In addition to the classical role of protein kinase C (PKC) as a mediator of transmembrane signals initiated at the plasma membrane, there is also significant evidence to suggest that a more sustained PKC activity is necessary for a variety of long term cellular responses. To date, the subcellular localization of PKC during sustained activation has not been extensively studied. We report here that long term activation of PKC (1 h) leads to the selective translocation of classical PKC isoenzymes, α and βII, to a juxtanuclear compartment. Juxtanuclear translocation of PKC required an intact C1 and C2 domain, and occurred in a microtubule-dependent manner. This juxtanuclear compartment was localized close to the Golgi complex but displayed no overlap with Golgi markers, and was resistant to dispersal with GTPase disrupting agents, brefeldin A and nocodazole. Further characterization revealed that PKCα and βII translocated to a compartment that colocalized with the small GTPase rab11, which is a marker for the subset of recycling endosomes concentrated around the microtubule-organizing center/centrosome. Analysis of the functional consequence of cPKC translocation on membrane recycling demonstrated a cPKC-dependent sequestration of transferrin, a marker of membrane recycling, in the cPKC compartment. These results identify a novel site for cPKC translocation and define a novel function for the sustained activation of PKCα and βII in regulation of recycling components.

Members of the protein kinase C (PKC) superfamily of lipid-dependent, serine-threonine kinases function as critical signaling intermediates in numerous signal transduction pathways and cellular regulatory processes. Currently, PKC consists of 11 distinct isoenzymes grouped into three subfamilies (classical, novel, and atypical) on the basis of lipid cofactor requirements and structural homology (1). The first PKC kinases to be identified were the calcium-dependent or classical PKC (cPKC) isoenzymes (α, βI, βII, and γ). Members of this subfamily contain a cysteine-rich zinc finger C1 domain, which is the site of sn-1,2-diacylglycerol (DAG) and phorbol ester binding, and a C2 domain which confers calcium-dependent phospholipid binding properties. The novel PKC (nPKC) isoenzymes (∆, ε, η, θ) also contain a C1 domain and bind DAG similar to the classical isoenzymes but display calcium independence due to a truncated, non-functional C2 domain. Lipid regulation of the atypical PKC (aPKC) subfamily (λ, ζ) is the most divergent due to a truncated, DAG-insensitive C1 domain and no C2 domain (1-3).

A key determinant of cellular PKC activation lies in the ability of individual isoenzymes, especially those of cPKC and less so that of nPKC, to translocate to the plasma membrane in response to DAG and tumor-promoting phorbol esters. At the plasma membrane, PKC becomes fully activated and gains access to specific substrates (4). Although this classical model of acute PKC activation (seconds to minutes) stands today as a paradigm for the study of lipid-dependent signal transduction, there is additional biochemical and immunocytochemical evidence to suggest that PKC may translocate to sites other than the plasma membrane (5). Using a variety of PKC agonists, including extracellular agonists and phorbol esters, PKC has been localized to the Golgi complex (6), nuclear envelope (7, 8), nucleus (9, 10) mitochondria (11), and/or cytoskeleton (12-14).

In the course of investigating the mechanisms of PKC translocation, we observed that following relatively long term (1 h) treatment with PMA (which we now distinguish as “sustained stimulation”), select PKC isoenzymes translocated to a singu lar, juxtanuclear location. Characterization of this compartment revealed PKCα and βI translocated to a rab11-positive subset of recycling endosomes found concentrated around the microtubule-organizing center (MTOC)/centrosome.

The endosomal recycling compartment consists primarily of a vesiculo-tubular system that carries proteins and lipids to various subcellular destinations, and also serves as a sorting center for lipids and proteins. It is also involved in the recycling of plasma membrane proteins by shuffling those proteins intracellularly and then back to the cell surface (15). Although PKC has been implicated in receptor desensitization and internalization, little is known regarding the possible roles for PKC in regulating recycling function (16-19).

We further show in this study that coincident with the translocation of cPKC to the juxtanuclear compartment was the sequestration of membrane-recycling components and that this occurred in a cPKC kinase activity-dependent manner. These results suggest a role for PKC in trapping/sequestering recycling components.

