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Effect of Pleiotropic Carbohydrate Mutations (*ctr*) on Tryptophan Catabolism[1]

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The pleiotropic *ctr* mutation has been shown to affect tryptophan uptake and tryptophanase formation. Genetic reversions are of two types: (i) complete, restoring to wild type, located at 46 to 47 min; (ii) partial, restoring only tryptophanase synthesis, located at 73 min. In some strains the effect of *ctr* mutations could be reversed by cyclic adenosine 3',5'-monophosphate (cAMP) plus tryptophan. A mutant producing tryptophanase constitutively was suppressed by a *ctr* mutation. Production of tryptophanase in this suppressed strain was not restored by the addition of cAMP, but required cAMP plus tryptophan.

Previous studies have shown that *ctr* mutations affect uptake and utilization of a wide variety of carbohydrates: fructose, glucose, glycerol, lactose, maltose, mannitol, mannose, melibiose, and succinate (20). In addition, it has been reported that these mutations are accompanied by a loss of enzyme I of the phosphoenolpyruvate-dependent phosphotransferase system (10; *manuscript in preparation*). Since several of the utilizations affected do not involve phosphorylation, namely, lactose, mannitol, and succinate, the mechanism by which *ctr* mutatons act remains unexplained. In this paper we report that *ctr* mutations also effect the synthesis of tryptophanase and one of the specific transport systems for tryptophan uptake. Preliminary notice of this has been made previously (H. G. Morse, R. J. Wang, and M. L. Morse, Bacteriol. Proc., p. 60, 1969).

MATERIALS AND METHODS

Cells used to assay tryptophanase and ^{14}C-tryptophan uptake were grown in minimal medium, D(M) (7). D(M) contains per liter: 5.7 g of Na_2HPO_4, 2 g of KH_2PO_4, 0.5 g of sodium citrate·$5H_2O$, 0.1 g of $MgSO_4 \cdot 7H_2O$, and 1 g of $(NH_4)_2SO_4$. Sodium pyruvate (10 g/liter) was added as a source of carbon. When required for growth of auxotrophic strains, amino acid and purines were added to 50 μg/ml, and thiamine and nicotinic acid were added to 2 μg/ml. Tryptophan was included in the growth medium at 10^{-3} M for the induction of tryptophanase.

Cholate-indicator agar (9), supplemented with 1% carbohydrate, was used to test for the metabolism of the individual carbohydrates. On these plates, cells giving rise to white colonies cannot metabolize the added carbohydrate, whereas cells capable of its metabolism give rise to red colonies.

One *ctr*$^-$ mutant, 2570Cl (*ctr10*), was induced with nitrous acid by the technique of Schwartz and Beckwith (16). The induction of the other *ctr*$^-$ mutants by nitrosoguanidine and their characterization were previously described (18-20). The *ctr*$^-$ mutants were isolated by spreading the mutagenized cells on cholate-indicator agar plates supplemented with 1% glucose and selecting white colonies. The pleiotropic defect in carbohydrate metabolism was established by streaking the Glu$^-$ cells on cholate-indicator agar supplemented with individual carbohydrates. The *ctr* mutants were generally Ara$^+$, Fru$^-$, Gal$^+$, Glp$^-$, Lac$^-$, Mal$^-$, Man$^-$, Mel$^-$, Mtl$^-$, and Suc$^-$.

Genetic mapping by P1 phage transduction was performed according to the method of Rothman (15).

Mal$^+$ and Lac$^+$ revertants of *ctr* mutants were isolated by spreading several hundred cells on cholate-indicator agar plates containing mannitol and lactose, respectively, and picking red papillae which appeared in the white colonies of ctr$^-$ cells. Revertants, which synthesize tryptophanase, were selected by spreading approximately 10^6 to 10^7 cells of the respective *ctr* mutant on D(M) agar plates in which 10^{-3} M tryptophan was substituted for $(NH_4)_2SO_4$ as the source of nitrogen.

Cells producing tryptophanase constitutively were selected by a modification of the method of Kessler and Rickenberg (6). After mutagenesis with nitrosoguanidine, cells were spread on D(M) agar plates in which $(NH_4)_2SO_4$ had been substituted with tryptophan at 200 μg/ml and contained α-methylglucoside at 2 μg/ml.

