Korean J. Zool. 33: 141–151, 1990

Immunocytochemical Localization of *raf* Protein Kinase in Cerebrum of *Geoclemys reevesii* (Gray)

Won Chul Choi and Hyun Keun Moon

Department of Biology, College of Natural Sciences, Pusan National University

Raf **protein kinases and protein kinase C belong to serine/threonine-specific proteins in the cytoplasm, and are similar to each other in functional structure and the aspect of the distribution of cell. The distribution of *raf* protein kinase in the cerebrum of *Geoclemys reevesii* was studied by using the antibodies against a-*raf* and c-*raf* protein kinase which induce the expression of *raf* family oncogenes. In general, *raf* protein kinases were distributed in such restricted regions as the general pallium, hippocampal formation, primordium hippocampi, nucleus of lateral olfactory tract, basal amygdaloid nucleus, and bed of stria terminalis. Immunological labeling of c-*raf* protein kinase was more widespread than that of a-*raf*.**

However, the intensity of the labeling of c-*raf* was lower than that of a-*raf*. The spherical cells of basal amygdaloid nucleus is a ring-like form, because only the cytoplasm was immunolabeled. Especially, c-*raf* protein kinase occurred in the cells which contained protein kinase C abundantly such as pyramidal cells and Purkinje cells. This suggests that a- and c-*raf* protein kinases may synegistically induce carcinoma with *myc* gene which is activated by protein kinase C.

KEY WORDS: *raf*-protein kinases, *raf* antibodies, Pyramidal cells.

The *raf* gene, a kind of oncogene, was found in murine sarcoma virus (MSV) 3611 by Rapp and coworkers (1983). This gene, v-*raf* is to be a transforming gene, and has the encode of serine/threonine-specific protein in cytosol (Beck *et al.*, 1987; Bonner *et al.*, 1986).

Recently, it was reported that the molecular weight of a-*raf* was 64 KD, and that of c-*raf* was 74 KD (Bonner *et al.*, 1985; Bonner *et al.*, 1986; Cleveland *et al.*, 1986; Moelling *et al.*, 1984).

These *rafs* induce primary human stomach cancer (Simizu *et al.*, 1985), human glioblastoma line (Fukui *et al.*, 1985), and rat hepatocellular carcinoma (Ishikawa *et al.*, 1985).

Genetic expression of *raf* gene is the cause of the activation of *raf* protein kinase. And the pathway of the activation of *raf* protein kinases has been studied recently (Rapp *et al.*, 1988).

It is very important to find out in what region of brain tissue protein kinases exist and in what kinds of cells they exist, as *raf* protein kinases cause tumors from the expression of *raf* oncogenes. However, there has not been an immunological investigation of the *raf* genes of the lower Reptilia which live in fresh water. This paper deals with the distribution of *raf* protein kinases by using antibodies against a-*raf* and c-*raf* protein kinases in the cerebrum of *Geoclemys reevesii*.

Materials and Methods

Adult *Geoclemys reevesii* were used as specimens.

Used specific binding antibodies against a- and c-*raf* protein kinases were obtained from Dr. U. Rapp of Viral Carcinogenesis Lab., NCI, NIH, USA.

The brains were removed and fixed for 12 hours at 4°C with a fixative solution containing 2% (wt/vol) formaldehyde, 1.5% lysine, and 0.2% sodium m-periodate in a sodium phosphate buffer, pH 7.4 (Gerfen *et al.*, 1987). The brains were then transferred to 0.1 M potassium phosphate buffer saline (KPBS, pH 7.4) for 12 hrs at 4°C. The brains were sectioned on a cryocut microtome. Sections were incubated in dilutions of polyclonal antibodies (each 10 μg/ml) directed against a-*raf* protein kinase or c-*raf* protein kinase in KPBS containing 2% normal goat serum and 0.5% Triton X-100 at 4°C for 24 hrs. Following incubation in the primary antibodies, sections were rinsed three times for 5 min each and reacted then using the avidin-biotin peroxidase technique (Hsu *et al.*, 1981). Sections were first incubated in a dilution of goat anti-rabbit conjugated biotin (2.5 μg/ml, BRL, USA) in KPBS containing 2% normal goat serum for 2 hrs at room temperature and then rinsed 2 times for 15 min in KPBS. They were incubated in dilution of streptavidin-conjugated peroxidase (2.5 μg/ml) in KPBS for 1 hr at room temperature and first rinsed for 5 min in 0.1 M sodium acetate buffer pH 6.0 (containing 5 mg of 3, 3'-diaminobenzidine, 20 mM dextran, 7.5 mM NH_4Cl, and 25 units of glucose oxidase) and then washed to a fresh solution for 30 min at room temperature. The sections were transferred in sodium acetate buffer containing 1.0% (v/v) formalin for 10 min, rinsed in KPBS, and mounted with balsam onto gelatin-coated slides.

