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Defect in phorbol acetate-induced translocation of diacylglycerol kinase in erbB-transformed fibroblast cells

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We here show that tetradecanoyl phorbol acetate (TPA) and 1-oleoyl 2-acetyl glycerol (OAG) cause the translocation of diacylglycerol (DG) kinase from the cytosol to the membrane fractions in chick embryo fibroblast (CEF) cells. However, this translocation is not marked in erbB-transformed chick embryo fibroblast (GEV) cells. The activities of phosphatidylinositol (PI) and phosphatidylinositol 4-phosphate (PIP) kinases in membrane fractions are not altered by TPA treatment in either CEF or GEV cells. Such reduced translocation of DG kinase by TPA is also observed in src-transformed cells, but not in myc-transformed cells. These results suggest that the defect in DG kinase translocation may result in failure to suppress the overactivation of protein kinase C in erbB-2 and src-transformed cells, which may lead to cell growth and transformation.

Diacylglycerol kinase; Translocation; Oncogene, erbB; Oncogene, src

1. INTRODUCTION

An avian retroviral oncogene, v-erbB, which is responsible for erythroblast and fibroblast transformation by avian erythroblastosis virus, has been shown to have extensive homology to the epidermal growth factor (EGF) receptor [1–3]. Therefore, the erbB gene product may induce a signal transduction system similar to that resulting from EGF receptor activation that leads to cell proliferation. Although for some growth factors the activation of inositol phospholipid metabolism has been suggested to play a role in the signal transduction that leads to cell proliferation [4–6], it has been reported that EGF does not stimulate inositol phospholipid metabolism in 3T3 fibroblasts [7]. However, EGF is able to increase inositol phospholipid metabolism in A431 cells in which EGF receptors are overproduced. Similarly, in erbB-transformed chick embryo fibroblast (GEV) cells, inositol phospholipid metabolism is markedly stimulated compared to that in normal chick embryo fibroblast (CEF) cells [8]. Moreover, the activities of three kinases that play important roles in inositol phospholipid metabolism, phosphatidylinositol (PI) kinase, phosphatidylinositol 4-phosphate (PIP) kinase and diacylglycerol (DG) kinase, are increased 3–5-fold in GEV cells, which is presumed to be related to cell growth enhancement in GEV cells.

DG, produced by the phosphodiesteratic cleavage of polyphosphoinositides, plays a crucial role as an intracellular second messenger through the activation of protein kinase C [9,10]. In several oncogene-transformed cells, DG accumulation and subsequent activation of protein kinase C have been demonstrated [8,11], suggesting the possibility that the alteration of DG attenuation causes cell transformation. The attenuation of DG is carried out by two pathways [12–15]: phosphorylation by DG kinase, and degradation by DG lipase. The predominant pathway of attenuation is considered

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to be via DG kinase. Therefore, DG kinase plays an important role in regulating the activity of protein kinase C. The tumor promoter, tetradecanoyl phorbol acetate (TPA), is thought to bind protein kinase C at the DG-binding site instead of DG leading to the activation of the kinase [9,10]. Recent studies in cultured cells suggest that translocation of protein kinase C from cytosol to membranes occurs in response to DG or TPA and that translocation may be an important event in signal transduction [16,17].

In this report, we examined the effect of TPA and DG, both protein kinase C activators, on the translocation of DG kinase [8], to clarify the regulatory mechanism of protein kinase C by DG kinase, and demonstrated that the translocation was relatively small in erbB- or src-transformed cells.

2. MATERIALS AND METHODS

2.1. *Cell cultures*

Chick embryo fibroblast cells (CEF) were transformed by infection with gag-erbB (GEV), gag-myc (MC 29), avian erythroblastosis virus (AEV) or the Schmidt-Ruppin strain of Rous sarcoma virus of subgroup A (SRA). The cells were cultured in Eagle's minimum essential medium supplemented with 10% heat inactivated fetal calf serum and 20 mM Hepes/NaOH buffer (pH 7.4).

2.2. *Preparation of cytosol and membrane fractions*

The cells ($1–2 \times 10^6$) were treated with 100 ng/ml of TPA or OAG at 37°C for the indicated time and then washed with phosphate-buffered saline (PBS) three times and harvested with a rubber policeman. The cells were collected by centrifugation at $1000 \times g$ for 5 min at 4°C and suspended in 50 mM Tris-HCl (pH 7.4) containing 1 mM ethylene glycol-bis-(β-aminoethyl-ether)-*N,N,N',N'*-methylsulfonylfluoride ($5–6 \times 10^6$ cells/500 μl) and then disrupted by sonication at 0°C. The supernatant and membrane fractions were separated by centrifugation at $100000 \times g$ for 60 min.

2.3. *Assay of PI, PIP, and DG kinase activities*

The activities of PI, PIP and DG kinase were measured as described previously [8]. They are indicated as pmol/min per mg protein.