Tryptophanase activity of toluenized cell was determined by a modification of the procedure of Pardee and Prestidge (10). Log-phase cells were harvested by

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centrifugation at 12,500 × *g* for 10 min, washed with 7 ml of saline, and resuspended in D(M). Each reaction mixture contained 0.05 optical density units of cells suspended in 0.4 ml of D(M) to which 0.05 ml of toluene was added and vigorously mixed. After a 5-min incubation at 37 C, 0.2 ml of the substrate mixture [5 mg of tryptophan per ml and 150 μg of pyridoxal phosphate per ml in 0.1 M tris(hydroxymethyl)aminomethane-hydrochloride, *p*H 7.5)] was added. To determine the rate of tryptophan hydrolysis by the production of indole, separate reaction mixtures were stopped at appropriate times by adding 1.8 ml of a solution containing 5% concentrated H_2SO_4 in *n*-butanol and 5% *p*-dimethylaminobenzaldehyde in 95% ethanol mixed in a ratio of 12:5, respectively. After 20 to 30 min for the development of color, the reaction mixtures were centrifuged at 12,500 × *g* to remove the lysed cells, and the optical density was measured at 568 nm. The specific activity of the tryptophanase (units of enzyme per milligram of protein) was calculated by assuming that, at 620 nm, 1 optical density unit of *Escherichia coli* cells is equal to 0.67 mg of protein (8). One unit of tryptophanase is defined here as the amount of enzyme which will produce 1 μmole of indole per minute from the hydrolysis of tryptophan.

Cells used to measure ^{14}C-tryptophan uptake were washed twice with equal volumes of D(M) and resuspended in D(M) at a concentration of 0.15 optical density units/ml in the presence of 50 μg of chloramphenicol per ml. After incubating at 37 C for 10 min, ^{14}C-tryptophan (54.5 mCi/mmole; Nuclear-Chicago) was added to give 0.02 μCi/ml. At appropriate times, 1-ml samples were removed, filtered through membrane filters (Millipore Corp.; 0.45 μm), and washed twice with 6 volumes of D(M). The filters were dried, and the ^{14}C retained was counted by liquid scintillation.

RESULTS

Effect of ctr mutations on tryptophanase production and tryptophan uptake. The levels of tryptophanase in different cultures of wild-type cells induced by 10^{-3} M tryptophan in minimal medium with pyruvate as carbon source vary over about a threefold range. Induction of *ctr* mutations in these strains causes both a reduction in the basal level of enzyme formation and in the amount of tryptophanase formed after induction. In Table 1 are shown the enzyme levels of several *ctr* mutants and their parental strains, both noninduced and induced by prior growth in tryptophan. Some of these mutants have been described previously (18–20). Not shown are the results of a study of *ctr1* and *ctr8* since the parental strains of these cultures lack tryptophanase.

Three apparently distinct tryptophan transport systems have been described in *E. coli*. Of the constitutive systems which transport tryptophan, one is a general transport system for aromatic amino acids, and the other transports tryptophan specifically (2). The third transport system which has been described is inducible and also transports tryptophan specifically (3). In the general system, each of the aromatic amino acids competitively inhibits the uptake of the others, whereas tryptophan uptake by the specific transport system is not affected by the presence of the other aromatic amino acids. Thus, it is possible to distinguish the various tryptophan transport systems by measuring tryptophan uptake in induced and noninduced cells in the presence or absence of relatively high concentrations of phenylalanine.

In Fig. 1 is shown the uptake of ^{14}C-L-tryptophan by a wild-type culture and a *ctr* mutant derivative of this culture when both have been induced with tryptophan. The data show that the *ctr* mutation affects transport by a tryptophan-specific transport system but does not prevent entry of tryptophan into the cell. Similar data, which are not presented, show that this tryptophan transport system, which is affected by the *ctr* mutation, is inducible since neither the wild type nor the mutant demonstrates the specific transport of tryptophan unless induced by prior growth on tryptophan. Therefore, under the conditions of these experiments these cells did not demonstrate a constitutive system which transports tryptophan specifically.

Reversions with tryptophanase activity. Genetic reversion of Ctr$^-$ to Ctr$^+$ can be detected by changes in phenotype on a number of carbohydrates including mannitol. Revertants selected on mannitol, subsequently shown to utilize the other carbohydrates affected by *ctr* mutations, have restored levels (Tna$^+$) of tryptophanase (Table 2). The levels may vary, suggesting a spectrum of revertant genotypes.

