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# Changes in protein kinase C activity are associated with the differentiation of Friend erythroleukemia cells

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**We investigated the activity and cellular distribution of protein kinase C during the dimethylsulfoxide (DMSO) and hypoxanthine-induced differentiation of Friend murine erythroleukemia cells. Most of the cellular protein kinase C activity was found in the soluble fraction of unstimulated Friend cells. Within 15 min of the addition of DMSO or hypoxanthine, protein kinase C underwent a dramatic and prolonged reversal of this distribution which was accompanied by a gradual decline in total cellular protein kinase C activity over the ensuing 5 days. The loss of total activity was found to be dose dependent although maximal translocation from soluble to insoluble components occurred at even lower concentrations of the inducers tested. Two clones of Friend cells, selected for their failure to differentiate in response to DMSO, showed alterations in protein kinase C activity and/or distribution following DMSO addition when compared to wild-type Friend cells. These data show that different inducers of Friend cell differentiation have similar effects on cellular protein kinase C, that the protein kinase C changes accompanying this process are immediate but prolonged, and that changes in protein kinase C activity and distribution are associated with Friend cell differentiation.**

## Introduction

Cellular differentiation involves the transformation of pluripotent stem cells into the basic specialized cell types required for organized functional tissues. It is known that some of the processes of differentiation include a cessation of cell division followed by de novo RNA transcription, high rates of protein and lipid synthesis, radical changes in membrane constituents, and eventual alterations in cellular morphology (see Refs. 1 and 2 foir a review). Each change requires a highly complex series of reactions which are tighly regulated with respect to the time of their initiation and duration. Friend murine erythroleukemia cells are widely used as a model system to study the cellular mechanism of erythroid differentiation, since this process can be induced by a variety of agents, including dimethylsulfoxide

Abbreviations: DMSO, dimethylsulfoxide; OAG, 1-oleoyl-2-acetylglycerol; PMA, phorbol 12-myristate 13-acetate; TPA, 12-*O*-tetradecanoylphorbol 13-acetate.

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and hypoxanthine (5-hydroxypurine).

The phosphorylation of protein and lipid molecules is now recognized as a common molecular mechanism used to regulate important cellular pathways. All animal cell types so far examined contain a $Ca^{2+}$-activated and phospholipid-dependent protein kinase (protein kinase C) which is currently thought to be one such cellular regulator [3]. Protein kinase C is found primarily among the soluble constituents of unstimulated cells such as neutrophils [4,5], platelets [6] and lymphocytes [3,7]. Protein kinase C activity becomes associated with insoluble cellular material, primarily the plasma membrane [8], when cells are stimulated by one of several different classes of agents, including phorbol esters (for which it is a cellular receptor, see Refs. 9 and 10), and chemotactic peptides such as *n*-formylmethionylleucylphenylalanine. This observation has been termed 'translocation' and may be necessary for the subsequent specific phosphorylation of a defined subset of proteins, such as the receptors for insulin and epidermal growth factor [11], glycogen synthase [12] and the 20 kDa light chain of myosin [13]. While there is no direct evidence that protein kinase C activity is required for specific cellular activities, there is a correlation between protein kinase C activity and both degranulation and superoxide anion production in some cases in the neutrophil [14] and with the activation of interleukin 2 receptors in the lymphocyte [15].

In this report, we present results which suggest that translocation and activation of protein kinase C from the soluble to the insoluble cellular fraction is correlated with DMSO- and hypoxanthine-induced Friend cell differentiation. In two DMSO-resistant cloned lines of friend cells, neither inducing agent caused differentiation (less than 2%) and protein kinase C activity was markedly different than in DMSO-sensitive cells. Protein kinase C activity in one clone was translocated before any challenge with inducer, and in both clones protein kinase C activity decreased dramatically with time and inducer concentration. These results suggest that protein kinase C translocation is correlated with the terminal differentiation of Friend cells, but that it is not itself sufficient to cause commitment.

## Materials and Methods

*Cells.* Friend cells (clone 745, Ref. 16) were grown at 37°C in $15 \times 100$ mm petri plates in Dulbecco's modified Eagles medium (Gibco) supplemented with 10% fetal calf serum, 2 mM glutamine, 100 $\mu$g/ml streptomycin and 100 units/ml penicillin. Experiments were performed in the log phase of growth and cells were used only at a viability of greater than 95%. DMSO-resistant clones were selected by first incubating $10^6$ Friend cells in 100 mm petri dishes with 1.5% DMSO for three weeks, and replating the cells in fresh medium as needed. After 3 weeks the cells which were still proliferating were (by definition) DMSO-resistant clones. Each petri plate was serially cloned into a single 96 well plate. Thus, each clone was derived from an independently arising DMSO-resistant cell. These cells were maintained continuously in 1.5% DMSO and in all cases demonstrated 0–5% spontaneously occurring benzidine positive cells.

