Calcium-mediated Translocation of Cytosolic Phospholipase A\textsubscript{2} to the Nuclear Envelope and Endoplasmic Reticulum*

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Cytosolic phospholipase A\textsubscript{2} (cPLA\textsubscript{2}) is activated by a wide variety of stimuli to release arachidonic acid, the precursor of the potent inflammatory mediators prostaglandin and leukotriene. Specifically, cPLA\textsubscript{2} releases arachidonic acid in response to agents that increase intracellular Ca\textsuperscript{2+}. In vitro data have suggested that these agents induce a translocation of cPLA\textsubscript{2} from the cytosol to the cell membrane, where its substrate is localized. Here, we use immunofluorescence to visualize the translocation of cPLA\textsubscript{2} to distinct cellular membranes. In Chinese hamster ovary cells that stably over-express cPLA\textsubscript{2}, this enzyme translocates to the nuclear envelope upon stimulation with the calcium ionophore A23187. The pattern of staining observed in the cytoplasm suggests that cPLA\textsubscript{2} also translocates to the endoplasmic reticulum. We find no evidence for cPLA\textsubscript{2} localization to the plasma membrane. Translocation of cPLA\textsubscript{2} is dependent on the calcium-dependent phospholipid binding domain, as a calcium-dependent phospholipid binding deletion mutant of cPLA\textsubscript{2} (ΔCII) fails to translocate in response to Ca\textsuperscript{2+}. In contrast, cPLA\textsubscript{2} mutated at Ser-505, the site of mitogen-activated protein kinase phosphorylation, translocates normally. This observation, combined with the observed phosphorylation of ΔCII, establishes that translocation and phosphorylation function independently to regulate cPLA\textsubscript{2}. The effect of these mutations on cPLA\textsubscript{2} translocation was confirmed by subcellular fractionation. Each of these mutations abolished the ability of cPLA\textsubscript{2} to release arachidonic acid, establishing that cPLA\textsubscript{2}-mediated arachidonic acid release is strongly dependent on both phosphorylation and translocation. These data help to clarify the mechanisms by which cPLA\textsubscript{2} is regulated in intact cells and establish the nuclear envelope and endoplasmic reticulum as primary sites for the liberation of arachidonic acid in the cell.

The 85-kDa cytosolic phospholipase A\textsubscript{2} (cPLA\textsubscript{2}), which selectively releases arachidonic acid from the sn-2 position of membrane phospholipids, is crucial to the initiation of the inflammatory response. cPLA\textsubscript{2} activity is stimulated by a wide variety of agents, including the proinflammatory cytokines interleukin 1 (1, 2) and tumor necrosis factor (3), macrophage colony-stimulating factor (4), thrombin (5, 6), ATP (7), mitogens (7–10), and endothelin (11). The release of arachidonic acid is the rate-limiting step in the generation of prostaglandins and leukotrienes, the principal inflammatory eicosanoids. Cleavage of arachidonoyl-containing phospholipids also results in the release of lysosphospholipid, the precursor of the inflammatory mediator platelet-activating factor (12).

cPLA\textsubscript{2} is expressed in many cell types. Many of these are associated with the inflammatory response, such as monocytes (4), neutrophils (13), and synovial fibroblasts (14). However, cPLA\textsubscript{2} is also expressed in kidney, spleen, heart, lung, liver, testis, and hippocampus (15). This diverse pattern of expression is consistent with accumulating evidence that in addition to its role in inflammation, cPLA\textsubscript{2} participates in signaling in processes such as platelet activation (6, 16), tumor necrosis factor-induced cytotoxicity (17), and cell proliferation (8, 10, 18).

cPLA\textsubscript{2} activity is regulated both transcriptionally and post-translationally. Post-translational activation is thought to occur by two mechanisms. One mechanism involves agonist-induced MAP kinase phosphorylation of cPLA\textsubscript{2}, resulting in stimulation of its intrinsic enzymatic activity (19, 20). The second involves a Ca\textsuperscript{2+}-dependent translocation of cPLA\textsubscript{2} from the soluble to the membrane fraction of cells (21–23), allowing cPLA\textsubscript{2} access to its arachidonoyl-containing phospholipid substrate. As discussed below, the results presented in this study establish that both mechanisms are important for the stimulation of cPLA\textsubscript{2}-induced arachidonic acid release.

