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TISSUE- AND CELL TYPE-SPECIFIC EXPRESSION OF mRNAS FOR FOUR TYPES OF INOSITOL PHOSPHOLIPID-SPECIFIC PHOSPHOLIPASE C

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The mRNA levels for four types of inositol phospholipid-specific phospholipase C (PLC) in various tissues and cell cultures have been studied by Northern analysis using cDNA probes for PLC isozyme I, II, and III [Sue, P.-G., Ryu, S.H., Moon, K.H., Sue, H.W., and Rhee, S.G. (1988) Proc. Natl. Acad. Sci. USA 85, 5419-5423 and Cell 54, 161-169], and the recently identified isozyme IV. All four types are ubiquitously expressed in rat tissues, but the levels of the mRNAs vary among tissues and cell lines. PLC-I mRNA levels are extremely high in brain and rat C6 glioma cells with lower levels in other tissues tested. PLC-II and -III have a more widespread distribution, with relatively high levels in brain, lung, spleen, thymus, and testis in the case of PLC-II, and in skeletal muscle, spleen, and testis for PLC-III. PLC-II and -III mRNAs were also detected in all cell lines examined except human promyelocytic HL60 cells. PLC-IV mRNA levels are extraordinarily high in spleen and HL60 cells. These results indicate that rat C6 glioma cells, together with most rat tissues, contain all four PLC isozymes. Other cultured cell types examined also contain two or three PLC isozymes except for HL60 cells, which contain only PLC-IV. The concomitant expression of PLC isozymes in cultured cells suggests a diverse function for PLC isozymes in single cells.

Inositol phospholipid-specific phospholipase C (PLC) catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to generate two second messenger molecules, inositol 1,4,5-trisphosphate and diacylglycerol, by activating a specific receptor for calcium mobilizing hormones and neurotransmitters (for reviews, see Refs. 1-3). A number of types of phospholipase C with different Mr values have been purified and characterized thus far from various tissues (for reviews, see Ref. 4), and cDNA clones for some

The abbreviations used are: PLC, inositol phospholipid-specific phospholipase C; SDS, sodium dodecyl sulfate; kb, kilobase(s); kbp, kilobase pair(s).

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of these isozymes have been obtained (5-10). The multiple isozymes of PLC implies heterogeneity of the pathway for transmembrane signaling. PLC isozymes may couple to diverse cellular components and be activated in different manners. Rhee and coworkers have purified and cloned three isozymes, designated PLC-I, -II, and -III (6,8). In addition, we have recently isolated a new cDNA clone and identified its corresponding PLC proteins in various rat tissues. Based on structural similarities between this new PLC and the three isozymes of Rhee, et. al., we refer to this new isozyme as PLC-IV[1]. In the present paper, we describe the differential mRNA distribution of the four PLC isozymes in various tissues and cultured cells examined by Northern analysis.

MATERIALS AND METHODS

Materials --- The multiprime labeling kit, the rapid hybridization kit, nylon filters (Hybond-N^{TM}), and [α-^{32}P]dCTP (3,000 Ci/mmol) were purchased from Amersham. The probes for PLC-I, -II, -III, and -IV were a 1.5-kbp fragment (base nos. 389-1876) of the PLC-I clone (6), a 1.6-kbp fragment (base nos. 1573-3123) of the PLC-II clone (8), a 1.1-kbp fragment (base nos. 653-1742) of the PLC-III clone (6), and a 3.3 kbp fragment of the PLC-IV clone, respectively. Rat pheochromocytoma PC12, glioma C6, and pituitary GH_4 cells were kindly donated by Drs. Y. Kaziro and H. Ito (Institute of Medical Science, Tokyo University, Tokyo, Japan). Rat pituitary GH_3 cells were kindly provided by Dr. T. Tsushima (Tokyo Women's Medical College, Tokyo, Japan). Human neuroblastoma SKNSH, and glioma T98 cells were the generous gifts of Drs. K. Toyoshima and K. Senba (Institute of Medical Science, Tokyo University, Tokyo, Japan). GH_3 and GH_4 cells were cultivated at 37 °C in 150-mm dishes containing F-10 medium supplemented with 12.5 % horse serum and 5 % fetal calf serum under an atomosphere of 95 % air-5 % CO_2. The other cultures were kept in Dulbecco's modified Eagle's medium supplemented with 10 % fetal calf serum and 2.5 % horse serum under the same conditions. Human promyelocytic HL60 and myelogenous K562 cells were the generous gifts of Dr. E. Huberman (Argonne National Laboratory, Argonne, IL) and were cultivated in RPMI 1640 supplemented with 10 % fetal calf serum.

