Protein Kinase C-mediated Interphase Lamin B Phosphorylation and Solubilization*

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Disassembly of the sperm nuclear envelope at fertilization is one of the earliest events in the development of the male pronucleus. We report that nuclear lamina disassembly in interphase sea urchin sperm cytosol is a result of lamin B phosphorylation mediated by protein kinase C (PKC). Lamin B of permeabilized sea urchin sperm nuclei incubated in fertilized egg G2 phase cytosolic extract is phosphorylated within 1 min of incubation and solubilized prior to sperm chromatin decondensation. Phosphorylation is Ca\(^{2+}\)-dependent. It is reversibly inhibited by the PKC-specific inhibitor chelerythrine, a PKC pseudosubstrate inhibitor peptide, and a PKC substrate peptide, but not by inhibitors of PKA, p34\(^{cdk2}\) or calmodulin kinase II. Phosphorylation is inhibited by immunodepletion of cytosolic PKC and restored by addition of purified rat brain PKC. Sperm lamin B is a substrate for rat brain PKC in vitro, resulting in lamin B solubilization. Two-dimensional phosphopeptide maps of lamin B phosphorylated by the cytosolic kinase and by purified rat PKC are virtually identical. These data suggest that PKC is the major kinase required for interphase disassembly of the sperm lamina.

The nuclear lamina consists of a polymeric network of intermediate filament molecules, the nuclear lamins, underlying the inner nuclear membrane. The lamina is a dynamic structure, undergoing expansion during interphase of the cell cycle, and depolymerization at mitosis upon breakdown of the nuclear envelope (NE)\(^{1}\) (1). Mitotic disassembly and reassembly of the lamina is regulated by reversible lamin phosphorylation and dephosphorylation (1). Interphase lamin phosphorylation has also been reported (2–6), but its significance is not fully understood.

Several lamin kinases have been identified that promote mitotic lamina solubilization or inhibit lamina assembly in vitro. They include cyclin B/p34\(^{cdk2}\) (7), S6 kinase II (8), protein kinase C (PKC) (4, 9), and the cAMP-dependent protein kinase PKA (10). Down-regulation of PKA has also been shown to be essential for mitotic lamina disassembly (11). Although not a lamin kinase, Ca\(^{2+}\)/calmodulin-dependent kinase II (CaM kinase II) is also involved in mitotic NE breakdown in sea urchin embryos (12). PKC has also been shown to phosphorylate chicken lamin B\(_{3}\) in interphase, a process thought to regulate lamin import into the nucleus (13). These observations imply that multiple kinases regulate the dynamics of the nuclear lamina during the cell cycle.

The transformation of the sea urchin sperm nucleus into a pronucleus at fertilization provides an opportunity to investigate NE assembly/disassembly during interphase. Sea urchin eggs are fertilized in G1 phase of the first cell cycle after completion of both meiotic divisions. At fertilization, the sperm NE vesiculates and a new NE reforms around the male pronucleus as the sperm chromatin decondenses (14). Male pronuclear formation has been duplicated in a cell-free system by incubating detergent-permeabilized sperm nuclei in fertilized egg extracts (15–19). Detergent-permeabilized sperm nuclei retain their lamina, which consists of a major 65-kDa B-type lamin (referred to as lamin B) and several minor uncharacterized lamin epitope-containing peptides (18). The first step of male pronuclear formation in vitro is the disassembly of the sperm nuclear lamina. The pronuclear lamina is reassembled only following formation of the nuclear membranes during nuclear swelling (19).

