INTRODUCTION

Eukaryotic chromosomes are spatially organized within the nucleus, the boundary of which is formed by the nuclear envelope. The envelope consists of a double membrane, nuclear pore complexes (NPCs), and the nuclear lamina (for reviews see Marshall and Wilson, 1997; Nigg, 1997). The outer membrane is continuous with the rough endoplasmic reticulum (ER), and joins the inner membrane to form pores for the NPCs. Several proteins anchored to the inner membrane interact with both the chromatin and the nuclear lamina, a polymeric network of intermediate filaments composed of A-type and B-type lamins (for reviews see Gerace and Burke, 1988; Moir et al., 1995; Gant and Wilson, 1997).

Although the nucleus provides a physical framework for chromosomal organization, it is highly dynamic (reviewed by Forbes and Johnson, 1995). The nuclear envelope of higher eukaryotes breaks down at the end of mitotic prophase, allowing for the events of mitosis, and is reconstructed around the chromosomes during anaphase and telophase, re-establishing the architecture of the functional nucleus. Disassembly and reassembly of the nuclear envelope is crucial for the progression of mitosis in higher eukaryotic cells. Membrane-associated proteins that bind chromosomes are proposed to play an important role in nuclear assembly (reviewed by Gant and Wilson, 1997). The chromatin binding properties of these proteins seems to be regulated by cell-cycle dependent modifications, such as phosphorylation (Pfaller and Newport, 1995; Moos et al., 1996), since the nuclear envelope does not assemble around metaphase chromosomes.

We determined the times when the nuclear membrane, nuclear pore complex (NPC) components, and nuclear import function were recovered during telophase in living HeLa cells. Simultaneous observation of fluorescently-labeled NLS-bearing proteins, lamin B receptor (LBR)-GFP, and Hoechst33342-stained chromosomes revealed that nuclear membranes reassembled around chromosomes by 5 minutes after the onset of anaphase (early telophase) whereas nuclear import function was recovered later, at 8 minutes. GFP-tagged emerin also accumulated on chromosomes 5 minutes after the onset of anaphase. Interestingly, emerin and LBR initially accumulated at distinct, separate locations, but then became uniform 8 minutes after the onset of anaphase, concurrent with the recovery of nuclear import function. We further determined the timing of NPC assembly by immunofluorescence staining of cells fixed at precise times after the onset of anaphase. Taken together, these results showed that emerin, LBR, and several NPC components (RanBP2, Nup153, p62), but not Tpr, reconstitute around chromosomes very early in telophase prior to the recovery of nuclear import activity.

Key words: Nuclear envelope, Mitosis, Live analysis, Nuclear transport, Chromosome decondensation

Live fluorescence imaging reveals early recruitment of emerin, LBR, RanBP2, and Nup153 to reforming functional nuclear envelopes

Tokuko Haraguchi1,2,*, Takako Koujin1, Tomohiro Hayakawa1,2, Toru Kaneda1,3, Chihiro Tsutsu1, Naoko Imamoto4, Chihiro Akazawa5, Jun Sukegawa6, Yoshihiro Yoneda4,7 and Yasushi Hiraoka1,2

1Kansai Advanced Research Center, Communications Research Laboratory, and CREST Research Project, Japan Science and Technology Corporation, 588-2 Iwaoka, Iwaoka-cho, Nishi-ku, Kobe 651-2492, Japan
2Department of Biology, Graduate School of Science, Osaka University, 1-16 Machikaneyama, Osaka 560-0043, Japan
3Olympus Optical Co., Ltd, Hachioji, Japan
4Department of Cell Biology and Neurobiology, Graduate School of Medicine, Osaka University, 2-2 Yamada-oka, Osaka 565-0871, Japan
5Department of Neurochemistry, National Institute of Neuroscience of Japan, NCNP, 4-1-1 Ogawahigashi, Kodaira 187-8502, Japan
6School of Medicine, Tohoku University, 2-1 Seiryou-cho, Aoba-ku, Sendai, Japan
7Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565-0871, Japan

*Author for correspondence (e-mail: tokuko@crl.go.jp)

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SUMMARY

We determined the times when the nuclear membrane, nuclear pore complex (NPC) components, and nuclear import function were recovered during telophase in living HeLa cells. Simultaneous observation of fluorescently-labeled NLS-bearing proteins, lamin B receptor (LBR)-GFP, and Hoechst33342-stained chromosomes revealed that nuclear membranes reassembled around chromosomes by 5 minutes after the onset of anaphase (early telophase) whereas nuclear import function was recovered later, at 8 minutes. GFP-tagged emerin also accumulated on chromosomes 5 minutes after the onset of anaphase. Interestingly, emerin and LBR initially accumulated at distinct, separate locations, but then became uniform 8 minutes after the onset of anaphase, concurrent with the recovery of nuclear import function. We further determined the timing of NPC assembly by immunofluorescence staining of cells fixed at precise times after the onset of anaphase. Taken together, these results showed that emerin, LBR, and several NPC components (RanBP2, Nup153, p62), but not Tpr, reconstitute around chromosomes very early in telophase prior to the recovery of nuclear import activity.

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Although the nucleus provides a physical framework for chromosomal organization, it is highly dynamic (reviewed by Forbes and Johnson, 1995). The nuclear envelope of higher eukaryotes breaks down at the end of mitotic prophase, allowing for the events of mitosis, and is reconstructed around the chromosomes during anaphase and telophase, re-establishing the architecture of the functional nucleus. Disassembly and reassembly of the nuclear envelope is crucial for the progression of mitosis in higher eukaryotic cells. Membrane-associated proteins that bind chromosomes are proposed to play an important role in nuclear assembly (reviewed by Gant and Wilson, 1997). The chromatin binding properties of these proteins seems to be regulated by cell-cycle dependent modifications, such as phosphorylation (Pfaller and Newport, 1995; Moos et al., 1996), since the nuclear envelope does not assemble around metaphase chromosomes.

Two integral inner membrane proteins, the lamin B receptor (LBR; Worman et al., 1988; Bailer et al., 1991) and lamin associated polypeptide2 (LAP2; Foisner and Gerace, 1993; Furukawa et al., 1995), bind chromosomes in vitro (Worman et al., 1990; Kawahira et al., 1997; Foisner and Gerace, 1993; Furukawa et al., 1997) and associate with chromosomes at the
earliest stage of nuclear envelope assembly (Bodoor et al., 1999). The sea urchin LBR homologue is likely to mediate targeting of membranes to sperm chromatin during assembly of the nuclear envelope of pronuclei, since depletion of the LBR homologue-containing vesicles significantly reduced nuclear membrane assembly around chromatin (Collas et al., 1996). Furthermore, vesicles reconstituted after immunodepletion of LBR are less active in binding chromosomes (Pyropasopoulou et al., 1996). However, LBR has not been directly demonstrated to mediate envelope assembly in mammalian somatic cells.

