The Karyopherin Kap142p/Msn5p Mediates Nuclear Import and Nuclear Export of Different Cargo Proteins

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Abstract. We have identified a novel pathway for protein import into the nucleus. Although the product of *Saccharomyces cerevisiae* gene *MSN5* was previously shown to function as a karyopherin (Kap) for nuclear export of various proteins, we discovered a nuclear import pathway mediated by Msn5p (also referred to as Kap142p). We have purified from yeast cytosol a complex containing Kap142p and the trimeric replication protein A (RPA), which is required for multiple aspects of DNA metabolism, including DNA replication, DNA repair, and recombination. In wild-type cells, RPA was localized primarily to the nucleus but, in a *KAP142* deletion strain, RPA was mislocalized to the cytoplasm and the strain was highly sensitive to bleomycin (BLM). BLM causes DNA double-strand breaks and, in *S. cerevisiae*, the DNA damage is repaired predominantly by RPA-dependent homologous recombination. Therefore, our results indicate that in wild-type cells a critical portion of RPA was imported into the nucleus by Kap142p. Like several other import-related Kap–substrate complexes, the endogenous RPA–Kap142p complex was dissociated by RanGTP, but not by RanGDP. All three *RPA* genes are essential for viability, whereas *KAP142* is not. Perhaps explaining this disparity, we observed an interaction between RPA and Kap95p in a strain lacking Kap142p. This interaction could provide a mechanism for import of RPA into the nucleus and cell viability in the absence of Kap142p. Together with published results (Kaffman, A., N.M. Rank, E.M. O'Neill, L.S. Huang, and E.K. O'Shea. 1998. *Nature.* 396:482–486; Blondel, M., P.M. Alepuz, L.S. Huang, S. Shaham, G. Ammerer, and M. Peter. 1999. *Genes Dev.* 13:2284–2300; DeVit, M.J., and M. Johnston. 1999. *Curr. Biol.* 9:1231–1241; Mahanty, S.K., Y. Wang, F.W. Farley, and E.A. Elion. 1999. *Cell.* 98:501–512) our data indicate that the karyopherin Kap142p is able to mediate nuclear import of one set of proteins and nuclear export of a different set of proteins.

Key words: *Saccharomyces cerevisiae* • karyopherin • nuclear transport • single-stranded DNA binding protein • bleomycin

Introduction

Eukaryotic cells are characterized by the existence of a double-membraned nuclear envelope, which separates the nucleus and the cytoplasm. Transport of molecules between these compartments occurs through nuclear pore complexes (NPCs)[1] that penetrate the nuclear envelope. NPCs form aqueous channels through which small molecules can pass by free diffusion. Macromolecules that are unable to pass efficiently through the NPC by free diffusion are transported into or out of the nucleus in an active manner via nuclear localization signals (NLSs) and nuclear export signals (NESs), respectively (for reviews see Pemberton et al., 1998; Wozniak et al., 1998; Görlich and Kutay, 1999; Nakielny and Dreyfuss, 1999). Signal-dependent transport pathways are mediated by recognition of these signals by soluble nuclear transport receptors known as karyopherins (Kaps; also called importins [Görlich et al., 1994], nuclear pore targeting complexes [PTACs; Imamoto et al., 1995], p97 [Chi et al., 1995], transportins [Pollard et al., 1996], and exportins [Stade et al., 1997]). Comparative sequence analysis revealed that in *Saccharomyces cerevisiae*, the Kap family is composed of 1 Kapα and 14 structurally related Kapβ proteins. Kapβ-related proteins carry cargo molecules into or out of the nucleus by binding to their substrates, the NPC and RanGTP. Kapα, Kap60p in yeast, functions as an adapter protein able to bind substrate and Kapβ1 (Kap95p in yeast) (Conti et al., 1998; Kobe, 1999). To date, it has been shown in yeast that nine Kaps function as import receptors and four Kaps function as export receptors (Enenkel et al., 1995; Aitchison et al., 1996; Pemberton et

