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REVIEW

Membrane Lipid Control of Cytokinesis

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ABSTRACT. **In the final stage of cell division, cytokinesis constricts and then seals the plasma membrane between the two daughter cells. The constriction is powered by a contractile ring of actin filaments, and scission involves rearrangement of the lipid bilayer of the cell membrane. We have shown that the lipid phosphatidylethanolamine (PE), which normally resides in the internal leaflet of the bilayer, is exposed on the external leaflet of the cleavage furrow as a result of enhanced transbilayer movement of the phospholipids during cytokinesis. To investigate the role of PE in cytokinesis, we employed two different approaches: manipulation of cell surface PE by a PE-binding peptide and establishment of a mutant cell line specifically defective in PE biosynthesis. Both approaches provide evidence that surface exposure of PE is essential for disassembly of the contractile ring at the final stage of cytokinesis. Based on these findings, we proposed that the transbilayer redistribution of PE plays a critical role in mediating coordinated movements between the contractile ring and the plasma membrane that are required for the proper progression of cytokinesis.**

Key words: phosphatidylethanolamine/contractile ring/phospholipid asymmetry/phospholipid-binding peptide

The function of cytokinesis is to create a membranous barrier between the two daughter cells. At the terminal phase of cytokinesis in eukaryotic cells, both the microtubules and the contractile ring of actin filaments at the cleavage furrow disassemble, which is followed by the fusion of opposing plasma membrane and cell separation. Thus, it is likely that the coordinated changes in the cytoskeletons and membrane lipids are essential for achieving successful cell division, but it remains unclear how these activities are temporally and spatially coordinated during cytokinesis.

Phospholipids in most biological membranes are arranged asymmetrically between the two leaflets of the bilayer. In eukayotic plasma membrane, aminophospholipids such as phosphatidylethanolamine (PE) and phosphatidylserine (PS) reside predominantly in the inner leaflet, while phosphatidylcholine (PC), sphingomyelin, and glycolipids are enriched in the outer leaflet (Schroit and Zwaal, 1991; Zachowski, 1993). This transbilayer distribution of membrane lipids is not a static situation, but is likely to be a result of the balance between the inward and outward translocation of phospholipids across the bilayer membranes (Bevers *et al*., 1999). In biological membranes, the transposition of phospholipids between leaflets proceeds much more rapidly than that in model membranes (Trotter and Voelker, 1994; Bevers *et al*., 1999). Since the translocation reaction requires ATP and is sensitive to sulfhydryl modifying reagents (Martin and Pagano, 1987), the lipid translocation is likely to be facilitated by proteins. Recent studies have identified several molecules involved in the transbilayer movement of phospholipids in an ATP-dependent manner; however, our understanding of the molecular mechanisms promoting the transbilayer movement of phospholipids as well as its physiological functions is still limited.

To study the molecular motion and the functions of membrane phospholipids, we have established various phospholipid-binding probes that include monoclonal antibodies against PC (Nam *et al*., 1990), PS (Umeda *et al*., 1989), and phosphatidylinositol 4,5-bisphosphate (PIP_2) (Miyazawa *et al*., 1988), a tetracyclic polypeptide of 19 amino acids (Ro09-0198) that binds specifically to PE (Aoki *et al*., 1994, Emoto *et al*., 1997), and lysenin, a sphingomyelin-specific

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Abbreviations: Ro, Ro09-0198; SA-Ro, streptavidin-Ro complex; CHO, Chinese hamster ovary; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLScR, phospholipid scramblase.

binding protein derived from the earthworm (Yamaji *et al*., 1998). Using PE-binding peptide, we have demonstrated that PE is exposed on the cell surface of cleavage furrow as a result of transbilayer movements of phospholipids at the final stage of cytokinesis (Emoto *et al*., 1996). Furthermore, addition of PE-binding peptide conjugated to streptavidin (SA-Ro) to the dividing cells prevents cytokinesis, suggesting that PE plays a role in completion of cytokinesis (Emoto and Umeda, 2000). To further examine the role of PE in cytokinesis, we next established and analyzed a mutant Chinese hamster ovary (CHO) cell line defective in PE biosynthesis (Emoto *et al*., 1999). The results obtained from these two distinct approaches have demonstrated that PE is required for proper progression of cytokinesis. In addition, our findings suggest that redistribution of PE on the furrow membrane plays a critical role in controlling the disassembly of the contractile ring.