Reversion to ability to grow on tryptophan as sole source of nitrogen can be selected on minimal medium containing tryptophan. Such revertants (Tna$^+$) are of two phenotypes on mannitol indicator agar: Mtl$^+$ and Mtl$^-$. The Mtl$^+$ phenotypes, when tested against the other carbohydrates affected by *ctr* mutations, are found to utilize them. The first of these reversions appear to be changes Ctr$^-$ to Ctr$^+$; the second, designated Mtl$^-$ since the mutants remain unable to utilize the carbohydrates of the *ctr* phenotype, are partial revertants (Tna$^+$Ctr$^-$). The Tna$^+$Ctr$^-$ reversions also have increased levels of tryptophanase, again with some variation from revertant to revertant (Table 3). All of the 12 Tna$^+$Ctr$^-$ revertants thus far tested have been found to be inducible for tryptophanase production.

Three independent revertants were also selected on lactose (phenotype Lac$^+$Mal$^+$Glp$^+$), and none of these revertants possessed tryptophanase activity.

The characteristics of tryptophan uptake were

TABLE 1. *Effect of ctr mutations on tryptophanase levels*

Parent	Mutant[a]	Tryptophanase[b]	
		Noninduced	Induced
911IV		3.7	166.7
	911IVA	0.0	0.0
2570		1.3	288.3
	2570C1	0.0	10.9
2092		8.3	291.3
	2092C	5.5	130.4
2547		0.0	223.1
	2547A	0.0	52.9
2570S		10.9	217.8
	ctr7	0.0	29.7

[a] Some of these mutants have been described previously: 911IVA (*ctr4*); 2092C, (*ctr2*); 2547A (*ctr5*). Newer mutants shown by transduction to be linked to the other mutants are 2570C1 (*ctr10*) and *ctr7*. Enzyme levels of from 1 to 10 units are not considered significant, and the culture is considered to be Tna^-. See Table 6 for assays of independent cultures and compare the levels of 2570C1 (Table 1 and Table 7) for variability. The effect of *ctr* mutations on tryptophanase synthesis is observable in broth-grown cultures. For example: 2547, has a specific activity of 121; 2547A, specific activity of 30; 911, specific activity of 138; 911IVA, specific activity of 2.6. Cultures of *ctr* mutants grown in minimal medium with succinate and acetate as carbon source also had very low or zero levels of tryptophanase.

[b] Units of enzyme per milligram of protein, multiplied by 10^3; in subsequent tables, designated minus tryptophan, plus tryptophan.

examined in one Tna^+Ctr^- revertant of *ctr10*. The experiments (*data not shown*) show that the Tna^+Ctr^- revertant had not recovered activity of the specific inducible tryptophan uptake system which was lost with the induction of the *ctr* mutation.

Genetic analysis of ctr revertants with tryptophanase activity. Genetic analysis of the complete Ctr^+ revertant phenotype shows that it is located at the *ctr* region and linked to *supN*. This result is shown by making transductions with phage P1 from a revertant donor ($Ctr^+Tna^+SupN^+$) to a *ilv188 ctr4* recipient and selecting for ability to grow in the absence of isoleucine and valine (Table 4). The mutation *ilv188* is suppressible by *supN* (4). The isoleucine-valine-independent $SupN^+$ transductants when tested on mannitol indicator are plus or minus and correspond to Ctr^+ and Ctr^- when tested completely. Test of such transductants shows that the ability to produce tryptophanase activity is *supN* linked and

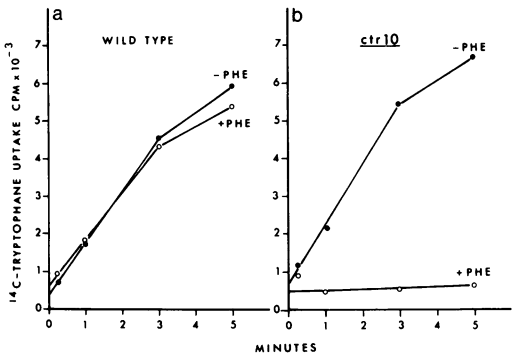

FIG. 1. *Uptake of ^{14}C-tryptophan (○); in the presence of 10^{-3} M phenylalanine (●). (a) Wild-type cells, (b) the ctr10 mutant. The data show that in the wild type, grown in tryptophan, uptake of tryptophan is not greatly affected by the presence of phenylalanine since the tryptophan-specific systems are active. In the ctr10 mutant there is little tryptophan transport in the presence of phenylalanine because the tryptophan-specific transport system is inactive, and the aromatic permease is inhibited by phenylalanine.*

TABLE 2. *Enzyme levels of Ctr^+ revertants selected on mannitol*[a]

Revertant	Minus tryptophan	Plus tryptophan
ctr5 rev 2	0.0	65.9
ctr5 rev 3		127.9
ctr5 rev 4		982.6
ctr10 rev 1		269.6
ctr10 rev 2		442.0

[a] Tryptophanase levels expressed as units of enzyme per milligram of protein, multiplied by 10^3. Induction in minimal medium plus pyruvate plus 10^{-3} M tryptophan.