In vitro studies have strongly suggested that the membrane binding function of cPLA\textsubscript{2} resides in its Ca\textsuperscript{2+}-dependent phospholipid binding (CaLB) domain (22, 24), a region similar to the C11 domain of protein kinase C. This domain has been shown to be necessary and sufficient for Ca\textsuperscript{2+}-dependent membrane binding (22, 24). In this study, we characterize the translocation of cPLA\textsubscript{2} in intact cells and show that this enzyme translocates to the nuclear envelope and endoplasmic reticulum. Deleting the CaLB domain abolishes the ability of cPLA\textsubscript{2} to translocate to these membranes, whereas a mutation at the MAP kinase phosphorylation site has no effect on translocation. Each of these mutations is shown to prevent cPLA\textsubscript{2}-induced arachidonic acid release. Our present data, together with recent reports localizing several arachidonic acid-metabolizing enzymes to the nuclear envelope and endoplasmic reticulum (25–29), establish these membranes as major sites of arachidonic acid production and metabolism in the cell.

EXPERIMENTAL PROCEDURES

Cell Culture and Antibodies—CHO cells were maintained as described previously (5). Monoclonal antibody 1.1.1 (19) was raised against human cPLA\textsubscript{2} purified from E5-CHO cells and was used for staining of parental and cPLA\textsubscript{2}-overexpressing CHO cells. Polyclonal antibody 790S, generated against cPLA\textsubscript{2} produced in Escherichia coli, was used for immunoblotting (22). PGHS-1-specific polyclonal antibody
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was generated against the N terminus of ovine PGHS-1, residues Ala-25 to Cys-35 (30). E-selectin monoclonal antibody was generated against soluble human E-selectin (31).

Immunofluorescence Staining—Cells grown on coverslips in growth medium were rinsed with serum-free medium prior to a 2-min treatment with 2 mM calcium ionophore A23187 (Sigma). Treated and untreated cells were washed twice briefly with Tris-buffered saline containing 0.01% Triton X-100. In the experiments presented in this paper, all washes and incubations of A23187-treated treated cells contained 1 mM CaCl₂ in an attempt to lessen the chance that translocated cPLA₂ would detach from membranes. Experiments performed in the absence of CaCl₂, however, yielded identical staining patterns (not shown). Cells were fixed for 2 min at room temperature in a 50:50 mixture of acetone and methanol. After two brief washes, blocking was performed in Tris-buffered saline, 20% goat serum for 1 h at 37°C. Cells were incubated with primary antibody (150 µg of cPLA₂ antibody/ml, 100 µg of E-selectin antibody/ml, or a 1:2 dilution of PGHS-1 antibody) for 1 h at 37°C and washed once briefly followed by 2 × 10-min washes. Incubations with secondary antibody were performed for 1 h at 37°C using a 1:100 dilution of fluorescein isothiocyanate-conjugated goat anti-mouse or anti-rabbit antibody (Zymed, San Francisco, CA) and washed as above. After a brief wash with water, coverslips were inverted onto slides with Slowfade bleaching retardant (Molecular Probes). The pattern of cPLA₂ localization was similar using a variety of staining procedures, including starving cells of serum overnight prior to staining, eliminating Triton from all wash buffers, or fixing the cells with 4% paraformaldehyde (followed by permeabilization with 0.2% Triton) (data not shown).

Fluorescence Confocal Microscopy—The subcellular location of cPLA₂ was visualized by fluorescence confocal microscopy. An Insight Bilateral scanning confocal microscope (Meridian Instruments, Okemos, MI) was used with an argon ion laser as the excitation source (32). The laser was used at a power ranging from 25–75 mW, depending on the level of fluorescence intensity. All images were photographed with a 100× objective. Untreated and A23187-treated samples for a given cell line were analyzed using identical instrument settings.

Fractionation and Immunoblotting—Cells were starved overnight in serum-free media and treated with 2 µM A23187 for 10 min. After washing with Tris-buffered saline, cells were collected by scraping and lysed in 1 ml lysis buffer (20 mM Hepes, pH 7.4, 1 mM EGTA, 0.34 M sucrose, 10 µg of leupeptin/ml, 2 µg phenylmethylsulfonyl fluoride, 5 µg dithiothreitol) by Parr bombing, 600 p.s.i. for 5 min. Lysates were centrifuged for 1 h at 100,000 × g and protein (5 µg) from the supernatant and pellet fractions was subjected to SDS-polyacrylamide gel electrophoresis. Proteins were transferred to nitrocellulose, blocked with anti-cPLA₂ polyclonal antibody 7905 (22), and developed by chemiluminescence following incubation with horseradish peroxidase-conjugated protein A (Amersham).