Preparation of Poly(A^+) RNA and Northern Analysis --- Adult male Fisher rats (300-400 g) were used as the source of various tissues. Tissues were extracted with 6 M guanidine thiocyanate and total RNAs were isolated by centrifugation through a bed of 5.7 M cesium chloride (11). poly(A^+) RNA was obtained by oligo$(dT)_{12-18}$-cellulose column chromatography (11). Poly(A^+) RNA (2 or 3 µg) was denatured and electrophoresed in 1 % agarose gels containing 3 % formaldehyde and transferred by the capillary blot method in 20 x SSC (1 x SSC; 0.15 M NaCl and 0.015 M sodium citrate) to nylon filters. Hybridization was performed using the Rapid Hybridization kit at 65 °C. Each probe was labeled with [α-^{32}P]dCTP using the multiprime labeling kit and was employed for hybridization at 5 x 10^5 cpm/ml. After 12-h hybridization, the filters were washed in 2 x SSC containing 0.1 % SDS twice at room temperature and

[1]Manuscript submitted for publication. The cDNA sequence of PLC-IV indicates that this PLC isozyme could correspond to the putative human PLC as reported by Ohta, S. et. al. (10).

twice at 65 °C. The filters with poly(A^+) RNAs derived from rat tissues and cells were further washed in 0.1 x SSC containing 0.1 % SDS at 65 °C twice. The filters were exposed to Fuji RX films with intensifying screens at -70 °C.

RESULTS AND DISCUSSION

Poly(A^+) RNAs were prepared from various rat tissues including brain, liver, kidney, lung, skeletal muscle, heart, small intestine, stomach, spleen, thymus, and testis. The mRNA levels of PLC isozymes were studied by Northern blot analysis using four cDNA clones as probes. As shown in Figure 1, different profiles were obtained for the four probes used. These profiles indicate that the four isozymes of PLC are widely distributed in rat tissues, although the cDNA probes for PLC-I, -II, and -III were originally obtained from brain and that for PLC-IV from skeletal muscle.

When a cDNA clone for PLC-I was used as a probe, two distinct bands were detected in brain (Fig. 1a). These bands probably derive from different poly

