Temporal control of nuclear envelope assembly by phosphorylation of lamin B receptor

Li-Chuan Tseng[a] and Rey-Huei Chen[a,b]
[a]Graduate Institute of Life Sciences, National Defense Medical Center, Taipei 114, Taiwan; [b]Institute of Molecular Biology, Academia Sinica, Taipei 11529, Taiwan

ABSTRACT The nuclear envelope of metazoans disassembles during mitosis and reforms in late anaphase after sister chromatids have well separated. The coordination of these mitotic events is important for genome stability, yet the temporal control of nuclear envelope reassembly is unknown. Although the steps of nuclear formation have been extensively studied in vitro using the reconstitution system from egg extracts, the temporal control can only be studied in vivo. Here, we use time-lapse microscopy to investigate this process in living HeLa cells. We demonstrate that Cdk1 activity prevents premature nuclear envelope assembly and that phosphorylation of the inner nuclear membrane protein lamin B receptor (LBR) by Cdk1 contributes to the temporal control. We further identify a region in the nucleoplasmic domain of LBR that inhibits premature chromatin binding of the protein. We propose that this inhibitory effect is partly mediated by Cdk1 phosphorylation. Furthermore, we show that the reduced chromatin-binding ability of LBR together with Aurora B activity contributes to nuclear envelope breakdown. Our studies reveal for the first time a mechanism that controls the timing of nuclear envelope reassembly through modification of an integral nuclear membrane protein.

INTRODUCTION

The nuclear envelope (NE) of eukaryotic cells serves to compartmentalize the cytoplasm and the nucleus. It is continuous with the endoplasmic reticulum (ER) and is composed of inner and outer membranes that are joined by the nuclear pore complex (NPC). Underneath the inner membrane is nuclear lamina made of a meshwork of the lamin protein, which maintains the structure of the nucleus and organizes the chromatin territories through interaction with inner membrane proteins (Hetzer *et al.*, 2005). In metazoans, the NE breaks down at the onset of mitosis and reassembles around the chromatin mass in late anaphase. These mitotic events must be coordinated spatially and temporally for proper segregation of the chromosomes.

Nuclear envelope breakdown (NEBD) begins with the stretching and tearing of the nuclear membrane by spindle microtubules (Beaudouin *et al.*, 2002; Salina *et al.*, 2002). Complete NEBD requires the depolymerization of nuclear lamina through phosphorylation of lamin by Cdk1 in early mitosis (Heald and McKeon, 1990; Peter *et al.*, 1990). In addition, NPC components and several inner nuclear membrane proteins are also the substrates of Cdk1 (Courvalin *et al.*, 1992; Macaulay *et al.*, 1995; Dechat *et al.*, 1998). Phosphorylation of these components is important for the disassembly of NPC and NE (Onischenko *et al.*, 2007; Laurell *et al.*, 2011). The breakdown of NE allows the spindle microtubules to capture and separate sister chromatids toward the opposite spindle poles. Subsequently, the NE assembles around the segregated chromatin mass to complete the nuclear division.

Reassembly of the NE has been best studied in vitro using extracts prepared from *Xenopus* and sea urchin eggs. These in vitro systems reveal that NE assembly involves chromatin recruitment of the precursor membrane vesicles, fusion of the vesicles on the chromatin surface, and insertion of the NPC (Vigers and Lohka, 1991; Drummond *et al.*, 1999; Sasagawa *et al.*, 1999). The binding of membrane vesicles to the chromatin is inhibited by Cdk1 and requires type 1 or 2A protein phosphatase (PP1/2A; Pfaller *et al.*, 1991; Ito *et al.*, 2007). The fusion of NE vesicles requires Ran GTPase, its nucleotide exchange factor RCC1 (Hetzer *et al.*, 2000; Zhang and Clarke, 2000), and the chaperone p97-Ufd1-Npl4 complex (Hetzer *et al.*, 2001). Despite the advances following from

these in vitro studies, the mechanism underlying NE assembly in living cells remains largely unknown. In mitotic cells the disassembled NE membrane and some of its associated proteins are retrieved to the ER network after NEBD instead of forming membrane vesicles (Ellenberg *et al.*, 1997; Yang *et al.*, 1997; Daigle *et al.*, 2001). During NE reassembly, the end of tubular ER first attaches to the chromatin, followed by the flattening and spreading of the membrane on the chromatin surface with the help of ER-shaping proteins reticulon and DP1 (Anderson and Hetzer, 2007, 2008). With the insertion of NPC, the membrane eventually fuses to enwrap the whole chromatin mass.

Some of the inner nuclear membrane proteins are integral membrane proteins, including lamin B receptor (LBR), MAN1, emerin, and lamin A–associated protein β (LAP2β; Hetzer *et al.*, 2005). These proteins remain associated with the ER after NEBD in mitosis (Ellenberg *et al.*, 1997; Yang *et al.*, 1997). RNA interference (RNAi) studies have shown that these proteins have redundant functions in NE assembly, as silencing any of these genes causes only a small delay, but the knockdown of multiple genes has an additive effect on NE assembly (Anderson *et al.*, 2009). However, the exact role of these proteins in NE reassembly is unclear.

LBR, originally identified as a binding protein of lamin B from the turkey erythrocyte, is a transmembrane protein residing on the inner nuclear membrane (Worman *et al.*, 1988). The protein contains a nucleoplasmic amino-terminal domain followed by eight transmembrane domains and a small carboxyl-terminal region (Ye and Worman, 1994). The nucleoplasmic domain is capable of interacting with lamin B, heterochromatin protein 1, importin β, and DNA (Ye and Worman, 1994; Ye *et al.*, 1997; Polioudaki *et al.*, 2001; Ma *et al.*, 2007). LBR has been shown to participate in NE assembly both in vitro and in vivo. The NE precursor vesicles containing LBR are able to associate with the sperm chromatin to initiate NE assembly in sea urchin egg extracts (Wilson and Newport, 1988; Collas *et al.*, 1996). Immunodepletion of LBR reduces chromatin recruitment of the NE vesicles in vitro (Pyrpasopoulou *et al.*, 1996), and RNAi of LBR delays NE assembly in vivo (Anderson *et al.*, 2009). LBR is phosphorylated in both interphase and mitosis. Phosphorylation at Ser-71 by Cdk1 in mitosis suppresses chromatin binding of the amino terminus of LBR in the egg extract (Takano *et al.*, 2004). However, the physiological significance of LBR phosphorylation in living cells is unknown.

In this study, we investigate the process of NE reassembly by time-lapse imaging of HeLa cells. We focus on how phosphorylation might affect the timing of NE reassembly at the end of mitosis. We also determine the functional significance of LBR phosphorylation in this process.

RESULTS
Nuclear envelope assembly begins in late anaphase
To understand the timing mechanism underlying NE reassembly at the end of mitosis in vivo, we followed this process in HeLa cells by time-lapse microscopy. To visualize nuclear envelope, we expressed green fluorescent protein (GFP)–tagged Sec61β as an ER marker because ER is the major source of the nuclear membrane for NE reassembly. We also expressed mCherry-tagged histone H2B for tracking chromosomes. At metaphase, the ER network was distributed in the whole cell except for the space occupied by the spindle and chromosomes (Figure 1A). In early anaphase, sister chromatids were separated and pulled toward the spindle poles without apparent contact with the ER membrane. The chromosome arms shortened gradually and became a compact mass in late anaphase (Mora-Bermudez *et al.*, 2007). Meanwhile, the ER membrane began to attach to the edge of the chromatin mass near the spindle poles

(Figure 1A, 6 min). Following the initial attachment, the membrane quickly extended toward the central surface of the chromatin mass, first the polar region and then the area facing the spindle midzone (Figure 1A). The NE completely enclosed the chromatin by 14 min (Figure 1A and Supplemental Video S1).

We also followed the inner nuclear membrane protein LBR that is dispersed in the ER during mitosis in HeLa cells (Ellenberg *et al.*, 1997). ER-labeled by GFP-LBR attached to the chromatin in late anaphase and extended in the same direction as observed with GFP-Sec61β (Figure 1B and Supplemental Video S1). LBR was preferentially sorted to inner nuclear membrane and thus formed a clear nuclear outline (Figure 1B, 14–22 min). The average time of initial ER attachment to the chromatin was 5.7 and 5.2 min in anaphase for GFP-Sec61β– and GFP-LBR–expressing cells, respectively (Table 1). The earlier attachment with GFP-LBR indicates that overexpression of LBR facilitates the recruitment of ER membrane.

Inhibition of Cdk1 causes premature ER attachment
Cdk1 is the major kinase that is activated to promote mitotic entry and inactivated to allow mitotic exit. Thus we tested the possibility that Cdk1 might be involved in the temporal control of NE reformation. We introduced a high concentration (200 μM) of the Cdk1 inhibitor roscovitine to cells in early metaphase during live-cell imaging in order to inhibit Cdk1 activity efficiently during the short window of anaphase. In vitro kinase assay shows that roscovitine only inhibits Cdk1 but not other mitotic kinases Aurora B and Plk1 at this concentration (Supplemental Figure S5). The addition of roscovitine caused massive chromosome bridges (Figure 2A and Supplemental Video S2). ER membrane labeled by GFP-LBR started to attach to several regions on the surface of the chromosomes in early anaphase (Figure 2A, 2 min). The attached ER membrane moved along with the segregating chromosomes and then covered the chromosome arms (Figure 2A). The NE formed quickly first on the outer surface of the chromatin and then extended to the inner surface along the chromosome bridges (Figure 2A and Supplemental Video S2). The average time of initial attachment was 2.2 min, which was much earlier than for the dimethyl sulfoxide (DMSO)–treated control cells (4.1 min; Figure 2B). Roscovitine also accelerated membrane attachment, as observed with GFP-Sec61β–expressing cells (Supplemental Figure S1 and Supplemental Video S2). Thus inhibition of Cdk1 activity leads to premature ER attachment, indicating that Cdk1 activity is important for the temporal control of NE assembly.