Lipid dynamics in cytokinesis

The cytokinetic process ends up with fusion of adjacent membranes, which would accompany the dynamics rearrangement of architecture of membrane lipids (Ellen *et al*., 1984; Zimmerberg *et al*., 1993). To investigate the role of lipid dynamics in cell division, we have developed a series of lipid-binding probes and analyzed the cell surface localization of lipid molecules in dividing cells. We first found that phosphatidylethanolamine (PE), which is present in much greater amounts in the inner as opposed to the outer leaflet of the plasma membrane, is transiently exposed on the cell surface of the furrow membrane in dividing CHO cells (Fig. 1) (Emoto *et al*., 1996). Concerning the source of PE on furrow surface, there remain several possibilities: PE could come from the inner leaflet by transbilayer movement, or it could become concentrated from elsewhere in the outer leaflet by lateral movements. Although we have not come to a final conclusion yet, we favor the former possibility based on the following observations. First, intense staining was observed only during late telophase and we detected no alterations in the surface distribution of PE during cytokinesis, suggesting that the latter possibility is unlikely. Second, Kobayashi and Pagano (1989) showed that the amount of PE in the outer leaflets of CHO cell plasma membranes increased about threefold when cells entered the late telophase. We recently found that the plasma membrane sphingomyelin, which is shown to be distributed on the cell surface uniformly in the interphase cells (Yamaji *et al*., 1998), was excluded from the furrow surface by staining with a sphingomyelin-specific binding protein, lysenin (unpublished data). These observations suggest that a unique PE-rich lipid domain may be formed in the outer leaflet of the cleavage furrow membrane during cytokinesis.

Molecular mechanisms for PE exposure during cytokinesis

The movement of phospholipids across the artificial membrane bilayers is an extremely slow process, because transport of the charged head group through the hydrophobic core of the lipid bilayer represents a high energy barrier (Dawidowicz, 1987). Thus, the transient exposure of PE at furrow membrane is likely to be facilitated by proteins. Recent studies have identified several molecules mediating the transbilayer movement of phospholipids. ABC (ATP-binding cassette) transporters are shown to play a crucial role in multidrug resistance (mdr) activity, the acquisition of simultaneous resistance to a wide variety of structurally and functionally unrelated compounds. Recently, several members of ABC transporters, such as mammalian P-glycoproteins (Ruetz and Gros, 1995; van Helvoort *et al*., 1996) and multidrug resistance protein 1 (MRP1) (Dekkers *et al*., 1998; Raggers *et al*., 1999), are shown to facilitate an ATP-dependent outward movement of phospholipid analogs as well as amphipathic drugs. In regard to the inward-transport, an integral membrane P-type ATPase, called Drs2p, was proposed to function as an aminophospholipid translocase, since disruption of yeast *DRS2* gene resulted in significant decrease of the internalization of fluorescent PS analogs across the plasma membrane (Tang *et al*., 1996). However, several groups have challenged this observation after having failed to detect a difference between wild-type and DRS2Δ cells in the translocation of fluorescent lipids across the plasma membrane (Siegmund *et al*., 1998; Marx *et al*., 1999), calling into

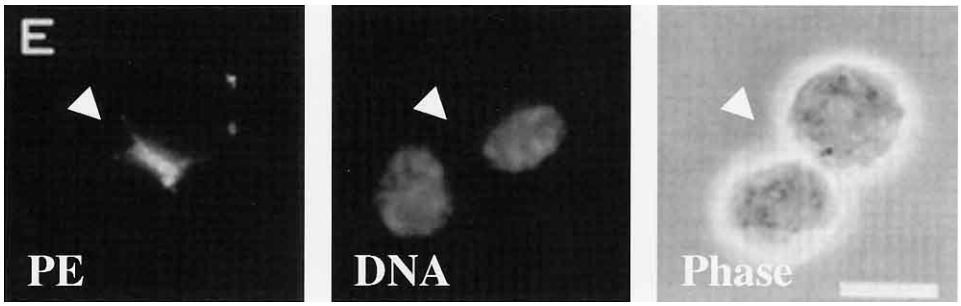

Fig. 1. Exposure of PE on the cell surface of dividing cells. Cell surface PE was detected by fluorecein-labeled SA-Ro. DNA was stained with DAPI.

