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Specificity of Gene Expression in Adipocytes

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During the differentiation of preadipose 3T3 cells into adipose cells, the mRNAs for three proteins increase strikingly in abundance. To determine the degree of cell-type specificity in the expression of these mRNAs, we estimated their abundances in several nonadipose tissues of the mouse. None of these mRNAs was strictly confined to adipocytes, but the ensemble of three mRNAs was rather specific to adipocytes. Insofar as is revealed by these three markers, the distinctive phenotype of adipocytes is the result of the enhanced expression of a number of genes, none of which is completely silent in all other cell types.

The specific structure and functions of each differentiated cell type are presumably the result of the particular set of proteins it possesses. Attention is frequently concentrated on proteins found only in a single cell type, such as globin in erythrocytes or ovalbumin in oviductal epithelial cells. The problem of differentiation is thereby condensed into the "turning on" of the corresponding gene.

This simplification obscures the fact that each differentiated cell type requires an increase in the abundance of not one or two but of a considerably larger number of proteins to express a unique phenotype. Even if most of these proteins are synthesized by other cell types as well, the ensemble of proteins must be unique.

Adipocytes of white adipose tissue are a very distinctive cell type. The specialized functions of the cell are the synthesis, storage, and hormonally controlled hydrolysis of triglyceride. To accomplish this unique set of functions, does the adipose cell require unique proteins? All enzymes of triglyceride synthesis must be present in the liver as well as in adipose tissue, since the liver synthesizes abundant triglyceride, not for storage, but for secretion in the form of lipoprotein particles. The triglyceride-hydrolyzing enzyme lipoprotein lipase is important to adipose cells, but it is also found in muscle and other tissues (16). Even the hormone-sensitive lipase, thought to be the enzyme of triglyceride metabolism most specific to the adipose cell (7), has recently been identified in bovine adrenal cortex, where it is involved in steroid production (5). Therefore, no enzyme of triglyceride metabolism has so far been found to be unique to adipose cells.

During the differentiation of preadipose 3T3 cells into adipose cells, numerous changes occur in the protein composition of the cells (18), some of these changes being due to the accumulation of known enzymes. The most convenient enzyme for following the differentiation is the stable isozyme of glycerophosphate dehydrogenase, which increases in activity by several orders of magnitude (10, 13, 19). This enzyme is not unique to adipose tissue, however; although absent from preadipose cells (19), it is present in several other cell types, including liver, brain, muscle, heart, and kidney (9, 14). Two other proteins, one of 13 kilodaltons (kDa), which from its amino acid sequence appears to be a lipid-binding protein (2; K. S. Cook, C. R. Hunt, and B. M. Spiegelman, J. Cell. Biol., in press), and another of 28 kDa also accumulate during adipose differentiation (18). cDNA clones encoding these three proteins have been isolated, and with their use it has been shown that the corresponding mRNAs increase over 100-fold during differentiation (17). These changes are at least in part determined at the level of gene transcription (Cook et al., in press; Djian, P., M. A. Phillips, and H. Green, submitted for publication). Using the cDNA probes, we examined a number of other tissues for the presence of these mRNAs.

3T3-F442A cells (6) were maintained as preadipocytes in the Dulbecco-Vogt modification of Eagle medium supplemented with 6% cat serum and 1% calf serum. Adipocytes were obtained from the same cell line after confluent cultures were allowed to differentiate for 10 days in medium supplemented with 10% fetal bovine serum and insulin at 5 μg/ml.

Total cellular RNA was prepared from tissues of CD-1 mice and from 3T3 adipocytes and preadipocytes by extraction in 6 M guanidine thiocyanate and pelleting through 5.7 M CsCl, essentially as described by Maniatis et al. (12). mRNA was selected by oligodeoxythymidylate cellulose chromatography (1) and quantitated by hybridization to [^{3}H]polyuridylate (3). Polyadenylated RNA was electrophoresed on formaldehyde-containing agarose gels (12) and transferred to GeneScreen (New England Nuclear Corp.). The filters were UV irradiated and hybridized to ^{32}P-labeled cDNA probes (15) by the method of Church and Gilbert (4), with the further addition of denatured salmon sperm DNA (100 μg/ml) to the hybridization solution. After washing (4), the filters were exposed to prefogged Kodak XAR-5 film (11) at −70°C with an intensifying screen.

All mRNA preparations gave rise to well-developed bands when probed with cDNA for actin (17), demonstrating the integrity of the mRNA preparations, including those which gave no signal with the three cDNA probes of interest. mRNA of adipose 3T3 cells hybridized strongly with all three probes (Fig. 1). The intensity of each band was assigned a value of 100 (Table 1). As described previously (17), preadipose cells contained no mRNA for glycerophosphate dehydrogenase or the 28-kDa protein, but they did possess mRNA for the 13-kDa protein at a level about 2% that of the adipose cells.

