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Tumor Promoter-Inducible Genes Are Differentially Expressed in the Developing Mouse

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TIS genes are rapidly and transiently induced by tetradecanoyl phorbol acetate in 3T3 cells. We analyzed the developmental appearance of a number of the TIS genes to determine whether, in a normal physiological context, these genes have common or distinct mechanisms of regulation. Each TIS gene has a distinct tissue specificity and/or developmental profile.

Tetradecanoyl phorbol acetate (TPA), the most potent of the phorbol ester tumor promoters, has a variety of effects on target cells. These include tumor promotion in animals, promotion of transformation in cell cultures, modulation of differentiation and cell division, induction of gene amplification, and inhibition of metabolic cooperation. To study the mechanisms of phorbol ester-induced mitogenesis, we cloned cDNAs for genes whose expression is induced by TPA in Swiss 3T3 cells (13). These TPA-induced sequences, which we call TIS genes, (i) are induced rapidly (increased TIS mRNA levels are present within minutes), (ii) are induced transiently (TIS mRNA levels peak between 30 and 120 min, and then decline to basal levels within 180 to 240 min), (iii) are "primary response genes" (16) (TPA-induced increases in TIS mRNAs do not require protein synthesis), and (iv) are "superinducible" by TPA in the presence of cycloheximide. Their rapid, transient induction and cycloheximide superinducibility are similar to the induced expression of the c-*fos* proto-oncogene (6, 7). Hybridization experiments demonstrated that the TIS28 cDNA is a partial clone of the c-*fos* gene (13).

c-*fos* gene expression is induced by many biological agents, including growth factors, hormones, and agents that stimulate differentiation. Thus, c-*fos* expression is induced in 3T3 cells by the mitogens epidermal growth factor and fibroblast growth factor and in PC12 pheochromocytoma cells by both nerve growth factor and depolarization (3, 7, 9). Similarly, epidermal growth factor and fibroblast growth factor stimulate the expression of the TIS genes in 3T3 cells (13), and nerve growth factor and depolarization induce the expression of a number of TIS genes in PC12 cells (10). These data suggest that common control mechanisms may regulate the expression of many of the TIS genes. We examine here the expression of the TIS genes in mice during postnatal development. If all TIS genes are expressed in parallel in developing animals, one might conclude that their modes of regulation are similar. On the other hand, if the expression of TIS genes show distinct tissue-specific and/or developmental patterns, there must exist gene-specific differences in the molecular mechanisms regulating their expression.

We first compared the expression of the TIS genes in various organs of adult Swiss Webster mice (Simonsen Labs). Animals were sacrificed by cervical dislocation, and frozen tissues were homogenized in 4 M guanidinium thiocyanate–25 mM sodium citrate (pH 7.0)–0.5% sodium sarcosine–0.1 M 2-mercaptoethanol. RNA was isolated by the method of Chomczynski and Sacchi (1), and equal amounts (15 μg) were separated by electrophoresis (13). Ethidium bromide (1 ng) was added to each sample before loading to allow quantitation of rRNA (15). Gels were illuminated with UV light and photographed. rRNA was quantitated by scanning the negative with an Ultroscan XL laser densitometer. Transfer of RNA to nitrocellulose and hybridization with TIS gene cDNAs were performed as described previously (13). Figure 1 shows an example of pairwise comparisons of TIS gene expression in organs of adult mice. TIS21 mRNA was expressed to a greater extent in adult thymus than in adult brain. In contrast, TIS1 mRNA expression was greater in brain than in thymus. The TIS21 and TIS1 genes, both isolated as TPA-inducible sequences in Swiss 3T3 cells, are differentially expressed in adult mouse brain and thymus.

One may also compare the developmental expression of TIS genes within a single type of tissue. Differences in developmental expression patterns for two TIS genes in a single type of tissue would demonstrate that the two genes are not regulated in the same way. For example, the TIS1 gene was more actively expressed in adult than in newborn testis (Fig. 2). In contrast, TIS21 was more actively ex-

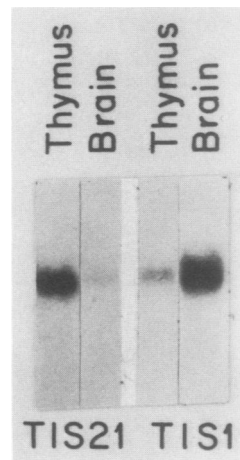

FIG. 1. Differential expression of TIS21 and TIS1 in adult mouse brain and thymus. RNA preparation, electrophoresis, transfer, and probing are described in the text.

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TABLE 1. Expression of TIS genes in mouse postnatal development[a]