**EXPERIMENTAL PROCEDURES**

**Materials**—Eagle’s minimal essential media, Dulbecco’s modified Eagle’s medium, and HEPES were from Invitrogen. The HeLa and DLD-1
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Cell line conditions were kindly provided by Dr. Dennis Watson (Medical University of South Carolina), and the CHO-K1 cell line was kindly provided by Justin Turner (Medical University of South Carolina). The HEK 293, HT-1080, COS-1, and A549 cell lines were all purchased from American Tissue Culture Collection (Manassas, VA). Primary antibodies: Anti-PKCα was described previously (20); anti-20 S proteasome (cat. 539145), 4β-phorbol-12-myristate-13-acetate (PMA), and 4α-phorbol were purchased from Calbiochem (La Jolla, CA); anti-pericentrin (cat. PRB-432C) and anti-giantin (cat. PRB-114C) were from Covance (Oakland, CA); anti-GM130 (cat. 610822), and anti-p230 (cat. 611280) were purchased from BD Transduction Labs (Lexington, KY); antirab11 (cat. 9020) and anti-ubiquitin (cat. FL-76) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Alexa Fluor® 594 Transferrin, mouse anti-rabbit, and secondary antibody conjugates were purchased from Molecular Probes, Inc (Eugene, OR). All other chemicals and reagents were from Sigma.

Cell Culture Conditions—HeLa, Cos-1, and A549 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM), HEK 293, and HT-1080 cells were maintained in Eagle’s minimal essential media (MEM), DLD-1 cells were maintained in RPMI 1640, and CHO-K1 cells were maintained in Ham’s F12K. All media (Invitrogen) were supplemented with 10% (v/v) fetal bovine serum (Summit), 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were grown at 37 °C in a humidified incubator with 5% CO2 and passed every 3–4 days to maintain logarithmic growth.

Constructs—The subcloning of full-length wild-type PKCζII, PKCθII-mt-C1, and PKCθII-JC2 into pBK-CMV-GFP were described previously (21, 22). GFP fusion constructs of PKCs and PKCζ were prepared using a similar method; cDNA was amplified with PCR and gene-specific primers synthesized with a 5′-EcoRI site. The amplified full-length human EST encoding wild-type atypical PKCζ was purchased from American Type Culture Collection and amplified with PCR. Atypical PKCζ was amplified with primers encoding a 5′-XhoI restriction site and a 3′-EcoRI site. The amplified full-length, wild-type PCR product was ligated into pEGFP-C3 vector (Clontech) and sequenced at the MUSC DNA Sequencing Facility. The platelet-derived growth factor β receptor was amplified from a fetal kidney cDNA library and subcloned into a FLAG-tagged pcDNA3.

Transient Transfection, Indirect Immunofluorescence, and Confocal Microscopy—Cells were plated onto 35-mm confocal dishes (MatTek) at a density of 2.5–5.0 × 104 cells/dish and grown for 24 h. Transient transfection of DNA (1.5 μg/dish) was performed with LipofectAMINE 2000 (Invitrogen) according to manufacturer’s recommendations. Twelve hours post-transfection, cells were treated and fixed with 3–3.7% paraformaldehyde with or without 10% methanol for 10 min at 37 °C or room temperature. Following fixation, cells were permeabilized with 20–100% w/v methanol for 5 min. The methanol was aspirated, and cells were allowed to air dry (∼5 min). Cells were washed three times with 1.5% PBS/PBS for 5 min each and then blocked in 2.5% FBS for 1 h at room temperature. All primary antibody incubations were performed in 1.5% FBS/PBS with 0.15% saponin at dilutions of 1:50–1:100 for 3 h at room temperature or overnight at 4 °C. Following incubation with the primary antibody, cells were washed three times with 1.5% PBS/PBS for 5 min each. Cells were incubated with secondary antibody dilute in 1.5% FBS/PBS and 0.15% saponin at a dilution of 1:50 to 1:100 for 1 h at room temperature. Cells were washed three additional times with 1.5% PBS/PBS for 5 min each. Confocal images were captured immediately following immunofluorescence processing with an Olympus IX-70 Spinning Disk Confocal Microscope and a P2000 (568-594 Trans) oil objective. Each micrograph represents a single image captured at the equatorial plane of the cell. All micrographs were processed for publication in Adobe Photoshop® 5.0.