For the isolation of cytosol and membrane fractions of the cell, the brain was finely chopped in the ice cold HAP (10 mM KH_2PO_4-NaOH buffer (pH 6.0) containing 5% glucose, 5% fructose, and 1% bovine serum albumin) medium. Trypsinization medium (HAP medium containing 1% trypsin, 10 mg/g tissue) was added to the pellet after centrifugation for 5 min at 140 xg, and then shaken for 90 min in the incubator of 37°C. After washing, the supernatant was discarded. The pellet was added to ice cold buffer calf serum (a mixture of 9:1 (v/v) calf serum and phosphate buffer, pH 6.0), and centrifuged for 5 min at 140 xg. Again, the supernatant was discarded. The pellet containing small pieces of tissue was washed two times in HAP meidum, place on the nylon bolting cloth of 150 mesh, and stroked with a glass bar. The cells which passed through the nylon bolting cloth was washed in HAP medium without trypsin, and then finally washed in KPBS. The pellet was ultrasonicated after adding 0.5% sodium deoxycholate. The pellet and supernatant were used as membrane fraction and as cytosol fraction respectively.

For the immunoblotting, the isolated cytosol and membrane fractions were electrophoresed on the polyacrylamide slab gel, and electrophoretically transferred to a nitrocellulose sheet in electrode buffer (20 mM/L Tris-HCl, 150 mM/L glycine, 20% methanol, v/v). The transferred nitrocellulose sheets were saturated with 2% BSA. They were then incubated overnight at 4°C with primary antibody (15 μg/ml). The nitrocellulose sheets were incubated in horseradish peroxidase conjugated anti-rabbit IgG for 4 hrs at 4°C, and then developed with 4-chloro-1-napthol and hydrogen peroxide. The nitrocellulose sheets were washed and dried.

Results

The diagram of the brain of *Geoclemys reevesii* beionging to Reptilia is shown in Fig. 1. For immunohistochemical observation, the brains were sectioned transversely as shown in Fig. 2. Fig. 3 is a diagram of the cerebral regions depicted in

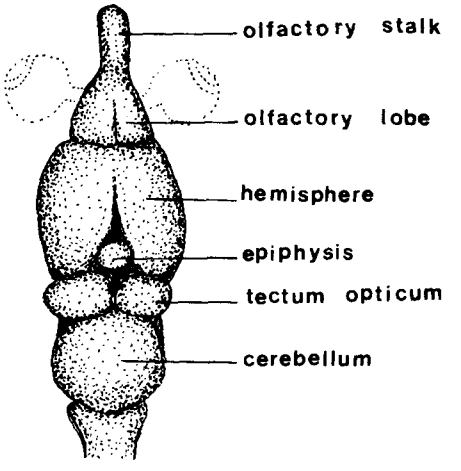

Fig. 1. Brain of *Geoclemys reevesii*.

Fig. 2. Transverse section through the middle part of cerebral hemisphere.

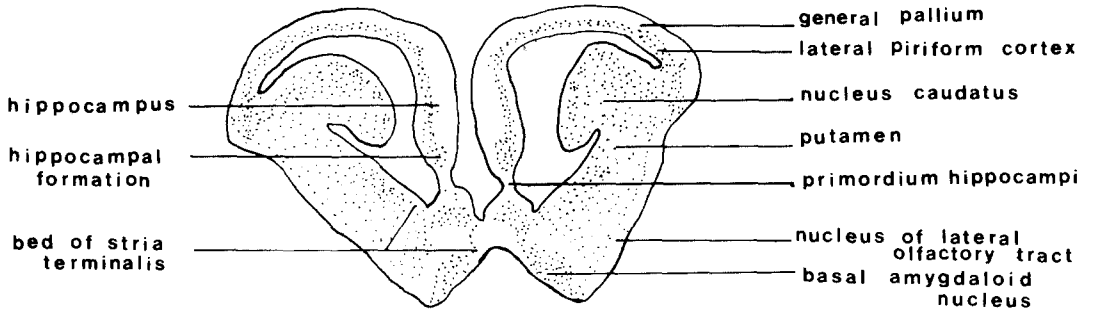

Fig. 3. Diargam of fig. 2.

Fig. 2.

Neuronal cells in the restricted area were labeled with each antibody, but antibodies preadsorbed with the heterologous antigen did not label on the sections. Both antibodies against a-*raf* and c *raf* produced specific labeling patterns in the brain of *Geoclemys reevesii*. The middle part of the cerebrum (a little upper part of epiphysis) was sectioned and treated with immunocytochemical technique.

In the general pallium, the immunoreactivity of a-*raf* (Fig. 4) and c-*raf* protein kinase (Fig. 5) appeared typically in some pyramidal cells among many constituent cells. A-*raf* protein kinase was more densely distributed and clearly seen than c-*raf* protein kinase. The labeling of the antibody, especially, against a-*raf* protein kinase occurred very densely in the neuronal cell bodies, but was not labeled in their nuclei. On the other hand, the c-*raf* protein kinase was more lightly stained than

the a-*raf* protein kinase.

In the primordium hippocampi, the property of immunoreactivity of a-*raf* protein kinase was relatively dense labeled at the connected portion with the fasciculated dendrite and at the opposite portion against at the dendrite portion of the cell body, and also stained on the long fasciculated dendrites of pyramidal cells (Fig. 6). The nucleus of this neuronal cell was not stained the same as in the general pallium. Therefore the cell was occurred in ring-shaped. The dendrite labeled with c-*raf* was not easily found because of the low level of c-*raf* protein kinase (Fig. 7).

