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ALT: A New Factor Involved in the Synthesis of RNA by *Escherichia coli*

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Summary. We have defined a new gene, *alt*, which affects RNA synthesis in *Escherichia coli*. Mutants for *alt* arise among revertants of strains lacking the CRP-cAMP system necessary for full expression of catabolite-sensitive operons. Studies on a temperature-sensitive *alt* mutant indicate that the *alt* gene product is necessary for the synthesis of an important class of messenger RNA molecules.

Introduction

The *lac* and *ara* operons of *Escherichia coli*, like other catabolite-sensitive (CS) operons, normally require for their transcription, adenosine-3′,5′-cyclic monophosphate (cAMP), and at least one protein factor, variously termed CAP, CRP, and CGA (Perlman and Pastan, 1968; Ullman and Monod, 1968; Zubay, Schwartz and Beckwith, 1970; Emmer, de Crombrugghe, Pastan and Perlman, 1970; Riggs, Reiness and Zubay, 1971). The CRP-cAMP system is involved in the initiation of *lac* transcription (Magasanik, 1970). Strains whose *lac* expression is no longer dependent on CRP or cAMP have mutations e.g. P^r_{UV5} in the *lac* promoter region (Silverstone, Arditti and Magasanik, 1970; Silverstone, Magasanik, Reznikoff, Miller and Beckwith, 1969). Purified RNA polymerase (holoenzyme) will transcribe *lac* DNA *in vitro* (de Crombrugghe, Chen, Anderson, Nissley, Gottesman, Pastan, and Perlman, 1971; Eron and Block, 1971). Transcription of normal *lac* DNA is dependent on CRP and cAMP whereas transcription of DNA from the promoter mutant P^r_{UV5} is largely independent of these components (Eron and Block, 1971). The purified system therefore mimics *lac* transcription *in vivo* and this suggests that transcription of the *lac* operon involves only three components; RNA polymerase, CRP and cAMP, in addition to the four nucleoside triphosphate substrates of the enzyme. Both biochemical and genetic studies suggest that the *lac* promoter contains at least two sites, one—the target for CRP and the other—the binding site for RNA polymerase (Eron and Block, 1961; de Crombrugghe *et al.*, 1971; Arditti, Grodzicker and Beckwith, in press).

We shall describe a new class of mutation, termed *alt*, which compensates for loss of the CRP-cAMP system. The *alt* mutants could have a changed RNA polymerase able to initiate transcription of CS operons without CRP intervention. Alternatively, they could have an altered transcription factor able to substitute

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for the CRP-cAMP system. In its unaltered form, such a factor could normally act in concert with the CRP-cAMP system, being required to initiate transcription of CS operons. In this case, the interpretation of the transcription studies mentioned above, would have to be revised. On the other hand, the factor may normally only be required for transcription of genes of some other class, unrelated to CRP.

Genetic and physiological studies show that *alt* mutations affect transcription of certain operons, suggesting that they alter a transcription factor, rather than the RNA polymerase itself. Our studies favour the conclusion that this factor normally specifies transcription of genes not using CRP.

Materials and Methods

Chemicals. Rifampicin was a gift from Gruppo Lepetit (Milan). Adenine-^{14}C (U) and uridine-5-^{3}H were obtained from Amersham, isopropyl-β-D-thio-galactoside (IPTG) and adenosine-3′,5′-cyclic monophosphoric acid (cAMP) from Sigma Biochemicals, BBOT scintillant from CIBA, and polyethyleneimine cellulose plates (PEI) from Merck (Darmstadt).

Bacterial Strains. E. coli K12 strains used in these experiments are described in Table 1.

