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trans Activation of Rat Phosphoenolpyruvate Carboxykinase (GTP) Gene Expression by Micro-Coinjection of Rat Liver mRNA in *Xenopus laevis* Oocytes

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To study the liver-specific *trans* activation of the rat phosphoenolpyruvate carboxykinase (PEPCK) gene, the PEPCK promoter was linked to a reporter gene and was microinjected into *Xenopus laevis* oocytes alone or in conjunction with rat liver poly(A)$^+$ RNA. The rat liver mRNA markedly enhanced the expression of the PEPCK-chimeric construct. This effect appeared to be sequence specific, as it was dependent on the presence of the intact promoter. Moreover, the RNA effect was limited to mRNA preparations from PEPCK-expressing tissues only. Finally, microinjection of size-fractionated liver mRNA revealed that the *trans*-acting factor(s) is encoded by RNA of 1,600 to 2,000 nucleotides, providing a direct bioassay for the gene(s) involved in this tissue-specific *trans*-activation process.

Mechanisms underlying tissue-specific gene expression have attracted attention in recent years. It is clear that defined *cis* regulatory elements, within the structural gene or its flanking regions, can confer tissue-specific expression of genes (11). However, much less is known about the *trans*-acting factors that interact with DNA and regulate selective modes of gene transcription.

We are studying the gene encoding the cytosolic form of rat phosphoenolpyruvate carboxykinase (EC 4.1.1.32) (PEPCK), which is expressed in several tissues arising from various embryonal origins (the liver from endoderm and the kidney cortex and adipose tissue from mesoderm) (for reviews on the tissue-specific expression of PEPCK, see references 1 and 16). Recently, using transient expression assays in transfected cells, we have shown that 597 base pairs (bp) of the PEPCK promoter is sufficient to direct cell-specific gene expression in hepatoma cells, adipocytes (2), and kidney epithelial cells (T. Shoshani, N. Benvenisty, and L. Reshef, unpublished results). Experiments by Hanson and colleagues, using transgenic mice, have likewise shown that this region of the PEPCK promoter is sufficient to confer tissue-specific gene expression in the liver and kidney (12). However, different elements within this promoter region confer enhanced expression in either hepatocytes or adipocytes (2).

We have examined the possibility of using *Xenopus laevis* oocytes to reconstitute *cis*- and *trans*-acting control of the PEPCK gene in ovo. The oocytes are capable of efficient transcription of foreign DNA (18) and translation of the RNA product (13). By virtue of these properties, this system has been instrumental in identifying (by using biological assays) cloned genes whose transcripts are rare (9, 10). Our approach was to coinject various promoter-containing expression vectors with RNA preparations encoding potential *trans*-acting factors and to monitor the resultant transcriptional efficiency of those promoters. The assumption that *trans*-acting factors, translated in the oocytes from microinjected RNA, will enter the nucleus and regulate transcription of a coinjected DNA derives support from recent experiments where microinjection of RNA from myeloma cells into *X. laevis* oocytes stimulated the expression of a chimeric gene that contained the immunoglobulin OCTA sequence (15).

To examine the possibility of reconstituting the transcriptional regulation of the PEPCK gene in *X. laevis* oocytes, we first used the plasmid 597-pck-CAT, in which the structural chloramphenicol acetyltransferase (CAT) gene, serving as a reporter, is linked to 597 bp of the rat PEPCK promoter and 69 bp of its first exon (2). When supercoiled 597-pck-CAT plasmid alone was microinjected into oocytes, negligible CAT activity could be detected in oocyte extracts 24 h postinjection. Coinjection of poly(A)$^+$ RNA from rat liver markedly stimulated CAT production driven by this plasmid (Fig. 1). To examine the specificity of the RNA-induced effect, we microinjected several additional CAT-containing plasmids whose expression levels have previously been examined by transient expression assays in cultured cells. These included pSV2cat, which contains the early promoter-enhancer of simian virus 40 (8) and is expressed in many cell types, including hepatocytes (2); pA10kE-CAT, which is myelocyte specific and contains the enhancer of the immunoglobulin kappa chain; pBR-rIns-CAT, which contains the endocrine pancreatic promoter-enhancer of the rat insulin gene (17); and pBR-rChym-CAT, which includes the exocrine pancreatic chymotrypsin promoter-enhancer (17). Of these plasmids, pSV2cat (which is active in hepatocytes) was the only one for which expression was stimulated by coinjection of the liver RNA, while the others were all marginally active whether in the absence or presence of coinjected liver poly(A)$^+$ RNA (Fig. 1). Thus, it seems that the liver RNA-dependent stimulation of CAT production directed by 597-pck-CAT is a promoter-specific phenomenon, rather than a general nonspecific effect. Despite the reproducibility of this RNA stimulation (10 out of 12 independent experiments), we have noted a threefold variation in its magnitude among separate experiments. The reason for the variation is not known; however, this might be associated with the quality of the poly(A)$^+$ RNA preparation and