Emerin is another integral inner membrane protein (Nagano et al., 1996; Manilal et al., 1996; Yorifugi et al., 1997) and is responsible for the human inherited disease called Emery-Dreifuss muscular dystrophy (Bione et al., 1994). The N-terminal region of emerin has sequence homology to the N-terminal region of LAP2 isoforms α, β, and γ (Manilal et al., 1996; Tsuchiya et al., 1999; Gant et al., 1999) which possess chromatin binding activity (Furukawa et al., 1997), but it is not known if emerin binds chromosomes. On the other hand, emerin is reported to bind lamins (Fairley et al., 1999). Biochemical nuclear fractionation suggested a tight binding between emerin and the nuclear lamina (Squarzoni et al., 1998). Emerin association with nuclear components is also suggested by the diffusional mobility analysis using emerin fused with green fluorescent protein (GFP; Östlund et al., 1999) and by the localization analysis of a series of truncated emerins (Tsuchiya et al., 1999). However, whether emerin is required for nuclear envelope assembly in telophase is unknown.

While the binding partners for emerin have not been reported, LBR was reported to bind human homologues of Drosophila HP1 (Ye and Worman, 1996; Ye et al., 1997), a heterochromatin protein originally identified as a suppressor of position effect varigation (James and Elgin, 1986; Eissenberg et al., 1990). LBR is now believed to play a role in attaching the nuclear envelope to heterochromatin during interphase through its binding to HP1s. In humans, three HP1 proteins that arise from different genes, termed HP1α, HP1β, and HP1γ, have been described (Singh et al., 1991; Saunders et al., 1993; Ye and Worman, 1996); these isoforms were designated HP1α, HP1β, and HP1γ, respectively. It is as yet unknown whether the human HP1 homologues are involved in the assembly of the nuclear envelope in telophase.

Reassembly of the NPCs during telophase re-establishes the nuclear envelope as a nucleo-cytoplasmic boundary, which selectively transports macromolecules between the nucleus and the cytoplasm (reviewed by Davis, 1995; Pante and Abei, 1993; Forbes, 1992; Gerace, 1992; Burke, 1990). Based on its large molecular mass of 125,000 kDa, the vertebrate NPC is believed to be composed of multiple copies of at least 50 distinct proteins (nucleoporins; Yang et al., 1998). Little is known about how NPCs assemble, or what constitutes the minimum essential structure for selective transport function.

Here we studied the properties of four nucleoporins: RanBP2, p62, Nup153, and Tpr. RanBP2 is a protein containing FG repeat motifs and four binding sites for the GTPase, Ran (Yokoyama et al., 1995). RanBP2 is localized to the cytoplasmic filaments of NPCs (Yokoyama et al., 1995; Wilken et al., 1995) and is believed to be essential for nuclear import activity since it provides the docking site for importin β complexed with an NLS-bearing substrate and importin α (Delphin et al., 1997). Interestingly, RanBP2 has eight zinc-finger motifs similar to those of Nup153, and these motifs bind DNA in a Zn2+-dependent manner (Yokoyama et al., 1995). Nucleoporin p62 is a glycoprotein localized to the central plug of the NPC and is required for translocation of the importin α/NLS complex through the NPC (Davis and Blobel, 1986, 1987; Finlay and Forbes, 1990; Sterne-Marr et al., 1992; Moor and Blobel, 1992; Paschal and Gerace, 1995). After translocation, the importin α/NLS complex detaches from the NPC whereas importin β remains associated with a basket structure on the nuclear side of the NPC (Ullman et al., 1999). Nup153, with repeats of a zinc finger motif (Sukeyawa and Blobel, 1993), is thought to be a component of this basket structure (Pante et al., 1994). Tpr, a large coiled-coil protein, is a ubiquitous component of the intranuclear NPC-attached filaments (Cordes et al., 1997), which may extend into the nuclear interior (Paddy, 1998). Although Nup153 and Tpr are both major physiological binding sites for importin β in the nucleus (Shah et al., 1998), it is currently unknown if either Nup153 or Tpr is required for nuclear import. On the other hand, it has been shown that both Nup153 and Tpr are important for nuclear export (Bastos et al., 1996; Bangs et al., 1998).

Nuclear envelope assembly has been extensively studied using Xenopus egg extracts, which support both disassembly and reassembly of nuclei in vitro (Wilson and Wiese, 1996). Our goal here was to explore how functional nuclear structures are re-established in living mammalian somatic cells. Using a fluorescence microscope system that is capable of recording multi-wavelength, three-dimensional images of living mammalian cells over time (Haraguchi et al., 1997, 1999), we examined nuclear reassembly and recovery of nuclear import activity in living HeLa cells. By imaging fluorescently labeled proteins in living cells, we can determine the temporal sequence of nuclear assembly, while simultaneously monitoring for nuclear functions such as nucleocytoplasmic transport. Two integral membrane proteins, LBR and emerin, fused with jellyfish green fluorescent protein (GFP) were used to mark the nuclear envelope, and NLS-bearing proteins conjugated with red (TRITC) fluorescence were used to detect nuclear transport activity. The temporal sequence of assembly of NPC components RanBP2, p62, Nup153, and Tpr was determined by immunofluorescence staining of cells that had been followed in the living state and fixed at precise times during live observation. Our results show that emerin, LBR, and all tested components of the NPC, except for Tpr, are incorporated into the nuclear envelope before import activity is detected.