In the hippocampal formation, the highest immunoreactivity arose treated with a-*raf* antibody (Fig. 8), while the relatively large pyramidal cells were not clearly labeled. The whole cell body of other constituent cells, small sized cells, were strongly labeled under light microscope, although nuclei were not labeled. Somethimes the labeled fasciculated dendrite of a large sized cell was observed, but the dendrites were failed to observe in the small sized cell. The labeling pattern of the antibody against c-*raf* was very low level in the big sized cell, which is streamlined shaped, and it did not occur in the small sized cell labeled with a-*raf* (Fig. 9).

In nucleus caudatus, only a few large sized cells were immunolabeled with a-*raf* and other constituent cells were not immunolabeled (Fig. 10). The pryamidal cells with fasciculated dendrites were more condensely labeled with c-*raf* antibody than the neighboring neuronal cells in this region (Fig. 11).

In the most putamen region, no immunoreactivity of both antibodies against a- and c-*raf* protein kinases was found. Rarely, a few small cells reacted with only the a-*raf* antibody (Fig. 12), but the cells labeled with the antibody of c-*raf* were not observed at all (Fig. 13).

In nucleus of lateral olfactory tract, the antibody of a-*raf* was densely labeled only in the fasciculated dendrite with the except of the cell body of pyramidal cell. Hence, it was seen similar to an irregular network in form (Fig. 14), but the labeled cells were not many distributed in this region. Fig. 15 shows the large sized cells labeled with the antibody of c-*raf*. It is not easy to find the labeled fasciculated dendrtie which stretches from the neuronal cell.

In the bed of stria terminalis, relatively large numbers of cells in small size were labeled with the antibody of a-*raf*. Especially, the spherical neuronal cell was composed of a large volume of nucleus among neighboring cells (Fig. 16), and the spherical cell was only labeled in cytoplasm. Therefore, immunolabeling seems ring-shaped in appearance.

The number of cells labeled with antibody against c-*raf* protein kinase was fewer than that of those labeled with the a-*raf* antibody. The immunoreactivity occurred in the relatively large cells (Fig. 17).

In the basal amygdaloid nucleus, cells of some different appearance were located and these reacted very strongly with the antibody of a-*raf*. It is an interesting phenomenon that the volume of the ring-shaped cell was larger than that of the constituent cells in the bed of stria terminalis (Fig. 18). The labeling pattern of antibody against c-*raf* had a lower intensity than that with a-*raf* (Fig. 19).

Fig. 20 shows the band of the a-*raf* protein kinase clearly occurred in the cytosol fraction, but it was not detected in the membrane fraction. But the band of c-*raf* protein kinase was not observed in cytosol and membrane fractions.

In view of the results so far achieved, the labeled cells with a- and c-*raf* antibodies were distributed in the general pallium, primordium hippocampi, hippocampal formation, nucleus of lateral olfactory tract, bed of stria terminalis, and basal amygdaloid nucleus. In these regions, generally, oval- or pyramidal-shaped cells were labeled with the antibodies in the cellular distribution of *raf* protein kinases.

Among the immunolabeled region with the both a- and c-*raf* antibodies, especially, the fasciculated dendrites of neuronal cells were densely immunolabeled with a-*raf* antibody in general pallium, primordium hippocampi, and nucleus of lateral olfactory tract. In the immunoreactivity, the labeling pattern of a-*raf*-positive system was more clearly defined than that of the c-*raf*-positive system when each antibody was treated in the same concentration.

