions of these operons could be obtained by altering the conditions employed when performing studies on the effect of the arginine holorepressor as described previously for the *argF* and *argI* operon (Sens et al., submitted for publication). From the results (Fig. 11), it is clear that there is competition between the RNA polymerase and holorepressor. Experiments performed with small quantities of holorepressor added together with a small proportion of RNA polymerase (both in the *argR*$^+$ S-30 cell extract) prior to the addition of the major proportion of RNA polymerase (contained in the *argR*$^-$ S-30) demonstrate that repression was considerably greater than when the reverse order of addition of holorepressor and RNA polymerase was employed. It may be postulated, therefore, that repression of these operons occurs by the arginine holorepressor preventing the binding of RNA polymerase at the respective promoters and the subsequent formation of mRNA polymerase initiation complex as reported by Squires et al. (44) for the tryptophan operon.

This work describes the study of two DNA templates, one carrying the *argA* operon and the other carrying the *argECBH* gene cluster. It has been shown that at least 86% of *argE*, 90% of *argCBH*, and 90% of *argA* gene expression is mediated by the arginine holorepressor at the level of transcription and that repression appears to result from the holorepressor interfering with the binding of RNA polymerase to the promoter site. The in vitro system described provides the basis for further study regarding repressor-corepressor interactions and will permit the development of studies aimed at determining whether or not other control sites are present as described by Bertrand et al. (2) for the *trp* operon, whether autoregulation occurs as has been postulated for other operons (45), and whether the presence or removal (by deletion of the gene in question) of other arginine enzymes has an effect on the particular arginine operon under study.

## ACKNOWLEDGMENTS

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## LITERATURE CITED

1. **Baumberg, S., D. F. Bacon, and H. J. Vogel.** 1965. Individually repressible enzymes specified by clustered genes of arginine biosynthesis. Proc. Natl. Acad. Sci. U.S.A. 53:1029-1032.
2. **Bertrand, K., L. Korn, F. Lee, T. Platt, C. L. Squires, C. Squires, and C. Yanofsky.** 1975. New features on the regulation of the tryptophan operon. Science 189:22-26.
3. **Bretscher, A. P., and S. Baumberg.** 1976. Control mutations within the divergently transcribed *argECBH* cluster of *E. coli* K-12 J. Mol. Biol. 102:205-220.
4. **Celis, T. F. R., and W. K. Maas.** 1971. Studies on the mechanism of repression of arginine biosynthesis in *Escherichia coli*. IV. Further studies on the role of arginine transfer RNA repression of the enzymes of arginine biosynthesis. J. Mol. Biol. 62:179-188.
5. **Cunin, R., D. Elseviers, G. Sand, G. Freundlich, and N. Glansdorff.** 1969. On the functional organization of the *argECBH* cluster of genes in *Escherichia coli* K-12. Mol. Gen. Genet. 106:32-47.
6. **Cunin, R., and N. Glansdorff.** 1971. Messenger RNA from arginine and phosphenol pyruvate carboxylase genes in *argR*$^+$ and *argR*$^-$ strains of *E. coli*. FEBS Lett. 18:135-137.
7. **Cunin, R., N. Kelker, A. Boyen, H. Yang, G. Zubay, N. Glansdorff, and W. K. Maas.** 1976. Involvement of arginine in *in vitro* repression of transcription of arginine genes C, B and H in *Escherichia còli* K-12. Biochem. Biophys. Res. Commun. 69:377-382.
8. **Cunin, R., P. Pouwels, N. Glansdorff, and M. Crabeel.** 1975. Parameters of gene expression in the biopolar *argECBH* operon of *E. coli* K-12. The question of translational control. Mol. Gen. Genet. 140:51-60.
9. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin $B_{12}$. J. Bacteriol. 60:17-28.
10. **Dickson, R. C., J. Abelson, W. Barnes, and W. S. Reznikoff.** 1975. Genetic regulation: the *lac* control region. Science 187:27-29.
11. **Elseviers, D., R. Cunin, N. Glasdorff, S. Baumberg, and E. Ashcroft.** 1972. Control regions within the *argECBH* gene cluster of *Escherichia coli* K-12. Mol. Gen. Genet. 117:349-366.
12. **Eron, L., and R. Block.** 1971. Mechanism of initiation and repression of *in vitro* transcription of the *lac* operon in *E. coli*. Proc. Natl. Acad. Sci. U.S.A. 68:1828-1832.
13. **Eshenbaugh, D. L., D. Sens, and E. James.** 1974. A solid phase radioimmune assay for ornithine transcarbamylase, p. 61-73. *In* R. B. Dunlap (ed.), Advances in experimental medicine and biology, vol. 42. Plenum Publishing Corp., New York.
14. **Gillespie, D., and S. Spiegelman.** 1965. A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. J. Mol. Biol. 12:829-842.
15. **Glansdorff, N., and G. Sand.** 1965. Coordination of enzyme synthesis in the arginine pathway of *Escherichia coli* K-12. Biochim. Biophys. Acta 108:308-311.
16. **Glansdorff, N., G. Sand, and C. Verhoef.** 1967. The dual genetic control of ornithine transcarbamylase synthesis in *Escherichia coli* K-12. Mutat. Res. 4:742-751.
17. **Gorini, L., W. Gunderson, and M. Burger.** 1961. Genetics of regulation of enzyme synthesis in the arginine biosynthetic pathway of *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. 26:173-182.
18. **Gottesman, S., and J. R. Beckwith.** 1969. Directed transposition of the arabinose operon: a technique for the isolation of specialized tranducing bacteriophage for any *Escherichia coli* gene. J. Mol. Biol. 44:117-127.
19. **Hradecna, A., and W. Szybalski.** 1967. Fractionation of the complementary strands of coliphage λ DNA based on the asymmetric distribution of the poly UG binding sites. Virology 32:633-643.
20. **Jacoby, G.** 1972. Control of the *argECBH* cluster in *Escherichia coli*. Mol. Gen. Genet. 117:337-348.
21. **Jacoby, G., and L. Gorini.** 1969. A unitary account of the repression mechanism of arginine biosynthesis in *Escherichia coli*. J. Mol. Biol. 39:73-87.
22. **James, P., D. Sens, W. Natter, S. K. Moore, and E. James.** 1976. Isolation and characterization of the specialized transducing bacteriophages φ80d*argF* and λh80cI857d*argF*: specific cleavage of arginine transducing deoxyribonucleic acid by the endonucleases *Eco*RI and *Sma*R. J. Bacteriol. 126:487-500.
23. **Krzyzek, R., and P. Rogers.** 1972. Arginine control of transcription of *argECBH* messenger ribonucleic acid in *Escherichia coli*. J. Bacteriol. 110:945-954.
24. **Krzyzek, R., and P. Rogers.** 1976. Dual regulation by arginine of the expression of the *Escherichia coli argECBH* operon. J. Bacteriol. 126:348-364.
25. **Krzyzek, R., and P. Rogers.** 1976. Effect of arginine on the stability and size of *argECBH* messenger ribonucleic acid in *Escherichia coli*. J. Bacteriol. 126:365-376.
26. **Lavallé, R., and G. Dehauwer.** 1970. Tryptophan messenger translation in *Escherichia coli*. J. Mol. Biol. 51:435-447.
27. **Leisinger, T., and D. Haas.** 1975. N-acetylglutamate synthetase of *Escherichia coli* regulation of synthesis and activity by arginine. J. Biol. Chem. 250:1690-1693.
28. **Lennox, E.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1:190-206.
29. **Maas, W. K.** 1961. Studies on repression of arginine biosynthesis in *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. 26:183-191.
30. **McGeoch, D., J. McGeoch, and D. Morse.** 1973. Cell-free synthesis of tryptophan operon mRNA. Nature

