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Granulocyte-Macrophage Colony-Stimulating Factor and Tetradecanoyl Phorbol Acetate Induce a Distinct, Restricted Subset of Primary-Response TIS Genes in Both Proliferating and Terminally Differentiated Myeloid Cells

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Induction of early-response genes (tetradecanoyl phorbol acetate [TPA]-induced sequences, or TIS genes; R. W. Lim, B. C. Varnum, and H. R. Herschman, Oncogene 1:263–270, 1987) by granulocyte-macrophage colony-stimulating factor (GM-CSF) and TPA was examined both in a factor-dependent murine cell line, 32D clone 3, and in mature human neutrophils. When GM-CSF-deprived 32D clone 3 cells were exposed to GM-CSF or to TPA, four TIS mRNAs (TIS7, TIS8, TIS10, and TIS11) were rapidly and transiently induced. However, neither GM-CSF nor TPA could induce accumulation of TIS1 mRNA in 32D clone 3 cells, even under superinducing conditions. Both GM-CSF and TPA also elicited rapid, transient expression of TIS8 and TIS11 mRNA in postmitotic human neutrophils. However, neither agent could induce accumulation of TIS1 mRNA in human neutrophils. TIS1 is a member of the nuclear receptor supergene family that codes for ligand-dependent transcription factors. Cell-type restriction of inducible transcription factors may contribute to developmental specification.

We have isolated a family of cDNA clones induced as primary responses (32) in Swiss 3T3 cells by the tumor promoter-mitogen tetradecanoyl phorbol acetate (TPA). We refer to these as TPA-inducible sequences, or TIS cDNAs (17). All of the TIS mRNAs are also induced in 3T3 cells by serum, epidermal growth factor (EGF), and fibroblast growth factor (18). Each TIS mRNA demonstrates a unique developmental profile (25), which suggests that each TIS gene has unique regulatory features. Several other laboratories have isolated similar sets of primary response genes from mitogen-stimulated 3T3 cells (1, 4, 15) and from nerve growth factor (NGF)-treated PC12 cells (19, 20, 26). It is likely that these gene products play important roles in signal transduction, regulating both cell division and differentiation.

TIS28 has previously been identified as c-*fos* (17). Sequence data (29) demonstrated that the TIS8 cDNA is identical to the serum-induced cDNA variously identified as egr-1 (24), KROX-24 (16), and zif/268 (3), a zinc finger-containing protein that presumably binds to DNA. We have recently completed the sequence of the TIS1 cDNA (data not shown). This cDNA has also been cloned as the NGF-inducible sequence NGF1B (20) and the serum-inducible clone nur77 (12). It encodes a protein with substantial similarity to the nuclear receptor gene superfamily that includes the glucocorticoid, estrogen, thyroid hormone, and retinoic acid receptors. The TIS1 protein is, like the other members of this family, likely to be a transcription factor. The sequence of the TIS7 cDNA has also been completed (B. C. Varnum, R. W. Lim, and H. R. Herschman, Oncogene, in press). A 3′ fragment of this cDNA was previously cloned as a Newcastle disease virus-inducible message (23). A rat homolog, PC-4, has also been cloned from an NGF-induced PC12 library (26). The properties of the predicted proteins are summarized in Table 1.

Expression of c-*fos*, the prototype TIS gene, is induced in a variety of cells in response to an extraordinarily wide range of extracellular signals (6). The independent isolation of TIS1, TIS7, and TIS8 as TPA (17, 29)-, serum (3, 16, 24)-, and NGF (19, 20, 26)-inducible sequences suggests that all TIS genes may demonstrate promiscuous induction. However, whereas the majority of TIS genes could be induced by TPA, EGF, or NGF in PC12 cells, TIS10 mRNA could not be detected (14), suggesting that cell type restriction of TIS gene expression may exist for subsets of these early-response genes. We did not, however, distinguish in that study between the possibilities of species specificity (PC12 cells are rat cells, and the TIS10 cDNA is from a murine mRNA) and cell-type-specific restriction of TIS10 expression. The protein synthesis inhibitor cycloheximide, when present with inducers, causes superinduction of TIS mRNAs in 3T3 cells (17). Even under superinducing conditions, PC12 cells were unable to accumulate any TIS10 mRNA after exposure to TPA or EGF (Fig. 1). In contrast, TIS1 mRNA was easily detected, demonstrating that RNA prepared from the PC12 cells was not degraded. However, the murine TIS10 cDNA probe hybridized to a cross-reacting mRNA in Rat-1 cells; had any TIS10 mRNA been present in the PC12 cells, it would have been detected. The ability of the TIS10 gene to be induced by a variety of agents (TPA, EGF, and NGF) is extinguished in PC12 cells.