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[1]*Abbreviations used in this paper:* 4NQO, 4-nitroquinoline *N*-oxide; BLM, bleomycin; DSB, double-strand break; GFP, green fluorescent protein; Kap, karyopherin; NER, nucleotide excision repair; NLS, nuclear localization signal; NPC, nuclear pore complex; nup, nucleoporin; PrA, protein A; RPA, replication protein A; ssDNA, single-stranded DNA; XRIP, *Xenopus* RPA-interacting protein.

al., 1997; Rosenblum et al., 1997; Rout et al., 1997; Schlenstedt et al., 1997; Stade et al., 1997; Albertini et al., 1998; Ferrigno et al., 1998; Hellmuth et al., 1998; Hood and Silver, 1998; Kaffman et al., 1998a,b; Senger et al., 1998; Solsbacher et al., 1998; Blondel et al., 1999; DeVit and Johnston, 1999; Mahanty et al., 1999; Pemberton et al., 1999; Titov and Blobel, 1999). No example of a Kap able to function as both an import receptor and an export receptor has been described, even though the Kaps themselves must be both imported and exported to complete a round of transport.

Directionality of the cargo molecules into or out of the nucleus is governed by several factors, including the RanGTPase system and NPC asymmetry (Moore, 1998; Rout et al., 2000). Ran can switch between a GDP-bound and a GTP-bound form. The nucleotide exchange factor for Ran (RanGEF/Prp20p in yeast) localizes to the nucleus, whereas the RanGTPase activating protein (RanGAP/Rna1p in yeast) localizes to the cytoplasm (Matunis et al., 1998; Talcott and Moore, 2000). This asymmetric distribution of Ran-regulating proteins, RanGEF that generates RanGTP in the nucleus, and RanGAP that excludes RanGTP from the cytoplasm, leads to high concentrations of RanGTP in the nucleus relative to the cytoplasm (Izaurralde et al., 1997; Moore, 1998). Among the proteins that compose the NPC are a set of Phe-Gly repeat–containing nucleoporins (FG nups). A subset of FG nups provide sites of docking or termination of transport for Kap–substrate complexes (Fontoura et al., 2000; Rout et al., 2000). A protein to be imported forms a complex via binding of its NLS to its cognate Kap. This complex first docks to a set of FG nups on the cytoplasmic side of the NPC. After translocation through the NPC into the nucleus, the import cargo–Kap complex is disassembled by the binding of RanGTP to the Kap, liberating the import cargo and Kap in the nucleus (Rexach and Blobel, 1995; Floer et al., 1997; Görlich et al., 1997; Chook and Blobel, 1999). Additional factors may be involved in the disassembly in vivo (Senger et al., 1998; Pemberton et al., 1999). On the other hand, an export cargo forms a trimeric complex via its NES with its cognate Kap and RanGTP in the nucleus and this complex docks at a set of FG nups on the nucleoplasmic side. The export cargo-Kap–RanGTP complex translocates through the NPC and docks to FG nups on the cytoplasmic side, where RanGTP is hydrolyzed to RanGDP by the action of RanGAP and RanBP1 (Yaseen and Blobel, 1999). This nucleotide conversion leads to disassembly of the export complex and allows export cargo, Kap, and RanGDP to be liberated in the cytoplasm (Fornerod et al., 1997; Kutay et al., 1997; Floer and Blobel, 1999).