Table 1. Bacterial strains

Strains	Pertinent genetic characteristics	Source or derivation
WZ11	F⁻ *metB⁻ lac⁺ cya⁺ crp⁺ alt⁺ str-r rel⁻*	Streptomycin resistant derivative of W1655. (Lederberg and Lederberg, 1953)
WZ22	F⁻ *metB⁻ lac⁺ cya*-855 *crp⁺ alt⁺ str-r rel⁻*	Ultraviolet mutagenesis of WZ11
WZ25	F⁻ *metB⁻ lac⁺ cya⁺ crp*-868 *alt⁺ str-r rel⁻*	Ultraviolet mutagenesis of WZ11
WZ30	F⁻ *metB⁻ lac⁺ cya*-855 *crp⁺ alt*-1 *str-r rel⁻*	Pleiotropic, sugar-positive temperature sensitive revertant of WZ22
WZ44	F⁻ *metB⁻ lac⁺ cya*-855 "*glpK*" *str-r rel⁻*	Glycerol-positive revertant of WZ22. The precise nature of the "glpK" mutation has not been established
WZ57	F⁻ *metB⁺ cya⁺ crp⁺ alt* 1 *str-r*	Met⁺ Lac⁺ recombinant of WZ30 mated with D8007
D8007	Hfr G10, *his⁻* (Fig. 1)	Matney, Goldschmidt and Erwin (1963)
ED1002	Hfr *lac proB*, integrated F′ts *lac⁺ spc-r str-r* (Fig. 1)	Masters and Broda (1971)
ED1009	Hfr *lac proB*, integrated F′ts *lac⁺ spc-r str-r* (Fig. 1)	Masters and Broda (1971)
KLF2	F′ *argG⁺ argR⁺/argG⁻ his⁺ leu⁺ metB recA⁻ strA*	Low (4968)

Genetic loci follow standard abbreviations (Taylor, 1970); *cya* is the genetic locus responsible for the production of an ATP-dependent adenyl cyclase (Yokota and Gots, 1970); *crp* is the locus responsible for production of a protein product necessary for transcription of catabolite sensitive operons (Schwartz and Beckwith, 1970; Perlman *et al.*, 1970); *alt* is the locus discussed in this text. Hfr origins and their direction of transfer are indicated in Fig. 1.

Growth Media. Nutrient broth (NB) was Oxoid NB2. Two liquid minimal salts media were used; MS (de Haan and Gross, 1962) and M63 (Pardee, Jacob, and Monod, 1959) to which glucose (0.2%), vitamin B_1 (1 μg/ml), and growth supplements (20 μg/ml) were added. Solid media were obtained by adding Davis New Zealand Agar at 1.25% (NB) or 1.75% (MS) to the appropriate liquid media.

Bacterial Crosses. Our mating methods are described in Scaife and Pekhov (1964).

Assay for β-D-Galactosidase. Exponential cells appropriately diluted in one ml of MS were added at 0° to a mixture of 0.2 ml of 0.1% solution of CeTB (Tyler and Magasanik, 1969), 0.8 ml of PM² buffer (the reducing buffer of Revel, Luria and Rotman, 1961), and 0.01 ml of 1% sodium deoxycholate (DOC). We assayed the samples (Revel *et al.*, 1961) using 1.0 M Na_2CO_3 in 8M urea to terminate the reaction.

Enzyme activity is expressed as the corrected change in OD_{420} per minute per ml of culture.

Measurement of Total RNA Synthesis. Labelling of RNA and inhibition of RNA synthesis is described by Pato and von Meyenberg (1970). Cultures grown in NB or supplemented M63 containing 40 μg/ml uridine were labelled with uridine tritiated at position 5 of the pyrimidine ring (2.5 μCi/ml, 40 μg/ml) to minimise the entry of label into DNA as thymine. Initiation of RNA synthesis was stopped with 200 μg/ml rifampicin. Samples were prepared for counting as described by Bremer and Yuan (1968), and counted in BBOT-toluene (4 g/l).

Cyclic AMP Assay. The assay we used was a combination of methods developed by S. Fogel and by G. Edlin (personal communications). It involves the fractionation, by two-dimensional chromatography, of labelled nucleotides from cells grown in ^{14}C-adenine. The PEI chromatography plates are washed according to Randerath (1966). Cells exponentially growing in supplemented M63 containing 50 μg/ml ^{14}C-Adenine (U) (50 μCi/ml) for 3–4 generations are concentrated on Millipore filters (10^7 cells), and then extracted in 2.5 ml of 0.3M perchloric acid at 0° C. The debris is removed from the extract by centrifugation and the supernatant is neutralized with two drops of EDTA (0.5M) + KOH (6M). The precipitate is removed by centrifugation and 40 μl of the supernatant, adjusted to a standard specific activity is spotted on the PEI cellulose plate with a 10 μl solution of cAMP (10 mM) as a marker. Each sample is fractionated by two dimensional LiCl chromatography (Cashel and Gallant, 1969). The cAMP marker shows up as a fluorescent spot under ultraviolet light. This spot is cut out of the chromatogram and its radioactivity measured in a gas-flow counter (Nuclear Chicago).