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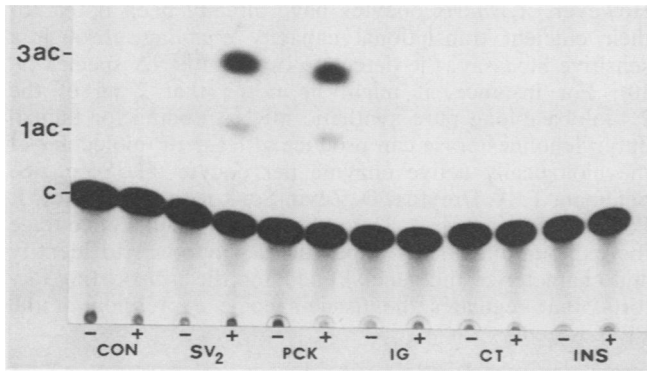

FIG. 1. Effect of liver poly(A)$^+$ RNA on the expression of CAT-chimeric constructs microinjected into *X. laevis* oocytes. Supercoiled plasmid DNA (50 ng per oocyte) and poly(A)$^+$ RNA (50 ng per oocyte) were microinjected into unfertilized *X. laevis* oocytes at stage 6, as detailed elsewhere (13, 14). The plasmids used contained the bacterial CAT structural gene linked to the enhancer-promoter region of either SV40 (pSV2cat) (8), SV2; rat PEPCK (597-pck-CAT) (2), PCK; mouse immunoglobulin kappa chain (pA10E-kappa-CAT immunoglobulin, obtained from Y. Bergman), IG; rat chymotrypsin B (pBR-rChym-CAT) (17), CT; or rat insulin I (pBR-rIns-CAT) (18), INS. Rat liver poly(A)$^+$ RNA was isolated by double oligo(dT)-cellulose chromatography (3) from RNA extracted by the guanidinium thiocyanate method, purified by centrifugation through a CsCl cushion (4). Following overnight incubation at 18°C, a 30-oocyte sample were homogenized in a buffer containing 0.25 M Tris (pH 7.5), 1 mM EDTA, and 0.5 mM phenylmethylsulfonyl fluoride. CAT activity was determined in supernatant extracted from 15 oocytes, as previously described (8). The products were separated by thin-layer chromatography. The plasmids were either injected with (+) or without (−) rat liver poly(A)$^+$ RNA (50 ng per oocyte). CON, No injection of plasmid; C, chloramphenicol; 1ac and 3ac, acetylated forms of chloramphenicol.