Disruption of the Golgi Complex with Nocodazole and Brefeldin A—The Golgi dissociating agents, nocodazole and brefeldin A were employed to probe cellular localization of GFP-CRC. Cells were plated in 35-mm confocal dishes (MatTek) at a density of 2.5–5.0×104 cells/dish. After 24 h of growth, cells were transiently transfected with GFP-PKCζ. Twelve hours post-transfection, cells were treated with 100 nM PMA for 1 h at 37 °C to induce the translocation of PKCζ to the pericentrum. Following this initial PKCζ stimulation, cells were then treated with concentrations of either nocodazole (5 μM) or brefeldin A (5 μM) in serum-free media for 2–3 h. Concentrations were previously (23, 24) determined to cause full Golgi dissociation, which was also verified in this study (Fig. 3, B and C).

Steady State Labeling with Transferrin—Cells were plated onto 35-mm confocal dishes at a density of 2.5–5.0 × 104 cells/dish, grown for 24 h, and then transiently transfected with GFP-PKCζ. Twelve hours post-transfection, visualization of total cellular recycling compartments was achieved by steady state labeling cells with 5–10 μg/ml Alexa Fluor® 594-conjugated transferrin (Molecular Probes) for 30 min in the presence of 0.01% MeSO or 100 nM PMA. All labeling was performed in the presence of complete culture media with 10% FBS.

Recycling Assay—Cells were plated onto 35-mm confocal dishes at a density of 2.5–5.0 × 104 cells/dish, grown for 24 h, and then transiently transfected with GFP-PKCζ. Twelve hours later, the recycling of membrane components was visualized by chase labeling of transfected cells with 10 μg/ml of unlabelled transferrin for 30 min following a 1 h steady state labeling of cells with 10 μg/ml Alexa Fluor-conjugated transferrin. The steady state labeling and subsequent chase with unlabelled transferrin were performed either in the presence of...
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RESULTS AND DISCUSSION

Endogenous and Overexpressed PKC Translocates to a Juxtanuclear Location When Stimulated in a Prolonged Manner— Whereas acute (5-10 min) stimulation of PKC with its agonist 4β-phorbol-12-myristate-13-acetate (PMA) results in translocation of PKC to the plasma membrane, longer PMA treatments (1 h) of HeLa cells resulted in the translocation of both PKC isoenzymes (1 h) of HeLa cells. Twelve hours post-transfection, cells were treated with 100 nM PMA for 1 h. D, transient transfection of HeLa cells with wild-type GFP-PKCβII (panel 1), GFP-PKCβII-mt-C1 (panel 2), isolated GFP-fused C1 domain (panel 3), and GFP-PKCβII-ΔC2 (panel 4). Twelve hours post-transfection cells were treated with 100 nM PMA for 1 h. E, time course of cells transiently transfected with GFP-cPKCβII and stimulated with PMA for the indicated time periods. Panel 5 was preincubated 30 min with 5 μM nocodazole followed by 100 nM PMA. Panel 6 was preincubated 30 min with 5 μM cytochalasin D.

Thus, PKCα undergoes translocation to a novel, juxtanuclear location upon prolonged stimulation with various PKC agonists. PKCα and βII Translocate to a Juxtanuclear Compartment—To determine if translocation of PKC to the juxtanuclear compartment was specific to PKCα, representatives of each PKC subfamily (classical, βII; novel, ε; atypical, ζ) were subcloned into pEGFP vector and transiently transfected into HeLa cells. Following 1 h exposure to PMA, only GFP-PKCβII was observed to translocate to both the plasma membrane and to the juxtanuclear site (Fig. 2A, panels 1 and 2). The DAG/PMA-sensitive and calcium-independent PKC isoenzyme GFP-PKCα was found only at the plasma membrane (Fig. 2B, panels 3 and 4), whereas the DAG-insensitive GFP-PKCε did not respond to PMA and exhibited no alteration in cytoplasmic localization (Fig. 2C, panels 5 and 6). These data suggest that the translocation of PKC to the juxtanuclear region involves a mechanism that appears restricted to some members of the calcium- and DAG/PMA-sensitive cPKC subfamily.