Except for spleen tissue, the mRNA for glycerophosphate dehydrogenase was present in all tissues examined (Fig. 1 and Table 1). This was expected, since previous studies by Kozak and colleagues had demonstrated that glycerophosphate dehydrogenase and, when examined, its mRNA were present in these tissues (8, 9, 14). The mRNA appeared to be of similar size in all of the tissues we examined.

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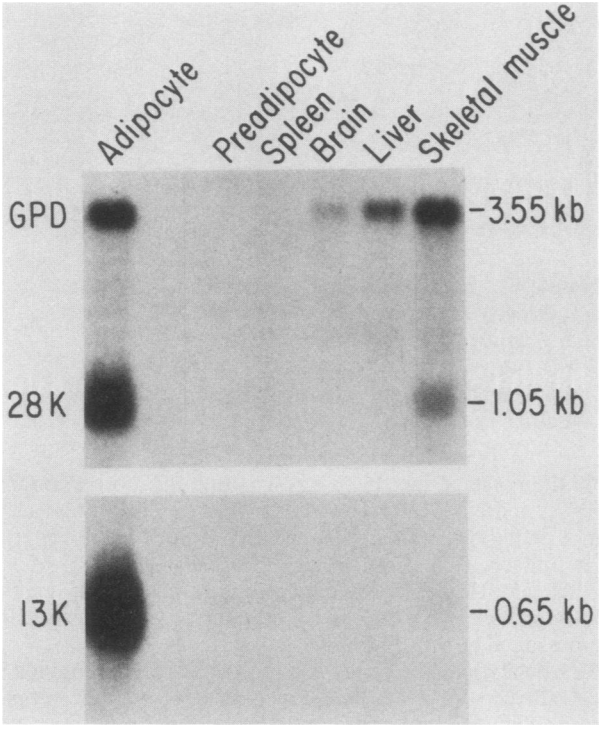

FIG. 1. Northern blot analysis showing mRNAs present in 3T3 adipocytes, preadipocytes, and various mouse tissues. Three micrograms of polyadenylated RNA was loaded per lane of a 1.4% agarose gel containing formaldehyde. After electrophoresis, RNA was transferred to GeneScreen and hybridized to the ^{32}P-labeled plasmids PGPD-1 and PAd-20 (17), which contain cDNA probes for the mRNAs of glycerophosphate dehydrogenase (GPD) and the 28-kDa protein, respectively. The blot was then dehybridized (4) and hybridized with plasmid PAd-5 (17), a probe for the mRNA of the 13-kDa protein.

The mRNA for the 28-kDa protein was present in significant amounts in skeletal muscle obtained from both legs (Fig. 1 and Table 1) and diaphragms. Trace amounts of the mRNA (≤1% that in adipose cells) were found in heart and kidney, but it is possible that this was due to contaminating adipose tissue. No tissue examined contained abundant mRNA for the 13-kDa protein, although it was detected in heart, kidney, and muscle in amounts similar to or a little higher than that in preadipose cells. Of the three mRNAs, the one for the 13-kDa protein appears to be the most specific to adipose tissue. Recent determination of the sequence of this protein from cloned cDNAs has shown that it possesses homology to the P2 protein of myelin, the fatty acid-binding proteins of liver and intestine, and the cellular retinoic acid-binding protein (2; Cook et al., in press). It is evidently present in small amounts in other cell types but is concentrated most specifically in adipocytes. The mRNA for the 28-kDa protein was the next most specific for adipocytes, and the combination of the two was elevated strongly in adipose cells alone. The mRNA of glycerophosphate dehydrogenase is so widely distributed that it adds comparatively little to the specificity of the adipocyte markers.

TABLE 1. Relative amounts of mRNA present in 3T3 preadipocytes, adipocytes, and various mouse tissues, as estimated by densitometric scans of Northern blots

Cell	Relative amt of mRNA for:		
	Glycerophosphate dehydrogenase	28-kDa protein	13-kDa protein
3T3-F442A cells			
Adipocytes	100	100	100
Preadipocytes	0[a]	0	2
Tissues			
Brain	9	0	0
Liver	18	0	0
Heart	4	1	9
Kidney	29	<1	3
Skeletal muscle	78	17	3
Spleen	0	0	0

[a] mRNA undetected, even on long exposures of the film.

The experiments described here support the concept that it is the assortment of active genes and their quantitative expression that gives adipocytes their unique character. There is still no example in this cell type of an active gene that is completely silent in other cell types.

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