TABLE 3. *Enzyme levels of revertants selected for ability to grow on tryptophan*[a]

Revertant	Minus tryptophan	Plus tryptophan
ctr10 A	0.6	103.3
ctr10 B	0.0	65.7
ctr10 C	0.0	17.7
ctr10 D	1.9	108.7
ctr10 E	1.1	65.7
ctr10 F	1.9	82.3

[a] As in Table 1.

TABLE 4. *Linkage of the Ctr^+Tna^+ phenotype to supN*

Donor	Recipient	$SupN^+$ transductants per 10^6 recipient cells	No. of supN transductants	
			Ctr^+Tna^+	Ctr^-Tna^-
supN Ctr^+[a]	*ilv188 ctr4*	3.2	63 (81.5%)	14

[a] *SupN* wild-type parent of *ctr5* described previously (5).

cotransduces with the Ctr$^+$ phenotype (Fig. 2).

Genetic analysis was also made of Ctr$^+$ revertants of *ctrl0* by using *ctrl0* as a recipient and selecting for mannitol-independent transductions. These were subsequently tested and shown to be Ctr$^+$. In every case the Tna$^+$ phenotype accompanied Ctr$^+$.

Revertants of the other class of Tna$^+$ revertants, Ctr$^-$, were also analyzed genetically and shown to be linked to *ilv*, which cotransduces at about 20% with the gene for tryptophanase (14). Transductions were made from Tna$^+$Ctr$^-$Ilv$^+$ revertants of *ctrl0* to a *ilv188 ctr4* recipient, selecting for Ilv$^+$ and classifying the transductants (all Ctr$^-$) for tryptophanase activity by either streaking them on minimal medium containing tryptophan as sole carbon source or assaying them enzymatically. The results of Table 5 show that two Tna$^+$Ctr$^-$ revertants of *ctrl0* are *ilv* linked.

Assay of a random sample of nine Tna$^+$Ilv$^+$ transductants, derived from two independent Tna$^+$ revertants, for tryptophanase activity showed that they were all inducible for the production of tryptophanase and that they varied widely with regard to their response to induction with 10^{-3} M tryptophan.

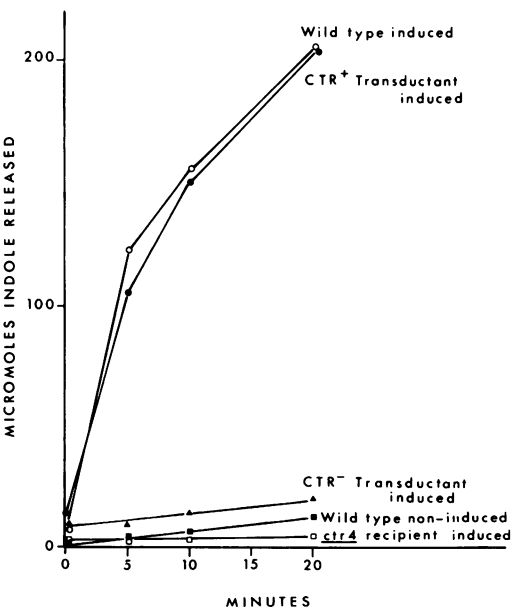

FIG. 2. *Evidence that genetic information at the ctr region affects tryptophanase production. Indole formation from tryptophan:* ○, *wild-type parent strain (911IV), induced;* ●, *wild-type parent strain, noninduced;* □, *a ctr mutant of 911IV, induced;* ■, *a Ctr$^+$ transductant from 911IV selected by SupN linkage, induced;* ▲, *a Ctr$^-$ transductant from 911IV, selected by SupN linkage, induced.*

TABLE 5. *Linkage of the Tna$^+$Ctr$^-$ phenotype to ilv*

Donor	Recipient	Frequency of Ilv$^+$ transductants per 10^6 cells plated	No. of Ilv$^+$ transductants	
			Tna$^+$	Tna$^-$
ctrl0 rev 1	*ilv188 ctr4*	11.3	12 (11%)	92[a]
ctrl0 rev 2	*ilv188 ctr4*	17.2	9 (20%)	36[b]

[a] Cotransduction of Tna$^+$ with Ilv$^+$ measured by streaking on minimal medium with tryptophan as sole carbon source.