Arachidonic Acid Release—Cells were plated onto 12-well clustered dishes at 1.5 × 10⁶ cells per well in growth medium. After a 20 h incubation, the medium was removed and replaced with 0.5 ml of a medium (lacking serum) (Life Technologies, Inc., Grand Island, NY) containing 0.5 µCi of [³²P]arachidonic acid (100 Ci/ml; New England Nuclear, Wilmington, DE) and incubated for another 20 h at 37°C. The cells were then washed three times with medium containing 0.1% bovine serum albumin and incubated with A23187 for 10 min. The medium was removed, and radioactivity was quantified by scintillation counting.

RESULTS

Ca²⁺ Ionophore Induces cPLA₂ Translocation to the Nuclear Envelope and Endoplasmic Reticulum—cPLA₂ can be induced to associate with natural membranes in the presence of physiologically relevant Ca²⁺ levels in vitro (21, 22). This Ca²⁺-dependent translocation is thought to reflect a translocation that occurs in vivo when cells are stimulated with agents that increase intracellular Ca²⁺. As the translocation of cPLA₂ in response to Ca²⁺ has not yet been characterized in intact cells, the identity of the membrane(s) to which cPLA₂ binds is not known. To address this issue, indirect immunofluorescence was performed using E5-4, a CHO cell line that overexpresses human cPLA₂ (5). In resting cells, cPLA₂ was found distributed throughout the cytoplasm. In contrast, after treatment with the Ca²⁺ ionophore A23187, significant cPLA₂ staining appeared as a discrete ring surrounding the nucleus. A distinct ring was not apparent in untreated cells, although a low level of perinuclear staining was detectable in some cells. This suggests that an increase in cytosolic Ca²⁺ induces a translocation of cPLA₂ from the cytoplasm to the nuclear envelope.

Interestingly, immunofluorescent staining of cPLA₂ in A23187-treated E5-4 cells was consistently more intense than that seen in untreated cells (quantitated in Fig. 1B). This is most likely due to a greater loss of soluble cPLA₂ than membrane-bound cPLA₂ throughout the staining procedure. A similar interpretation was offered for the results observed upon immunogold labeling of the arachidonic acid-metabolizing enzyme 5-lipoxygenase, which also translocates to the nuclear envelope in response to A23187 (27). In that study, 5-lipoxygenase was undetectable in unstimulated cells but was apparent at the nuclear envelope after ionophore treatment. Alternatively, binding of cPLA₂ to membranes may result in a better exposure of the epitope for the monoclonal antibody, resulting in more efficient antibody binding. In either case, the observed increase in cytoplasmic staining intensity suggests that cPLA₂ translocates not only to the nuclear envelope but also to a cytoplasmic membrane structure, most likely the endoplasmic reticulum. Indeed, in some experiments ionophore treatment resulted in an increase in reticular cytoplasmic fluorescence, consistent with endoplasmic reticulum staining.

The plasma membrane was consistently found to be devoid of cPLA₂ staining. To ensure that fixation had not disrupted the integrity of the plasma membrane, CHO cells overexpressing the plasma membrane protein E-selectin were stained. As shown in Fig. 2, E-selectin antibody was clearly able to label the plasma membrane. No labeling of the nuclear envelope was observed in these cells. As a control for the specificity of the anti-cPLA₂ monoclonal antibody, immunofluorescence was also...
performed on the parental CHO cells used for these studies. Only faint staining was observed (Fig. 2), similar to that seen in controls in which the monoclonal antibody was eliminated from the staining protocol (data not shown). The lack of endogenous cPLA2 staining is likely to be due both to the low level of cPLA2 expressed in these cells and the failure of this antibody to recognize murine (and by inference, hamster) cPLA2 efficiently. Fig. 2 also shows staining of CHO cells overexpressing PGHS-1, which metabolizes arachidonic acid to prostaglandin. Similar staining results were obtained with a CHO line expressing the isoform PGHS-2 (not shown). Both PGHS isoforms have been localized to the endoplasmic reticulum and nuclear envelope (25, 29). As expected, neither E-selectin nor PGHS-1 staining patterns were affected by ionophore treatment.

