Regulation of Binding of Lamin B Receptor to Chromatin by SR Protein Kinase and cdc2 Kinase in Xenopus Egg Extracts*

Makoto Takano‡, Yuhei Koyama§, Hiromi Ito, Satomi Hoshino, Hiroshi Onoghi, Masatoshi Hagiwara*, Kazuhiro Furukawa†, and Tsuneyoshi Horigome‡

Department of Chemistry, Faculty of Science, Niigata University, Igarashi-2, Niigata 950-2181, Japan

Received for publication, August 11, 2003, and in revised form, December 19, 2003

Published, JBC Papers in Press, January 12, 2004, DOI 10.1074/jbc.M308854200

Participation of multiple kinases in regulation of the binding of lamin B receptor (LBR) to chromatin was suggested previously (Takano, M., Takeuchi, M., Ito, H., Furukawa, K., Sugimoto, K., Omata, S., and Horigome, T. (2002) Eur. J. Biochem. 299, 943–953). To identify these kinases, regulation of the binding of the nucleoplasmic region (NK, amino acid residues 1–211) of LBR to sperm chromatin was studied using a cell cycle-dependent Xenopus egg extract in vitro. The binding was stimulated on specific phosphorylation of the NK fragment by an S-phase egg extract. Protein depletion with beads bearing SF2/ASF, which binds SR protein kinases, abolished this stimulation, suggesting that an SR protein kinase(s) is responsible for the activation of LBR. This was confirmed by direct phosphorylation and activation with recombinant SR protein-specific kinase 1. The binding of the NK fragment to chromatin treated with an S-phase extract was suppressed by incubation with an M-phase extract. Enzyme inhibitor experiments revealed that multiple kinases participate in the suppression. One of these kinases was shown to be cdc2 kinase using a specific inhibitor, roscovitine, and protein depletion with beads bearing p13, which specifically binds cdc2 kinase. Experiments involving a mutant NK fragment showed that the phosphorylation of serine 71 by cdc2 kinase is responsible for the suppression.

The nuclear envelope separates the nucleoplasm from the cytoplasm and thereby organizes the nuclear architecture. The nuclear envelope consists of two lipid bilayers (inner and outer), nuclear pore complexes, and the nuclear lamina and undergoes repeated dynamic assembly and disassembly in every cell cycle in higher eukaryotes. Studies on the molecular mechanisms underlying nuclear envelope assembly and disassembly are important to understand the mechanisms underlying cell division and prenucleus formation. Studies on the organization of the nuclear architecture by the nuclear envelope during the cell cycle are also important to understand how mutants of nuclear envelope proteins, i.e. lamin A/C (1), emerin (2), and lamin B receptor (LBR) (3), cause genetic diseases (4).

The assembly and disassembly of the nuclear envelope in the cell cycle accompany association and dissociation of the inner nuclear membrane with and from chromatin, respectively. All major inner nuclear membrane proteins, such as the lamina-associated polypeptide 2 (LAP2) family, LBR, and emerin, are known to bind to chromatin in vitro (5, 6) and to accumulate on the surface of chromatin during the anaphase-telophase transition in vivo (7). Therefore, the binding of these proteins to chromatin is thought to support the binding of the inner nuclear membrane to chromatin. Indeed, LBR and LAP2 have been demonstrated to play functional roles in the interaction of the nuclear envelope and chromatin through in vitro experiments as follows. Depletion of LBR from nuclear envelope precursor vesicles suppresses nuclear assembly (8), neutralization of LBR by antibodies in a sea urchin cell-free system inhibits targeting of nuclear envelope precursor vesicles to the surface of chromatin (9), and the addition of an amino-terminal fragment of LAP2 inhibits nuclear envelope formation (10).

