Diacylglycerol Induces Fusion of Nuclear Envelope Membrane Precursor Vesicles*

Purified membrane vesicles isolated from sea urchin eggs form nuclear envelopes around sperm nuclei following GTP hydrolysis in the presence of cytosol. A low density subfraction of these vesicles (MV1), highly enriched in phosphatidylinositol (PtdIns), is required for nuclear envelope formation. Membrane fusion of MV1 with a second fraction that contributes most of the nuclear envelope can be initiated without GTP by an exogenous bacterial PtdIns-specific phospholipase C (PI-PLC) which hydrolyzes PtdIns to form diacylglycerides and inositol 1-phosphate. This PI-PLC hydrolyzes a subset of sea urchin membrane vesicle PtdIns into diglycerides enriched in long chain, polyunsaturated species as revealed by a novel liquid chromatography-mass spectrometry analysis. Large unilamellar vesicles (LUVs) enriched in PtdIns can substitute for MV1 in PI-PLC induced nuclear envelope formation. Moreover, MV1 prehydrolyzed with PI-PLC and washed to remove inositols leads to spontaneous nuclear envelope formation with MV2 without further PI-PLC treatment. LUVs enriched in diacylglycerol mimic prehydrolyzed MV1. These results indicate that production of membrane destabilizing diglycerides in membranes enriched in PtdIns may facilitate membrane fusion in a natural membrane system and suggest that MV1, which binds only to two places on the sperm nucleus, may initiate fusion locally.

At the end of each mitosis in eukaryotes, the nuclear envelope is typically reconstituted by membrane fusion, forming the nuclear compartment and segregating the chromosomes from the cytoplasm. A similar process encloses sperm chromatin in egg cytoplasm following fertilization. A number of studies emphasizing the role of proteins have addressed the mechanism of nuclear envelope assembly, many utilizing cell-free systems derived from eggs or somatic cells (1–4). However, relatively little attention has been paid to the essential role(s) played by membrane lipids in this process.

Male pronuclear or somatic nuclear envelope formation involves binding of nuclear membrane precursors to the chromatin surface followed by fusion to create a double membrane enclosing the chromatin (1, 5–7). We have previously reported that envelope formation in a cell-free system derived from sea urchin eggs requires the fusion of three egg membrane vesicle populations and remnants of the sperm nuclear envelope at the tip and base of the conical nucleus (8–10).

One of the egg vesicle populations (MV1) is particularly unusual. It is a low density fraction highly enriched in the membrane lipid phosphatidylinositol (PtdIns) (9, 11). MV1 binds at the tip and base of the sperm nucleus and is required for nuclear envelope formation, which can be induced by addition of GTP or a bacterial PtdIns-specific phospholipase C (PI-PLC) (9, 12). The endogenous sea urchin PI-PLC activity probably resembles a typical eukaryotic enzyme whose substrate is PtdIns(4,5)P_2. GTP-initiated envelope formation is inhibited by GTPγS and by the PI 3-kinase inhibitors, wortmannin and LY294002 (12, 13). Initiation of the fusion process by exogenous bacterial PI-PLC or human recombinant PI-PLCγ can be inhibited by the PI-PLC inhibitors ET-18-OCH_3 or U73122 (12, 14).

PtdIns hydrolysis is best known as an intermediate step in G-protein signaling pathways in which PtdIns(4,5)P_2 is hydrolyzed by PI-PLC to form diacylglycerol (DAG) and inositol 1,4,5-triphosphate (InsP_3). Typically, such signaling occurs in membranes containing 3–10% PtdIns with much lower amounts of PtdIns(4,5)P_2 (15). However, the large amount of PtdIns present in MV1 (up to 80% of the phospholipid) suggested to us that the PtdIns hydrolysis may be important in altering membrane structure rather than in initiating a signaling pathway (12). Since MV1 binds to the tip and base of the nucleus, hydrolysis at these points might lead to fusion initiation through localized formation of DAG. The hydrolysis products of PtdIns catalyzed by the bacterial PI-PLC are diglycerides (normally DAG) and inositol 1-phosphate (from the intermediate D-myoinositol-1,2-cyclic phosphate) (16). DAG produced enzymatically by phospholipases acting on synthetic membranes has been shown to be membrane destabilizing and induce membrane fusion (17–20).

