Korean J. Zool. 33: 266–275, 1990

Immunocytochemical Localization of c-*raf* Protein Kinase in EC-4 Cell

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c-*raf* protein kinase, a kind of oncogene, is a cytoplasmic serine/threonine-specific protein and is activated by mitogenic or oncogenic signals. The structure and functions of c-*raf* protein kinase are considered very similar to those of protein kinase C. Using immunocytochemical approach, the time course of singal transduction of c-*raf* protein kinase in EC-4 cell was examined with 12-0-tetradecanoylphorbol-13-acetate (TPA) as tumor promotor and platelet-derived growth factor (PDGF) as mitogenic factor. Immunoreactive c-*raf* was initially bound to the perinuclear membrane and then moved into the nucleus. The effect of the long-term treatment with TPA or PDGF was taken place down regulation at different time point. These results indicate that TPA and PDGF give rise to the translocation of c-*raf* protein kinase through the two different pathways.

KEY WORDS: c-*raf* protein kinase, TPA, PDGF, Signal transduction, Down regulation

c-*raf* gene is a kind of *raf* oncogene, and has the cellular homologue of v-*raf* which is the transforming gene of the murine sarcoma virus 3611 (Rapp *et al.*, 1983). This C-*raf* containing the human homologous of v-*raf* (Bonner *et al.*, 1985) is one of the protooncogenes, and encoded by exon 10 through 16 in a carboxyl-terminal domain. It is 30% homologous to the *src* tyrosine kinase domain (Mark and Rapp, 1984). The C-*raf* protein kinase also contains a characteristic cystein-rich sequence similar to that of protein kinase C (Rapp *et al.*, 1987). The characteristic constitution of protein kinase C and C-*raf* protein kinase such as cystein-rich domain and serine/threonine-specific protein (Huang *et al.*, 1986; Iwashita and Fox, 1984; Mark and Rapp, 1984; Moelling *et al.*, 1984; Ohno *et al.*, 1987; Sutrave *et al.*, 1984; Walton *et al.*, 1987) is similar to each other. These serine/threonine-specific protein kinase are activated by mitogenic and oncogenic signals (Iwashita and Fox, 1984). And C-*raf* encodes not only a serine/threonine-specific protein, but also a 74 kd cytoplasmic protein. C-*raf* induces, generally, some human and rodent carcinomas (Fukui *et al.*, 1985; Ishikawa *et al.*, 1985; Shimizu *et al.*, 1985). Very few attempt has been made to determine the translocation of c-*raf* protein kinase since c-*raf* protein kinase has been known to exist in cytoplasm only, and appears to be cascaded (Rapp *et al.*, 1987). Also, the signal transduction of *raf* protein kinase (Rapp *et al.*, 1988) and protein kinase C (Nishizuka, 1984; 1986) has been recently studied.

It is of importance to investigate whether the localization of the c-*raf* protein kinase may alter from the cytoplasm to the nucleus. Therefore, the present paper attempts to elucidate the precise time course of c-*raf* protein kinase following treatments of 12-0-tetradecanoylphorbol-13-acetate (TPA) which is one of tumor promotors or platelet-derived growth factor (PDGF) using immunocytochemical procedure.

Materials and Methods

Cell Culture

The *raf*-transformed EC-4 cells, a subline of NIH3T3 cells, were kindly provided by Drs.

This investigation was supported by grant from Korea Science and Engineering Foundation (891-042-028-1).

Wayne Anderson (Cellular Oncology Lab., NCI, NIH) and Ulf Rapp (Viral Carcinogenesis Lab., NCI, NIH). Cells were cultivated as described previous paper (Rapp *et al.*, 1988). The cells were grown in Dulbecco's Modified Eagles Medium (DMEM, GIBCO) supplemented with 10% bovine fetal calf serum (Hyclone) and 10 μl of 100x penicilline-stereptomycine-Fungizone mixture (Whittaker Bioproducts Inc.).

Immunocytochemical Localization studies

EC-4 cells (1×10^2) were seeded into wells (1 cm^2) in Lab-Tek chamber slides (Nunc, INc.) allowing to attach and grow for 24 hrs in the above mentioned culture medium. The attached cells were washed three times with serum-free DMEM in order to reduce the effect of serum. The serum-starved cells were incubated in 200 nM of 12-0-tetradecanoylphorbol-13-acetate (TPA) (Pharmacia P-L Biochemicals) or 5 ng/ml of platelet-derived growth factor (PDGF)(PDGF, Inc.) according to the time course (5 min, 10 min, 20 min, 30 min, 1 hr, 6 hrs, and 12 hrs). And then, the cells were washed with PBS-washing buffer containing 0.005% Tween-20, 0.014% $CaCl_2.2H_2O$ and 0.01% $MgCl_2.6H_2O$ three times for 5 min each. They were fixed with 2% formaldehyde in PBS without Tween-20, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ at room temperature. Then washed three times for 15 min with PBS and permeabilized with cold absolute metanol for 5 min. They were washed with PBS three times for 15 min. They were blocked with 2% bovine serum albumin (BSA) in PBS for 1 hr. They were incubated at 4°C for 24 hrs with rabbit anti c-*raf* SP63 (1 : 300 dilution) antisera in PBS containing 2% normal goat serum and 0.5% Triton X-100. Following this incubation, they were washed with PBS four times for 20 min. And they were reacted according to the avidin-biotin-Texas Red method (Bayer and Wilchek, 1979; Guesdon *et al.*, 1979). They were reacted with biotinylated goat anti-rabbit IgG (2.5 mg/ml,BRL) in PBS containing 1% BSA and 0.05% Tween-20 for 2 hrs at room temperature. They were washed with PBS three times for 15 min and then reacted with 0.15% streptoavidin-Texas Red in PBS containing 1% BSA and 0.05% Tween-20, and washed with PBS three times for 15 min. They were washed finally with 1% BSA in PBS. Slides were mounted with 50% glycerol in PBS and photographed with P800/1600 Kodak Ektachrome film under an Olympus BH-2 microscope at x400.