Results

The Isolation of Alt Mutations. We have derived *alt* mutants from two different starting strains. One of these is WZ22 which has a mutation, *cya*-855, which reduces the intracellular concentration of cAMP to less than 5 per cent of the normal level (Table 2). The mutation *cya*-855 maps between *metE* and *ilv* (Silverstone, Tsuji and Scaife, unpublished), the region containing the gene for adenyl cyclase (Yokota and Gots, 1970). The expression of catabolite-sensitive operons is much reduced in WZ22 (Table 2), a defect which we attribute to inactivation of the CRP-cAMP system. As expected, the defect can be reversed by exogenous cAMP (Table 2).

The second strain giving rise to *alt* mutants, WZ25, has a different mutation, *crp*-868, also reducing the expression of CS operons. However, the mutation does not affect the cAMP concentration in WZ25, and its effect on CS operons cannot be reversed by exogenous cAMP (Table 2). The mutation therefore has the phenotype expected for a mutant lacking the CRP factor. Strong support for this conclusion comes from our observation that the *lac* promoter mutant, p^r_{UV5}, which is transcribed in the absence of the CRP-cAMP system (Schwartz and Beckwith, 1970), can be transcribed in *crp*-868 (data not shown). As expected,

Table 2. β-galactosidase synthesis and cyclic AMP levels in wild type and mutant strains

Strain	Relevant genotype	β-D-Galactosidase synthesis			cAMP level	
		30° C		42° C	Experiment 1	Experiment 2
		− cAMP	+ cAMP	− cAMP		
WZ11	*cya*$^+$*alt*$^+$	100	100	100	197	231
WZ22	*cya*-855 alt$^+$	3.2	103	3.3	28	33
WZ25	*crp*-868 *alt*$^+$	2.7	2.6	3.0	201	213
WZ30	*cya*-855 *alt*-1	15	33	3.2	37	36
WZ57	*cya*$^+$ *alt*-1	47	48	46	—	—
Blank	—	—	—	—	16	24

(a) Cyclic AMP was assayed in cultures grown at 30° C from which perchloric acid extracts were prepared as described in Materials and Methods. After fractionation on PEI cellulose with LiCl, the amount of label in the spot corresponding to cyclic AMP was counted as described. Values given are the number of counts per minute per spot. The blank value was obtained by adding label to a perchloric acid extract of unlabelled WZ11.
(b) Each value for β-galactosidase represents the increase of enzyme activity synthesised per unit increase in OD (Klett units, red filter) of the assayed culture. These values are expressed as a percentage of the wild type activity in that growth condition.
(c) Cultures of the wild type grown with cAMP (10^{-3} M) show almost twice the β-galactosidase activity found in an equivalent culture without cAMP. This is due to relief of catabolite repression in these glucose-grown cultures (Perlman *et al.*, 1969; deCrombrugghe and Pastan, 1969).
(d) Note. We find β-galactosidase synthesis by WZ30 to be unchanged after it has been transduced to rel$^+$.

crp-868 is cotransducible with *strA* (Perlman, Chen, de Crombrugghe, Emmer, Gottesman, Varmus and Pastan, 1970).

Revertants of WZ22 and WZ25 occur at a frequency of 10^{-6} to 10^{-7}. They are readily selected on minimal agar containing arabinose as sole carbon source, since utilisation of this sugar requires the CRP-cAMP system (de Crombrugghe, Perlman, Varmus and Pastan, 1969). Some Ara$^+$ revertants continue to express other CS operons at a reduced level. They could have a mutation in the *ara* promoter circumventing its requirement for the CRP-cAMP system. The remainder regain function of the other CS operons. These pleiotropic revertants have therefore acquired a mutation which either restores or compensates for the CRP-cAMP system defective in these parent strains.

It is a remarkable fact that about 5% of the pleiotropic revertants are unable to grow at 42° C, either on minimal-glucose, or nutrient medium. We call these *alt* mutants (alternative to CRP-cAMP). Their properties form the basis of this investigation.

