J. Mol. Biol. (1973) **79**, 149–162

Different Cyclic AMP Requirements for Induction of the Arabinose and Lactose Operons of *Escherichia coli*

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(*Received 13 March 1973*)

Three different techniques demonstrated that higher 3′,5′-cyclic AMP levels are required for induction of the arabinose operon than are required for induction of the lactose operon of *Escherichia coli*: (1) an *in vitro* DNA-directed protein synthesis system indicated that half-maximal induction of the arabinose operon required a twofold higher concentration of cyclic AMP than the lactose operon; (2) an *in vivo* synthesis of the enzymes of the respective operons in an adenyl cyclase minus strain, made permeable by EDTA treatment, also showed the same result; and (3) in a completely *in vivo* synthesis of the enzymes under conditions of catabolite repression, where intracellular cyclic AMP is limiting, inducibility of the arabinose operon was dramatically (7·5-fold) depressed relative to inducibility of the lactose operon.

1. Introduction

In *Escherichia coli* the 3′,5′-cyclic AMP receptor protein and cAMP† are necessary for normal induction of operons in many catabolic pathways including the lactose and arabinose operons (Emmer *et al.*, 1970; De Crombrugghe *et al.*, 1969). *A priori* the functioning of the cAMP system in these operons could be expected to be identical. However, here we show this is not the case, for the levels of cAMP that are required to induce the positively controlled L-arabinose operon (Sheppard & Englesberg, 1967; Greenblatt & Schleif, 1971) are higher than those needed to induce the negatively controlled lactose operon.

Studies on the *in vitro* regulation of the *ara* and *lac* operons hinted that induction of the two operons could require different levels of cAMP. The cAMP concentration required for half-maximal induction of the *ara* operon, 6×10^{-4} M (Greenblatt & Schleif, 1971), was significantly higher than that required for half-maximal induction of the *lac* operon, 2×10^{-4} M, or a later value of 5×10^{-5} M, all measured in transcription–translation systems, or the value of 5×10^{-6} M measured in a purified transcription system (Chambers & Zubay, 1969; Zubay *et al.*, 1970; De Crombrugghe *et al.*, 1971). Hitherto our studies on the arabinose operon used C protein and *ara* DNA template derived from strain B/r in a K12-derived protein synthesis extract. Meaningful comparisons of the response to cyclic AMP of the two operons require first, a homologous *in vitro* system consisting entirely of components from one strain, and second, in light of the variability of the *lac* results, rigorous parallelism between the *ara* and *lac* operons in the execution of experiments. In this paper we report a

† Abbreviation used: cAMP, 3′,5′ cyclic AMP.

comparison of the inducing role of cAMP in the *ara* and *lac* operons carried out under essentially identical conditions. Three different systems showed that higher cAMP levels are required for induction of the *ara* operon than are required for induction of the *lac* operon. These were: (1) the DNA-directed *in vitro* synthesis of the enzymes of the two operons in the presence of varying amounts of added cAMP, (2) the *in vivo* synthesis of the enzymes in an adenyl cyclase minus strain with cAMP supplied and varied externally, and (3) the *in vivo* synthesis with cAMP internally synthesized. In the last system we used the fact that some nutrients in the growth medium generate a catabolite repression mediated, at least in part, by a decrease in intracellular cAMP levels (Perlman *et al.*, 1969; Ullmann & Monod, 1968; Makman & Sutherland, 1965).

Although cAMP and cAMP receptor protein are required for induction of both operons, the levels of cAMP required for detectable or half-maximal induction of the *ara* operon are at least twofold higher than the levels required for induction of the *lac* operon. Thus, the function of cAMP receptor protein and cAMP may be the same in both operons, but the details of its interactions in the two systems cannot be identical.

2. Materials and Methods

(a) *Bacteria and bacteriophages*

All of the strains used were derived from *E. coli* K12.

(b) *Chemicals, media, centrifugations and enzyme assays*

(i) *Media and chemicals*

As in Schleif (1969) except where noted. Phosphoenol pyruvate was obtained from Calbiochem, adenosine 3′,5′-cyclic monophosphate from Sigma, nucleotide triphosphates from P. L. Biochemicals, and calcium leucovorin from Lederle. TB broth is described by Gottesman & Yarmolinsky (1968); YT broth is described by Schleif (1969).

(ii) *Centrifugations*

Unless otherwise specified centrifugations were carried out using the Sorvall SS34 rotor for volumes <40 ml and the GSA rotor for volumes >40 ml. Centrifugations in swinging-bucket and angle rotors were carried out in the Beckman model L2 ultracentrifuge.

(iii) *Assays*

Arabinose isomerase was assayed as in Schleif *et al.* (1971).

β-galactosidase was assayed as outlined by Craven *et al.* (1965).

β-galactosidase synthesized *in vitro* was assayed as described by Zubay *et al.* (1970).

Ribulokinase synthesized *in vitro* was assayed in the following manner. After the 70 min of *in vitro* protein synthesis, 50 μl of kinase assay mixture is added to each 50 μl of protein synthesis reaction. The kinase assay mixture consists of 200 mM-potassium phosphate (pH 7·8), 1 mM-potassium-EDTA, 10 mM-magnesium acetate, 20 mM-ATP, 10 mM-NaN_3, 4 mM-dithiothreitol, 1·0 mg streptomycin/ml, 500 μg chloramphenicol/ml, 20 mM-NaF, 1·33 mM-[^{14}C]arabinose (0·14 mCi/mmol) and $1{\cdot}25\times10^{14}$ molecules of the hexameric arabinose isomerase in a ribulokinase-free crude extract. The isomerase is in the supernatant of a 30,000 ***g*** spin for 30 min, prepared from an *ara* deletion strain RFS696, in which $\lambda daraC^{+}$-$B^{-}hy80$ was heat-induced. The cells were grown for 3 h in YT broth plus 0·2% arabinose, spun down, ground with alumina and resuspended in 10 mM-Tris·acetate (pH 7·8), 14 mM-magnesium acetate, 60 mM-KCl, and 0·1 mM-dithiothreitol, and dialyzed against the resuspension buffer. This extract can be stored at 4°C for up to 6 months. The kinase assay is incubated for 20 h at 30°C. [^{14}C]ribulose-5-phosphate is separated from [^{14}C]ribulose and [^{14}C]arabinose by ascending chromatography on Whatman DE81 paper strips, 1·5 cm × 17·0 cm. The strips are folded in half along their length, then folded back 3 cm from one end and pushed into the tube of incubated mixture. After several seconds they absorb the liquid (conveniently applying the sample to a region centered 3 cm from the end) and are removed, straightened, and immediately developed with water. In approximately 80 min the water front reached 0·5 cm from the top, the strips were dried and the radioactivity in the bottom 9 cm was determined in toluene-based scintillation fluid.