*Induction of differentiation.* Cells were plated into fresh medium at a concentration of $10^5$ cells/ml. For dose-response experiments, an appropriate concentration of inducer was added for the specified time period. For time course studies, inducer was added to an aliquot of cells for 5 days. Aliquots of cells from each plate were stained for hemoglobin using the benzidine reaction [17]. DMSO-resistant clones were grown continuously in 1.5% DMSO. To study the effect of DMSO or hypoxanthine on protein kinase C, cells were pelleted by centrifugation, washed in DMSO-free medium, and grown for 5–7 days in the absence of DMSO.

*Cell fractionation and protein kinase C assay.* Cells were harvested into 50 ml conical polypropylene tubes (Corning), centrifuged at $1000 \times g$ for 6 min and the pellet resuspended at $5 \cdot 10^7$ cells/ml in extraction buffer (50 mM Tris (pH 7.5), 2 mM EGTA, 0.2 mM phenylmethylsulfonylfluoride, 50 mM 2-mercaptoethanol and 0.05% Triton X-100). Cells were disrupted by sonication (30 s at setting 33 with a Fischer model 3000 dismembrator), centrifuged at $500 \times g$ for 5 min to remove nuclei and unbroken cells, and then centrifuged at $100\,000 \times g$ for 60 min. The supernatant material was decanted and is defined

as the soluble fraction; the pelleted material was resuspended by sonication in an equal volume of extraction buffer and is defined as the insoluble fraction.

The activity of protein kinase C was monitored in a cell-free assay as previously described [14,18] using lysine-rich histone as a substrate. 50 μl of either the soluble or insoluble fraction were added to 180 μl of an assay mixture containing final concentrations of 50 mM Tris (pH 7.5), 0.6 mM $Ca^{2+}$, 150 μg/ml histone (type IIIs), 50 μM [γ-$^{32}$P]ATP (3 Ci/mM; Amersham), 20 μg/ml phosphatidylserine and 0.01% Triton X-100. 100 ng/ml phorbol 12-myristate 13-acetate (PMA) or 2 μg/ml 1-oleoyl-2-acetylglycerol (OAG) served as stimuli in the assay. OAG was prepared as a 1 mg/ml stock solution in chloroform, an aliquot of which was dried under nitrogen and resuspended in water by sonication. The assay was allowed to procede for 10 min at 30°C with constant agitation, and then stopped by the rapid addition of 1.5 ml ice-cold 25% trichloroacetic acid plus 0.2 mg bovine serum albumin. Precipitates were collected onto 0.45 μm membrane filters (HAWP, Millipore), dried, and counted in 10 ml hydrofluor (National Diagnostics, Somerville, NJ) in a Searle Isocap model 300 scintillation counter. Each experiment contained control conditions including assays at 4°C using boiled sample, in the absence of sample or in the absence of calcium, activator and phosphatidylserine; background (nonspecific) kinase activity was measured in the presence of calcium, but without phosphatidylserine or activator. Results are expressed as the average of triplicate samples ± S.E., and the significance of the data was assessed using the Student's *t*-test (paired samples). The specific activity of protein kinase C is defined as pmol of histone phosphate produced per $10^6$ cell equivalents, and which was dependent upon the presence of calcium and phospholipid.

All reagents were obtained from Sigma (St. Louis, MO) unless otherwise specified.

## Results

We have used both DMSO and hypoxanthine to induce Friend cells to terminally differentiate. The activity of protein kinase C as well as the appearance of hemoglobin were monitored in these cells during a 5 day incubation period after the addition of inducer. We also studied these activities in two cloned cell lines which do not differentiate in the presence of either DMSO or hypoxanthine.

*Time course of differentiation and protein kinase C activity in DMSO-sensitive cells*

DMSO-sensitive 745 Friend cells were incubated for varying times with either 1.5% DMSO or 5 mM hypoxanthine. These inducer concentrations have been shown by previous studies to be optimal to induce Friend cells to differentiate into benzidine-positive mature erythroid progenitors. Cells were homogenized and soluble and insoluble fractions were prepared by centrifugation and then tested for protein kinase C activity. The results of this study are presented in Fig. 1. Under these conditions, benzidine-positive cells were not detected until 36–48 h after the addition of inducer; 50% of the cells had differentiated by approx. 2.5 days, and more than 80% of the cells by 5 days in the presence or either inducing agent.