The binding of these proteins to chromatin has to be regulated precisely throughout the cell cycle. The binding of LAP2 to chromatin was shown to be regulated by phosphorylation using HeLa cell extracts (5). LAP2β is phosphorylated by protein kinase C (PKC) (11). We demonstrated that the binding of LBR to chromatin is regulated by phosphorylation of LBR in the nucleoplasmic region by cell cycle-specific Xenopus egg cytosol fractions (6). It is also known that LBR is phosphorylated by cdc2 kinase (12, 13), SR protein-specific kinase (SRPK) (14), protein kinase A (12), calmodulin-dependent protein kinase II (12), and casein kinase II (12). However, in the cases of all inner nuclear membrane proteins, the protein kinase responsible for the regulation of the binding to chromatin has not yet been identified.

In this study, we analyzed kinases responsible for regulation of the binding of LBR to chromatin using cell cycle-specific Xenopus egg cytosol fractions. It was shown that the stimulation of the binding of LBR to chromatin by an S-phase cytosol fraction (SC) was caused by phosphorylation by SRPK. On the other hand, suppression of the binding of LBR to chromatin by

* This work was supported by a grant from the Biodiseg research Project and a grant for Project Research from Niigata University. 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.

† To whom correspondence should be addressed: Dept. of Chemistry, Faculty of Science, Niigata University, Igarashi-2, Niigata 950-2181, Japan. Tel.: 81-25-262-6160; Fax: 81-25-262-6160; E-mail: thori@chem.sc.niigata-u.ac.jp.
an M-phase cytosol fraction (MC) was caused by multiple kinases, one of which was identified as cdc2 kinase. Based on these findings and others, regulation of the binding of LBR to chromatin is discussed.

**EXPERIMENTAL PROCEDURES**

**Materials**—Okadaic acid and a phosphatase inhibitor, I-2, were purchased from Wako (Osaka, Japan) and Biomol Research Laboratories Inc. (Plymouth Meeting, PA), respectively. Protein kinase inhibitors, i.e. A3, K252b, and calphostin C, were obtained from Calbiochem-Novabiochem. A protein kinase A inhibitor (P0300), apyrase, staurosporine, 6-dimethylaminopurine (DMAP), and DNA-cellulose (double-stranded) were from Sigma. cdc2 kinase affinity-purified from a Xenopus egg extract using p13 resin (15) was purchased from Calbiochem-Novabiochem. PKC, trypsin, and glutathione-Sepharose 4B were from Molecular Probes (Eugene, OR), Promega Co. (Madison, WI), and Amersham Biosciences, respectively.

**Buffer**—The extraction buffer comprised 50 mM Hepes-KOH (pH 7.7), 250 mM sucrose, 50 mM KCl, and 2.5 mM MgCl2; and the PP1 reaction buffer comprised 50 mM Tris-HCl (pH 7.0), 0.1 mM EGTA, 0.5 mM dithiothreitol, and 1 mg/ml bovine serum albumin (BSA).

**Preparation of Demembranated Xenopus Sperm Chromatin and Xenopus Egg Cytosol Fractions**—S-phase and M-phase Xenopus egg cytosol fractions and demembranated Xenopus sperm chromatin were prepared as described previously (6).

**Chromatin Binding Assay**—The nucleoplasmic region of human LBR (NK, amino acid residues 1-211) and the arginine-serine-repeat-containing region within NK (RS, amino acid residues 53-89) were expressed in *Escherichia coli* as glutathione S-transferase (GST) fusion proteins and then bound to glutathione-Sepharose beads as described previously (6). In some experiments, a mutant GST-NK, GST-NK(S71A), was used. In this mutant, serine 71 is substituted by alanine. Using the thus prepared beads, the chromatin binding assay was carried out as described previously (6). Briefly, Xenopus sperm chromatin, which was demembranated with lysolecithin and subsequently decondensed with heated Xenopus egg cytosol (6), in 20 μl of extraction buffer (750 mM salt) was added to 4 μg of GST-fused protein-bound to 2 μl of glutathione-Sepharose 4B beads suspended in 10 μl of extraction buffer. After incubation at 4 °C for 10 min, the binding reaction was stopped by pipetting 15-μl samples onto glass slides spotted with 12 μl of a fixing solution (3% formaldehyde, 6 μg/ml Hoescht dye 33342, 50 mM KC1, 15 mM NaCl, 50% glycerol, and 15 mM Pipes, pH 7.2). The fixed samples were observed by phase-contrast and fluorescence microscopy.