MATERIALS AND METHODS

Cells and reagents
HeLa cells (Gey et al., 1952) were obtained from the Riken Cell Bank (Tsukuba Science City, Tsukuba, Japan). Hoechst 33342 was purchased from Calbiochem (La Jolla, CA). Emerin-GFP fusion constructs were prepared as previously described (Nagano et al., 1996; Tsuchiya et al., 1999). Rhodamine-NLS-BSA, NLS-allophycocyanin (NLS-APC; Imamoto et al., 1995), and GST-NLS-GFP were also prepared by methods reported previously (Yokoya et al., 1999).
Fluorescein-conjugated nucleoplasmin and rhodamine-conjugated nucleoplasmin were prepared by a minor modification of methods described previously: briefly, nucleoplasmin was purified from Xenopus laevis oocytes using the method described by Dingwall et al. (1982), except that the extraction by organic solvent was omitted; the purified nucleoplasmin was then conjugated with fluorescein isothiocyanate or rhodamine succinimidyl ester (Newmeyer et al., 1986). Two monoclonal antibodies were obtained commercially: mAb414 (Babco, Richmond, CA), against the XAFXFG motif-containing domain common to certain nucleoporins (Davis and Blobel, 1986; Sukegawa and Blobel, 1993), and mAb203-37 (Matritech, Cambridge, MA), against Tpr (Cordes et al., 1997). Rabbit polyclonal antibody 552 (Yokoyama et al., 1995) against RanBP2 was the generous gift of Dr T. Nishimoto (Kyushu University, Japan). Anti-emerin antibodies (Yorifuji et al., 1997) were generous gifts from Drs Arahata and Tsuchiya (National Institute of Neuroscience of Japan, Japan). Rabbit polyclonal antibody RF28 against Nup153 was prepared by injecting rabbits with the bacterially-expressed zinc finger domain of Nup153 (Sukegawa and Blobel, 1993) that was purified using HisBind Resin (Novagen, Madison, WI). Antibodies to Nup153 were affinity purified by using AminoLink Coupling Gel (Pierce, Rockford, IL) immobilized with the protein. The purified antibody was dialeyzed against Tris-buffered saline (20 mM Tris-HCl, pH 7.5, 150 mM NaCl) before use.

Construction of lamin B receptor-GFP fusion

The coding region of the lamin B receptor was cloned from mRNA isolated from HeLa cells by RT-PCR using the SuperScript Plasmid System Kit (Gibco BRL, Rockville, MD). Complementary DNA was produced from the mRNA fraction using the following primers: CGTCTCGAGATGCGAGAATTGAC and GCCGATCCGTAGATGTAGGAAATACG. Prior to PCR amplification, the DNA sequence of the lamin B receptor cDNA isolated from HeLa cells was determined by DNA sequencer ABI377 (Applied Biosystems, Norwalk, CT). The DNA sequence of the fusion plasmid was confirmed using ABI377 DNA sequencer (Applied Biosystems, Norwalk, CT).

Microscope system setup

For fluorescence imaging of living cells, a Delta Vision (Applied Precision Inc. Seattle, WA) microscope system was used. Details of the microscope system have been described previously (Haraguchi et al. 1997); briefly, a Pelletier-cooled CCD camera (Photometrics Ltd, Tucson, Arizona), with a 1317×1035 pixel CCD chip (KAF1400), is attached to the Olympus inverted microscope IX70; microscope lamp shutter, focus movement, CCD data collection, and filter combinations are controlled by a Silicon Graphics Indigo2 workstation. For temperature control during microscopic observation, the microscope system is located in a custom-made, temperature-control room where the temperature can be controlled in a range from 10°C to 50°C with a precision of 0.1°C. The computer and other control units are placed outside the room and control the microscope remotely. The microscope is also equipped with micromanipulator 5171 and microinjector 5242 (Eppendorf, Hamburg, Germany) for microinjecting cells.

Fluorescence microscopy in living cells

Preparation of fluorescently-stained living cells for microscopic observation was described previously (Haraguchi et al., 1997, 1999): Briefly, cells plated on a 35 mm glass-bottom culture dish were stained with 100 ng/ml of Hoechst 33342 (a DNA specific fluorescent dye) for 5 to 30 minutes to stain chromosomes, then cultured in phenol red-free DME medium supplemented with 10% fetal bovine serum in a CO2 incubator for at least 30 minutes before microscopic observation. For microscopic observation, Hepes pH 7.3 (final concentration, 20 mM) was used to avoid the need for CO2 gas. Fluorescently tagged NLS-bearing proteins were microinjected into Hoechst 33342-stained cells to monitor nuclear envelope transport activity and chromosome behavior simultaneously. To fluorescently stain the nuclear envelope, plasmid DNA bearing either the lamin B receptor-GFP or GFP-emerin coding sequence was microinjected into nuclei one day before microscopic observation. Microinjection into the nucleus gave better results than several chemical transfection methods, including Superfect, both in expression of the GFP fusion construct and in cell morphology. However, recent experiments with new transfection methods such as Lipofectamin Plus (Gibco BRL) and Cell Fect (Pharmacia Biotech, Sweden) resulted in relatively higher efficiency of transfection and good cell morphology, suitable for conducting live analysis.

Fluorescently stained living cells were imaged using an Olympus oil immersion objective lens with a high numerical aperture (0.95/NA=1.35). Each pixel represents 0.17 μm in the specimen plane. For wavelength switching during data collection, excitation and barrier filters are mounted on revolving wheels controlled by a Silicon Graphics workstation. A single dichroic mirror with quadruple-band pass properties (Chroma Technology, Brattleboro, Vermont) was used to eliminate significant displacement of images during wavelength switching, and thus no further alignment was necessary (Hiraoka et al., 1991). An excitation filter with a narrow peak at 380 nm (Chroma Technology, Brattleboro, Vermont) was used for live imaging of cells stained with Hoechst 33342.

Indirect immunofluorescence staining of the cells after live observation

Live observation of fluorescently stained cells was performed as described above; after the metaphase-anaphase transition live images were collected every one minute until fixation. We tested several fixation methods and compared images of fluorescent NLS-bearing proteins taken immediately before and after the addition of fixative. Glutaraldehyde mixed with formaldehyde fixed cells quickly, whereas fixation using formaldehyde alone required several minutes. Methanol also fixed cells quickly. Consequently, we used glutaraldehyde plus formaldehyde for immunofluorescence staining with all anti-NPC antibodies except the anti-Nup153 antibody, which was fixed with methanol. Briefly, a mixture of 37% formaldehyde and 2% glutaraldehyde was added to the culture medium to a final concentration of 3.7% and 0.2%, respectively, just after the last live image was taken, and the cells were incubated in the fixative for 10 minutes at room temperature. The fixed cells were then treated twice with 0.1% sodium borohydride for 15 minutes to block the active residues of unreacted glutaraldehyde. Finally the cells were permeabilized with 0.2% Triton X-100 in PBS for 5 minutes at room temperature. Some of the antibodies gave a higher background staining with this method than with formaldehyde alone or methanol fixation. Methanol fixation was used for anti-Nup153 antibody, since immunofluorescent signals were not obtained with glutaraldehyde/ formaldehyde fixation. In this case, the culture medium was removed before adding cooled methanol (~30°C), and the cells were incubated in methanol for 5 minutes at room temperature; the sodium borohydride blocking and Triton permeabilization steps were omitted.