To further examine the question of restricted TIS gene induction, we turned to cells of the myeloid lineage. Granulocyte-macrophage colony-stimulating factor (GM-CSF), a

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TABLE 1. Characteristics of TIS genes

Gene	Characteristic(s)	Other isolate(s)	Additional reference(s)
TIS1	DNA-binding domain; member of nuclear receptor gene superfamily	NGF1B, nur77	12, 20
TIS7	Some sequence similarity to interferons	MIF 20/11, PC-4	23, 26, 30
TIS8	Zinc finger-containing protein; presumptive DNA-binding protein	egr-1, KROX-24, zif/268, NGF1A	3, 16, 19, 24
TIS10	Sequence not finished	Unique	
TIS11	No sequence motif indicating function	Unique	29
TIS28	Transcriptional modulator	c-*fos*	17

glycoprotein produced by activated T lymphocytes and by certain fibroblasts and endothelial cells exposed to specific cytokines (21, 31), stimulates the proliferation of bone marrow progenitors that give rise to monocytes as well as neutrophilic and eosinophilic granulocytes (27, 28). The biological activity of GM-CSF appears to be mediated by a single class of high-affinity receptors (7, 10). Orlofsky and Stanley (22) previously demonstrated GM-CSF induction of *fos*, *myc*, and JE mRNA in a murine factor-dependent cell line, BAC1.2F5.

32D clone 3 cells, a cell of myeloid lineage (11), require GM-CSF or interleukin-3 (multi-CSF) for continued proliferation. To examine the effect of GM-CSF and TPA on the expression of TIS genes in 32D clone 3 cells, cultures were first incubated overnight in the absence of GM-CSF and then treated with GM-CSF or TPA. The GM-CSF concentration used in these experiments was fivefold greater than that required to maximally restimulate proliferation of 32D clone 3 cells (unpublished observations). TIS8, TIS10, and TIS11 mRNAs were virtually undetectable in control, untreated cultures and were rapidly and transiently induced by both GM-CSF and TPA (Fig. 2A). Untreated 32D clone 3 cells expressed detectable levels of TIS7 mRNA. Both TPA and GM-CSF induced modest expression of this gene. In marked contrast to the results for the other four TIS genes, no induction of TIS1 expression could be detected in 32D clone 3 cells in response to either GM-CSF or TPA.

TIS1 can be superinduced in 3T3 cells (17) and in PC12 cells (Fig. 1) in the presence of cycloheximide. To examine at greater sensitivity the lack of TIS1 expression in 32D clone 3 cells, we combined GM-CSF and TPA treatments with cycloheximide exposure. TIS11 expression in response to both GM-CSF and TPA was superinduced by cycloheximide in 32D clone 3 cells (Fig. 2B). In contrast, TIS1 mRNA was undetectable, even under conditions of cycloheximide superinduction. RNA from TPA-induced 3T3 cells was analyzed on the same gel and subjected to the same blotting procedures and hybridization analysis (Fig. 2B, lane 7). Had

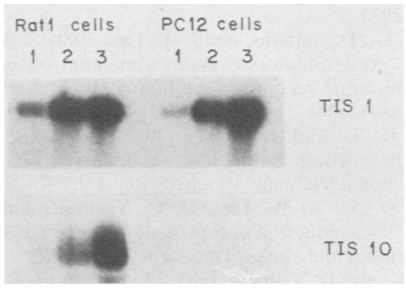

FIG. 1. Superinduction analysis of TIS10 and TIS1 mRNA accumulation in Rat-1 and PC12 cells. Cells were exposed to cycloheximide (Sigma Chemical Co.) alone (10 μg/ml; lane 1), cycloheximide plus TPA (Pharmacia, Inc.) (50 ng/ml; lane 2), or cycloheximide plus EGF (Collaborative Research, Inc.) (5 ng/ml; lane 3) for 3 h. TPA was dissolved in acetone at a concentration of 1 mg/ml. Rat-1 and PC12 cells were grown and treated with growth factors as described previously (14, 17). Total RNA was prepared and analyzed by Northern (RNA) analysis (14, 17). Each lane contained 10 μg of RNA.