Isolation of Nuclei

The cells were cultured in serum-free DMEM for 24 hrs and isolated from culture flasks. The cells were incubated with 200 nm of TPA for 30 min after washing with the serum-free DMEM and then, centrifuged at 10,000 xg for 3 min. The pellet was suspended and stayed in swollen buffer (10 mM Tris-HCl, pH 7.4 containing 10 mM NaCl, 1.5 mM $MgCl_2.6H_2O$ and 0.25 M) for 20 min. The swollen cells were homogenized 8-10 up-and down strokes at 2,000 rpm with Potter-Elvehjam homogenizer. Homogenized cells were centrifuged at 1,000 xg for 20 min. The pellet were suspended and washed in 0.25 M sucrose in buffer A (10 mM Tris-Hcl, pH 7.4 containing 3 mM $CaCl_2.2H_2O$) at 1,000 xg with centrifuge for 20 min. And crude nuclear pellet and crude cytosol were obtained. The crude nuclear pellet was homogenized in 2.2 M sucrose, centrifuged at 40,000 $\times$ g for 1 hr and homogenized in 0.25 M sucrose in buffer A containing 1% Triton X-100. After centrifugation at 1,000 $\times$ g for 20 min, the nuclear pellet was washed twice with 0.25 M sucrose in buffer A without Triton X-100 to obtain the pruified nuclei.

Immunoblotting

The isolated cytosol and nuclei fractions were electrophoresed on the polyacrylamide slab gel, and transferred to nitrocellulose paper in electrode buffer (20 mM Tris-HCl, 150 mM glycine and 20% methanol). The transferred nitrocellulose papers were saturated with 2% BSA. They were incubated overnight at 4°C with primary antibody (15 μg/ml), and with horseradish peroxide as secondary antibody for 4 hrs at 4°C. They were developed with 4-chloro-1-napthol and hydrogen peroxide. The nitrocellulose papers were washed and dried.

Results

EC-4 cell line is a retrovirus expressing full length of c-*raf*-1 cDNA. To determine the effect

of signal translocation, the TPA and PDGF were treated to EC-4 cells. Figs. 1a and 2a show the distributions of c-*raf* protein kinase in the control groups treated with TPA and PDGF, respectively. C-*raf* protein kinase is scattered throughout the cell in normal condition. Especially, it is clear that the c-*raf* protein kinase was significantly distributed in the cytoplasm. Figs. 1b and 2b are the phase contrast photographs of Figs. 1a and 2a.

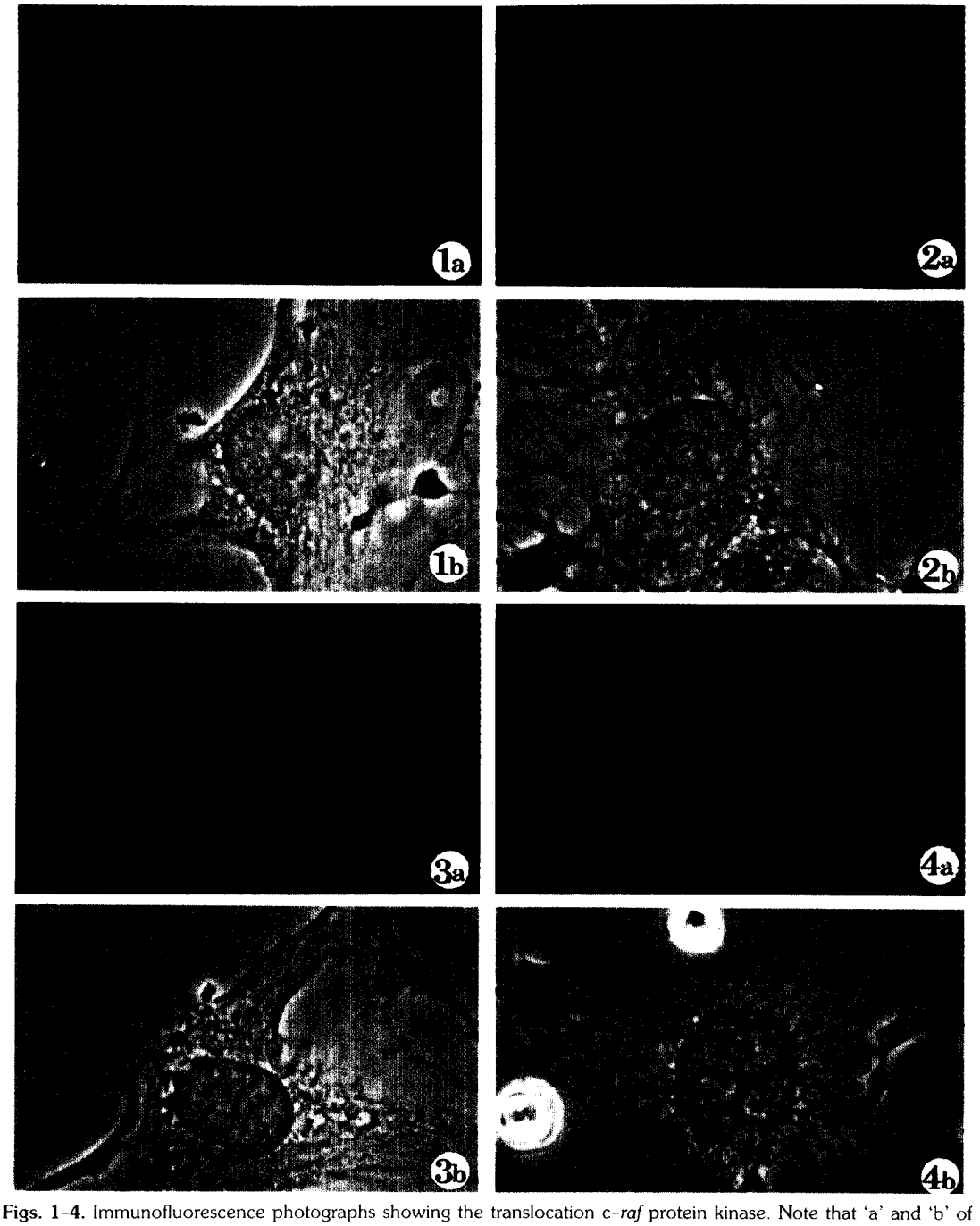
In the TPA-treated cell for 5 min, c-*raf* protein kinase was retained not only in the cytoplasm and the perinuclear membrane but in the nucleus staining very weakly as shown in immunoreacted cells under fluorescence (Fig. 3a) and phase-contrast optics (Fig. 3b). The cells treated with PDGF for 5 min (Figs. 4a and 4b) showed that PDGF induced the translocation of c-*raf* into the nucleus was more rapidly induced by PDGF (Fig. 4a) then TPA (Fig. 3a). The enzyme was found to be largely associated with the perinuclear membrane and the nucleus (Fig. 4a), while it began to migrate into the nucleus in only very small quantity after TPA treatment.