Total cellular protein kinase C activity (e.g., the

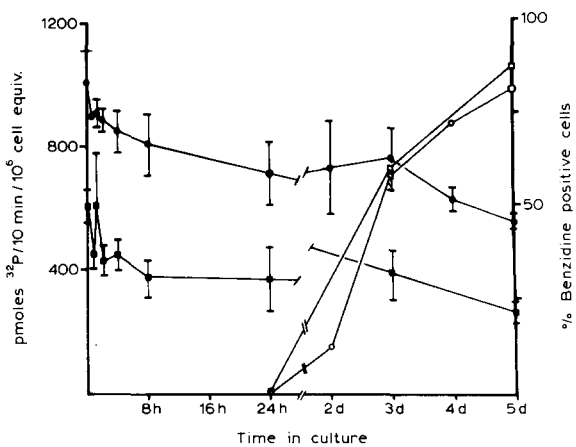


Fig. 1. The effect of DMSO and hypoxanthine on total cellular protein kinase C activity in Friend cells. Cells were incubated in culture with either 1.5% DMSO (●) or 5 mM hypoxanthine (■). Cells were periodically withdrawn from culture, and assayed for protein kinase C activity using a cell-free assay system as detailed in Materials and Methods. Cells were also stained for hemoglobin using the benzidine test (○, DMSO, □, hypoxanthine). Values are expressed as the mean of three experiments (± S.E.). Total cellular protein kinase C activity is defined as the sum of soluble and insoluble activities at a given time.

summation of soluble and insoluble activities) in untreated cells averaged 1006 ± 46 pmol/10 min per $10^6$ cells ($n = 9$). 8 h after the addition of DMSO or hypoxanthine total cellular protein kinase C activity had decreased by 20% ($P < 0.03$) and 38% ($P < 0.01$), respectively (Fig. 1). After 5 days in culture, total cellular protein kinase C activity had decreased by 45% and 57%, respectively ($P < 0.01$).

Cells were also tested for the distribution of protein kinase activity in soluble and insoluble fractions at various times during the incubation

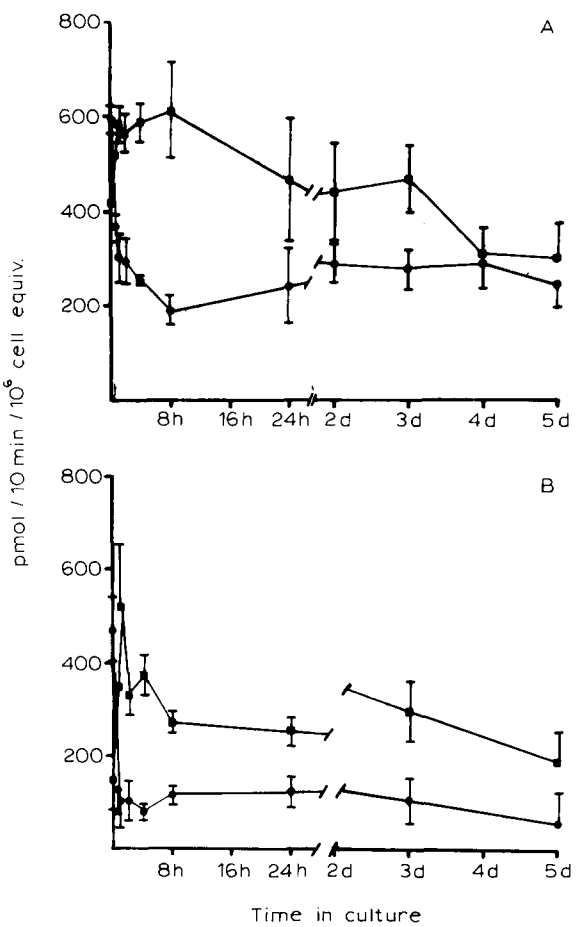


Fig. 2. The effect of DMSO and hypoxanthine on the distribution of cellular protein kinase C during clone 745 Friend cell differentiation. Cells were incubated with either 1.5% DMSO (A) or 5 mM hypoxanthine (B). Cells were withdrawn from culture after intervals of up to 5 days and assayed for protein kinase C activity in the soluble (●) and insoluble (■) cellular fractions. Results are the means of at least three experiments ± S.E.