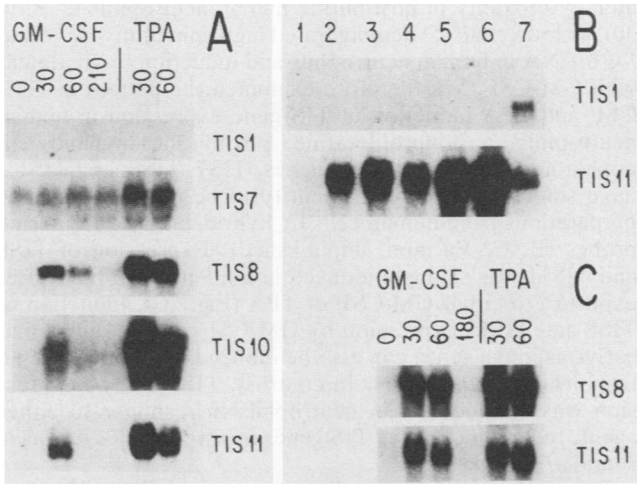

FIG. 2. (A) Induction of TIS genes by GM-CSF and TPA in 32D clone 3 cells. Factor-dependent 32D clone 3 cells were maintained in Iscove modified Dulbecco medium supplemented with 10% fetal calf serum and recombinant murine GM-CSF prepared by transfection of COS cells with the murine GM-CSF cDNA in the pXM vector. COS supernatant containing murine GM-CSF was diluted 1:1,000 to maintain cell growth. Cells were washed extensively and incubated overnight in the absence of factor before initiation of the experiment. The next day, cells were treated with GM-CSF (1 nM) or TPA (50 ng/ml) for the times shown (in minutes). RNA was isolated by the method of Holmes and Bonner (13) and subjected to electrophoresis. After transfer to nitrocellulose, the blots were hybridized with labeled probes for detection of TIS mRNAs. The zero time point is an untreated culture. Each lane contained 10 μg of RNA. (B) Superinduction analysis of the TIS1 and TIS11 genes in 32D clone 3 cells. Lanes: 1, control; 2, GM-CSF (1 nM) for 30 min; 3, TPA (50 ng/ml) for 30 min; 4, cycloheximide (10 μg/ml) for 3 h; 5, GM-CSF plus cycloheximide for 3 h; 6, TPA plus cycloheximide for 3 h; 7, RNA from 3T3 cells treated with TPA. Each lane contained 10 μg of RNA. RNA samples for the two hybridizations were run on a common gel and blotted to a single filter. The filter was then cut in half for hybridization with the TIS1 and TIS11 probes. (C) Induction of TIS genes by GM-CSF and TPA in human neutrophils. Cells were prepared by layering heparinized blood from normal donors over mono-poly-resolving media (Flow Laboratories, Inc.). The gradients were centrifuged at 1,000 × *g* for 30 min, and a band consisting of ≥95% neutrophils was isolated. Cells were treated with GM-CSF (1 nM) or TPA (50 ng/ml) for the times shown (in minutes). RNA was isolated by the method of Holmes and Bonner (13). Each lane contained 15 μg of total RNA.

TABLE 2. Inducibility of TIS genes

Gene	Inducibility in:									
	3T3 cells			PC12 cells			32D clone 3 cells		Human neutrophils	
	TPA	EGF	FGF	TPA	EGF	NGF	TPA	GM-CSF	TPA	GM-CSF
TIS1	+	+	+	+	+	+	−	−	−	−
TIS7	+	+	+	+	+	+	+	+	NT[a]	NT
TIS8	+	+	+	+	+	+	+	+	+	+
TIS10	+	+	+	−	−	−	+	+	NT	NT
TIS11	+	+	+	+	+	+	+	+	+	+

[a] NT, Not testable.

TIS1 mRNA been present in 32D clone 3 cells treated with either TPA or GM-CSF, it would have easily been detected.

GM-CSF also causes changes in forward light scatter, degranulation, enhanced chemotaxis, phagocytosis of a variety of microorganisms, and antibody-dependent cell-mediated cytotoxicity in postmitotic human neutrophils (2, 8, 9, 30). Colotta et al. (5) demonstrated high constitutive levels of *fos* mRNA in human neutrophils and induction by treatment with GM-CSF. We therefore examined the pattern of GM-CSF and TPA induction of TIS gene expression in human neutrophils, a nonproliferating, postmitotic myeloid cell population. Three of the TIS genes, TIS1, TIS8, and TIS11, have sufficient sequence similarity to be detected in RNA preparations from human cells by hybridization with murine probes (B. C. Varnum, unpublished). Expression of TIS8 and TIS11 was strongly induced in human neutrophils after exposure to either GM-CSF or TPA (Fig. 2C). Induction of TIS8 and TIS11 expression by GM-CSF clearly shows that early-response genes can also be induced in a nonmitogenic response to such factors. In contrast, TIS1 mRNA expression was not induced in neutrophils in response to either agent. Inducibility of the TIS genes in several cell systems is summarized in Table 2.

The TIS1 gene appears to be silenced in both proliferative and postmitotic myeloid cells and cannot be induced, even if the cell surface receptor for GM-CSF is bypassed and the protein kinase C second-messenger system is directly activated. In contrast, a different gene, TIS10, is silenced in PC12 cells and cannot be induced by a variety of growth factors or by TPA. Clearly, TIS10 expression is not required for PC12 proliferation, and TIS1 expression is not essential for 32D clone 3 proliferation or for neutrophil function.

Our data with PC12 cells, 32D clone 3 cells, and human neutrophils demonstrate that the subset of TIS genes induced varies from one cell type to another after activation of a second-messenger pathway common to all of these cells. These restrictions in TIS gene response may reflect cell-specific differences in either chromatin structure or nuclear factors necessary for expression of specific TIS genes. Coordinate expression of specific subsets of TIS genes may play an important role in the different responses of distinct cell types to a variety of hormones, growth factors, and other biological response modifiers. The silencing of the TIS1 gene, which codes for a potential transcription factor likely to activate a subset of secondary-response genes after its induction, may reflect a part of the specification of myeloid cells during development.

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