In the TPA-treated cell for 10 min (Figs. 5a and 5b), the high-leveled enzyme in quantity was immunolabeled throughout the cytoplasm and nucleoplasm. At this time, the immunoreactivity of perinuclear membrane was more intensed (Fig. 5a) than that of TPA-treated cell for 5 min as seen (Fig. 4a). In the PDGF-treated cell for 10 min (Figs. 6a and 6b), the immunoreactivity was occurred in the highest level in the nucleus. The enzyme was also thickly accumulated around perinuclear membrane, as shown in Fig. 6a. And the enzyme also associated with the cytoplasmic compartments around the nucleus.

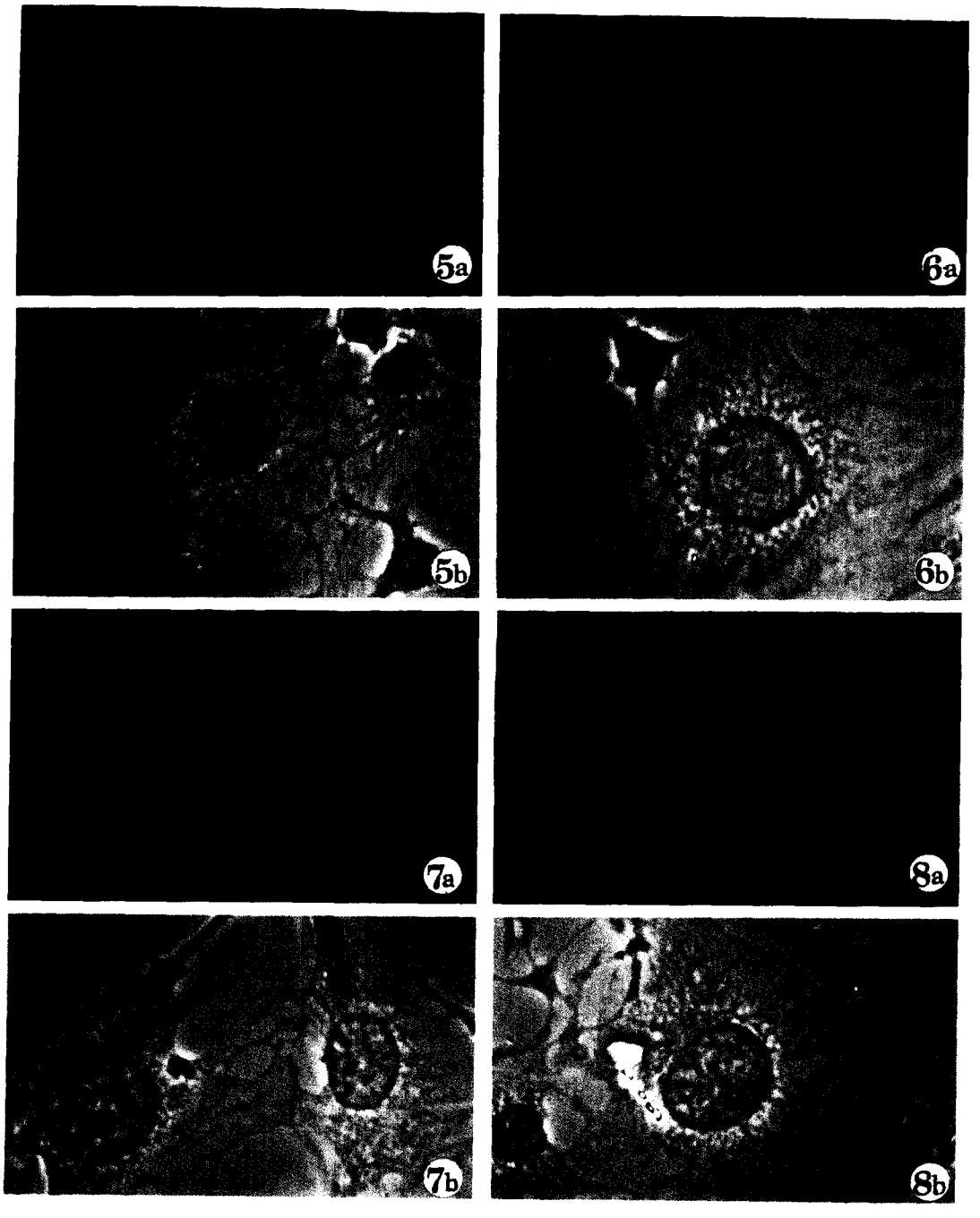
In the immunolabeled cell examined for 20 min incubated with TPA (Figs. 7 a and 7b), the immunoreactivity was the highest level which was seen to correspond with the case of PDGF-treated cell for 10 min in Fig. 7a. And the translocated c-*raf* protein kinase was maintained in high quantity in the nucleus of the cell incubated with PDGF. The highest level of the enzyme in the nucleus was maintained for two durations; One is from at 10 min until 20 min (Figs. 8a and 8b) with PDGF treatment, and the other is from at 20 min until 30 min (Figs. 9a and 9b) with TPA treatment. There were no evidences of the changes of the translocation for both durations. Hence, the translocation in the PDGF-treated cell always took place a step ahead than that of the TPA-treated cell. In the PDGF-treated cell for 30 min (Figs. 10a and 10b), the enzyme began to derease in the cytoplasm.