The Genetic Identity of Alt Mutants. We have studied 75 independent *alt* mutants isolated as revertants of *cya*-855 and *crp*-868 strains. Mating experiments show (Table 3) that the *alt* mutation in every case is located in the small sector of the *E. coli* chromosome between *metC* (59 minutes) and *spc* (64 minutes).

The *alt* gene is transferred early by Hfr ED1009 but not by Hfr ED1002. It is carried by the chromosomal fragment of KLF2 (Table 3). The *alt*-1 mutation does not cotransduce with *argG* or *pnp* (data not shown), indicating that the *alt*

Table 3. The mapping of *crp* and *alt* mutations by conjugation

Recipient genotype	No. of isolates tested	Donors yielding wild-type recombinants		
		KLF2	ED1002	ED1009
metB ***crp-868*** (WZ25)	1	−	+	+
metB cya-855 ***alt-1*** (WZ30)	1	+	−	+
metB crp-868 ***alt:*** *metB cya*-855 ***alt***	74	+	−	+

In each cross the donor marker tested for transfer is indicated in bold type.
(a) *The Hfr crosses.* From each cross we selected *met*B$^+$ recombinants, which were then tested for the *crp*$^+$ or *alt*$^+$ character. + = more than 70% of met$^+$ inheriting the donor marker. − = less than 1%.
(b) *The KLF2 crosses.* The *alt*$^+$ derivatives were selected directly at 42° on minimal plates containing methionine and glucose. The KLF2 donor yields 10^4 *alt*$^+$ derivatives per ml of mating mixture (parent input $\Omega 2 \times 10^8$ cells per ml). In a control cross the same donor yields 5×10^4 *arg*G$^+$ derivatives per ml. The *alt*$^+$ derivatives transfer KLF2 and segregate *alt*$^-$ at a low frequency (10^{-3}).

gene is very close to either *metC* or *spc*. In the case of one of the mutants, *alt*-1, we have shown that a single lesion is responsible for temperature sensitivity and the restoration of CS operon expression. Spontaneous temperature-resistant revertants were selected from WZ30 *cya*-855 *alt*-1. All of these (660) concomitantly became arabinose negative. If the two phenotypes were due to separate lesions, most, if not all our derivatives should have remained Ara$^+$.

Do Alt Mutations Restore the CRP-cAMP System? The *alt*-1 mutation does not change either of the known factors (CRP and cAMP) required for normal transcription of CS operons. If the mutation changed the CRP protein enabling it to act without cAMP, *alt*-1 should map in the *crp* gene. The *crp* gene is located close to *strA* (Perlman *et al.*, 1970) and is transferred early by Hfr ED1002 (Table 3). As we have seen Hfr ED1002 does not transfer *alt* early. In addition we can show that *alt*-1 does not open a new route for cAMP synthesis. The strain WZ30 *cya*-855 *alt*-1 contains no more detectable cAMP than WZ22, its *alt*$^+$ parent (Table 2). Moreover *alt*-1, isolated as a suppressor of *cya*-855, will also suppress the *crp*-868 mutation. Suppression of both *cya* and *crp* mutations would not be expected if *alt*-1 affected cAMP synthesis.

Since we find that *alt*-1 has no direct effect on CRP or cAMP, we conclude that it provides an alternative mechanism enhancing the expression of CS operons.

Does Alt-1 Affect Messenger Synthesis from CS Operons? Strains defective for the CRP-cAMP system synthesise a low amount of β-galactosidase (Table 2). The effect of the *alt*-1 mutation is to increase 3–5 fold the β-galactosidase synthesised by such strains (Table 2). The increment disappears when a *cya*-855 *alt*-1 culture is raised to 42° C (Table 2 and Fig. 2). Thus, the process permitting excess enzyme synthesis is temperature sensitive. We can ascribe the excess synthesis either to more efficient translation of the residual *lac* messenger in *cya*$^-$ strains or to an increase in the amount of *lac* messenger.