3. RESULTS

In normal chick embryo fibroblast cells (CEF) and also in erbB-transformed cells (GEV), most of the PI and PIP kinase activities are present in the membrane fraction. DG kinase activity, however, is detected in both the soluble and membrane fractions. Whether both activities arise from the same enzyme species is not known, although the properties of membrane-bound and soluble kinases are reported to be similar [18]. Activities of these kinases in GEV cells were higher than those in CEF cells as reported earlier [21]. TPA treatment did not change the activities of PI and PIP kinase in either GEV or CEF cells (table 1). However, DG kinase activity in the membrane fraction was dramatically increased (4.2-fold) in TPA-treated CEF cells. On the other hand, in GEV cells the activity was altered less significantly (1.6-fold) than in CEF cells.

Fig.1 shows the alterations in the distribution of DG kinase brought about by TPA treatment. In normal CEF cells, TPA increased the activity of DG kinase in the membrane fraction very markedly, while DG kinase activity in the cytosol was decreased. In addition, total DG kinase activity in homogenate fraction (data not shown) was not altered. The results suggest that TPA causes the translocation of DG kinase from the cytosol to the membrane fraction. This translocation reaches a maximum 30 min after TPA treatment. At 30 min, DG kinase activity in the membrane fraction was increased 7-fold while that of the cytosol was decreased to 40% of the activity of untreated controls. On the other hand, TPA caused a slight translocation of DG kinase in GEV cells. The translocation of DG kinase occurred in a dose-dependent manner in CEF cells (fig.2A). High concentrations of TPA (100 ng/ml) caused a 6-fold increase in DG kinase activity in the membrane fraction. However, in GEV cells, DG kinase activity in the membrane fraction was not so evident as

Table 1

Effect of TPA on the activities of PI kinase, PIP kinase and DG kinase in membrane fraction

Cells	TPA treatment	PI kinase	PIP kinase	DG kinase
CEF	–	1025 ± 18	182 ± 9	71 ± 8
	+	1059 ± 21	178 ± 8	431 ± 13
GEV	–	2279 ± 29	407 ± 18	240 ± 10
	+	2523 ± 32	425 ± 19	378 ± 14

Cells were treated with 100 ng/ml of TPA for 30 min at 37°C and then membrane fractions were isolated. Each kinase activity was assayed by incubating at 37°C for 3 min (PI kinase), 10 min (PIP and DG kinase). Activity expressed as pmol/min per mg protein

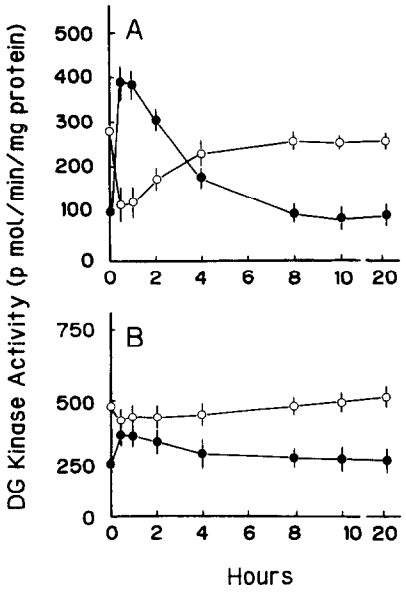

Fig.1. Time course of the increase in membrane-bound DG kinase activity caused by TPA. CEF and GEV cells were treated with TPA (100 ng/ml) at 37°C for the indicated time and then washed with phosphate-buffered saline (PBS) three times and harvested with a rubber policeman. The cells were collected and then disrupted by sonication at 0°C. The cytosol and membrane fractions were prepared as described and the DG kinase activity was measured. (A) DG kinase activity in cytosol (○) and membrane fractions (●) of TPA-treated CEF cells. (B) DG kinase activity in cytosol (○) and membrane fractions (●) of TPA-treated GEV cells.

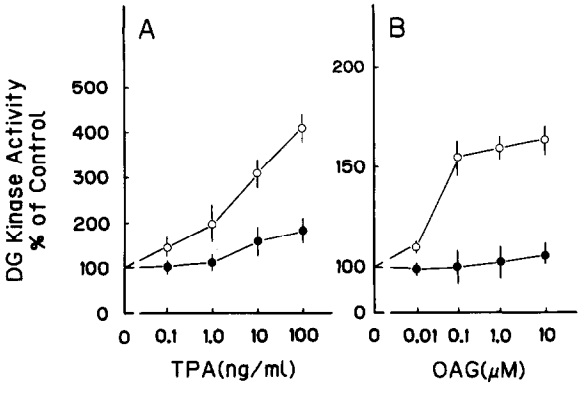

Fig.2. Effect of TPA or DG kinase activity in membrane fractions. Cells were treated with various concentrations of TPA (A) or OAG (B) for 30 min at 37°C. After membrane fractions were isolated from CEF (○) or GEV (●) cells, DG kinase activity was measured. The activity of non-treated cells was taken as 100%.

