Ca\textsuperscript{2+}-regulated exocytosis is required for rapid resealing of disrupted plasma membranes. It has been previously demonstrated that repeated membrane disruptions reseal more quickly than the initial wound and that this facilitated response requires the transcription factor cAMP-response element-binding protein (CREB). This study examines the signaling pathway between membrane disruption and CREB-dependent gene expression in 3T3 fibroblasts. A reporter gene assay using pCRE-d2EGFP revealed that membrane disruption induced CRE-mediated transcription. Immunofluorescence observations suggested that membrane disruption activated CREB, p38 mitogen-activated protein kinase (p38 MAPK), and MAPK kinase3/6, the kinase responsible for activation of p38 MAPK. CREB phosphorylation upon membrane disruption was inhibited by a specific p38 MAPK inhibitor, SB203580. Both CRE-mediated transcription and long-term potentiation of membrane resealing and wound-induced exocytosis were suppressed when cells were wounded in the presence of either SB203580 or Gö-6976, a specific protein kinase C (PKC) inhibitor. Furthermore, activation of MAPK kinase3/6 was impaired by PKC inhibition during membrane disruption. These results suggest that PKC mediates the stimulation of CREB-dependent gene expression through a p38 MAPK pathway upon membrane disruption.

Mechanical stress induces disruptions of plasma membranes in many animal tissues under physiological conditions, and a cell survives these disruptions by rapidly resealing its cell membrane (1). Small disruptions on the order of micron-diameter evoke the Ca\textsuperscript{2+}+-dependent exocytosis of vesicles near the wound site, which is essential for successful membrane resealing (2–8). The recruitment of vesicles to docking sites near the wound so that subsequent cell membrane disruptions reseal more quickly (6, 15). It has also been indicated that the potentiation of membrane resealing and wound-induced exocytosis last at least 24 h after the initial wound and require CREB because a dominant-negative CREB mutant inhibited these processes (15). Furthermore, it has been demonstrated that inhibition of PKA during the initial wound does not affect long-term potentiation of membrane resealing (15), suggesting that other, PKA-independent pathways are used to activate CREB upon membrane disruption.

The aim of the present study was to define which signaling pathways are involved in CREB activation and, therefore, CRE-mediated transcription after membrane disruption. To address this issue, a vector, pCRE-d2EGFP, containing a reporter gene encoding a destabilized variant of the enhanced green fluorescent protein (d2EGFP) under the control of a cAMP-response element promoter was transfected into 3T3 fibroblasts, and the changes of fluorescent intensity of d2EGFP upon membrane disruption were analyzed. Immunofluorescent analysis was also used to evaluate the phosphorylation state of CREB, p38 MAPK, and MAPK kinases (MEKs) upon membrane disruption. Membrane disruption was found to induce CREB phosphorylation and CRE-mediated transcription via a PKC- and p38 MAPK-dependent pathway. This signaling pathway was required for long-term potentiation of membrane resealing and wound-induced exocytosis.

EXPERIMENTAL PROCEDURES

Cell Culture—Swiss 3T3 fibroblasts were cultured in Dulbecco’s modified Eagle’s medium (Invitrogen) containing 10% calf serum (Invitrogen) and 100 units/ml penicillin and 0.1 mg/ml streptomycin (Invitrogen) at 37 °C in a 5% CO\textsubscript{2}, humidified atmosphere.

Transfection—Vectors pCRE-d2EGFP and pPUR were purchased from BD Biosciences Clontech (Palo Alto, CA). These vectors were transduced into 3T3 fibroblasts. In order to examine the phosphorylation of CREB, MAPK, and MAPK kinases, Swiss 3T3 fibroblasts were transfected with pCRE-d2EGFP. After 24 h, the cells were transfected with pPUR. The cells were then subjected to mechanical stress using a cell rupture plate (Nipro) and incubated for 24 h. The cells were then harvested, and the phosphorylation state of CREB, MAPK, and MAPK kinases was analyzed.
co-transfected into 3T3 cells using LipofectAMINE Plus (Invitrogen) according to the manufacturer’s protocol. After the transfected cells were grown in Dulbecco’s modified Eagle’s medium containing 10% calf serum and 10% fetal-calf serum without antibiotics for 48 h, the selective antibiotic puromycin (BD Biosciences Clontech) was added at 5 μg/ml. Culture medium containing puromycin was changed every third day. After 2 weeks, transfected clones were isolated and maintained with 5 μg/ml puromycin.