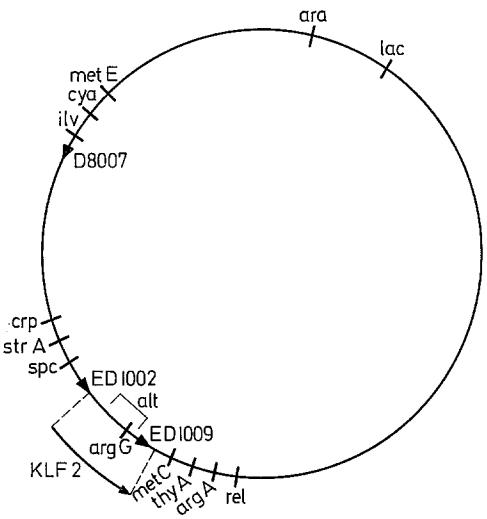

Fig. 1. A genetic map of *E. coli* showing the markers, Hfrs and F-prime factors used in this study. The two Hfr strains were formed by integration of F_{ts} lac into the bacterial chromosome (Masters and Broda, 1971). The F′ factor, KLF2, is also called F102. Its chromosomal fragment does not extend as far as *str*-A (Low, 1968; Goman, unpublished). This confirms the separate identities of the *alt* and *crp* genes, since *crp* maps beyond *str*-A (Goman, unpublished) and KLF2 carries *alt*

Results reported below show that the synthesis of certain messengers is temperature sensitive in *alt*-1 strains. Thus it is reasonable to infer that *alt*-1 stimulates β-galactosidase synthesis by increasing the synthesis of *lac* messenger.

A convenient technique for detecting the total messenger RNA synthesised by a bacterial culture has recently been devised (Pato and von Meyenberg, 1970). The culture is challenged with rifampicin, which rapidly prevents further initiation of RNA chains. Chains initiated before the drug is added complete their synthesis. They are labelled with ^{3}H-uridine, added at the same time as the drug. A fraction of this label is contained in unstable molecules, presumably messenger, which can be seen to disappear during further incubation of the culture. The remaining label is in stable RNA (Fig. 3).

At 42° C, a broth culture of WZ30 *cya*-855, *alt*-1, stops making detectable unstable RNA, while stable RNA continues to be made (Fig. 3). Such a culture ceases to grow (OD) within one generation after the shift. These observations suggest that a step necessary for messenger, but not stable RNA synthesis is rendered temperature sensitive by *alt*-1. We can be sure that this temperature sensitivity is caused by *alt*-1 since WZ22, the *cya*-855 *alt*$^+$ parent of WZ30 can make unstable RNA in broth at 42° C (Fig. 3 A).

Not all messengers require the *alt*-1 product for their synthesis. They can be detected in a minimal-grown culture of WZ30. In such a culture synthesis of detectable unstable RNA remains virtually unchanged for more than an hour (1.2 generations) after the shift to 42° C (Fig. 3 D), and growth stops only after 4 generations. This difference can be explained if we recall that growth in glucose-minimal medium demands derepression of all biosynthetic pathways; it seems