PP1/2A is important for NE assembly
Our results suggest that phosphorylation of some Cdk1 substrates prevents premature ER attachment to the chromatin until late anaphase. Therefore, dephosphorylation of these substrates is probably required for the recruitment of ER membrane in late anaphase. The protein phosphatases 1 and 2A (PP1/2A) have broad substrate specificity and are known to be involved in several aspects of mitosis (De Wulf *et al.*, 2009). To test whether PP1/2A activity might be required for NE reformation in cells, we inhibited the PP1/2A activity with calyculin A, which has good permeability for the cells (Namboodiripad and Jennings, 1996). Because the PP1/2A activity is required for establishing metaphase (Sassoon *et al.*, 1999), we added calyculin A to the culture medium immediately after anaphase onset during live-cell imaging. On calyculin A treatment, most of the cells failed to assemble a complete NE, as observed with GFP-LBR or GFP-Sec61β (Figure 3, Supplemental Figure S2, and Supplemental Videos S3 and S4). In the most severe cases, ER membrane attached only to the edge of the chromatin mass, whereas the regions facing

A GFP-Sec61β H2B-mCherry
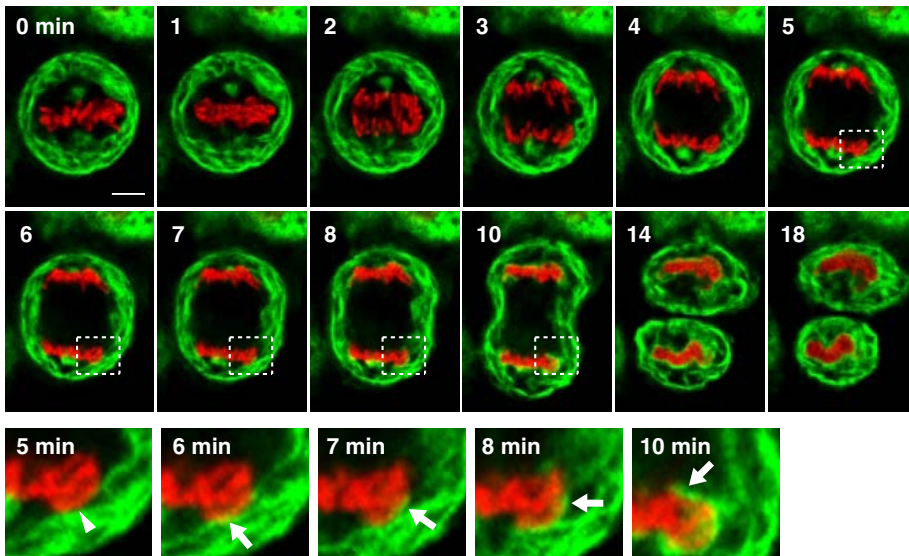
B GFP-LBR H2B-mCherry
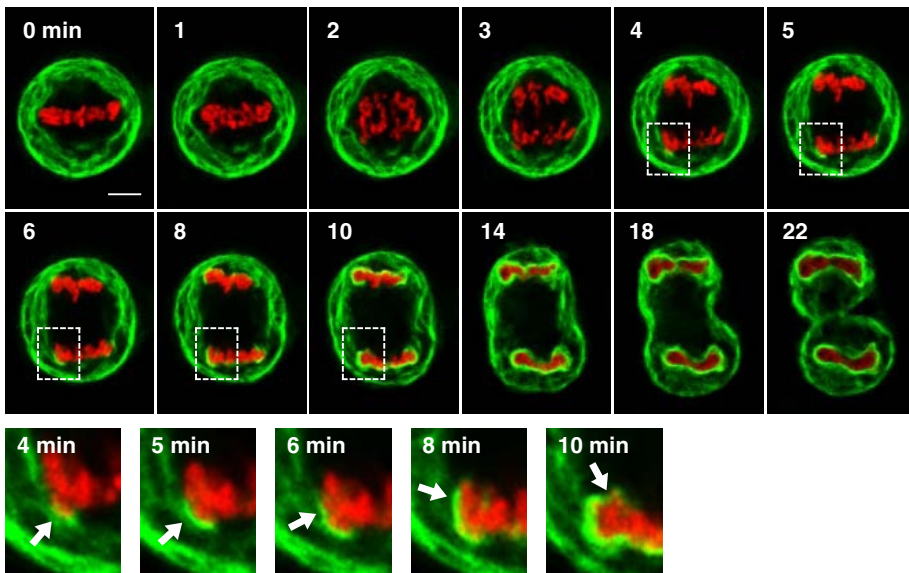
FIGURE 1: ER attachment to the chromatin initiates in late anaphase. (A) Time-lapse images of a HeLa cell transfected with the ER marker GFP-Sec61β (green) and chromosome marker H2B-mCherry (red). Images were acquired every min, and the last image before anaphase onset was set as t = 0. Outlined areas are enlarged below. The arrowhead indicates the chromatin devoid of ER membrane at 5 min. From 6 min, the membrane attached to and spread on the chromatin surface (arrows). (B) Time-lapse images of a cell expressing GFP-LBR and H2B-mCherry. Outlined areas are enlarged below to show ER attachment (arrows). Bar, 5 μm. The time series is presented in Supplemental Video S1.

the spindle poles or midzone were devoid of membrane (Figure 3A, a, and Supplemental Figure S2A, a). In other cells, NE assembled but never completely covered the entire chromatin mass (Figure 3A, b, and Supplemental Figure S2A, b). In addition, calyculin A treatment delayed the initial ER attachment in comparison with DMSO control (Figures 3C and S2C). During continuous incubation with calyculin A, the partially formed NE disassembled (Figure 3A, 20 min). These results indicate that the PP1/2A activity is required for NE reassembly.

LBR phosphorylation mutant causes premature ER attachment

LBR is a known substrate of Cdk1 (Nikolakaki et al., 1997) and is involved in membrane recruitment during nuclear envelope formation (Pyrpasopoulou et al., 1996). We wondered whether phosphorylation of LBR might affect the timing of NE assembly. The amino-terminal nucleoplasmic region (amino acids 1–208) of LBR contains four Ser/Thr-Pol motifs (Ser-71, Ser-86, Thr-118, and Thr-200) that are possible Cdk1 phosphorylation sites. To determine the phosphorylation, we generated recombinant proteins with glutathione S-transferase (GST) fused to the nucleoplasmic domain of LBR. The four possible phosphorylation sites of Cdk1 were completely replaced with alanine (4A) or in combinations of three (3A). Consistent with the previous finding that Ser-71 is a target for Cdk1 (Takano et al., 2004), the wild type and the 3A/71S (71S 86A 118A 200A) mutant were phosphorylated to similar levels by Cdk1 in vitro (Figure 4, A and B), indicating that Ser-71 is the major phosphorylation site of Cdk1. In addition, 3A/86S (71A 86S 118A 200A) was phosphorylated by Cdk1 to ~40% of the wild-type level, whereas 3A/T118 and 3A/T200 were barely phosphorylated by Cdk1 (Figure 4, A and B). Therefore, both Ser-71 and Ser-86 are phosphorylation sites of Cdk1. Live imaging of GFP-tagged LBR[AA] mutant (S71A S86A) shows that the ER membrane attached to the segregating chromosomes in early anaphase (Figure 5A, 3 min), which was similar to that in roscovitine-treated cells. However, unlike roscovitine treatment, the expression of GFP-LBR[AA] rarely caused chromosome bridges (Figure 5A and Supplemental Video S5). About 60% of GFP-LBR[AA]–expressing cells began NE assembly within 4 min after anaphase onset, whereas <10% of GFP-Sec61β and 33% of GFP-LBR cells had ER attachment at this time (Figure 5B). The average time of initial ER attachment in GFP-LBR[AA]–expressing cells was ~4 min (Table 1), which was significantly earlier than that of GFP-Sec61β or GFP-LBR cells. The result suggests that phosphorylation of LBR by Cdk1 may contribute to the temporal control of nuclear envelope assembly.

Globular II domain of LBR negatively regulates chromatin attachment

Phosphorylation of LBR in the nucleoplasmic domain might regulate the chromatin-binding activity of LBR. The nucleoplasmic domain of LBR consists of two globular domains separated by a linker sequence (Figure 4C; Ye et al., 1997). To determine which region is important for recruiting ER to the chromatin, we expressed mutants deleted for the globular I (GFP-LBR[ΔGI], lacking amino acids 1–60) or the globular II domain (GFP-LBR[ΔGII], lacking amino acids 105–208;

	Time (min)	n	p
Sec61β	5.7 ± 1.1	51	<0.05
LBR	5.2 ± 1.3	54	n/a
LBR^{71A86A}	4.3 ± 1.1	50	<0.0005
LBR$^{\Delta GI}$	6.0 ± 1.2	50	<0.005
LBR$^{\Delta GI/71A86A}$	5.4 ± 1.2	50	0.435
LBR$^{\Delta GII}$	2.1 ± 1.5	50	<0.0005
LBR$^{\Delta GII/71A86A}$	1.6 ± 1.1	50	<0.0005

The initial time of ER attachment is shown as mean ± SD. n indicates the total number of cells. The p values compared with LBR were calculated by the *t* test. n/a, not applicable.

TABLE 1: Initial time of ER attachment.