After fixation, cells were incubated for 1 hour at room temperature with blocking buffer (1% BSA dissolved in PBS) to prevent non-specific binding of the antibody. Then, a primary antibody was added to cell culture dilution of 1:300 and incubated for 20 hours at 4°C; the primary antibodies used were polyclonal antibodies specific for Ran BP2 and Nup153, a monoclonal antibody specific for Tpr (mAb203-
...and another monoclonal antibody for p62 and two other O-GlcNAc-modified nucleoporins (mAb414). Cells were then washed four times with 2 ml PBS and stained with a fluorescent secondary antibody, Texas Red-conjugated anti-rabbit IgG or Texas Red-conjugated anti-mouse IgG, at a dilution of 1:200 for 3-4 hours at room temperature. Finally, the cells were washed three times with PBS, incubated with Hoechst 33342 (0.5 μg/ml) for 10 minutes, and then incubated sequentially with PBS containing 2.5% 1,4-diazabicyclo-2,2,2-octane (DABCO) and 20, 40, 60, 80, and 90% glycerol. The cells were mounted in 95% glycerol, containing 1 mg/ml para-phenylene diamine as an anti-fading reagent, and imaged by fluorescence microscope as described below.

The details of the microscope system have been described previously (Hiraoka et al., 1991): briefly, fluorescent microscopic images were obtained with an Olympus microscope IMT-2 using an oil immersion objective lens (SPlanApo 60UV, NA=1.4) and high-selectivity filters for Hoechst 33342, fluorescein, rhodamine (or Texas Red), and Cy-5 (Chroma Technology). Serial optical section data (15-30 focal planes at 0.5 μm intervals) for DNA, the NLS-bearing proteins (GFP-NLS or NLS-APC), and the NPC components were collected on a Peltier-cooled charge-coupled device (Photometrics) and computationally processed by a three-dimensional deconvolution method (Agard et al., 1989).

Western blotting
HeLa cells (1×10⁴) were collected by scraping and dissolved in a 1% SDS sample buffer in the presence of protease inhibitors (1 mM PMSF, 1 mM EDTA, 10 μg/ml leupeptin, 10 μg/ml pepstain, 1 μg/ml aprotinin and 10 μg/ml N-tosyl-L-phenylalanine chloromethyl ketone (TPCK)) by boiling for 5 minutes. Ten micrograms of total protein from the HeLa cell extract was loaded onto each lane of an SDS 10-20% gradient polyacrylamide gel. After electrophoresis, proteins were transferred to a PVDF membrane using a semi-dry blotting system (Atto Corp., Tokyo, Japan) and stained using the Immunoblotting ABC-POD kit (Wako, Osaka, Japan).

RESULTS
Visualization of nuclear import of NLS-bearing proteins in living cells
We first examined the behavior of two well-characterized NLSs, the monopartite SV40 T-antigen NLS and the bipartite nucleoplasmin NLS, in living HeLa cells using fluorescence microscopy as described in Materials and Methods. The monopartite NLS, located on a GST-GFP chimera (designated NLS-GFP), was microinjected into the cytoplasm of living HeLa cells previously stained with the DNA specific fluorescent dye Hoechst 33342. Double-wavelength fluorescence imaging showed that NLS-bearing proteins migrated into the nucleus immediately after microinjection (Fig. 1A). Similar results were obtained using two other constructs with the monopartite NLS (rhodamine-NLS-BSA and NLS-APC) and with constructs bearing the bipartite NLS (fluorescein-nucleoplasmin and rhodamine-nucleoplasmin; data not shown). On the other hand, GST-GFP constructs that lacked an NLS remained in the cytoplasm (Fig. 1B). In addition, microinjection of wheat germ agglutinin (WGA), an inhibitor of active nuclear transport (Yoneda et al., 1987), retained the NLS-GFP in the cytoplasm (Fig. 1C). These results demonstrated that the nuclear fluorescence which accumulated in living cells was due to NLS-dependent active nuclear transport and not to passive diffusion of the proteins into the nucleus.

NLS-bearing proteins were also imported successfully in cells treated with vinblastine at a concentration of 10 μg/ml for 10.5

Fig. 1. NLS-dependent nuclear migration of fluorescently-labeled protein in living cells. (A) NLS-GFP was microinjected into the cytoplasm of living HeLa cells and imaged every 30 seconds at 37°C. (B) GFP without an NLS was microinjected into the cytoplasm of living HeLa cells. Images were obtained every 30 minutes at 37°C; the images at 0 and 90 minutes after microinjection are shown. (C) NLS-GFP and 2 mg/ml WGA were microinjected into the cytoplasm of living HeLa cells. Images were obtained every 10 minutes at 26°C; the image at 90 minutes after microinjection is shown. The image data are representative of 6 independent experiments for A, 3 for B and 3 for C. Bar, 10 μm.

Fig. 2. The nuclear envelope localization of LBR-GFP in HeLa cells. HeLa cells transiently expressing LBR-GFP (middle) were fixed with 3.7% formaldehyde and co-stained for chromosomes with Hoechst33342 (left) and lamin B (right) by indirect immunofluorescence staining. Bar, 10 μm.
Reformation of functional nuclear envelopes

hours. This treatment depolymerizes microtubules and produces tubulin paracrystals, resulting in complete removal of tubulin dimers from the cytoplasm (Haraguchi et al., 1997; Takanari et al., 1990; Starling, 1976; Fujiwara and Tilney, 1975). Under these conditions, NLS-GFP or fluorescein-nucleoplasmin microinjected into the cytoplasm migrated into the nucleus at the same rate as in untreated cells (data not shown), demonstrating that the nuclear migration of NLS-bearing proteins does not require cellular activities mediated by microtubules.