period (Fig. 2). Untreated cells had an average of 70% of their total cellular protein kinase C activity associated with the soluble material, with the remaining activity associated with the insoluble fraction. Within 15 min after the addition of inducer, we observed a rapid and dramatic translocation of protein kinase C activity to the insoluble fraction, which accounted for 62% of the total activity with DMSO and 79% with hypoxanthine. Protein kinase C activity in the insoluble fraction increased significantly over control (time = 0) levels by 8 h in culture with DMSO (from 30% to 75%, $P < 0.01$; Fig. 2a), and displayed similar kinetics with hypoxanthine (Fig. 2b). The relative amount of insoluble protein kinase C activity decreased after 8 h in culture with DMSO but remained relatively constant over this time with hypoxanthine (Fig. 2). However, both inducers caused a similar and large decrease in the spec. act. of insoluble protein kinase C (decreasing from 589 to 311 pmol/10 min per $10^6$ cells for DMSO and from 381 to 195 pmol/10 min per $10^6$ cells for hypoxanthine from 8 h to 5 days). With either inducer, both the spec. act. and the relative percentage of protein kinase C activity in the soluble fraction immediately and dramatically decreased and remained relatively constant over the entire 5-day period; soluble activity typically was below 50% of control levels (Fig. 2). Therefore, the decrease in total protein kinase C activity (Fig. 1) can be explained completely on the basis of a decrease in insoluble activity over time. These data indicate that both DMSO and hypoxanthine cause a rapid and prolonged translocation of protein kinase C activity followed by a decrease in total activity over the 5-day culture.

*The effect of inducer concentration on differentiation and protein kinase C activity in DMSO-sensitive cells*

Cells were incubated with various concentrations of each inducing agent for 5 days, the time required to obtain maximal differentiation (Fig. 1). Under these conditions, as little as 0.6% DMSO or 0.07 mM hypoxanthine stimulated a significant population of cells to differentiate (Fig. 3).

We observed a biphasic response in total cellular protein kinase C activity with respect to DMSO concentration (Fig. 3). Concentrations less than

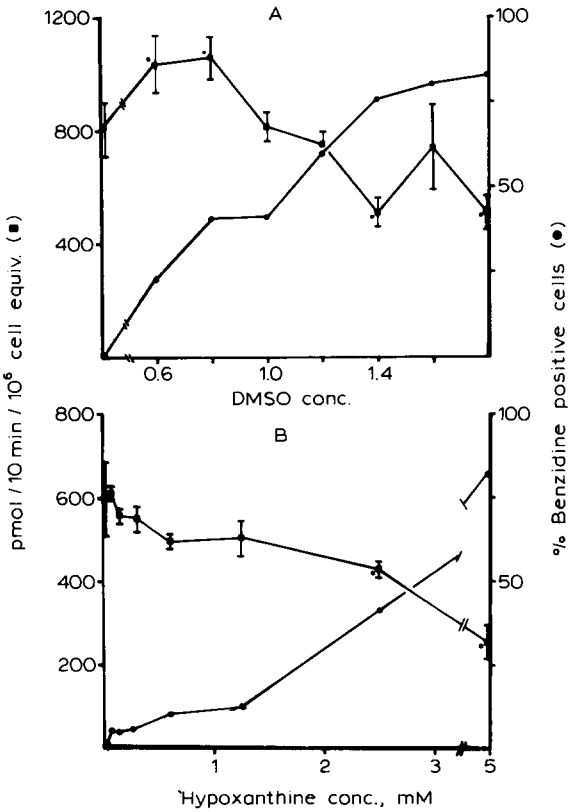


Fig. 3. The effect of inducer concentration on total cellular protein kinase C activity in Friend cells. Cells were incubated in culture with various doses of either DMSO (A) or hypoxanthine (B) for 5 days. At the end of the incubation period cells were assayed for total cell protein kinase C activity (■). As an indication of cellular differentiation aliquots of cells were monitored for the presence of heme proteins using the benzidine reaction (●); the extent of differentiation is expressed as the percentage of the total population of cells which were benzidine positive. Protein kinase C activity is expressed as the mean ± S.E. of at least three experiments. *, indicates data significantly different from the control value ($P < 0.04$).