**Binding Assay of LBR Fragments to DNA-cellulose**—The purified GST and GST-fused LBR fragments (2 μg of proteins) were incubated with 30 μl of a 10% suspension of double-stranded DNA-cellulose in 500 μl of DNA-binding buffer (20 mM Hepes-KOH (pH 7.6), 0.2 mM KC1, 0.5 mM EDTA, 0.1% Triton X-100, 0.1 mM phenylmethanesulfonyl fluoride, 1 μg/ml aprotinin, 20% glycerol, and 1 mg/ml BSA, at 4 °C for 6 h with rotation. Then, the DNA-cellulose was washed three times with the binding buffer and once with the binding buffer without BSA. LBR fragments bound to the cellulose were eluted with a 2% SDS solution, separated by SDS-PAGE, and visualized by staining with Coomassie Brilliant Blue and with polyclonal antibodies against GST as described previously (17).

**Statistical Analysis**—The significance of the pretreatment of beads bearing LBR fragments with various reagents in the chromatin binding assay was evaluated by means of Student's t test (n = 3).

**RESULTS**

We showed previously that the binding of GST-NK to chromatin is stimulated by phosphorylation of GST-NK in the RS region by a Xenopus egg S-phase cytosol fraction (Fig. 1) (6). To identify the kinase responsible for this stimulation, we first examined protein kinase A and showed that it could cause the stimulation (6). However, an inhibitor specific for the kinase could not suppress the stimulation by the cytosol (6). Then we examined cdc2 kinase, calmodulin-dependent kinase II, and casein kinase II, which have been shown to phosphorylate LBR (12). However, they did not stimulate the binding (data not shown). It has been reported that a nuclear envelope-associated protein kinase phosphatase LBR (18) and that its substrate specificity is identical to that of SR protein-specific kinase I (12). Then, we treated GST-NK (amino acid residues 1-211 of LBR) with SRPK1 and found that the enzyme clearly stimulates the binding of GST-NK to chromatin (Fig. 1, column 6). Stimulation of the binding by pretreatment with SC and SRPK1 was similarly inhibited by kinase inhibitors having broad specificities such as A3, K202b (Fig. 1), and staurosporine (data not shown). The 50% inhibition value for both stimulating kinases, i.e., the kinase in SC and SRPK1, with A3 was 0.1 μM (data not shown). These results suggested that the kinase responsible for the stimulation of the binding of GST-NK to chromatin in SC is SRPK. When GST-RS (amino acid residues 53-89 of LBR) was used instead of GST-NK to narrow the phosphorylation site, similar stimulation on treatment with SC and SRPK1 was observed (Fig. 1, columns 10-13). These results suggested that the stimulation is caused by phosphorylation of residues in the RS region. To verify the
participation of SRPK in SC in the stimulation, affinity-depletion experiments on SRPK were carried out. When SC was affinity-depleted of SRPK using SF2/ASF beads, the SC lost the ability to stimulate the binding of GST-NK to chromatin (Fig. 2, column 5). Subsequent addition of purified SRPK1 to the SRPK-depleted SC restored its ability to stimulate the binding (Fig. 2, column 6). These data clearly indicate that the stimulation of the binding of GST-NK to chromatin is caused by SRPK in SC. The phosphorylation sites of GST-NK treated with SC, SC depleted of SRPK, and SRPK1 itself were compared by phosphopeptide mapping. As can be seen in Fig. 3, the two maps obtained for GST-NK treated with SC and SRPK1 were very similar to each other. Moreover, all spots except spot 1 disappeared on depletion of SRPK from SC (Fig. 3, Dep.SC). A part of the complex patterns of the phosphopeptide maps of SC and SRPK was possibly caused by alternative phosphorylation of serines within RS motifs (arginine-serine repeats), as suggested previously for chicken LBR (Fig. 3) (13, 18). Incomplete digestion by trypsin may also have been involved. GST itself was not phosphorylated at all (data not shown). Thus, we concluded that SRPK in SC phosphorylates GST-NK and stimulates the binding to chromatin.