We show here that under fusion-stimulating conditions, bacterial PI-PLC treatment of sea urchin egg membranes results in large increases of a small subset of diradylglyceride (diacylglycerol, alkylacylglycerol, and alkenylacylglycerol) molecular species, in particular, DAG 18:0/20:4. To test whether fusion of natural membranes induced by PI-PLC in our cell-free system might result from localized production of DAG, we took two complementary approaches. First, MV1 was hydrolyzed with PI-PLC and washed to remove the water-soluble inositol.

---

* This work was partially supported by Fundação para a Ciência e a Tecnologia PRAXIS XXI Grant BD-19568-99 (to T.B.), by an Amherst College Faculty Research Award of the H. Axel Schupf ’57 Fund for Intellectual Life (to D. L. P.), and by The Welcome Trust (to M. J. O. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 These authors contributed equally to this work.

2 To whom correspondence should be addressed. Tel.: 413-542-2198; Fax: 413-542-7955; E-mail: dpocci@amherst.edu.

3 The abbreviations used are: MV, membrane vesicle population; DAG, diacylglycerol; DRG, diradylglyceride; PtdIns, phosphatidylinositol; PtdCho, phosphatidylcholine; InsP_3, inositol 1,4,5-triphosphate; PI-PLC, PtdIns-specific phospholipase C; LC-MS, liquid chromatography-mass spectrometry; LUV, large unilamellar vesicle; GTPγS, 5-guanosine-5’-γ-thio)triphosphate; ET-18-OCH_3 or ET, 1-Octadecyl-2-O-methyl-sn-glycerol-3-phosphorylcholine; LB, egg lysis buffer; S10, cytosolic egg extract 10,000 g supernatant; S150, cytosolic egg extract 150,000 g supernatant; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N,N,N’’N’-tetraacetic acid; NE, nuclear envelope.
products. The resulting prehydrolyzed MV1 when added to a cell-free system containing other nuclear envelope precursor MVs and cytosol led to nuclear envelope formation with no added inducer. Second, we used synthetic large unilamellar membrane vesicles (LUVs) to substitute for MV1 and varied the phospholipid composition of these membranes. LUVs containing 75% PtdIns mimicked MV1. In the presence of cytosol these LUVs bound to the tips of nuclei and initiated fusion when exogenous PI-PLC was added. Furthermore, LUVs containing 75% DAG led to fusion without exogenously added PI-PLC, mimicking prehydrolyzed MV1.

These results indicate that production of substantial amounts of diglycerides from the PtdIns of MV1 can lead to nuclear envelope formation and offer a possible role for PtdIns-rich membranes in local initia-
tion of nuclear envelope formation.

MATERIALS AND METHODS

Buffers and Reagents—Egg lysate buffer (LB), nuclear preparation buffer, membrane wash buffer (MWB), and Tris/HCl, pH 7.4, buffer (TN) were prepared as previously described (21). *Lytechinus pictus* and *Paracentrotus lividus* (sea urchins) were obtained from Marinus (Long Beach, CA) and Universidade Lusófona, Portugal, respectively (*L. pictus* was used for all experiments unless otherwise stated). The ATP generation system was 1 mM ATP, 20 mM creatine phosphate, and 1 mg/ml creatine kinase (Type I), all from Sigma in LB. GTP (Sigma, Type II) stock was 1 mg/ml in LB buffer. The stock of lipophilic membrane probe 3,3′-dihexylocarbocyanine iodide (DiOC₆) from Molecular Probes was 0.1 mg/ml in methanol. Synaptojanin1 (Syn1-Spase) phosphatase construct (S470-R962; lacking the Sac phosphatase and proline-rich domains) from R. Woscholski (Imperial College London). PI-PLC from *Bacillus cereus* was from Sigma. PI-PLC stocks (3.14 units/ml) were made by dissolving 0.8 mg in 1 ml of 144 mM NaCl, 10 mM Tris/HCl, pH 7.4, 0.02% (w/v) bovine serum albumin, and stored at 4°C for 2 h at room temperature. Total lipids were extracted from each using 150 µl of chloroform/methanol (2:1 v/v), transferred into a 1.5 ml of MWB buffer and homogenized by passing twice vigorously through a 22-gauge needle. The lysate was cleared at 10,000 × g for 10 min in a 5417R Eppendorf microcentrifuge at 4°C. The recovered supernatant, referred to as cytoplasmic extract or S10, includes cytosol and cytoplasmic membrane vesicles. S10 was used directly or frozen and stored in small aliquots at −80°C.