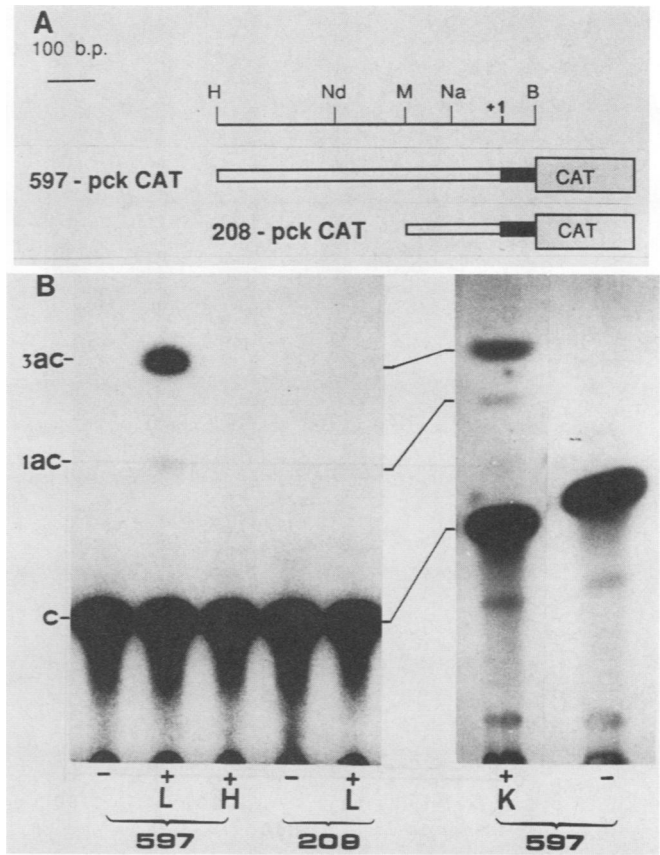

FIG. 2. Sequence and RNA specificity of the *trans*-acting control of the PEPCK promoter in *X. laevis* oocytes. (A) Schematic illustration of PEPCK-CAT chimeric genes. The 5′ end and flanking region of the PEPCK gene is presented with some of its restriction enzyme sites. H, *Hin*dIII; Nd, *Nde*I; M, *Mst*II; Na, *Nae*I; B, *Bgl*II. The transcription start site is indicated by +1. □, PEPCK gene 5′-flanking region; ■, 69 bp of the 5′ end of PEPCK gene; ▩, CAT structural gene. The size of the 5′-flanking sequences of the PEPCK gene in each construct is specified by the number of base pairs (for details, see reference 2). (B) *X. laevis* oocytes were microinjected with either 597-pck-CAT or 208-pck-CAT (2), without (−) or with (+) poly(A)$^+$ RNA from rat liver (L), mouse hybridoma of BALB/c myeloma NSI/1 with spleen cells (5) (H), or rat kidney (K). For details, see the legend to Fig. 1.

with variable translational efficiencies in different batches of oocytes (13).

While the 597 bp of the PEPCK promoter included in the 597-pck-CAT plasmid is sufficient to confer hepatocyte-specific expression, deletion to position −208 with respect to the transcription start site (Fig. 2) has been shown to decrease the expression about 10-fold in cultured cells (2). In the oocytes, the effect of coinjected liver poly(A)$^+$ RNA on the plasmid 208-pck-CAT (harboring the deleted PEPCK promoter to position −208) decreased 5- to 10-fold. Thus, the *trans*-activating factor associated with liver RNA appears to recognize a specific sequence at a distance. Moreover, previous transient expression assays in hepatocytes demonstrated that the deleted sequence bears the domain required for enhanced expression of the PEPCK gene in hepatocytes (2). Thus, the stimulatory effect of the liver RNA on expression of PEPCK chimeric genes in oocytes requires the presence of the hepatocyte-specific *cis* regulatory promoter element.

To examine the specificity of mRNA induction of the *trans*-activation process, we compared the effect of coinjection of the 597-pck-CAT plasmid with poly(A)$^+$ RNA from PEPCK-expressing tissues (rat liver and kidney) and PEPCK-nonexpressing cells (mouse A52 cells, hybridoma of myeloma and spleen cells) (Fig. 2). While the poly(A)$^+$ RNA from both liver (10 out of 12 experiments) and kidney (6 out of 8 experiments) stimulated CAT production driven by 597-pck-CAT (Fig. 2), the myeloma RNA failed to do so (in all experiments) (Fig. 2). Also, we found that the myeloma RNA stimulated expression driven by pSV2cat, although by only about threefold (results not shown). In this respect, it has been previously found that myeloma mRNA from immunoglobulin-producing cells also stimulates in oocytes the expression of a coinjected chimeric gene containing the OCTA sequence from the immunoglobulin gene (15). Therefore, the *X. laevis* oocyte system actually reconstitutes the liver-specific mode of regulation of the PEPCK promoter, encompassing both the *cis* and *trans* factors that control the tissue-specific PEPCK gene expression. An appreciation of this powerful approach to the reconstitution of biological effects in microinjected oocytes can also be gleaned from previous experiments, where injection of the histone H3 gene, together with total cellular sea urchin RNA into *X. laevis* oocytes, complemented a deficient factor needed to achieve a correct 3′ termination of the transcripts (7).