Interphase lamin disassembly requires ATP hydrolysis, consistent with the involvement of protein kinase(s) (18). One kinase activated at fertilization in the sea urchin is PKC. Fertilization stimulates phospholipase C in the egg plasma membrane, releasing diacylglycerol and inositol 1,4,5-trisphosphate from phosphoinositides. Increased inositol 1,4,5-trisphosphate triggers an intracellular release of Ca\(^{2+}\), which together with diacylglycerol activates PKC (20). Activated soluble PKC has been shown to translocate to the plasma membrane (21). Translocation of activated PKC to non-plasma membranes, such as the NE, has also been reported, as PKC moves to the nucleus of cultured mammalian cells upon mitogenic stimulation (9, 22, 23). A sea urchin PKC isoform (suPKC1) has been cloned (24) and several substrates proposed (21, 25). However, no lamin kinase activity has been attributed to fertilization-activated PKC.

We report here that phosphorylation of sperm nuclear lamin B precedes its solubilization, in an interphase egg cytosolic extract, and provide evidence that this phosphorylation is mediated by PKC. Lamin B phosphorylation and solubilization...
Interphase Sperm Lamin Phosphorylation and Solubilization

EXPERIMENTAL PROCEDURES

Reagents and Antibodies—1-2-Bis-(2-aminophenoxy)ethane-N,N,N',N'-tetraacetate acid (BAPTA), 6-diethylaminopurine (DMPA), staurosporine, chelerythrine, and the PKA inhibitor PKI were from Sigma. The PKC pseudosubstrate inhibitor peptide (PKC peptide-(19–31), p13 3'-beads, and purified rat brain apo PKC were from Upstate Biotechnology (Lake Placid, NY). Autocamtide-3 and the PKC substrate peptide (Ser-Cys-Cys-Phe-Leu-Cys-Glu-Lys-Thr-Ser-Leu-Glu) were from Life Technologies (Bethesda, MD). [32P]ATP was from DuPont NEN (Brussels, Belgium). The p42/p44 kinase inhibitors olomoucine and roscovitine were gifts from Dr. Laurent Meijer (20). The antibody W3-1 (a gift from Dr. Jon Holy), is a chicken polyclonal antibody raised against a fusion protein encoded by a sea urchin lamin lamin B cDNA clone (27). W3-1 was previously characterized (27), and recognizes a 65 kDa B-type lamin (p65) on Western blots of Lactichesus pect us sperm and male pronuclei (18, 19). The anti-sea urchin PKC antibody, a gift from Dr. Sheldon Wenn, was raised in rabbits against the NH2-terminal domain of L. pect us PKC (suPKC1) (24).

Sperm Nuclei and Egg Cytosolic Extracts—L. pect us sperm heads were demembranated by extraction with 0.1% Triton X-100 in nuclear bu er containing 250 mM sucrose, 250 mM potassium acetate, 0.5 mM spermidine, 0.15 mM spermine, 50 mM Hepes, pH 7.2, and demembranated nuclei were washed and resuspended in NB to 105 nuclei/ml as described previously (16). Demembranated nuclei retain their lamina, including all p65 (18), as well as two lipolytic structures, which represent detergent-resistant NE specializations at each end of the nucleus, in the centriolar and acrosomal fossa regions (16).

Mature L. pect us eggs, arrested in G2, after completion of both meiotic divisions, were fertilized and cytosolic extracts prepared 10–15 min postinsemination as described elsewhere (19). Briefly, eggs were homogenized, the lysate cleared at 10,000 g, and the supernatant centrifuged at 150,000 g for 3 h at 4 °C to yield a cytosolic extract. To be able to detect solubilized sperm lamin B, cytosols were immunodepleted of endogenous lamin B using the W3-1 antibody as described previously (16) prior to lamina disassembly reactions. Immunodepletion of PKC from the cytosol was done using a 1:25 dilution of the anti-PKC polyclonal antibody and protein A-Sepharose-bound anti-rabbit IgG antibodies.