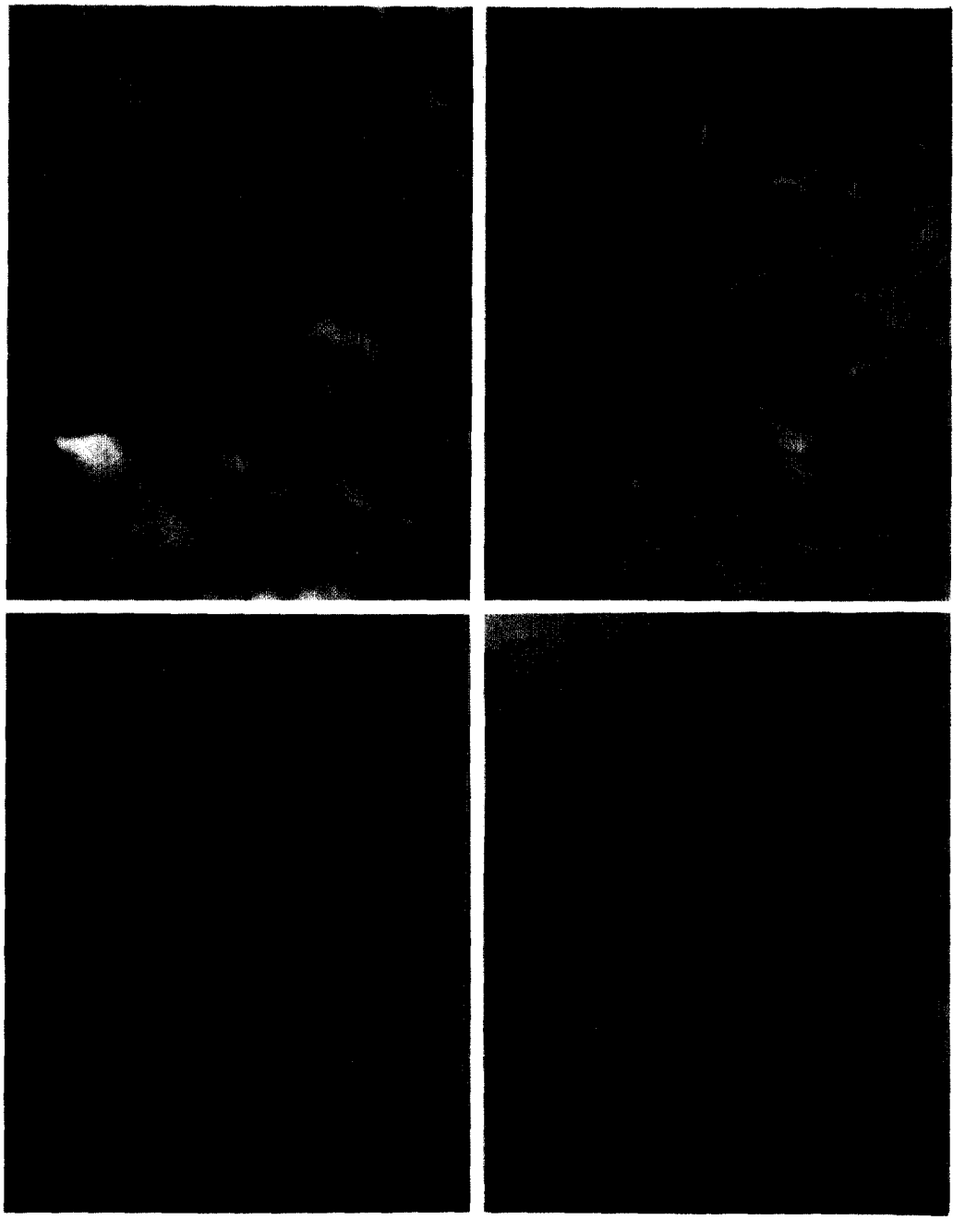

Fig. 4. In general pallium, cytoplasm is strongly labeled for the immunoreactivity of a-*raf* protein kinase.
Fig. 5. c-*raf* protein kinase occurs with little lower immunoreactivity than a-*raf* protein kinase of Fig. 4. in general pallium.
Fig. 6. Pyramidal cells with fasciculated dendrites are densely labeled with a-*raf* protein kinase antibody in the primordium hippocampi.
Fig. 7. No immunoreactivity of c-*raf* protein kinase is observed on the fasciculated dendrite in primordium hippocampi.

