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Transcriptional Control of the Galactose Operon by the *capR* (*lon*) and *capT* Genes

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Mutations in *capR* or *capT* cause derepression of the enzymes of the *gal* operon. The *gal*-specific messenger ribonucleic acid is directly proportional to the *gal* enzyme levels in wild type, *capR*, and *capT* strains. These results indicate that *capR* and *capT* control the *gal* operon at the transcriptional level.

Recent reports have discussed the control of the *gal* operon of *Escherichia coli* by the *galR* gene as well as the *capR* (*lon*) and *capT* genes (7, 10). The enzymes of the *gal* operon are elevated by a mutation in any one of these genes. It has been known for some time that *galR* codes for a repressor (3, 13, 14). When this repressor is inactivated either by mutation or combination with an inducer (D-fucose) both *gal* operon enzymes (*galE*, epimerase, EC 5.2.3.2., *galT*, transferase, EC 2.7.10; and *galK*, galactokinase, EC 2.7.1.6.) and *gal*-specific messenger ribonucleic acid (mRNA) increase (2). These results suggest that *galR* controls enzyme synthesis at least partly at the level of transcription. Mackie and Wilson (10) reported similar findings with a *capR6* mutant. However, their observed increase in *gal*-specific mRNA did not account for the increase in *gal* enzymes. The question still remains, therefore, whether *capR* actually controls enzyme synthesis entirely at the transcriptional level or not.

Hua and Markovitz (7) have already established that mutations in *capR* and *capT* cause derepression of the enzymes of the *gal* operon. They proposed, therefore, that *capR* and *capT* acted independently of *galR* to cause derepression. In the present report, this derepression is demonstrated to be at the level of transcription.

All strains of bacteria used in the experiments (Table 1) were derivatives of *E. coli* K-12. The properties of the strains employed were published elsewhere (7).

Preparation of cell-free extracts for the measurement of enzyme was as described by Hua and Markovitz (7), except that 10^{-2} M mercaptoethanol was used. Epimerase was assayed at 25 C by the method described by Kalckar et al. (8). Protein was determined by the method of Lowry et al. (9).

The level of epimerase is expressed as micromoles of uridine 5'-diphosphate (UDP)-glucose formed per hour per milligram of protein.

DNA was extracted from purified λp*gal8c1857Sam7* (4) by the method of Thomas and Abelson (17). ^{3}H-labeled RNA was prepared as described by Okamoto et al. (12) and modified by Varmus et al. (18). Since the cultures were growing in minimal glucose at 23 C, the ^{3}H-UdR pulse was usually 8 min in order to get RNA of 100,000 counts per min per μg or higher. The actual specific radioactivity of the RNA varied from culture to culture. Nitrocellulose filters (25 mm; Schleicher & Schuell, type B6) were loaded with 11.76 μg of λ or λp*gal* deoxyribonucleic acid (DNA) as described by Gillespie and Spiegelman (5). Filters (5 mm) containing 1 μg of DNA each were punched out of the 25-mm filters. ^{3}H-labeled DNA was used to determine the amount of DNA remaining on the filters after some experiments. Less than 10% of the DNA fixed to the filters is lost during the entire hybridization and posthybridization procedure. A typical hybridization involved incubation of 1 μg of DNA fixed to a filter with 1 μg of ^{3}H-RNA (or 2 μg of ^{3}H-RNA in the case of *capR*$^+$) in a final volume of 0.3 ml of 4 × SSC (0.15 M NaCl plus 0.015 M sodium citrate) for 20 h at 74 C. The procedure, including the final ribonuclease step, is essentially the same as found by Varmus et al. (18). In one experiment (*data not shown*), unlabeled RNA from *gal* deletion strain S165 (deleted for *galE*, *galT*, and most of *galK* [15]) was used as competitor, but there was no change in the result.

The results presented in Table 1 compare the derepression of epimerase caused by mutations in *capR*, *capT*, and *galO* to the increase in *gal*-specific messenger. Galactokinase was also measured, and its derepression is similar to

TABLE 1. *Level of UDP-galactose-4-epimerase compared to gal-specific mRNA*

Strain	Relevant alleles	UDP-galactose-4-epimerase[a]	Fold derepression of epimerase	*gal*-Specific mRNA hybridized × 10^2 (%)		Fold derepression of calculated *gal* mRNA level
				Observed[b]	Calculated[c]	
MC129	*capR*$^+$	3.75	1.0	0.89	0.18	1
HC1002	*capR9*	23.6	6.3	1.98	1.27	7
HC1003	*capT*	20.1	5.4	1.96	1.25	7
HC1022	*capR*$^+$, GalO^c	50.6	13.5	2.65	1.94	11
HC1023	*capR9*, GalO^c	115	30.5	6.06	5.35	30

[a] Results are the averages of two separate experiments in which the variability never exceeded 8%.

[b] For each experiment, the percent hybridized to λDNA was subtracted from the percent hybridized to λ*pgal* DNA to give "*gal*-specific mRNA." The *gal*-specific counts per minute ranged from 24 to 75 counts/min. The values reported here are the average of two separate experiments, and duplicate samples were used in all experiments. The two experiments (three experiments for *capR*$^+$) varied by 21% for *capR*$^+$, 2.5% for *capR9*, 10% for *capT*, 19% for *capR*$^+$, GalO^c, and 1% for *capR9*, GalO^c.