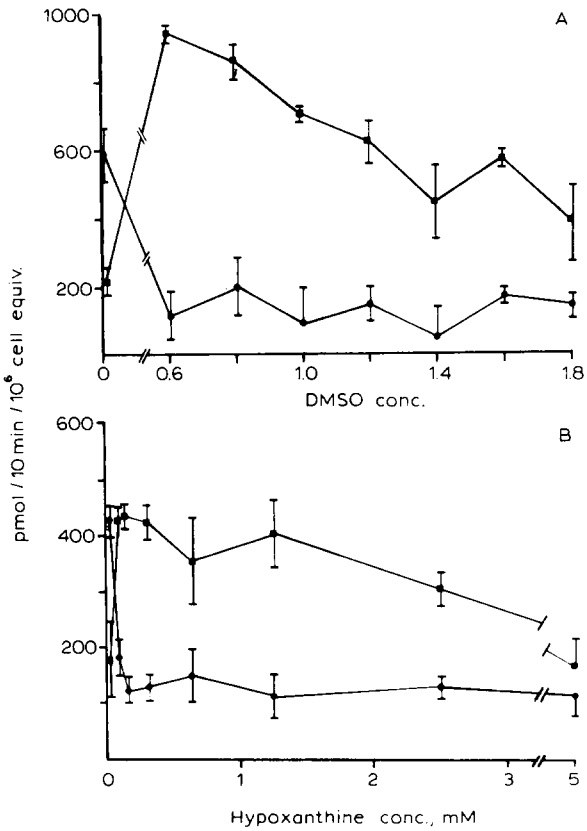


Fig. 4. The effect of DMSO and hypoxanthine on the distribution of total cellular protein kinase C activity in differentiating Friend cells. Cells were incubated with increasing concentrations of either DMSO (A) or hypoxanthine (B) for 5 days. At the end of the incubation period, the cells were removed from culture and assayed for protein kinase C activity in the soluble (●) and insoluble (■) fractions. Results are expressed as the means (± S.E.) of at least three experiments.

0.6% stimulated little total cellular protein kinase C activity; with 0.6% to 0.8% there was an increase in protein kinase activity over control levels ($P < 0.04$), eventually achieving 1072 pmol/10 min per $10^6$ cells (130% of control; Fig. 3a). Higher concentrations of DMSO caused a dose-dependent decrease in activity, reaching a low of 531 pmol/10 min per $10^6$ cells at 1.4% ($P < 0.02$ for 1.4% and 1.6% DMSO). 0.2–0.4% DMSO concentrations actually inhibited total cellular protein kinase C activity (31% less than control values, data not shown). In contrast, we observed a continual dose-dependent decrease in protein kinase C activity from 0.15 mM to 2.5 mM hypoxanthine (Fig. 3b). Higher concentrations of the inducer were toxic to the cells.

We next observed the distribution of protein kinase C activity as a function of inducer concentration (Fig. 4). Even the lowest tested concentrations of DMSO (0.2%) and hypoxanthine (0.07 mM) caused the translocation of most of the protein kinase C activity to the insoluble fraction (data not shown). The relative percentage of total cellular protein kinase C activity which was associated with the insoluble fraction decreased over

the entire concentration range for DMSO (Fig. 4a), and decreased by 23% for hypoxanthine (Fig. 4b). Soluble protein kinase C activity also remained constant and less than 50% of control (no inducer) levels over the entire concentration range for both inducers.

*Protein kinase C activity in DMSO-resistant cell lines*

Several DMSO-resistant clones of Friend cells were derived from the parental 745 cell line. These clones grew well in the continuous presence of 1.5% DMSO but did not differentiate with this inducer or with 5 mM hypoxanthine (less than 2% differentiated cells). The activity and distribution of protein kinase C was assessed in two of these lines, designated clones 1 and 7, after exposure to 1.5% DMSO. Prior to DMSO addition, each clone was first grown in its absence for 5–7 days. The results of total cellular protein kinase C measurements on these cells are summarized in Fig. 5. Compared to DMSO-sensitive 745 cells, clone 1 total cellular protein kinase C activity decreased immediately and dramatically, such that within 4 h it was only 39% of the pre-treatment value ($P < 0.01$). Activity continued to decrease until 24 h, where it remained low and stable at about 15–20% of 745 cell values (Fig. 6a). In contrast, total cellular protein kinase C activity in clone 7 was generally equivalent to DMSO-sensitive cells in response to DMSO before dropping below 745 cell values after 3 days. As expected, less than 2% of the cells from both clone 1 and 7 had differentiated in response to DMSO treatment (data not shown).

The distribution of protein kinase C activity in DMSO-resistant cells was monitored over the 5-day time course. Clone 7 cells (Fig. 6a), much like DMSO-sensitive 745 cells (Fig. 2), had most of their protein kinase C activity associated with the soluble fraction before challenge with inducer (more than 57%). Protein kinase C activity trans-

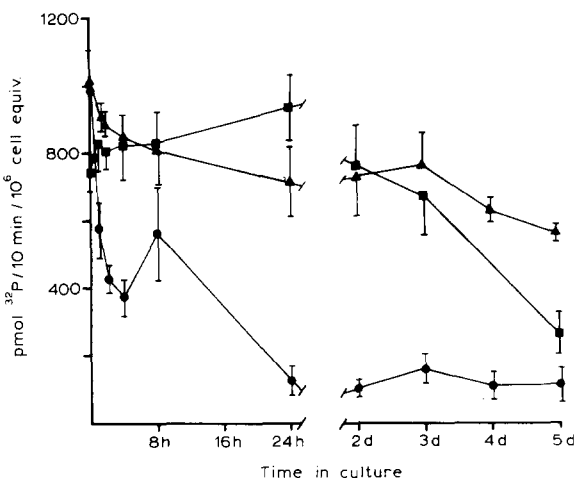


Fig. 5. Effect of DMSO on total cellular protein kinase C activity in 745 DMSO-sensitive and two DMSO-resistant clones. Cells were incubated in 1.5% DMSO for intervals of up to 5 days, and assayed for total cellular protein kinase C activity. Total cellular activity is defined as the sum of the soluble plus insoluble activities at a given time point. Activity is expressed as the mean values (±S.E.) of at least three experiments. DMSO-resistant clones 1 and 7 were cultured in the absence of DMSO for at least 5–7 days before incubation with inducer. In every experiment less than 2% of the DMSO-resistant cells differentiated in the presence of inducer. DMSO-sensitive 745 cells (▲); DMSO-resistant clone 1 (●); DMSO-sensitive clone 7 (■).