Replication protein A (RPA) is a heterotrimeric complex that has a high affinity for single-stranded DNA (ssDNA). This complex is highly conserved in all eukaryotic cells and in yeast is composed of three subunits of ~70, 30, and 14 kD, referred to as Rpa1p, Rpa2p, and Rpa3p, respectively. Each of the genes encoding yeast RPA subunits is essential for viability (Brill and Stillman, 1991). RPA was originally identified as a factor required for the initiation and elongation of SV40 DNA replication in vitro (Wold and Kelly, 1988; Brill and Stillman, 1989). Subsequently, RPA has been shown to be required for multiple processes in eukaryotic DNA metabolism, including DNA replication, DNA repair, and recombination through specific physical interactions with other proteins and with ssDNA (for reviews see Wold, 1997; Waga and Stillman, 1998; Iftode et al., 1999). In *S. cerevisiae*, these proteins include chromosomal replication protein, DNA polymerase α-DNA primase, DNA recombination and repair proteins, Rad52p and *RTH1* nuclease, and a cell cycle–regulating protein, Mec1p (Longhese et al., 1994; Firmenich et al., 1995; Brush et al., 1996; Biswas et al., 1997; Hays et al., 1998; New et al., 1998). Recently, it was shown that the *Xenopus* Kapβ1 and *Xenopus* RPA-interacting protein α (XRIPα) complex was able to import RPA into the nucleus of permeabilized HeLa cells (Jullien et al., 1999). However, it is not known what is the predominant import pathway for RPA in *Xenopus* in vivo. In this complex, XRIPα is thought to act analogously to Kapα. XRIPα orthologues are found in humans and in *Drosophila*, but not in yeast. The absence of an XRIPα in yeast suggests that yeast and *Xenopus* use divergent pathways to import RPA.

MSN5/KAP142 was originally isolated as a multicopy suppressor in yeast mutants defective in the Snf1p protein kinase and subsequently as a gene required for the calcineurin-dependent induction of *PMC1* (Estruch and Carlson, 1990, 1993; Matheos et al., 1997). Recent studies have shown multiple functions of *MSN5*, including regulation of carbohydrate metabolism, cell stress response, mating response, signal transduction control, cell cycle control, and pseudohyphal differentiation of diploid cells (Akada et al., 1996; Lorenz and Heitman, 1998; Alepuz et al., 1999). By using mutant strains that are missing individual Kaps, it was shown recently that Msn5p mediates several cellular responses through functioning as a nuclear export receptor of various proteins, including transcription factors, Pho4p and Mig1p; inhibitor of G1 cyclin–Cdk complex, Far1p; as well as a scaffold protein of a mitogen-activated protein kinase cascade, Ste5p (Kaffman et al., 1998a; Blondel et al., 1999; DeVit and Johnston, 1999; Mahanty et al., 1999). Each of these substrates is exported only in certain conditions, including high phosphate or low glucose in the growth medium, or mating response. It is not known whether Kap142p has any activities independent of environmental stimulation. (To reflect the primary function of Msn5p, we hereafter refer to it as Kap142p. For nomenclature issues and open reading frame details, see http://genome-www4.stanford.edu/cgi-bin/SGD/locus.pl?locus=s0002743.)

Here we show that Kap142p is a Kap that mediates not only nuclear export, but also nuclear import. We have identified the ssDNA-binding protein complex RPA as an import substrate for Kap142p. *KAP142* is not essential for viability, whereas all three *RPA* genes are essential. This led us to identify Kap95p as a Kap likely to be involved in a secondary import pathway for RPA.

Materials and Methods

Yeast Strains

All strains were derived from *S. cerevisiae* DF5 (Finley et al., 1987). All yeast manipulations were performed according to described protocols (Aitchison et al., 1995). *KAP142* and *YBR137W* were deleted by integrative transformation of *HIS5* (*Schizosaccharomyces pombe*). The *HIS5* re-

