*ENDOGENOUS INDUCTION OF THE GALACTOSE OPERON IN ESCHERICHIA COLI K12**

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Although the pathway for the dissimilation of galactose in *E. coli* is well understood,[1] the problem of its genetic regulation by means of repression or derepression of the synthesis of the enzymes involved in this pathway has not yet been completely clarified. Galactose is taken up by the cell from the medium by a specific active transport system called galactose permease.[2,3] It is thereupon acted on by a series of three enzymes—galactokinase, galactose-1-phosphate uridyl transferase, and UDPGal-4-epimerase—for the conversion of galactose into glucose-1-phosphate. The structural genes of these three enzymes are linked and constitute the so-called galactose operon. The synthesis of these three enzymes is under the control of a regulator gene, R_{gal},[4] and is coordinately induced by galactose or D-fucose. Enzymes responsible for the biosynthesis of UDP-glucose, such as UDP-glucose pyrophosphorylase, are also essential for the catabolism of galactose as well as for the biosynthesis of glucosyl or galactosyl compounds. However, the synthesis of UDP-glucose pyrophosphorylase is independent of the regulation of the galactose operon.[5,6]

One of the unique features of the galactose system is the fact that the basal levels of these enzymes are rather high in the absence of an exogenous inducer. These levels can be further increased only 10- to 15-fold in the presence of the inducer. The question arises as to whether an endogenous inducer plays a role in this system.

It has been shown previously that a number of galactokinaseless mutants of *E. coli* K12 synthesize Gal-1-P uridyl transferase and UDPGal-4-epimerase at derepressed levels.[7,8] Recent work has indicated that the unlinked galactose permease also becomes constitutive in galactokinaseless mutants.[2,3] The pleiotropic nature of the galactokinase defect is supported by the striking correlation between the functional galactokinase deficiency and the constitutivity of the galactose operon, in both forward and back mutations. Like the derepression due to a defective regulator gene (R_{gal}^-), the galactokinaseless constitutivity of the galactose operon, $K^-[Gal^c]$, is recessive to galactokinase positive inducibility, K^+ $[Gal^i]$, in heterozygous strains. However, it differs in two aspects from the derepressed state in regulator gene defective, R_{gal}^-. $K^-[Gal^c]$ is repressed by methyl-thio-β-D-galactopyranoside which has little effect on R_{gal}^-; moreover, $K^-[Gal^c]$ exhibits greater catabolite repression by glucose than R_{gal}^-.[9,10] The recessiveness of the $K^-[Gal^c]$ and the fact that the unlinked galactose permease is also constitutive in galactokinaseless mutants exclude the possibility of an O^c type of constitutivity. All the evidence taken together suggests that the $K^-[Gal^c]$ is due to an accumulation of an endogenous inducer rather than to a lack of repressor.

If an endogenous inducer is being formed, it should possess certain characteristics. It has to be an inducer for the galactose operon. Moreover, since the inducer mani-

fests itself only in mutants with defective galactokinase, it must react with the native galactokinase, either directly or indirectly, in such a way as to be rendered inactive. If the internal inducer were *galactose itself*, or a galactoside compound, it might be made inactive by phosphorylation. Galactose is known to be generated from other compounds via the UDP-glucose pathway. In this paper, we shall present experimental evidence to show that the UDP-glucose pathway is essential for the manifestation of the galactokinaseless constitutivity of the galactose operon.

Materials and Methods.—Bacterial strains: Many of the strains used in the present study are galactose-negative mutants derived from a wild-type strain of *E. coli* K12, W3100. They were kindly provided by Drs. E. and J. Lederberg. Strains W3092c (K^-) and W4597 ($UDPGPP^-$) were described before.[5, 7] Strain W4597 K^- was obtained from W4597 by Dr. T. A. Sundararajan. Strain 316 E^- was an epimeraseless derivative of W3100, selected after P^{32} decay by Dr. K. Ebisuzaki. Strain PL-2, a thiamine-requiring epimeraseless mutant derived from Hfr H, was kindly provided by Dr. G. Buttin through Dr. M. B. Yarmolinsky. Strain C7M was an epimeraseless mutant derived from *E. coli* C7 by Dr. I. Fukutoma. For the purpose of the present study, we have obtained double mutants with the genotype of $K^-T^+E^-$ from all three E^- strains mentioned above, 316 E^-, PL-2, and C7M.