As the next step, we examined the change in the binding affinity of LBR to chromatin on the transition from S-phase to M-phase. As can be seen in Fig. 4, the stimulated binding of GST-NK to chromatin on treatment with SC was suppressed by subsequent treatment with MC (compare columns 2 and 3 in Fig. 4). We suggested previously that the suppression is caused by phosphorylation (6). Protein phosphatase inhibitor okadaic acid had no effect on the suppression (data not shown). To clarify the kinase(s) participating in the suppression, we examined various kinase inhibitors. The kinase(s) responsible for the suppression was sensitive to inhibitors with broad specificity, i.e. staurosporine and DMAP (Fig. 4, columns 4 and 5). The 50% inhibition value for the suppression by staurosporine was 5 ± 2 nM (n = 3, data not shown). Complete depletion of ATP from the reaction mixture by pretreatment with apyrase also inhibited the suppression (data not shown). When specific inhibitors of cdc2 kinase, protein kinase A, and PKC were examined, a cdc2 kinase inhibitor, roscovitine, showed partial inhibition of the suppression, although the other inhibitors had no effect (Fig. 4, columns 6–8). An inhibitor of calmodulin-dependent protein kinase II, i.e. EGTA, had no effect on the suppression (data not shown). These results suggested that multiple inhibitors have a role in the suppression of the binding of GST-NK, although the precise role of each kinase is not clear.
kinases including cdc2 kinase participate in the suppression. Then, to further clarify the participation of cdc2 kinase in the suppression, a mutant GST-NK, GST-NK(S71A), of which the serine 71 kinase is responsible for a part of the suppression of the binding of GST-NK to chromatin (compare columns 11 and 12 in Fig. 4). These results supported the idea that phosphorylation of serine 71 by cdc2 kinase participates in the suppression of the binding of GST-NK to chromatin. To verify the participation of cdc2 kinase, affinity-depletion experiments on cdc2 kinase were carried out. As can be seen in Fig. 5, when MC was affinity-depleted of cdc2 kinase using p13 beads, the SC completely restored its ability to suppress the binding of GST-NK to chromatin (compare columns 3 and 6 in Fig. 5A). Subsequent addition of authentic cdc2 kinase to the cdc2 kinase-depleted MC restored its ability to suppress the binding (Fig. 5A, column 7). These results clearly indicate that cdc2 kinase is responsible for a part of the suppression of the binding of GST-NK to chromatin.