placement cassette was generated by PCR amplification with primers that contained 60 nucleotides flanking the *KAP142* or *YBR137W* open reading frames. Heterozygous diploids were sporulated and tetrads were dissected to generate *KAP142* deletion (Δ) or *YBR137W*Δ haploid strains. Deletion of genes was confirmed by PCR on genomic DNA with internal and external 3′ noncoding primers. The *KAP120Δ* strain (Chaves and Blobel, personal communication) was also generated using the integrative transformation of *HIS5*. The *HIS5* replacement cassette was generated by PCR amplication with primers that contained 60 nucleotides flanking the *KAP120* open reading frames. Heterozygous diploids were sporulated and tetrads dissected to generate *KAP120Δ* strain. Deletion of genes was confirmed by PCR on genomic DNA with internal and external 3′ noncoding primers. COOH-terminal genomic *KAP142-PrA* or *YBR137W-PrA* was constructed by integrative transformation of PCR-amplified cassettes containing four and a half IgG-binding domains of protein A (PrA; *Staphylococcus aureus*) immediately after 60 nucleotides of 3′ sequence of *KAP142* or *YBR137W* and including a *HIS5* selection marker (these templates were kindly donated by Dr. R. Beckmann, Institut für Biochemie, Charité Berlin-Mitte, Berlin, Germany). Heterozygous diploids were sporulated and tetrads were dissected to generate *KAP142-PrA* and *YBR137W-PrA* haploid strains. *RPA1-PrA* and *RPA2-PrA* strains were created by direct integration of a PCR product into the DF5α haploid strain. *RPA1-PrA* and *RPA2-PrA*/*KAP142*Δ strains were constructed by direct replacement of *KAP142* with *URA3* in *RPA1-PrA* and *RPA2-PrA* strains. *RPA2-PrA*/*YBR137W*Δ strain was constructed similarly to the *RPA2-PrA*/*KAP142*Δ strain. Correct integration was verified by PCR and immunoblotting.

Green fluorescent protein (GFP)-tagged fusion proteins were expressed by transforming plasmid pYX242-GFP in which human La, *YLA1*, or *PHO4* was cloned into wild-type or *KAP142* deletion haploid strains. pYX242-GFP, pYXhsLa-GFP, and pYXYla1p-GFP were kindly provided by Dr. J.S. Rosenblum (Rockefeller University). *PHO4* was amplified from *S. cerevisiae* genomic DNA (Promega) and cloned into pYX242-GFP using primer-encoded BamHI and HindIII sites to generate pYXPho4p-GFP.

Immunofluorescence Microscopy

PrA-tagged cells were fixed in 3.7% formaldehyde for 10 min or as indicated. Immunofluorescence microscopy on yeast spheroplasts was done as described previously (Wente et al., 1992). PrA tags were visualized using rabbit anti–mouse IgG (preadsorbed against formaldehyde-fixed wild-type yeast cells) followed by Cy-3–conjugated donkey anti–rabbit IgG (Jackson ImmunoResearch Laboratories). Nuclei were visualized with DAPI staining. Direct fluorescence was used to visualize live, unfixed cells transformed with a plasmid-encoding GFP fusion protein. All images were viewed under the 63× oil objective on a ZEISS Axiophot microscope; images were collected in a video imaging system and manipulated in the computer program Adobe Photoshop®.

Cell Fractionation and Immunoisolation

Fractionation and immunoisolation of PrA fusion proteins were performed as described previously (Aitchison et al., 1996). In brief, 300 ml of postnuclear postribosomal cytosol was prepared from 10 liters of the *KAP142-PrA* strain and 50 ml of cytosol was prepared from 2 liters of *RPA2-PrA*, *RPA2-PrA/KAP142*Δ, and *YBR137W-PrA* strains. All strains were grown to an OD_{600} of 1.6. Kap142p-PrA, Rpa2p-PrA, or Ybr137wp-PrA and associated proteins were immunoisolated by overnight incubation of cytosol at 4°C with 50 μl of rabbit IgG–Sepharose (made from coupling CNBr-activated Sepharose 4B [Amersham Pharmacia Biotech] to rabbit IgG [Cappel]) per 50 ml cytosol. The IgG-Sepharose was isolated and washed extensively by TB (20 mM Hepes/Cl, pH 7.5, 110 mM KOAc, 2 mM $MgCl_2$, 1 mM DTT, and 0.1% Tween 20) with protease inhibitors (1 mM 4-[2-aminoethyl]-benzenesulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin A) before elution with a step gradient of 50–4,500 mM $MgCl_2$ or with SDS sample buffer. Proteins eluted with $MgCl_2$ were precipitated with methanol and chloroform before separation by SDS-PAGE on a 4–20% gradient acrylamide gel (Invitrogen). Coomassie-staining bands were excised and proteins were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Fernandez et al., 1994; Gharahdaghi et al., 1996). Peptide masses were compared with several databases (http://prospector.ucsf.edu/ucsfhtml3.4/msfit.htm or http://prowl.rockefeller.edu/cgi-bin/ProFound). Anti-Kap60p antibody was a generous gift of Dr. M. Rexach (Stanford University, Stanford, CA).