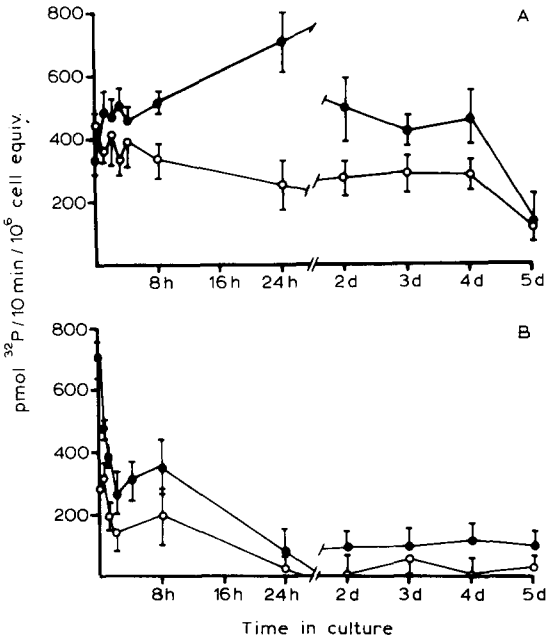


Fig. 6. The distribution of protein kinase C activity in two DMSO-resistant Friend clones after exposure to DMSO. Cells were incubated in the presence of 1.5% DMSO, removed at intervals up to 5 days and assayed for protein kinase C activity in the soluble and insoluble cell fractions. Activity is expressed as the means (±S.E.) of at least three experiments. Soluble fraction (○); insoluble fraction (●); A, clone 7; B, clone 1.

located to the insoluble fraction within 30 min after the addition of DMSO, and activity values for both fractions were roughly comparable for clone 7 and DMSO sensitive 745 cells over the 5-day time course. Clone 1 cells, however, had a very different protein kinase C activity profile (Fig. 6b). Protein kinase C activity before DMSO addition was found primarily in the insoluble fraction (72% of the total), and retained essentially the same distribution over the entire experimental time course. Both fractions showed a decrease in protein kinase C activity over time, which again was different than that observed for the parental cell line. Therefore, although DMSO exerted a profound effect on the total protein kinase C activity in clone 1 cells, it failed to affect protein kinase C distribution, presumably because translocation had already occurred prior to the addition of DMSO. These data indicate that DMSO-resistant clones may display different protein kinase C distributions and activities when compared to DMSO-sensitive cells.

**Discussioin**

The transformation of hematopoietic stem cells into fully differentiated and circulating erythrocytes is a highly complex and little understood process. We have shown that in Friend cells this process was accompanied by a rapid and prolonged translocation of protein kinase C activity from the soluble to the insoluble fraction. Translocation was associated with an initial increase in total cellular protein kinase C activity and with a rise in protein kinase C activity associated with the insoluble fraction. The total activity then gradually decreased over time to near control levels.

As little as 0.2% DMSO or 0.07 mM hypoxanthine caused a translocation of protein kinase C activity from the soluble to the insoluble fraction (Fig. 4) even though these inducer concentrations were not sufficient to stimulate a significant number of cells to differentiate. However, high concentrations of both inducers (more than 0.4% DMSO or 0.62 mM hypoxanthine) had similar effects on differentiation and on both protein kinase C activity and translocation. Similar results were obtained using *N*,*N*-dimethylacetamide as an inducer (data not shown). Low concentrations of DMSO (less than 0.5%) were inhibitory to total cellular protein kinase C activity, an effect not seen with hypoxanthine (Fig. 3). This phenomenon probably reflects a condition of the entire population of cells at the 5-day time point, since cells tested after 4 h of incubation showed no such inhibition at these concentrations, and in fact a slight increase in activity was observed (unpublished observation). The biphasic nature of the effect of DMSO on protein kinase C activity has been observed with other activators, namely the non-phorbol mezerein. This agent activates protein kinase C at very low concentrations, and is a very potent inhibitor of the enzyme at higher concentrations (see Ref. 14). What is more, both mezerein and PMA stimulate neutrophil degranulation and superoxide anion production. Two conclusions can be drawn from these data. First, the threshold concentration of inducer needed to commit cells toward differentiation is higher than that which stimulates protein kinase C activity. This implies that protein kinase C translocation and high protein kinase C activity in the insoluble fraction are associated with differentiation but are probably involved in many other cellular processes not directly related to differentiation as well. Second, that structurally unrelated inducers of Friend cell differentiation demonstrated the same effects on protein kinase C indicates that protein kinase C may be part of a common pathway through which these agents act.