Cytosol (S150) was prepared by fractionating the S10 at 150,000 × g for 3 h in a Beckman Ti50 rotor at 4°C. S150 supernatant was used immediately or frozen in aliquots at −80°C. The pellet of membrane vesicles (MV0) was washed twice in MWB with phenylmethylsulfonyl fluoride added freshly to a final concentration of 1 mM for 10 min at 45,000 × g in a Ti50 rotor. MV0 was resuspended in 0.10 of the volume of the original S10 and used immediately or quick frozen in aliquots at −80°C.

To prepare MV1 and MV2, MV0 from 2.5 ml of packed eggs was carefully resuspended in 100 µl of TN, and then 0.90 µl of MWB buffer was added. Complete suspension was achieved by passing through a series of increasingly smaller plastic micropipette tips (1 ml to 20 µl). MVs were stained with DiOC₆ at a final concentration of 10 µM/ml and observed using a Zeiss Neofluar 100× objective (>20 nm) by positive electrospray ionization on a Shimadzu QP8000a single quadrupole MS (probe voltage, 1 kV; nebulizer gas, 4 liters/min N₂; curved desolvation line temperature, 275°C).

**Sperm Nuclei Permeabilization, Fertilized Egg Extracts, and MV Preparation—Sperm nuclei of *L. pictus* were permeabilized with 0.1% Triton X-100 as described previously (21). Demembranated nuclei were washed and resuspended at 10³ nuclei/ml. Nuclei were diluted 1:25 and added to egg extracts to a final ratio of approximately one sperm nucleus per egg equivalent. Eggs and sperm were collected and eggs fertilized as described (21). Fertilized eggs were washed twice in Millipore HAWP-filtered sea water at 100 × g for 1 min in a 5403 Eppendorf swinging bucket microcentrifuge at 15°C. At 13 min post-fertilization, 2.5 ml of packed eggs was washed twice with an equal volume of cold LB buffer and homogenized by passing twice vigorously through a 22-gauge needle. The lysate was cleared at 10,000 × g for 10 min in a 5417R Eppendorf microcentrifuge at 4°C. The recovered supernatant, referred to as cytoplasmic extract or S10, includes cytosol and cytoplasmic membrane vesicles. S10 was used directly or frozen and stored in small aliquots at −80°C.

Avoid any repetition. No extra content was added for the blank regions.
the appropriate inducer was added. Samples were incubated for 2 h. Nuclear envelope formation was scored as a continuous fluorescent rim, in contrast to the patchy appearance of bound MVs.

GTP inducer was added from the stock solution to a final concentration of 0.25 mM. PI-PLC was added to a final concentration of 0.07 unit/ml. Inhibition reactions were performed by addition of inhibitors to S150. The final concentration of ET-18-OCH₃ (Sigma) was 19.6 μM and of wortmannin (Sigma) was 25 nM. Each experiment was repeated at least three times, 100 nuclei were counted in each sample and standard deviations calculated.

For the synaptojanin1 experiments, MVs from S10 were assembled around chromatin in the presence of ATP for an hour. Fusion was induced with 1 mM GTP for 2 h. Alternatively, nuclei were treated with 1 μg/ml of the Syn1-5ptase protein for 15 min prior to the addition of GTP, and a further 1 μg/ml Syn1-5ptase was added simultaneously with GTP. An average of 24 nuclei were scored on three independent occasions and the mean and S.E. of these results calculated.

Pretreatment of MV1—MV1 was diluted in MWB, and either the inducer alone or the inducer and inhibitor was added to the reaction mix as above. Reactions were incubated for 1 h at room temperature. Vesicles were stained with DiOC₆ as described above and were pelleted by centrifugation at 45,000 g for 15 min. The supernatant was removed, and the pellet was washed in MWB and resuspended. The pretreated MV1 was added to MV2 and S150 in presence of the ATP-generation system and decondensed nuclei. The reaction was incubated for 2 h and observed as described.