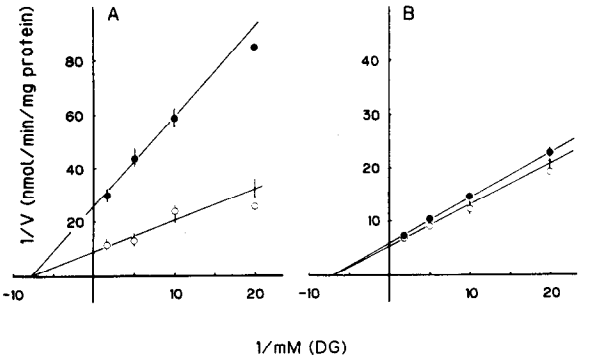

Fig.3. Lineweaver-Burk plots of DG kinase activity in membrane fractions. CEF (A) and GEV (B) cells were treated with (○) or without TPA (●) (100 ng/ml) for 30 min at 37°C. After membrane fractions were isolated, DG kinase activity was measured with the various concentrations of DG.

that in CEF cells, even at high concentrations of TPA. Similarly, the translocation of DG kinase was induced by OAG in a dose-dependent manner in CEF cells, although its effect was fairly weak compared to that by TPA (fig.2B). Also in this case, OAG did not induce a marked translocation in GEV cells. Lineweaver-Burk plots (fig.3) show that the V_{max} value of DG kinase was increased in TPA-treated CEF cells compared to GEV cells without a change in the K_m value, suggesting the increase in DG kinase protein rather than the modulation of DG kinase.

To ascertain whether such a low translocation of DG kinase is also observed in other oncogene-induced transformed cells or not, we examined the effect of TPA on DG kinase translocation in src-, and myc-transformed cells (table 2). Consistent

Table 2

Effect of TPA on the activities of DG kinase in various cells

Cell strain	Onco-gene	Membrane		Cytosol	
		Control	TPA	Control	TPA
CEF		71 ± 8	431 ± 13	288 ± 9	179 ± 5
GEV	v-erbB	240 ± 10	378 ± 14	444 ± 11	325 ± 9
AEV	v-erbB	264 ± 9	394 ± 14	435 ± 12	304 ± 10
SRA	v-src	233 ± 8	324 ± 6	336 ± 8	255 ± 4
MC29	v-myc	121 ± 6	588 ± 12	264 ± 5	139 ± 4

Cells were treated with TPA (100 ng/ml) for 30 min at 37°C and then membrane and cytosol fractions were separated. Activity expressed pmol/min per mg protein

with the above observation, translocation of DG kinase by TPA was not as evident in v-erbB or v-src-transformed cells as in normal cells. However, the translocation was marked in v-myc-transformed cells. These results suggest that transformed cells whose oncogene products are present in plasma membrane (erbB and src) may have some defect in translocation of DG kinase which is usually caused in response to DG formed in the membrane.

4. DISCUSSION

We have shown that TPA causes a marked translocation of DG kinase from the cytosol to membrane fraction in CEF cells but not in erbB- and src-transformed cells. Recently, Besterman et al. [19] have reported that DG kinase is translocated from cytosol to the membrane fraction by the addition of DG. We have also found that DG kinase is translocated in response to TPA in neutrophils [29]. Therefore, TPA may cause translocation by a similar mechanism.

Considering that protein kinase C competes with DG kinase for DG and TPA, DG kinase may play an important role in the regulation of protein kinase C activity. If this is the case, the translocation of DG kinase to the membrane fraction may be necessary to suppress the overactivation of protein kinase C, since protein kinase C also moves to membranes after DG or TPA treatment. Therefore, a defect in the translocation of DG kinase may result in the failure to suppress protein kinase C, which may lead to cell growth and transformation. Indeed, the activity of membrane-associated protein kinase C was found to be increased in erbB-transformed cells compared to that in untransformed cells in accordance with an increase in DG content [8]. However, DG kinase activities both in membrane and cytosol fractions were found to be increased in src- and erbB-transformed cells as shown in table 2. This discrepancy suggests that the increment in DG kinase is not sufficient to eliminate DG increased by transformation, resulting in the accumulation of DG. Recently, we have found that in ras-transformed cells, DG kinase activity in membrane fractions is decreased and that in cytosol fractions is increased with no change in total activities [21]. Therefore, the increase in total DG kinase activity in src- and erbB-transformed cells may be due to oncogene products which have tyrosine kinase activity. From these results, we conclude that DG kinase translocation plays an important role in regulation of protein kinase C activity but in transformed cells, it does not function normally resulting in a sustained activation of protein kinase C.

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