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Transient Expression of Foreign Genes in Lymphoid Cells Is Enhanced by Phorbol Ester

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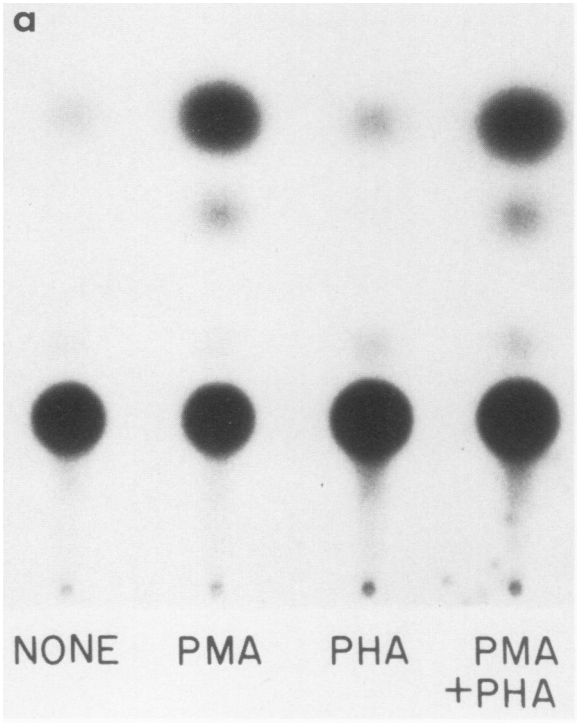
In lymphoid cells and an erythroleukemia cell line, phorbol myristate acetate significantly enhanced (up to 90-fold) expression of chloramphenicol acetyltransferase driven by four unrelated viral transcriptional units. The results suggest that phorbol myristate acetate acts via a general mechanism to enhance gene expression in these cells and provides a convenient means for increasing transient expression of foreign DNA.

Phorbol myristate acetate (PMA) is known to influence gene expression in numerous biologic systems (8, 11, 15). In particular, treatment of T lymphocytes with PMA and phytohemagglutinin (PHA) results in their activation characterized by changes in rate of cellular proliferation or induction of T-cell-specific genes including interleukin 2 and the interleukin 2 receptor (7, 13). We investigated the influence of these compounds on transient expression of foreign DNA in T cells as well as in other cell types. We began by examining whether activation would permit a general increase in expression of foreign genes in the human T-cell line Jurkat (3). In Jurkat cells, PHA treatment alone results in a slight degree of activation as measured by induction of interleukin 2 (3). PMA alone does not result in activation of Jurkat cells but greatly increases the activation seen with PHA (3).

We examined the effects of PHA and PMA, alone or in combination, on expression of pSV2-CAT. pSV2-CAT contains the simian virus 40 (SV40) promoter and enhancer elements linked to the coding sequence of the bacterial chloramphenicol acetyltransferase (CAT) gene (6). Jurkat cells (10^7 per treatment) were transfected with 5 μg of pSV2-CAT by the DEAE-dextran procedure of Queen and Baltimore (12) with minor modifications (8a). PMA (10 ng/ml) and PHA (1 μg/ml) were added immediately after transfection, and cells were assayed for CAT activity 48 h later (8a). The results (Fig. 1a) showed that PHA had little effect on the expression of pSV2-CAT. In contrast, PMA treatment resulted in a significant increase (greater than 10-fold) in CAT activity from pSV2-CAT. The combination of PMA plus PHA resulted in CAT activity similar to that seen with PMA alone. To optimize the PMA effect, dose-response and time course studies were performed. Maximum enhancement was achieved with PMA concentrations between 20 and 100 ng/ml (Fig. 1b). Thus, we empirically selected a concentration of 25 ng of PMA per ml for use in further experiments. Time course studies (Table 1) showed that the effect of PMA was rapid, resulting in greater than 10-fold enhancement with as little as 6 h of exposure to PMA (added 42 h after transfection and only 6 h before CAT determination). Maximum enhancement was achieved with 24 h of exposure to PMA, but longer treatment resulted in decreased CAT gene expression, presumably because of toxic effects of the compound. Thus, for the remainder of the