**Scratch Wound Protocol**—pCRE-d2EGFP-transfected 3T3 cells were plated on coverslips and grown for 2–3 days before use. During wound experiments, the cells were maintained in 1.8 mM Ca2+ Ringer’s solution. The 3T3 cells were rinsed with 1.8 mM Ca2+ Ringer’s solution at 37 °C before adding the above rhodamine solution at 37 °C along with various protein kinase inhibitors. Then the cells were wounded by slowly scratching the coverslips multiple times with a sterile 27-gauge needle (16). The cells were allowed to stand for 5 min and then were returned to normal culture conditions. Fluorescent images of d2EGFP were taken on a Leica DMR fluorescence microscope equipped with a SPOT RT camera (Diagnostic Instruments, Sterling Heights, MI). Exposure time and gain level of the camera remained constant during experiments.

**Immunofluorescent Staining**—Anti-phospho-CREB antibody was purchased from Calbiochem-Novabiochem. Antibodies against phospho-p38 (D-8) and phospho-MEK1/2 (Ser-217/221), phospho-SEK1/MKK4 (Ser-257/Thr-261), and phospho-MKK7 (Ser-271/Thr-275) were purchased from Cell Signaling Technology (Beverly, MA). Non-transfected 3T3 cells were plated on the coverslips and were grown for 2–3 days before use. They were wounded by scratching in the presence of 1.0 mg/ml fluoro-emerald (fixable fluorescein-dextran, 10,000 MW; Molecular Probes) instead of TMR-dextran as described above and were fixed with 4% formaldehyde in phosphate-buffered saline for 30 min. The cells were permeabilized with 0.1% Triton X-100 in phosphate-buffered saline for 5 min and immunostained with primary antibodies for 1 h at room temperature or overnight at 4 °C. TMR-conjugated goat anti-rabbit IgG or anti-mouse IgG (Molecular Probes) was used for detection. Immunostaining was photographed using the Leica DMR fluorescence microscope and SPOT RT camera described above.

**Image Analysis**—The fluorescent intensities of d2EGFP and immunostaining in digitized images were evaluated by Imaged 1.30, public domain image processing software from the National Institutes of Health. A 25 × 25 pixel box was positioned over the fluorescent image of each cell, and the average intensity within the selection (the sum of the intensities of all the pixels in the selection divided by the number of pixels) was measured. Statistics were calculated by Student’s t test using Instat 2 (GraphPad Software, San Diego, CA).

**Assay of Exocytosis by FM1–43 Destaining**—Non-transfected 3T3 cells were plated on glass-inserted plastic dishes (Asahi Techno Glass, Tokyo, Japan) and were grown for 2–3 days before use. These cells were both wounded and marked by scratching in the presence of 1.0 mg/ml fluorescein-dextran, 10,000 MW (Molecular Probes) in 1.8 mM Ca2+ Ringer’s solution and were incubated with fresh culture medium containing 4 μM FM1–43 (Molecular Probes) overnight. FM1–43-loaded cells were then wounded again 24 h after the initial wound with a glass needle using an Eppendorf Injectman 5179 and Transjector 5246 mounted on an Olympus IX71 inverted microscope at room temperature (28 °C). Fluorescence changes were analyzed as described previously (9) using MetaFluor 6.1 software (Universal Imaging, Downingtown, PA).

**Membrane Resealing Assay**—Non-transfected 3T3 cells, plated on glass-inserted plastic dishes, were wounded and marked by scratching in the presence of 1.0 mg/ml fluorescein-dextran, 10,000 MW in 1.8 mM Ca2+ Ringer’s solution. These cells were loaded with fura-2/AM (Molecular Probes) 24 h after the wound. Fura-2-loaded cells were then wounded with a glass needle, and membrane resealing was monitored as described previously (9).