question whether Drs2p functions as an aminophospholipid translocase at the plasma membrane. In addition to these molecules, Sims and his colleagues recently isolated a 35-kDa erythrocyte membrane protein, named phospholipid scramblase (PLScR), and suggested that this molecule is involved in the rapid movement of PS from inner-to-outer plasma membrane leaflets in response to elevated cytosolic calcium concentration (Zhou *et al*., 1997; Zhao *et al*., 1998). We have examined the localization of these lipid transporters during cytokinesis and found that PLScR1 becomes concentrated in the furrow membrane at the final stage of cytokinesis, although PLScR1 is uniformly distributed on the cell surface in early mitosis (Fig. 2). Thus, PLScR1 is a possible candidate for PE translocation during cytokinesis so far. In addition, we have recently identified a novel membrane protein involved in PE translocation by establishment and analyses of budding yeast mutants with altered sensitivity to PE-binding peptide (manuscript in preparation). Since this molecule is highly conserved from yeast to mammalian cells, this molecule would also be the candidate molecule involved in the PE translocation during cytokinesis. This possibility is now being investigated.

Inhibition of contractile ring disassembly by PE-binding peptide

To study the role of PE exposure in cytokinesis, we examined the effects of Ro peptide, a PE-binding peptide, on cytokinesis (Emoto *et al*., 1996; Emoto and Umeda, 2000). When mitotic CHO-K1 cells were incubated for 2 h with Ro peptide conjugated with streptavidin (SA-Ro), most cells could not complete cell division. These cells remained connected up to 16 h after the initiation of cytokinesis (Fig. 3). Time-lapse observations revealed that the SA-Ro treated cells formed cleavage furrows normally, and fully contracted to produce daughter cells, but the cells could not separate and remained connected by a cytoplasmic bridge (Fig. 3). No significant effect on cytokinesis was detected by using other phospholipid-specific probes, including annexin V that binds to PS, and anti-phospholipid mAbs specific for either PS, PC, or PIP_2. To further analyze the effect of Ro peptide on cytokinesis, we examined the distribution of SA-Ro bound to the arrested cell membrane. SA-Ro bound predominantly to the surface of the cytoplasmic bridge. In addition, the actin filaments concentrated in the cytoplasmic bridge co-localized just underneath the plasma membrane to which the SA-Ro bound. When SA-Ro treated cells were incubated in tissue culture medium containing 10 μM PE liposomes, the contraction in the cytoplasmic bridge was gradually released and eventually the daughter cells fused with each other to form binucleated cells within 2 h. No significant binding of SA-Ro to the binucleated cells was observed, which suggests that the cell-surface bound SA-Ro was absorbed by the PE-liposomes. The contractile ring remaining in the cytoplasmic bridge disappeared, and stress fibers were formed throughout the binucleated cells. Myosin II and radixin that had been concentrated in the cytoplasmic bridge translocated to cytoplasm in the binucleated cells. These results demonstrate that the inhibition of contractile ring disassembly is reversible upon re-