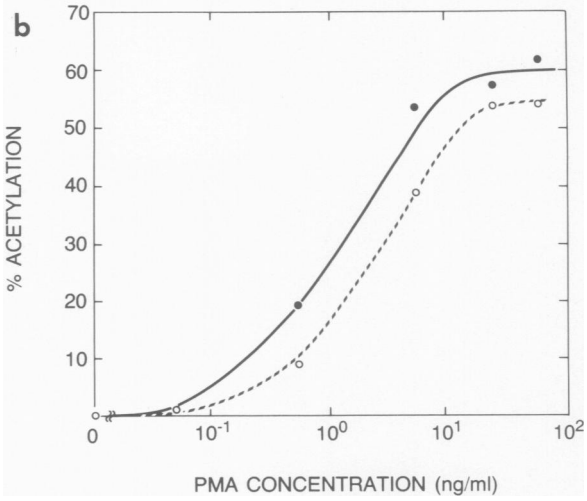


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TABLE 1. Effect of time of exposure to PMA on transient expression of pSV2-CAT in Jurkat cells

Expt no. and time of PMA addition (h posttransfection)	Total h of PMA treatment	Relative pSV2-CAT expression[a]
1		
No PMA	0	1
48	0	0.9
43	5	32
38	10	43
24	24	51
0	48	9
2		
No PMA	0	1
42	6	13
24	24	42
0	48	14

[a] The values shown are ratios of CAT activity seen for the given times of PMA treatment relative to that seen in the absence of PMA treatment.

experiments, cultures were treated with PMA 24 h after transfection.

The ability of PMA to enhance pSV2-CAT expression in other cell types was examined (Table 2). The effect of PMA was not restricted to T cells but also occurred in the B-cell line Raji and the erythroleukemia cell line K562. Slight enhancement was observed with the human hepatoma cell line HepG2, but PMA had no effect on either of the two fibroblast cell lines or HeLa cells.

To examine the regulatory sequence specificity of the PMA effect, transfection experiments were performed with several other viral vectors. These included pRSV-CAT, pHTLVI-CAT, and pGALVSEATO-CAT, which contain the long terminal repeats (and therefore promoter and enhancer elements) of Rous sarcoma virus (5), human T-cell lymphotropic virus type I (14), and the SEATO strain of gibbon ape leukemia virus (GALV) (8a) linked to the CAT gene. The results (Table 3) showed that in hematopoietic cells the effect of PMA was not restricted to vectors containing SV40 regulatory sequences. CAT gene expression from all of the recombinant vectors tested was increased by PMA to some degree. Interestingly, enhancement of expression by PMA for different vectors varied considerably from one cell line to the next and most likely reflects interaction with as yet unidentified cell-specific transcriptional factors. In HepG2 cells, pHTLVI-CAT expression, like that of pSV2-CAT, was slightly increased by PMA. In HeLA cells, however, no enhancement of expression was seen with any of the recombinant plasmids.

Evidence that the increased CAT activity seen in transfected cells is associated with increased accumulation of CAT gene-specific mRNA is shown in Fig. 2. Poly(A)$^+$ RNA from K562 cells transfected with pSV2-CAT or pHTLVI-CAT and subsequently treated with or without PMA was analyzed by Northern analysis. A radiolabeled probe prepared from a purified fragment containing CAT gene cDNA sequences was used to identify CAT gene-specific mRNA. As with CAT activity, CAT gene mRNA levels were significantly increased in pHTLVI-CAT- and pSV2-CAT-transfected cells treated with PMA. We obtained similar results with Jurkat cells.

To determine whether the PMA enhancement was dependent on enhancer regions, the plasmids listed in Table 4 were transfected into MLA 144 cells, and CAT gene expression was determined with or without PMA treatment. Little CAT expression was seen with the plasmid pA10CAT, containing the SV40 promoter deleted of its enhancer (10), regardless of PMA treatment. However, with plasmids containing the SV40 promoter and either the SV40 enhancer (pSV2-CAT)

FIG. 1. Expression of pSV2-CAT is enhanced by PMA. (a) Jurkat cells (10^7 per treatment) were transfected with 5 μg of pSV2-CAT. Replicate cultures were either left untreated (NONE) or treated with PHA (1 μg/ml), PMA (10 ng/ml), or both PHA and PMA for 48 h, after which cells were harvested and the level of CAT gene expression was determined. Shown are the results of a typical CAT assay. Lower spots represent unacetylated chloramphenicol; higher spots are acetylated derivatives. (b) Dose-response curve for PMA enhancement. Replicate cultures of Jurkat cells were treated (24 h after transfection) with various doses of PMA. CAT activity was determined after an additional 24 h. Results are shown for two separate experiments.