[b] Cotransduction of Tna$^+$ with Ilv$^+$ measured by enzymatic assay.

Genetic suppression of the effects of ctr mutations. It has been shown previously (20) that *ctr* mutants of strains forming the proteins of the *lac* operon constitutively are not Lac$^-$; however, they produce lesser amounts of β-galactosidase and transport β-galactosides at a reduced rate compared to their *ctr*$^+$ parent (5). The same also appears true for the glycerol-metabolizing system of *E. coli* (1).

It was of interest to ascertain the effect of *ctr* mutations on tryptophanase production to determine whether constitutive synthesis of tryptophanase was suppressible by *ctr* mutations. A mutant producing tryptophanase constitutively was selected by the technique of Kessler and Rickenberg (6), which employs the inhibition of enzymatic induction by α-methylglucoside. From populations of cells producing tryptophanase only after induction, constitutive mutants can be selected by using a mixture of α-methylglucoside and tryptophan where tryptophan is made the limiting factor for growth. Under these conditions, constitutive mutants have a selective advantage over inducible mutants.

Five *ctr* mutants were isolated (after nitrosoguanidine treatment) in a mutant producing tryptophanase constitutively. The enzyme levels of these mutants are shown in Table 6, where it is clear that *ctr* mutations suppress enzyme levels of tryptophanase below the basal levels found in the constitutive parent and in the wild type.

Biochemical suppression of the effects of ctr mutations. Pastan and Perlman have shown that cyclic adenosine 3′,5′-monophosphate (cAMP) stimulates tryptophanase induction in *E. coli* (12). In addition, they have shown that induction of the *lac* operon, which is defective in mutants lacking enzyme I of the phosphotransferase system, was reparable by the addition of cAMP (11). It was therefore of interest to see what effect cAMP might have upon tryptophanase induction in *ctr* mutants.

In Table 7 are shown the effects of cAMP with and without tryptophan on the production of tryptophanase by the wild type, *ctr* mutants,

TABLE 6. *Tryptophanase activity of a constitutive culture and the effect of ctr mutations on tryptophanase activity*

Culture	Tryptophanase	
	Minus tryptophan	Plus tryptophan
2570 Constitutive[a]		
1	32.2	121.1
2	26.5	107.6
2570 Constitutive *ctr a*[a]		
1	0.0	1.21
2	0.0	3.8
2570 Constitutive *ctr c*	0.0	0.0
2570 Constitutive *ctr d*	7.7	27.7
2570 Constitutive *ctr e*	0.0	0.0
2570 Constitutive *ctr f*	0.0	0.6

[a] Assays on independent cultures in each case. Units of enzyme per milligram of protein, multiplied by 10^3.

and *ctr* mutants induced in a strain producing tryptophanase constitutively. These results show that cAMP partially restores tryptophanase synthesis in two *ctr* mutants grown in its presence and in the presence of inducer. However, the response cannot be easily quantitated since the cultures involved vary in their ability to grow in the presence of cAMP. Synthesis of tryptophanase was suppressed by cAMP in the strain producing tryptophanase constitutively, and this suppression was at least relieved by growth in the presence of tryptophan.

The five independent *ctr* mutants induced in the strain producing tryptophanase constitutively did not respond by producing tryptophanase when 5×10^{-3} M cAMP was added to the growth medium. However, they did respond, variably, when tryptophan and cAMP were added.

DISCUSSION

It has been shown in previous studies that the principal ability of the *E. coli* cell affected by *ctr* mutations was the ability of the cell to take up and concentrate carbohydrates. In this paper it is shown that the uptake of an amino acid and the synthesis of an enzyme hydrolyzing it are also affected by *ctr* mutations.

The effect on tryptophan uptake was confined to a specific inducible transport system for tryptophan. Since tryptophan is still transported into mutant cells by other transport systems, the effect of *ctr* mutations on the ability to grow on tryptophan as sole source of carbon is not because of failure to transport or concentrate molecules, but for other reasons. This observation is confirmed by observations (Wang, Dahl, and Morse, *manuscript in preparation*) on growth on lactose and induction of the lactose operon without the involvement of either active or passive transport.