In the TPA-treated cell for 1 hr (Figs. 11a and 11b), there was observed the low level of immunolabeled enzyme. The immunoreactivity in Fig. 11a seemed to correspond with the begining of the enzyme degradation in the nucleus of PDGF-treated cell as shown in Fig. 10a. Although the most of the immunolabeled enzymes had been degraded in the nucleus, the cytoplasmic immunolabeled enzymes were increased as seen in Fig. 11a. In the case of the PDGF-treated cell for 1 hr (Figs. 12a and 12b), the enzyme was largely increased on the framework of the cytoplasmic components. While the degradation of the enzyme was going on in the nucleus.

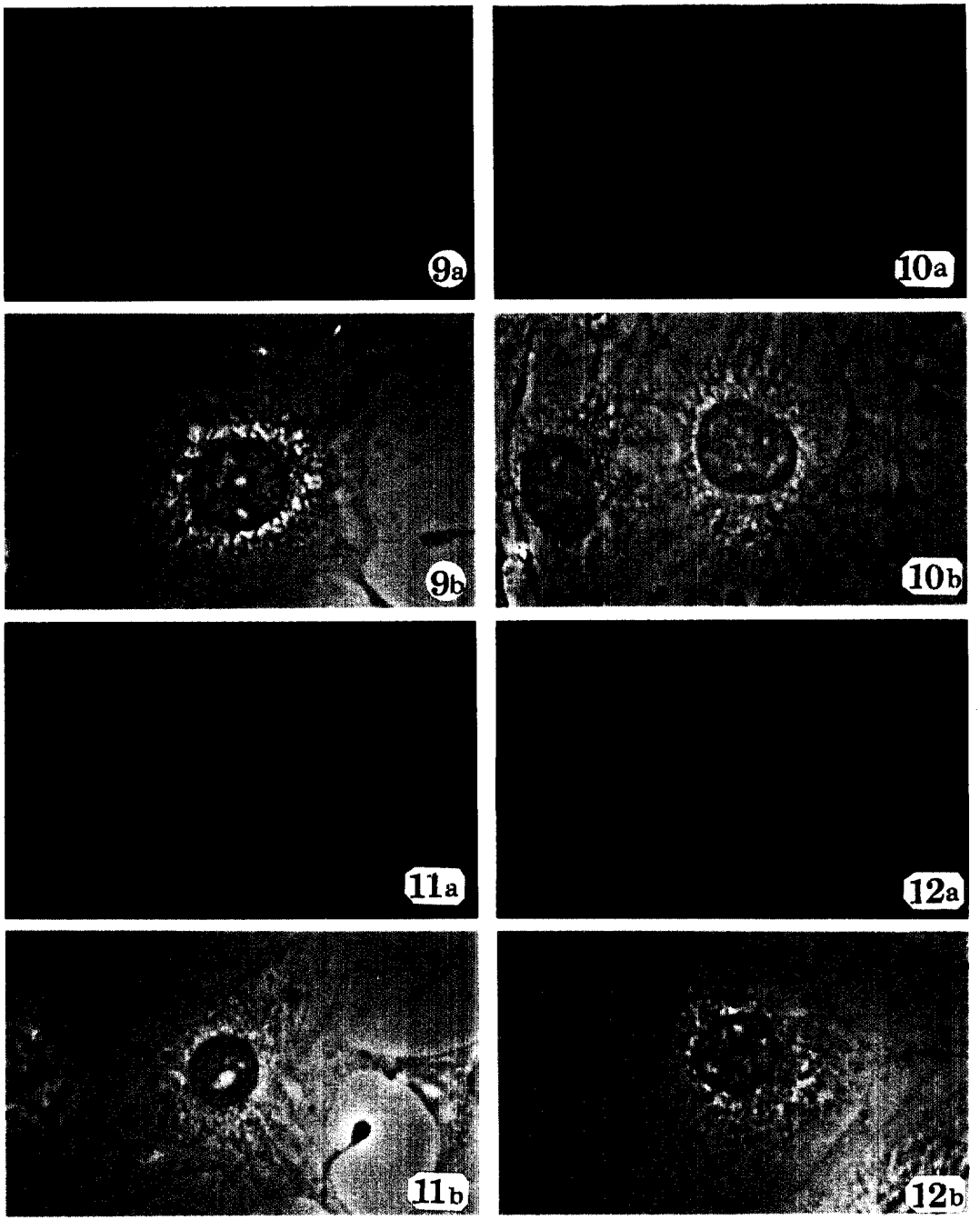
In the TPA-treated cell for 6 hrs (Figs. 13a and 13b) or 12 hrs (Figs. 15a and 15b), the immunoreactivity of the enzyme was more confined to the limited area which was more compacted around nuclei (Figs. 13a and 15a) than in the earlier stage of the TPA-treated cell. In the PDGF-treatment for 6 hrs (Figs. 14a and 14b) or 12 hrs longer (Figs. 16a and 16b), there was no immunoreactivity in the nucleus. But the immunolabeled enzymes were condensed in the very limited area around the nucleus (Fig. 14a) or the one side onto the perinuclear membrane (Fig. 16a). At this time, the enzyme in the nucleus began to degrade in many cases, and at the same time, the enzymes were trapped within the cytoplasmic framework around the nucleus from the enzyme scattering throughout the cytoplasm. Fig. 17 shows the bands of c-*raf* protein kinase after TPA-treatment for 30 min by using immunoblotting. The band of the c-*raf* protein kinase occurred in the control group of whole cell extraction without TPA-treatment (Fig. 17, lane 1) and of the cytosol fraction treated with TPA for 30 min (Fig. 17, lane 3). The appearance of the band in the control group was clearly indicated that EC-4 cell was transfected with 10 fold overexpression of c-*raf*-1 from retrovirus vector than normal cell, so



