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CYCLIC AMP-INDUCED EXPRESSION OF THE MOUSE LACTATE DEHYDROGENASE-A PROMOTER-CAT FUSION GENE IN CHINESE HAMSTER OVARY WILD-TYPE CELLS, BUT NOT IN cAMP-DEPENDENT PROTEIN KINASE MUTANT CELLS

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The promoter region of mouse lactate dehydrogenase-A gene was fused with the chloramphenicol acetyltransferase gene of Escherichia coli, and the expression of this fusion gene was induced by cyclic AMP in Chinese hamster ovary wild-type cells, but not in mutants affecting the regulatory or catalytic subunit of cAMP-dependent protein kinase.

In mammals, lactate dehydrogenase (LDH) isozymes are encoded by LDH-A (muscle), B (heart) and C (testis) genes, and the expression of these three genes is developmentally regulated and tissue-specific (1). The synthesis of LDH-A mRNA and polypeptide in rat C6 glioma cells has been shown to be induced by isoproterenol or dibutyryl cAMP (2). However, the mechanisms by which cAMP regulates eukaryotic gene expression remain to be elucidated. We have already described the exon-intron organization of the mouse LDH-A gene (3) and identified its putative promoter region (4). In this report, the promoter region of the mouse LDH-A gene was fused to Escherichia coli cat gene encoding chloramphenicol acetyltransferase, and the expression of this fusion gene was shown to be induced by 8-bromo-cAMP in Chinese hamster ovary (CHO) wild-type cells, but not in cAMP-dependent protein kinase (cADepPK) mutant cells.

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Abbreviations:
LDH: Lactate dehydrogenase; CHO: Chinese hamster ovary; cADepPK: Cyclic AMP-dependent protein kinase; CAT: Chloramphenicol acetyltransferase.

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Material and Methods

The Escherichia coli cat gene was used as reporter to monitor the promoter function of mouse LDH-A gene. The HindIII 2.4Kb fragment containing the promoter region of the cloned mouse LDH-A gene (3,4) was inserted into the unique HindIII site of plasmid pSV0-cat provided by Dr. B. H. Howard (5). The orientation of the LDH-A promoter in the recombinant plasmid DNAs was determined by restriction endonuclease cleavage and Southern blotting (6), probing with the LDH-A promoter and the cat gene (data not presented). Plasmid pLA1-cat contains the mouse LDH-A promoter in the proper orientation for expressing the cat structural gene and plasmid pLA2-cat possesses the promoter in the reverse orientation.

The CHO cells were chosen as transfection host, since the CHO cells appear to synthesize only the LDH-A subunit (E. W. Hou, unpublished). The CHO X3/5 cell line was provided by Dr. K. R. Tindall (7), while cell lines 10001, 10215, 10248 and 10260 were by Dr. M. M. Gottesman (8). The CHO cells were maintained in Ham's F-12 medium supplemented with 10% heat-inactivated fetal calf serum with 10% CO_2 at 37°C (7). At 24 hrs before transfection was performed, $5x10^5$ CHO cells per 100-mm dish were seeded in the medium containing 100 unit/ml of penicillin and 100 μg/ml of streptomycin. Fresh medium was supplied 2 hrs before adding the super-coiled plasmid DNA (10 μg) coprecipitated with calcium phosphate (9). The transfected cell culture was incubated at 37°C for 6 hrs, at which time the medium was removed and the cells were washed twice with phosphate-buffered saline before fresh medium containing gentamicine (25 μg/ml) was supplied. After 40 hrs, the CAT enzyme activity of the transiently expressed cat gene was determined by the procedure of Gorman, Moffat and Howard (5).

For the cAMP-induced expression of the LDH-A promoter-cat fusion gene, the CHO wild-type cells and mutants affecting either Type I regulatory or catalytic subunit of cADepPK were cotransfected with pLA1-cat and pSV2-gpt provided by Dr. P. Berg (10). After the CHO cells were incubated without selection at 37°C for two days, the confluent cells were trypsinized and dispensed 1:10 onto 100-mm dishes. The cells were grown without selection for two-three days, followed by two-three weeks under selection conditions (aminopterin, mycophenolic acid, xanthine, adenine, thymidine; 1.3, 10, 250, 25, 12 μg/ml, respectively). $5x10^5$ CHO cells stably cotransfected with pLA1-cat and pSV2-gpt were seeded in nonselective medium containing various concentrations of 8-bromo-cAMP, and incubated for two days with one change of medium containing the same concentration of 8-bromo-cAMP. The CAT enzyme activity was assayed as described (5), and the relative amounts of chloramphenicol and its acetylated derivatives were determined by scintillation counting. The extent of conversion was calculated, and the cAMP-induction (%) was expressed over the control without cAMP added.

Results and Discussion

Figure 1 shows the results of transient expression of pLA1-cat, pLA2-cat, pSV2-cat or PSV0-cat. The LDH-A promoter oriented properly with respect to the cat gene in Plasmid pLA1-cat was able to promote expression of the reporter cat gene, while the LDH-A promoter in reverse orientation in plasmid pLA2-cat was not. Further, the CHO cells transfected with plasmid pSV2-cat showed high CAT enzyme activity, confirming a strong SV40 early promoter. The cat gene in plasmid pSV0-cat was not expressed because of the absence of a promoter. Similar results were also obtained from the stable expression of these four plasmid DNA.