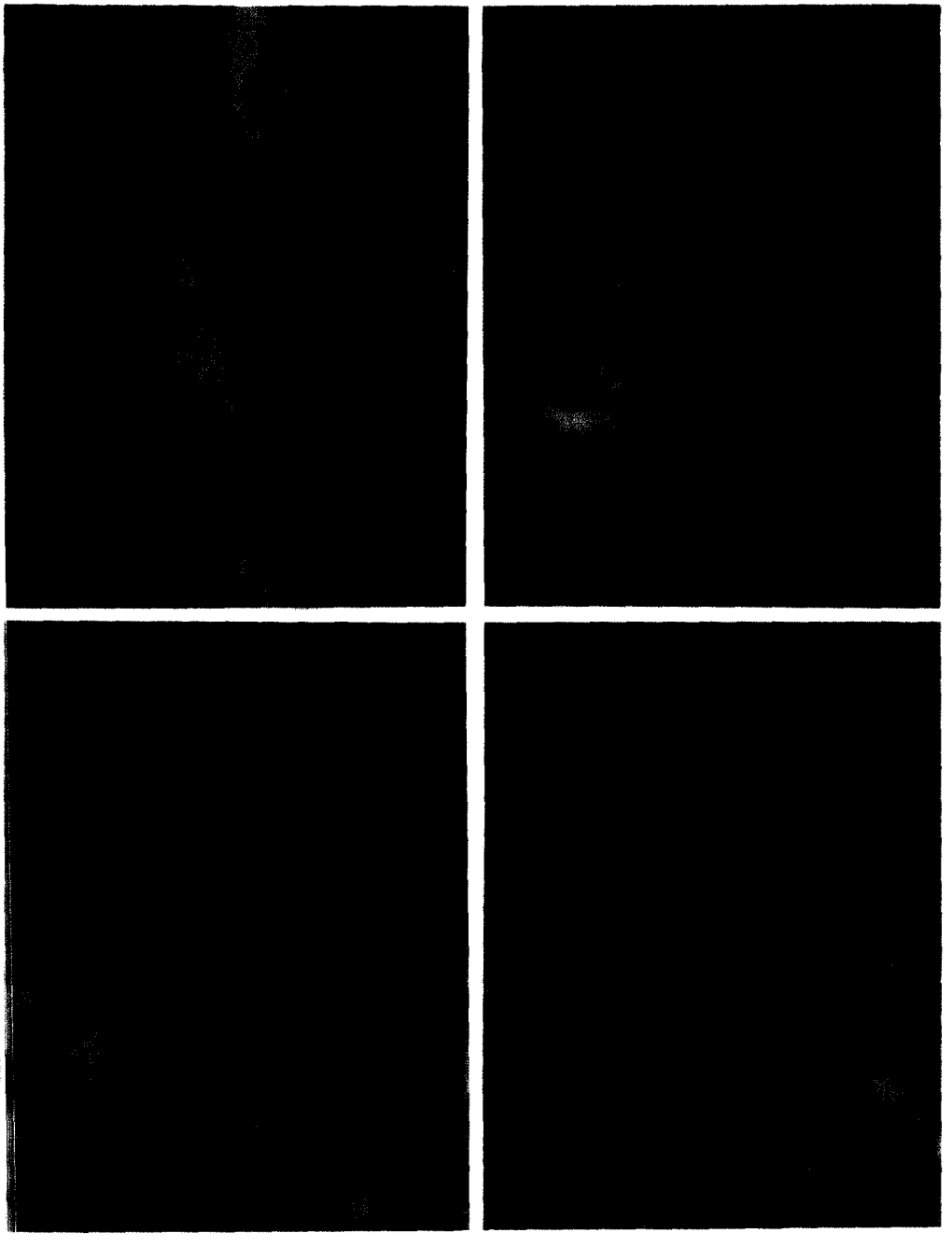

Fig. 8. The small cells in hippocampal formation are densely labeled with a-*raf* protein kinase antibody.
Fig. 9. Pyramidal cells in the same region of Fig. 8 are labeled with c-*raf* protein kinase antibody.
Fig. 10. In few cells, immunoreactivity of a-*raf* protein kinase appeared in the nucleus caudatus.
Fig. 11. Nuleus caudatus region is immunoreacted with c-*raf*. Pyramidal cell with a fasciculated dendrite is more clearly labeled than are neighboring neurons.

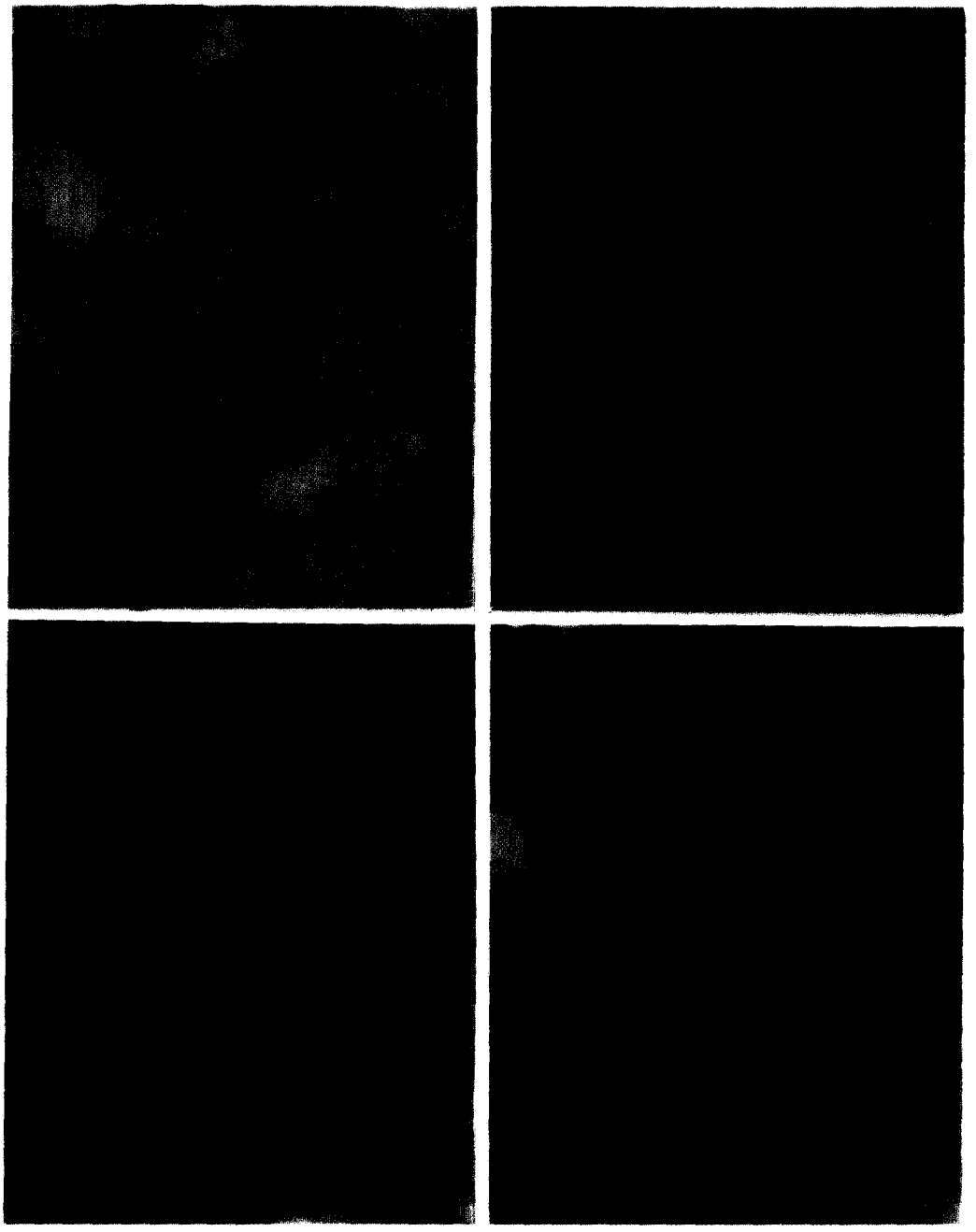

Fig. 12. Cells in the most putamen region do not react with a-*raf* protein kinase antibody. Very few small cells show immunoreactivity.
Fig. 13. No cells in the putamen react with the c-*raf* protein kinase antibody.
Fig. 14. In nucleus of lateral olfactory tract, some cells are labeled with a-*raf* protein kinase antibody, and many dendrites reacted.
Fig. 15. Some cells except their dendrites are labeled with c-*raf* protein kinase antibody in nucleus of lateral olfactory tract.