Several cultured cell lines have been shown to differentiate and/or cease to proliferate upon treatment with protein kinase C-stimulating agents, such as 1-oleoyl-2-acetylglycerol and phorbol 12-*O*-tetradecanoylphorbol 13-acetate (TPA). For example, TPA stimulates the differentiation of HL-60 cells into macrophages and also stimulates protein kinase C activity and cellular protein phosphorylation [19–21]. In addition, *sn*-1,2-dioctanoylglycerol, an agent which competes with [$^3$H]phorbol 12,13-dibutyrate for partially purified protein kinase C from HL-60 cells, also caused activation of protein kinase C and differentiation of HL-60 cells [21]. PMA and OAG also induce differentiation in 70Z/3 murine pre-B lymphocytes [22,23]. The hypothesis that proliferation is dependent upon protein kinase C activity is strengthened by data which show that a de-

crease in extracellular calcium was associated with a decrease in protein kinase C activity in the particulate fraction of Balb/c 3T3 cells, and that changes in calcium concentration parallel similar changes in protein kinase C activity and DNA synthesis [24]. However, DMSO-induced Friend cells were reported to have no apparent change in intracellular calcium concentration after at least 40 h of incubation with the inducer [25]. While these data appear to argue against protein kinase C activity being a requirement in differentiation, local and relatively small changes in calcium concentration within the cell may be enough to stimulate sufficient protein kinase C translocation and activity such that certain necessary regulatory functions are accomplished. However, it must first be demonstrated directly that protein kinase C activity is a necessary function in the differentiation process.

The conclusion from previous studies of several cell types, and from the present work, is that protein kinase C activation and translocation are correlated with cell differentiation. Instrumental in drawing this conclusion from the present study was the use of DMSO-resistant clones of Friend cells. First, low doses of all inducers were able to stimulate the activity and translocation of protein kinase C in 745 cells, but were not sufficient to cause the appearance of hemoglobin or the cessation of cell division that accompanies Friend cell differentiation (Figs. 3 and 4). Second, DMSO-resistant clone 1, incubated with 1.5% DMSO, showed both an immediate decrease in protein kinase C activity and less than 2% differentiation (Fig. 5). Clone 7 cells showed protein kinase C distribution characteristics which closely paralleled the parental line although the total cellular protein kinase C activity was not affected until late in the experimental time course. Clone 1 displayed unusual responses to DMSO compared to the other cell lines tested, in that the translocation of protein kinase C activity was observed before challenge with inducer. These observations probably indicate a cellular defect in this clone reflected by an initial stimulation of protein kinase C activity during the clonal selection process, but an inability to carry out subsequent steps in differentiation both at that time and after a second challenge with inducer. Although there is no direct evidence, the fact that two independently derived DMSO-resistant clones demonstrated abnormalities in the behavior of protein kinase C argues in favor of a role for protein kinase C in the differentiation of Friend cells. In addition, other work in our laboratory has demonstrated that H-7, an inhibitor of protein kinase C [26], also inhibits DMSO-induced friend cell differentiation (unpublished observation).

Phorbol esters have been shown to inhibit the DMSO-induced differentiation of Friend cells [27–29], an effect presumably mediated by the ability of these agents to stimulate protein kinase C [3]. However, all three agents (e.g., phorbol, DMSO and hypoxanthine) independently cause protein kinase C translocation. The recent observation that DMSO or hypoxanthine treatment reduces the levels of diacylglycerol in Friend cells [30] is consistent with our results, since diacylglycerol has widely been shown to stimulate protein kinase C. The apparent inconsistency between the effects of phorbol and either DMSO or hypoxanthine on differentiation may be resolved by considering each agent's specific effect in the mechanism for inducing this event. For example, all three agents cause protein kinase C translocation but phorbol esters may activate other physiological pathways than do the other agents, such as stimulating the phosphorylation of different substrates by protein kinase C. Thus, the antagonistic effects of Friend inducers and phorbol esters may be related to their opposite effects on total cellular protein kinase C activity.