Tryptic Phosphate Kinase Analysis of Phosphorylated Lamin B—Two-dimensional phosphopeptide mapping of lamin B phosphorylated in the presence of [γ-32P]ATP was performed as described previously (30). Phosphorylated lamin B immobilized on nitrocellulose was incubated at 25 °C overnight in 50 mM NH4HCO3 containing 100 μg/ml trypsin. Efficiency of trypsinization was determined by Cerenkov counting of the supernatant and was routinely greater than 90%. Two-dimensional phosphopeptide mapping was performed on Kodak cellulose thin layer plates. Electrophoresis was carried out in pH 1.9 buffer (formic acid/glacial acetic acid/deionized water, 50:15:65) for 18 min and rinsed in 1,000 V followed by chromatography for 3 h in isobutyric acid buffer (isobutyric acid/1-butanol/pyridine/glacial acetic acid/deionized water, 65:2.5:3.29, v/v). Tryptic phosphopeptide maps were visualized by autoradiography at ~70 °C.

RESULTS

Phosphorylation and Solubilization of Lamin B in Interphase Fertilized Egg Cytosolic Extract—A peculiarity of sea urchin eggs is that they are fertilized in interphase (G1), after completion of both meiotic divisions. Previous studies have shown that the sea urchin sperm lamina is disassembled in G1 phase cell-free extracts (18). To further document this interphase lamina disassembly, we examined the time course of sperm lamin B solubilization in fertilized egg cytosolic extract. Demembranated sperm nuclei that still contain a lamina (Fig. 1A, Input) were incubated in cytosol for 5 and 10 min, and examined by immunofluorescence using anti-lamin B antibodies. All detectable lamin labeling disappeared within 10 min, first from the lateral aspects of the chromatin, then from the nuclear poles (Fig. 1A). These data were confirmed by immunoblotting, thus eliminating antigen-masking artifacts. Concomitant with disassembly from nuclei, lamin B appeared progressively in the cytosol (Fig. 1B). Furthermore, the apparent molecular mass of sperm lamin B was shifted from 65 to 68 kDa prior to solubilization (Fig. 1B, upper panel), suggesting rapid phosphorylation. Alkaline phosphatase treatment of nuclei harboring the 68-kDa lamin restored its migration to a 65 kDa form (data not shown), confirming that the 68-kDa lamin (designated p68) is a phosphorylated form of lamin B (p65).

Phosphorylation of lamin B was directly demonstrated by rapid (1 min) 32P incorporation into sperm lamin B (Fig. 1C, upper panel), immediately followed by the release of phosphorylated lamin into the supernatant (Fig. 1C, lower panel). Identity of the 68-kDa 32P-labeled protein from lamin B was verified by immunoprecipitation of both components from the cytosol by anti-lamin B antibodies (Fig. 1D). These results show that the only phosphorylated component of the 68-kDa protein in egg cytosol is lamin B, and indicate that sperm lamin B is phosphorylated prior to being solubilized in interphase egg cytosol.

Lamin B Phosphorylation and Solubilization Are Ca2+-
dependent.
Release of intracellular Ca\textsuperscript{2+} has been shown to trigger mitotic NE breakdown in the sea urchin (31). To determine whether Ca\textsuperscript{2+} was required for lamin solubilization in interphase fertilized egg cytosol, sperm nuclei were incubated in cytosol containing 5 mM of the Ca\textsuperscript{2+} chelator BAPTA (a concentration that inhibits NE growth associated with completion of male pronuclear formation in vitro) (32) and lamin B solubilization was examined by immunofluorescence. BAPTA blocked lamin solubilization and chromatin decondensation (Fig. 2A). This inhibition was reversible since incubation of BAPTA-treated nuclei in fresh cytosol restored both processes (Fig. 2A, Wash). These results were verified by immunoblotting analysis of nuclei (data not shown). BAPTA also prevented most lamin B phosphorylation and solubilization, as judged by autoradiography of cytosolic proteins (Fig. 2B), indicating that both processes are Ca\textsuperscript{2+}-dependent.