TABLE 2. PMA enhances transient expression of pSV2-CAT in a variety of cell types

Cell line[a]	Description	Range and mean of relative CAT activity (fold enhancement by PMA)[b]	
		Range	Mean
Jurkat	Human T cell	35–97	60 (8)
MLA144	Ape T cell	7–24	15 (4)
EL-4	Murine T cell	10–13	11 (3)
Raji	Human B cell	2–3	2.8 (4)
K562	Human erythroleukemia	14–64	34 (4)
HepG2	Human hepatoma	2–4	2.5 (5)
HeLa	Human epithelial	0.6–0.7	0.6 (3)
CV-1	Simian fibroblast	0.7–2.0	1.4 (4)
3T3	Murine fibroblast	0.6–1.3	1.0 (3)

[a] Jurkat, EL-4, Raji, and K562 cells were transfected as described in the text. MLA 144 cells were transfected by the modified DEAE-dextran procedure of Gopal (4). HepG2, HeLa, CV-1, and 3T3 cells were transfected by the calcium phosphate precipitation method (5).

[b] Relative CAT activity refers to the ratio of CAT activity seen with PMA treatment to that seen in the absence of PMA. Numbers in parentheses indicate the number of separate experiments from which the mean values were derived. With one-half of the total extract from 10^7 transfected cells in a 3-h CAT assay, the mean percentages of conversion of [^{14}C]chloramphenicol to its acetylated derivatives in the absence of PMA were as follows: Jurkat, 1.4%; MLA 144, 0.3%; El-4, 2.5%; Raji, 16.5%; and K562, 0.8%. With one-fifth of the total extract from a 60-mm (diameter) dish of transfected cells in a 20-min CAT assay (60 min for 3T3 cells), the mean percentages of conversion were as follows: HepG2, 18%; HeLa, 29%; CV-1, 48%; and 3T3, 27%.

TABLE 3. Enhancement of transient gene expression with different transcriptional control elements linked to the CAT gene

Cell line	Relative CAT activity[a]			
	pSV2-CAT	pRSV-CAT	pHTLVI-CAT	pGALVSEATO-CAT
Jurkat	60	4.2	4.4	44
MLA144	15	5.3	34	1.0
K562	34	2	24	1.2
HepG2	2.5	1.7	2.4	0.8
HeLa	0.6	0.7	ND	0.8

[a] CAT activity is expressed as mean fold enhancement obtained with PMA treatment relative to untreated cultures. Values were derived from a minimum of three separate experiments. Conditions of assay were varied to obtain values within the linear range of activity. Cells: Jurkat, 50% extract and 3-h assay for all vectors; MLA 144, 50% extract and 3-h assay for pSV2-CAT and pRSV-CAT but 20% extract and 60-min assay for pHTLVI-CAT and 20% extract and 20-min assay for pGALVSEATO-CAT; K562, 50% extract and 3-h assay for pSV2-CAT and pRSV-CAT but 20% extract and 1- to 2-assay for pHTLVI-CAT and pGALVSEATO-CAT. ND, Not done.

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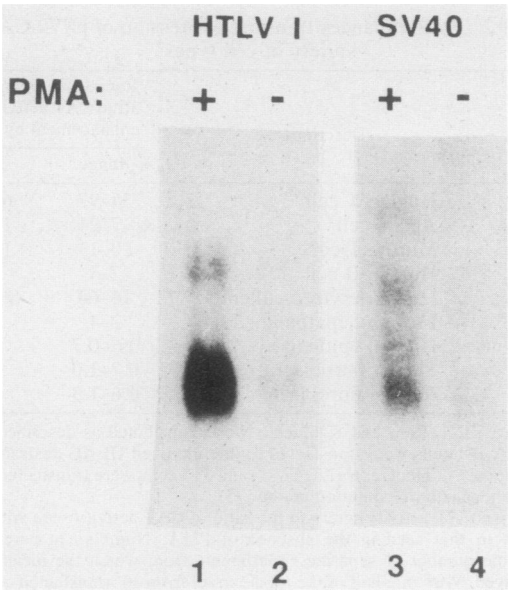

FIG. 2. Northern transfer of poly(A)$^+$ RNA (20 μg) from K562 cells transfected with pHTLVI-CAT and pSV2-CAT hybridized with a CAT gene probe. Cells were treated with PMA 24 h after transfection. RNA was prepared 24 h later. Densitometric analysis showed a greater than 50-fold increase in CAT gene-specific mRNA for pHTLVI-CAT and a 20-fold increase in CAT gene-specific mRNA for pSV2-CAT. CAT activity measured in the same experiment was enhanced by PMA 40-fold for pHTLVI-CAT and 30-fold for pSV2-CAT (data not shown).

or the GALV enhancer (p1405) (8a) region, there was marked increase in CAT gene expression. We obtained similar results with other plasmid constructions containing the GALV enhancer in the opposite orientation or positioned 3′ to the CAT gene. With the plasmid p1419 (8a), containing the GALV enhancer but deleted of promoter sequences, no CAT gene expression was observed. Thus, whereas a promoter element is clearly necessary to direct CAT gene expression, the elevated levels seen with PMA are dependent on the presence of enhancer regions.