**RESULTS**

GTP-induced Nuclear Envelope Formation Is Blocked by Depletion of the Putative Substrate of the Endogenous PI-PLC—Nuclear envelope formation in a cell-free system can be induced by adding GTP or PI-PLC (12). GTP induction of nuclear envelope formation is inhibited by the PI-PLC inhibitor U73122 (14). To further demonstrate that GTP induction of nuclear envelope formation in the cell-free system requires endogenous PI-PLC activity, GTP was added to cytoplasmic extracts depleted of the PI-PLC substrate phosphatidylinositol bisphosphate.

We used a construct of synaptojanin1 (Syn1-5ptase) phosphatase (S470-R962; lacking the Sac phosphatase and proline-rich domains), which has a strong specificity for the D5-phosphate of the inositol ring. Enzyme kinetics have shown that this construct has the greatest preference for PtdIns(4,5)P₂18:0/20:4 over other phosphoinositide substrates (22). Nuclear envelope formation by GTP was severely inhibited by treatment with this phosphatase indicating that an endogenous PI-PLC is required for GTP induction (Fig. 1).

**Bacterial PI-PLC Treatment of Sea Urchin Membrane Vesicles Produces a Subset of Diacylglycerol Species**—Bacterial PI-PLC, which hydrolyzes unphosphorylated PtdIns and has been reported to have no activity toward PtdIns(4,5)P₂ (16), was chosen for our experiments to minimize complications of rates of PtdIns phosphorylation upon the kinetics of DAG production from PtdIns(4,5)P₂. In addition this enzyme does not produce inositol 1,4,5-trisphosphate and therefore a resulting increase in free Ca²⁺ associated with this effector. Hydrolysis of sea

*Figure 1. Nuclear envelope formation is inhibited by Syn1 5-phosphatase. After binding of membrane vesicles to sperm nuclei in cytoplasmic extracts (S10) in the presence of an ATP-generating system, aliquots were untreated or treated with a construct of the 5-phosphatase domain of synaptojanin1. GTP was added to one treated and one untreated aliquot and nuclear envelope formation scored. The phosphatase, specific for the D₅-phosphate of the inositol ring, severely blocked nuclear envelope formation induced by GTP.*

*Figure 2. Mass spectrometry analysis of diacylglycerol species present in isolated sea urchin cytoplasmic membrane vesicles before and after bacterial PI-PLC treatment. P. lividus MV0 was isolated from 1.0 ml of S10 and resuspended in 400 μl of LB. Half was untreated, and half was treated with 0.16 unit/ml bacterial PI-PLC for 2 h at room temperature. Total lipid extracts were made and 64 diglyceride species analyzed as described under “Materials and Methods.” Species that contributed less than 2% of total diglycerides in control and treated samples have been grouped as “others.” Data are presented as mean mole % of the total DAG pool ± S.E. (n = 4).*
Diacylglycerol and Nuclear Envelope Formation

**TABLE ONE**

Nuclear envelope formation in vitro requires MV1

| Membranes present | Inducers | Envelope formation | Inhibitors | Envelope formation |
|-------------------|----------|--------------------|------------|--------------------|
| MV0               | GTP      | 95 ± 3%            | ET-18-OCH₃ | <1%                |
|                   | PL-PLC   | 98 ± 2%            | Wortmannin | <1%                |
| MV1 + MV2         | GTP      | 95 ± 6% (Fig. 3A)  |            | <1% (Fig. 3B)      |
|                   | PL-PLC   | 93 ± 6% (Fig. 3C)  |            | <1% (Fig. 3D)      |
| MV2               | GTP      | 9% ± 7             |            |                    |
|                   | PL-PLC   | 15% ± 7            |            |                    |

These result show that under the conditions used here, bacterial PI-PLC produces a large increase in a minor subset of diglycerides present in sea urchin membrane vesicles, in particular DAG 18:0/20:4.

**Pretreatment of MV1 with PI-PLC Renders It Fusogenic**—We have previously hypothesized that, upon hydrolysis, high levels of PtdIns in MV1 provide sufficient DAGs to facilitate fusion of nuclear envelope precursors (12). We tested this idea by pretreating MV1 with PI-PLC to produce diglycerides, then washing the membranes to remove soluble inositol phosphates and incubating pretreated MV1 in extracts containing the remaining egg MV nuclear envelope precursors (collectively termed MV2).