Ribulokinase of whole cells was assayed by the above procedure. To prepare the cells for the assay, cultures were centrifuged at 5000 revs/min for 10 min, resuspended in an equal volume of M9 medium (Schleif, 1969), recentrifuged as above to wash out arabinose, and resuspended in 0·1 ml of 0·01 M-Tris·HCl, pH 7·6, 0·01 M-dithiothreitol. Cells were made permeable by the addition of 5 μl of toluene, mixing for 15 s, and placing the mixtures in an ice bath for 20 min. Resuspended cells (1 to 5 μl) were added to 50 μl of assay mixture at 37°C to initiate the assay. The basal level of kinase in 5×10^8 cells of uninduced strain RFS1 produces 1000 cts/min above a background of 100 cts/min in a 90-min incubation.

(c) *Isolation of the λ*dara *phage used as a DNA template*

Strain RFS825 was grown at 34°C to 3×10^8 cells/ml in 600 ml of 0·5 concentration YT broth plus 0·4% maltose. $MgSO_4$ was added to 0·01 M and $\lambda cI_{857}S_7$ phage was added at a multiplicity of infection of 3. After 20 min at 30°C, the temperature was raised to 42°C for 20 min, then lowered to 34°C for 90 min, and the culture was centrifuged for 5000 revs/min for 10 min. The cell pellet was resuspended in 2·0 ml of 0·01 M-$MgSO_4$, lysed by addition of 0·25 ml of chloroform, and cell debris removed by centrifugation at 7000 revs/min for 15 min. One sixth of this lysate (3×10^{10} phage) was used to transduce 1×10^{10} cells of strain RFS726, which had been grown to 3×10^8 cells/ml in M9/glycerol medium, centrifuged and resuspended in 0·01 M-$MgSO_4$. A total of 15 transduced colonies arose on minimal arabinose plates. The purified candidates were pooled and recycled through the above transduction using $\frac{1}{150}$ as many cells. More than 1000 *ara*$^+$ transductants were obtained and several were tested for their ability to produce a high frequency transducing lysate both with and without helper phage. A suitable double lysogen was labeled JTL80. The arabinose transducing phage of JTL80 was lysis defective, S_7 (Goldberg & Howe, 1969) and was labeled λd*ara101* (arabinose transducing phage isolated in this laboratory will be numbered from 101). The two periods of growth beyond the normal lysis time of λS^+ selected phage having acquired by recombination the lysis defective S_7 allele.

(d) *Isolation of the λ*daraC$^+$B$^-$

The induced ribulokinase in a number of F′ *araB* mutants (Schleif, 1972) was measured and one with an undetectable level was found, RFSF′53. This episome was transferred to strain RFS825 and following growth to stationary phase in YT broth cells were diluted and spread on a tetrazolium arabinose plate, and one *ara*$^-$ colony, presumably of the structure F′ *araB53*/*araB53* was picked, JTL106. This strain was used, in a manner similar to the above isolation of λd*ara101*, to isolate an *araC*$^+$*B*$^-$ transducing phage. JTL106 was grown in 500 ml of TB broth plus 0·4% maltose at 34°C to 3×10^8 cells/ml and infected with $\lambda cI_{857}S_7$ at a multiplicity of infection of 3 for 20 min at room temperature. The temperature was then raised to 42°C for 15 min and lowered to 34°C for 95 min. Cell debris was removed by centrifugation at 6000 revs/min for 10 min. Chloroform (1 ml) was added to the supernatant. Before transducing with this lysate the excess $CHCl_3$ was removed by centrifugation and much of the remaining $CHCl_3$ was removed by a 30-min vacuum aspiration. 200 ml of the supernatant at 3×10^9 phage/ml were used to infect 1×10^{11} cells of JTL103 that had been grown in TB broth plus 0·4% maltose to 1 to 2×10^9 cells/ml, centrifuged, and resuspended in 0·01 M-$MgSO_4$. After 50 min adsorption at 34°C, the cells were concentrated by centrifugation, resuspended in 1 ml of 0·01 M-$MgSO_4$, and spread on a minimal arabinose plate. The 100 transductants were pooled and recycled through the above transducing procedure using 0·001 as many cells. Strain JTL41 was lysogenized with λd*araC*$^+$*B*$^-$ by infecting cells (that had been grown to 5 to 10×10^8 cells/ml in TB broth plus 0·4% maltose and starved in 0·01 M-$MgSO_4$ for 2 h) at a high multiplicity of infection with λd*araC*$^+$*B*$^-$ and $\lambda cI_{857}S_7$ helper and plating on a YT plate spread with 5×10^9 λcIb_2. Survivors able to grow on a minimal arabinose plate after mating with RFSF′2 (F′ $C^-B^+/\Delta ara$) were further tested for their ability to make an *araC* high frequency transducing lysate. This lysogen was labeled JTL107 and its transducing phage was labeled λd*ara102*.