Fig. 3. Dynamics of chromosomes, the nuclear envelope, and NLS-bearing proteins during anaphase and telophase in living HeLa cells. Living cells were fluorescently labeled with Hoechst33342 (left), LBR-GFP (second from the left), and NLS-APC (third from the left) to stain chromosomes, the nuclear envelope, and NLS-bearing proteins, respectively. The merged images (right) represent chromosomes (blue), LBR-GFP (green), and NLS-APC (red). The numbers to the left of each image show the time in minutes after the metaphase-anaphase transition. Live data were obtained at 37°C. The image data are representative of 5 independent experiments. See text for details. Bar, 10 μm.

Recovery of the nuclear envelope and its nuclear import activity during telophase

To examine the cell cycle behavior of nuclear proteins, fluorescent NLS-bearing proteins were imaged in living HeLa cells during mitosis. Chromosomes were stained with Hoechst 33342 to monitor the cell cycle stage. Four different fluorescently labeled NLS-bearing proteins (NLS-GFP, NLS-APC, rhodamine-NLS-BSA, and rhodamine-nucleoplasmin) were used to visualize nuclear import activity. The dynamic
Fig. 4. Inhibition of telophase nuclear import by WGA. The behavior of NLS-GFP in the absence (A) and presence (B) of WGA. NLS-GFP, with or without WGA (2 mg/ml) in PBS, was microinjected into living HeLa cells. Microinjection and observation were performed at 26°C. The numbers to the left of each image show the time in minutes after microinjection. The image data are representative of 2 independent experiments for (A) and 6 for (B). Bar, 10 μm.

Fig. 5. Quantitation of nuclear import activity after the metaphase-anaphase transition. Fluorescence intensity of NLS-bearing fluorescent probes in the nuclear (open circle) and cytoplasmic (triangle) regions (5 square pixels) was determined from live images, such as those depicted in Fig. 3. The subtracted intensity (nucleus minus cytoplasm; closed circles) was plotted to show the net intensity of NLS-bearing fluorescent probes in the nuclear region. The axial bar represents fluorescence intensity and the horizontal bar represents the time in minutes after the metaphase-anaphase transition. (A) Quantitation based on image data in Fig. 3. (B) Quantitation from an independent experiment in which rhodamine-conjugated BSA-NLS was used as the NLS substrate; corresponding image data are not shown. (C and D) Quantitation from two independent experiments in which rhodamine-conjugated nucleoplasmin was used as the fluorescent NLS-bearing transport substrate; corresponding image data are not shown.
behavior of chromosomes and nuclear proteins was followed throughout the mitotic cell cycle: fluorescent NLS-bearing proteins microinjected into the cytoplasm rapidly migrated into the nucleus and were retained there during interphase of the cell cycle (Fig. 1A); the fluorescent NLS-bearing proteins were located in the cytoplasm during metaphase, as expected, and were visibly re-concentrated around the chromosomes after cytokinesis began (Fig. 3). All four NLS-bearing proteins examined behaved similarly. We concluded that the accumulation of fluorescent NLS-bearing proteins in reassembling telophase nuclei was an accurate assay for the assembly of functional nuclear envelopes.

To determine when nuclear import activity was recovered with respect to nuclear envelope reformation, we simultaneously imaged a third molecule, the inner nuclear membrane protein LBR fused to GFP (LBR-GFP). The plasmid encoding the LBR-GFP fusion was microinjected into the nucleus; the expressed LBR-GFP was correctly localized to the nuclear envelope as marked by lamin B (Fig. 2), although some LBR-GFP was present in the cytoplasm, possibly associated with the endoplasmic reticulum (Fig. 2). By time-lapse imaging at one minute intervals, we precisely determined the time of the metaphase-anaphase transition, defined by the onset of chromosome segregation, and then followed nuclear envelope assembly and nuclear transport activity as the cells went through telophase and cytokinesis. LBR-GFP was visibly concentrated around the chromosomes 4 minutes after the metaphase-anaphase transition (arrow in Fig. 3), whereas the apparent nuclear transport of NLS-allophycocyanine was first evident 7-8 minutes after the metaphase-anaphase transition (arrowhead in Fig. 3). Similar results were found using rhodamine-NLS-BSA (data not shown), with nuclear import activity (detectable at 8-9 minutes) occurring several minutes after LBR-GFP colocalized with the chromosomes (5 minutes;
Chromosome decondensation occurred only after the recovery of nuclear import activity (not visible in these timepoints, data not shown).

To eliminate the possibility that the NLS-bearing proteins were sequestered into the reforming nucleus rather than actively imported through the NPC, we examined the nuclear accumulation of NLS-GFP in the presence of WGA. Living HeLa cells stained with Hoechst 33342 were microinjected with NLS-GFP in interphase, and then placed on the microscope stage where they underwent mitosis; WGA was microinjected into the cells during anaphase before nuclear accumulation of NLS-GFP was detected. These experiments were carried out at a lower temperature, 26°C, because the WGA solution was too viscous to be microinjected through an injection needle at 37°C; thus, the progression of mitosis was slower than in experiments done at 37°C. In the absence of WGA, NLS-GFP was imported into the nucleus in telophase, albeit at a slower rate (Fig. 4A, 20min; compare with Fig. 3), and chromosomes decondensed in the subsequent interphase (Fig. 4A, 60min). On the other hand, nuclear import of NLS-GFP was not detected up to 90 minutes in the cells that were microinjected with WGA (Fig. 4B). Therefore, we concluded that the observed accumulation of NLS-bearing proteins into the reforming nucleus was due to active transport through the NPC. Intriguingly, chromosomes remained condensed in those cells with no nuclear import activity (Fig. 4B, 90min).

Nuclear import was also measured quantitatively by plotting the fluorescence intensity of the NLS-bearing protein in the

Fig. 7. The ‘core’ localization of endogenous emerin in telophase nuclei. Three examples (A-C, D-G, and H-L) of dividing HeLa cells are shown. HeLa cells were fixed with methanol at −30°C for 10 minutes (A-C and H-L) or with 3.7% formaldehyde for 10 minutes at room temperature (D-G), and stained by indirect immunofluorescence method with an emerin-specific polyclonal antibody as the primary antibody and rhodamine-conjugated (B) or Cy3-conjugated (F and J) anti-rabbit antibody as the secondary antibody; chromosomes were stained with Hoechst 33342 (A, D and H); LBR was stained by transient expression of the LBR-GFP fusion construct (E and I); microtubules were stained with an anti-α-tubulin antibody TAT1 (Woods et al., 1989) as the primary antibody and Cy5-conjugated anti-mouse IgG as the secondary antibody. In the merged images (C, G and L), green represents emerin, blue represents LBR-GFP, and red represents chromosomes stained with Hoechst 33342 in C or microtubules in L. Bar, 10 μm.
cytoplasm (open triangle) and nucleus (open circle), as a function of time after the metaphase-anaphase transition. Accumulation in the nucleus (closed circles) was estimated by subtracting the cytoplasmic values from the nuclear values. NLS-APC and rhodamine-NLS-BSA accumulated in the nucleus at 8 minutes (Fig. 5A) and 9 minutes (Fig. 5B), respectively, after the metaphase-anaphase transition, consistent with our non-quantitative imaging results (Fig. 3). A bipartite NLS-bearing protein, nucleoplasmin conjugated with rhodamine, gave similar results (Fig. 5C,D). The statistical analysis of the timing of nuclear accumulation of NLS-bearing proteins in live cells is summarized in Table 1. We concluded that the timing of the recovery of nuclear