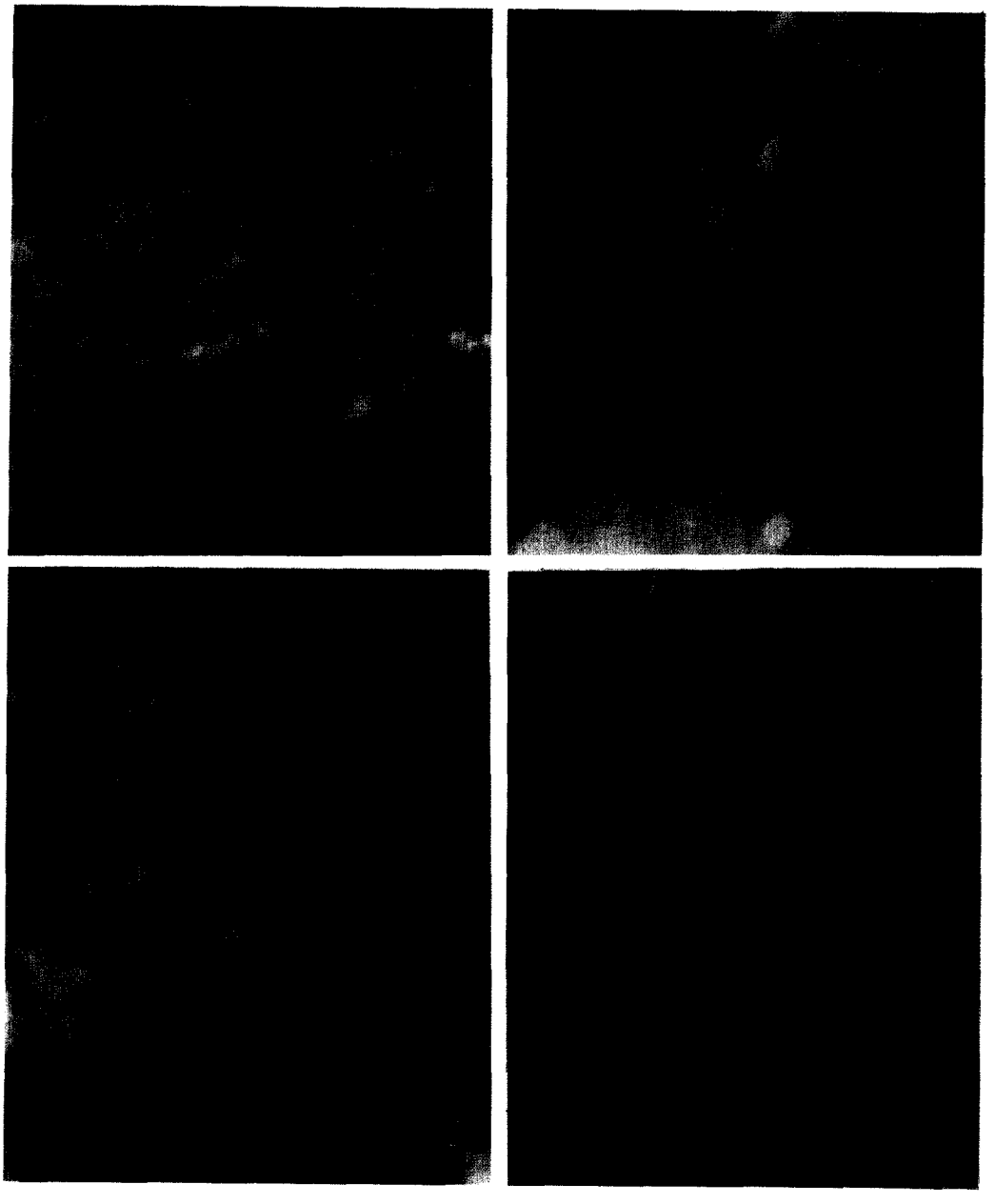

Fig. 16. Cells in bed of stria terminalis are densely labeled with a-*raf* protein kinase antibody.
Fig. 17. In bed of stria terminalis, cells are weakly labeled with c-*raf* protein kinase antibody.
Fig. 18. cytoplasm of cell in basal amygdaloid nucleus is densely labeled with a-*raf* protein kinase antibody.
Fig. 19. Cells in basal amygdaloid nucleus are very light labeled with c-*raf*. *All magnifications of Fig. 4 through Fig. 19 are 660 ×.

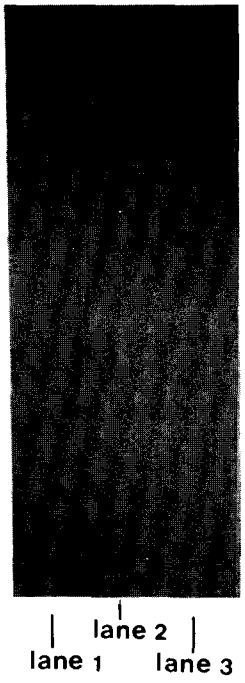

Fig. 20. The band of a-*raf* protein kinase on the nitro cellulose paper after immunoblotting. Lane 1: crude extraction. Lane 2: cytosol fraction. Lane 3: membrane fraction. *Note: the band is not detected in lane 3.