(e) In vitro *protein synthesis*

(i) *Preparation of DNA*

Cells (strain JTL81 or V5009) are grown at 33°C in 2 × concentrated YT broth to 3×10^8 cells/ml, induced at 42°C for 15 min, and grown an additional 3 to 4 h with vigorous shaking at 33°C. Cells are centrifuged and resuspended in $\frac{1}{50}$ vol. of λ suspension medium (0·01 M-Tris·HCl (pH 7·4), 0·005 M-$MgSO_4$, 0·1 M-NaCl). The cell pellets were occasionally frozen at −10°C for several days before purification of the phage. 0·05 ml of chloroform and 0·5 μg of pancreatic DNAase are added per ml of resuspended cells. A 15-min incubation at 37°C completes lysis of the cells and debris is removed by centrifugation at 7000 revs/min for 15 min. The lysate is layered on a block gradient consisting of a 2-ml 20% sucrose layer and 3, 2-ml CsCl layers of density 1·3, 1·5† and 1·7, all of which are in λ suspension medium, and spun for 90 min at 22,000 revs/min in the SW25·1 rotor. The phage banding at density 1·5 are collected. 1 vol. of phage is mixed with 2 vol. of λ suspension medium and layered on a second block gradient of the 3, 1-ml CsCl layers and centrifuged in the SW50 rotor at 22,000 revs/min for 90 min. The collected phage band is then centrifuged to equilibrium in CsCl of density 1·5, in the Spinco 40 angle rotor. The phage band corresponding to the transducing phage is collected; for JTL81 it is the top band, for V5009 it is the bottom band.

The phage DNA is purified by first diluting to an A_{260} = 16·0 and dialyzing into 0·05 M-NaCl, 0·01 M-Tris·HCl (pH 7·8), 0·001 M-sodium-EDTA. Recrystallized sodium dodecyl sulfate is added to 0·5% and the solution is incubated for 15 min at 65°C. KCl is added to 0·5 M and the suspension is chilled at 0°C for 15 min before removing the precipitate by centrifugation at 7000 revs/min for 15 min. The supernatant is dialyzed against 0·1 M-NaCl, 0·01 M-Tris·HCl (pH 7·8), and 0·001 M-EDTA with 4 buffer changes, first at room temperature for 2 h and then 3 at 4°C for a total of 40 h. The final dialysis is into 0·01 M-Tris·acetate, pH 7·8.

(ii) *Preparation of the S-30 extract*

The procedure was modified from Zubay *et al.* (1970). Strain RFS726 is grown at 33°C in 12 g nutrient broth (Difco)/l, 0·5% glucose, and 10 μg thiamin/ml to 2×10^8 cells/ml. The culture is quickly chilled in ice water and centrifuged at 4°C (unless stated otherwise all operations are carried out at 0 to 4°C). The cells are washed in 14 ml of buffer I (10 mM-Tris·acetate (pH 7·8), 14 mM-magnesium acetate, 60 mM-KCl, and 0·1 mM-dithiothreitol), centrifuged at 20,000 revs/min in the Spinco 40 angle rotor for 15 min, and again washed in buffer I and centrifuged. Cells are weighed into a mortar chilled to 0 to 4°C, covered with 2 weights of 0 to 4°C alumina, ground for 3 to 4 min, and 2 weights of buffer II (same as buffer I except 60 mM-potassium acetate substituted for KCl) are slowly mixed with the ground cells. The suspension is centrifuged at 17,500 revs/min in the 40 angle rotor for 20 min. All the supernatant is poured off, gently mixed to homogeneity, and mixed with an appropriate volume of preincubation mixture as described by Nirenberg (1963), except that 0·1 mmol of dithiothreitol was substituted for the β-mercaptoethanol. This mixture is incubated for 80 min at 37°C in the dark, then dialyzed against 10 mM-Tris·acetate (pH 7·8), 14 mM-magnesium acetate, 30 mM-potassium acetate, and 1 mM-dithiothreitol for only 4·5 h with a single buffer change, 0·2 to 0·5 ml portions are frozen rapidly in an acetone–solid CO_2 bath and stored at −70°C. All operations are carried out as rapidly as possible following chilling of the cells.

(iii) *Preparation of* araC *protein extract*

Strain JTL107 is grown at 33°C to 5×10^8 cells/ml in 2 × concentrated YT broth. After a 10-min induction at 42°C, cells are grown for 40 min at 33°C. The culture is chilled in ice water to 4°C and centrifuged at 5500 revs/min for 15 min. Cells are ground vigorously for 7 min with 2 weights of alumina, resuspended in 1·9 vol. of 50 mM-Tris·acetate

† A density of 1·45 is better for purifying phage from JTL81 strain.

(pH 7·8), 300 mM-potassium acetate, 10 mM-magnesium acetate, 10 mM-β-mercaptoethanol, 100 μg phenylmethylsulfonyl fluoride/ml, 5 mM-potassium EDTA, and centrifuged at 36,000 revs/min in the 40 angle rotor for 1 h. The supernatant is dialyzed against 250 ml of 10 mM-Tris·acetate (pH 7·8), 60 mM-potassium acetate, 14 mM-magnesium acetate, 0·1 mM-potassium-EDTA, and 0·1 mM-dithiothreitol for 2 h, rapidly frozen in 0·2-ml portions, and stored up to 6 months at −70°C. The protein concentration of the C protein extract used in the experiments reported here was 26 mg/ml, as determined by the Biuret method using bovine serum albumin as a standard.

(iv) In vitro *protein synthesis*

The *in vitro* protein synthesis was modified from Zubay *et al.* (1970). We found $CaCl_2$ unnecessary for ribulokinase and β-galactosidase synthesis, as was found for β-galactosidase (Emmer *et al.*, 1970) and tryptophan enzymes (Pouwels & Van Rotterdam, 1972). The optimum magnesium acetate concentration was determined for each S-30 extract, usually being 15·5 to 18 mM. DNA was used at 80 μg/ml, trisodium phosphoenol pyruvate at 24·7 mM, protein from the S-30 extract at 7500 μg/ml, and potassium acetate at 43·3 mM. It was unnecessary to add tRNA for ribulokinase and β-galactosidase synthesis. This was also found for the tryptophan enzymes (Pouwels & Van Rotterdam, 1972). Tetrahydrofolate was added as calcium leucovorin, 1·5 μg/50 μl reaction mixture. The *in vitro* synthesis was carried out in a total volume of 50 μl in disposable test tubes. The tubes were washed in chromic acid, rinsed with sodium citrate, and extensively rinsed with distilled, deionized water. Each reaction mixture contained 1·5 μl of *araC* protein extract and L-arabinose at 4 mM.