Juxtanuclear Translocation of cPKC Requires a C1 and C2 Domain—Members of the classical PKC subfamily are distinguished by the presence of the highly conserved calcium-dependent, acidic phospholipid-binding, C2 domain. To investigate the requirement of the C2 and/or C1 domain in translocation of PKC isoenzymes to the juxtanuclear compartment, a series of cPKC mutants were generated encoding either a mutant of the C1 domain (PKCβII-mt-C1) that disrupts binding of DAG/PMA, a full deletion of the C2 domain (PKCβII-ΔC2), and an isolated C1 domain (GFP-C1) of PKCβII. One hour treatment with 100 nM PMA led to the translocation of wild-type GFP-PKCβII to both the plasma membrane and juxtanuclear location, whereas the diffuse cytoplasmic localization of PKCβII-mt-C1 remained unchanged following PMA treatment (Fig. 2D, panels 1 and 2). These data demonstrate that PKC translocation to both the plasma membrane and juxtanuclear compartment requires a functional interaction with...
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its agonist at the C1 domain. Importantly, GFP-C1 exhibited translocation to the plasma membrane as has been previously reported (27) but there was no translocation to the juxtanuclear region evident (Fig. 2D, panel 3). This suggested that C1 domain binding to the phorbol ester alone at the plasma membrane was not sufficient to induce translocation and that translocation to the juxtanuclear compartment requires other conserved domains (e.g. C2 domain) and/or a full-length protein. To that end, similar to the isolated C1 domain, the full length GFP-PKCaII-ΔC2 exhibited translocation to only the plasma membrane (Fig. 2D, panel 4). These results suggest that unlike translocation of PKC to the plasma membrane in response to PMA, wherein a C1 domain is sufficient, translocation to the juxtanuclear compartment requires both the C1 and C2 domains. This requirement for the C2 domain may account for the cPKC subfamily-specific translocation. Of note, in subsequent studies, the PKCα and βII isoforms were found to behave very similarly in parallel experiments, and therefore, for extended investigation we chose to focus on the cPKCα isoenzyme.

Translocation of PKC to the Juxtanuclear Compartment Is Dependent upon Microtubules—To investigate the mechanism of cPKC accumulation at the juxtanuclear compartment, a time course was performed with 100 nM PMA. At 5 min PMA, a large percentage of the GFP-tagged PKCα was observed to translocate to the plasma membrane (Fig. 2E, panel 1). Beginning at 15 min of PMA, filamentous structures began to appear in the cytosol and these seemed to radiate from the cell periphery (Fig. 2E, panel 2). At later time points these filaments were observed to coalesce into denser filamentous structures (Fig. 2E, panel 3). At 60 min PMA, PKC was observed localized at both the plasma membrane and at the juxtanuclear compartment with relatively little filaments observed (Fig. 2E, panel 4). The time-dependent appearance of these cytoplasmic filaments suggested the possibility that PKC was translocating to the juxtanuclear compartment in a cytoskeletal-dependent manner. To investigate this further, cells were preincubated with either the actin poison, cytochalasin D (28), or the microtubule depolymerizing agent nocodazole (24) and then treated with PMA for 1 h. As seen in Fig. 2E, panel 3, in the presence of nocodazole, PKC was able to translocate to the plasma membrane but there was no subsequent translocation to the juxtanuclear region. On the other hand, cytochalasin D did not block PKC translocation to the juxtanuclear location but did appear to have an effect on the compartment itself suggesting that actin may be involved in the structure of the compartment (Fig. 2E, panel 4). Taken together, these data suggest that at early time points of stimulation, PKC translocates to the plasma membrane and that this occurs in a microtubule-independent manner. Upon more long term stimulation, PKC then requires microtubules for further translocation to the juxtanuclear compartment.