Phosphopeptide mapping was carried out to compare the phosphorylation sites of GST-NK after treatment with SC, MC, SC followed by MC, cdc2 kinase, and PKC (Fig. 6). When GST-NK was treated with cdc2 kinase (Fig. 6, cdc2), two major spots (c and e) and three minor ones (a, b, and d) were observed. On the other hand, when GST-NK(S71A) was phosphorylated with cdc2 kinase (Fig. 6, cdc2/S71A), the two major spots observed for cdc2 disappeared. Therefore, the two major spots that disappeared should represent tryptic peptides containing serine 71, and the serine should be phosphorylated. Based on the amino acid sequence of LBR, the relative positions of spots c and e (Fig. 6), and the substrate specificity of trypsin, spots c and e could be considered to be peptides phosphorylated at serine 71 comprising amino acid residues 65–75, GGSTSSSSPSRR, and residues 65–74, GGSTSSSSPS, of LBR, respectively. Furthermore, when autoradiography films of cdc2 and MC (Fig. 6) obtained on the same day were superposed, spots a, b, c, d, and e completely coincided with spots 1, 3, 4, 6, and 8, respectively (data not shown). Therefore, we concluded that these matching spots contain the same phosphopeptides. The phosphopeptide map of GST-NK treated with PKC (Fig. 6, PKC) showed a single spot, which overlapped spot 6 in Fig. 6 (MC). Phosphopeptide maps obtained for GST-NK treated with MC (Fig. 6, MC) and with SC and subsequently MC (SC-MC) were very similar to each other, although the patterns were very complicated. Spot 7 (Fig. 6) appeared irregular. When the patterns of SC-MC and SC (Fig. 6) were compared, spots 4, 6, 8, 10, 11, and 12 seemed to be relatively enhanced by the treatment with MC. For SC-MC (Fig. 6), spots 4 and 8, which correspond to spots c and e for cdc2, indeed incorporated a little more 32P than in the case of SC. Moreover, when MC and SC-MC were compared with SC (Fig. 6), MC and SC-MC treatments were seen to clearly stimulate 32P incorporation into spots 12 and 10. When GST-NK was treated with MC in the presence of a specific inhibitor of cdc2 kinase (Fig. 6, MC+Rosco.), spots 12 and 10 disappeared. Moreover, when GST-NK(S71A) was treated with MC (Fig. 6, MC/S71A), spot 12 disappeared, and 32P incorporation into spot 10 decreased to the level in SC. These results suggested that the peptides corresponding to spots 12 and 10 (Fig. 6) contained serine 71 and were phosphorylated by cdc2 kinase in MC. The differences in the positions of major phosphopeptides observed on treat-
ment with authentic cdc2 kinase, i.e. spots c and e for cdc2 (Fig. 6), compared with those obtained with cdc2 kinase on MC treatment, i.e. spots 12 and 10 for MC and SC-MC, could be explained as follows. The peptides in spots c and e (Fig. 6), which contained phosphorylated serine 71, incorporated a second phosphate group, and thus these peptides shifted to spots 12 and 10, respectively.

From the results of the above experiments involving protein kinase inhibitors and mutant GST-NK, it was suggested that SRPK in SC and cdc2 kinase in MC participate in the stimulation and suppression of the binding of GST-NK to chromatin, respectively. Then we verified these results by reconstitution of the regulation system with the purified enzymes (Fig. 7). When GST-NK was treated with purified SRPK1 instead of SC, a similar increase in the chromatin binding was observed (compare columns 2 and 3 in Fig. 7). On the other hand, when GST-NK pretreated with SC and SRPK1 was subsequently treated with purified cdc2 kinase, a similar decrease in the chromatin binding was observed (compare columns 5 and 6 in Fig. 7). These results clearly show that the stimulation and a part of the suppression of the binding of GST-NK to chromatin by SC and SC-MC can be reproduced with purified SRPK1 and cdc2 kinase, respectively.

We showed previously that LBR binds to the DNA moiety of chromatin. Then, we examined whether the binding of LBR to DNA is regulated in a cell cycle-dependent manner or not by the DNA-cellulose method (Fig. 8). Pretreatment of GST-NK with SC had little effect on the binding to DNA-cellulose (compare columns 1 and 2 in Fig. 8). Pretreatment of GST-NK with SC-MC treatment, as in the case of chromatin binding (compare Fig. 8, A3 and A4). The results obtained in experiments involving a cdc2 kinase inhibitor, i.e. roscovitine, suggested that cdc2 kinase is responsible for a part of the