Dissociation Experiments

Yeast Ran (Gsp1p) was expressed in bacteria, purified, and loaded with GTP as described previously (Floer and Blobel, 1996). RanGTP was incubated with the yeast RanGAP (Rna1p) overnight at room temperature to convert RanGTP to RanGDP as described previously (Albertini et al., 1998). Kap142p-PrA and bound proteins were immunoisolated by IgG-Sepharose as described above. After extensive washing, the IgG-Sepharose was divided into three siliconized tubes and incubated with buffer alone, RanGDP, or RanGTP (final concentration 5 μM) in a volume of 100 μl TB with protease inhibitors for 20 min at room temperature. After incubation, dissociated proteins were collected in the supernatant. Proteins bound to the Sepharose were eluted with 50 and 250 mM $MgCl_2$. All fractions were precipitated and separated by SDS-PAGE, followed by immunoblotting with rabbit polyclonal antibody against Rpa1p. Antibodies against Rpa1p and Rpa2p were kindly provided by Dr. B. Stillman (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

Drug Sensitivity Assay

Stock solutions of bleomycin (BLM; Sigma-Aldrich) were prepared with sterile water. Assays measuring BLM sensitivity were carried out with YED medium containing 0.5% yeast extract and 1% dextrose. BLM concentrations were varied between 0.5 and 2 μg/ml. For the BLM sensitivity assay, wild-type strain and Kap mutants including *KAP104*Δ (Aitchison et al., 1996), *KAP108*Δ (Rosenblum et al., 1997), *KAP111*Δ (Pemberton et al., 1997), *KAP114*Δ (Pemberton et al., 1999), *KAP119*Δ (Albertini et al., 1998), *pse1-1* (Seedorf and Silver, 1997), *KAP122*Δ (Titov and Blobel, 1999), *KAP123*Δ (Rout et al., 1997), *xpo1-1* (Stade et al., 1997), *KAP142*Δ (this study), *KAP120*Δ (Chaves and Blobel, personal communication), and *YBR137W*Δ (this study) were grown to an OD_{600} of 0.5–0.8 with YED medium, diluted, and 7 μl of cell cultures was dropped onto YED medium solidified by 2% agar (YEDA). Cells grown in the absence or in the presence of various concentrations of BLM were incubated at 30°C for 1–4 d. For survival tests, cells grown in YED medium at 30°C to an OD600 of 0.5–0.8 were diluted and 100 μl of cell cultures were spread on YEDA in the presence and the absence of BLM. Cells were incubated at 30°C for 1–4 d and colonies were counted. 4-nitroquinoline *N*-oxide (4NQO) (Sigma-Aldrich) was prepared in 50% DMSO. Assays measuring 4NQO sensitivity were carried out with YEDA containing 1 μM 4NQO. Drop test was performed analogously to the BLM sensitivity assay. Cells in the presence of 4NQO were incubated at 30°C for 4 d.

Results

Immunolocalization of Kap142p-PrA

To determine the subcellular localization of Kap142p, we generated a strain expressing Kap142p fused to four and a half IgG-binding repeats of PrA. The haploid strain (*KAP142-PrA*), which expresses Kap142p-PrA from the endogenous promoter of Kap142p, did not show any changes in growth rate relative to wild-type cells at 30°C. The resulting fusion protein was used to determine the localization of Kap142p by indirect immunofluorescence.