PKCα Translocates to a Singular, Juxtanuclear Location Distinct from the Golgi Complex—In an effort to identify the juxtanuclear compartment to which cPKC translocated, an extensive colocalization study was undertaken. As expected,
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Fig. 4. Accumulation of GFP-cPKCα at the juxtanuclear location is not due to aggregation of PKC. A, indirect immunofluorescence for proteasome 20 S and B, ubiquitin in HeLa cells following 1 h MeSO (0.01%) or 100 nM PMA. C, immunoblot for endogenous PKCα in cells treated for 0, 6, 24, and 48 h with 100 nM PMA. Bar represents 10 μm.

Given that cPKC translocates to a singular location in the center of the cell, the mitochondria-specific dye, MitoTracker, and an ER-targeted protein, pDS-Red-ER, all failed to display any overlap with GFP-PKCα translocated to the juxtanuclear region (data not shown). Translocation of cPKC to a central region of HeLa or HEK 293 cells suggested that under conditions of prolonged stimulation, PKCα may associate with an organelle that can also be found as a single compartment such as the Golgi complex. This was thought to be a strong possibility since the PKCα isoenzyme was suggested to localize to the golgi complex in the NIH 3T3 cell line following long term activation with PMA (6). In addition, some DAG/PMA-sensitive, non-kinase proteins, including munc13 (29) and chimerains (30) have also been reported to translocate to the Golgi complex following activation with phorbol esters. Using antibodies to GM130 (31), giantin (32), and p230 (33), as markers of the cis, medial, and trans compartments of the Golgi complex, respectively, GFP-PKCα was observed to localize close to the Golgi complex at low magnification (Fig. 3A, panels 1–3). However at higher magnification, it was evident that the pattern of cPKC translocation was separable from the Golgi complex (Fig. 3A, panels 4–6). Analysis of the high magnification micrographs revealed that GFP-PKCα translocated to a region of the cell that was surrounded by the Golgi complex but displayed only minimal overlap with that organelle.

In order to conclusively rule out any association of PKCα with the Golgi, the Golgi complex was disrupted with 5 μM nocodazole (BFA) (23). Total disruption of the Golgi complex was indicated by dispersal of GM130 visualized by indirect immunofluorescence (Fig. 3B, panels 1–2). However, BFA had little effect on PKCα translocated to the juxtanuclear site (Fig. 3B, panels 3–4). It has been reported that BFA may incompletely disrupt the Golgi complex and that the cis/medial Golgi dissociates, but the trans-Golgi remains intact (34). To address this possibility, cells were treated with 5 μM nocodazole, which effectively disrupts all aspects of the Golgi complex following 1 h PMA (24)(Fig. 3C, panels 1–2). The administration of nocodazole had no effect on the GFP-PKCa associated with the juxtanuclear site (Fig. 3C, panels 3–4). Quantitation of the effect of both nocodazole and BFA on cPKC translocation showed that there was only modest effects on the number of cells exhibiting juxtanuclear translocation (Fig. 3D). Thus, the apparent resistance of this compartment to BFA and nocodazole-induced disruption suggests that PKCα translocated to an organelle distinct from the Golgi complex and whose structure was independent of microtubules. These results argue against the localization of PKCα to the Golgi complex upon prolonged activation. Interestingly, these results also reveal that although PKCα may localize to microtubules to translocate to the juxtanuclear compartment, the compartment itself once it is formed is largely microtubule-independent.