Our results suggest that after 5 days in culture, which is the time period necessary to cause a differentiation of more than 75% of the cells with an optimal dose of inducer, Friend 745 cells show a dose-dependent decrease in protein kinase C activity. We interpret these data to indicate that there is an early and continual requirement for protein kinase C activity once a particular cell has committed toward differentiation, and that the need for protein kinase C in the associated processes falls off about halfway into the differentiation period. Of course, the observed decrease in protein kinase C activity may possibly reflect only the decrease in total cellular protein which accompanies Friend cell differentiation. However, this interpretation is strengthened by the finding

that protein kinase C translocation is immediate but prolonged over the entire 5-day period. The commitment to differentiate into specific cell types must necessarily be followed by high metabolic activity in which protein kinase C may play some role.

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**References**

1 Sabin, F.R. (1928) Physiol. Rev. 888, 191–244
2 Nat. Cancer Inst. Monogr. (1964) 14, 119
3 Nishizuka, Y. (1984) Nature 308, 693–698
4 Balazovich, K.J., Smolen, J.E. and Boxer, L.A. (1984) Blood 64, 64a (abstract)
5 Wolfson, M., McPhail, L.C., Nasarallah, V.N. and Snyderman, R. (1985) J. Immunol. 135, 2057–2062
6 Kawahara, Y., Takai, Y., Minakuchi, R., Sano, K. and Nishizuka, Y. (1980) J. Biochem. 88, 913–916
7 Kraft, A.S. and Anderson, W.B. (1982) J. Biol. Chem. 257, 13193–13196
8 Kraft, A.S. and Anderson, W.B. (1983) Nature 301, 621–623
9 Castagna, M., Takai, Y., Kaibuchi, K., Sno, K., Kikkiwa, U. and Nishizuka, Y. (1982) J. Biol. Chem. 257, 7841–7851
10 Niedel, J.E., Kuhn, J.L. and Vandenbark, G.R. (1980) Proc. Natl. Acad. Sci. USA 80, 36–40
11 Jacobs, S., Sahyoun, N.E., Saltiel, A.R. and Cuatrecasas, P. (1983) Proc. Natl. Acad. Sci. USA 80, 6211–6213
12 Ahmad, Z., Lee, F.T., DePaoli-Roach, A. and Roach, P.J. (1984) J. Biol. Chem. 259, 8743–8747
13 Naka, M., Nishikawa, M., Adelstein, R.S. and Hidaka, H. (1983) nature 306, 490–492
14 Balazovich, K.J., Smolen, J.E. and Boxer, L.A. (1986) Blood, 68, 810–817
15 Farrar, W.L. and Taguchi, M. (1985) Lymphokine Res. 4, 87–93
16 Friend, C., Scher, W., Holland, J.G. and Saito, T. (1971) Proc. Natl. Acad. Sci. USA 68, 378–382
17 Orkin, S.H., Harosi, F.I. and Leder, P. (1975) Proc. Natl. Acad. Sci. USA 72, 98–102
18 Kuo, J.F., Andersson, R.G.G., Wise, B.C., Mackerlova, L., Solomonsson, I., Brackett, N.L. and Katoh, N. (1980) Proc. Natl. Acad. Sci. USA 77, 7039–7043
19 Kreutter, D., Caldwell, A.B. and Morin, M.J. (1985) J. Biol. Chem. 260, 5979–5984
20 Durham, J.P., Elmer, C.A., Butcher, F.R. and Fontana, J.A. (1985) FEBS Lett. 185, 157–161
21 Ebeling, J.G., Vandenbark, G.R., Kuhn, L.J., Ganong, B.R., Bell, R.M. and Niedel, J.E. (1985) Proc. Natl. Acad. Sci. USA 82, 815–819
22 Rosoff, P.M. and Cantley, L.C. (1983) Proc. Natl. Acad. Sci. USA 80, 7547–7550
23 Rosoff, P.M., Stein, L.F. and Cantley, L.C. (1984) J. Biol. Chem. 259, 7056–7060
24 Donnely, T.E., Jr., Sittler, R. and Scholor, E.M. (1985) Biochem. Biophys. Res. Commun. 126, 741–747
25 Faletto, D.L. and Macara, I.G. (1985) J. Biol. Chem. 260, 4884–4889
26 Hidaka, H.M., Inagaki, S., Kawamoto, S. and Sasaki, Y. (1984) Biochemistry 23, 5036
27 Yamasaki, H., Martel, N., Fusco, A. and Ostertag, W. (1984) Proc. Natl. Acad. Sci. USA 81, 2075–2079
28 Yamasaki, H., Fibach, E., Nudel, U., Weinstein, I.B., Rifkind, R.A. and marks, P.A. (1977) Proc. Natl. Acad. Sci. USA 74, 3451–3455
29 Rovera, G., O'Brien, T. and Diamond, C. (1977) Proc. Natl. Aacd. Sci. USA 74, 2874–2898
30 Faletto, D.L., Arrow, A.S. and Macara, I.G. (1985) Cell 43, 315–325