FIG. 6. Tryptic phosphopeptide mapping of LBR fragments treated with SC, MC, SC-MC, cdc2 kinase, and PKC. Beads bearing 20 μg of GST-NK were incubated with 20 μl of SC (SC), MC (MC), 1 mm roscovitine (MC+Rosco), 4 units of cdc2 kinase (cdc2), or 0.03 μg of PKC in extraction buffer (PKC) supplemented with 2 μl of 3.3 μCi [γ-32P]ATP (110 TBg/mmole) at 23 °C for 1 h. Beads bearing 20 μg of GST-NK were subsequently treated with 25 μl of extraction buffer (1) and SC (2–9) at 23 °C for 1 h. Beads bearing 20 μg of GST-NK (cdc2/S71A) were treated in the presence of radioactive ATP and subsequently treated with MC in the presence of radioactive ATP as above. The thus prepared samples were subjected to tryptic phosphopeptide mapping as in Fig. 3. Spots a, b, c, d, and e in cdc2 correspond to spots 1, 3, 4, 6, and 8 in MC, respectively.

FIG. 7. Regulation of the binding of GST-NK to chromatin by purified kinases. GST-NK beads were pretreated with 25 μl of extraction buffer (1), SC (2, 4, and 5), and 0.025 μg of SRPK1-containing extraction buffer (3 and 6) in the presence of an ATP-regenerating system at 23 °C for 1 h. After washing, these beads were treated with extraction buffer (1), SC (2), 0.025 μg of SRPK1 (3), MC (4), and 4 units of cdc2 kinase (5 and 6) under the same conditions. Then the binding to chromatin was examined as in Fig. 1. Each column shows the mean ± S.D. (vertical bar) for three experiments. * indicates a significant difference from the respective control (p < 0.05).

FIG. 8. Binding of LBR fragments to DNA-cellulose. Beads bearing LBR fragments GST-NK (A), GST-RS (B), and GST-NK (S71A) (C), or control GST (D) were treated with extraction buffer (1) and SC (2–9) at 23 °C for 1 h. The beads were subsequently treated with MC (4), MC + 14 units/ml apyrase (Apy.) (5), MC + 10 μM staurosporine (Sta.) (6), MC + 1 mm roscovitine (Rosco.) (7), MC + 15 μM calphostin C (Cal.C) (8), and MC + 15 μM calphostin C + 1 mm roscovitine (9) as above. The thus treated LBR fragments were eluted from the beads with glutathione and then incubated with DNA-cellulose. LBR fragments bound to the DNA-cellulose were eluted, electrophoresed, transferred to a nitro-cellulose membrane, and detected with anti-GST antibodies. For other details, see “Experimental Procedures.”
suppression of the binding of GST-NK to DNA-cellulose (Fig. 8, A5–A9). Calphostin C, a PKC inhibitor, had no effect on the binding (compare Fig. 8, A4 and A8). Similar results were obtained using GST-RS (amino acid residues 53–89) instead of GST-NK (amino acid residues 1–211) (compare Fig. 8, A and B).

These results suggested that the binding of NK to DNA through the RS region was regulated by MC treatment. When GST-NK(S71A) was used instead of wild-type GST-NK, SC-MC treatment did not cause complete suppression of the binding to DNA-cellulose (Fig. 8, C4). All these results are consistent with the idea that the phosphorylation of serine 71 by cdc2 kinase in MC is responsible for a part of the suppression of the binding of GST-NK to DNA-cellulose.