Figure 4C). Of interest, ER attachment to the chromatin was much accelerated in cells expressing GFP-LBR$^{\Delta GII}$ (Figure 6 and Supplemental Video S6). This process began by 4 min in anaphase in more than 68% of the cells and even before anaphase in 26% of the cells (Figure 6C), indicating that the globular II domain might negatively

A

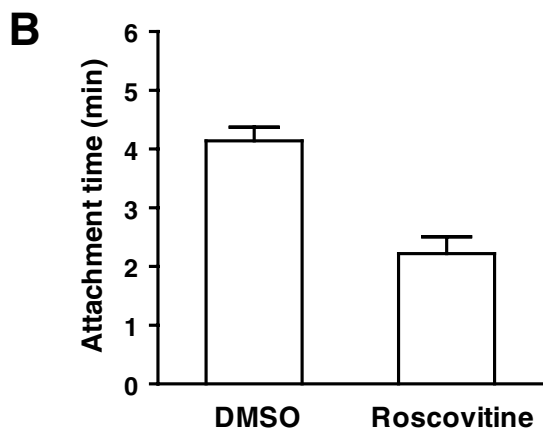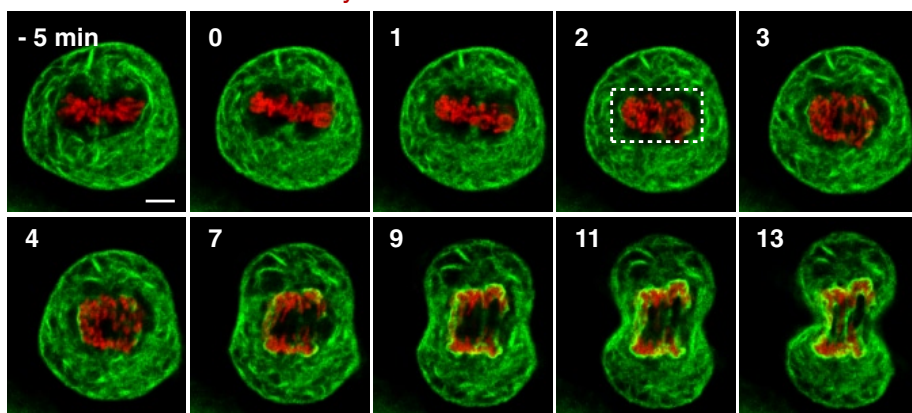GFP-LBR H2B-mCherry + Roscovitine

2 min

B

FIGURE 2: Inhibition of Cdk1 activity causes premature ER attachment to the chromatin. (A) Time-lapse images of GFP-LBR and H2B-mCherry in a cell treated with roscovitine in early metaphase during live-cell imaging. The outlined area in the image of 2 min is enlarged below. The arrows indicate initial attachment. Bar, 5 μm. The time series is presented in Supplemental Video S2. (B) The average times of initial ER attachment for DMSO and roscovitine are 4.1 ± 0.2 and 2.2 ± 0.3 (mean ± SEM) min, respectively. n > 20 for both; p < 0.0001.

regulate the chromatin-binding activity. On the other hand, the majority of the GFP-LBR$^{\Delta GI}$ cells initiated NE assembly between 5 and 7 min (Figure 6C). The average times of initial attachment were 2.1 and 6 min for GFP-LBR$^{\Delta GII}$ and GFP-LBR$^{\Delta GI}$, respectively (Table 1).

To determine how phosphorylation might affect the globular domains, we expressed the deletion mutants with additional mutations at the phosphorylation sites (Figure 4C). The results show that the times of ER attachment for GFP-LBR$^{\Delta GI-AA}$ (5.4 min) and GFP-LBR$^{\Delta GII-AA}$ (1.6 min) were only slightly earlier than that of the deletion mutants with intact phosphorylation sites (Figure 6C and Table 1). These results suggest that phosphorylation of LBR has little effect once the globular II domain is deleted. Thus phosphorylation might regulate the chromatin-binding ability of LBR through the globular II domain.

Expression of GFP-LBR$^{\Delta GI}$ shows a slight delay in ER attachment (average initial attachment time, 6 min; Table 1) in comparison with the expression of wild-type protein (time, 5.2 min; Table 1), suggesting that LBR$^{\Delta GI}$ might be impaired in chromatin binding. In fact, in vitro chromatin binding assay with purified recombinant 6His-LBR-mCherry proteins shows that wild-type, LBR^{AA}, and LBR$^{\Delta GII}$ bound sperm chromatin to similar levels, whereas the binding was much reduced with LBR$^{\Delta GI}$ (Figure 7A). To determine whether chromatin binding is affected by Cdk1 phosphorylation, wild-type and LBR^{AA} proteins were first incubated with either interphase or mitotic egg cytosol prior to the in vitro chromatin-binding reactions. The result shows that wild-type LBR treated with mitotic, but not interphase, cytosol was impaired in chromatin binding, whereas LBR^{AA} was not affected by either cytosol (Figure 7B). Consistent with the previous study (Takano *et al.*, 2004), our results suggest that mitotic phosphorylation of LBR inhibits its chromatin-binding ability.

Aurora B promotes the dissociation of NE membrane from chromosomes in early mitosis

Overexpression of GFP-LBR$^{\Delta GII}$ or inhibition of Cdk1 caused premature attachment of ER to metaphase chromosomes in a small fraction of cells, whereas most cells exhibited premature attachment after anaphase onset. Live imaging showed that GFP-LBR$^{\Delta GII}$ cells can undergo normal NEBD with complete dissociation of nuclear membrane from chromosomes before anaphase (Figure 8). Thus the association of ER membrane with metaphase chromosomes was a consequence of premature ER attachment rather than incomplete dissociation of NE membrane after NEBD. Because GFP-LBR$^{\Delta GII}$ seems to bypass the inhibitory effect of Cdk1 (Figure 6), we suspect that another mitotic kinase besides Cdk1 might also inhibit premature ER attachment in early mitosis. A good candidate is Aurora B, which has multiple functions in mitosis and is located on prophase chromosomes in mammalian cells (Ruchaud *et al.*, 2007). It was shown that Aurora B must be extracted from the chromosomes to allow NE assembly in frog

A GFP-LBR H2B-mCherry + Calyculin A

8/21

10/21

3/21

B GFP-LBR H2B-mCherry + DMSO

20/20

C
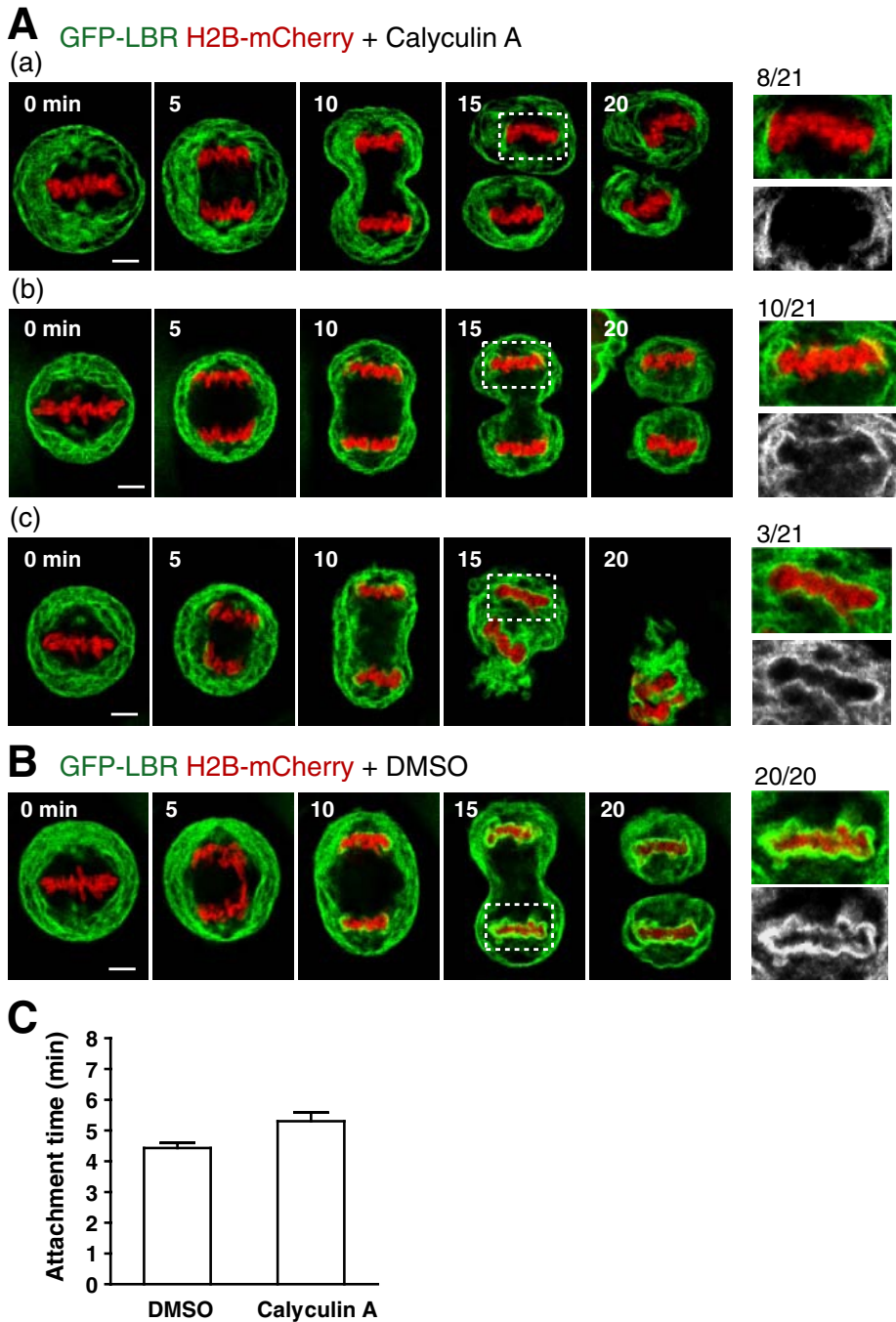
FIGURE 3: Inhibition of PP1/2A activity disrupts NE reassembly. (A) Time-lapse images of GFP-LBR and H2B-mCherry in cells treated with calyculin A immediately after anaphase onset. The outlined areas in the images at 15 min are magnified (right), showing that NE assembly was affected to different degrees. The numbers on top of the magnified images represent the fraction of cells in each category. (a) Almost no or only small patches of membrane on chromosomes (8 of 21 cells). (b) Incomplete enclosure of the chromatin (10 of 21 cells). (c) Complete enclosure the chromatin (3 of 21 cells). The arrows indicate areas devoid of the NE. Bar, 5 μm. The time series is presented in Supplemental Video S3. (B) Time-lapse images of GFP-LBR and H2B-mCherry in a cell treated with DMSO immediately after anaphase onset. (C) The average times of initial attachment for DMSO and calyculin A treatment are 4.4 ± 0.2 and 5.3 ± 0.3 (mean ± SEM) min, respectively. p < 0.05.