To estimate the size of the liver-specific mRNA species responsible for stimulating transcription directed by the PEPCK promoter, we fractionated liver poly(A)$^+$ RNA by sucrose gradient centrifugation (6). Various fractions were

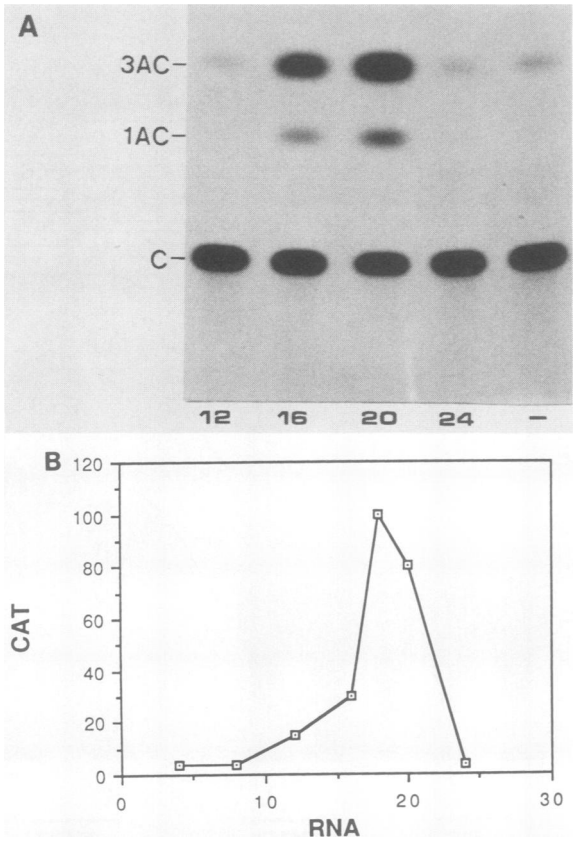

FIG. 3. Effect of sucrose gradient fractions of liver RNA on the expression of PEPCK-CAT chimeric gene in *X. laevis* oocytes. (A) CAT activities, driven by the PEPCK promoter, alone (−) or coinjected with various sucrose gradient fractions of liver poly(A)$^+$ RNA (as previously described [6, 14]), specified by the number below. C, Chloramphenicol; 1ac and 3ac, acetylated forms of chloramphenicol. (B) Densitometry of the 3-acetyl chloramphenicol spots, in arbitrary units, representing the CAT activity measured in oocytes coinjected with various RNA fractions of the sucrose gradient whose numbers (from bottom to top of the gradient, see reference 6) are indicated on the abscissa. Data are the mean of two separate experiments.

coinjected with 597-pck-CAT DNA into oocytes, and CAT production was determined. This analysis demonstrated 25-fold stimulation of PEPCK promoter-driven CAT production by a single-fraction mRNA corresponding to a population of transcripts 1,600 to 2,000 nucleotides long (Fig. 3). Thus, it appears that this single RNA fraction is by itself capable of stimulating gene expression. The identification of an RNA fraction that stimulates transcription directed by the PEPCK promoter should facilitate further isolation and study of the factor(s) that activates PEPCK gene expression.

Regarding the ability of the *X. laevis* oocyte system to be of general use, it should be kept in mind that proteins encoded by the foreign mRNA have to be synthesized in the oocyte cytoplasm and transported into the nucleus, where they presumably participate in the formation of the chromatin conformation required of the coinjected promoter DNA to facilitate transcription. The amount of *trans*-acting proteins that can be produced in the oocytes depends on numerous factors, including the abundance and stability of their corresponding RNAs, and on the stability of the protein product itself. Thus, it is possible that this approach favors the detection of more stable and abundant RNAs or proteins. However, *X. laevis* oocytes have already been noted for their efficient translational capacity enabling, through a sensitive bioassay, the detection of rare mRNA species (9, 10). For instance, it might be noted that 2 ng of the 2.4-kilobase-long pure synthetic mRNA coding for human butyrylcholinesterase can produce at least 10^8 molecules of the biologically active enzyme per oocyte (H. Soreq, S. Seidman, T. A. Dreyfus, D. Zevin-Sonkin, and H. Zakut, J. Biol. Chem., in press). Thus, the present results encourage the exploitation of this experimental approach to identify and characterize the gene(s) encoding the *trans*-acting factor(s) that regulates the tissue-specific expression of the PEPCK gene.

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