**Lamin B Phosphorylation Is Inhibited by the PKC-specific Inhibitor Chelerythrine and by Immunodepletion of Cytosolic PKC**—Several lamin kinases have been identified, including p34\textsuperscript{CDC2}, PKC, and PKA (7, 9, 10, 33). In an attempt to determine which kinase(s) mediates interphase sperm lamin B phosphorylation, sperm nuclei were incubated in egg cytosol containing increasing concentrations of the following kinase inhibitors: the nonspecific kinase inhibitors DMAP and staurosporine, the p34\textsuperscript{CDC2}-specific inhibitors olomoucine and roscovitine, PKI, or the PKC-specific inhibitor chelerythrine (34). Nuclear proteins were immunoblotted using anti-lamin B antibodies and amounts of lamin B quantified by densitometry. DMAP and staurosporine inhibited lamin B solubilization with an IC\textsubscript{50} of 1.5 mM and 12 \mu M, respectively, whereas olomoucine, roscovitine, or PKI were ineffective (Fig. 3A). The most effective inhibitor of lamin B solubilization was the PKC-specific inhibitor chelerythrine (IC\textsubscript{50} 0.17 \mu M; Fig. 3A). As observed with Ca\textsuperscript{2+} chelation, these inhibitions were reversible. Immunofluorescence observations verified these results and showed a parallel between prevention of lamin solubilization by kinase inhibition and prevention of chromatin decondensation (Fig. 3B).

To determine whether the effects of these inhibitors on lamin solubilization were due to inhibition of lamin phosphorylation, sperm nuclei were added to cytosol containing [\gamma\textsuperscript{32}P]ATP and kinase inhibitors. The presence of phosphorylated, solubilized lamin B in the cytosol was determined by autoradiography. As shown in Fig. 3C, lamin B phosphorylation was inhibited by DMAP (2 mM), staurosporine (100 \mu M), and chelerythrine (10 \mu M), but was insensitive to olomoucine, roscovitine, or PKI. These results suggest a role of PKC in sperm lamin B phosphorylation resulting in solubilization in interphase egg cytosol.

To investigate further the involvement of PKC in interphase sperm lamin B phosphorylation and solubilization, nuclei were added to cytosol preincubated with 50 \mu M of either a highly selective PKC inhibitor peptide or a PKC substrate peptide, in the presence of [\gamma\textsuperscript{32}P]ATP. Lamin B phosphorylation was examined by autoradiography of cytosolic proteins, and relative amounts of phosphorylated and solubilized lamin B were determined by densitometry of duplicate autoradiograms. Both peptides prevented lamin B phosphorylation and solubilization (Fig. 4A). These inhibitor concentrations were the minimal concentrations completely abolishing lamin B phosphorylation (data not shown) (30). In contrast, preincubation of cytosol with p13\textsuperscript{autoact—agarose beads (0.25 \mu g/\mu l cytosol), which specifically bind p34\textsuperscript{CDC2}, or 50 \mu M autacamide 3, a CaM kinase II-specific substrate peptide, did not inhibit cytosolic lamin kinase activity (Fig. 4A). Together with previous data, these results suggest a role for PKC in phosphorylating and solubilizing sperm lamin B in interphase cytosol.

To determine if PKC was the only enzyme responsible for interphase sperm lamin B phosphorylation, cytosol was immunodepleted of endogenous PKC using a 1:25 dilution of a polyclonal antibody against the NH\textsubscript{2}-terminal end of suPKC1 (24) (Fig. 4B). This antibody reacted with a 71-kDa protein on immunoblots of egg cytosol (Fig. 4B, arrow), and occasionally with 52- and 84-kDa uncharacterized proteins in some cytosol preparations (not shown) (24). Lamin B phosphorylation in PKC- or mock-depleted cytosol showed that lamin B kinase activity was abolished by ~90% by immunodepletion of PKC from the cytosol (as determined by densitometry of the autoradiogram shown in Fig. 4C). When examined by immunofluorescence using anti-lamin B antibodies, nuclei incubated in PKC-depleted cytosol exhibited peripheral lamin labeling similar to that of input nuclei, while the chromatin remained condensed (data not shown). Subsequent addition of purified rat brain a\beta PKC (100 \mu g/\mu l) to PKC-depleted cytosol restored lamin B phosphorylation and solubilization (Fig. 4C), and promoted chromatin decondensation. These results argue that sperm lamin B phosphorylation and solubilization in interphase cytosol are mediated by PKC.