Reversion to the ability to grow on tryptophan as sole carbon source was studied and found to occur at two sites: (i) at the site of the initial mutation (*ctr* region, 46 to 47 min) and restoring the complete wild-type phenotype; (ii) at a site linked to *ilv* (74 min), 27 min from the *ctr* region. This region is close to the location of the structural gene for tryptophanase and its regulatory gene (17). The later reversions were partial reversions and remained unable to utilize the carbohydrates affected by the initial *ctr* mutation. This observation, partial reversion of *ctr* mutations at distant sites, parallels that described previously for reversion to utilization of maltose and lactose in *ctr* mutants (20).

Both types of revertants, those selected on mannitol (Ctr^+Tna^+) and those selected on tryptophan (Ctr^-Tna^+) were inducible for tryptophanase production. In addition, the transductants from each class of revertant, selected for a marker linked to the site of the reversion, were also inducible for tryptophanase production.

The induction of a *ctr* mutation in a strain producing tryptophanase constitutively suppressed the synthesis of tryptophanase. Addition of cAMP by itself to these *ctr* mutants did not produce tryptophanase, nor did the addition of tryptophan. A combination of cAMP plus tryptophan did induce tryptophanase.

Addition of cAMP plus inducer in the growth medium induced tryptophanase in a number of *ctr* mutants. However, the response was variable,

TABLE 7. *Effects of cyclic 3',5'-adenosine monophosphate on tryptophanase levels[a] in ctr mutants*

Culture	Plus tryptophan	Plus cAMP[b]	Plus cAMP and tryptophan
2570	207.5		168.1
2570C1 (*ctr10*)	3.0		145.4
2570 Constitutive[c]	121.1	20.8	173.8
2570 Constitutive *ctr a*	1.2	7.1	124.2
2570 Constitutive *ctr c*	0.0	0.0	36.8
2570 Constitutive *ctr d*	27.7	5.8	143.6
2570 Constitutive *ctr e*	0.6	1.3	94.5
2570 Constitutive *ctr f*	0.0	2.6	128.2
911IV	246.2		275.0
911IVA (*ctr4*)	0.0		21.2

[a] As in Table 2.

[b] Cyclic 3',5'-adenosine monophosphate (cAMP) was added to give a final concentration of 5×10^{-3} M in all cases except those of 911IV and 911IVA in which the cAMP concentration was reduced to 10^{-3} M because higher concentrations inhibited growth.

[c] See Table 6 for tryptophanase levels of noninduced 2570 constitutive and the respective *ctr* derivatives.

since some *ctr* mutants were inhibited by cAMP at the concentrations necessary for effect in other mutants which responded to cAMP.

Pastan and Perlman (13) have made extensive study of the effect of cAMP on the induction of a number of enzyme systems in *E. coli*. In addition, they have shown that mutants lacking components of the phosphoenolpyruvate-dependent phosphotransferase system (enzyme I and HPr) are more sensitive in terms of β-galactosidase induction to catabolite repression. This latter observation they believe is owing to loss or lack of cAMP in such mutants. β-Galactosidase induction in the phosphotransferase mutants they studied was reparable by the addition of high concentrations of cAMP.

From various bits of evidence, Pastan and Perlman proposed a model of involvement of cAMP in enzyme induction and synthesis. In the case of tryptophanase, they postulated that cAMP is necessary for the translation of the tryptophanase messenger ribonucleic acid (RNA). If this model were true, then it would be expected that the *ctr* mutations would suppress the expression of even constitutive production of tryptophanase. Unlike the case with lactose and glycerol where constitutive synthesis was not completely suppressed by *ctr*, *ctr* mutations in a strain producing tryptophanase constitutively suppressed all synthesis of tryptophanase, which accords with the model proposed by Pastan and Perlman. However, if the simple model proposed were true, addition of cAMP to the culture producing tryptophanase messenger RNA but not translating it should produce enzyme synthesis almost immediately. The results presented here, requirements for both tryptophan and cAMP for enzyme formation in *ctr* mutants induced in a strain producing tryptophanase constitutively, indicate that the simple model does not hold. Higher order interactions between inducer, repressor, cAMP, and other factors could provide an explanation. Perhaps the nature of the constitutive mutation should also be taken into consideration, whether it is a regulator gene change or an operator change, since the involvement of cAMP in tryptophanase gene expression is not completely elucidated.

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