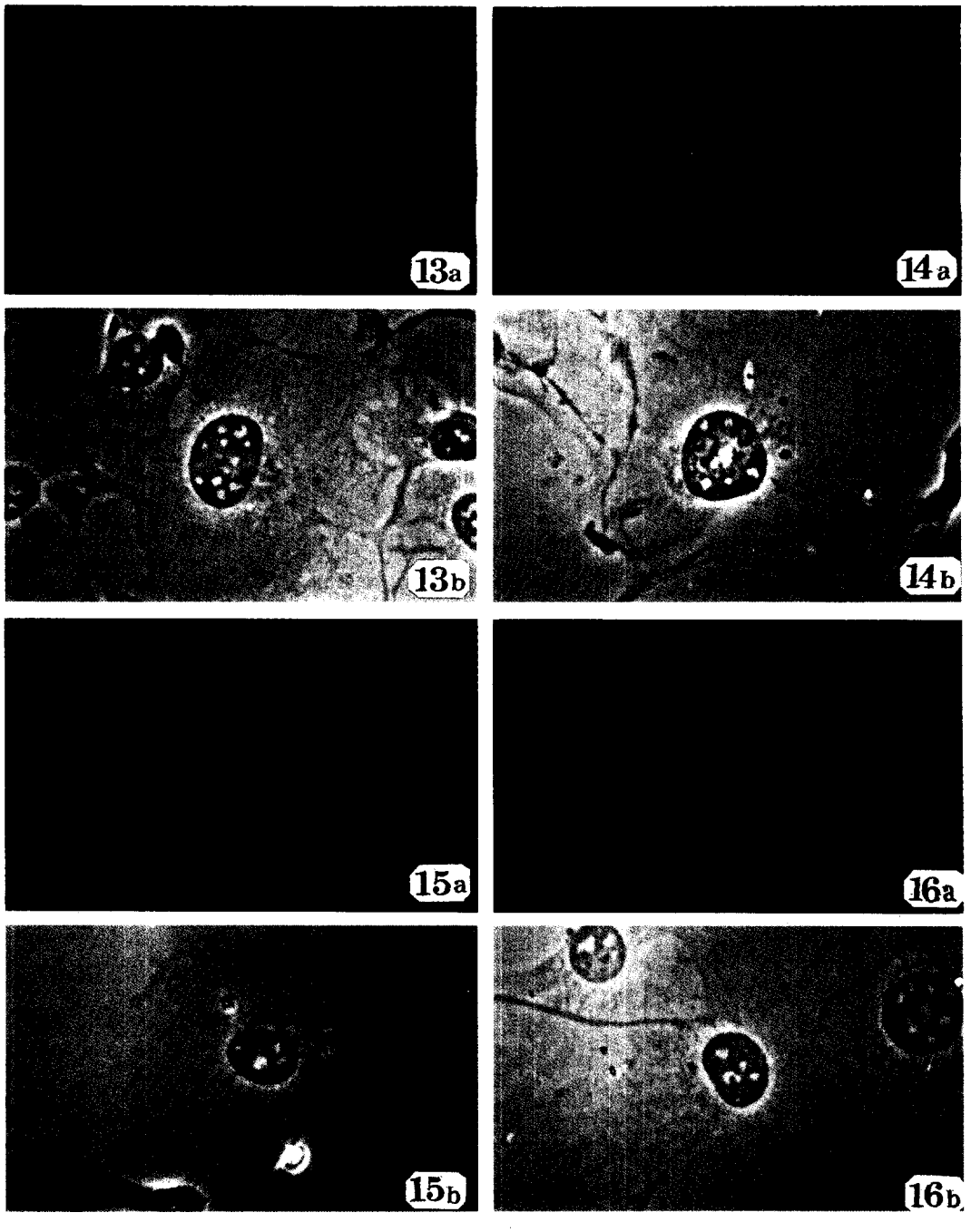
Figs. 1-4. Immunofluorescence photographs showing the translocation c-*raf* protein kinase. Note that 'a' and 'b' of all figures designate immunofluorescence photographs and phase contrast photographs. In the control group (Figs. 1a and 1b) of c-*raf* protein kinase without TPA, the enzyme is weakly intensed throughout the cytoplasm. But no enzyme is located in the nucleus. Control group (Figs. 2a and 2b) of c-*raf* protein kinase without PDGF. After TPA-treated cell for 5 min (Figs. 3a and 3b), the enzyme is still located in the cytoplasm and appears onto perinuclear membrane. And after PDGF-treated cell for 5 min (Figs. 4a and 4b), c-*raf* protein kinase begins to migrate into the nucleus.