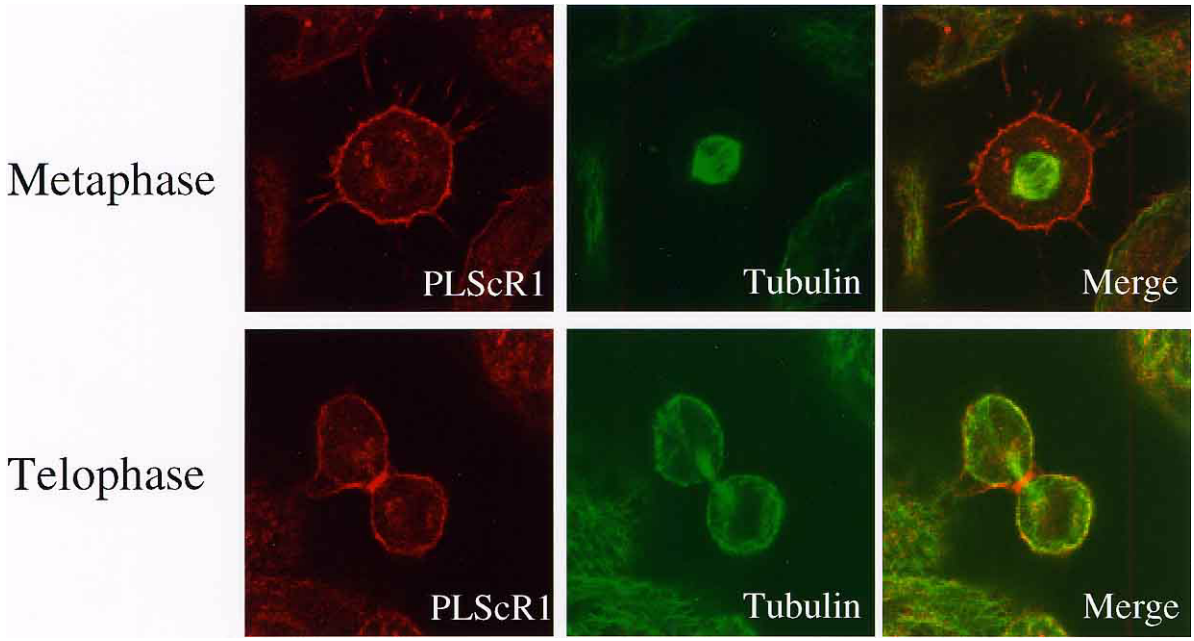

Fig. 2. Cellular distribution of PLScR1 in dividing Hela cells.

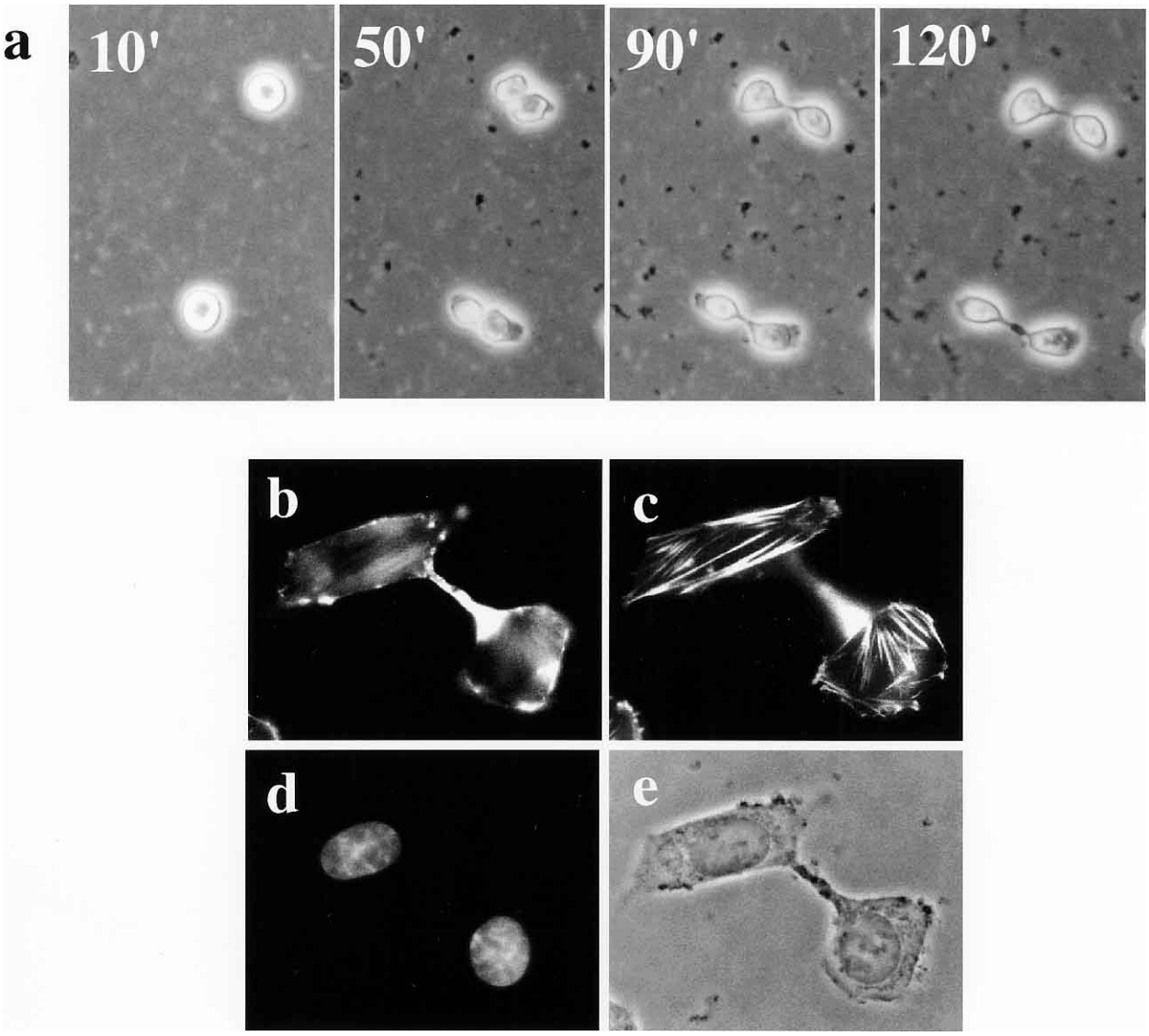

Fig. 3. SA-Ro treatment blocks disassembly of the contractile ring. (a) shows the time-lapse observation of cytokinesis of cells treated with SA-Ro. Lower panel shows the focal plane of the cytoplasmic bridge where actin bundles remain concentrated (b), and the focal plane of the bottom layer of the daughter cell where stress fibers are seen (c) in SA-Ro (50 μg/ml) treated cells. DAPI staining (d) and phase-contrast (e) are also shown.