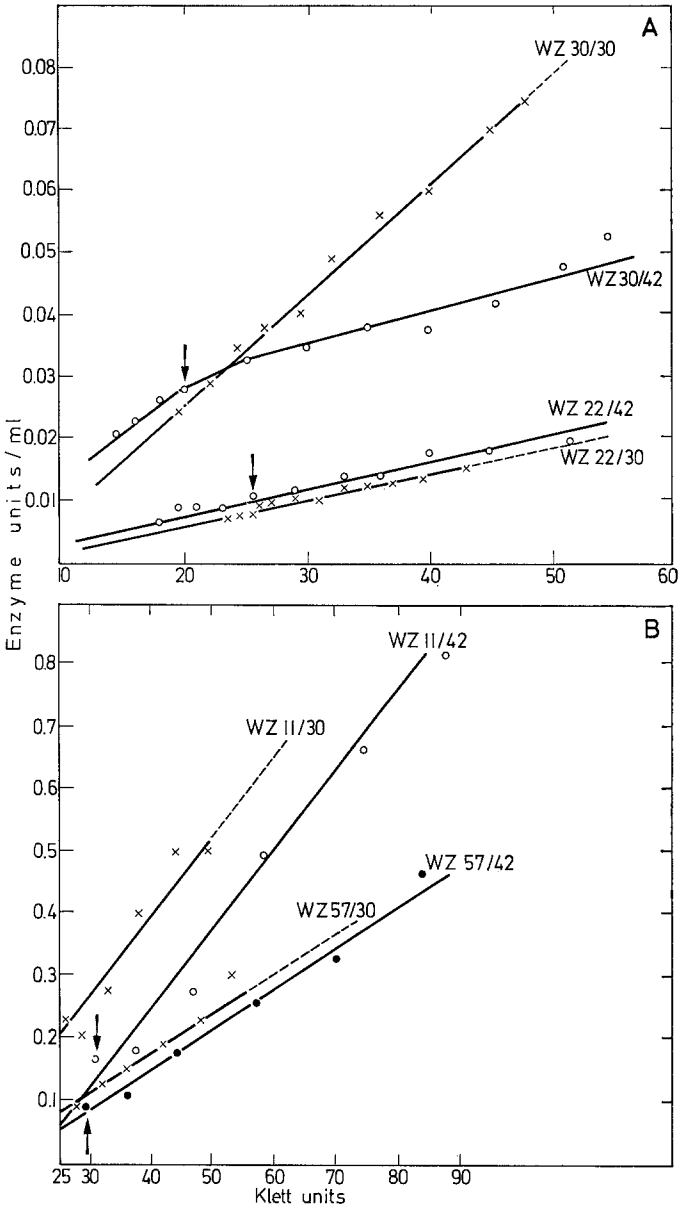

Fig. 2A and B. β-galactosidase synthesis at 30° C and 42° C in wild type, *cya*-855, and *alt*-1 strains. A, Strains WZ22 *cya*-855 and WZ30 *cya*-855 *alt*-1. B, Strains WZ11 *cya*$^+$ *alt*$^+$ and WZ57 *cya*$^+$ *alt*-1. Cultures were pregrown in supplemented MS-glucose $+10^{-3}$ M IPTG at 30° C. They were then diluted in the same medium at 30° C, allowed to resume exponential growth to the Klett values indicated, and then sampled and assayed as described in Materials and Methods. Cultures were shifted to 42° C at the optical density indicated by ↓. 30° C (×); 42° C (○, ●)

that synthesis of messenger from biosynthetic operons does not require the *alt* product.