**RESULTS**

**Cell Membrane Disruption Induces CRE-mediated Transcription in 3T3 Fibroblasts**—In a previous study, it was demonstrated that repeated cell membrane disruptions reseal more quickly than the initial wound and that this long-term potentiation of membrane resealing requires a transcription factor, CREB (15). The present study investigates the time course of CRE-mediated transcription in wounded cells using a reporter gene, pCRE-d2EGFP. As d2EGFP has a half-life of about 2 h, it reports transient activation of CRE-mediated transcription. pCRE-d2EGFP-transfected cells were wounded by scratching in the presence of the marker dye TMR-dextran in 1.8 mM Ca2+ Ringer’s solution. They were observed live under a fluorescent microscope at various intervals after wounding. Up-regulation of d2EGFP expression was localized to cells containing TMR-dextran, and green fluorescence protein was observed uniformly distributed in wounded cells (Fig. 1A). Fig. 1B summarizes the results of a kinetic study of CRE-mediated transcription after membrane disruption. The fluorescent intensity of d2EGFP increased to 69.1 ± 5.6 (n = 7) from 25.0 ± 1.2 (n = 17) 1 h after membrane disruption. The intensity remained high for 12 h after
membrane disruption and started to decrease after that. At 48 h after membrane disruption, the level of fluorescent signal from d2EGFP decreased to close to initial levels (32.6 ± 5.6, n = 4). The fluorescent intensity of d2EGFP in non-wounded cells plated on another coverslip did not show any significant changes throughout the experimental period (Fig. 1B), indicating that the increase in d2EGFP intensity observed in this study was induced by membrane disruption.

**The p38 MAPK Inhibitor SB203580 Suppresses CRE-mediated Transcription upon Membrane Disruption—**CRE-mediated transcription can be activated through a variety of signaling pathways including PKA, CaMK, and MAPK (11–14). To identify the signaling pathways for CRE-mediated transcription after the disruption of plasma membrane, pCRE-d2EGFP-transfected 3T3 cells were wounded in the presence of kinase inhibitors, KT5720 (10 μM), SB203580 (10 μM), or SB202474 (10 μM), and observed 3.5 h after membrane disruption. Cells that were wounded and survived plasma membrane disruption show cytosolic labeling with the marker dye, TMR-dextran. These cells show up-regulated d2EGFP expression, but SB203580 treated cells do not. Comparison of d2EGFP intensities of cells wounded under various conditions. Values are mean ± S.E. C, immunofluorescence of phospho-CREB. Cells were wounded by scratching in the presence of fluoro-emerald and SB203580 (10 μM) and fixed 1 h after wounding. In a control experiment, phosphorylation of CREB is induced by membrane disruption as indicated by increased immunostaining of the nuclear region with an anti-phospho-CREB antibody (arrow), whereas immunostaining is absent in non-wounded cells (arrowheads). Phosphorylation of CREB after membrane disruption is inhibited by SB203580 (asterisk). D, quantitative analysis of phospho-CREB immunostaining intensity of cell nuclei. Values are mean ± S.E.

![Image](image_url)
and was significantly higher than that of non-wounded cells (p = 0.0012, Student’s t test). This is in agreement with a previous report (15) that showed that PKA was not involved in long-term potentiation of membrane resealing. In contrast, cells wounded in the presence of SB203580 (10 μM), a specific inhibitor of p38 MAPK that blocks its ATP binding site (17), showed only a slight increase in the intensity of d2EGFP to 35.3 ± 3.4 (n = 10), which is not significantly different from non-wounded cells (p = 0.0619, Student’s t test). When cells were wounded in the presence of SB20474 (10 μM), an analogue of SB203580 that does not inhibit p38 MAPK activity, there was a significant increase in d2EGFP fluorescent intensity after membrane disruption. The fluorescent intensity of d2EGFP increased to 54.8 ± 3.4 (n = 10) (p < 0.0001, Student’s t test). The effects of CaMK inhibitors could not be investigated in this study, as membrane resealing itself is dependent on CaMK activity (2). These results suggest that p38 MAPK is required for the activation of CRE-mediated transcription after membrane disruption.