CaLB, but Not Ser-505 Phosphorylation, Is Required for Translocation—In cell-free systems, the CaLB domain is required for the association of cPLA2 with membranes (24). To confirm the importance of the CaLB domain for cPLA2 translocation in intact cells, immunofluorescence was performed on a CHO line (ΔCII) that overexpresses a cPLA2 deletion mutant lacking the CaLB domain (amino acids 1-134) (24). As shown in Fig. 3A, no increase in nuclear envelope staining was observed, indicating that this truncated cPLA2 is unable to translocate in response to ionophore. Consistent with this result, ionophore did not induce an increase in cytoplasmic staining intensity in these cells (Fig. 3B). This result establishes the increase in staining intensity as a CaLB-dependent event (most likely, as discussed above, a result of membrane association). The effect of a mutation at the MAP kinase phosphorylation site, Ser-505, was also investigated. MAP kinase phosphorylation at this residue has previously been shown to increase the intrinsic enzymatic activity of cPLA2 (19, 20). To determine whether phosphorylation at this site was involved in regulating translocation, immunofluorescence staining of CHO cells expressing SA505-cPLA2, containing a serine-to-alanine mutation at this residue, was performed. The translocation of SA505-cPLA2 in response to ionophore was indistinguishable from that observed with wild type cPLA2 (Fig. 4). The increase in staining intensity seen with wild type cPLA2 upon ionophore treatment was also observed in the SA505 line, further supporting the ability of this mutant to translocate to membranes.

To confirm that deletion of the CaLB domain, but not the mutation of Ser-505, abolished cPLA2 translocation, subcellular fractionation was performed using cells expressing these mutants. After ionophore treatment, cell lysates were centrifuged at 100,000 × g, and the soluble and particulate fractions were immunoblotted for cPLA2. As shown in Fig. 5, both cPLA2 and the SA505-cPLA2 mutant redistributed to the particulate fraction upon ionophore treatment, whereas the ΔCII mutant did not. The insert shown below the ΔCII portion of the figure represents a longer exposure of the blot, revealing the translocation of endogenous cPLA2 in the ΔCII line. These data confirm that phosphorylation at Ser-505 is not required for cPLA2 translocation. Conversely, translocation is not required for Ser-505 phosphorylation, as evidenced by the presence of a cPLA2 doublet in the ΔCII but not the SA505 line. The upper band of the cPLA2 doublet has previously been established to result from phosphorylation at Ser-505 (19). These data establish that translocation and phosphorylation occur independently.

ATP and thrombin have previously been shown to induce arachidonic acid release in CHO cells that overexpress cPLA2 (5). However, in fractionation experiments using these agents, we were unable to detect the appearance of cPLA2 in the pellet fraction (data not shown). This suggests that translocation...
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**Fig. 4. Translocation of SA505-cPLA<sub>2</sub>.** Cells stably overexpressing SA505-cPLA<sub>2</sub>, which contains a serine-to-alanine mutation at the Ser-505 MAP kinase phosphorylation site, were probed with antibody to cPLA<sub>2</sub>. Indirect immunofluorescence was performed as described in the legend to Fig. 1.

**Fig. 5. Fractionation of lysates from wild type (WT), ΔCII, and SA505 cells.** Cells overexpressing each cPLA<sub>2</sub> construct were stimulated with 2 mM ionophore for 10 min (+) or left untreated (−). Lysates were spun at 100,000 × g, and 9 μg of protein from the supernatant (S) and pellet (P) fractions were subjected to SDS-polyacrylamide gel electrophoresis. Proteins were transferred to nitrocellulose and immunoblotted for cPLA<sub>2</sub>. Development was by chemiluminescence. The positions of full-length cPLA<sub>2</sub> and ΔCII are indicated. The panel below the ΔCII portion of the blot represents a longer exposure of these lanes, demonstrating that endogenous cPLA<sub>2</sub> translocates normally in the ΔCII line. The band in the ΔCII pellet fractions represents a small amount of insoluble ΔCII. * indicates a cross-reactive species of unknown origin.