As shown in TABLE ONE, nuclear envelope formation can be induced by either GTP or PI-PLC in a complete system containing decondensed sperm nuclei, cytosol (S150), an ATP generating system and MV0 (cytoplasmic MVs, which include MV1, MV2, and other unnecessary MV fractions (9)). Similar levels of NE formation were seen in a more defined system when purified MV1 and MV2 were substituted for MV0. If MV1 was omitted, basal levels of envelope formation were detected, indicating that MV1, although it contributes a minor fraction of total nuclear envelope, is required for envelope formation. GTP-induced fusion was blocked by wortmannin (a PI 3-kinase inhibitor), and PI-PLC induced fusion by ET-18-OCH₃ (a PI-PLC inhibitor).

If the relevant activity of exogenous PI-PLC is the production of diglycerides in MV1, pretreatment of MV1 should make subsequent PI-PLC treatment unnecessary. As shown in TABLE TWO, MV1 prehydrolyzed with PI-PLC led to nuclear envelope formation even in the absence of the PI-PLC inducer. Envelope assembly using pretreated MV1 was no longer sensitive to ET-18-OCH₃ inhibition during incubation in extract, although ET-18-OCH₃ blocked the effect of PI-PLC pretreatment. MV2 was required, since it provides most of the nuclear envelope (9). Representative nuclei corresponding to some experiments in TABLE ONE (and keyed there) are shown in Fig. 3.

If the relevant activity of exogenous PI-PLC is the production of diglycerides in MV1, pretreatment of MV1 should make subsequent PI-PLC treatment unnecessary. As shown in TABLE TWO, MV1 prehydrolyzed with PI-PLC led to nuclear envelope formation even in the absence of the PI-PLC inducer. Envelope assembly using pretreated MV1 was no longer sensitive to ET-18-OCH₃ inhibition during incubation in extract, although ET-18-OCH₃ blocked the effect of PI-PLC pretreatment. MV2 was required, since it provides most of the nuclear envelope (9). Representative nuclei corresponding to some experiments in TABLE ONE (and keyed there) are shown in Fig. 3.

These data indicate that PI-PLC pretreatment renders MV1 fusogenic and are consistent with the notion that exogenous PI-PLC acts to induce fusion in the cell-free system by production of diglycerides in the PtdIns-rich MV1 membrane fraction at sufficient levels to induce fusion.

**PtdIns-rich LUVs Can Substitute for MV1**—To better define the role of MV1 in the formation of nuclear envelopes, we prepared protein-free model membranes with a phospholipid composition mimicking MV1 (75% PtdIns/25% PtdCho (w/w)). These vesicles, when added to a system containing cytosol, an ATP-generating system and sperm nuclei but no MV2, bound to the nuclei at two positions corresponding to the sites of the sperm nuclear envelope remnants, thus mimicking MV1.
Binding did not occur in LB buffer (data not shown), presumably because proteins in the S150 (cytosol) are necessary to mediate specific binding.

That these LUVs are capable of assuming the role of MV1 in membrane fusion was tested as shown in TABLE THREE. TABLE ONE shows that induction by PI-PLC requires the MV1 fraction. TABLE THREE shows that when MV1 is substituted by PtdIns-rich LUVs, either GTP or PI-PLC will initiate fusion, each subject to the appropriate inhibition. No envelope formation was seen when the LUV, MV2, or inducer were omitted. Some representative examples of induction of nuclear envelope formation by GTP and PI-PLC of PtdIns-rich LUVs in the presence of MV2 are shown in Fig. 5, B–E (keyed in TABLE THREE).

These data indicate that synthetic membranes of 75% PtdIns/25% PtdCho can mimic many properties of MV1. These vesicles bound to the same regions as MV1 and conferred GTP or PI-PLC regulation of nuclear envelope formation.