Strain W3092c was mutagenized with N-methyl-N′-nitro-N-nitrosoguanidine (0.7 mg/ml) at 37°C in 0.2 *M* acetate buffer at pH 5.2 for 2 hr. After intermediate growth in nutrient broth, the survivors were screened for derivatives with defective UDP-glucose pyrophosphorylase, based on sensitivity to C21 phage,[11] failure to be complemented with $F'Gal^+/Gal^+$ strain W4520, and ability for host-induced modification of bacteriophage T4.[12] Strains CN 30-2, CN 35-A, and CN 39-1, all with the genotype of $K^-UDPGPP^-$, were thus obtained from W3092c.

Media, growth of bacteria, and preparation of enzyme extract; Since nutrient broth frequently contains significant amounts of glucose and galactose, minimal medium A[13] with 0.45% sodium succinate was used throughout this study. At the early logarithmic phase of growth, the inducer was added, and the cells were harvested $2^1/_2$ hr later, at about 9×10^8 cells/ml. Cells were collected by centrifugation at 0°C, washed once with 0.05 *M* Tris-HCl at pH 7.5, and then suspended in 1–2 ml of the same buffer. For the assay of galactokinase, cells were suspended in 0.02 *M* potassium phosphate buffer pH 7.0, containing 0.01 *M* mercaptoacetic acid and 0.001 *M* EDTA. The suspended cells were disrupted by treatment in a Branson sonifier at 0°C for 3 min. The resulting suspension was centrifuged at $20,000 \times g$ for 20 min at 0°C and the cell-free supernatant was used for enzyme assays.

For the assay of β-galactosidase activity, toluenization of the bacterial cells was carried out at 37°C for 20 min. Bacterial culture was treated with toluene and 0.1% sodium deoxycholate in respective volume ratios of 50:1:0.5.

Enzyme assays; Gal-1-P uridyl transferase and UDPGal-4-epimerase were assayed according to the methods of Kalckar *et al.*[7] The two-step method was used for the epimerase assay, the concentration of UDP-galactose being 10^{-3} *M* during the first-step incubation. Galactokinase activity was measured according to the method of K. Ebisuzaki.[14] The incubation mixture contains, in a final volume of 0.5 ml, 2.5 μmoles of D-galactose-1-C^{14} (about 5×10^4 cpm), 100 μmoles of Tris-HCl pH 7.8, 2.5 μmoles of $MgCl_2$, 12.5 μmoles of NaF, and 5 μmoles of 2-mercaptoethanol. After incubation, 0.2 ml of the heat-inactivated reaction mixture was put on a small column of Dowex-1-formate, washed with six 1-ml aliquots of deionized water, and eluted with 3 ml of ammonium formate buffer consisting of equal volumes of 1 *M* ammonium formate and 0.4 *M* formic acid. The eluates were plated on planchets and counted in a low-background gas-flow counter. Incubations for the galactokinase and epimerase assays were carried out at 25°C, while the transferase assay was performed at room temperature. Protein was measured by the Biuret method.[15]

Assay of the β-galactosidase activity was performed on the toluenized bacterial suspensions by the method of Novick and Weiner.[16] One unit of β-galactosidase activity is defined as the amount of enzyme which hydrolyzes 1 mμmole of o-nitrophenyl-β-D-galactopyranoside per min at 28°C in 0.1 *M* sodium phosphate at pH 7.0. The activities of galactokinase, transferase, and epimerase are expressed as μmoles of substrate converted per mg protein per hr, whereas the β-galactosidase activity is expressed as units per unit optical density of bacterial culture at 650 mμ.