Figs. 5-8. The enzyme is located both in the cytoplasm and the nucleus (Figs. 5a and 5b) for 10 min TPA-tratment. But most of c-*raf* protein kinase migrated into the nucleus after PDGF-treatment for 10 min (Figs. 6a and 6b). In the TPA-treated cell for 20 min (Figs. 7a and 7b), the enzyme is located more abundantly than that of 10 min-treated cell. In the PDGF-treated cell for 20 min (Figs. 8a and 8b), the enzyme begins to redistribute into the cytoplasm.



Figs. 9-12. In the TPA-treated cell for 30 min (Fgis. 9a and 9b), most of c-*raf* protein kinase translocated into the nucleus. The enzyme is decreased in the nucleus (Figs. 10a and 10b) in the PDGF-treated cell for 30 min. In the TPA-treated cell for 1 hour (Figs. 11a and 11b), c-*raf* protein kinase redistributed in the cytoplasm. C-*raf* protein kinase is redistributed in the nucleus (Figs. 12a and 12b).



Figs. 13-16. c-*raf* protein kinase begins to condense around perinuclear membrane (Figs. 13a and 13b) in the TPA-treated cell for 6 hours. And c-*raf* protein kinase condensed in a confirmed area around nucleus (Figs. 14a and 14b) in the PDGF-treated cell for 6 hours. In the TPA-treated cell for 12 hours, the enzyme is more condensed than that of the 6 hour-treated cell with TPA (Figs. 15a and 15b). Condensed mass of c-*raf* protein kinase appears at the one-side of perinuclear membrane (16a and 16b).

called NIH3T3 Clone-7 cell. But the isolated nuclei without TPA-treatment had not been occurred in any band of c-*raf* protein kinase (Fig. 17, land 2). It means that the enzyme was not translocated into the nucleus in this case. The band occurred in the isolated nuclei which were treated with TPA (Fig. 17, lane 4). It appears then that the enzyme was translocated into the nucleus only after TPA-treatment.

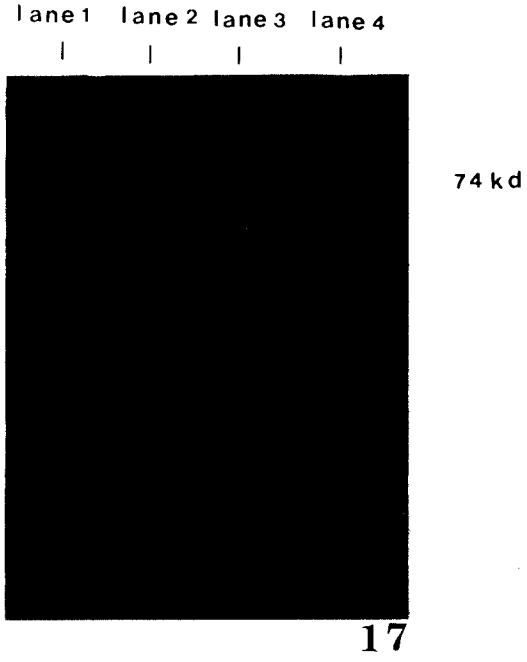


Fig. 17. Immunoblotting after TPA treatment for 30 min. Lane 1: Control of the crude extraction. Lane 2: Isolated nuclei without TPA. Lane 3: Isolated cytosol with TPA. Lane 4: Isolated nuclei with TPA.

Discussion

Protein kinase C (PKC) is activated by the presence of both 1,2-diacylglycerol (DAG) which was produced from the hydrolysis of inositol phospholipids (Galizzi *et al.*, 1987; Takai *et al.*, 1985) and Ca^{++} (Pozzen *et al.*, 1984). And PKC has a mechanism to transduct into the cell from extracellular signals (Bell, 1986; Shoji *et al.*, 1986; Takai *et al.*, 1985) and induces mitogenic event. Both PKC and c-*raf* protein kinase are not only serine/threonine-specific protein but also they are similar to each other in their amino acid sequences and functions.

In the earlier paper (Rapp *et al.*, 1988), we demonstrated that c-*raf* protein kinase was involved in a singal transduction in the cell. The translocational phenomenon of c-*raf* protein kinase appeared in the cells which were treated with TPA as tumor promotor or PDGF as growth factor according to the time-course experiment. The translocation of PDGF-treated cell; The maximal amount of enzyme was translocated into the nucleus after 30 min treated with TPA, while the enzyme of the PDGF-treated cell was reached there in maximum quantity after 10 min. This difference in time of the translocation between TPA and PDGF-treated cells may explain as follows. The PDGF bound with receptor on the plasmic membrane activated phospholipase C (PLC) which produces DAG and inositol 1,4,5-triphosphate (IP_3) from breakdown of phosphatidylinositol 4,5-bisphosphate (PI_2)(Charest *et al.*, 1985; Nosek *et al.*, 1986).