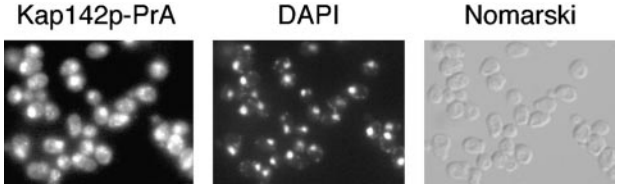
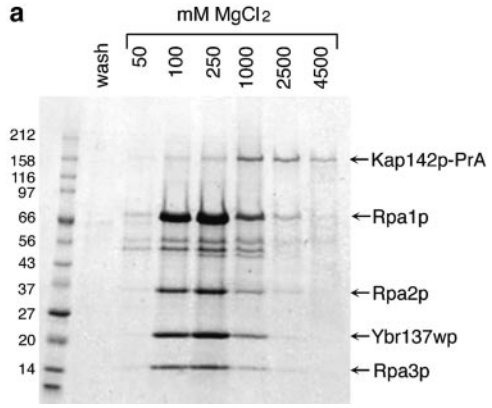


Figure 1. Kap142p-PrA localizes both to the cytoplasm and the nucleus. Haploid cells whose endogenous Kap142p was tagged with PrA were examined by indirect immunofluorescence of the PrA tag (left), DNA was stained with DAPI (middle), and whole cells were visualized by Nomarski optics (right).



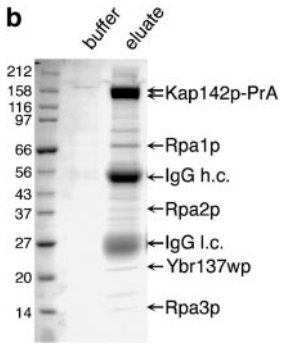


Figure 2. (a) Immunoisolation of cytosolic proteins interacting with Kap142p-PrA. The cytosol from a haploid *KAP142-PrA* strain was incubated with IgG-Sepharose. The last wash fraction and fractions eluted with a stepwise $MgCl_2$ gradient were separated by SDS-PAGE and stained with Coomassie blue. Kap142p-PrA eluted between 1,000 and 4,500 mM $MgCl_2$. Major bands migrating at ~70, 37, and 14 kD were identified by mass spectrometry as Rpa1p, Rpa2p, and Rpa3p, respectively. The band migrating to ~20 kD was identified as Ybr137wp. Relative molecular mass standards are indicated on the left. (b) Kap142p-PrA is insoluble at 4.5 M $MgCl_2$ but soluble in SDS-PAGE sample buffer. The cytosol of a haploid *KAP142-PrA* strain was incubated with IgG-Sepharose. The proteins bound to the IgG-Sepharose were eluted by buffer (lane 1) and eluted with SDS sample buffer (lane 2). The proteins were separated by SDS-PAGE and then stained with Coomassie blue. Kap142p-PrA appeared as doublet bands (double arrows). h.c., heavy chain; l.c., light chain.

Fixation of the cells for 2.5 min revealed Kap142p-PrA to be localized to both the nucleus and the cytoplasm (Fig. 1). In addition, some of the cells had strong immunofluorescent intensity in the nucleus. The result that Kap142p-PrA localized to both the nucleus and the cytoplasm was consistent with the localization of other PrA-tagged Kaps.

Identification of Proteins Interacting with Kap142p

Kaps that function in protein import form stable complexes with their substrates in the cytoplasm where the RanGTP concentration is low. To isolate proteins that interact with Kap142p, a postnuclear, postribosomal cytosol fraction from our *KAP142-PrA* strain was prepared and incubated with IgG-Sepharose.

Bound proteins were eluted with a stepwise gradient of 50–4,500 mM $MgCl_2$. Eluted proteins were precipitated and separated by SDS-PAGE followed by staining with Coomassie blue. SDS-PAGE analysis revealed the existence of four major bands eluting between 100 and 1,000 mM $MgCl_2$ (Fig. 2 a). The elution properties of these proteins were similar to those of previously described nuclear import substrates bound to their cognate Kaps. This was in contrast to the Kap-PrA, which forms a very stable complex with the IgG and is only eluted at very high $MgCl_2$ concentrations. Kap142p-PrA was eluted above 1,000 mM $MgCl_2$ but at high $MgCl_2$ concentrations was mostly insoluble (see below and Fig. 2 b).