DISCUSSION

It was suggested in this study that SRPK in SC is responsible for the stimulation of the binding of GST-NK to chromatin, as judged with affinity depletion of SRPK from SC (Fig. 2), the addition of authentic SRPK to the SRPK-depleted SC (Fig. 2), and kinase inhibitors (Fig. 1). A specific inhibitor of SRPK also suppressed the stimulation of the binding of GST-NK to chromatin and the incorporation of 32P into GST-NK on treatment with SC. Phosphorylation of GST-NK by SC and authentic SRPK caused similar stimulation of the binding and gave similar phosphopeptide maps (Figs. 1, 3, and 7). From these results we concluded that the kinase in SC responsible for the stimulation of the binding of GST-NK to chromatin is SRPK. When the chicken LBR amino-terminal region (cGST-wtNt), which corresponds to the human GST-NK used in this study, was treated with either SRPK1 or an SR protein kinase, a very similar phosphopeptide map to that of GST-NK treated with SC or SRPK1 was obtained (14). Phosphorylation of cGST-wtNt by SRPK1 and the SR protein kinase was completely suppressed for the stimulation of the binding of GST-NK to chromatin, whereas phosphorylation of LBR by SRPK may regulate or maintain interactions of p32/p34 (18). On the other hand, phosphorylation by a similar SRPK in an Xenopus egg extract stimulated the binding of LBR to chromatin (Figs. 1 and 2). Therefore, phosphorylation of LBR by SRPK may regulate or maintain interactions in vivo among components in the LBR complex containing chromatin.

Phosphorylation of GST-NK by cdc2 kinase in MC caused partial suppression of the binding to chromatin (Figs. 4 and 5). Direct phosphorylation of serine 71 in LBR by cdc2 kinase in MC could be explained by the phosphopeptide maps, although the patterns were complicated (Fig. 6). Phosphorylation of GST-NK by SRPK in MC was also observed (compare Figs. 3 and 6). Suppression of the binding of LBR to chromatin on treatment with MC could not be fully explained by cdc2 kinase, suggesting the presence of another kinase(s) that participates in the suppression (Figs. 4 and 5). Which spot in the phosphopeptide maps in Fig. 6 corresponds to the phosphorylation by the kinase is not clear. However, one possible explanation is as follows; spots 10 and 12 (Fig. 6) contain peptides having two molar phosphate groups/molar peptide, and their phosphate groups are transferred by cdc2 kinase and the other kinase.

The kinase of interest is sensitive to staurosporine and DMAP (Fig. 4). Pfaller and Newport (23) reported that both cdc2 kinase and “membrane release kinase” are necessary to release nuclear membrane vesicles from the chromatin surface in a Xenopus egg extract system. They partially purified the kinase (23). The membrane release kinase phosphorylates an approximately 60-kDa protein in a Xenopus egg membrane fraction (23). The kinase is inhibited by DMAP but not by specific inhibitors for known kinases such as protein kinase A, PKC, and calmodulin-dependent protein kinase (23). All these characteristics are the same as those of our kinase, that is, our kinase phosphorylates 60-kDa nuclear envelope protein LBR and is inhibited by DMAP (Fig. 4) but not by specific inhibitors for protein kinase A, PKC, and calmodulin-dependent kinase (Fig. 4). Moreover, the membrane release kinase acts with cdc2 kinase in vesicle release (23), and our kinase suppresses the binding of GST-NK to chromatin with cdc2 kinase. Therefore, the kinase working with cdc2 kinase for suppression of the binding of GST-NK to chromatin is very likely the same enzyme as this membrane release kinase.

In living cells, LBR, LAP2, emerin, and other inner nuclear membrane proteins have to dissociate from chromatin in the prometaphase to early anaphase of the cell cycle. It was suggested in this study that the binding of LBR to chromatin is suppressed by the phosphorylation of serine 71 by cdc2 kinase and some other residue by a kinase similar to membrane release kinase in MC. However, the kinases responsible for the regulation of the binding of other inner nuclear membrane proteins to chromatin are not yet known. Identification of these kinases is very important for understanding the molecular mechanisms underlying nuclear envelope disassembly.