Fig. 8. (A) Proposed localization of each antigen (reviewed by Nigg, 1997). (B) Western immunoblotting of HeLa cell proteins was performed using specific antibodies to distinct NPC components: (1) mAb414 against the p62 complex, (2) anti-552 against RanBP2, (3) RF28 against Nup153, and (4) mAb203-37 against Tpr. The bars to the left of each lane represent, from the top, 200, 116, 97.4, 66, 45, 31, and 21.5 kDa.

Fig. 9. Indirect immunofluorescence staining after in situ fixation of cells observed in the living state. (Upper panel) Time-lapse fluorescence images of living cells, visualizing chromosomes stained with Hoechst 33342 (left frames) and NLS-GFP (right frames). Living cells were cultured on a microscope stage at 37°C; images were taken every minute after the metaphase-anaphase transition until the cells were fixed at 5 minutes. The images at 0-5 minutes were taken before fixation; the bottom row (unmarked) shows the same cell immediately after fixation. The numbers to the left of each image show the time in minutes after the metaphase-anaphase transition. (Lower panel) The same cell shown in the upper panel was further analyzed by immunofluorescence staining using antibodies against the p62 complex.
transport activity in HeLa cells is relatively constant; both monopartite and bipartite NLS-bearing proteins began to accumulate in the nucleus approximately 8 minutes after the metaphase-anaphase transition.

Three-dimensional analysis of LBR-GFP and GFP-emerin in living cells

We then determined when GFP-emerin and LBR-emerin were incorporated into assembling nuclear envelopes and visualized this process in three dimensions in living HeLa cells. Fig. 6A shows an example of time-lapse, three-dimensional images of LBR-GFP recorded in a living cell. LBR-GFP was localized densely to the peripheral regions of the chromosomes 4 minutes after the metaphase-anaphase transition, but sparsely to the central chromosome core regions close to the mitotic spindle (shown by the arrow in Fig. 6A). At 7-8 minutes after the metaphase-anaphase transition, LBR-GFP was evenly distributed around the chromosomes, suggesting that by this time the chromosomes were completely enclosed by the nuclear membrane. The timecourse of GFP-emerin recruitment to chromosomes was as rapid as that of LBR-GFP (Fig. 6B). GFP-emerin appeared around chromosomes at 4 minutes, and was evenly distributed around the chromosomes by 7-8 minutes after the metaphase-anaphase transition. However, unlike LBR-GFP, GFP-emerin was rather enriched in the central chromosome core region in which LBR-GFP was relatively sparse (compare 5 and 6 minute timepoints in Fig. 6A and B). Interestingly, LBR-GFP and GFP-emerin became uniformly distributed around the chromosomes at the same time as nuclear transport activity was recovered.

The distinct localization of emerin at early timepoints was also seen for endogenous native emerin by indirect immunofluorescence staining (Fig. 7B,F,I); basically the same results were obtained in cells fixed with methanol and formaldehyde. Simultaneous staining of emerin and LBR confirmed their differential localization around the chromosomes in early telophase (Fig. 7E and F, I and J). The region of emerin enrichment relative to the mitotic spindle is shown in Fig. 7J and K. Microtubules do not seem to be necessary for localization of emerin behind the spindle as the emerin enrichment was observed when microtubules were depolymerized during anaphase (data not shown).

Reassembly of nuclear pore complexes during telophase

We wanted to explore the assembly pathway for NPCs and find out which major structural elements were associated with transport activity. We therefore examined four proteins located on very distant parts of the NPC (see Fig. 8A): p62, Ran BP2, Nup153, and Tpr (Nigg et al., 1997; Davis, 1995). Except for the anti-RanBP2 antibody, our antibodies each reacted with one major band on western blots of HeLa cells (Fig. 8B): anti-p62, anti-RanBP2, anti-Nup153, and anti-Tpr antibodies recognized bands of approximately 60 kDa, 360 kDa and 180 kDa, 170 kDa, and 270 kDa, respectively, as expected. These molecular

Table 1. Timing of nuclear import

<table>
<thead>
<tr>
<th>NLS-bearing protein</th>
<th>Minutes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monopartite SV40-NLS</td>
<td>8.0±0.4</td>
</tr>
<tr>
<td>GST-NLS-GFP</td>
<td>(n=13)</td>
</tr>
<tr>
<td>NLS-BSA-rhodamine</td>
<td>8</td>
</tr>
<tr>
<td>NLS-allophycocyanine</td>
<td>7</td>
</tr>
<tr>
<td>Bipartite NLS</td>
<td></td>
</tr>
<tr>
<td>Nucleoplasmin-rhodamine</td>
<td>7.5±0.6</td>
</tr>
</tbody>
</table>

Fluorescent NLS-bearing proteins were microinjected into interphase cells in an asynchronous population, and microinjected cells were placed on the microscope stage, where they underwent mitosis. Cell cycle stages were monitored by the morphology of chromosomes stained with Hoechst 33342; when a cell reached metaphase, images of that cell were taken every minute after the metaphase-anaphase transition. The cells were maintained at 37°C throughout the experiment.

*Time in minutes after the onset of anaphase chromosome segregation (average ± standard deviation). The value n in parentheses is the number of independent experiments; one cell was examined in each of the individual experiments.

RanBP2 antibody, our antibodies each reacted with one major band on western blots of HeLa cells (Fig. 8B): anti-p62, anti-RanBP2, anti-Nup153, and anti-Tpr antibodies recognized bands of approximately 60 kDa, 360 kDa and 180 kDa, 170 kDa, and 270 kDa, respectively, as expected. These molecular
masses are consistent with values reported previously (Davis and Blobel, 1986; Yokoyama et al., 1995; Sukegawa et al., 1993; Cordes et al., 1997).