In the presence of both Ca^{++} and phsopholipid, PKC is activated by DAG (Rozengurt, 1986). In the other side, Ca^{++} in the endoplasmic reticulum (ER) is mobilized into the cytoplasm by IP_3. Subsequently, the concentration of Ca^{++} is finally increased in the cytosol (Charest *et al.*, 1985; Nosek *et al.*, 1986). The enzyme may to be activate and induces then some responses of targeting proteins in the cell.

The functional reaction of TPA which is similar to that of DAG arose PKC activation. For this reason, we may easily misunderstand that the reaction of TPA may more rapidly take place than that of PDGF. But, the cell-responsed time (translocation time of c-*raf* protein kinase or PKC) to TPA is delayed than that to PDGF. Because TPA does not directly stimulate or induce IP_3 which stimulated Ca^{++}-releasing from ER. Therefore, I suggest that Ca^{++} is necessary for the translocation of c-*raf* protein kinase into the nucleus from the cytoplasm. Increase of Ca^{++} concentration in the cytoplasm activated the protease. And then, this activated protease may give conformational change of c-*raf* protein kinase or PKC in turn.

PKC activated *myc* gene in the nucleus, and the

activated *myc* gene reactes with *raf* protein kinase synergistically. But cytophysiological mechanism is abnormally broken down by long term exposure to TPA or PDGF. In this situation, most cells require down regulation to keep normal state from abnormal mechanism. C-*raf* protein kinase is activated very high and redistributed in the cytoplasm when the degradation rate of the enzyme is elevated by TPA or PDGF in the cell. The activated enzyme plays inhibition of PLC reaction (Bianca *et al.*, 1986; Brock *et al.*, 1985; Labarc *et al.*, 1984; Rittenhouse and Sassen, 1985), Ca^{++} mobilization (Kariya and Takai 1987), and desensitization of epidermal growth factor (EGF) receptor (Brown *et al.*, 1979; Cochet *et al.*, 1984; Iwashita and Fox, 1984) for the reduction of overexpressed mechanism. Successive down regulation which is induced by long time exposure of TPA or PDFG may take place the phenomenon of condensed c-*raf* protein kinase around perinuclear membrane, and may also influence on cell organells. Ultimately, the enzyme may be abnormally associated with cell organells and condensed as a mass around nucleus membrane by cytophysical phenomenon.

References

Bayer, E. A. and M. Wilchek, 1979. The biotin transport system in yeast. *Methods in Biocehm. Anal.* **26:**1-45.

Bell, R. M, 1986. Protein kinase C activation by diacylglycerol second messemgers. *Cell* **45:**631-632.

Bianca, V. D., K. Greskowiak, M. A. Cassatella, L. Zeni, and F. Rossi, 1986. Phorbol 12-myristate 13-acetate potentiates the respiratory burst while inhibits phosphoinositide hydrolysis and calcium mobilization by formyl-methionyl-leucyl-phenylalanine in human neutrophils. *Biochem. Biophys. Res. Commun.* **135:**556-565.

Bonner, T. I., S. B. Kerby, R. Strave, M. A. Gunnel, G. Mark, and U. R. Rapp, 1985. Structure and biological activity of human homologs of the *raf/mil* oncogene. *Mol. Cell. Biol.* **5:**1400-1407.

Brock, T. A., S. E. Rittenhouse, C. W. Powers, L S. Ekstein, M. A. Gimbrone, Jr., and R. W. Alexander, 1985. Phorbol ester and 1-oleyol-2-acetylglycerol inhibit angiotensin activation of phospholipase C in cultured vascular smooth muscle cells. *J. Biol. Chem.* **260:**14158-14162.

Charest, R., V. Pripic, J. H. Exton, and P. F. Blackmore, 1985. Stimulation of inositol triphosphate formation in hepatocytes by vasopressin, adrenalin and angiotensin II and its relationship to changes in cytosolic free Ca^{++}. *Biochem. J.* **227:**79-90.

Fukui, M., T. Yamaoto, S. Kawai, K. Maruo, and K. Toyoshima, 1985. Detection of a *raf*-related and two other transforming DNA sequences in human tumors maintained in nude mice. *Proc. Natl. Acad. Sci. USA* **82:**5954-5958.

Galizzi, J. P., J. Qar, M. Fosset, C. V. Renterghem, and M. Lazdunski, 1987. Regulation of calcium channels in aortic muscle cells by protein kinase C activators-(diacylglycerol and phorbol esters) and by peptides(vasopressin and bombesin) that stimulate phosphoinositide breakdown. *J. Biol. Chem.* **262:**6947-6950.

Guesdon, J. L., T. Termynck, and S. Averemeas, 1979. The use of avidin-biotin interaction in immunoenzymatic techniques. *J. Histochem. Cytochem.***27:**1131-1138.

Hwang, K. P., H. Nakabayashi, and F. L. Hwang, 1986. Isozymic forms of rat brain Ca^{++}-activated and phospholipid-dependent protein kinase. *Proc. Natl. Acad. Sci. USA* **83:**8335-8539.

Ishikawa, F., F. Takaku, M. Ochiai, K. Hayasi, S. Hirohashi, M. Terads, S. Takayama, M. Nagao, and T. Sugimura, 1986. Activation of rat c-*raf* during transfection of heptocelular carcinoma DNA. *Proc. Natl. Acad. Sci. USA* **83:**3209-3212.

Iwashita, S., and C. F. Fox, 1984. Epidermal growth factor and potent phorbol tumor promoters induce epidermal growth factor receptor phosporylation in a similar but distinctively different manner in human epidernoid carcinoma A431 *cells. J. Biol. Chem.* **259:**2559-2567.