Recent reports suggest that the excess production of misfolded proteins may result in the aggregation of proteins in an MTOC-associated structure which the authors named the aggresome (35, 36). The aggresome consists of aggregated proteinaceous pools of ubiquitin and proteasome subunits to aid in the degradation of misfolded proteins (37). In our studies, no evidence was found that overexpression of GFP-tagged cPKCα isoenzymes and/or long term treatment with PKC agonists could modulate the subcellular location of ubiquitin or the 20 S proteasome (Fig. 4, A and B), and thus, PKCα did not colocalize with either ubiquitin or 20 S proteasome. Further, no PKC degradation was detected up to 6 h for either endogenous
PKC Translocation to a Compartment That Is Closely Associated with the Centrosome—The centrosome or the central body named by Theodor Boveri more than 100 years ago can be found at the center of interphase cells where it functions as the primary MTOC (38). Due to its central location, the centrosome became a candidate for the novel compartment defined by cPKC translocation. Indirect immunofluorescence with markers of the centrosome demonstrated that GFP-cPKCα translocated to a compartment that was centrally defined by pericentrin (39) and γ-tubulin (40) (Fig. 5, A and B). However, it was evident that GFP-PKCα localization extended significantly beyond the centrosome. These observations suggested that with long term stimulation PKCα translocates to a novel compartment located in a central region of the cell and that this compartment was closely associated with the centrosome.

PKCα Translocates to a Subset of Transferrin-positive Recycling Endosomes—Based on the above evidence, we evaluated compartments/cellular functions that show pericentriolar behavior. Although the endosomal recycling compartment (ERC) usually shows a diffuse tubulo-vesicular morphology, in some cells a subcomponent of the recycling compartment adopts a singular pericentriolar distribution, closely related to the Golgi complex and MTOC (41, 42); however, this distribution has not been shown to be associated with any known specific functions or regulation of the recycling compartment (15, 42). To determine if PKCα localized to any aspect of the endosomal recycling system, cells were steady state-labeled with a fluorescent-conjugated transferrin (AF-Tf) ligand, in the presence of PMA. As shown in Fig. 5C, sustained stimulation of PKC resulted in localization of PKCα to a subset of transferrin-positive recycling endosomes concentrated in a central area of the cell, suggesting that cPKC translocated to the subcomponent of the recycling system associated with the MTOC/centrosome.

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![Fig. 6. cPKC regulates the sequestration of recycling components in a juxtanuclear compartment. A, steady state labeling (60 min) of HEK 293 cells with 5–10 μg/ml Alexa Fluor® (594)-conjugated transferrin (AF-Tf) in absence (panel 1) or presence (panel 2) of 100 nM PMA. A, panel 3, cells were steady state-labeled with AF-Tf in the presence of PMA and then treated 30 min with 3 μM Go6976. B, 30-min chase with unlabeled transferrin following 1 h AF-Tf steady state labeling in the absence of PMA. C, 30-min chase with unlabeled transferrin following 1 h AF-Tf steady state labeling in the presence of 100 nM PMA. Micrographs are representative images from three independent experiments. Bar represents 10.0 μm.](image)

![Fig. 7. Schematic representation of cPKC translocation. A, steady state labeling of cells with transferrin results in the labeling of all recycling endosomal compartments, which in the absence of PKC stimulation are found in a diffuse, cytosolic pattern but with a slight concentration in the pericentriolar region. The pericentriolar region is characterized by the presence of the small GTPase, rab11 and pericentrin. B, short term (5 min) stimulation of PKC leads to the recruitment of cPKC to the plasma membrane. C, long term stimulation of PKC results in two events: 1) a reorganization of transferrin-positive compartments to a concentrated pattern in the pericentriolar region; and 2) the translocation of PKCα to the pericentriolar region in a compartment that overlaps with rab11 and a subset of transferrin-positive vesicles. Both of these events require cPKC activity.](image)
Long Term Stimulation of cPKC Results in the Sequestration of Membrane-recycling Components—Next we explored the biological consequences of the translocation of PKCs to this compartment. The endosomal system is known to function as a sorting center for proteins and lipids destined for degradation in lysosomes, trafficking to other organelles, or recycling back to the plasma membrane (15, 45). Since the trafficking of transferrin has been the subject of extensive study (41), we examined the effects of PKC stimulation and inhibition of cPKC kinase activity on the localization of AF-Tf. As seen in Fig. 6A, steady state labeling of cells with AF-Tf in the absence of PMA resulted in transferrin that was found localized in a somewhat diffuse cytoplasmic pattern but with some concentration of transferrin in the juxtanuclear region. In contrast, steady state labeling the cells in the presence of 100 nM PMA led to a concentration of fluorescent transferrin in the juxtanuclear location (Fig. 6A, panel 2). Interestingly, these results suggested that long term stimulation of PKC may have an effect on the organization of recycling endosomes. This was further supported when cells were steady state labeled in the presence of phorbol esters for 1 h and then treated with 3 μM cPKC specific kinase inhibitor, Gö 6976. In the absence of cPKC kinase activity (basal and PMA-stimulated), the juxtanuclear pattern of endosomes that was seen with long term PKC stimulation was lost, and transferrin was found localized throughout the cytoplasm (Fig. 6A, panel 3).