egg extracts (Ramadan *et al.*, 2007). To test the possibility that Aurora B might be involved in the reassembly of the NE in mammalian cells, we added the specific Aurora B inhibitor ZM447439 to cells

that had just begun anaphase during live imaging. ZM447439 only inhibits Aurora B, but not Cdk1 or Plk1, at the concentration used in this study (Supplemental Figure S5). The addition of ZM447439 did not affect the timing of ER attachment as observed with GFP-Sec61β or GFP-LBR (Supplemental Figure S3 and Supplemental Video S7). The aberrant chromosome segregation and cytokinesis indicate that Aurora B was indeed inhibited (Supplemental Figure S3). Because Aurora B locates on the chromosomes only in prophase and translocates to the centromere in prometaphase, the protein might be involved in NEBD rather than NE assembly. We tested this possibility by adding ZM447439 to prophase cells. For the control of DMSO treatment, all of GFP-LBR and 88% of GFP-LBR[AA] cells completely dissociated NE from chromosomes within 10 min after NEBD, and 81% of GFP-LBR[ΔGII] cells finished this process within 20 min (Figure 8 and Supplemental Video S8). GFP-LBR[ΔGII-AA] showed further delay, with 52% of the cells finishing this process within 20 min and 24% of the cells retaining patches of membrane on chromosomes even after 40 min (Supplemental Figure S4). ZM447439 treatment caused a small delay in the complete dissociation of NE membrane in both GFP-LBR and GFP-LBR[AA] compared with DMSO control (Figure 8). Of interest, most of GFP-LBR[ΔGII] and GFP-LBR[ΔGII-AA] cells treated with ZM447439 retained patches of nuclear membranes on chromosomes even at 40 min after NEBD (Figure 8 and Supplemental Figure S4). Measurement of the average distance between chromatin and ER/NE membrane in cells at 20 min after NEBD shows that ZM447439 treatment decreased the distance in GFP-LBR[ΔGII] cells (0.8 μm), and the effect was less dramatic with GFP-LBR[AA] (0.92 μm) compared with wild type (1.3 μm) (Figure 8C). These results show that Aurora B activity becomes important for complete NEBD in the presence of LBR[ΔGII] or LBR[AA] mutant that partially bypasses the inhibitory effect of Cdk1 on chromatin binding.

DISCUSSION

Our study elucidates the temporal control of NE reassembly in late mitosis. The process of NE assembly has been best studied using the in vitro reconstitution system derived from frog egg extracts. However, the question of how NE assembly is coordinated with other mitotic events cannot be easily addressed in the in vitro system. In this study, we used live imaging of HeLa cells to investigate the role of mitotic regulators Cdk1, PP1/2A, and Aurora B that control multiple aspects of mitosis. By delivering their inhibitors to cells at specific mitotic stages, we demonstrated that these regulators
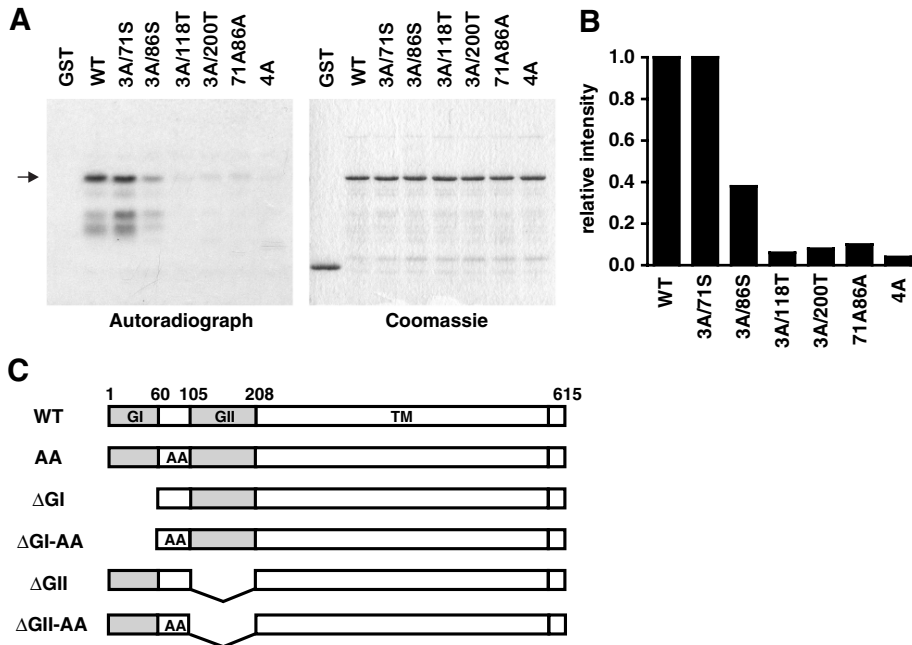
FIGURE 4: Cdk1 phosphorylates LBR at Ser71 and Ser86. (A) In vitro kinase assay of Cdk1 using the substrates GST or GST-fused N-terminal region (residues 1–208) of LBR as indicated on the top: wild type (WT), 3A/71S (71S 86A 118A 200A), 3A/86S (71A 86S 118A 200A), 3A/118T (71A 86A 118T 200A), 3A/200T (71A 86A 118A 200T), 71A86A (71A 86A 118T 200T), and 4A (71A 86A 118A 200A). The autoradiograph (left) and Coomassie staining (right) of the gel are shown. The arrow indicates the position of GST-LBR$^{1-208}$. The lower species are the degraded products. (B) Quantification of the phosphorylation. The data were normalized with the protein levels as determined by Coomassie staining and compared with the phosphorylation signal of the wild type, which is set as 1. (C) The diagram illustrates the domains in LBR and the mutants used in this study. AA, alanine substitutions at the Cdk1 phosphorylation sites; GI, globular I domain; GII, globular II domain; TM, transmembrane domain.

are important for the disassembly and reformation of the NE during mitosis and that phosphorylation of inner nuclear membrane protein LBR contributes to the temporal control of NE assembly.

Cdk1 prevents premature ER attachment to the chromatin

NE reassembly must be coordinated with sister chromatid segregation to ensure genome stability. Our time-lapse imaging shows that Cdk1 activity prevents premature ER attachment in early anaphase and that PP1/2A is required for NE reassembly. These results suggest that the balance between the activities of Cdk1 and PP1/2A determines the timing of NE reassembly. It was shown that PP1 is involved in the dephosphorylation of Cdk1 substrates after anaphase and that inactivation of Cdk1 at the end of mitosis induces PP1 activity through a feedback mechanism (Wu et al., 2009). Thus PP1 may be responsible for initiating NE assembly at the end of mitosis. Because the protein phosphatase inhibitor calyculin A used in our study is effective toward both PP1 and PP2A, we cannot rule out the possibility that PP2A may also be involved in this process. So far, it is unclear whether NE reassembly is a consequence of mitotic exit or Cdk1 inactivation is directly involved in NE reassembly. Our result of premature ER attachment upon inhibition of Cdk1 suggests a direct role for Cdk1 in the temporal control of NE assembly.

Phosphorylation of LBR by Cdk1 inhibits ER attachment

Overexpression of integral NE membrane proteins is known to expedite NE assembly (Anderson et al., 2009). Consistently, overexpression of wild-type LBR slightly accelerates ER attachment compared with that of Sec61β overexpression (Table 1). Overexpression of Cdk1 phosphorylation-site mutant LBR^{AA} further advances ER attachment, indicating that Cdk1 phosphorylation negatively regulates the chromatin-binding ability of LBR. This notion is supported by the in vitro chromatin-binding assay (Figure 7; Takano et al., 2004). However, Lu et al. (2010) recently proposed that phosphorylation of Ser71 is important for LBR to associate with importin β, which targets LBR to Ran-GTP on chromatin, implying a positive role of Ser71 phosphorylation in NE assembly. Their finding contradicts with our results and those of Takano et al. (2004). It is worth noting that LBR is capable of chromatin binding on its own (Ulbert et al., 2006). Thus the recruitment of LBR-containing membrane to chromatin may not require importin β in vivo. Furthermore, Lu et al. (2010) also showed that LBR^{S71A} is not incorporated into the NE and perturbs NE assembly in cells. However, we found that LBR^{AA} assembles into the NE and even advances NE formation during anaphase. One explanation for the discrepancy is that Lu et al. (2010) expressed Xenopus LBR in human cells in their studies. The nucleoplasmic domains of human and Xenopus LBR share only 36% identity, raising the possibility that Xenopus LBR may not behave normally in human cells.

Analysis of the nucleoplasmic domain of LBR shows that LBR$^{\Delta GII}$ markedly advances ER attachment, indicating that globular II domain normally inhibits the chromatin-binding activity of LBR. The ER attachment time of LBR$^{\Delta GII-AA}$ is only slightly earlier than LBR$^{\Delta GII}$, suggesting that phosphorylation may inhibit chromatin binding of LBR through the globular II domain (Figure 9). The average time of initial ER attachment for LBR$^{\Delta GII}$ (2.1 min in anaphase) is earlier than that of LBR^{AA} (4.3 min; Table 1), suggesting that additional modification or mechanism is required to completely remove the inhibitory effect of the globular II domain. The Cdk1 phosphorylation might help to keep LBR in a conformation that masks the chromatin-binding site in the nucleoplasmic region. In late anaphase, LBR is then dephosphorylated to expose the chromatin-binding site. The process of Cdk1 inactivation through cyclin B degradation and the subsequent PP1 activation may provide enough time lag between anaphase onset and LBR dephosphorylation so that the NE does not reform until late anaphase.