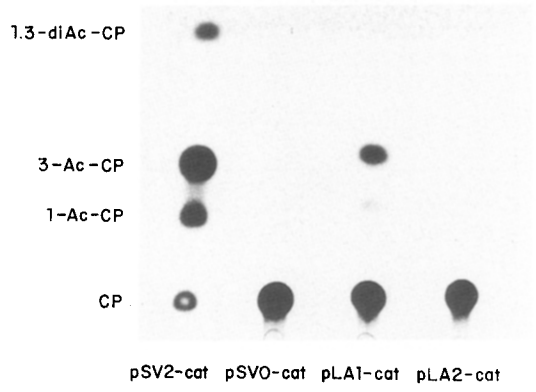

Figure 1. Thin-layer chromatographic analysis (TLC) of CAT enzyme activity from CHO cells transiently expressing the mouse LDH-A promoter-cat fusion gene.

The transfected CHO cells were scraped off, washed twice with Tris-EDTA-NaCl (40 mM, 1mM, 150 mM), freeze-thawed three times, and ultrasonicated with a microtip in 150 µl of 0.25M Tris-Cl, pH 7.8 at room temperature for 30 seconds. After spinning in a microfuge, the protein present in the supernatant fraction of the cell extract was determined, and 100 to 250 µg of protein was added to the CAT reaction mixture consisting of 0.4 µCi ^{14}C-chloramphenicol (Amersham), 4 mM acetyl CoA (Pharmacia) in 0.25M Tris-Cl, pH 7.8. The reaction was stopped after 5 hrs incubation at 37°C by extracting (Vortex) with 1 ml of ethyl acetate (Pierce, sequanal grade) at room temperature for 45 seconds. Chloramphenicol (CP) and its (1-Ac-CP; 3-Ac-CP; 1,3-diAc-CP) present in the organic phase were concentrated using the SpeedVac (Savant) and chromatographed onto a Silica TLC plate in chloroform/methanol (95:5, v/v). The plate was exposed to XAR-5 Kodak film with light-plus intensify screen for 4 to 20 hrs at -70°C.

For the cAMP-induction of the LDH-A promoter-cat fusion gene, CHO wild-type and mutant cells were stably cotransfected with pLA1-cat and pSV2-gpt (10), and the results of the induction by 8-bromo-cAMP are summarized in Table I. In CHO wild-type cells of both X3/5 and 10001, 8-bromo-cAMP (0.1, 0.5 and 1.0 mM) increased two to three folds the expression of LDH-A promoter-cat fusion gene. In mutant 10260 cells, low concentration (0.1 mM) of 8-bromo-cAMP seemed to have some induction, although higher concentrations (0.5 and 1.0 mM) of 8-bromo-cAMP exhibited inhibitory effect. The recessive mutant 10260 has only a very small amount of Type II cADepPK because of the much reduced (less than 10%) C subunit (8). In the cADepPK-mutants containing the altered catalytic (mutant 10215) or Type I regulatory (mutant 10248) subunit, the 8-bromo-cAMP inhibited strongly the expression of LDH-A promoter-cat gene. As described previously (8,11), the dominantly altered C subunit of mutant 10215 exhibits increased affinity for RI subunit and, similarly, the dominantly altered RI subunit of mutant 10248 is tightly bound to the C subunit. Thus, in mutants 10215 and 10248, the RII subunit exists as "free" form and

TABLE I. Induction (%) of the mouse LDH-A promoter-cat fusion gene expression by 8-bromo-cAMP in CHO cells

Cell Lines	cADepPK	8-bromo-cAMP (mM) 0	0.1	0.5	1.0
X3/5	wild-type	100	-	-	381
		100	340	241	299
10001	wild-type	100	-	-	418
		100	312	312	299
10260	Reduced C	100	-	-	70
		100	203	62	75
10215	altered C	100	-	-	25
		100	58	22	28
10248	altered RI	100	-	-	56
		100	9	6	7

there is no Type II cADepPK activity. These results suggest the requirement of Type II cADepPK for cAMP induction. It is of interest to note that similar results on the cAMP-induced expression of rat somatostatin gene were obtained in neuroendocrine PC12 wild-type cells and mutant A126-1B2 possessing markedly reduced Type II cADepPK activities. It has been reported that the phosphorylated form of Type II regulatory subunit appears to be transported into the nucleus and its topoisomerase activity may regulate gene expression by altering chromatin structure near/within the responsive genes (12).

In this report, we demonstrate that the expression of mouse LDH-A promoter-cat fusion gene was induced by 8-bromo-cAMP in the Chinese hamster ovary wild-type cells, but not in cAMP-dependent protein kinase mutant cells. The nucleotide sequence of the promoter/regulatory region of the mouse LDH-A gene has been determined (4), and a palindrome cAMP-responsive sequence (5 CTGACGTCAG 3) defined by deletion-analysis of the rat somatostatin gene (13) was found at the corresponding positions -49 to -40 of mouse LDH-A gene. The precise sequence of the cAMP-regulatory element of the mouse LDH-A gene remains to be demonstrated experimentally.

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