To determine the precise timing of NPC assembly, we combined time-lapse analysis of fluorescent NLS-bearing proteins in living cells with fixation and indirect immunofluorescence staining for NPC components in the same cells (Figs 9-12). Cells undergoing live observation were fixed at precise times after the metaphase-anaphase transition and then stained by indirect immunofluorescence. This method is illustrated for a cell fixed 5 minutes after the metaphase-anaphase transition with glutaraldehyde/formaldehyde (Fig. 9); cells and chromosomal structures were fixed within one minute after the addition of fixative (compare the fluorescence images that were taken before and immediately after fixation at 5 minutes; see Materials and Methods for comparison of fixation methods). Fixing cells with methanol gave similar results except that NLS-GFP could not be fixed. In contrast, when formaldehyde alone was used for fixing cells, M-phase events were not arrested until 2-3 minutes after addition of the fixative. Thus, we used glutaraldehyde or methanol to fix cells in situ.

Using in situ fixation after live observation, we examined the temporal behavior of p62, RanBP2, Nup153, and Tpr. At 5-6 minutes after the metaphase-anaphase transition, fluorescent NLS-bearing proteins had not yet accumulated in the nucleus (Figs 10B, 11D and 12B), consistent with the results shown in Figs 3 and 9. Nevertheless, p62, Ran BP2, and Nup153 were already detectable as punctate staining around the chromosomes (Figs 10C and 11B; data not shown for p62). At all later timepoints shown, when nuclear transport was active, the signals for p62, Ran BP2, and Nup153 at the chromosome rim intensified (see Fig. 10F and I for an example of RanBP2; Fig. 11F and J for an example of Nup153). These results suggest that these three spatially-separate components of the NPC (p62, RanBP2, and Nup153) start assembling around the chromosomes coincident with the assembly of membranes containing LBR-GFP and GFP-emerin. In contrast to these NPC components, Tpr did not localize to the perichromosomal region until 9-11 minutes after the metaphase-anaphase transition (Fig. 12C, F, and I), well after nuclear transport was functional (Fig. 12E and H). The signal for Tpr at the nuclear rim intensified at later timepoints (Fig. 12L). These results strongly suggest that Tpr assembly is not required for the import of NLS-bearing proteins in the early telophase nucleus.

**DISCUSSION**

We demonstrated that proteins which harbor two different types of NLSs, the SV40 T antigen NLS (monopartite NLS) and the nucleoplasmin NLS (bipartite NLS), migrate into reassembling telophase nuclei at the same rate; we have also shown that their rate of migration into interphase nuclei is the same (Table 1 and Fig. 1A). This finding is consistent with monopartite and bipartite NLSs sharing common import machinery (reviewed by Nigg, 1997). The nuclear accumulation of fluorescently-tagged proteins provided a clear microscopic assay for functional NPCs reassembled in an enclosed nuclear envelope.

Using lamin B receptor-GFP and GFP-emerin, we then demonstrated that assembly of the nuclear envelope started at late anaphase to very early telophase, just after the contractile ring started pinching the plasma membrane. We also demonstrated that the p62 complex, RanBP2, and Nup153, but not Trp, started to assemble around chromosomes at the same time as LBR-GFP begin to accumulate around the chromosomes. And finally, our results show that nuclear import function, as determined by the nuclear accumulation of NLS-bearing proteins, appeared about 2-3 minutes later, coincident with apparent enclosure of chromosomes by the reformed nuclear envelope. This re-assembly timecourse of the telophase nuclear envelope is diagrammed in Fig. 13.

**LBR and emerin are recruited early to the reforming nuclear envelope**

Several lines of evidence support LBR playing a major role in targeting nuclear envelope precursor vesicles to chromosomes (Chaudhary and Courvalin, 1993; Poccia and Collas, 1997; Pyrpasopoulou et al., 1996; Ye and Worman, 1994). Using full length LBR fused with GFP (LBR-GFP), we found that assembly of the nuclear envelope started immediately after anaphase; similar results were obtained using a truncated form of LBR-GFP in COS-7 cells (Ellenberg et al., 1997). These results indicate that the chromosome-targeting of LBR-containing membranes is a very early event in telophase, and support the idea that LBR is important in the initial steps of membrane targeting to chromosomes.

Surprisingly, emerin may also have a role early in assembly since it associated with chromosomes as early as did LBR (Fig. 6). A previous immunofluorescence study showed that an emerin-related protein, LAP2β, also assembled around chromosomes in very early telophase (Feiser and Gerace, 1993; Furukawa et al., 1997). Emerin has significant amino acid sequence homology with LAP2 (Furukawa et al., 1995; Gant et al., 1999; Harris et al., 1995): 39 residues near the N-terminus contain 16 identities and 34 residues on the C terminus contain 14 identities (Dechat et al., 1998). It remains unknown whether emerin binds chromosomes in the early assembly of the nuclear envelope. Emerin seems to associate with intranuclear structures, since its diffusional mobility during interphase is slower in nuclear membranes than in ER membranes (Östlund et al., 1999).

We found that LBR-GFP and GFP-emerin associated with chromosomes at the same times: punctate staining in a portion of the perichromosomal region was visible starting at 5-6 minutes after the metaphase-anaphase transition, with uniform staining throughout the perichromosomal region seen about 2 minutes later (Fig. 6). Significantly, the initial localization of GFP-emerin on the assembling nuclear envelope differed from that of LBR-GFP: during cytokinesis, GFP-emerin was enriched in the central core region behind the spindle, whereas LBR-GFP was rather sparse in this region. Interestingly, the N-terminal 65 amino acids of emerin were sufficient for this ‘core’ localization (T. Haraguchi, unpublished observation). Both LAP2α and β, which share 39-residues with the N-terminal region of emerin, are also enriched at this ‘core’ region, as shown in previous reports (Dechat et al., 1998; Bodoor et al., 1999). We therefore deduce that the distinct localizations of LBR and emerin at early timepoints may reflect a non-uniform distribution of their binding partners on the
chromosome; emerin’s partners may be enriched at the central core region of chromosomes and LBR’s partners may be excluded from this region. This prediction can be tested in the future when the binding partners for emerin are identified.

