appendix b-4

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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expression in hepatoma cells, adipocytes (2), and kidney epithelial cells (T. Shoshani, N. Benvenisty, and L. Reshef, unpublished results).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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