(London) New Biol. 245:137-140.

31. **McLellan, W. L., and H. J. Vogel.** 1970. Translational repression in the arginine system of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 67:1703-1709.
32. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 423. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
33. **Miller, J. H., K. Ippen, J. G. Scaife, and J. R. Beckwith.** 1968. The promoter-operator region of the *lac* operon of *Escherichia coli*. J. Mol. Biol. 38:413-420.
34. **Nakanishi, S., S. Adhya, M. E. Gottesman, and I. Pastan.** 1973. *In vitro* repression of the transcription of *gal* operon by purified *gal* repressor. J. Biol. Chem. 248:5937-5942.
35. **Panchal, C., S. Bagchee, and A. Guha.** 1974. Divergent orientation of transcription from the arginine gene *ECBH* cluster of *Escherichia coli*. J. Bacteriol. 117:675-680.
36. **Pannekock, H., R. Cunin, A. Boyen, and N. Glansdorff.** 1975. *In vitro* transcription of the *argECBH* gene cluster. FEBS Lett. 51:143-145.
37. **Parkinson, J., and R. Huskey.** 1968. Isolation of deletion mutants of bacteriophage lambda. J. Mol. Biol. 56:369-384.
38. **Pouwels, P., R. Cunin, and N. Glansdorff.** 1974. Divergent transcription in the *argECBH* cluster of genes in *Escherichia coli* K12. J. Mol. Biol. 83:421-424.
39. **Press, R., N. Glansdorff, P. Miner, J. Devries, R. Kadner, and W. K. Maas.** 1971. Isolation of $\phi$80 transducing particles carrying different regions of the *E. coli* genome. Proc. Natl. Acad. Sci. U.S.A. 68:795-798.
40. **Rogers, P., T. Kaden, and M. Toth.** 1975. Repression of *arg* mRNA synthesis by L-arginine in cell-free extracts of *Escherichia coli*. Biochem. Biophys. Res. Commun. 65:1284-1291.
41. **Sens, D., D. L. Eshenbaugh, and E. James.** 1975. Resolution of the DNA strands of the specialized tranducing bacteriophage λ*h*80cI857*dargF*. J. Virol 16:85-93.
42. **Sens, D., and E. James.** 1975. Regulation of *argF* mRNA synthesis, performed *in vitro*. Biochem. Biophys. Res. Commun. 64:169-174.
43. **Shimada, K., R. Weisberg, and M. E. Gottesman.** 1972. Prophage lambda at unusual chromosomal locations. I. Location of the secondary attachment sites and properties of the lysogens. J. Mol. Biol. 80:297-314.
44. **Squires, C. L., F. Lee, and C. Yanofsky.** 1975. Interaction of the *trp* repressor and RNA polymerase with the *trp* operon. J. Mol. Biol. 92:93-111.
45. **Umbarger, H. E.** 1975. Regulation of amino acid biosynthesis in microorganisms, p. 13-18. *In* H. R. V. Arnstein (ed.), Synthesis of amino acids and proteins. International review of science and biochemistry, series I. vol. 7. University Park Press, Baltimore.
46. **Williams, L. S.** 1973. Control of the arginine biosynthesis in *Escherichia coli*: role of arginyl-transfer ribonucleic acid synthetase in repression. J. Bacteriol. 113:1419-1432.