The data presented here establish that in intact cells, cPLA<sub>2</sub> translocates to membranes in response to an increase in intracellular Ca<sup>2+</sup>. Specifically, cPLA<sub>2</sub> binds to the nuclear envelope and the endoplasmic reticulum. The translocation of cPLA<sub>2</sub> to these sites is consistent with previous work examining the subcellular distribution of cPLA<sub>2</sub> in Ca<sup>2+</sup>-ionophore-stimulated macrophages (26). In this study, cPLA<sub>2</sub> from stimulated cells was detected both in the nuclear fraction and in the membrane fraction lacking nuclei, consistent with its presence in both the nuclear envelope and the endoplasmic reticulum. In another study (23), fractionation of neutrophils stimulated with granulocyte/macrophage colony-stimulating factor and the chemotactic peptide fMet-Leu-Phe revealed cPLA<sub>2</sub> to be in the membrane fraction, even after nuclei were removed by centrifugation. Although the translocation of cPLA<sub>2</sub> to the nuclear envelope could not be addressed in this study (the presence of cPLA<sub>2</sub> in the nuclear fraction was not examined), its presence in the membrane fraction supports the translocation of cPLA<sub>2</sub> to the endoplasmic reticulum. Taken together with our immunofluorescence results, including the lack of cPLA<sub>2</sub> staining at the plasma membrane, these data implicate both the nuclear envelope and endoplasmic reticulum as the primary sites for arachidonic acid production in the cell. Interestingly, PGHS-1 and -2 (25, 29), the enzymes responsible for metabolizing arachidonic acid to prostaglandins, are also localized at these sites. In addition, 5-lipoxygenase, which metabolizes arachidonic acid to leukotrienes, as well as its activating protein, FLAP, are localized to the nuclear envelope (27). These data suggest that arachidonic acid is both produced and metabolized at the nuclear envelope and endoplasmic reticulum.

In the work presented in this paper, the localization of cPLA<sub>2</sub> was examined by immunofluorescent staining of cPLA<sub>2</sub>-overexpressed in CHO cells. This approach was chosen to assess the
effects of the ΔCII and SA505 mutations on cPLA₂ translocation. Caution must be taken with this approach, however, as it is possible that overexpression of cPLA₂ may result in a wider pattern of expression than occurs physiologically. Interestingly, cPLA₂ translocation to the nuclear envelope was recently demonstrated in rat mast cells stimulated with A23187 or IgE/antigen (34). In this study, no obvious staining of the endoplasmic reticulum was observed. This observation raises the possibility that the localization of cPLA₂ to the endoplasmic reticulum is a consequence of overexpression. However, it is also possible that the relative proportion of cPLA₂ in the nuclear envelope and endoplasmic reticulum varies between cell types. Further studies will be necessary to clarify this issue. In any case, the observed lack of plasma membrane staining, both in mast cells and in cells overexpressing cPLA₂, strongly suggests that cPLA₂ does not translocate to this membrane in response to increases in intracellular calcium.

Translocation of cPLA₂ is abolished upon deletion of the CaLB domain but not upon mutation of Ser-505. This suggests that translocation and phosphorylation regulate cPLA₂ independently, a conclusion supported by the observation that ΔCII is phosphorylated in CHO cells (as evidenced by the doublet in Fig. 5). Both translocation and phosphorylation are critical for A23187-induced arachidonic acid release. The observation that these regulatory mechanisms function independently strongly supports a model in which phosphorylation at Ser-505 serves primarily to activate the enzymatic activity of cPLA₂ (19, 20), and translocation allows access of the enzyme to its substrate.

This discussion focuses on the localization of cPLA₂ to the nuclear envelope and endoplasmic reticulum. How cPLA₂ is selectively localized to these sites is not known. The translocation of cPLA₂ to the nuclear envelope is consistent with previous data indicating that arachidonic acid is preferentially released from the nuclear envelope, as observed in pulse-chase experiments using [14C]arachidonate-labeled HSDM1 C1 cells stimulated with bradykinin (35). Interestingly, electron microscopic studies using [3H]arachidonic acid have shown that the nuclear membrane is the preferred site of initial arachidonic acid incorporation in these cells (36). Arachidonic acid is also incorporated rapidly into the endoplasmic reticulum; in contrast, transit to the plasma membrane is slow. This study shows that although arachidonic acid is preferentially incorporated at particular sites, it becomes evenly distributed throughout the cell. Thus, a correlation exists between the cellular localization of cPLA₂ and the sites at which arachidonic acid is initially incorporated into the cell. The significance of this correlation, however, is not yet known.