TABLE 1

EFFECT OF SUPERIMPOSED *UDPGPP* DEFECT ON $K^-[Gal^c]$

Strain	Genotype	Parent	Galactose-1-P Uridyl Transferase* Unin-duced	In-duced	UDPGal-4-Epimerase* Unin-duced	In-duced	UDPG-Pyro-phosphorylase*
W3100	$K^+T^+E^+UDPGPP^+$	Wild type	0.67	6.77	1.69	22.94	2.17
W3092c	$K^-T^+E^+UDPGPP^+$	W3100	6.86	7.28	44.25	41.20	2.71
CN 30-2	$K^-T^+E^+UDPGPP^-$	W3092c	0.98	5.80	5.29	40.45	0.37
CN 35-A	$K^-T^+E^+UDPGPP^-$	W3092c	1.06	6.69	5.80	46.80	0.32
CN 39-1	$K^-T^+E^+UDPGPP^-$	W3092c	1.15	5.47	7.84	42.70	0.32
W4597K⁻	$K^-T^+E^+UDPGPP^-$	W4597 (from W3100)	1.46	8.92	5.79	36.61	0.25
KE 20-6	$K^-T^+E^+UDPGPP^{\pm}$	W4597K⁻	3.45	8.97	13.49	36.98	0.58

* Cells were grown at 37°C in minimal A medium + 0.45% sodium succinate. D-fucose at final concentration of 5×10^{-3} *M* was added during early logarithmic phase of growth as inducer. Cells harvested $2^1/_2$ hr after inducer was added. Enzyme activities are expressed as μmoles/mg protein/hr.

Results.—Effect of defective UDP-hexose pathway on the constitutivity of the galactose operon in galactokinaseless mutants: Strain W4597 K⁻ was found to be inducible with respect to both transferase and epimerase, despite its lack of galactokinase activity. This was the first indication to us that a superimposed UDPG pyrophosphorylase defect might abolish the constitutivity of the galactose operon in galactokinaseless mutants. A partial revertant of the UDPG pyrophosphorylase defect, strain KE 20-6, was obtained from W4597 K⁻ after mutagenesis with ethyl methanesulfonate.[17] It permitted a significantly faster uninduced synthesis of both transferase and epimerase than its parental strain W4597 K⁻, and hence approached the state of full constitutivity (Table 1).

It was considered more desirable, however, to start with a K⁻ strain known to manifest full $[Gal^c]$, and examine subsequently the status of the gal-operon constitutivity in derivatives with defective UDPG pyrophosphorylase. As can be seen from Table 1, these double mutants have lost their $[Gal^c]$. It seems therefore clear that a block in the UDP-glucose pathway abolishes the galactokinaseless constitutivity of the galactose operon.

The finding of the dependence of $K^-[Gal^c]$ on the unimpaired synthesis of UDP-glucose suggested that the activity of UDPGal-4-epimerase might also be essential in the manifestation of this pleiotropic effect of the galactokinase mutation. Double mutants of the genotype of $K^-T^+E^-$ were obtained from a number of independent $K^+T^+E^-$ strains. All of them showed lack of constitutivity of transferase biosynthesis (Table 2). One of them, PL-2-7, was mated with F′ *Gal* $K^-T^+E^+/K^-T^+E^+$ derived from W3092c, strain C3-3. It gave rise to an F-ductant with the genotype of $K^-T^+E^+/K^-T^+E^-$, which was found to possess the phenotype of $K^-[Gal^c]$ (Table 3). It should also be noted that $K^-[Gal^c]$ was likewise found in the recombinants arising from the F-ductant. These results strongly support the notion that the lack of constitutivity of the galactose operon in the $K^-T^+E^-$ double mutants is due to the failure in the generation of the endogenous inducer in mutants with defective UDPGal-4-epimerase. The dominance of constitutivity over inducibility in the merozygote is probably the result of the dominance of the epimerase positive gene in the episome over the defective epimerase gene of the host.