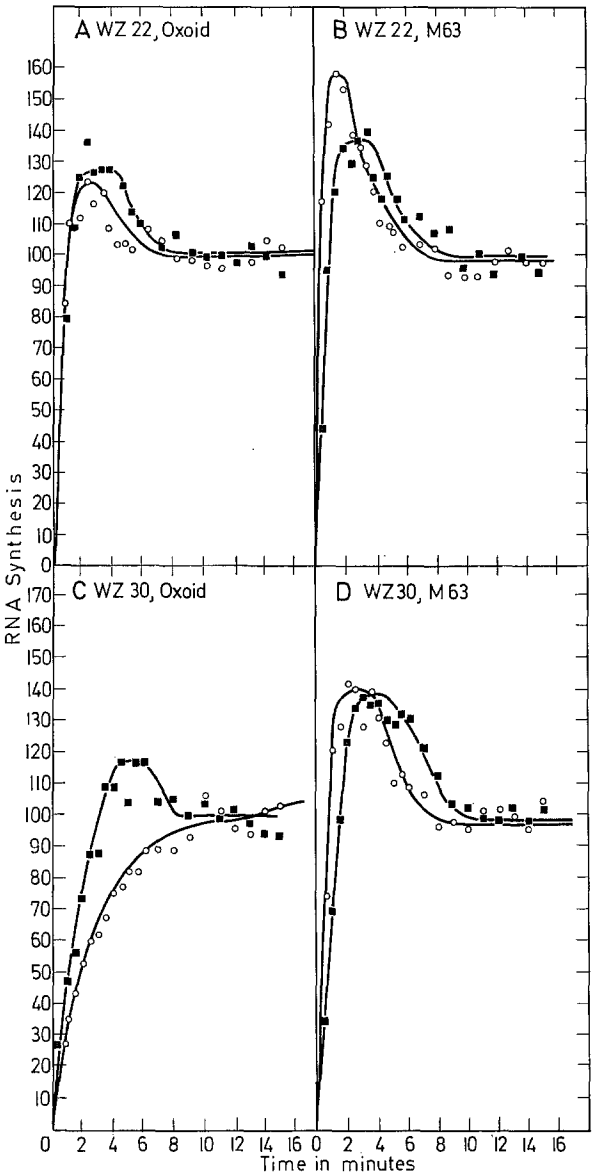

Fig. 3 A—D. Measurement of total RNA synthesis by WZ22 and WZ30. Cultures were maintained in the exponential phase at 30° C, and incubated at 42° C for 1–1.6 generations. An aliquot (2.5 ml) was taken from each culture and exposed to rifampicin (200 μg/ml) and ^{3}H-5-Uridine at 0 minutes. Samples (0.1 ml) were taken at the times indicated, prepared as described in Materials and Methods, and counted. Values for RNA synthesised are counted as a percent of the average plateau value for the particular experiment. 30° C control culture (▪); 42° C culture (○)

Our pulse-label experiments have enabled us to detect a class of messengers whose synthesis requires the *alt*-1 product. We therefore conclude that in selecting strains regaining CS operon activity, we have isolated mutants in which tran-

scription of CS operons is restored. This renewed transcription is determined by the *alt*-1 product.

The Mode of Action of the Alt Product. The evidence we have presented indicates that the *alt*-1 product is a factor which permits transcription of CS operons. In order to test whether the *alt* product normally participates in CS operon transcription we have examined the behaviour of a strain combining a normal CRP-cAMP system with the temperature sensitive *alt*-1 product. We have assayed synthesis of β-galactosidase in a culture of WZ57 *cya*$^+$ *crp*$^+$ *alt*-1 shifted from 30° to 42° C. We find that the temperature shift has no effect on the relative rate of β-galactosidase synthesis (Fig. 2 B). The simple interpretation of this result is that the *alt* product (in its heat-labile, *alt*-1 form in this strain) does not normally participate in CS operon transcription.

The Character of Alt-1 Mediated Transcription. Our mutant WZ22 *cya*-855, which contains little or no cAMP, expresses its CS operons much less efficiently than its *cya*$^+$ parent. This agrees with the conclusion (de Crombrugghe *et al.*, 1969; Zubay *et al.*, 1970) that maximum CS operon expression depends on the cAMP concentration in the bacterial cell. The residual enzyme synthesis in *cya*$^-$ strains is resistant to transient and catabolite repression, effects attributed in *cya*$^+$ strains to the reduction of the intracellular cAMP concentration. This resistance is shown in the following way.

The strain WZ47 is a glycerol specific revertant of strain WZ22. It can grow on glycerol and glucose-minimal media, but it cannot grow on other carbon sources such as lactose or arabinose. The mutation conferring this new property maps near the *glpK* locus (Silverstone, unpubl.) and is probably similar to the *lac* p^r_{UV5} mutation or the *glpK* mutation described by Berman and Lin (1971). Addition of glucose to a glycerol-growing culture of strain WZ47 does not affect the differential rate of β-galactosidase synthesis detectably (Fig. 4). On the other hand, addition of glucose to a glycerol-growing culture of strain WZ30 *cya*-855 *alt*-1, results in the immediate arrest of β-galactosidase synthesis known as transient repression (Magasanik, 1970). After one half a generation, β-galactosidase synthesis then resumes at half the differential rate in glycerol growing cells (catabolite repression). Therefore, the new system for transcription of CS operons appears to be subject to the glucose effects of catabolite and transient repression, although there is no detectable cAMP in the cells. We conclude that catabolite and transient repression can be mediated in *E. coli* by a substance or substances other than cAMP. This conclusion is reminiscent of the recent finding (Prival and Magasanik, 1971) that *Klebsiella aerogenes* has a system other than the CRP-cAMP system, which can promote initiation of transcription of some CS operons in response to changes in growth conditions.