Stimulation of CRE-mediated transcription requires phosphorylation of CREB at Ser-133. To observe CREB phosphorylation in wounded cells, non-transfected cells were wounded by scratching in the presence of the marker dye fluoro-emerald (fixable fluorescein-dextran) in 1.8 mM Ca2+ Ringer’s solution, fixed 1 h later, and then immunostained with anti-phospho-CREB antibody, which recognizes the amino acid sequences surrounding phosphorylated Ser-133 of CREB. Cell nuclei were immunopositive for anti-phospho-CREB antibody in wounded cells (Fig. 2C, arrow), whereas cell nuclei of non-wounded cells were not immunopositive (Fig. 2C, arrowheads). On the other hand, nuclear staining of anti-phospho-CREB antibody was absent when cells were wounded in the presence of 10 μM SB203580 (Fig. 2C, asterisk). As shown in Fig. 2D, membrane disruption resulted in a 2.4-fold increase in immunostaining intensity of phospho-CREB in the nuclear region in control experiments (p < 0.0001, Student’s t test). On the other hand, when cells were wounded in the presence of 10 μM SB203580, the observed increase in immunostaining intensity was not significant (p = 0.2204, Student’s t test). These results imply that CREB activation is achieved by the p38 MAPK pathway upon membrane disruption.

Membrane Disruption Activates p38 MAPK Pathway in a PKC-dependent Manner—To observe the active form of p38 MAPK in wounded cells, non-transfected cells were wounded by scratching in the presence of fluoro-emerald in 1.8 mM Ca2+ Ringer’s solution, fixed 30 min later, and immunostained with anti-phospho-p38 antibody (Fig. 3A). Cell nuclei were immunopositive for anti-phospho-p38 antibody in wounded cells (Fig. 3A, arrows). In control experiments, intensities of immunostaining were 24.6 ± 2.0 (n = 7) and 13.6 ± 2.0 (n = 10) in wounded and non-wounded cells, respectively (p = 0.0016, Student’s t test) (Fig. 3B). When cells were wounded in the presence of the p38 MAPK catalytic site inhibitor SB203580 (10 μM), the immunostaining intensity was also significantly different between wounded and non-wounded cells (p < 0.0001, Student’s t test). Intensities of immunostaining were 21.9 ± 1.9 (n = 13) and 13.2 ± 0.9 (n = 22) in wounded and non-wounded cells, respectively. These results suggest that phosphorylation of p38 MAPK is induced by membrane disruption and that p38 is not autophosphorylated (18) but transphosphorylated by the up-stream kinases MEK3, MEK4, or MEK6 (19).

To observe the activation of MEKs, non-transfected cells were wounded by scratching in the presence of fluoro-emerald in 1.8 mM Ca2+ Ringer’s solution, fixed 30 min later, and immunostained with antibodies against phospho-MEK3/6, phospho-MEK4, phospho-MEK1/2, or phospho-MEK7. Phosphorylated MEK3/6 was mainly detected in the nuclear region in wounded cells (Fig. 4A, arrow), whereas it was absent in the nuclear region in non-wounded cells. Membrane disruption resulted in a significant increase in immunostaining intensity of phospho-MEK3/6 from 20.3 ± 0.6 (n = 75) to 32.9 ± 1.6 (n = 29) (p < 0.0001, Student’s t test) (Fig. 4B). On the other hand, nuclear localization of phospho-MEK3/6 was inhibited when cells were wounded in the presence of a specific PKC inhibitor, G6-6976 (20). When cells were wounded in the presence of G6-6976 (1 μM), the immunostaining intensity was increased only slightly to 21.9 ± 1.2 (n = 19) (p = 0.2008, Student’s t test) (Fig. 4B). Furthermore, PKC stimulation by phorbol ester, phorbol-12-myristate-13-acetate (PMA), induces phosphorylation of MEK3/6 (Fig. 4, A and B).

There was no immunostaining with anti-phospho-MEK4 antibody upon membrane disruption, whereas cells stimulated with 0.5 M sorbitol showed phospho-MEK4 staining mainly in the cytoplasm (Fig. 4C). Nuclear staining of phospho-MEK1/2 and phospho-MEK7 were induced by stimulation with PMA (100 nM); however, membrane disruption did not show any detectable nuclear staining (Fig. 4, D and E). These results suggest that membrane disruption preferentially activates MEK3/6 and that PKC regulates the activation of the p38 MAPK, most likely through MEK3 and -6, upon membrane disruption.