moval of the surface-bound SA-Ro, and suggest that exposure of PE on the cleavage furrow membrane plays a crucial role in the regulation of contractile ring disassembly.

A PE-deficient mutant cell line exhibits a defect in cytokinesis

To further study the role of PE in cytokinesis, we established a CHO mutant cell line with a specific defect in PE biosynthesis (Emoto *et al.*, 1999). The mutant, designated as R-41, was isolated as a variant resistant to the cytotoxicity of Ro peptide. The mutant cell was shown to be a unique mutant in which only cellular PE content was decreased to about half that of the parent cells because of a defective translocation of PS into mitochondrial inner membrane where PS is converted to PE by PS decarboxylase. When R-41 mutant cells were cultured in normal medium, the cells exhibited a partial cytokinetic defect in which the late telophase of cytokinesis was extensively prolonged. Further decrease in the PE level of the mutant cells by culturing them in ethanolamine-deficient medium blocked the contractile ring disassembly without affecting the initial formation of the contractile ring or its contraction, resulting in cell division arrest in late telophase (Emoto and Umeda, 2000). In this culture condition, no significant exposure of PE on the cleavage furrow surface was detected by the fluorescein-la-

beled PE-binding peptide in the mutant cells. Addition of PE or ethanolamine, a precursor of PE, restored cellular PE levels and normal cell division. These results demonstrate a good correlation between PE exposure on the cleavage furrow membrane and successful cytokinesis, and provide further support for the proposal that redistribution of PE on the cleavage furrow membrane may play a crucial role in completion of cytokinesis.

Regulation of actin filament assembly by transbilayer redistribution of PE

To further study the role of redistribution of cell surface PE in the cytoskeletal reorganization, we examined the effect of SA-Ro on actin filament organization in interphase cells (Umeda and Emoto, 1999). The addition of SA-Ro caused a rapid morphological change in which most cells lost ruffling membrane structures within 15 min, and after 30 min they became refractile and rounded, leaving their beaded dendritic processes attached to the plates. Electron microscopic observations showed that most of the SA-Ro treated cells lost their microvilli structures. Staining of actin filaments with fluorescence-labeled phalloidin showed that the incubation with SA-Ro for 15 min induced the disruption of existing actin stress fibers and led to the polymerization of actin filament underneath the plasma membrane. This SA-Ro-induced morphological change and reorganization of actin filaments was fully reversible, since removal of surface-bound SA-Ro by incubating with 10 μM PE-containing medium resulted in a gradual restoration of their normal morphology and an accompanying disassembly of cortical actin filaments followed by the formation of stress fibers. Double immuno staining showed that the staining of cortical actin filaments was co-localized with the cell surface staining of SA-Ro, suggesting that the cortical actin filaments assembled to the local area of plasma membrane where SA-Ro bound to the surface. These observations clearly demonstrated that redistribution of plasma membrane PE in both dividing and interphase cells directly affect the organization of actin filaments. Based on these observations, we propose that the transbilayer assembly state of plasma membrane PE via yet unidentified molecule(s) which transmits information on the PE assembly state to disassemble the actin cytoskeletal system. These continuous crosstalks between plasma membrane and underlying actin cytoskeletons may play a pivotal role in the coordinated movement of cytoskeletal and membrane systems (Fig. 4).