Labarc, R., A. Janowsky, J. Patel. and S. Paul, 1984. Phorbol esters inhibit agonist-induced [^{3}H] inositol-1-phosphate accumulation in rat hippocampal slice. *Biochem. Biophys. Res. Commun.* **123:**703-709.

Melling, K., B. Heimann, P. Beimling, U. R. Rapp, and T. Sander, 1984. Serine- and threonine-specific protein kinase activates of purified *gag-mal and gag-raf* proteins. *Nature* **312:**558-561.

Nosek, T. M., F. W. Mark, S. T. Zeigler, and R. E. Godt, 1986. Inositol triphosphate enhances calcium release in skinned cardiac and skeletal muscle. *American Physiological Society* **33:**c807-c811.

Nishizuka, Y., 1984. Turnover of inositol phospholipids and signal transduction. *Science* **225:**1365-1370.

Nishizuka, Y., 1986. Studies and prospectives of protein kinase C. *Science* **233:**305-312.

Ohno, S., H. Kawasaki,S. Tmajoh, and K. Suzuki, 1987. Tissue-specific expression of three distinct types of

rabbit protein kinase C. *Nature* **325:**161-166.

Pozzan, T., G. Gatti, N. Dozio, L. M. Vicentini, and J. Meldolesi, 1984. Ca^{2+}-dependent and -independent release of neurotransmitters from PC 12 cells: a role for protein kinase C activation. *J. Cell Biol.* **99:**628-638.

Rapp, U. R., L. J. Cleveland, S. M. Storm, T. W. Beck, and M. Huleihel, 1987. Transformation by *raf* and *myc* oncogenes. *In* : Oncogene and Cancer, *VNU Sci. Press., Tokyo/Utrecht, pp.* 55-74.

Rapp, U. R., G. Heidecker, M. Huleihel, J. L. Cleveland, W. C. Choi, T. Pawson, J. N. Ihle, and W. B. Anderson, 1988. *raf* Family serine/threonine protein kinases in the mitogen signal transduction. *Cold Spring Harbor Symposia on Quantitative Biology* LII:173-183.

Rapp. U. R., M. D. Goldsborough, G. E. Mark, T. I. Bonner, J. Groffen, F. H. Reynold, Jr., and F. R. Stephenson, 1983a. Structure and biological activity of *v-raf,* a unique oncogene transduced by a retovirus. *Proc. Natl. Acad. Sci. USA* **80:**4218-4222.

Rittenhouse, S. E. and J. P. Sassen, 1985. Mass changes in myoinositol triphosphate in human platelets stimulated by thrombin. *J. Biol. Chem.* **260:**8657-8660.

Rozengurt, E., 1986. Early signals in the mitogenic response. *Science* **234:**161-166.

Shimizu, K., Y. Nakatsu, M. Sekiguchi, K. Hokamura, K. Tanaka, M. Tarada, and T. Sugimura, 1985. Molecular coloning of an activated human oncogene, homologous to *v-raf,* from primary stomach cancer. *Proc. Natl. Acad. Sci. USA* **82:**5641-5645.

Shintaro, I. and C. F. Fox, 1984. Epidermal growth factor and potent phorbol tumor promotors induce epidermal growth factor receptor phosphorylation in a similar but distinctively different manner in human epidermoid carcinoma A431 cells. *J. Biochem.* **259:**2559-2567.

Shoji, M., P. R. girard, G. J. Mazzei, W. R. Vogler, and J. F. Kuo, 1986. Immunocytochemical evidence for phobol-ester induced protein kinase C translocation in HL60 cells. *Biochem. Biophys. Res. Commun.* **135:**1144-1149.

Sutrave, P., T. I. Bonner, U. R. Rapp, H. W. Jansen, and K. Bister, 1984. Nucleotide sequences of avian retrovial oncogene *v-mil:* homologue of murine retroviral oncogene *v-raf. Nature* **309:**85-88.

Takai, Y., K.Kaibichi, T. Tsuda,and M. Hoshijima, 1985. Roles of protein kinase C in transmembrane signalling. *J. Cell. Biochem.* **29:**143-155.

Walion G. M., P. J. Bertics, L. G. Hudson, T. S. Vedvick-,and G. N. Gill, 1987. A three-step purification procedure for protein kinase C: characterization of the purified enzyme. *Anal. Biochem.* **161:**425-437.

(Accepted April 30, 1990)

EC-4 세포에 있어서 c-*raf* Protein Kinase의 면역세포화학적 위치

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Oncogene의 일종인 c-*raf* protein kinase는 세포질 속에 존재하는 serine / threonine-specific protein이며, 이것은 mitogenic signal에 의해 활성화된다. c-*raf* protein kinase의 구조와 기능은 protein kinase C (PKC)와 매우 유사한 것으로 생각된다. 면역세포화학적으로 c-*raf* protein kinase의 signal transduction을 조사하기 위하여 EC-4 세포에 tumor promotor인 12-0-tetradecanoylphorbol-13-acetate (TPA)와 mitogenic factor인 platelet-derived growth factor (PDGF)로 time-course에 따라서 처리하였다. Translocation되는 c-*raf*는 먼저 perinuclear membrane에 모이고 그 후에 핵내로 이동되었다. 그런데 TPA와 PDGF로 처리한 c-*raf*의 translocation은 각각의 다른 경로를 가짐을 알 수 있었다. TPA와 PDGF을 장기간 처리하였을 때, c-*raf* protein kinase의 down regulation이 유도됨을 알 수 있었다.