PKC Is Involved in CRE-mediated Transcription and Long-term Potentiation of Wound-induced Exocytosis and Membrane Resealing—The effect of PKC inhibition on CRE-mediated transcription is shown in Fig. 5. Cells transfected with pCRE-d2EGFP were wounded in the absence or presence of a specific

![Membrane disruption activates p38 MAPK](http://www.jbc.org/)
PKC inhibitor, Go–6976 (1 μM), and up-regulation of d2EGFP expression was observed 3.5 h after membrane disruption. When non-treated cells were wounded as a control, the fluorescent intensity of d2EGFP was increased to 57.8 ± 3.7 (n = 40) from 24.8 ± 1.0 (n = 29). When cells were wounded in the presence of Go–6976 (1 μM), the increase in the intensity of d2EGFP was suppressed to 33.1 ± 3.3 (n = 24). These results indicate that CRE-mediated transcription is activated by PKC upon membrane disruption.

To investigate the effect of PKC inhibition on long-term potentiation of wound-induced exocytosis, cells were initially wounded and marked with fura-dextran. The cells were then loaded with 4 μM FM1–43 overnight. FM1–43 intercalates into the outer leaflet of lipid bilayers and is much more fluorescent in hydrophobic than in hydrophilic environments (21). When cells are incubated with the dye and later washed, dye remaining in the plasma membrane rapidly diffuses away, leaving only dye that is trapped in the endocytosed membrane. Subsequent delivery of the FM1–43 into the plasma membrane by exocytosis allows diffusion of the dye into the external medium and results in a loss of cellular fluorescence near the wound site. As shown in Fig. 6A, a previous wound increased the amount of exocytosis at a second wound when measured 24 h later. The amount of local FM1–43 destaining at the second

FIG. 4. Membrane disruption preferentially activates MEK3/6 in a PKC-dependent manner. A, immunofluorescence of phosphorylated MEK3/6. Cells were wounded by scratching in the presence of fluoro-emerald and a specific PKC inhibitor, Go–6976 (1 μM). Accumulation of phosphorylated MEK3/6 into the nuclear region is induced by the membrane disruption as indicated by increased immunostaining of nuclei with an anti-phospho-MEK3/6 antibody (arrow), whereas immunostaining is absent in nuclei of non-wounded cells. Phosphorylation of MEK3/6 upon membrane disruption is inhibited by Go–6976 (asterisk). PKC stimulation by PMA induces phosphorylation of MEK3/6. B, quantitative analysis of phospho-MEK3/6 immunostaining intensity of cell nuclei. Values are mean ± S.E. C–E, immunofluorescence of phospho-MEK4, phospho-MEK1/2, and phospho-MEK7. Cells were wounded by scratching in the presence of fluoro-emerald, fixed 30 min after wounding, and immunostained either with antibodies against phospho-MEK4 (C), phospho-MEK1/2 (D), or phospho-MEK7 (E).
wound at 24 h increased to 9.7 ± 1.2% (n = 7) from 5.1 ± 0.78% (n = 9) for initial wounds. When cells were initially wounded in the presence of PKC inhibitor Go-6976 (1 μM) or p38 MAPK inhibitor SB203580 (10 μM), the amounts for a second wound were suppressed to 4.4 ± 0.85% (n = 7) or 4.1 ± 0.73% (n = 7), respectively.

To investigate whether PKC inhibition suppresses long-term potentiation of membrane resealing at the second wound, cells were initially wounded and marked with the dye fluorescein-dextran. Then membrane resealing was monitored 24 h after the initial wound as described previously (9). Briefly, a membrane disruption (Fig. 6B, arrow) was indicated by a rise in the emission ratio of fura-2 excited at 380 and 360 nm, and the loss of the dye resulted in a decrease in the fluorescent intensity excited at 360 nm. When the cell membrane resealed, the decrease in fluorescent intensity stopped (Fig. 6B, bar). To compare the timing of membrane resealing in each condition, the resealing rate was defined as the inverse of the resealing time in seconds. For cells that failed to reseal, the rate was defined as zero. The resealing rate for a second wound increased to 0.1 ± 0.02 (n = 11) in control experiments (Fig. 6C) as reported previously (15). When cells were initially wounded in the presence of Go-6976 (1 μM) or SB203580 (10 μM), the rates for a second wound were suppressed to 0.035 ± 0.004 (n = 12) or 0.036 ± 0.004 (n = 13), respectively. In the separate dishes, when unlabeled cells were initially wounded, the resealing rate was 0.042 ± 0.004 (n = 10). These results indicate that both p38 MAPK and PKC are indeed involved in the activation of CRE-mediated transcription. A, cells were transfected with a reporter gene, pCRE-d2EGFP. Wounded cells show cytosolic labeling of TMR-dextran (left panels). In paired images, these cells are seen to have up-regulated d2EGFP expression in a control experiment (arrow), whereas PKC inhibitor Go-6976 (1 μM) suppressed up-regulation of d2EGFP expression (arrowheads). Cells were observed 3.5 h after membrane disruption. B, comparison of d2EGFP intensities of cells wounded in the presence or absence of Go-6976. Values are mean ± S.E. by guest on July 24, 2018http://www.jbc.org/Downloaded from
Disruption of plasma membrane is a common form of cell injury in many animal tissues. Cells survive this disruption by delivering vesicles to the damaged plasma membrane through exocytosis, a ubiquitous Ca\(^{2+}\)-triggered response to membrane disruption (1). It has been previously demonstrated that repeated membrane disruption reveals long-term potentiation of Ca\(^{2+}\)-regulated exocytosis in 3T3 fibroblasts, which is closely correlated with faster membrane resealing rates at repeated wounds (15). This potentiation of exocytosis and membrane resealing is PKA-dependent in the early stages (minutes), in the intermediate-term (hours) requires protein synthesis, and for the long-term (24 h) depends on gene expression that is mediated by the transcription factor CREB.