Discussion

Our studies permit us to draw a number of firm conclusions about the *alt* function. The product is almost certainly a protein, since our *alt* mutants are temperature sensitive. It is a product which is vital to the cell, since, all tested *alt*-1 strains are unable to grow on any medium at 42°, while an *alt*-1/KLF2 *alt*$^+$ partial diploid can grow at this temperature. The *alt*$^+$ product mediates in the synthesis of certain RNA species and we can explain the temperature-sensitivity

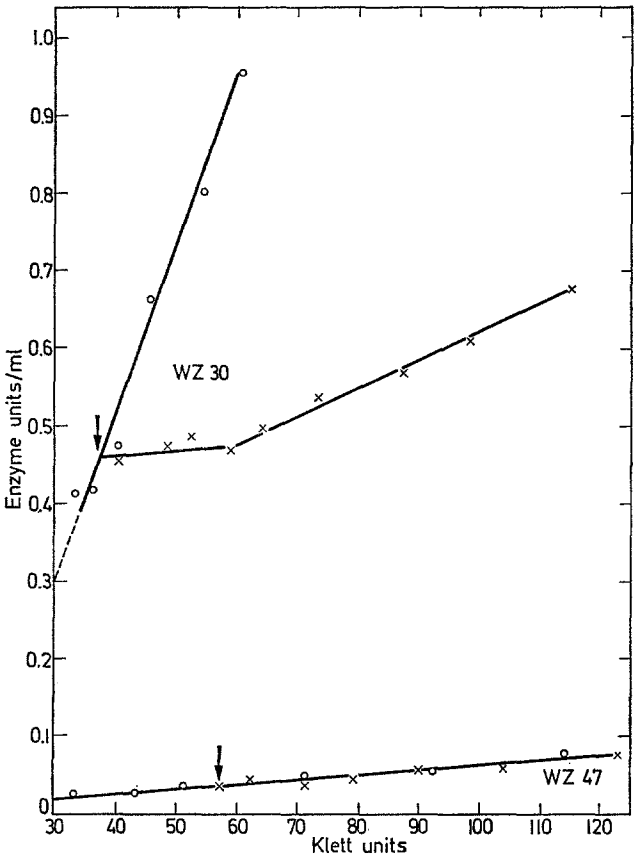

Fig. 4. Catabolite and transient repression of WZ30 and WZ47. Cultures were grown in MS-glycerol at 30° C and assayed as described in Fig. 2. Glucose (0.2%) was added at the optical density indicated (↓). Glycerol grown cultures (∘); Cultures to which glucose is adedd (×)

of *alt*-1 strains if we assume that at least one of these RNA species is vital to the cell.

We can draw some general conclusions about the nature of the RNA species whose synthesis requires the *alt* function. Firstly, we can be sure that the *alt* function is used for the synthesis of some CRP-independent RNA species, because a broth-grown *cya*$^-$ *alt*$^+$ strain (WZ22) synthesises unstable RNA which cannot be found in WZ30 *cya*$^-$ *alt*-1 at 42° (compare Fig. 3 A and C). The transcription of CRP-independent species may be the exclusive function of the *alt*$^+$ product. In this case we must assume that *alt*-1 is a mutation which permits the *alt* protein to recognise CS promoters without abolishing its original specificity. The fact that *lac* expression ceases to be temperature-sensitive in *crp*$^+$ *cya*$^+$ *alt*-1 strain supports this conclusion. However, we would like to emphasise that this result does not exclude the possibility that *alt*$^+$ does normally participate in *lac* transcription, that the *alt*-1 mutation enables it to promote *lac* expression in the absence of CRP or cAMP and that the *alt*-1 protein is stabilised on CS promoters by an intact CRP-cAMP system.

We can envisage several possible functions for the *alt* protein. The simplest hypothesis is that the *alt* protein is a factor which enables RNA polymerase to initiate transcription of certain genes, for example by causing the enzyme to bind to their promoters. Such a mechanism has been proposed for CRP action (Travers, Kamen and Schleif, 1970). There are, however, other possibilities. The *alt* protein could have an enzymatic function, modifying either the polymerase or promoters, to facilitate the initiation of transcription. Otherwise it could be a subunit of RNA polymerase. The subunit would determine, directly or indirectly, the promoter-affinity of the enzyme, and in the *alt*-1 mutant would show its temperature-sensitivity only on certain promoters.

Recent genetical and biochemical studies (Eron and Block, 1971; Arditti, Grodzicker and Beckwith, in press) indicate that the *lac* promoter contains a specific CRP target site, deleted in the p^- mutation L1. Experiments are in progress to determine whether *alt*-1 acts at this site, or at the RNA polymerase binding site characterised by the *lac* promoter mutation p^r_{UV5}.

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