Perspectives

PE is commonly used as a dominant membrane lipid from bacterial to mammalian cells. Interestingly, a mutant *E.coli* strain that completely lacks PE has a specific defect in cytokinesis. In *E.coli*, cell division is mediated by the formation of a septal ring, the FtsZ ring, which circumferentially invaginates the cell wall to cleave the cell into two (Bi and Luthenhaus, 1991). In the PE-deficient strain, FtsZ protein and other essential division proteins are recruited to the division site, but the FtsZ ring failed to constrict (Mileykovskaya *et al.*, 1998). Thus, it is likely that PE is

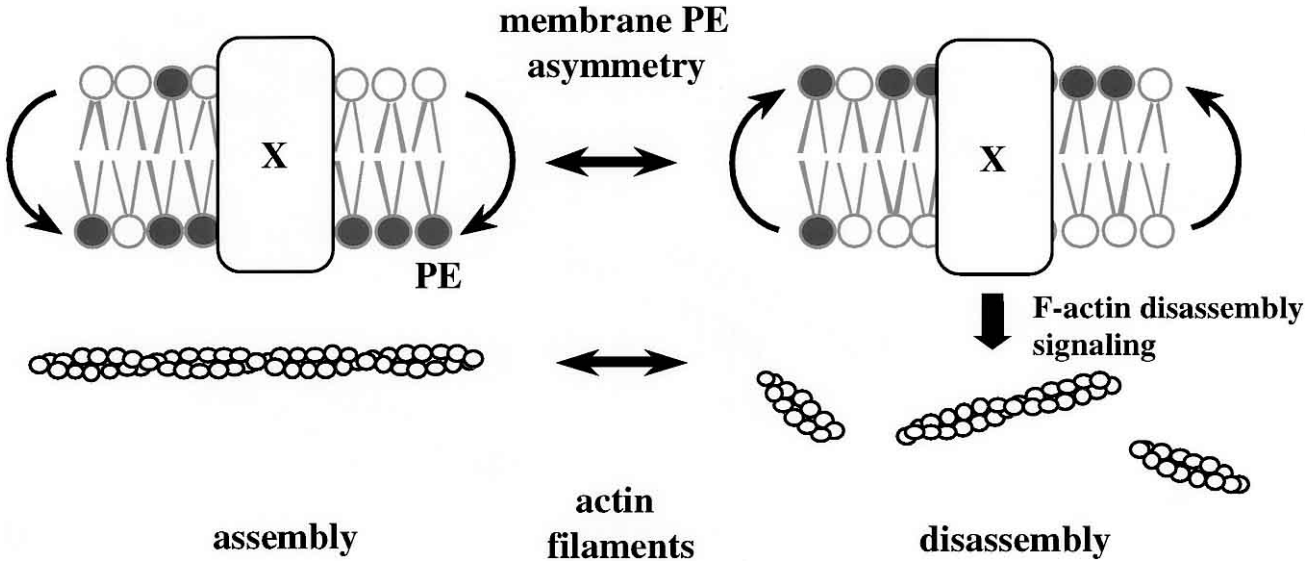

Fig. 4. A model for regulation of actin filament assembly by transbilayer redistribution of membrane PE. Disassembly of actin filaments at the base of plasma membrane is accelerated when transbilayer movement of PE from the inner to outer leaflet is activated. This redistribution of PE may affect the function of an unknown protein that transmits a signal to disassemble the components of the actin cytoskeletal system. Relocation of PE from the outer to inner leaflet may provide an appropriate membrane surface where actin filaments tend to assemble. Local change in transbilayer distribution of PE in plasma membrane may provide spatio-temporal signals to coordinate the movement between cytoskeletal and membrane systems.

essential for cytoskeletal organization in the completion of cytokinesis in prokaryotic cells as well as mammalian cells.

Recent studies have shown that several lipid molecules, such as psychosine (Kanazawa *et al*., 2000; Im *et al*., 2001) and PIP_2 (Brill *et al*., 2000), are implicated in the rearrangement of actin filaments at the final stage of cytokinesis. It is of interest to investigate how these lipid productions and localizations are coordinated in the dividing cell membrane.

The molecular mechanisms of how membrane phospholipids regulate the actin cytoskeleton and what are the downstream events that lead to cytoskeletal reorganization remains largely unknown. One possibility is that the PE-rich domains formed on the cleavage furrow membrane provides a specific milieu for activation or assembly of certain molecules regulating disassembly of the contractile ring, because PE-rich membranes tend to form a nonbilayer hexagonal structure that has been shown to regulate various membrane-bound enzymes such as the calcium pump (Yeagle and Sen, 1986), protein kinase C (Bazzi *et al*., 1992), and phospholipase D (Nakamura *et al*., 1996). Recently, we have obtained evidence that PE-rich domain is required for the membrane assembly and/or activation of Rho, a small GTPase implicated in the reorganization of the contractile ring (unpublished data). Thus, the redistribution of PE on the plasma membrane may regulate the local assembly and/or activation of Rho GTPase, which result in the spatial reorganization of the actin cytoskeleton.

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