While human HP1α and γ were reported as putative binding partners of LBR in interphase (Ye and Worman, 1996), they seem not to interact with LBR early in telophase because neither HP1α nor γ showed early recruitment to chromosomes in telophase; instead, HP1β, which has not been reported as a binding partner of LBR, appeared on chromosomes concurrently as LBR, implicating HP1β as having a possible role in reassembly of LBR early in telophase (T. Koujin and T. Haraguchi, unpublished results). However, HP1β distributed uniformly, and did not correlate with the non-uniform distribution of LBR observed at 5-7 minutes after the onset of chromosome segregation. The non-uniform distribution of LBR may be influenced by LBR binding to a different partner.

Because the distributions of LBR and emerin became uniform at the same time as nuclear transport activity was recovered, their uniform distribution may reflect a functionally mature state of the nuclear envelope. This leads to the possibility that LBR-containing membranes and emerin-containing membranes are initially targeted to separate regions of anaphase/telophase chromosomes and only become uniformly distributed after these membranes have fused completely to form the functional envelope. Alternatively, the uniform redistribution of LBR and emerin may depend on their subsequent association with late-assembling uniformly-distributed nuclear components such as lamins.

RanBP2, p62 and Nup153 are assembled in the NPC at very early telophase

By imaging living cells that contained fluorescent substrates of nuclear transport, we addressed when the reassembling NPC becomes functional for nuclear import. Surprisingly, NPC components which localize to distinct regions of the NPC complex (Fig. 8A) – the p62 complex (located at the central core), RanBP2 (forms the cytoplasmic filaments), and Nup153 (distal end of nucleoplasmic basket) – all associated visibly with chromosomes very early in telophase, at 5 minutes after the metaphase-anaphase transition. This timing of assembly is concurrent with the time when LBR-GFP begins to appear around the chromosomes, suggesting that major components of the NPC become concentrated into the nuclear envelope presumably by assembling into NPCs. However, despite the presence of a substantial amount of p62, RanBP2, and Nup153, nuclear import activity was not detectable until 7-8 minutes after the metaphase-anaphase transition. The delay between the appearance of these NPC components at the chromosome rim, and detectable import activity, could be explained as follows: first, the assembling nuclear membrane could have an inactive NPC, this NPC being activated a little later in telophase by some signal such as phosphorylation (Pfaller and Newport, 1995; Moos et al., 1996) or assembly of an additional component(s); or second, the assembling nuclear membrane could have active NPCs but the imported substrates could not be retained by the non-enclosed nucleus. The factor(s) that effect the initiation of nuclear import activity are not known so far.

We have determined the precise time, based on the timing of the onset of chromosome segregation, when RanBP2, p62,
Reformation of functional nuclear envelopes

and Nup153 assemble. The relative timing of various NPC components was recently reported, showing that assembly of NPC components occurs in the order of Nup153, POM121, p62, CAN/Nup214, and gp210/Tpr (Bodoor et al., 1999). Our results are consistent with theirs, including the conclusion that a portion of Nup153 seemed to associate with chromosomes beginning at metaphase (data not shown), suggesting that Nup153 is assembled earlier than the others. Interestingly, RanBP2 is also assembled very early, by 5 minutes after the onset of chromosome segregation. The biological significance and the structural basis of early recruitment of RanBP2 is unknown. One possible explanation is that RanBP2 may bind DNA utilizing its zinc finger motifs in this particular stage of the cell cycle, since the motifs bind DNA in vitro (Yokoyama et al., 1995). Note that these zinc finger motifs share homology with Nup153, although there is no direct evidence that early recruitment of Nup153 depends on these motifs. Another explanation is that the NPC components assemble rapidly in telophase of somatic cell division. It is not straightforward to compare our live data directly with the proposed structural intermediates of NPCs imaged in high resolution electron microscopy (Goldberg et al., 1997); however, these authors reported that after an apparent lag time ‘mature’ NPC structures assemble quite rapidly, in as little as 7 minutes in vitro (Wiese et al., 1997). The most puzzling result of the Bodoor et al. study is that gp210 accumulated in the peri-chroimosomal region as late as Tpr, raising questions

Fig. 12. Assembly timecourse of Tpr. Time-lapse analysis and the subsequent immunofluorescence staining were performed as described in the legend of Fig. 9 with glutaraldehyde/formaldehyde fixation. Images in each row were obtained from the same cell, representing chromosomes stained with Hoechst 33342 (A,D,G,J,M), NLS-GFP (B,E,H,K,N), and Tpr (C,F,I,L,O). The numbers to the left of each row show the time in minutes after the metaphase-anaphase transition. Bar, 10 μm.

Fig. 13. Summary of the timing of the nuclear reassembly process during telophase. The time line represents minutes after the metaphase-anaphase transition.

Order of Nuclear Reassembly

1. metaphase-anaphase transition
2. LBR, emerin, Ran BP2, p62 complex, Nup153
3. nuclear import, LBR/emerin uniform
4. Tpr
about its, and Tpr’s, role in the NPC. Our results show that Tpr localized to the newly assembled nucleus well after the appearance of nuclear import activity, consistent with the model that Tpr is not necessary for import activity (our results do not rule out the possibility that Tpr may enhance import activity or be involved in regulating import activity), and in fact may depend on functional NPCs to be imported and assembled. Thus, while Tpr is reported to bind importin α and importin β (Shah et al., 1998), suggesting that it may be involved in nuclear import, our observations, along with reports that Tpr is required for RNA export (Bangs et al., 1998) suggest that Tpr may be involved in nuclear export rather than, or in addition to, import.

Conclusions

In this report, we determined the precise timing, rather than the relative order, of nuclear events in telophase: nuclear envelope assembly, NPC assembly, and nuclear import recovery. Membrane targeting to the chromosomes is detectable at 5 minutes, NPC assembly at 5-7 minutes, and nuclear import activity at 8 minutes, after the onset of chromosome segregation (Fig. 13). Our results show that emerin and LBR are recruited early to reforming nuclear envelopes, initially as patches and later uniformly. Our results further show the early assembly of NPC components, as seen for other nuclear envelope components such as LBR and emerin; RanBP2 assembles into the NPC as rapidly as p62 or Nup153, whereas Tpr assembles after nuclear import activity is recovered. The rapid assembly of a transport-competent nuclear envelope supports a model in which nuclear import function is required for further nuclear reorganization such as chromosome decondensation and the telophase-G1 transition (Benavente et al., 1989).

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REFERENCES


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