Several arachidonic acid-independent mechanisms could also explain the localization of cPLA₂. Although Ca²⁺ can induce purified cPLA₂ to bind membranes in the absence of additional protein (24), it remains possible that a “docking protein” localized to the nuclear envelope and endoplasmic reticulum allows more efficient binding of cPLA₂ at these sites. It is unlikely that a spatially localized release of Ca²⁺ is responsible for the targeting of cPLA₂, since our experiments revealed no translocation of cPLA₂ to the plasma membrane with A23187, whose effects are thought to include the trigger- ing of a Ca²⁺ influx across the plasma membrane. Another possibility is that some feature of the plasma membrane excludes cPLA₂, and cPLA₂ simply translocates to all cellular membranes that it is capable of binding. Interestingly, the plasma membrane is known to be enriched in sphingolipids compared to the nuclear envelope and endoplasmic reticulum. It is conceivable that the increase in phospholipid packing density that results from a high sphingolipid content may prevent cPLA₂ binding. Consistent with this idea, Leslie and Channon (21) have shown that both the activity and calcium sensitivity of cPLA₂ are inhibited by the increased substrate packing density induced by sphingolipids. Clearly, a better understanding of the lipid and protein components of distinct cellular membranes will greatly facilitate our understanding of the mechanism(s) governing the localization of cPLA₂.

As discussed above, the localization of cPLA₂ is similar to that reported for PGHS-1 and -2, which metabolize arachidonic acid to prostaglandins (25, 29). PGHS-1 is expressed constitutively and is thought to perform certain physiological “housekeeping” functions. PGHS-2, whose expression is induced by cytokines, has been implicated in inflammation (37, 38). cPLA₂-mediated arachidonic acid release can couple to PGHS-2, since antisense oligonucleotide inhibition of cPLA₂ decreases the level of endotoxin-stimulated PGE₂ in monocytes (without affecting PGHS-2 activity) (39), and PGE₂ release in endotoxin-stimulated monocytes has been shown to be dependent on PGHS-2 activity even in the presence of PGHS-1 (40). However, in platelets, known from various studies to contain only PGHS-1, inhibition of cPLA₂ using arachidonoyl trifluoromethyl ketone blocks thromboxane B₂ formation, suggesting that cPLA₂ can also couple to PGHS-1 (41). Indeed, the association of cPLA₂ with the nuclear envelope and the endoplasmic reticulum correlates well with the localization of both PGHS-1 and PGHS-2. Although it is not yet clear which factors govern the coupling of cPLA₂ with each PGHS isoform, the colocalization of cPLA₂ with these enzymes and 5-lipoxygenase is certain to ensure the efficient utilization of arachidonic acid upon agonist stimulation, consistent with a central role for cPLA₂ in the agonist-induced biosynthesis of prostaglandins and leukotrienes.