**DAG-rich LUVs Are Fusigenic**—The experiments in TABLE TWO suggest that diglycerides in the hydrolyzed MV1 fraction are responsible for fusion. To directly test this idea, we made LUVs in which DAG was quantitatively substituted for PtdIns. When 75% DAG/25% PtdCho (w/w) LUVs were added to a system containing MV2, cytosol, and an ATP-generating system, envelope formation occurred with or without the inducers GTP or PI-PLC (TABLE THREE). The percent of nuclei showing fusion was in all cases well above the background levels in the absence of LUVs or MV1 (TABLE ONE). No envelopes were formed without MV2 so that the LUVs by themselves did not form an envelope. Fusion was not altered by the inclusion of inhibitors of the normal inducers. Fig. 6 shows some representative examples (keyed in TABLE FOUR). These data indicate that DAG vesicles are fusigenic in a natural membrane system and suggest a mechanism for the fusion of PtdIns-rich vesicles upon hydrolysis by PI-PLC.

### DISCUSSION

A cell-free system derived from fertilized sea urchin eggs supports nuclear envelope assembly on added membrane-stripped sperm nuclei induced by GTP hydrolysis (4, 10). Exogenously added bacterial PI-PLC can also induce nuclear membrane formation (12, Fig. 7). Each is dependent on a minor membrane fraction MV1, highly enriched in PtdIns (12). MV1 contributes only 10% of the nuclear membrane vesicle precursor population and binds exclusively to the regions of the sperm nucleus containing remnants of the sperm nuclear envelope (9). Most of the nuclear membrane is contributed by the major fraction MV2 enriched in a marker enzyme of the endoplasmic reticulum (9).

The biological function of MV1 is not known. A natural membrane fraction so rich in PtdIns had never been reported previously (11). MV1...
binds via peripheral proteins to two specific regions of the sperm nucleus (9) containing that portion of the sperm nuclear membrane that does not break down following fertilization (23) and that is characterized by an unusual underlying osmiophilic "cup" (10). In contrast, the major MV2 subfraction binds all around the nuclear periphery through lamin B receptor, a chromatin-binding intrinsic inner nuclear membrane protein (24).

The high concentration of PtdIns in MV1 prompted us to hypothesize that its role was to generate, upon PI-PLC catalyzed hydrolysis, a local enrichment of membrane destabilizing DAG leading to initiation of fusion with the other membrane vesicles that make up the bulk of the membrane (Fig. 2). DAG, by destabilizing membranes and inducing negative curvature (26, 27), and/or by recruiting non-PKC C1 domain containing proteins to the nuclear envelope, induces fusion of MVs. This final step also has a low (\( \sim 50 \) nM) calcium dependence (4, 14). Fusion is subject to inhibition by GTP\(_\Sigma\) and BAPTA, exogenous DAG kinase, and the 5-phosphatases SopB and Syn1, which remove DAG and PtdIns(4,5)P\(_2\), respectively, from the system (Ref. 14 and Fig. 1). In a further step, nuclei can be induced to swell in the presence of additional ATP (13), in a process requiring lamin B import to the nucleus (data not shown) (28).

### TABLE THREE

PtdIns-rich LUVs can substitute for MV1 in nuclear envelope assembly

| LUVs          | MV2 | Inducers | Envelope formation | Inhibitors       | Envelope formation |
|---------------|-----|----------|--------------------|------------------|-------------------|
| 75% PtdIns/25% PtdCho | −   | −        | <1% (Fig. 5A)      | −                | −                 |
| +             | GTP | 90 ± 9%  | −                  | +                | <1% (Fig. 5C)     |
| −             | GTP | <1%      | −                  | −                | −                 |
| +             | PI-PLC | 93 ± 5%  | −                  | −                | <1% (Fig. 5D)     |
| −             | PI-PLC | <1%      | −                  | −                | −                 |

### TABLE FOUR

DAG-containing LUVs are fusigenic

| LUVs          | MV2 | Inducers | Envelope formation | Inhibitors       | Envelope formation |
|---------------|-----|----------|--------------------|------------------|-------------------|
| 75% DAG/25% PtdCho | −   | −        | 93 ± 6% (Fig. 6A)  | −                | −                 |
| +             | GTP | 95 ± 5%  | −                  | +                | 93 ± 5% (Fig. 6B) |
| −             | GTP | <1% (Fig. 6C) | −         | −                | −                 |
| +             | PI-PLC | 97 ± 4%  | −                  | −                | 90 ± 6% (Fig. 6D) |
| −             | PI-PLC | <1%      | −                  | −                | −                 |
envelope precursors (10, 12). Several lines of evidence presented here support this hypothesis.