To determine the functional consequences of sustained cPKC activity on the kinetics of membrane recycling, chase experiments were performed with non-fluorescent transferrin. In the absence of PKC stimulation, a 30-min chase following steady state labeling with AF-Tf resulted in the recycling of all transferrin back to the plasma membrane and a complete loss of fluorescence from the cell (Fig. 6B). These results are consistent with published data that have established the rapid recycling of transferrin and recycling endosomes (46–48). In stark contrast, a 30-min chase in the presence of sustained PKC stimulation resulted in the retention of AF-Tf in the PKC-positive juxtanuclear compartment (Fig. 6C). These observations demonstrate that cPKC can effectively sequester recycling components in this juxtanuclear compartment.

Concluding Remarks—This study shows that unlike the classical model of PKC activation, in which PKC responds to the acute elevation of lipid second messengers with a reversible (30–120 s) translocation to the plasma membrane, a more prolonged stimulation can induce translocation to a site which is centered on the centrosome but extends beyond it. This compartment, most closely related to a previously recognized variant of the endocytic recycling compartment (ERC) which can be found in some cells concentrated in an area around the MTOC/centrosome (41).

This compartment, though close to the Golgi apparatus, was clearly distinguishable from it when examined at high magnification and with Golgi-disrupting agents. The current results raise the possibility that previous studies demonstrating localization of PKCγ, n-Chimaerin, and munc-13 to the Golgi may also involve this compartment (6, 29, 30). Also, previous studies, using subcellular fractionation, have suggested that PKCα may localize to the nucleus (7). The current results raise the intriguing possibility that the juxtanuclear compartment may fractionate with nuclei.

Although no specific functions for the pericentriolar subcompartment of the recycling endosomes has been proposed, the current results show that PKC regulates the dynamics of endocytosis and trafficking of transferrin through its sequestration in this compartment. This novel function of PKC may be hypothesized to extend to other cargo and components of the recycling endosomes, and it may have relevance to many events dependent on the availability of membrane components (such as receptors, channels, and transporters) and subdomains (such as rafts) at the plasma membrane (Fig. 7). According to this hypothesis, sustained stimulation of PKCα results in its translocation to the pericentriolar compartment along with a subset of recycling endosomes. Maintenance of PKC activity then may affect trapping or sequestration of these components in the pericentriolar region. When PKC is turned off, the sequestered components are released and could re-enter recycling pathways. That this function may extend beyond transferrin (as shown in this study), is supported by some observations in the literature. For example, it has been shown that activation of PKC regulates the availability of the dopamine transporter at the plasma membrane (49) although the location of this event and its relationship to the endocytic compartment were not investigated. It would be interesting to determine if these effects on the dopamine transporter (and other membrane components) are due to retention in this pericentriolar recycling subcompartment of recycling endosomes, in a mechanism similar to that seen with transferrin.

In addition to such potential consequences of cPKC-regulated sequestration of components of the recycling endosomes, it is also possible that the concentration/sequestration of these components may serve to exert compartment-specific effects. Thus, the current results demonstrate novel translocation and function for select cPKCs that may emerge as a key regulator of the dynamics of plasma membrane/endosomal recycling and function.

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