(f) In vivo *cyclic AMP stimulation of β-galactosidase and arabinose isomerase synthesis*

A culture of CA7902, an adenyl cyclase minus strain, was grown exponentially at 37°C for more than 7 doublings in M9 medium plus 0·2% succinate, containing 1 mM-AMP to decrease the growth advantage of cyclase plus revertants. At 5×10^8 cells/ml, the culture was centrifuged at 5500 revs/min for 7 min, resuspended in 5 vol. of prewarmed M9/succinate medium, and grown for 2 doublings. Cells were then centrifuged at 5500 revs/min for 7 min and made permeable by the EDTA treatment of Lieve (1965). Following the treatment, warmed M9/succinate medium was added to bring the cell density to 3×10^8 cells/ml. 0·5 ml of cells was added to warmed, scrupulously cleaned culture tubes in a shaking 37°C water bath. The tubes contained varying amounts of cAMP and the cells were preincubated with cAMP for 6 min. A solution of arabinose and isopropyl-β-D-galactoside was added to each tube, giving final concentrations of 27 mM and 0·5 mM, respectively. After a 20-min induction at 37°C, the cells were assayed for arabinose isomerase and β-galactosidase.

3. Results

(a) *Isolation and properties of λd*ara *phage*

Induction of a λ lysogen yields *bio* and *gal* transducing phage at a frequency of 10^{-6} to 10^{-7} (Wollman, 1963; Morse *et al.*, 1956) by an abnormal excision event, in which bacterial genes adjacent to the λ integration site are excised with the prophage (Campbell, 1962). The arabinose operon is too distant from the λ integration site for similar production of λ*ara* transducing phage. However, Shimada *et al.* (1972) have isolated a λ lysogen that has λ integrated into the leucine genes, adjacent to the arabinose genes, by forcing λ to lysogenize a strain deleted of the normal λ integration site. As described in Materials and Methods, this strain was used to isolate a λd$araC^+B^+A^+$, the source of K12 *ara* DNA template for the *in vitro* synthesis.

In the preparation of λd$araC^+B^+A^+$ phage, helper phage was to be removed by isopycnic centrifugation in CsCl. Since the λd*ara* phage possessed the density of

TABLE 1

Bacterial strains used

Strain no.	Genotype	Comments
KS73	Hfr: *Δ(gal attλ bio uvrB) leu73*	From R. Weisberg. HfrH $\lambda c\mathrm{I}_{857}$ inserted into leucine. Shimada *et al.* (1972).
RFS1	Hfr: *thi*	Strain HfrH, *thi*$^-$.
RFS696	F$^-$: *ara498 leu498 thi* (λd*araB696* $c\mathrm{I}_{857}S_7h80$)	Heat inducible, lysis defective *araC*$^+$*B*$^-$*A*$^+$ lysogen. The chromosome contains a deletion of *leu* and *araC*, *B*, and *A*.
RFS726	F$^-$: *ara498 leu498 lac74 thi*	Deletion of leucine and at least *araC*, *B*, and *A*, deletion of *lac*.
RFS817	F$^-$: *ara498 leu498 lac74 thi str*	From RFS726, *str*^{r}.
RFS825	F$^-$: *leu73 Δ(gal attλ bio uvrB)*	*Ara*$^+$ *str*^{r} product of KS73 × RFS817.
CA7902	F$^-$: *cya7902*	From J. Beckwith, adenyl cyclase minus.
V5009	F$^-$: *lac74 thi* $\phi 80^r$ ($\lambda c\mathrm{I}_{857}S_{t68}h80$, λd*lac*$c\mathrm{I}_{857}S_{t68}h80$)	From E. Signer. Double lysogen containing a helper phage and a more dense *lac* transducing phage. Both phages are lysis defective and heat inducible.
RFSF′2	F′ : *thr*$^+$ *araC2 leu*$^+$/*ara498 leu498 thr thi lac74*	R. Schleif (1972).
RFSF′20	F′ : *thr*$^+$ *araB20 leu*$^+$/*ara498 leu498 thr thi lac74*	R. Schleif (1972).
RFSF′53	F′ : *thr*$^+$ *araB53 leu*$^+$/*ara498 leu498 thr thi lac74*	R. Schleif (1972).
RV	F$^-$: *lac74 thi*	From M. Malamy. Deletion of *lac* genes.
JTL28	F$^-$: *thr leu lac74 tsx28*	Derived from RV by phage P1 transduction of *thr*$^-$ *ara*$^+$ and *leu*$^-$ *ara*$^+$ of C600 to *ara*$^-$ derivatives of RV. Also T6^r.
JTL41	F$^-$ *ara498 leu498 lac74 tsx28*	*ara498* deletion was transduced into JTL28 by phage P1.
JTL78	F$^-$: *araB20 tsx28*	Missense *araB* of RFSF′20, like JTL28 but *thr*$^+$ *leu*$^+$ *araB20* and *lac*$^+$ by phage P1.
JTL80	F$^-$: *ara498 lac74* (λd*ara101* $c\mathrm{I}_{857}S_7$, $\lambda c\mathrm{I}_{857}S_7b_2$)	RFS726 transduced to *ara*$^+$, this work.
JTL81	F$^-$: *ara498 lac74* (λd*ara101* $c\mathrm{I}_{857}S_7$, $\lambda c\mathrm{I}_{857}S_7b_2$) *lam81*	Like JTL80 but resistant to λ*vir*.
JTL103	F$^-$: *araC1022 leu1022*	*araC1022 leu1022*, a deletion of *araC* and *leu* Schleif (1972), transduced into JTL28 by phage P1 to *thr*$^+$, by N. Nathanson.
JTL106	F′ : *araB53/araB53 Δ(gal att bio uvrB) leu73 lac74 str*	RFSF′53 was mated with RFS825 and an *ara*$^-$ homozygote was selected.
JTL107	F$^-$: *ara498 leu498 lac74 tsx28* (λd*ara102* $c\mathrm{I}_{857}$, $\lambda c\mathrm{I}_{857}S_7$)	This work.