First, PI-PLC-mediated fusion requires that MV1 be present. Second, pretreatment of MV1 with bacterial PI-PLC renders it fusigenic. These observations are consistent with the notion that exogenous PI-PLC acts to induce fusion in the cell-free system by production of DAG in the MV1 membrane fraction. Use of synthetic membranes of known composition permitted a third test. LUVs of 75% PtdIns/25% PtdCho mimic MV1, binding to the same regions as MV1 and conferring both GTP and PI-PLC induced fusion in the presence of MV2. Moreover, similar membrane models containing 75% DAG are also fusigenic in the absence of inducers.

Although the relevant endogenous PI-PLC activities required for nuclear envelope formation (which are blocked by the inhibitor U73122 but not its inactive analog U73343 (14)) would act upon PtdIns(4,5)P_2, and the bacterial enzyme acts upon PtdIns, both produce membrane diglycerides (16). Their soluble products are InsP_3 or inositol 1-phosphate, respectively. To avoid the classic signaling pathway involving InsP_3 production and kinetic complications of kinase activities required for PtdIns(4,5)P_2 production, we chose to use the prokaryotic PI-PLC (16). Since prehydrolyzed and washed MV1 was capable of initiating nuclear envelope formation, the effects of soluble inositols were eliminated. Use of DAG-rich LUVs also ruled out a role for production of these soluble inositols from MV1 in nuclear envelope formation in the cell-free system.

Since MV2 hydrolyzed with PI-PLC was unable to form nuclear envelopes in the absence of MV1, despite containing (typically low levels of) PtdIns (11) and DAG, a requirement for MV1 may be understood on the basis of its lipid ratios. We propose that high levels of PtdIns, either in a membrane domain or in a separate set of vesicles, may provide sufficient DAG upon PtdIns hydrolysis to locally initiate membrane fusion. DAG, by virtue of its physical properties, facilitates the phase transition of the lipid bilayer from lamellar to hexagonal II. This type of phase transition induces a localized destabilization of the membrane structure, which in turn favors membrane fusion. That DAG can lead to membrane fusion is supported by several reports using synthetic membrane systems treated with PLCs (17, 19, 20).

The fatty acid chains of the PtdIns and DAG species may play an additional role. The new method for quantification of DAGs by LC-MS presented here permitted a detail of analysis and sensitivity previously unattained in a natural membrane fusion system. That the PI-PLC preferentially produces diglycerides of long chain, polyunsaturated fatty acid content is intriguing, since these are expected to have major effects on increased membrane fluidity and alteration of other structural properties that could facilitate or fine-tune membrane fusion processes.

Our current and previous work emphasizes a role for DAG that is distinct from the classical pathways of signaling utilizing protein kinase C or other C-1 domain containing receptors, which typically involve low levels of DAG (Ref. 14 and Fig. 7). Although DAG can be generated in several ways, such as through PC-PLC hydrolysis of PtdCho or PLD pathways starting with PtdCho, it is worth noting there are no known eukaryotic PC PLCs. Moreover, we have neither been able to induce NE formation with bacterial PC-PLC nor inhibit it with D609 (29), a compound that inhibits both PC-PLC and PLD activities (30). Furthermore, the molecular species composition of PtdIns changes during fusion, whereas PtdCho is identical before and after binding and hydrolysis (14). Since PI-PLC pretreatment of MV1 is sufficient to lead to NE formation in the absence of further inducers in the cell-free system, it is unlikely that other sources of DAG are necessary.

We additionally suggest an important role for lipid modification in biological membrane fusion reactions. We propose that at high DAG levels, alterations of structure of natural membranes in localized regions can affect fusion events.