**Purified Mammalian PKC and Egg Cytosolic Kinase Phosphorylate Lamin B on Identical Sites**—Sea urchin lamin B contains several consensus PKC phosphorylation sites ((S/T)-(K/R); see Holy et al. (27) for Strongylocentrotus purpuratus and Lytechinus variegatus lamin sequences). To determine whether sperm lamin B is a substrate for phosphorylation by PKC, nuclei were incubated in PKC phosphorylation medium containing purified rat a\beta PKC or human \beta\textsubscript{II} PKC (33), and the reaction supernatant analyzed by autoradiography for the
presence of phosphorylated and solubilized lamin B. Lamin B was phosphorylated and solubilized by both kinases (Fig. 5A). Phosphorylation did not occur in the absence of PKC (indicating the absence of endogenous nucleus-associated lamin kinase activity) or in the presence of the PKC pseudosubstrate inhibitor peptide (50 μM), the PKC substrate peptide (50 μM), or the PKC-specific inhibitor chelerythrine (100 μM) (Fig. 5A). These results indicate that sperm lamin B is a substrate for purified PKC. Immunofluorescence analysis of nuclei incubated with rat αβγ PKC (Fig. 5B) or human βII PKC (not shown) showed that all detectable lamin B had disassembled from nuclei. However, although lamins were solubilized, the chromatin remained condensed (Fig. 5B), indicating that disassembly of the sperm nuclear lamina was not sufficient to promote chromatin decondensation in the absence of cytosol.

Final characterization of the interphase cytosolic lamin kinase was carried out by comparing the lamin B phosphorylation sites of the cytosolic kinase and of purified rat αβγ PKC. Tryptic digests of lamin B phosphorylated by both kinase preparations were subjected to two-dimensional thin layer chroma-
tography and autoradiography. Lamin B phosphorylated by the
cytosolic kinase generated 13 phosphopeptides that migrated 
with a pattern similar to 13 out of 14 phosphopeptides pro-
duced by purified αβγ PKC (Fig. 6, compare peptides 1–13 in 
left and middle panels). The identity of these 13 phosphopep-
tides was ascertained by their comigration when both tryptic 
digests were run on the same chromatogram (Fig. 6, Mix). As 
expected from our previous data, no phosphopeptides were 
detected when PKC was omitted from the phosphorylation 
reaction (data not shown). These results indicate that the cy-
tosolic interphase lamin kinase accounting for the lamin phosphopeptides detected is PKC.

**DISCUSSION**

PKC-mediated Interphase Lamin Phosphorylation and Solu-
bilization—We report in this study that sea urchin sperm nu-
clear lamina disassembly in interphase egg cytosol is a result of 
lamin B phosphorylation mediated by PKC. The following 
evidence supports our conclusions: (i) sperm lamin B phospho-
rylation is Ca^{2+}-dependent; (ii) phosphorylation is inhibited by 
the PKC-specific inhibitor chelerythrine, but not by inhibitors 
of PKA, p34cdc2 or CaM kinase II; (iii) lamin B phosphorylation 
is also inhibited by highly specific PKC inhibitors of different 
compositions, specificities and modes of action, such as a PKC 
inhibitor peptide and a PKC substrate peptide; (iv) sperm lamin B can be phosphorylated and solubilized by purified 
mammalian PKC in vitro; and (vi) finally, two-dimensional phosphopeptide maps of lamin B phosphorylated by the inter-
phase cytosolic kinase and by purified mammalian PKC are 
virtually identical.