Genotype	
$\lambda c\mathrm{I}_{60}b_2$	From D. Fr
$\lambda c\mathrm{I}_{857}S_7$	From E. Sig *S* gene respo
$\lambda c\mathrm{I}_{857}S_7b_2$	From a cros
λd*ara101* S_7	A defective defective.
λd*ara102* $c\mathrm{I}_{857}$	A defective sensitive rep
λ*vir*	From E. Sig grow on λ ly

wild type λ, a less dense helper, λ
the phage, induced from the doub
the phage densities were interchar
lighter λb_2 density, and the plaqu
this occurred in three independe
property of the phage. We propose
by λd*araC*$^+$*B*$^+$*A*$^+$ and helper λb_2,
nose region of the transducing ph

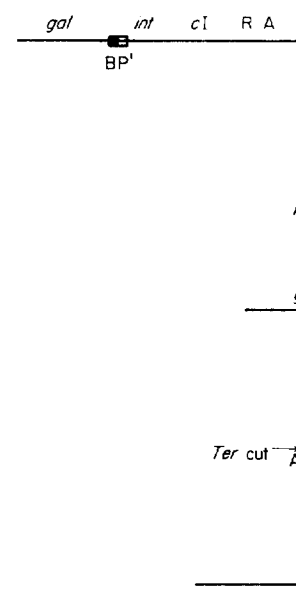

FIG. 1. The probable structure of the
upon induction. The proposed structu
complemented by λb_2, which provides
structure (Manly *et al.*, 1969). In addit
the bacterial substitution extends thro
in Results.

TABLE 2

Bacteriophage used

Genotype	Comments
$\lambda c\mathrm{I}_{60}b_2$	From D. Freifelder. λ clear, deletion b_2.
$\lambda c\mathrm{I}_{857}S_7$	From E. Signer. Temperature sensitive repressor, amber mutation in *S* gene responsible for lysis, suppressible by *suIII*.
$\lambda c\mathrm{I}_{857}S_7b_2$	From a cross of $\lambda c\mathrm{I}_{60}b_2 \times \lambda c\mathrm{I}_{857}S_7$.
λd*ara101* S_7	A defective λ carrying the $araC^+B^+A^+$ genes from HfrH, lysis defective.
λd*ara102* $c\mathrm{I}_{857}$	A defective λ carrying the $araC^+B^-$ genes from HfrH, temperature sensitive repressor.
λ*vir*	From E. Signer *via* Ira Herskowitz. Defective early operators, able to grow on λ lysogens.

wild type λ, a less dense helper, $\lambda c\mathrm{I}_{857}S_7b_2$ was used. However, after CsCl banding of the phage, induced from the double lysogen (λd*arac*$\mathrm{I}_{857}S_7$, $\lambda c\mathrm{I}_{857}S_7b_2$), we found that the phage densities were interchanged! The arabinose transducing phage was at the lighter λb_2 density, and the plaque-forming phage was at the wild type density. Since this occurred in three independent double lysogens, it appears to be an intrinsic property of the phage. We propose the following explanation: following lysogenization by λd$araC^+B^+A^+$ and helper λb_2, the b_2 region of the helper is adjacent to the arabinose region of the transducing phage. The b_2-*ara* region is a poor substrate for the λ

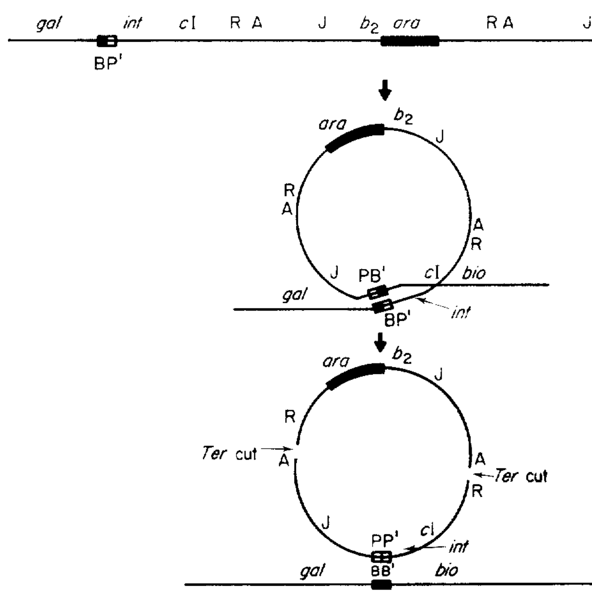

FIG. 1. The probable structure of the double lysogen formed with λd*ara* and λb_2, and its excision upon induction. The proposed structure is based on the fact that like λ*bio*, λd*ara* integration is complemented by λb_2, which provides not only *int* gene product but also a suitable attachment structure (Manly *et al.*, 1969). In addition, rare single λd*ara* lysogens lack λ immunity, suggesting the bacterial substitution extends through the λcI gene. The mechanism of excision is described in Results.

excision system, and on induction excision of the lysogen generates the double circle shown in Figure 1. The production of A-R cuts by the *ter* function (Mousset & Thomas, 1969) produces an *ara* transducing phage with b_2 density and a plaque-forming phage with wild type density.

To complete the homologous K12 *in vitro* protein synthesizing system, a concentrated source of *araC* protein was needed. In the past, transducing phage carrying the desired bacterial genes have been used to synthesize large quantities of gene product *via* a gene dosage effect during growth of the phage (Müller-Hill *et al.*, 1968; Abelson *et al.*, 1970). In addition, if the bacterial gene happens to become fused to a strong phage promotor, an additional increase in the synthesis of the protein may occur (Schleif *et al.*, 1971). A $\lambda araC$ phage would therefore be useful, but it must not carry an active *B* gene (ribulokinase), since kinase is the enzyme to be synthesized *in vitro* subject to regulation by the added C protein. Therefore, a non-leaky *araB* mutation was crossed into the arabinose operon in the strain containing λ in the leucine genes. An *araC* transducing phage was then isolated lacking a functional *araB* gene. A strain deleted of all arabinose genes was then lysogenized by the phage and was used to make *araC* protein extracts.