LBR$^{\Delta GI-AA}$ does not cause premature ER attachment, indicating that the globular I domain is important for chromatin binding. Indeed, deletion of the globular I domain (LBR$^{61-208}$) impairs the chromatin-binding ability in vitro (Figure 7). However, previous study showed that residues 53–89 bind chromatin (Takano et al., 2002). It is possible that chromatin binding of this region is inhibited by the globular II domain in LBR$^{61-208}$. Although the globular I domain is important for chromatin binding, expression of LBR$^{\Delta GI}$ does not block ER attachment. It is possible that other integral NE membrane proteins can still mediate ER recruitment, as NE formation in vivo involves multiple redundant interactions
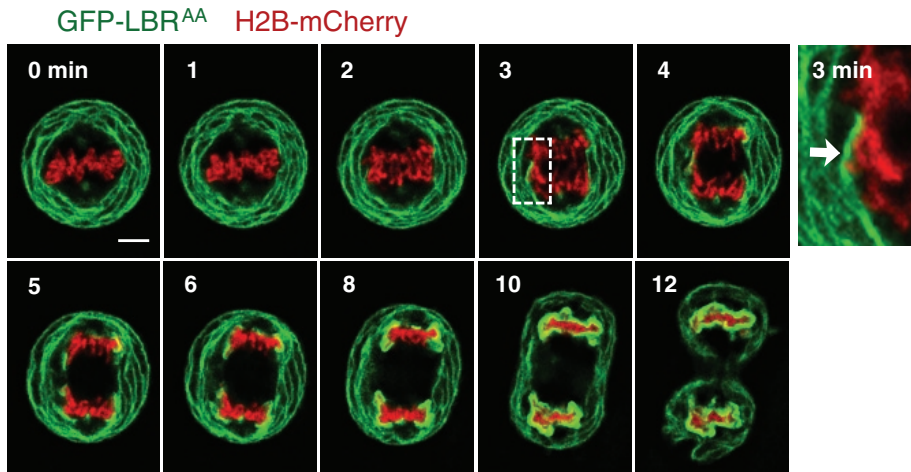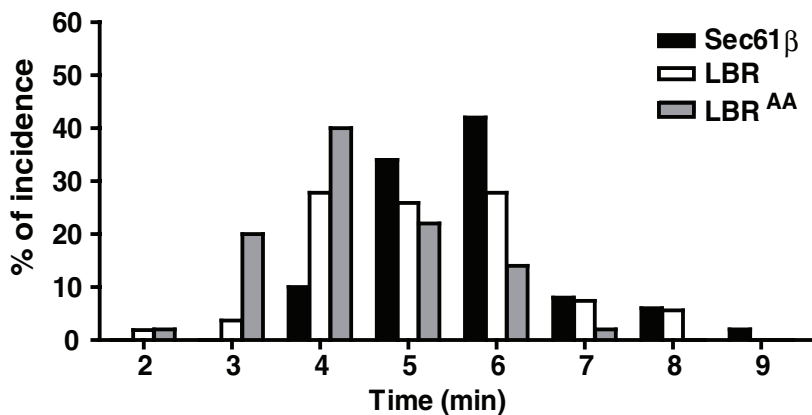
FIGURE 5: LBR^AA causes premature ER attachment. (A) Time-lapse images of GFP-LBR^AA and H2B-mCherry. The outlined area is enlarged (right), and the arrow indicates ER attachment. Bar, 5 μm. The time series is presented in Supplemental Video S5. (B) Frequency of initial ER attachment at each time point after anaphase onset (Sec61β, n = 51; LBR, n = 54; LBR^AA, n = 50).

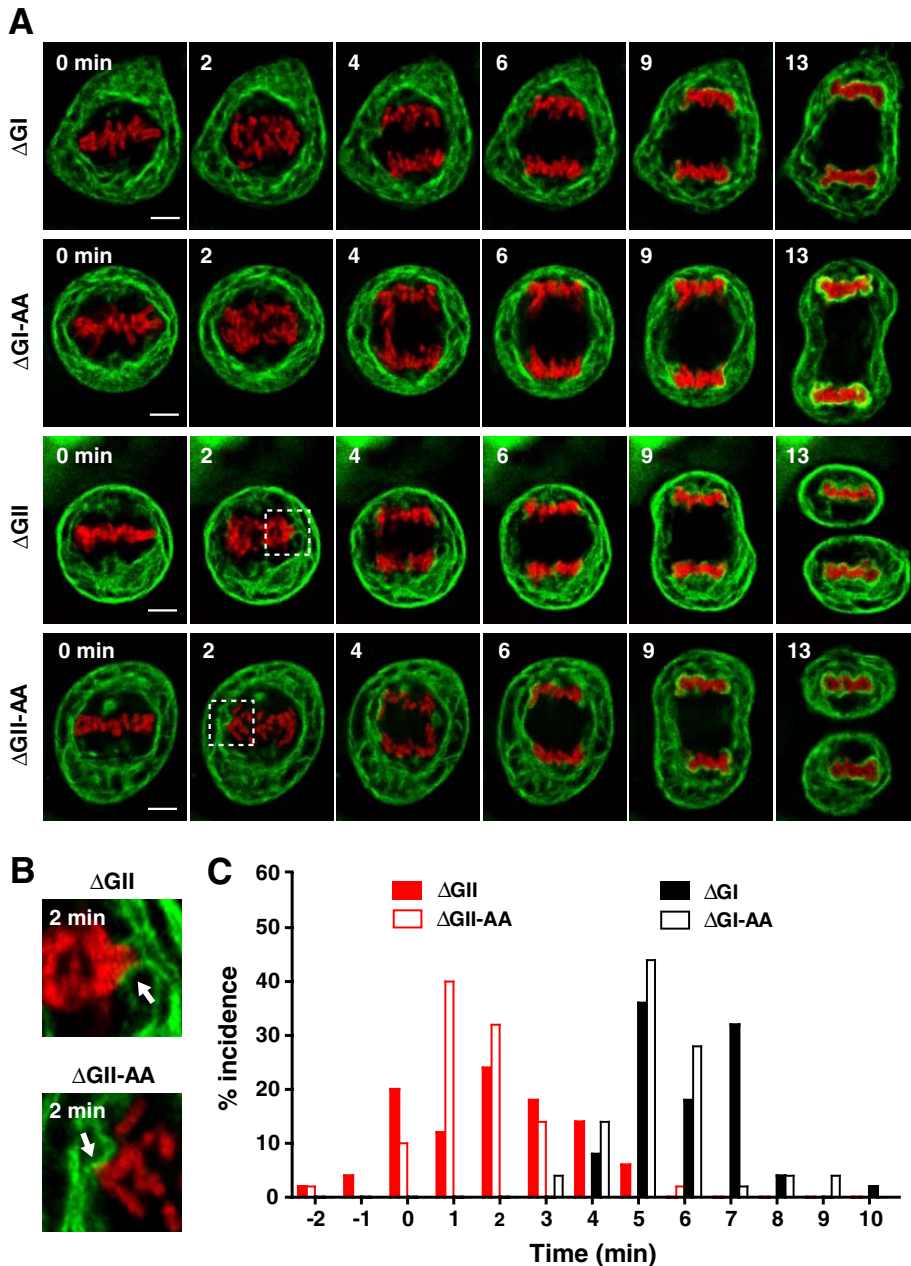cating that the inhibitory effect, if any, of the Aurora B substrates can be overcome by the LBR mutant.

Aurora B and LBR phosphorylation contribute to NE disassembly

Inhibition of Aurora B at prophase in cells expressing LBR^ΔGII or LBR^AA delays the complete dissociation of NE membrane from the chromatin after NEBD, whereas inhibition of Aurora B or expression of LBR^ΔGII alone affects this process to a lesser degree. These results are consistent with the notion that association of NE membrane with the chromatin involves multiple interactions. Aurora B residing on the prophase chromatin may phosphorylate chromatin proteins, which disrupts its interaction with some unknown inner nuclear membrane proteins, whereas phosphorylation of LBR by Cdk1 also reduces its chromatin binding (Figure 9). Sustaining both interactions causes retention of residual NE on the chromatin after NE ruptures. Thus NEBD involves initial NE rupture mediated by microtubules, depolymerization of nuclear lamina through phosphorylation of lamin, and loss of membrane–chromatin interaction through phosphorylation by Cdk1 and Aurora B. Taken together, our data show that the control of chromatin-binding ability of LBR, partly through Cdk1 phosphorylation, is important for both NEBD and NE reassembly.

Full-length Sec61β sequence was amplified from HeLa cDNA library (Invitrogen, Carlsbad, CA) by PCR using primers 5′ACGAATTCTATGCCTG-GTCCGACCCC3′ and 5′CCGGTACCCTACGAACGAGTGTACT-TGC3′. Both Sec61β and human LBR (IMAGE clone 3925138; Open Biosystems, Thermo Biosystems, Huntsville, AL) were fused with GFP at the amino terminus in pAcGFP1-C1 vector (Clontech, Mountain View, CA). Human H2B (IMAGE clone 40002352; Open Biosystems) was fused with mCherry at the carboxyl terminus in the vector pGW1myc2a-mCherry. Alanine mutations at Ser-71, Ser-86, Thr-118, and Thr-200 of LBR were generated with the QuikChange Multi Site-Directed Mutagenesis Kit (Stratagene, Santa Clara, CA). Deletion of the globular I domain in LBR was generated by PCR using primers 5′GGCGTTAGATCTAG-GCAAAGGAAAGGTGGC3′ and 5′GGGAATTCGAGCATTAGTA-GATGTATGG3′. Deletion of the globular II domain in LBR was generated by two steps of PCR. The first step amplified the amino-terminal region (amino acids 1–104) using primers 5′GGCA-GATCTATGCCAAGTAGGAAATTTGC3′ and 5′CACCAGGTACTC-CTCCAAAGGCCTGGTGGGAAGCAGAAGC3′, as well as the carboxyl-terminal region (amino acids 209–615) using primers 5′GCTTCTGCTTCCCACCAGGCCTTTGGAGGAGTACCTGGTG3′ and 5′GGGAATTCGAGCATTAGTAGATGTATGG3′. Both PCR products were then mixed and used as the template in the second

Aurora B is not involved in temporal control

Previous study using the in vitro nuclear assembly system of *Xenopus* egg extracts showed that Aurora B kinase activity inhibits NE formation and that removal of Aurora B from the chromatin is required for chromosome decondensation and NE assembly (Ramadan et al., 2007). We find that inhibition of Aurora B in early anaphase has no apparent effect on the timing of initial ER attachment to the chromatin, indicating that Aurora B is not important for the temporal control of NE reformation. On the other hand, it is possible that Aurora B substrates remain phosphorylated after blocking Aurora B activity in early anaphase, which may contribute to the inhibition of NE assembly. Aurora B resides on chromosomes in early mitosis and translocates to centromeres at metaphase and to the spindle midzone after anaphase (Ruchaud et al., 2007). Thus the maintenance of histone H3 phosphorylation and chromosome condensation does not require continuous presence of Aurora B on the chromosome. Despite the phosphorylation, overexpression of LBR^ΔGII mutant causes premature ER attachment in early anaphase, indi-

between several inner nuclear membrane proteins and chromatin (Anderson et al., 2009).