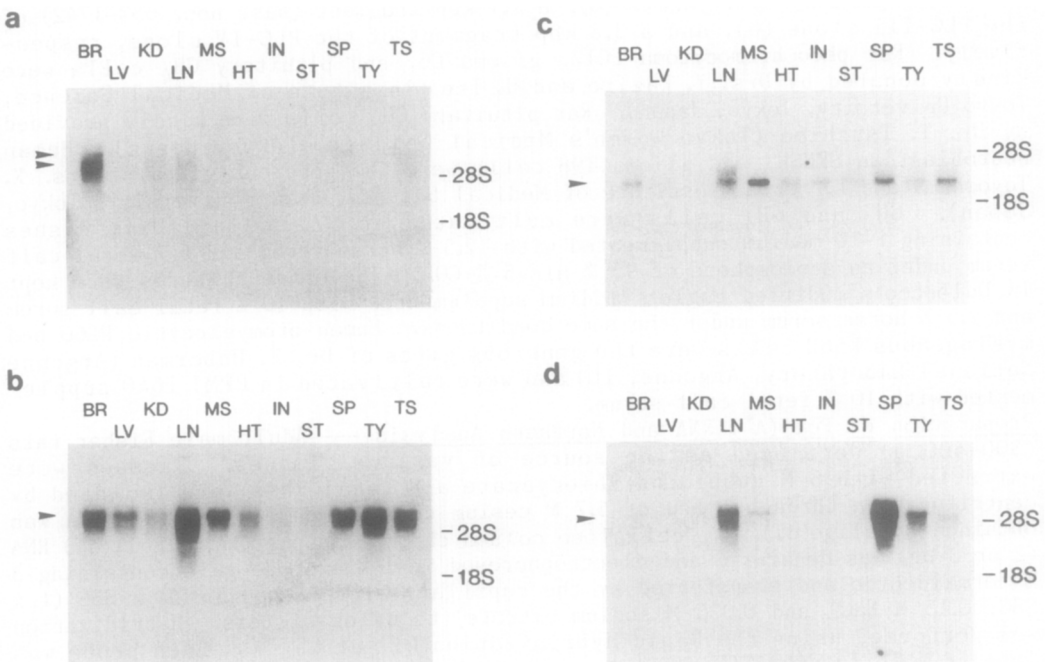

Figure 1. Northern blot analysis of mRNAs for four PLC isozymes from various rat tissues. Poly(A^+) RNAs (3 µg each) were electrophoresed on 1 % agarose gels and blotted to nylon filters. RNAs were hybridized with radiolabeled PLC-I (a), PLC-II (b), PLC-III (c), and PLC-IV (d) cDNA probes. Filters were exposed for 36 h (a,b) or 24 h (c,d). BR, brain; LV, liver; KD, kidney; LN, lung; MS, skeletal muscle; HT, heart; IN, small intestine; ST, stomach; SP, spleen; TY, thymus; TS, testis. Ribosomal RNAs (28S and 18S) were used as size markers. Locations of radioactive bands are indicated by arrows.

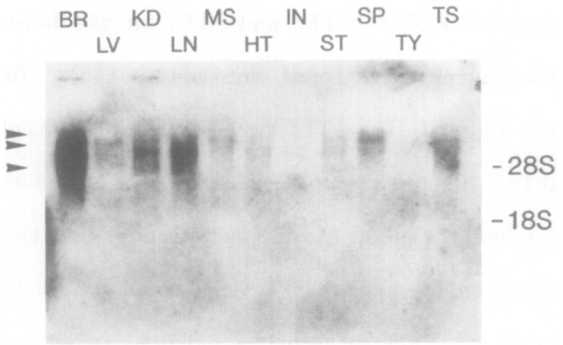

Figure 2. Visualization of trace amounts of PLC-I mRNAs. The filter from Figure 1a was exposed for 6 days. The other details are the same as described in Fig. 1.

A sites as observed by Rhee and coworkers (6). When the same filter used in Fig. 1a was exposed for a longer period, radioactive bands appeared for other tissues including liver, kidney, lung, skeletal muscle, heart, stomach, spleen, and testis (Fig. 2). In addition, a third band below the two main bands, also observed in brain, was found in kidney, lung, skeletal muscle, heart, stomach, and spleen. This band may result from the presence of multiple forms of PLC-I molecules (4,12-14). PLC-II and PLC-III mRNAs are widely expressed, but at different levels, in a variety of tissues (Fig. 1, b and c). The PLC-II and -III probes produced mainly 6.5 kb and 3.5 kb mRNA bands, respectively, in agreement with previous reports (6,8). Relatively large amounts of PLC-II mRNA were observed in brain, lung, skeletal muscle, spleen, thymus, and testis. PLC-III mRNA was most abundant in skeletal mucsle. PLC-IV mRNA with a length of 4.5 kb was also expressed ubiquitously, but its level varied significantly among tissues. The content of PLC-IV mRNA was extremely high in spleen and relatively high in lung, thymus, skeletal muscle, and testis, but only trace amounts were detected in brain, liver, and kidney.

Although it is difficult from these data to compare the content of the four isozymes in each tissue, the order of the mRNA levels seemed to be PLC-I > -II >> -III > -IV in brain, PLC-II > -IV >> -III > -I in lung, and PLC-IV > -II >> -III > -I in spleen. It is also noteworthy that the PLC-III mRNA is the predominant isozyme in the small intestine and stomach. Suh *et. al.*

determined the contents of PLC-I, -II, and -III in the homogenates from a number of bovine tissues using monoclonal antibodies (15). They reported the contents of PLC-I and -II (PLC-I > -II) to be extremely high in brain with PLC-III also detectable. PLC isozymes were also observed to be present in other tissues including heart, lung, liver, and kidney, but their amounts were relatively lower than in brain. The mRNA levels observed in this study appear consistent with these quantitative results. Thus, it is possible that PLC isozymes, including PLC-IV, are present proportionally to their mRNA levels in all tissues.