We have shown that PMA enhances transient expression of the CAT gene from four unrelated viral sequences in a variety of lymphoid cells, as well as in the erythroleukemia cell line K562. It was without effect in HeLa cells and two fibroblast lines but showed minimal enhancement (2.5-fold) of pSV2-CAT expression in HepG2 cells. Recently, Imbra and Karin (9) observed a similar but somewhat greater effect (fivefold enhancement) of PMA in these cells. They reported that PMA specifically induces the activity of the SV40 transcriptional enhancer element; because of the vectors tested, only those containing the SV40 enhancer showed significantly elevated expression after PMA treatment. We show here that expression of pHTLVI-CAT is also enhanced two- to threefold by PMA in these cells. With hematopoietic cells we found that, like that of pSV2-CAT, expression of three unrelated retroviral vectors, pRSV-CAT, pHTLVI-CAT, and pGALVSEATO-CAT, was significantly enhanced by PMA treatment, and the degree of enhancement was considerably greater than that seen in HepG2 cells. Thus, the ability of PMA to enhance gene expression appears to be a more general effect in hematopoietic cells.

The effect of PMA on gene activity is at least in part dependent on the presence of a functional enhancer, as

TABLE 4. Enhancement of transient gene expression by PMA requires an enhancer element

Treatment	% Conversion of [^{14}C]chloramphenicol (fold enhancement by PMA)[a]			
	pA10CAT (−E; SV40 P)[b]	pSV2-CAT (SV40 E; SV40 P)	p1405 (GALV E; SV40 P)[c]	p1419 (GALV E; −P)[d]
Control	0.14	1.4	14	<0.1
PMA	0.23 (1.6)	24 (17)	62 (4.4)	<0.1

[a] Abbreviations: E, enhancer; P, promoter.
[b] pA10CAT contains the SV40 early promoter deleted of its functional enhancer.
[c] p1405 contains the GALV enhancer positioned 5′ to pA10CAT.
[d] p1419 contains the GALV enhancer 5′ to the CAT gene with no promoter element.

shown with the GALV enhancer in these studies (Table 4). Imbra and Karin (9) also noted such a dependence for the SV40 enhancer in HepG2 cells. They proposed the presence of a *cis*-acting element in the SV40 enhancer region which serves as a specific target for the PMA effect. Elsholtz et al. (2) recently provided evidence for a PMA response element within the Moloney murine leukemia virus enhancer. Transcriptional activity of the long terminal repeat sequences of human T-cell lymphotropic virus type I and Rous sarcoma virus are also increased by PMA. Although it might be predicted that all of these genes would have a similar sequence responsible for their increased transcription, sequence comparisons have revealed no obvious homologies common to these viruses within the proposed *cis*-acting sequences.

The magnitude of enhanced expression seen with PMA treatment relates to specific plasmid-host cell interactions. For example, expression of pGALVSEATO-CAT was not influenced by PMA in MLA 144 or K562 cells but was markedly enhanced in Jurkat cells (Table 3). Relative to other cell types, pGALVSEATO-CAT was expressed at exceptionally high levels in MLA 144 and K562 cells (8a; Holbrook et al., in press; Table 3, footnote), and further enhancement by PMA may simply not be possible. However, when the GALV enhancer region was combined with the SV40 promoter element (a weaker promoter in these cells), strong enhancement of CAT gene expression was achievable in MLA 144 cells (Table 4). Thus, it is likely that the differences noted from cell type to cell type with different vectors reflect the relative strengths of both the promoter and enhancer elements in a particular cell.

The mechanism whereby PMA increases the level of expression of transfected DNA is not known. However, increased CAT activity in the presence of PMA is correlated with increased accumulation of CAT gene-specific mRNA (Fig 2; 2, 9). In general, PMA is thought to exert its effect through activation of protein kinase C (1, 15), but it is not known whether the enhancement observed in our studies requires such activation. Perhaps protein kinase C provides a common or general pathway whereby a number (perhaps a family) of different *trans*-activating factors are regulated, allowing them to interact with specific DNA sequences.

For practical purposes, treatment with PMA provides a convenient and reproducible method for enhancing short-term expression of exogenous DNA in a variety of hematopoietic cells. Therefore, addition of PMA treatment to transfection protocols could prove useful for a variety of situations in which low efficiency of transfection and low-level expression of transfected DNA have precluded the use of transient gene expression assays in hematopoietic cells.

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