FIGURE 6: Globular II domain of LBR prevents premature NE assembly. (A) Time-lapse images of GFP-tagged LBR mutants as indicated on the left and H2B-mCherry. (B) Enlargements of the outlined areas in the images of cells expressing GFP-LBR$^{\Delta GII}$ or GFP-LBR$^{\Delta GII-AA}$ at 2 min . The arrows indicate ER attachment to chromosomes. Bar, 5 µm. The time series is presented in Supplemental Video S6. (C) Frequency of initial ER attachment at times before (negative values) or after (positive values) the metaphase–anaphase transition.

Cell culture

HeLa cells were grown in DMEM containing 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin (Invitrogen) at 37°C in a humidified incubator supplemented with 5% CO_2. For live imaging, cells were seeded on the glass coverslip coated with 1 µg/ml poly-L-lysine (Sigma-Aldrich, St. Louis, CA). On the following day the cells were transfected with the expression plasmids using Effectene transfection reagent (Qiagen, Valencia, CA). Two days after transfection, the glass coverslip with cells was set up in a POC-R chamber (LaCon, Staig, Germany) for microscopy. For treatment of inhibitors during live-cell imaging, the prewarmed medium containing 200 µM roscovitine (CalBiochem, La Jolla, CA), 80 nM calyculin A (CalBiochem), 20 µM ZM447439 (Tocris Bioscience, Ellisville, MO), or an equivalent volume of the solvent DMSO was injected into the chamber through a perfusion tubing, and the existing medium in the chamber was simultaneously withdrawn through a second tubing.

Image acquisition and processing

Images were acquired with Observer Z1 microscope (Carl Zeiss, Jena, Germany) equipped with 63 × LCI Plan-Neofluor numerical aperture–1.3 water objective and AxioCam MR charge-coupled device camera controlled by AxioVision, release 4.8, software (Carl Zeiss). The cells in the POC-R chamber were maintained with 5% CO_2 at 37°C during time-lapse imaging. For NE reassembly, Z-stack images (14 sections, 1–1.5 µm apart) were collected every minute. For membrane dissociation after NEBD, Z-stack images (12 sections, 1–1.2 µm apart) were collected every 2 min. The images were processed by Huygens deconvolution software. The initial attachment of ER was defined as the first time point that ER contacted with the chromatin, which extended on the chromatin surface on the following time points and eventually became a part of the NE. The distance between chromatin surface and NE/ER membrane was measured as described previously (Muhlhausser and Kutay, 2007) for the central four sections of the Z-stack. Briefly, a radial grid of 20 lines with an angle of 18° between lines was placed in the center of the chromatin mass, and the distance between chromatin and membrane was manually measured along the grid lines by AxioVision, release 4.8, software.

PCR using primers 5′GGCAGATCTATGCCAAGTAGGAAATTTGC3′ and 5′GGGAATTCGAGCATTAGTAGATGTATGG3′. For the generation of GST-fused recombinant proteins, amino-terminal fragment of LBR (amino acids 1–208) and its variants were amplified from the full-length constructs in pAcGFP1-C1 vector by PCR using primers 5′GGCAACGGATCCATGCCAAGTAGGAAATTTGC3′ and 5′GGTTGAATTCCTCCAAGTCCTTTGCCCG3′ and were cloned into pGEX-2T at *Bam*HI and *Eco*RI sites. For the generation of recombinant proteins with N-terminal 6His and C-terminal mCherry fusions, LBR fragments were subcloned into pET28a vector at *Bam*HI and *Eco*RI sites and mCherry cloned at *Eco*RI and *Xho*I sites.

Purification of recombinant proteins

The expression constructs of pGEX-2T-LBR were transformed into *Escherichia coli* strain BL21-DE3 RIL. The bacteria were cultured in 1 l of Luria-Bertani (LB) medium to 0.6 OD_{600} before inducing the protein expression with 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 2.5 h. The cells were then lysed
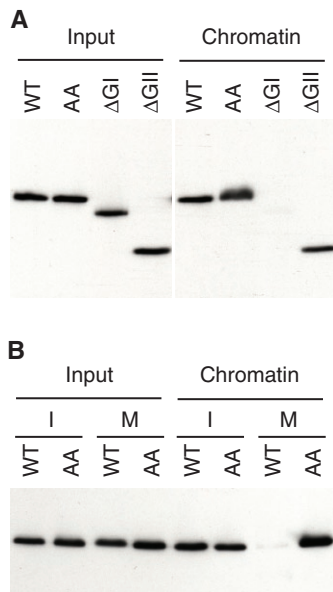
FIGURE 7: Globular I domain is involved in chromatin binding in vitro. (A) Frog sperm nuclei were decondensed in interphase egg cytosol and then incubated with indicated wild-type 6His-LBR-mCherry (WT) or its variants. Chromatin was then isolated, and its associated proteins were subjected to Western blot analysis with anti-mCherry antibody. (B) Recombinant WT or 6His-LBR^AA-mCherry (AA) proteins were first incubated with either interphase or mitotic cytosol before adding to the in vitro chromatin-binding reactions. Equivalents to 1/80 of the input and 1/10 of the chromatin fraction were analyzed by Western blots.

by sonication in lysis buffer A (phosphate-buffered saline [PBS] containing 50 mM EDTA, 10 µg/ml each of leupeptin, pepstatin, and chymostatin, 1 mM phenylmethylsulfonyl fluoride [PMSF]), followed by the addition of 1% Triton X-100. The GST-fusion proteins were purified through 1 ml of glutathione agarose and eluted with 10 mM glutathione in 50 mM Tris (pH 8.0). The purified proteins were concentrated by dialysis in PBS containing 50% glycerol.

For 6His-LBR-mCherry, the plasmids were transformed into BL21 strain. The protein expression was induced in 1 l of culture with 0.2 mM IPTG at 25°C for 4 h. The cells were lysed by sonication in lysis buffer B (50 mM sodium phosphate, pH 8, 300 mM NaCl, 10 µg/ml each of leupeptin, pepstatin, and chymostatin, and 1 mM PMSF), followed by the addition of 0.5% Triton X-100. The proteins were purified through 1.5 ml of Ni-NTA (Qiagen, Valencia, CA). After washing with wash buffer (50 mM sodium phosphate, pH 6, 300 mM NaCl, and 0.5% Triton X-100) containing 25 mM imidazole, the proteins were eluted with 0.3 M imidazole in wash buffer. The purified proteins were concentrated by dialysis in PBS containing 50% glycerol.

In vitro phosphorylation of LBR

The purified recombinant GST-LBR[1-208] proteins and GST were used as the substrates for Cdk1. The assay was performed in the kinase buffer (25 mM Tris-HCl, pH 7.5, 10 mM MgCl$_2$, 0.1 mM Na$_3$VO$_4$, 5 mM β-glycerophosphate, 2 mM dithiothreitol) supplemented with 50 µM ATP, 1 ng/µl Cdk1 (Cell Signaling Technology, Beverly, MA), 0.2 µg/µl substrate, and 1 µCi [γ-^{32}P]ATP at 30°C for 20 min. The reaction was resolved by 10% SDS–PAGE. The gel was stained by Coomassie blue (Sigma-Aldrich) and dried for autoradiography. The ^{32}P incorporation was quantified by Typhoon FLA 9000 (GE

Healthcare, Piscataway, NJ) with ImageQuant TL 7.0 software (GE Healthcare), and protein levels were determined with the ImageJ software (National Institutes of Health, Bethesda, MD).

Chromatin-binding assay

The extracts from *Xenopus* eggs were prepared as described (Murray, 1991) with the following modifications. For interphase cytosol, dejellied eggs were activated with 10 µM calcium ionophore A23187 (Sigma-Aldrich) in 0.2 × MMR (0.2 M NaCl, 4 mM KCl, 2 mM MgSO$_4$, 4 mM CaCl$_2$, 10 mM 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid [HEPES], 0.2 mM EDTA, pH 7.8). Eggs were then washed several times with 0.2 × MMR and then with extraction buffer (50 mM HEPES, pH 7.8, 250 mM sucrose, 50 mM KCl, 2.5 mM MgCl$_2$, and 10 µg/ml each of leupeptin, pepstatin, and chymostatin,). Packed eggs were centrifuged at 12,000 × g for 15 min in an SW55Ti rotor (Beckman Coulter, Brea, CA) at 2°C. The crude cytoplasmic fraction from the centrifugation was then centrifuged at 200,000 × g for 90 min in a TLS55 rotor (Beckman Coulter) to separate the cytosol from the membrane fractions. The cytosol was then centrifuged again at 200,000 × g for 30 min to remove residual membrane. The resulting high-speed supernatant (HSS) was then used for the chromatin-binding assay or for phosphorylating LBR proteins. The in vitro chromatin-binding assay was performed as described (Takano *et al.*, 2002). Briefly, HSS was first heated at 95°C for 10 min, cooled on ice for 5 min, and then centrifuged at 20,000 × g for 15 min to remove denatured proteins. The heated cytosol containing nucleoplasmin was then used to decondense demembranated frog sperm nuclei that were prepared as described (Murray, 1991). For each chromatin-binding reaction, 40 µl of heated cytosol were incubated with sperm nuclei at a density of ~15,000 per µl of cytosol for 30 min at 25°C, followed by incubation with 0.5 µg of 6His-LBR-mCherry proteins for 30 min on ice. The samples were then diluted with nine volumes (360 µl) of ice-cold extraction buffer containing 0.1% Triton X-100 and layered over 1 ml of 30% sucrose made in extraction buffer containing 0.1% Triton X-100. The samples were centrifuged at 15,000 × g for 15 min in a JS13.1 rotor (Beckman Coulter). Chromosomal pellets were washed in 500 µl of the sucrose solution and spun again for 5 min. The pellets were solubilized by heating at 95°C for 5 min in SDS–PAGE sample buffer for immunoblot analysis with anti-mCherry antibody (provided by Chao-Wen Wang, Academia Sinica, Taipei, Taiwan).