REFERENCES

1. Lohka, M., and Masui, Y. (1984) J. Cell Biol. 98, 1222–1230
2. Burke, B., and Gerace, L. (1986) Cell 44, 639–652
3. Ulitzur, N., and Gruenbaum, Y. (1989) FEBS Lett. 259, 113–116
4. Cameron, L. A., and Poccia, D. L. (1994) Dev. Biol. 162, 568–578
5. Voges, P. A. G., and Lohka, M. J. (1991) J. Cell Biol. 112, 545–556
6. Boman, A. L., Delamont, N. M., and Wilson, K. L. (1992) J. Cell Biol. 115, 281–294
7. Collas, P., and Poccia, D. (2000) Subcell. Biochem. 34, 273–302
8. Collas, P., and Poccia, D. (1995) Dev. Biol. 169, 123–135
9. Collas, P., and Poccia, D. (1996) J. Cell Sci. 109, 1275–1283
10. Poccia, D., Barona, T., Collas, P., and Larijani, B. (2002) in Nuclear Envelope Assembly in Embryos and Somatic Cells (Collas, P., ed) pp. 111–130, Kluwer/Plenum, New York
11. Larijani, B., Poccia, D. L., and Dickinson, L. C. (2000) Lipids 35, 1289–1297
12. Larijani, B., Barona, T. M., and Poccia, D. L. (2001) Biochem. J. 356, 495–501
13. Collas, P., and Poccia, D. (1995) Mol. Reprod. Dev. 42, 106–113
14. Byrne, R. D., Barona, T., Garnier, M., Koster, G., Katan, M., Poccia, D. L., and Larijani, B. (2005) Biochem. J. 387, 393–400
15. Vanhaesebroeck, B., Leevers, S., Ahmadi, K., Timans, J., Katso, R., Driscoll, P. C., Wooscholski, R., Parker, P. J., and Waterfield, M. D. (2001) Annu. Rev. Biochem. 70, 535–602
16. Griffith, O. H., and Ryan, M. (1999) Biochim. Biophys. Acta 1441, 237–254
17. Basanez, G., Ruiz-Arguelles, M. B., Alonso, A., Goni, F., Karlsson, G., and Edwards, K. (1997) Biophys. J. 72, 2630–2637
18. Plena, J. L., Goni, F. M., and Alonzo, A. (1989) Biochemistry 28, 7364–7367
19. Villar, A. V., Alonso, A., and Goni, F. M. (2000) Biochemistry 39, 14012–14018
20. Villar, A. V., Alonso, F. M., and Alonso, A. (2001) FEBS Lett. 494, 117–120
21. Collas, P., and Poccia, D. (1998) Methods Cell Biol. 53, 417–452
22. Schmid, A. C., Wise, H. M., Mitchell, C. A., Nussbaum, R., and Wooscholski, R. (2004) FEBS Lett. 576, 9–13
23. Longo, F. J., and Anderson, E. (1968) J. Cell Biol. 39, 339–368
24. Collas, P., Courvalin, J.-C., and Poccia, D. (1996) J. Cell Biol. 135, 1715–1725
25. Stephans, S., Beyer, B., Balbazar-Stahelin, U., Duncan, R., Kostacos, M., Lukom, M., Green, G. R., and Poccia, D. (2002) Mol. Reprod. Dev. 62, 496–503
26. Das, S., and Rand, R. P. (1984) Biochem. Biophys. Res. Commun. 124, 491–496
27. Allan, D., Thomas, P., and Micheli, R. H. (1978) Nature 276, 289–290
28. Collas, P., Pinto Correia, C., and Poccia, D.L. (1995) Exp. Cell Res. 219, 687–698
29. Larijani, B. (1999) Role of Lipid Signaling Pathways in an Intra- and Extracellular Phenomenon. Ph.D. thesis, University of Massachusetts, Amherst, MA
30. Kiss, Z., and Tomono, M. (1995) Biochem. Biophys. Acta 1259, 105–108
Membrane Transport, Structure, Function, and Biogenesis: Diacylglycerol Induces Fusion of Nuclear Envelope Membrane Precursor Vesicles

Teresa Barona, Richard D. Byrne, Trevor R. Pettitt, Michael J. O. Wakelam, Banafshe Larijani and Dominic L. Poccia

J. Biol. Chem. 2005, 280:41171-41177.
doi: 10.1074/jbc.M412863200 originally published online October 10, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M412863200

Find articles, minireviews, Reflections and Classics on similar topics on the JBC Affinity Sites.

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 27 references, 5 of which can be accessed free at http://www.jbc.org/content/280/50/41171.full.html#ref-list-1