To examine cell type-specific expression of PLC isozymes in detail and to investigate whether single cells contain only one isozyme or multiple forms of PLC, we next determined mRNA levels of PLC isozymes in a variety of cultured cells using the same four cDNA probes. As shown in Figure 3, the pattern of the radioactive bands varied among the four probes. Expression of PLC-I was

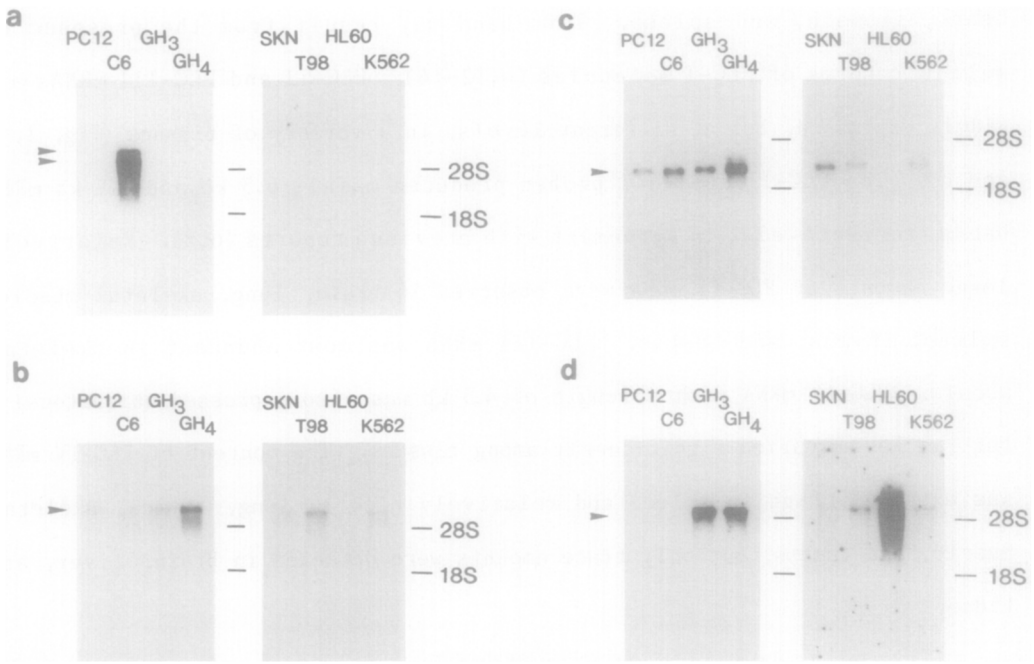

Figure 3. Northern blot analysis of mRNAs for four PLC isozymes from rat and human cell cultures. Poly(A^+) RNAs (2 µg each) were hybridized with radiolabeled PLC-I(a), PLC-II (b), PLC-III (c), and PLC-IV (d) cDNA probes. Filters on the left in (a)-(d) were exposed for 36 h; filters on the right in (a)-(c) were exposed for 2 days; the filter on the right in (d) were exposed for 5 days. The left panels show rat PC12, C6, GH_3, and GH_4 cells. The right panels show human SKNSH (SKN), T98, HL60, and K562 cells. Details are the same as described in "MATERIALS AND METHODS" and for Fig. 1.

detected only in C6 rat glioma cells (Fig. 3a), while PLC-II and PLC-III mRNAs were detected in all of the cells examined except HL60 cells (Fig. 3, b and c). PLC-IV mRNA was extremely high in HL60 cells, abundant in GH_3 and GH_4 cells, and present at lower levels in C6 (hardly seen in the figure), T98 and K562 cells (Fig. 3d). The length of the mRNAs detected in cultured cells were apparently the same as in tissues.

The expression of the individual PLC isozymes varies in each culture cell line. C6 cells contain all four PLC isozymes and GH_3 and T98 cells also contain three of them, whereas HL60 cells contain only PLC-IV. The expression of different PLC isozymes in the same cell line, as observed for PC12, C6, GH_3, GH_4, T98, and K562 cells, implies that PLC isozymes may be involved in different signal transduction pathways. PLC-I mRNA is localized mainly in brain and C6 cells. Although C6 cells are originally derived from a glial cell tumor, histochemical observations using monoclonal antibodies and synthetic oligonucleotides as probes suggest that PLC-I plays a role in signal transduction in postsynaptic membranes (16,17). The results shown in Fig. 2 further suggest that PLC-I may be involved in signal transduction at peripheral sites. On the other hand, since PLC-III mRNA is widespread among almost all tissues and cells and its mRNA levels are highest in skeletal muscle, PLC-III may be involved in some rather fundamental cellular process such as intracellular Ca^{2+} regulation.