For prior treatment with cytosol, 6His-LBR-mCherry proteins were first bound to Ni-NTA beads and then incubated with mitotic or interphase HSS for 75 min at 25°C. The beads were then washed four times with wash buffer (50 mM sodium phosphate, pH 6, 300 mM NaCl, and 0.5% Triton X-100), and the proteins were eluted with 0.3 M imidazole in wash buffer.

Kinase assays

HeLa cells were synchronized at mitosis with 100 ng/ml of nocodazole for 16 h. Mitotic cells were harvested by physical shake-off, replated, and treated with DMSO, roscovitine (200 µM), or ZM447439 (20 µM) for 20 min in a 37°C humidified incubator. The cells were lysed with lysis buffer (10 mM potassium phosphate, pH, 7.2, 1 mM EDTA, 5 mM ethylene glycol tetraacetic acid, 50 mM β-glycerophosphate, 1 mM sodium vanadate, 10 mM MgCl$_2$, 0.5% Triton X-100, 0.1% sodium deoxycholate, 10% glycerol, 1 mM PMSF, and 10 µg/ml each of leupeptin, pepstatin, and chymostatin). A total of 300 µg of lysate was used for immunoprecipitation with anti-Cdc2 antibody (sc-54; Santa Cruz Biotechnology, Santa Cruz, CA), anti–AIM-1 antibody (BD Transduction Laboratories, BD Biosciences, San Diego, CA) and anti-Plk antibody (N-19) (Santa Cruz Biotechnology), with 20 µl of
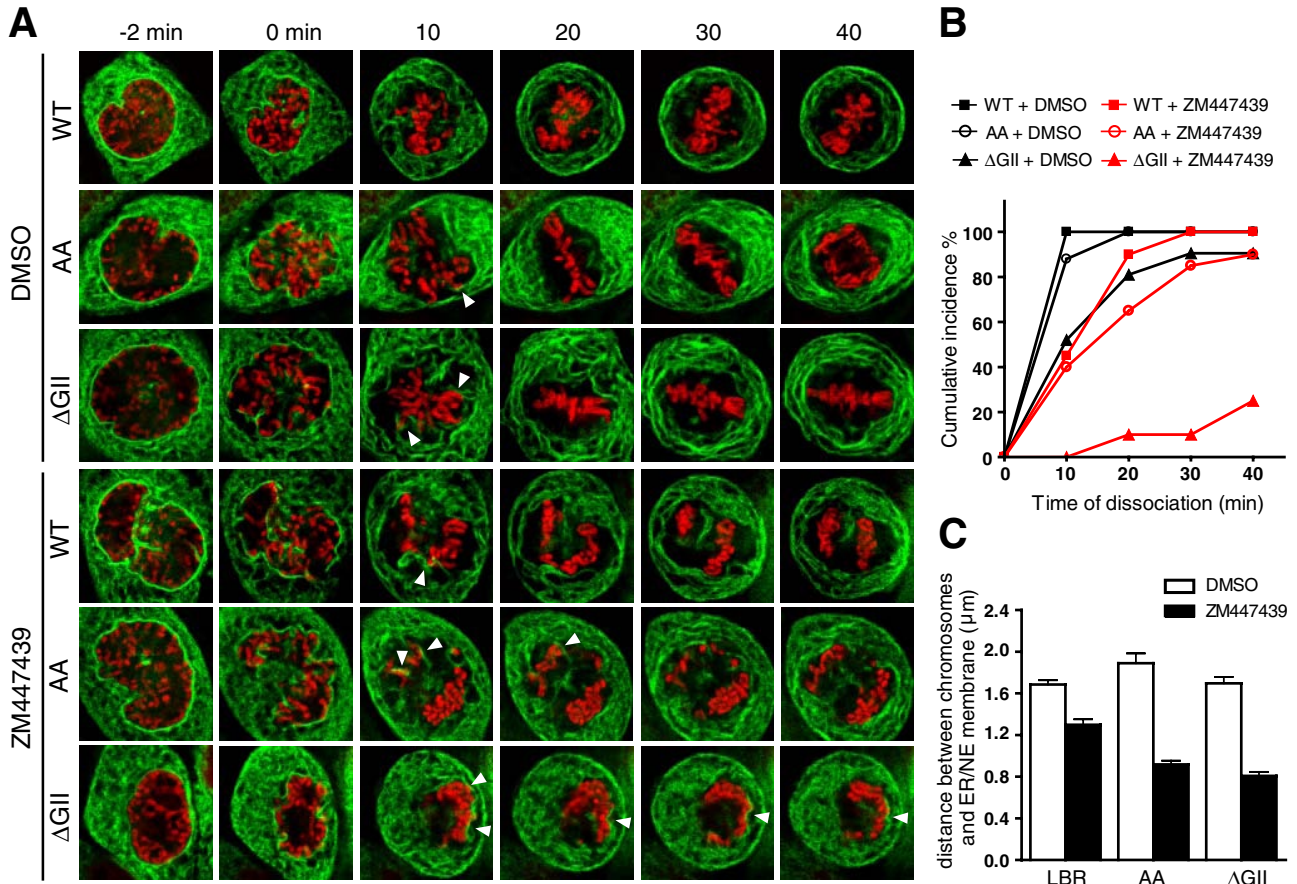
FIGURE 8: Aurora B is involved in the dissociation of NE membrane after NEBD. (A) Time-lapse images of cells expressing GFP-tagged WT or mutant LBR proteins as indicated on the left and H2B-mCherry. The cells were treated with DMSO or the Aurora B inhibitor ZM447439 at prophase. Images were acquired every 2 min. Time t = 0 corresponds to the time point of NEBD onset as defined by the loss of smooth and continuous line corresponding to the NE. The arrowheads indicate NE association with chromosomes. Bar, 5 μm. The time series is presented in Supplemental Video S8. (B) Cumulative frequency of complete dissociation of NE membrane at times after NEBD. Cells that completely dissociated NE membrane after 40 min or never completed this process before NE reassembled were not included in the graph (n > 20 for each). (C) Distance between chromatin and ER/NE membrane. The cells at 20 min after NEBD were analyzed with 20 measurements for each of the central four sections in a Z-stack. The graph shows the average distance in micrometers, and the error bars represent standard errors. *p < 0.0001.
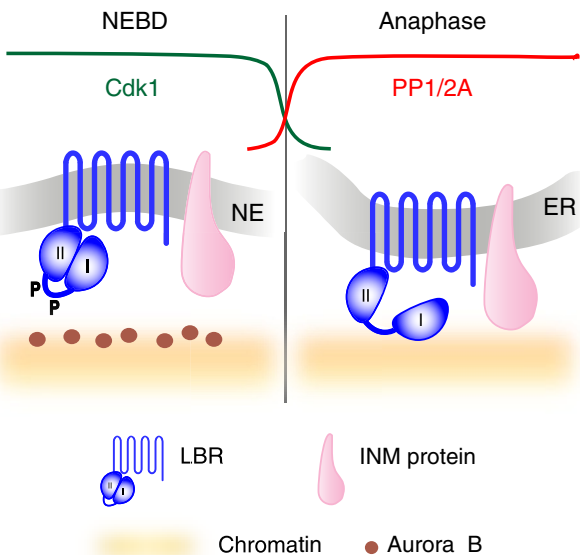
protein G–Sepharose (GE Healthcare), for 1 h. The immunoprecipitates were washed four times with lysis buffer and once with kinase buffer. The kinase assay was performed in 10-μl reaction in kinase buffer containing 50 μM ATP, 1 μCi [γ-^{32}P]ATP, and substrates (2 μg of histone H1, 2 μg of histone H3, and 0.6 μg of casein for the kinase assay of Cdk1, Aurora B, and Plk1, respectively) at 30°C for 20 min.

FIGURE 9: A model for the dissociation and reassembly of NE during mitosis. The schematic representation shows the status of LBR and the chromatin during NEBD (left) and in late anaphase (right). During NEBD Cdk1 phosphorylates LBR and inhibits its chromatin-binding ability through the globular II domain. Aurora B residing on the chromosomes also promotes nuclear membrane dissociation by unknown mechanisms. In late anaphase, the increased PP1/2A activity dephosphorylates LBR and Aurora B substrates, which allows LBR and other inner nuclear membrane (INM) proteins to associate with the chromatin. The inhibitory effect of Aurora B is lost because of its absence on anaphase chromosomes.

Western blotting

HeLa cells were transfected with plasmids expressing GFP or GFP-LBR variants along with H2B-mCherry as described for live imaging. The cells were harvested 2 d after transfection and lysed in lysis buffer. The Western blots were probed with anti-LBR rabbit monoclonal antibody (E398L) (Abcam, Cambridge, MA), anti–α-tubulin antibody (Upstate, Millipore, Billerica, MA), and anti-GFP antibody (provided by Chao-Wen Wang, Academia Sinica).

REFERENCES

Anderson DJ, Hetzer MW (2007). Nuclear envelope formation by chromatin-mediated reorganization of the endoplasmic reticulum. Nat Cell Biol 9, 1160–1166.

Anderson DJ, Hetzer MW (2008). Reshaping of the endoplasmic reticulum limits the rate for nuclear envelope formation. J Cell Biol 182, 911–924.