(b) *Comparison of* ara *and* lac *in* vitro *protein synthesis in response to cyclic AMP*

The *in vitro* synthesis of ribulokinase of the arabinose operon and β-galactosidase of the lactose operon was measured in the presence of varying amounts of cAMP (Fig. 2). The conditions for synthesis of the two enzymes were identical, the only difference being the DNA template added. β-galactosidase synthesis reaches 10% of maximum at 5×10^{-5} M-cAMP, whereas a 10% increase in ribulokinase synthesis

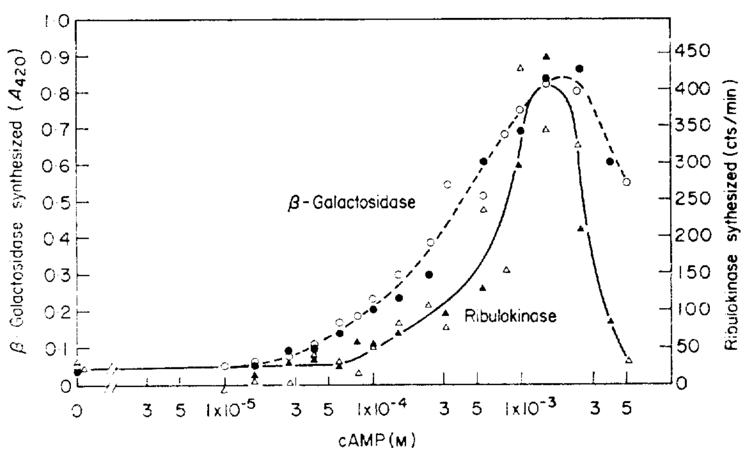

FIG. 2. The *in vitro* synthesis of β-galactosidase and ribulokinase as a function of cAMP concentration. The synthesis was carried out as described in Materials and Methods. The data from 2 independent experiments are presented, β-galactosidase synthesized from experiment I (○) and from experiment II, (●), are compared to ribulokinase synthesis from experiment I, (△), and from experiment II, (▲). β-galactosidase from experiment II was normalized to experiment I by multiplying by 1·3. Ribulokinase assay backgrounds of 190 cts/min have been subtracted from all kinase assays. In these measurements the composition of the synthesis reaction mix for the arabinose and lactose operons was identical, with only the DNA templates different.

occurs at $1{\cdot}4\times10^{-4}$ M. Similarly, the cAMP concentrations required for half-maximal response also differ, $3{\cdot}2\times10^{-4}$ M for *lac* and $6{\cdot}4\times10^{-4}$ M-cAMP for *ara*. The effects seen are reproducible and the data of Figure 2 are from two independent measurements.

(c) *Comparison of* ara *and* lac *in vivo protein synthesis in response to cyclic AMP provided externally*

Although much information and many *in vivo* consistencies have been obtained with *in vitro* systems, their fallibility in reconstructing the *in vivo* condition has been documented. Wetekam *et al.* (1972) found that expected polar effects in the *gal* operon were absent *in vitro*. Therefore, we sought verification of the cAMP results in conditions closer to those present *in vivo*. Adenyl cyclase minus cells, made permeable by EDTA treatment (Lieve, 1965), were preincubated with varying amounts of exogenously supplied cAMP and then induced for both operons simultaneously. Arabinose isomerase and β-galactosidase levels were then measured after 20 minutes of induction, limiting the induction period to the interval during which EDTA-treated cells remain permeable. Stimulation of β-galactosidase synthesis occurs with 10% of maximum stimulation at $1{\cdot}3\times10^{-4}$ M-cAMP, whereas arabinose isomerase synthesis requires $2{\cdot}2\times10^{-4}$ M-cAMP to produce 10% of maximum response (Fig. 3). The cAMP concentrations required for half-maximal stimulation also differ, with values of $3{\cdot}8\times10^{-4}$ M and $8{\cdot}6\times10^{-4}$ M for β-galactosidase and arabinose isomerase, respectively. Although there is an easily detected difference in the response of the two

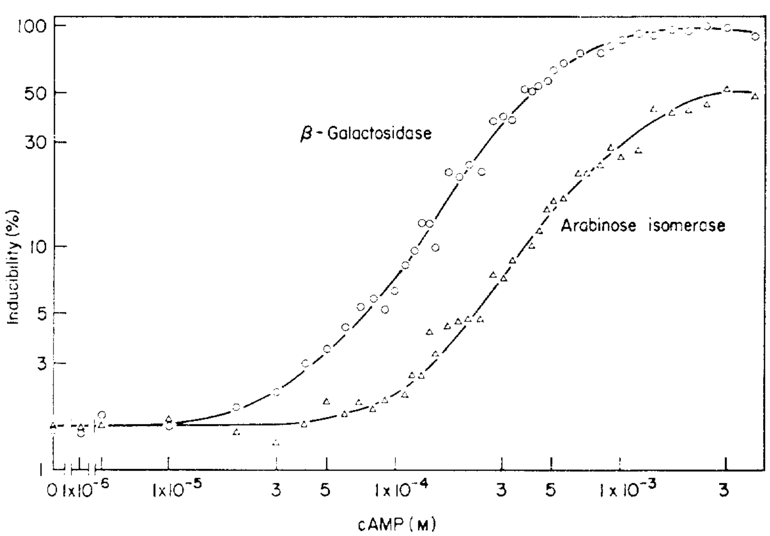

FIG. 3. *In vivo* synthesis of β-galactosidase and arabinose isomerase in adenyl cyclase minus, EDTA-treated cells as a function of cAMP concentration. The experiment was carried out as described in Materials and Methods. β-galactosidase levels, (○), are plotted as a percentage of the maximum level. Arabinose isomerase data, (△), was plotted to have the same level as β-galactosidase at zero cAMP. The value of 100% is equivalent to 1620 monomers/cell of β-galactosidase and 19,400 monomers/cell of arabinose isomerase. The number of monomers was calculated from the specific activity of pure β-galactosidase, taking the subunit molecular weight to be 135,000 and the molar extinction of *o*-nitrophenol to be 21,300. The number of isomerase monomers was calculated from the specific activity of pure isomerase (Patrick & Lee, 1968) with a subunit molecular weight of 60,000 (Patrick & Lee, 1969) and values were corrected for the 0·54 activity that isomerase has in our buffers compared to its activity in Lee's buffers.

operons to cAMP, the absolute values of the cAMP concentrations are not necessarily typical cellular concentrations. Added cAMP must contend not only with phosphodiesterase degradation but also with cell impermeability. Although the cells have been made more permeable by the EDTA treatment, the intracellular concentration does not necessarily equal the extracellular concentration (Lieve, 1965).