REFERENCES

1. Sullivan, T., Escalante-Alcalde, D., Bhatt, H., Anver, M., Bhat, N., Nagashima, K., Stewart, C. L., and Burke, B. (1999) J. Cell Biol. 147, 913–919
2. Nagano, A., Kogo, R., Ogawa, M., Kurano, Y., Kawada, J., Okada, R., Hayashi, Y. K., Teukahara, T., and Arakawa, K. (1996) Nat. Genet. 12, 254–259
3. Hoffmann, K., Dreger, C. K., Olin, A. L., Olin, D. E., Shultz, L. D., Lucke, B., Karl, H., Kaps, R., Muller, D., Vayu, A., Amzar, J., Ware, R. E., Sotelo Cruz,
Regulation of Binding of LBR to Chromatin

13271

N., Lindner, T. H., Herrmann, H., Reis, A., and Sperling, K. (2002) Nat. Genet. 31, 410–414
4. Burke, B., and Stewart, C. L. (2002) Nat. Rev. Mol. Cell Biol. 3, 575–585
5. Foisner, R., and Gerace, L. (1993) Cell 73, 1267–1279
6. Takano, M., Takeuchi, M., Ko, H., Furukawa, K., Sagimoto, K., Omata, S., and Horigome, T. (2002) Eur. J. Biochem. 269, 943–953
7. Haraguchi, T., Koujin, T., Hayakawa, T., Kaneda, T., Tsutsumi, C., Imamoto, N., Akazawa, C., Suhegawa, J., Yoneda, Y., and Hirai, Y. (2000) J. Cell Sci. 113, 779–784
8. Pyrpaospoulou, A., Meier, J., Maison, C., Simos, G., and Georgatos, S. D. (1996) EMBO J. 15, 7108–7119
9. Collas, P., Courvalin, J.-C., and Pecia, D. (1996) J. Cell Biol. 135, 1715–1725
10. Shumaker, D. K., Lee, K. K., Tanhehco, Y. C., Craigie, R., and Wilson, K. L. (2001) EMBO J. 20, 1754–1764
11. Dreger, M., Otto, H., Neubauer, G., Mann, M., and Hucho, F. (1999) Biochemistry 38, 2426–2434
12. Kawahire, S., Tachibana, T., Umemoto, M., Yoneda, Y., Imai, N., Saito, M., Ichimura, T., Omata, S., and Horigome, T. (1996) Exp. Cell Res. 222, 385–394
13. Nikolakaki, E., Meier, J., Simos, G., Georgatos, S. D., and Giannakouros, T. (1997) J. Biol. Chem. 272, 6208–6213
14. Papoutsopoulou, S., Nikolakaki, E., and Giannakouros, T. (1999) Biochem. Biophys. Res. Commun. 255, 602–607
15. Kusubata, M., Tokui, T., Matsuoka, Y., Okumura, E., Tachibana, K., Hisanaga, S., Kishimoto, T., Yasuda, H., Kami, M., Ohba, Y., Tsujimura, K., Yanai, R., and Inagaki, M. (1992) J. Biol. Chem. 267, 20937–20942
16. Keizumi, J., Okamoto, Y., Onogi, H., Mayeda, A., Krainer, A. R., and Hagiwara, M. (1999) J. Biol. Chem. 274, 11125–11131
17. Furukawa, K., Glass, C., and Kondo, T. (1997) Biochem. Biophys. Res. Commun. 238, 240–246
18. Nikolakaki, E., Simos, G., Georgatos, S. D., and Giannakouros, T. (1996) J. Biol. Chem. 271, 8365–8372
19. Duband-Goulet, I., and Courvalin, J.-C. (2000) Biochemistry 39, 6483–6488
20. Gui, J.-F., Lane, W. S., and Fu, X.-D. (1994) Nature 369, 678–682
21. Kursryanagi, N., Onogi, H., Wakabayashi, T., and Hagiwara, M. (1998) Biochem. Biophys. Res. Commun. 242, 357–364
22. Wang, H.-Y., Lin, W., Dyck, J. A., Yeakley, J. M., Songyang, Z., Cantley, L. C., and Fu, X.-D. (1998) J. Biol. Chem. 273, 737–750
23. Pfarr, R., and Newport, J. W. (1995) J. Biol. Chem. 270, 19666–19672