Anderson DJ, Vargas JD, Hsiao JP, Hetzer MW (2009). Recruitment of functionally distinct membrane proteins to chromatin mediates nuclear envelope formation in vivo. J Cell Biol 186, 183–191.

Beaudouin J, Gerlich D, Daigle N, Eils R, Ellenberg J (2002). Nuclear envelope breakdown proceeds by microtubule-induced tearing of the lamina. Cell 108, 83–96.

Collas P, Courvalin JC, Poccia D (1996). Targeting of membranes to sea urchin sperm chromatin is mediated by a lamin B receptor-like integral membrane protein. J Cell Biol 135, 1715–1725.

Courvalin JC, Segil N, Blobel G, Worman HJ (1992). The lamin B receptor of the inner nuclear membrane undergoes mitosis-specific phosphorylation and is a substrate for p34cdc2-type protein kinase. J Biol Chem 267, 19035–19038.

Daigle N, Beaudouin J, Hartnell L, Imreh G, Hallberg E, Lippincott-Schwartz J, Ellenberg J (2001). Nuclear pore complexes form immobile networks and have a very low turnover in live mammalian cells. J Cell Biol 154, 71–84.

De Wulf P, Montani F, Visintin R (2009). Protein phosphatases take the mitotic stage. Curr Opin Cell Biol 21, 806–815.

Dechat T, Gotzmann J, Stockinger A, Harris CA, Talle MA, Siekierka JJ, Foisner R (1998). Detergent-salt resistance of LAP2alpha in interphase nuclei and phosphorylation-dependent association with chromosomes early in nuclear assembly implies functions in nuclear structure dynamics. EMBO J 17, 4887–4902.

Drummond S, Ferrigno P, Lyon C, Murphy J, Goldberg M, Allen T, Smythe C, Hutchison CJ (1999). Temporal differences in the appearance of NEP-B78 and an LBR-like protein during Xenopus nuclear envelope reassembly reflect the ordered recruitment of functionally discrete vesicle types. J Cell Biol 144, 225–240.

Ellenberg J, Siggia ED, Moreira JE, Smith CL, Presley JF, Worman HJ, Lippincott-Schwartz J (1997). Nuclear membrane dynamics and reassembly in living cells: targeting of an inner nuclear membrane protein in interphase and mitosis. J Cell Biol 138, 1193–1206.

Heald R, McKeon F (1990). Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. Cell 61, 579–589.

Hetzer M, Bilbao-Cortes D, Walther TC, Gruss OJ, Mattaj IW (2000). GTP hydrolysis by Ran is required for nuclear envelope assembly. Mol Cell 5, 1013–1024.

Hetzer M, Meyer HH, Walther TC, Bilbao-Cortes D, Warren G, Mattaj IW (2001). Distinct AAA-ATPase p97 complexes function in discrete steps of nuclear assembly. Nat Cell Biol 3, 1086–1091.

Hetzer MW, Walther TC, Mattaj IW (2005). Pushing the envelope: structure, function, and dynamics of the nuclear periphery. Annu Rev Cell Dev Biol 21, 347–380.

Ito H, Koyama Y, Takano M, Ishii K, Maeno M, Furukawa K, Horigome T (2007). Nuclear envelope precursor vesicle targeting to chromatin is stimulated by protein phosphatase 1 in Xenopus egg extracts. Exp Cell Res 313, 1897–1910.

Laurell E, Beck K, Krupina K, Theerthagiri G, Bodenmiller B, Horvath P, Aebersold R, Antonin W, Kutay U (2011). Phosphorylation of Nup98 by multiple kinases is crucial for NPC disassembly during mitotic entry. Cell 144, 539–550.

Lu X, Shi Y, Lu Q, Ma Y, Luo J, Wang Q, Ji J, Jiang Q, Zhang C (2010). Requirement for lamin B receptor and its regulation by importin {beta} and phosphorylation in nuclear envelope assembly during mitotic exit. J Biol Chem 285, 33281–33293.

Ma Y, Cai S, Lv Q, Jiang Q, Zhang Q, Sodmergen, Zhai Z, Zhang C (2007). Lamin B receptor plays a role in stimulating nuclear envelope production and targeting membrane vesicles to chromatin during nuclear envelope assembly through direct interaction with importin beta. J Cell Sci 120, 520–530.

Macaulay C, Meier E, Forbes DJ (1995). Differential mitotic phosphorylation of proteins of the nuclear pore complex. J Biol Chem 270, 254–262.

Mora-Bermudez F, Gerlich D, Ellenberg J (2007). Maximal chromosome compaction occurs by axial shortening in anaphase and depends on Aurora kinase. Nat Cell Biol 9, 822–831.

Muhlhausser P, Kutay U (2007). An in vitro nuclear disassembly system reveals a role for the RanGTPase system and microtubule-dependent steps in nuclear envelope breakdown. J Cell Biol 178, 595–610.

Murray AW (1991). Cell cycle extracts. Methods Cell Biol 36, 581–605.

Namboodiripad AN, Jennings ML (1996). Permeability characteristics of erythrocyte membrane to okadaic acid and calyculin A. Am J Physiol 270, C449–C456.

Nikolakaki E, Meier J, Simos G, Georgatos SD, Giannakouros T (1997). Mitotic phosphorylation of the lamin B receptor by a serine/arginine kinase and p34(cdc2). J Biol Chem 272, 6208–6213.

Onischenko EA, Crafoord E, Hallberg E (2007). Phosphomimetic mutation of the mitotically phosphorylated serine 1880 compromises the interaction of the transmembrane nucleoporin gp210 with the nuclear pore complex. Exp Cell Res 313, 2744–2751.

Peter M, Nakagawa J, Doree M, Labbe JC, Nigg EA (1990). In vitro disassembly of the nuclear lamina and M phase-specific phosphorylation of lamins by cdc2 kinase. Cell 61, 591–602.

Pfaller R, Smythe C, Newport JW (1991). Assembly/disassembly of the nuclear envelope membrane: cell cycle-dependent binding of nuclear membrane vesicles to chromatin in vitro. Cell 65, 209–217.

Polioudaki H, Kourmouli N, Drosou V, Bakou A, Theodoropoulos PA, Singh PB, Giannakouros T, Georgatos SD (2001). Histones H3/H4 form a tight complex with the inner nuclear membrane protein LBR and heterochromatin protein 1. EMBO Rep 2, 920–925.

Pyrpasopoulou A, Meier J, Maison C, Simos G, Georgatos SD (1996). The lamin B receptor (LBR) provides essential chromatin docking sites at the nuclear envelope. EMBO J 15, 7108–7119.

Ramadan K, Bruderer R, Spiga FM, Popp O, Baur T, Gotta M, Meyer HH (2007). Cdc48/p97 promotes reformation of the nucleus by extracting the kinase Aurora B from chromatin. Nature 450, 1258–1262.

Ruchaud S, Carmena M, Earnshaw WC (2007). Chromosomal passengers: conducting cell division. Nat Rev Mol Cell Biol 8, 798–812.

Salina D, Bodoor K, Eckley DM, Schroer TA, Rattner JB, Burke B (2002). Cytoplasmic dynein as a facilitator of nuclear envelope breakdown. Cell 108, 97–107.

Sasagawa S, Yamamoto A, Ichimura T, Omata S, Horigome T (1999). In vitro nuclear assembly with affinity-purified nuclear envelope precursor vesicle fractions, PV1 and PV2. Eur J Cell Biol 78, 593–600.

Sassoon I, Severin FF, Andrews PD, Taba MR, Kaplan KB, Ashford AJ, Stark MJ, Sorger PK, Hyman AA (1999). Regulation of Saccharomyces cerevisiae kinetochores by the type 1 phosphatase Glc7p. Genes Dev 13, 545–555.

Takano M, Koyama Y, Ito H, Hoshino S, Onogi H, Hagiwara M, Furukawa K, Horigome T (2004). Regulation of binding of lamin B receptor to chromatin by SR protein kinase and cdc2 kinase in Xenopus egg extracts. J Biol Chem 279, 13265–13271.

Takano M, Takeuchi M, Ito H, Furukawa K, Sugimoto K, Omata S, Horigome T (2002). The binding of lamin B receptor to chromatin is regulated by phosphorylation in the RS region. Eur J Biochem 269, 943–953.

Ulbert S, Platani M, Boue S, Mattaj IW (2006). Direct membrane protein-DNA interactions required early in nuclear envelope assembly. J Cell Biol 173, 469–476.

Vigers GP, Lohka MJ (1991). A distinct vesicle population targets membranes and pore complexes to the nuclear envelope in Xenopus eggs. J Cell Biol 112, 545–556.

Wilson KL, Newport J (1988). A trypsin-sensitive receptor on membrane vesicles is required for nuclear envelope formation in vitro. J Cell Biol 107, 57–68.

Worman HJ, Yuan J, Blobel G, Georgatos SD (1988). A lamin B receptor in the nuclear envelope. Proc Natl Acad Sci USA 85, 8531–8534.

Wu JQ, Guo JY, Tang W, Yang CS, Freel CD, Chen C, Nairn AC, Kornbluth S (2009). PP1-mediated dephosphorylation of phosphoproteins at mitotic exit is controlled by inhibitor-1 and PP1 phosphorylation. Nat Cell Biol 11, 644–651.

Yang L, Guan T, Gerace L (1997). Integral membrane proteins of the nuclear envelope are dispersed throughout the endoplasmic reticulum during mitosis. J Cell Biol 137, 1199–1210.

Ye Q, Callebaut I, Pezhman A, Courvalin JC, Worman HJ (1997). Domain-specific interactions of human HP1-type chromodomain proteins and inner nuclear membrane protein LBR. J Biol Chem 272, 14983–14989.

Ye Q, Worman HJ (1994). Primary structure analysis and lamin B and DNA binding of human LBR, an integral protein of the nuclear envelope inner membrane. J Biol Chem 269, 11306–11311.

Zhang C, Clarke PR (2000). Chromatin-independent nuclear envelope assembly induced by Ran GTPase in Xenopus egg extracts. Science 288, 1429–1432.