Tissue	Age	Expression of:					
		TIS1	TIS7	TIS8	TIS10	TIS11	TIS21
Brain	Newborn	—	0.59	0.15	—	—	—
	5 days	—	0.44	—	—	—	—
	10 days	—	0.37	—	—	—	—
	21 days	0.97	0.40	1.00	—	—	—
	Adult	0.73	0.66	0.80	0.34	—	0.11
Heart	Newborn	0.40	—	0.12	—	0.42	0.12
	5 days	0.35	—	—	—	0.33	0.19
	10 days	0.12	—	—	—	0.27	0.29
	21 days	0.20	—	—	—	0.21	0.35
	Adult	—	—	—	—	0.18	—
Thymus	Newborn	0.56	0.54	0.40	1.00	1.00	1.00
	5 days	0.53	0.66	0.45	0.14	0.74	0.98
	10 days	0.71	0.73	0.30	0.18	0.59	0.92
	21 days	0.89	0.15	0.22	—	0.33	0.78
	Adult	0.22	—	0.12	—	0.25	0.42
Spleen	Newborn	—	0.15	—	—	—	0.20
	10 days	—	0.31	—	—	—	0.26
	21 days	—	0.22	—	—	0.40	0.22
	Adult	—	0.11	—	—	0.14	0.20
Lung	Newborn	—	0.35	—	—	—	0.19
	5 days	—	0.27	0.14	—	0.10	0.43
	10 days	—	0.22	0.15	—	0.25	0.31
	21 days	—	0.44	0.23	0.16	0.38	—
	Adult	—	0.40	—	0.23	0.12	—
Muscle	Newborn	—	0.22	0.14	—	—	—
	10 days	—	—	—	—	—	—
	21 days	0.22	0.16	—	—	0.13	—
	Adult	0.95	0.21	—	—	0.15	—
Kidney	Newborn	0.11	0.13	—	0.15	0.17	0.29
	10 days	0.13	0.10	0.31	—	0.20	0.26
	21 days	ND	ND	0.27	—	0.26	0.20
	Adult	—	0.15	0.10	—	0.13	0.14
Liver	Newborn	—	0.25	—	—	0.18	0.37
	Adult	—	—	—	—	0.27	—
Testis	Newborn	—	0.48	0.16	—	—	0.23
	10 days	—	0.54	0.33	0.10	—	0.38
	21 days	0.18	0.60	—	0.13	0.11	0.11
	Adult	1.00	1.00	—	0.21	0.14	—
3T3 cells		10.9	0.54	1.92	1.35	1.34	0.85

[a] TIS gene expression was determined by quantitative densitometry of autoradiograms and normalized relative to the staining intensity of rRNA. Values are relative expression levels in each tissue, with 1.00 being the highest in vivo expression level. The levels for mitogen-stimulated 3T3 cells are given as references. Values are averages from two to four different experiments. ND, Not done. —, Either no mRNA could be observed or a barely detectable level was present.

pressed in newborn than in adult testis (Fig. 2). Clearly, TIS21 and TIS1 are expressed according to different programs during testicular development. Similarly, the expression of TIS11 and TIS21 differs during liver development. TIS11 was expressed in essentially equal levels in newborn and adult liver, while TIS21 expression was greater in newborn than in adult liver (Fig. 2).

A quantitative comparison of the developmental expression of the TIS1, TIS7, TIS8, TIS10, TIS11, and TIS21 genes is presented in Table 1. TIS8 was maximally expressed in 21-day-old brain. Both TIS1 and TIS7 were maximally expressed in adult testis. However, the patterns of expression of these two genes in other tissues (e.g., brain, heart, and muscle) differed substantially. TIS10, TIS11, TIS21 were all maximally expressed in newborn thymus. TIS10 expression could not be observed in heart, spleen, or muscle at any time point; TIS11 and TIS21 expression could easily be detected in these tissues. Although TIS11 expression and TIS21 expression appeared more similar to one another than did that of any other two TIS genes (e.g., low at all times in brain, highest in newborn thymus, low in muscle), the developmental profiles of these genes in lung (Table 1) and liver (Table 1 and Fig. 2) differed. The expression of each TIS gene has unique developmental and tissue distribution profiles.

Other laboratories searching for mitogen-responsive (2, 4, 5, 11, 12) or nerve growth factor-responsive (14) genes have also described transiently induced primary response genes.

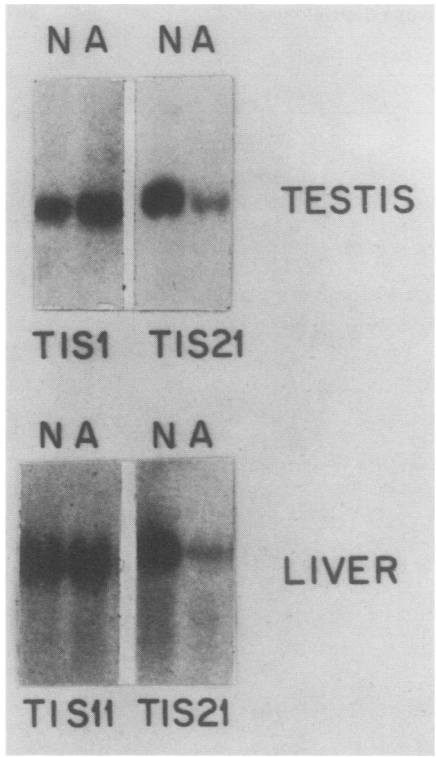

FIG. 2. Distinct developmental profiles of TIS1, TIS21, and TIS11. RNA preparation, electrophoresis, transfer, and probing are described in the text. N, Newborn; A, adult.

The TIS genes, the "competence" genes (2), and the "immediate-early" genes (11, 12) show some similarities in the kinetics of expression and ligand responsiveness, suggesting that these may be overlapping families. Johnson et al. (8) isolated a TPA-inducible clone, TPA-S1, from C3H10T1/2 cells. TPA-S1 is not a member of the TIS gene family. TPA-S1 mRNA reaches maximal levels 9 h after exposure to TPA. The TIS genes are maximally expressed by 0.5 to 2 h (13). Induction of TPA-S1 is not superinduced in the presence of cycloheximide (8), in contrast to the TIS genes (13). Sequence analysis suggests that TPA-S1 is closely related to two other recently cloned cDNAs (8). The developmental appearance of TPA-S1, the competence genes, and the immediate-early genes has not been described.

The TIS genes were first described as members of a family induced in response to a single agent (13). They demonstrate similar patterns of response to several inducing agents in two cell culture systems (10, 13). However, they clearly are not coordinately regulated during murine development. Each TIS gene has a unique pattern of tissue specificity and/or developmental profile. Although elucidation of the mechanism of expression of one TIS gene (e.g., c-*fos*) may prove useful in understanding the regulation of the others, each TIS gene apparently has unique regulatory components.

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