The identity of the 13 phosphopeptides of lamin B phospho-
ylated in interphase egg cytosol and by purified PKC argues 
that PKC is the only kinase required for phosphorylation and 
solubilization of sperm lamin B. Lamin phosphorylation and 
solubilization by PKC alone is unprecedented, as mitotic lamin 
solubilization in somatic cells appears to be elicited by multiple 
kineses (35). Although only one sea urchin PKC isozyme has 
been cloned (suPKC1) (24), several PKC isoforms may exist and 
may phosphorylate lamin B. Nonetheless, inhibition of lamin B 
phosphorylation in cytosol immunodepleted of PKC using an 
antibody against suPKC1 suggests that interphase sperm 
lamin B phosphorylation is elicited by a single PKC isozyme.

Nuclear Translocation and Activation of PKC—Sea urchin 
eggs are fertilized at the pronuclear stage, so the female pro-
nucleus is fully formed and remains intact as the sperm NE 
successively disassembles and reassembles to form the male 
pronuclear envelope. Thus an unresolved issue is how the in-
tegrity of the female pronucleus is maintained during sperm NE disassembly. One possibility is that the female pronucleus 
contains a different set of lamins that would not be a substrate 
for PKC (36). This is suggested by the lack of reactivity of 
female pronuclei by immunofluorescence and immunoblotting 
using several anti-lamin antibodies (27), whereas sperm nuclei 
are highly reactive (18). Alternatively, the female pronucleus 
can contain the same lamins, but with different covalent mod-
fications or specific lamin-associated proteins that might affect 
phosphorylation and solubilization by PKC. A third possibility 
may be the lack of translocation of PKC to the female pronu-
Sperm histones SpH1 and SpH2B are phosphorylated during decondensation, which is the phosphorylation of sperm histones. This process is critical for chromatin decondensation and takes place in eggs treated with DMAP, indicating that the sperm histone kinase is DMAP-insensitive (41). Sperm histones SpH1 and SpH2B are phosphorylated in vivo within 3 min of fertilization (43), as well as in vitro, albeit at a slower rate (29). Interestingly, the conversion of Sp histones to phosphorylated forms takes place in eggs treated with DMAP, indicating that the sperm histone kinase is DMAP-insensitive (41). The sperm histone kinase is thus likely to be distinct from the mitotic histone kinase p34cdc2 (44) and from PKC, which are both inhibited by DMAP (45) (this study). Chromatin decondensation therefore appears as a multistep process involving PKC for lamin phosphorylation and lamin disassembly and an as yet unidentified DMAP-insensitive kinase for histone phosphorylation.

**REFERENCES**

1. Gerace, L., and Blobel, G. (1980) Cell 19, 277–287

2. Ottaviano, Y., and Gerace, L. (1985) J. Biol. Chem. 260, 624–632

3. Smith, D. E., Gruenbaum, Y., Berrios, M., and Fisher, P. A. (1987) J. Cell Biol. 105, 771–780

4. Hornbeck, P., Huang, K. P., and Paul, W. E. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 2279–2283

5. Stuurman, N., Maus, N., and Fisher, P. A. (1995) J. Cell Sci. 108, 3137–3144

6. Kill, I. R., and Hutchinson, C. J. (1995) FEBS Lett. 377, 36–30

7. Peter, M., Nakagawa, J., Derrée, M., Labbé, J.-C., and Nigg, E. A. (1990) Cell 61, 591–602

8. Ward, G. E., and Kirschner, M. W. (1990) Cell 61, 561–577

9. Fields, A. P., Petit, G. R., and May, W. S. (1988) J. Biol. Chem. 263, 8253–8260

10. Stuurman, N. (1997) FEBS Lett. 401, 171–174

11. Lamb, N. J. C., Cavadore, J.-C., Labbé, J.-C., Mauer, R. A., and Fernandez, A. (1991) EMBO J. 10, 1525–1533

12. Baitinger, C., Alderton, J., Poccia, J.-C., Labbé, J.-C., Mauer, R. A., and Fernandez, A. (1991) EMBO J. 10, 1525–1533