(d) *Physiological consequences of the different cyclic AMP requirements of* ara *and* lac; *a completely* in vivo *comparison*

The two preceding experiments indicate that higher concentrations of cAMP are required for induction of the *ara* operon than are required for the induction of the *lac* operon. Since intracellular cAMP levels vary from one growth medium to another (Makman & Sutherland, 1965; Ullmann & Monod, 1968), then a medium could exist leading to an intracellular cAMP concentration too low for significant induction of the *ara* operon, but sufficient for appreciable induction of the *lac* operon. Yeast extract/tryptone broth was found to possess this property. In addition, the catabolites causing repression in this broth were found to be consumed during growth of *E. coli* and a transition occurs at approximately 4×10^{7} cells/ml from catabolite repressed to

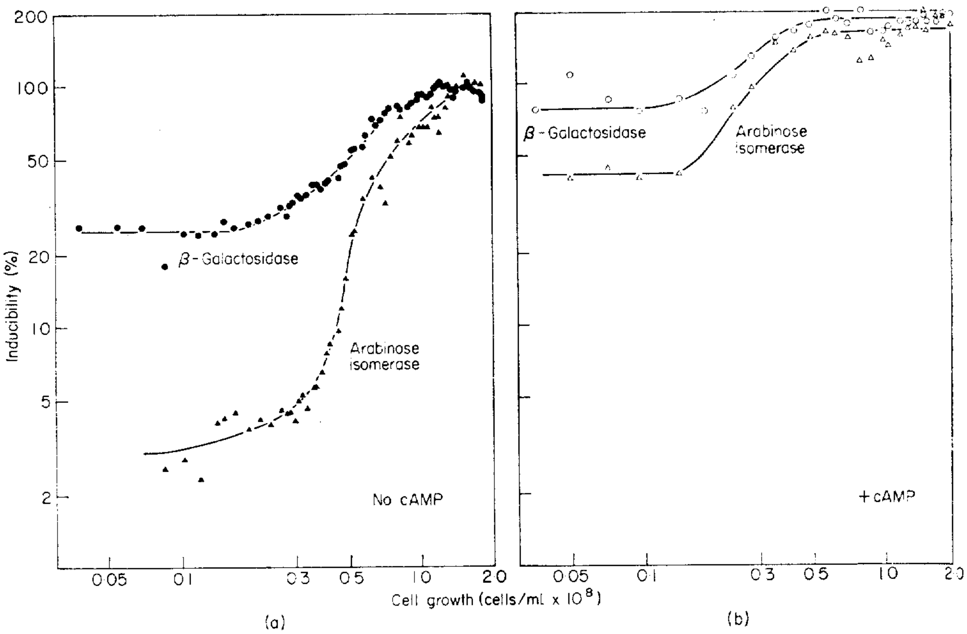

FIG. 4. Arabinose isomerase and β-galactosidase levels of cells growing in yeast extract/tryptone broth.

(a) Cells were grown at 37°C in a shaking water bath for 9 doublings in yeast extract broth containing 27 mM-arabinose and 5×10^{-4} M-isopropyl-β-D-thiogalactoside. Samples were harvested at a number of cell densities and chilled on ice, and arabinose isomerase (▲) and β-galactosidase (●) enzyme levels were measured at each cell density (care was taken to maintain the culture at 37°C during removal of samples). The cell density was determined as described in the legend to Fig. 5.

(b) A repeat of the above experiment was carried out in which cAMP was added to the culture at a final concentration of 6 mM, 4 to 5 doublings before taking the first sample. Arabinose isomerase (△) and β-galactosidase (○) levels were measured at each cell density.

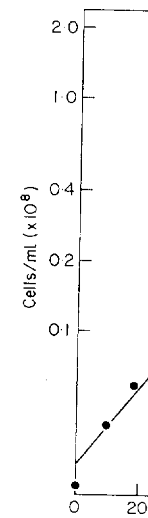

FIG. 5. Cell growth in YT broth. T
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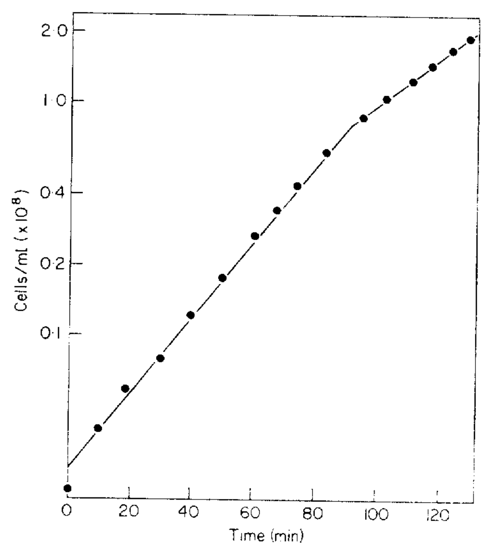

FIG. 5. Cell growth in YT broth. The growth was determined during the experiment described in Fig. 4 by measuring the turbidity at 550 nm. The turbidity was converted to cell destiny by multiplying turbidity by a conversion factor obtained with the Petroff–Hausser counting chamber. 6 mM-cAMP did not change the growth rate in any region of the growth curve by more than 20%.

non-repressed enzyme synthesis rates (Fig. 4 (a)). A shift to a slower growth rate also accompanies the transition in rates of enzyme synthesis (Fig. 5).

Wild type *E. coli* K12 grown exponentially in yeast extract/tryptone broth plus 27 mM-(0·4%) arabinose and 0·5 mM-isopropyl-β-D-thiogalactoside for more than eight doublings to a density of $2{\cdot}5\times10^7$ cells/ml possesses only 810 isomerase monomers and 1200 β-galactosidase monomers per cell. However, on further growth to $1{\cdot}5\times10^8$ cells/ml, isomerase increases 30-fold, whereas β-galactosidase increases less than fourfold (Fig. 4(a)). Thus repression by yeast extract/tryptone broth at low cell densities is 7·5 times more severe for the arabinose operon than for the lactose operon.

An increase or a decrease in the concentration of YT broth correspondingly shifts the transitions to higher and lower cell densities. The transition in synthesis rates per cell as cell density increases is not a condition resulting directly from a high cell density. A constant level of 100,000 monomers of isomerase and 11,200 monomers per cell of β-galactosidase was observed in a cell density range of 2×10^7 to 3×10^8 cells/ml with strain JTL78 grown in M9 medium plus 1×10^{-5} M-$MnCl_2$, 0·2% glycerol, 27 mM-arabinose and 0·5mM isopropyl-β-D-thiogalactoside. The high enzyme levels are probably due to the absence of catabolite repression and "self-catabolite" repression in this B^- missense strain grown in M9-glycerol medium (Katz & Englesberg, 1972).