Induction of lac operon with exogenously supplied galactose in K⁻ mutants: It was found previously[8, 9] that W3092c had a much lower threshold of induction of β-galactosidase synthesis by exogenously supplied galactose than that of the wild-

TABLE 2

ABSENCE OF TRANSFERASE CONSTITUTIVITY IN K^- STRAINS WITH EPIMERASE DEFECTS

Strain	Genotype	Parent	Galactokinase*		Gal-1-P Uridyl Transferase*		UDPGal-4-Epimerase*	
			Uninduced	Induced	Uninduced	Induced	Uninduced	Induced
316 E⁻#20	$K^+T^+E^-UDPGPP^+$	W3100	0.46	5.31	2.98	24.50	<0.005	<0.005
316 #20-6	$K^-T^+E^-UDPGPP^+$	316E⁻ #20	<0.01	<0.01	2.27	22.81	<0.005	<0.005
316 E⁻#1	$K^+T^+E^-UDPGPP^+$	W3100	0.48	5.31	2.62	26.70	<0.005	<0.005
316 #1-4	$K^-T^+E^-UDPGPP^+$	316E⁻ #1	<0.01	<0.01	2.41	26.50	<0.005	<0.005
C7M	$K^+T^+E^-UDPGPP^+$	*E. coli* C7	0.56	6.45	3.29	25.90	<0.005	<0.005
C7M-3	$K^-T^+E^-UDPGPP^+$	C7M	<0.01	<0.01	2.68	26.70	<0.005	<0.005
PL-2	$K^+T^+E^-UDPGPP^+$	Hfr H	0.64	9.13	3.23	28.35	<0.005	<0.005
PL-2-7	$K^-T^+E^-UDPGPP^+$	PL-2	<0.01	<0.01	3.73	17.58	<0.005	<0.005

* Conditions of growth and assay same as Table 1. Enzyme activities are expressed in μmoles/mg protein/hr.

TABLE 3

DEPENDENCE OF $K^-[Gal^c]$ ON EPIMERASE ACTIVITY

Strain		Genotype	Gal-1-P Uridyl Transferase*		UDPGal-4-Epimerase	
			Uninduced	Induced	Uninduced	Induced
Recipient	PL-2-7	$K^-T^+E^-$	1.57	17.15	<0.01	<0.01
Donor	C3-3	F′*Gal* $K^-T^+E^+/K^-T^+E^+$	18.63	21.50	81.80	62.40
Parental strain	W3092c	$K^-T^+E^+$	11.02	11.11	46.20	45.80
F-ductant and recombinants	PC-1†	"F′*Gal* $K^-T^+E^+/K^-T^+E^-$"	38.30	30.30	102.40	84.20
	PC-124†	"F′*Gal* $K^-T^+E^+/K^-T^+E^+$"	39.50	37.80	161.50	118.20
	PC-2†	"$K^-T^+E^+$"	18.10	15.90	85.70	65.70

* Growth and enzyme assays same as in Table 1. Enzyme activities are expressed as μmoles/mg/hr.
† Cross was made between C3-3 *Sms Meth⁻ Cyt⁻ F′Gal* $K^-T^+E^+/K^-T^+E^+$ and PL-2-7 *Smʳ B₁⁻ Hfr H* K^- T^+E^-. Markers selected were E^+ and *Smʳ*; unselected markers found to be *Cyt⁺ B₁⁻ Meth⁺*. The genotypes designated here are tentative, based on phage typing, complementation test, and enzyme levels.