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REFERENCES

1. Lin, L.-L., Lin, A. Y., and DeWitt, D. L. (1992) J. Biol. Chem. 267, 23451–23454
2. Skolnik, C. G., Vervoort, M., Molski, T., and Towler, P. S. (1993) FEBS Lett. 333, 339–343
3. Hocke, W. G., Ramesha, C. S., Chang, D. J., Fan, N., and Heller, R. A. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 4475–4479
4. Nakamura, T., Lin, L.-L., Harkanda, S., Knopf, J., and Kufe, D. W. (1992) EMBO J. 11, 4917–4922
5. Lin, L.-L., Lin, A. Y., and Knopf, J. L. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 6147–6151
6. Kramer, R., Roberts, E. F., Manetta, J. V., Hyslop, P. A., and Akubovski, J. A. (1993) J. Biol. Chem. 268, 26796–26804
7. Durr, J., and Rozengurt, E. (1993) J. Biol. Chem. 268, 8927–8934
8. B. Kast, R., Forstenberger, G., and Marks, F. J. (1993) J. Biol. Chem. 268, 16795–16802
9. Chen, H. S., D. A., and Jimenes, S. A. (1994) J. Biol. Chem. 269, 21786–21792
10. Barnett, R. L., Ruffini, L., Hart, D., Mancuso, P., and Nord, E. P. (1994) Am. J. Physiol. 266, F46–F56
11. Hanahan, D. J. (1986) Annu. Rev. Biochem. 55, 483–509
12. Ramesha, C. S., and Ives, D. L. (1993) Biochem. Biophys. Acta 1168, 37–44
13. Hulker, K. I., Hope, W. C., Chen, T., Anderson, C. M., Coffey, J. W., and Morgan, D. W. (1992) Biochem. Biophys. Res. Commun. 184, 712–718
14. Sharp, J. D., and White, D. L. (1993) J. Lipid Med. 5, 183–189
15. Takayama, K., Kudo, I., Kim, D. K., Nagata, K., Nozawa, T., and Inoue, K. (1991) FEBS Lett. 282, 326–330
16. Hayakawa, M., Ishida, N., Takeuchi, K., Shiba, K., Hori, T., Oku, N., Ito, T., and Tsujimoto, M. (1993) J. Biol. Chem. 268, 11290–11295
17. Gil, J., Higgins, T., and Rozengurt, E. (1991) J. Cell Biol. 113, 943–950
18. Lin, L.-L., Wartman, M., Lin, A. Y., Knopf, J. L., Seth, A., and Davis, R. J. (1993) Cell 72, 269–276
19. Nemenoff, R. A., Winitz, S., Qian, N.-X., Van Putten, V., Johnson, G. L., and Hasleby, L. E. (1993) J. Biol. Chem. 268, 1960–1964
20. Channon, J. Y., and Leslie, C. C. (1990) J. Biol. Chem. 265, 5409–5413
21. Clark, J. D., Lin, L.-L., Kuehl, W. V., and Tsujimoto, M. (1993) J. Biol. Chem. 268, 339–343
22. Nalefski, E. A., Sultzman, L. A., Martin, D. M., Kizil, R. W., Towler, P. S.,
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Knopf, J. L., and Clark, J. D. (1994) J. Biol. Chem. 269, 18239–18249
25. Regier, M. K., DeWitt, D. L., Schindler, M. S., and Smith, W. L. (1993) Arch. Biochem. Biophys. 301, 439–444
26. Peters-Golden, M., and McNish, R. W. (1993) Biochem. Biophys. Res. Commun. 196, 147–153
27. Woods, J. W., Evans, J. F., Ethier, D., Scott, S., Vickers, P. J., Hearn, L., Heben, J. A., Charleson, S., and Singer, I. I. (1993) J. Exp. Med. 178, 1935–1946
28. Brock, T. G., Paine, R. L., III, and Peters-Golden, M. (1994) J. Biol. Chem. 269, 22059–22066
29. Morita, I., Schinder, M., Regier, M. K., Otto, J., Hori, T., DeWitt, D. L., and Smith, W. (1995) J. Biol. Chem. 270, 10902–10908
30. Otto, J. C., and Smith, W. L. (1994) J. Biol. Chem. 269, 18668–18675
31. Larkin, M., Ahern, T. J., Stoll, M. S., Shaffer, M., Sako, D., O’Brien, J., Yuen, C.-T., Lawson, A. M., Childs, R. A., Barone, K. M., Langer-Safer, P. R., Hasegawa, A., Kiso, M., Larsen, G. R., and Feliz, T. (1992) J. Biol. Chem. 267, 13661–13668
32. Wade, M. H., De Feijter, A. W., Frame, M. K., and Schindler, M. (1993) Bioanalyt. Instrumen. 37, 117–141
33. Lowndes, J. M., Gupta, S. K., Osawa, S., and Johnson, G. L. (1991) J. Biol. Chem. 266, 14193–14197
34. Glover, S., Bayburt, T., Jonas, M., Chi, E., and Gelb, M. H. (1995) J. Biol. Chem. 270, 15359–15367
35. Capriotti, A. M., Furth, E. E., Arrasmith, M. E., and Laposata, M. (1988) J. Biol. Chem. 263, 10029–10034
36. Neufeld, E. J., Majerus, P. W., Krueger, C. M., and Saffitz, J. E. (1985) J. Cell Biol. 101, 573–581
37. Masferrer, J. L., Zwiefel, B. S., Manning, P. T., Hauser, S. D., Leaphy, K. M., Smith, W. G., Isakson, P. C., and Seibert, K. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 3228–3232
38. Seibert, K., Zhang, Y., Leaphy, K., Hauser, S., Masferrer, J., Perkins, W., Lee, L., and Isakson, P. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 12013–12017
39. Roshak, A., Sathe, G., and Marshall, L. A. (1994) J. Biol. Chem. 269, 25999–26005
40. Reddy, T. S., and Herschman, H. R. (1994) J. Biol. Chem. 269, 15473–15480
41. Riendeau, D., Guy, J., Weech, P. K., Laliberte, F., Yergey, J., Li, C., Desna-ras, S., Perrier, H., Liu, S., Nicol-Griffith, D., and Street, I. P. (1994) J. Biol. Chem. 269, 15619–15624
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