CREB activation is mediated by a variety of signaling cascades (11–14), and cell membrane disruption has been known to activate at least three protein kinases, CaMK, PKC, and PKA. CaMK is involved in wound-induced exocytosis and therefore in the membrane resealing itself (2). Both PKC and PKA are involved in short-term potentiation of membrane resealing and wound-induced exocytosis (6, 15). It has been demonstrated that inhibition of PKA during the initial wound does not affect long-term potentiation of membrane resealing, suggesting that other, PKA-independent pathways are used to activate CREB upon membrane disruption (15). The present study has identified the signaling pathway that converts the information of cell membrane disruption into CREB-dependent gene expression, i.e. PKC mediates CREB activation via a p38 MAPK pathway (Fig. 7).

Nuclear accumulation of phosphorylated MEK3/6 (the kinases responsible for p38 MAPK activation) was observed in wounded cells. Inhibition of PKC function impaired the activation of MEK3/6 upon membrane disruption, and stimulation of PKC by PMA induces the phosphorylation of MEK3/6. In contrast, other MEKs, MEK1/2, MEK4, and MEK7, were not phosphorylated upon membrane disruption. These results suggest that PKC isoforms can indeed mediate wound-induced stimulation of MEK3/6-p38 MAPK pathway. It has also been demonstrated that PKC activates p38 MAPK in other systems (23), although the mechanisms by which PKC isoforms activate p38 MAPK have not yet been established.

p38 MAPK has been reported to participate in the cellular responses to stress as well as in the control of survival of many cell types as both a positive and negative regulator (13, 19, 24, 25). The present study clearly indicates that p38 MAPK potentiates membrane resealing for long term. The phosphorylation of CREB at Ser-133 is not catalyzed directly by a MAPK family member, but other protein kinases that are activated by MAPK are involved in CREB phosphorylation (13). Although the downstream signaling of the p38 MAPK pathway leading to CREB phosphorylation upon membrane disruption remains to be demonstrated, it has been reported that mitogen- and stress-activated protein kinases MSK1 and MSK2 and MAPK-activated protein kinase-2 are activated by MAPK and then phosphorylate CREB in other systems (26–28).

Previously it has been reported that CREB-dependent long-term potentiation of membrane resealing appears 6 h after membrane disruption and that the intermediate-term (3 h) potentiation of membrane resealing and wound-induced exocytosis is not dependent on CREB (15). However, the present study shows that CREB-mediated transcription is already active 1 h after membrane disruption. One explanation for this discrepancy is that CREB-mediated transcription is already active 1 h after membrane disruption. One explanation for this discrepancy is that CREB-mediated transcription is already active 1 h after membrane disruption. One explanation for this discrepancy is that CREB-mediated transcription is already active 1 h after membrane disruption. One explanation for this discrepancy is that CREB-mediated transcription is already active 1 h after membrane disruption.

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Long-term Potentiation of Wound-induced Exocytosis and Plasma Membrane Repair Is Dependant on cAMP-response Element-mediated Transcription via a Protein Kinase C- and p38 MAPK-dependent Pathway
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