TABLE 4

PROPERTIES OF W3092i AS COMPARED TO W3100 AND W3092c

Strain	Galactokinase		Gal-1-P Uridyl Transferase*		UDPGal-4-Epimerase*		β-Galactosidase† at Exogenous Gal. Conc. of:			
	Uninduced	Induced	Uninduced	Induced	Uninduced	Induced	10^{-3} *M*	10^{-4} *M*	10^{-5} *M*	10^{-6} *M*
W3100	0.32	3.52	0.67	6.77	1.69	22.94	8.65	10.60	6.82	4.68
W3092c	<0.05	<0.05	6.86	7.28	44.25	41.20	824.1	303.7	100.5	11.8
W3092i	<0.05	<0.05	1.06	6.97	3.31	22.67	129.2	9.92	5.04	4.1

Cells were grown in minimal A medium + 0.45% sodium succinate.
* Enzymes of the galactose operon were induced by 5×10^{-3} *M* D-fucose for 2½ hr; activities are expressed in μmoles/mg protein/hr.
† β-galactosidase was induced with various concentrations of galactose for 3 hr; activities are expressed in units per unit optical density at 650 mμ of bacterial culture.

type W3100. This finding was confirmed in the present study, and furthermore, the induction of the synthesis of β-galactosidase was found to be accompanied by the induction of the rest of the lac operon, the β-galactoside permease and thio-β-galactoside transacetylase.[10] Strains with normal galactokinase required more than 10^4 times higher galactose concentrations in order to elicit an induction of β-galactosidase synthesis. The minimal concentration of external galactose required to induce a significant synthesis of β-galactosidase in W3092c was found to be around 10^{-5} M. Several independent galactokinaseless mutants of *E. coli* K12, besides W3092c, showed this characteristic of low threshold of the induction of the lac-operon by galactose, as well as the constitutivity of the galactose operon.

However, an interesting exception to these two characteristic properties of galactokinaseless mutants was noted. A variant of W3092c, arising spontaneously and still defective in galactokinase, was found to have lost the character [Gal^c] (Table 4). This variant will be called W3092i and may well be identical to W3092A described previously.[9] The variant W3092i differs from W3092c in another respect. The threshold level of exogeneous galactose necessary to induce the lac operon in W3092i was found to be considerably higher than that of W3092c. W3092i required a minimal concentration of exogenous galactose for β-galactosidase induction at the order of 10^{-3} M. In contrast, the inducible double mutants described above, both $K^-T^+E^-$ and K^-UDPGPP$^-$, were found to preserve this characteristic of W3092c, i.e., the low threshold of exogenous galactose for the induction of β-galactosidase formation (Table 5). Hence this characteristic is independent of the presence or absence of the constitutivity of the galactose operon. It should also be noted that strain W4597 K^- was also found to have a lower threshold of exogenous galactose for the induction of the galactose operon than that of W3092i.[10]

TABLE 5

RELATIONSHIP BETWEEN THE GAL-OPERON CONSTITUTIVITY AND THE GALACTOSE THRESHOLD FOR LAC-OPERON INDUCTION IN K^- MUTANTS

Strain	Genotype	Parent	Gal operon	β-Galactosidase* formed at external galactose concentration of 10^{-5} M	Induction period (min)
W3100	$K^+T^+E^+UDPGPP^+$	Wild type	Inducible	6.82	180
W3092c	$K^-T^+E^+UDPGPP^+$	W3100	Constitutive	100.48	180
W3092i	$K^-T^+E^+UDPGPP^+$	W3092c	Inducible	5.04	180
CN 30-2	$K^-T^+E^+UDPGPP^-$	W3092c	Inducible	140.5	160
W4597 K$^-$	$K^-T^+E^+UDPGPP^-$	W4597	Inducible	80.5	90
316 E$^-$#1	$K^+T^+E^-UDPGPP^+$	W3100	Inducible	13.99	180
316#1-4	$K^-T^+E^-UDPGPP^+$	316E$^-$#1	Inducible	115.16	180
B78A	$K^+T^+E^+UDPGPP^+R_{gal}^-$	C600	Constitutive	3.52	90

Media: minimal A medium + 0.45% sodium succinate.
* Assay of β-galactosidase activity was performed on toluenized cells; activities are expressed in units per unit optical density at 650 mμ.