The primary structures of PLC-II and PLC-IV are the most similar among the four PLC isozymes. Both contain a regulatory domain homologous to the N-terminus of src-related tyrosine kinases (5,8), along with domains for possible association with cellular components (18,19). Thus, it seems possible that the activation of PLC-II and PLC-IV is regulated in a similar manner and that they exhibit similar functions. Since it has been suggested that PLC-II might be involved in EGF- or PDGF-dependent cell growth (20-22), it is possible that PLC-IV also exhibits a central role in cell growth. However, their different tissue and cell distributions (Figs. 1b,d and 3b,d) also suggest that PLC-II and -IV may be involved in similar but distinct pathways of cellular activation.

REFERENCES

1. Nishizuka, Y. (1984) Science 233, 305-312
2. Berridge, M.J., and Irvine, R.F. (1984) Nature 312, 315-321
3. Majerus, P.W., Connolly, T.M., Deckmyn, H., Ross, T.S., Bross, T.E., Ishii, H., Bansal, V.S., and Wilson, D.W. (1986) Science 234, 1519-1526
4. Rhee, S.G., Sue, P.G., Ryu, S.H., and Lee, S.Y. (1989) Science 244, 546-550
5. Stahl, M.L., Ferenz, C.R., Kellche, K.L., Kriz, R.W., and Knopf, J.L. (1988) Nature 332, 269-272
6. Sue, P.G., Ryu, S.H., Moon, K.H., Suh, H.W., and Rhee, S.G. (1988) Cell 54, 161-169
7. Katan, M., Kriz, R.W., Totty, N., Philp, R., Meldrum, E., Aldape, R.A., and Knopf, P.J. (1988) Cell 54, 171-177
8. Sue, P.G., Ryu, S.H., Moon, K.H., Suh, H.W., and Rhee, S.G. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 5419-5423
9. Bennett, C.F., Malcarek, J.M., Varrichio, A., and Crooke, S.T. (1988) Nature 334, 268-270
10. Ohta, S., Matui, A., Nazawa, Y., and Kagawa, Y. (1988) FEBS Lett. 242, 31-35
11. Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982) in Molecular Cloning. A laboratory manual (Cold Spring Harbor) pp. 194-205
12. Ryu, S.H., Cho, K.S., Lee, K.-Y., Suh, P.-G., and Rhee, S.G. (1987) J. Biol. Chem. 262, 12511-12518
13. Katan, M., and Parker, P.J. (1987) Eur. J. Biochem. 168, 413-418
14. Lee, K.-Y., Ryu, S.H., Suh, P.-G., Choi, W.C., and Rhee, S.G. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 5540-5544
15. Suh, P.-G., Ryu, S.H., Choi, W.C., Lee, K.-Y., and Rhee, S.G. (1988) J. Biol. Chem. 263, 14497-14504
16. Gerfen, C.R., Choi, W.C., Suh, P.G., and Rhee, S.G. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 3208-3212
17. Ross, C.A., MacCumber, M.W., Glatt, C.E., and Snyder, S.H. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 2923-2927
18. Vogel, U.S., Dixon, R.A., Schaber, M.D., Diehl, R.E., Marshall, M.S., Scolnick, E.M., Sigel, I.S., and Gibbs, J.B. (1988) Nature 335, 90-93
19. Wasenius, V.M., Saraste, M., Salven, P., Eramaa, M., Holm, L., and Lehto, V.P. (1989) J. Cell Biol. 108, 79-93
20. Wahl, M.I., Nishibe, S., Suh, P.-G. Rhee, S.G., and Carpenter, G. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 1568-1572
21. Margolis, B., Rhee, S.G., Felder, S., Mervic, M., Lyall, R., Levitzki, A., Ullrich, A., Zilberstein, A., and Schlessinger, J. Cell, (1989) 57, 1101-1107
22. Meisenhelder, J., Sue, P.-G., Rhee, S.G., and Hunter, T. (1989) Cell, 57, 1109-1122