Discussion

In *raf* family oncogene, there are v-, a-, b-, c-, and d-*rafs*. C-*raf* is the progenitor gene of v-*raf*, and a-*raf* is a v-*raf* related gene which originated from v-*raf*. Also, *raf* genes have specific chromosomal locations in murine and human genomes (Bonner *et al.*, 1984). They were also found in various carcinomas in murine and human. A-*raf* occurs in long adenocarcinoma, epidermoid vulva carcinoma, and epididymis, etc. C-*raf* appears not only in these tumors induced by a-*raf*, but are also widespread in various tumors (Rapp *et al.*, 1987a).

In the previous study (Rapp *et al.*, 1988), it was demonstrated that the location of c-*raf* protein kinase is changed after adding platelet-derived growth factor (PDGF) as mitogen, and 12-0-tetradecanoylphorbol-13-acetate (TPA) as tumor promotor to NIH3T3-Clone 7 cells respectively. The phenomenon of translocation of *raf* protein kinases is similar to that of protein kinase C (Anderson *et al.*, 1985; Castagna *et al.*, 1982; Nishizuka, 1986; Pelech, 1986). Moreover, Worley and coworkers (1986) described that protein kinase C is densely located in pyramidal cells in rat brains. This localization pattern and some biochemical properties of *raf* protein kinase labeled by the immunocytochemical procedure are very similar to those of protein kinase C. Therefore, the possibility of their directed interactions is, firstly, diacylglycerol stimulates protein kinase C to activation (Bell, 1986), and then this activated protein kinase C is to make the high level of *myc* gene expression (Rapp *et al.*, 1988). Then *raf* and *myc* are synergically induced carcinoma (Cochran *et al.*, 1983; Jansen *et al.*, 1984; Kelly *et al.*, 1983; Rapp *et al.*, 1985; Rapp *et al.*, 1987a, 1987b).

In the second possibility, although some investigators (Rapp *et al.*, 1987a; Smith *et al.*, 1986) suggest that *ras* stimulates *raf* protein kinase, other biochemical pathways of protein kinase C may activate *raf* protein kinase. *Raf* and protein kinase C are located together in pyramidal cells or in other kinds of neuronal cells. Perhaps protein kinase C may directly or indirectly activate *raf* protein kinase through other biological mechanisms.

The biochemical properties are similar between a-*raf* and c-*raf* protein kinases, but their labeling intensities of antbodies show different appearance in the brain. In the cerebrum of *Geoclemys reevesii*, the cells which are labeled by the antibody of a-*raf* protein kinase are easily detected in the cells of some restricted regions in cerebrum. That is, a- and c-*raf* protein kinases are only distributed in the cells which are located in general pallium, primordium hippocampi, hippocampal formation, nucleus of lateral olfactory tract, bed of stria terminalis, and basal amygdaloid nucleus. However, most restricted regions in the cerebrum of *Geoclemys reevesii* are much higher immunoreacted with a-*raf* than c-*raf*. While in the most mammalian brains, the immunoreactivity of c-*raf* antibody is much more densely labeled than a-*raf*. This is very interesting phenomenon that the immunoactivity of a-*raf* changes gradually into that of c-*raf* according to the progress from the lower verte-

brates (unpublished data in our Lab.) to the upper vertebrates. Consequently, in *Geoclemys reevesii*, a-*raf* protein kinase is distributed in the spherical cells or pyramidal cells of rather restricted regions. And immunoreactivity of a-*raf* is particularly dense in the cell body and their fasciculated dendrites.

C-*raf* protein kinases are more widespreaded in mammalian tumors, whereas these enzymes in the normal tissues of cerebrum are primarily localized to pyramidal cell with fasciculated dendrites and some restricted neuronal cells. A-*raf* protein kinase is markedly enriched in hippocampal formation and general pallium of cerebral cortex. Although our knowledges of the functional localization between *raf* protein kinases and protein kinase C are incomplete, it seems likely that they play synergically a major role each other in pyramidal or spherical cells.

References

Anderson, W. B., A. Estival, H. Tapiovaara, and R. Gopalakristna, 1985. Altered subcellular distribution of protein kinase C (a phorbolester receptor). *Adv. Cycl. Nucl. Prot. Phos. Res.* **19**:287-306.

Beck, T. W., M. Huleihel, M. Gunnell, T. I. Bonner, and U. R. Rapp, 1987. The complete coding sequence of the human a-*raf* carrying retrovirus. *Nucl. Acids Res.* **15**:595-609.

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

Bonner, T. I., S. J. O'Brien, W. G. Nash, C. Morton, P. Leder, and U. R. Rapp, 1984. The human homologs of the *raf/mil* oncogene are located on human chromosome 3 and 4. *Science* **223**:71-74.

Bonner, T. I., S. B. Kerby, R. Sutrave, M. A. Gunner, 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.

Bonner, T. I., H. Opermann, P. Seeburg, S. B. Kerby, M. A. Gunnell, A. C. Young, and U. R. Rapp, 1986. The complete coding sequence of the human *raf* oncogene and the corresponding structure of the c-*raf*-1 gene. *Nuc. Acids Res.* **14**:1009-1015.