Discussion.—The inhibition by methyl-thio-β-D-galactopyranoside (MTG) of the constitutivity of the galactose operon in galactokinaseless mutants, contrasted with the lack of effect of MTG on the constitutivity in the R_{gal}^- mutant, suggests that an endogenous induction is operating in the former case, whereas the latter is due to a lack of repressor.[9] The demonstration of the dependence of K^-[Gal^c] on an intact UDP-glucose pathway not only further distinguishes K^-[Gal^c] from

R_{gal}^-[Gal^c], but also suggests the metabolic basis of the generation of the endogenous inducer. In view of the fact that the synthesis of the galactose enzymes could still be induced with a trace amount of exogenous galactose or D-fucose in the double mutants, $K^-UDPGPP^-$ and $K^-T^+E^-$, it is reasonable to assume that the essential role of the UDP-glucose pathway in the expression of K^-[Gal^c] lies in generating the internal inducer rather than affecting its action. The most plausible candidate for the supposed endogenous inducer would seem to be free galactose or a galactosyl compound derived from UDP-galactose. Alternatively, metabolites formed from galactose through pathways different from that regulated by the galactose operon should be considered.

Previous statements[6, 18] concerning the constitutivity of the galactose operon in double mutants of $K^-UDPGPP^-$ are in disagreement with the present data, for reasons which may be related to the use of nutrient media rather than synthetic media. Nutrient broth frequently contains significant amounts of galactose. Since we have observed that galactose at the concentration of 10^{-6} M elicited the induction of the galactose operon in strain W4597 K^-,[10] we are inclined to attribute the observed constitutivity of the galactose operon in $K^-UDPGPP^-$ to the presence of trace amounts of galactose in the nutrient broth. We have ourselves observed such an effect of nutrient media.[10]

The double mutants $K^-T^+E^-$ and $K^-UDPGPP^-$ have, interestingly enough, preserved the low galactose threshold for the induction of the lac operon, despite the fact that they have lost the [Gal^c]. This shows that these two phenotypic characteristics of strain W3092c, both resulting from the loss of the galactokinase activity, are nevertheless separable. The constitutivity of the galactose operon in K^- mutants depends on the production of the endogenous inducer, provided conditions exist which permit the retention of the inducer inside the cell. On the other hand, the sensitivity of the lac operon toward the exogenously supplied galactose appears to be independent of the endogenous production, but likewise may require the ability to accumulate galactose in the cell.

In contrast, the presumably single-step mutation from W3092c to W3092i brings about not only a loss of [Gal^c], but also a marked increase in the threshold for galactose induction of the lac operon. Since W3092i was not impaired in its UDP-hexose pathway, an obvious explanation is that it may have an increased capacity to dispose of the endogenously generated inducer. This could not be via phosphorylation, however, since both W3092c and W3092i contain less than 2 per cent of the wild-type galactokinase activity even when fully induced. The special character of W3092i is at present under investigation both from the genetic as well as from the biochemical points of view, with special emphasis on the study of the transport mechanisms of galactose. Preliminary results[10] have indicated that W3092i has a defective transport system for galactose, while this system is present constitutively in W3092c. This defective permease is probably identical to one of those reported by Rotman.[19, 20]

Internal induction of a catabolic pathway resulting from a mutation in one of the structural genes in the particular pathway may prove to be quite common. A recent report has indicated such a role for urocanate as an internal inducer for the histidine degradative pathway in *Aerobacter aerogenes*.[21] It is interesting to note that urocanate also possesses an active transport system for its intracellular accumula-

tion.[22] It seems very likely that there are three prerequisites for endogenous induction: (*i*) a defect in the enzyme which normally is directly responsible for the consumption of the inducer, (*ii*) a competent pathway for its endogenous generation, and (*iii*) conditions which permit a retention of the endogenously generated inducer. The observation that W3092i became $K^-[Gal^c]$ phenotypically when it was confluently grown[23] may be consistent with this view of a faulty retention mechanism for the internally generated inducer in W3092i.