13. Hennet, H., Peter, M., and Nigg, E. A. (1993) J. Cell Biol. 120, 1293–1304

14. Longo, F. J., and Anderson, E. (1968) J. Cell Biol. 39, 315–368

15. Cameron, L. A., and Poccia, D. L. (1991) Dev. Biol. 142, 58–68

16. Collas, P., and Poccia, D. L. (1994) Exp. Cell Res. 219, 607–608

17. Collas, P., Pinto-Correia, C., and Poccia, D. L. (1995) Cell 61, 1715–1725

18. Fields, A. P., Petit, G. R., and May, W. S. (1988) J. Biol. Chem. 263, 8253–8260

19. Fields, A. P. (1994) J. Biol. Chem. 269, 464–478

20. Poccia, D. L., and Green, G. R. (1992) Trends Biochem. Sci. 17, 225–227

21. Green, G. R., Collas, P., Burrell, A., and Poccia, D. L. (1995) Semin. Cell Biol. 6, 219–227

22. Hocevar, B. A., Burns, D. J., and Fields, A. P. (1993) J. Biol. Chem. 268, 7545–7552

23. Steinhardt, R. A., and Alderton, J. (1988) Nature 332, 364–366

24. Collas, P., and Poccia, D. L. (1995) Mol. Reprod. Dev. 42, 106–113

25. Goos, V. L., Hocevar, B. A., Thompson, L. J., Strutton, C. A., Burns, D. J., and Fields, A. P. (1994) J. Biol. Chem. 269, 19074–19080

26. Herbert, J. M., Augereau, J. M., Gleye, J., and Mafrad, J. P. (1999) Biochem. Biophys. Res. Commun. 172, 993–999

* FIG. 6. Interphase sperm lamin B phosphorylation in egg cytosol is mediated by PKC. Autoradiogram showing two-dimensional phosphopeptide mapping of 32P-labeled sperm lamin B phosphorylated by egg lamin kinase (left panel) and by purified rat brain PKC (middle panel). Running both samples on the same chromatogram (Mix) reveals that the 13 lamin B phosphopeptides generated by the egg lamin kinase and by purified PKC are identical. Cathode is on the left and anode on the right. ○ refers to the site where samples were applied.

* P. Collas, manuscript in preparation.
35. Fields, A. P., and Thompson, L. J. (1995) in Progress in Cell Cycle Research (Meijer, L., Guidet, S., and Tung, H. Y. L., eds) Vol. 1, pp. 271–286, Plenum Press, New York
36. Schatten, G., Maul, G. G., Schatten, H., Chaly, N., Simerly, C., Balecon, R., and Brown, D. L. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 4727–4731
37. Terasaki, M., and Jaffe, L. A. (1991) J. Cell Biol. 114, 929–940
38. Murray, N. R., Burns, D. J., and Fields, A. P. (1994) J. Biol. Chem. 269, 21385–21390
39. Dicheva, N., Banfic, H., and Irvine, R. F. (1993) Cell 74, 405–407
40. Banfic, H., Zizak, M., Dicheva, N., and Irvine, R. F. (1993) Biochem. J. 290, 633–636
41. Poccia, D. L., Pavan, W., and Green, G. R. (1990) Exp. Cell Res. 188, 226–234
42. Cothren, C. C., and Poccia, D. L. (1993) Exp. Cell Res. 205, 126–133
43. Green, G. R., and Poccia, D. L. (1985) Dev. Biol. 106, 235–245
44. Arum, D., Meijer, L., Britzuela, L., and Beach, D. (1986) Cell 55, 371–378
45. Meijer, L., and Pondaven, P. (1988) Exp. Cell Res. 174, 116–129