Adding 6 mM-cAMP to yeast extract/tryptone broth increases the level of arabinose isomerase 11-fold and β-galactosidase threefold at low cell densities (Fig 4(b)). Thus, cAMP largely reverses the catabolite repression generated by YT broth. At cell densities above $1{\cdot}5\times10^8$ cells/ml, when catabolites have been consumed, cAMP produces less than a twofold stimulation in enzyme levels.

4. Discussion

Three types of experiments demonstrated that induction of the L-arabinose operon requires higher cAMP levels than the lactose operon. These experiments were carried out under identical conditions for the two operons. In the cell-free synthesis of ribulokinase and β-galactosidase the initial cAMP concentrations were easily varied and a difference between the two operons in response to cAMP was demonstrated. The results from such measurements are not without ambiguities, however, for phosphodiesterase in the cell-free bacterial extract can reduce the cAMP levels (Monard *et al.*, 1969), and thus the *in vitro* cAMP concentrations producing induction may not equal the actual *in vivo* concentrations required. Furthermore, although DNA-directed, cell-free protein synthesis closely resembles many known *in vivo* processes, the degree of correspondence is as yet unknown.

The *in vitro* system was inhibited by high levels of cAMP. Such inhibition has been observed previously in the *lac* operon (Emmer *et al.*, 1970; De Crombrugghe *et al.*, 1971). De Crombrugghe *et al.* (1971) used a purified transcription system, thereby localizing the effect to RNA initiation or elongation. Since only one molecule of cAMP binds to the dimeric cAMP receptor protein at cAMP concentrations below 6 μM (Anderson *et al.*, 1971), perhaps the higher concentrations of cAMP force a second cAMP molecule onto cAMP receptor protein, thereby reducing its activity. (This possibility was suggested to us by Ira Pasten.) Consistent with this explanation and the behavior of the operons at low cAMP levels is the fact that high levels of cAMP inhibit induction of the arabinose operon more than the lactose operon (Fig. 2).

As a step closer to the true *in vivo* situation, the arabinose and lactose enzymes were induced *in vivo*, but the cAMP concentration was varied from outside the cell. This required adenyl cyclase negative cells that are unable to synthesize cAMP and their being made permeable by EDTA treatment to allow externally added cAMP to enter the cells more easily. Variation of the external concentration of cAMP again showed that induction of the arabinose operon requires higher cAMP levels than the lactose operon. Absolute concentrations of cAMP are again not meaningful, since the internal and external concentrations of cAMP may not be equal. Also the EDTA treatment itself could damage essential cell components and alter their cAMP response. However, such damage seems unlikely to affect one operon preferentially.

Finally, in an *in vivo* system using untreated, freely growing cells we also find a difference between the arabinose and lactose operons attributable to different cAMP requirements. At approximately 0·5 to 1 $\times$ 10^8 cells/ml in yeast extract/tryptone broth, the growth of cells measured turbidometrically shows a transition from a doubling time of 20 minutes to one of 32 minutes. The arabinose operon can be induced one-thirtieth as well before the shift as after the shift, whereas the lactose operon can be induced one-quarter as well before the shift as after the shift. Thus the arabinose operon is affected 7·5-fold more than the lactose operon by the presence of some nutrient, which is consumed when cells reach a density of 5 $\times$ 10^7 cells/ml. This repression could result from lower intracellular cAMP levels at cell densities below 5 $\times$ 10^7 cells/ml, the cAMP concentration being such that the *ara* operon is barely inducible, whereas the *lac* operon is almost fully inducible. Consistent with this view is the fact that inclusion of cAMP in the broth largely restores full inducibility at all cell densities (Fig. 4(b)).

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This work was supported in part b
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Some of the yeast extract/tryptone broth repression is not overcome by the addition of 6 mM-cAMP to the medium, and the resulting transition in enzyme inducibilities with growth to higher cell densities is 2·5 to fourfold for both β-galactosidase and arabinose isomerase (Fig. 4(b)). The inability of cAMP to totally reverse strong catabolite repression has been observed by others (Ullmann & Monod, 1968). The transition we observe in inducibility in the presence of added cAMP is accompanied by a change in doubling time from 24 to 32 minutes. This reduction in growth rate may free many RNA polymerase molecules from ribosome synthesis for relatively more active messenger synthesis. Indeed, Rose & Yanofsky (1972) suggested this to explain their finding that the tryptophan operon messenger initiation frequency increases with growth rate, reaches a maximum in minimal glucose, but falls sharply when ribosome synthesis is most active in rich medium. Thus the YT broth repression may be mediated not only by lowering the intracellular cAMP concentration, but also by a reduction in the intracellular concentration of free RNA polymerase capable of initiating messenger RNA synthesis.

Although measurements of arabinose isomerase levels have been made in YT broth on previous occasions (Schleif *et al.*, 1971), the cell density was always 2 to 4 $\times 10^8$ cells/ml. At these densities the catabolite repressors have been consumed and very little variation in enzyme per cell is observed. Other workers have noted that reproducible measurements of the cellular glycerol operon enzymes required harvesting cultures at the same cell density (Cozzarelli *et al.*, 1968). The importance of such a precaution is vividly demonstrated in the arabinose isomerase per cell transition between 3×10^7 and $1{\cdot}5 \times 10^8$ cells/ml of induced cells growing in yeast extract/tryptone broth (Fig. 4(a)).

A plausible explanation for the evolution of different cAMP sensitivities by the arabinose and lactose operons is that the *lac* operon must remain induced in the presence of glucose generated by the cleavage of lactose, whereas the *ara* operon must contend only with the weaker "self-catabolite" repression (Katz & Englesberg, 1972).

This work was supported in part by the National Institute of General Medical Sciences, National Institutes of Health (research grant GM18277 and Career Development award K4GM38797 to R. F. S.). Publication no. 907 from the Department of Biochemistry, Brandeis University.

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RNA-directed DNA P
Initiation of Synthesis

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