Moreover, it is conceivable that in order to permit the generation and subsequent accumulation of the endogenous inducer, the negative feedback control by the endogenous inducer on its biosynthesis must be weak or absent. In the case of K^- $[Gal^c]$, such a negative feedback control by the endogenous inducer, if it exists at all, might be counterbalanced by the inductive effect on the synthesis of epimerase. Such a seemingly positive "feedback" effect would occur because of the essential role of epimerase in the production of the endogenous inducer as well as the control of the synthesis of epimerase by this inducer. However, it does not necessarily follow that endogenous induction operates autocatalytically, since the level of the rate-limiting UDP-glucose pyrophosphorylase is not under the control of this inducer.

The physiological role of such an internally generated inducer in regulating a catabolic pathway remains obscure. If the generation of the endogenous galactose via the UDP-hexose pathway were of physiological importance in regulating the uninduced synthesis of the galactose enzymes in *E. coli* K12, one would expect to find the following correlations. Uninduced galactokinase and transferase levels should be particularly low in strains $K^+T^+E^-$ as compared with $K^+T^+E^+$. Likewise, in strain $K^+T^+E^+$ with a UDP-glucose pyrophosphorylase defect, uninduced levels of galactokinase, transferase, and epimerase should be greatly lowered. As seen from previous data,[5, 6] as well as from Table 2, neither of these predictions was realized. Hence one is led to consider the possibility of the existence of other endogenous inducers independent of the UDP-glucose pathway in their generation, or to attribute the appreciable uninduced synthesis of the enzymes programmed by the galactose operon to the presence of unstable repressors or insufficient levels of corepressors.

Summary.—Endogenous induction of the galactose operon in galactokinaseless mutants of *E. coli* K12 has been examined. It has been found that this pleiotropic phenomenon depends not only on a defective galactokinase, but also on additional factors. An unimpaired UDP-glucose/UDP-galactose pathway is a prerequisite for the formation of endogenous inducer. This is borne out by the fact that galactokinaseless mutants with an additional defect of either UDPG pyrophosphorylase or UDPGal-4-epimerase lose their constitutivity of the galactose operon. An efficient retention mechanism for the endogenous inducer seems also to be of importance in the manifestation of endogenous induction.

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Abbreviations used: Genotypes of strains used: *K*, *T*, and *E* denote the cistrons determining primary structures of galactokinase, Gal-1-P uridyl transferase, and UDPGal-4-epimerase, respectively; R_{gal} represents the gene regulating the expression of the galactose operon; *UDPGPP* denotes the gene for the enzyme UDPG pyrophosphorylase. Phenotype $[Gal^c]$ indicates the

constitutivity of the galactose operon; i.e., $K^-[Gal^c]$ represents the constitutivity of the synthesis of transferase and epimerase in galactokinaseless mutants. PEP stands for phosphoenolpyruvate.

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*ALTERED REPRESSION OF SOME ENZYMES OF SULFUR UTILIZATION IN A TEMPERATURE-CONDITIONAL LETHAL MUTANT OF NEUROSPORA**

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Temperature-conditional lethal mutants of microorganisms have long been known,[1] but only rather recently has evidence been gained about the metabolic nature of such lesions.[2–7] Previous work has shown that an ethionine-resistant, temperature-sensitive irreparable mutant (*r-eth-1*) of *Neurospora crassa* is characterized by a loss of control over methionine synthesis;[8, 9] it was inferred, but not proved, that the defect in *r-eth-1* involves an alteration of repression, rather than of end-product inhibition. The data presented below show that in *r-eth-1*