[c] The calculated value was determined by subtracting the percent non-*gal* RNA background from the observed value of percent RNA binding for each strain. The percentage of non-*gal* RNA was calculated by using the observed values for percent *gal*-mRNA and the fold depression of epimerase for *capR*$^+$ and *capR9*, GalO^c as follows: 0.0606% − non-*gal* RNA/30.5 = 0.0089% − non-*gal* RNA; non-*gal* RNA = 0.0071%.

epimerase in all of the strains (*data not shown*). An analysis of the effect of the various mutations on the *gal* enzyme levels has been published (7). It is clear (Table 1) that the *gal*-specific mRNA level increases with an increase in epimerase. In fact, after a small correction for non-*gal* RNA background, the derepression of epimerase is directly proportional to the derepression of *gal*-specific mRNA. Such a direct relationship between specific mRNA and enzyme levels has been obtained in only one other case, the *lac* operon (1). The results indicate, therefore (Table 1), that *capR* and *capT* control the *gal* operon at the transcriptional level.

The non-*gal* RNA that was binding in the hybridizations might have been transcribed from the "wrong" strand of the *gal* DNA duplex. This possibility was eliminated by hybridizing to the separated strands of λ*pgal* DNA prepared as described by Szybalski et al. (16). It was determined (*data not shown*) that the non-*gal* RNA background was due to transcription of the *l* strand of λ*pgal* DNA, the same strand on which transcription of the *gal* operon occurs (6).

Finally, the half-life of *gal*-specific mRNA in wild-type, *capR9*, and *capT* cultures has been measured. The technique for measuring the decay of mRNA was the same as that used by Miller et al. (11). There did not appear to be any significant difference in the half-lives of *gal* mRNA which varied between 6.5 and 7.5 min at 23 C in minimal medium.

Our results show that *gal*-specific mRNA is directly proportional to the *gal* enzyme levels in wild-type, *capR*, and *capT* strains, indicating that *capR* and *capT* control the *gal* operon at the transcriptional level.

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LITERATURE CITED

1. Adesnik, M., and C. Levinthal. 1970. The synthesis and degradation of lactose operon messenger RNA in *E. coli*. Cold Spring Harbor Symp. Quant. Biol. **35**:451-459.
2. Attardi, G., S. Naono, J. Rouviere, F. Jacob, and F. Gross. 1963. Production of messenger RNA and regulation of protein synthesis. Cold Spring Harbor Symp. Quant. Biol. **28**:363-372.
3. Buttin, G. 1963. Mecanismes regulateurs dans la biosynthese des enzymes du metabolisme du galactose chez *Escherichia coli* K-12. II. Le determinisme genetique de la regulation. J. Mol. Biol. **7**:183-205.
4. Feiss, M., S. Adhya, and D. L. Court. 1972. Isolation of plaque-forming, galactose-transducing strains of phage lambda. Genetics **71**:189-206.
5. Gillespie, D., and S. Spiegelman. 1965. A quantitative assay for DNA-RNA hybrids with DNA immobilized on a membrane. J. Mol. Biol. **12**:829-842.
6. Guha, A., M. Tabaczynski, and W. Szybalski. 1968. Orientation of transcription for the galactose operon as determined by hybridization of *gal* mRNA with the separated DNA strands of coliphage λdg. J. Mol. Biol. **35**:207-213.
7. Hua, S., and A. Markovitz. 1972. Multiple regulator gene control of the galactose operon in *Escherichia coli* K-12. J. Bacteriol. **110**:1089-1099.
8. Kalckar, H. M., K. Kurahashi, and E. Jorden. 1959. Hereditary defects in galactose metabolism in *Escherichia coli* mutants. I. Determination of enzyme activities. Proc. Nat. Acad. Sci. U.S.A. **45**:1776-1786.
9. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.

10. Mackie, G., and D. B. Wilson. 1972. Regulation of the *gal* operon of *Escherichia coli* by the *capR* gene. J. Biol. Chem. **247**:2973–2978.
11. Miller, Z., H. E. Varmus, J. S. Parks, R. L. Perlman, and I. Pastan. 1971. Regulation of *gal* messenger ribonucleic acid synthesis in *Escherichia coli* by 3′,5′-cyclic adenosine monophosphate. J. Biol. Chem. **246**:2898–2903.
12. Okamoto, K., Y. Sugino, and M. Nomura. 1962. Synthesis and turnover of phage messenger RNA in *E. coli* infected with bacteriophage T4 in the presence of chloromycetin. J. Mol. Biol **5**:527–534.
13. Parks, J. S., M. Gottesman, K. Shimada, R. A. Weisberg, R. L. Perlman, and I. Pastan. 1971. Isolation of the *gal* repressor. Proc. Nat. Acad. Sci. U.S.A. **68**:1891–1895.
14. Saedler, H., A. Gullon, L. Fiethen, and P. Starlinger. 1968. Negative control of the galactose operon in *E. coli*. Mol. Gen. Genet. **102**:79–88.
15. Shapiro, J. A., and S. L. Adhya. 1969. The galatose operon of *E. coli* K-12. II. A deletion analysis of operon structure and polarity. Genetics **62**:249–264.
16. Szybalski, W., H. Kubinski, Z. Hradecna, and W. C. Summers. 1971. Analytical and preparative separation of the complementary DNA strands, p. 383–413. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 21. Academic Press Inc., New York.
17. Thomas, C. A., Jr., and J. Abelson. 1966. The isolation and characterization of DNA from bacteriophage, p. 553–561. *In* G. L. Cantoni and D. R. Davies (ed.), Procedures in nucleic acid research. Harper and Row, New York.
18. Varmus, H. E., R. L. Perlman, and I. Pastan. 1970. Regulation of *lac* messenger ribonucleic acid synthesis by cyclic adenosine 3′,5′-monophosphate and glucose. J. Biol. Chem. **245**:2259–2267.