Castagna, M., Y. Takai, K. Kaibuchyi, V. Kikkawa, and Y. Nishizuka, 1982. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor promoting phorbol esters. *J. Biol. Chem.* **257**:7847-7851.

Cleveland, J. L., H. W. Jansen, K. Bister, T. N. Fredrickson, H. C. Morse III, J. N. Ihle, and U. R. Rapp, 1986. Interaction between *raf* and *myc* oncogenes in transformation in vivo and in vitro. *J. Cell Biochem.* **30**:195-218.

Cochran, B. H., A. C. Reffel, and C. D. Stiles, 1983. Molecular cloning of gene sequences regulated by platelet-derived growth factor. *Cell* **33**:939-947.

Fukui, M., T. Yamamoto, 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. U.S.A.* **81**:5954-5958.

Gerfen, C. R., W. C. Choi, P. B. Suh, and S. G. Rhee, 1987. Phospholipase C I and II brain isozymes: Immunohistochemical localization in neuronal system in rat brain. *Proc. Natl. Acad. Sci. U.S.A.* **85**:3208-3212.

Hsu, S. M., L. Raine, and H. Fanger, 1981. The use of avidinbiotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J. Histochem. Cytochem.* **29**:577-580.

Ishikawa, F., F. Takaku, M. Ochiai, K. Hayashi, S. Hirohashi, M. Terada, S. Takayama, M. Nasao, and T. Susimura, 1985. Activated c-*raf* gene in a rat hepatocellular carcinoma induced by 2-amino-3-methylimidazo [4.5.-F] quinoline. *Biochem. Biophys. Res. Commun.* **132**:186-192.

Jansen, H. W., R. Lurz, K. Bister, T. Bonner, G. Mark, and U. R. Rapp, 1984. Homologous cell-derived oncogenes in avian carcinoma virus MH2 and murine sarcoma virus 3611-MSV. Nature **307**:281-284.

Kelly, K., B. H. Stiles, and P. Leder, 1983. Cell-specific regulation of the c-*myc* gene by lymphocyte mitogens and platelet-derived growth factor. *Cell* **35**:603-610.

Moelling, K., B. Heimann, P. Beimling, U. R. Rapp, and T. Sander, 1984. Serine- and threonine-specific protein kinase activities of purified *gag-mill* and *gaf-raf* protein. *Nature* **312**:558-561.

Nishizuka, Y., 1986. Studies and perspectives of protein kinase C. Science **233**:305-311.

Pelech, S. L., K. E. Meier, and E. G. Krebs, 1986. Rapid microassay for protein kinase C translocation in swiss 3T3 cells. *Biochem.* **25**:8348-8353.

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

Rapp, U.:R., J. L. Cleveland, T. N. Frederickson, K. L. Holmes, H. C. Morse III, H. W. Jansen, T. Patchinsky, and K. Bister, 1985. Rapid induction of hemopoietic neoplasms in newborn mice by a *raf* (*mil*)/*myc* recombinant murine retrovirus. *J. Virol.* **55**:23-33.

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

Rapp, U. R., J. L. Cleveland, and T. I. Bonner, 1987b. The *raf* oncogene family. In: P. Reddy, T. Curran, and A. Skalka ed. Elsevier Pub. Co Amsterdam.

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 mitogen signal transduction. *Cold Spring Harbor Symp. Quanti. Biol.* **LIII:**173-183.

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

Smith, M. R., S. J. DeGudicibus, and D. W. Stacey, 1986. Requirement for c-*ras* proteins during viral oncogene transformation. *Nature* **320:**540-543.

Worley, P. F., J. M. Baraban, E. B. De Souza, and S. H. Snyder, 1986. Mapping second messenger system in the brain; differential localizations of adenylate cyclase and protein kinase C. *Proc. Natl. Acad. Sci. U.S.A.* **83:***4053*-4057.

(Accepted February 25, 1990)

남생이(***Geoclemys reevesii***) 대뇌에 있어서 *raf* Protein Kinase의 면역세포화학적 분포

최원철 · 문현근(부산대 자연대 생물학과)

Raf protein kinase와 protein kinase C는 세포질내 serine/threonine-specific protein에 속한다. 그리고 기능적인 구조와 세포내의 분포 양상은 서로 비슷 하다. *Raf* family oncogene을 발현 시키는 a-*raf*와 c-*raf* protein kinase에 대한 antibodies로써 남생이 대뇌의 *raf* protein kinase의 분포를 조사하였다. 일반적으로 *raf* protein kinase는 제한된 지역에서 즉, general pallium, hippocampal formation, primordium hippocampi, nucleus of lateral olfactory tract, basal amygdaloid nucleus와 bed of stria terminalis에서 나타났으며, c-*raf* protein kinase의 면역학적 labeling은 a-*raf*보다 그 범위가 넓었다. 그렇지만 labeling되는 intensity는 오히려 a-*raf*보다 낮았다.

그런데 a-*raf*에서 가장 명확한 좋은 예는 basal amygdaloid nucleus내의 구형 모양의 세포인데, 이 세포는 세포질이 매우 강하게 labeling되어 지므로 종국에는 ring모양과 같이 나타났다. 특히 c-*raf*는 protein kinase C가 많이 나타나는 pyramidal 세포나 purkinje세포에 많이 존재하는 것을 볼 때 protein kinase에 의하여 활성화 되는 *myc*와 서로 상협작용을 유도한다고 제안하는 바이다.