To identify the potential import substrates, the bands were excised and the proteins were digested with trypsin and analyzed by mass spectrometry. The proteins migrating at ~70, 37, and 14 kD corresponded to the three subunits of RPA, Rpa1p, Rpa2p, and Rpa3p, respectively. 17 matching peptides derived from the 70-kD band covered 31% of Rpa1p, 8 matching peptides from the 37-kD band covered 49% of Rpa2p, and 5 matching peptides from the 14-kD band covered 63% of Rpa3p. The proteins that were eluted by 250 mM $MgCl_2$ were additionally immunoblotted with antibodies that specifically recognize Rpa1p or Rpa2p, confirming the identification of these two proteins (data not shown). None of these proteins have been identified in similar experiments with other Kap-PrA fusions. The RPA protein complex binds ssDNA and is required for eukaryotic DNA metabolisms, such as DNA replication, DNA repair, and recombination. The protein migrating at ~20 kD corresponded to Ybr137wp, a protein of unknown function (Kucharczyk et al., 1999). Since RPA and Ybr137wp formed a complex with Kap142p in cytosol, these proteins might be import substrates for Kap142p.

The amount of Kap142p-PrA seemed substoichiometric relative to the amount of eluted RPA (Fig. 2 a). It is possible that when Kap142p-PrA was eluted with high concentrations of $MgCl_2$ and precipitated with chloroform and methanol, it remained mostly insoluble in SDS sample buffer and therefore only a small amount entered the gel. To test this possibility, the amount of Kap142p-PrA bound to IgG-Sepharose was examined. Proteins that bound to the resin after incubation with cytosol from *KAP142-PrA* strain were eluted by adding SDS sample buffer to the resin directly and proteins were analyzed by SDS-PAGE. By Coomassie blue staining, doublet bands that corresponded to Kap142p-PrA by mass spectrometry appeared as well as RPA, Ybr137w protein, and IgG heavy and light chains (Fig. 2 b). This result may explain the apparent substoichiometry of Kap142p-PrA in Fig. 2 a. The significance of the migration of Kap142p-PrA as a doublet remains to be investigated.

Rpa2p-PrA Forms a Cytoplasmic Complex with Rpa1p and Rpa3p and This Complex Binds to Kap142p and Ybr137wp

From the results shown above in Fig. 2, a and b, RPA subunits formed a complex with Kap142p-PrA in cytosol. To examine whether RPA exists as a trimeric complex in the yeast cytoplasm, proteins bound to Rpa2p-PrA were immunoisolated from cytosol of an *RPA2-PrA* strain by IgG-Sepharose affinity chromatography (Fig. 3). After extensive washing (lane 1), proteins bound to IgG-Sepharose were eluted with SDS sample buffer, boiled, and separated by SDS-PAGE followed by staining with Coomassie blue (lane 2). There were several bands in addition to IgG heavy and light chains. The band migrating at ~70 kD was immunoreactive with an anti-Rpa1p antibody, and was therefore Rpa1p. From its stoichiometry to Rpa1p and its migration, the 14-kD band was most likely Rpa3p. The band migrating

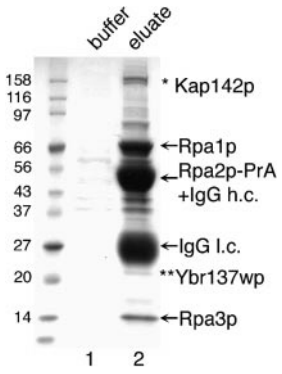


Figure 3. Immunoisolation of cytosolic proteins interacting with Rpa2p-PrA. Cytosol of an *RPA2-PrA* haploid strain was incubated with IgG-Sepharose and bound proteins were eluted and analyzed as in the legend to Fig. 2 a. The proteins bound to the IgG-Sepharose were eluted with buffer (lane 1) and eluted with SDS sample buffer (lane 2). The proteins were separated by SDS-PAGE and then stained with Coomassie blue. The bands migrating to ~150 kD (*) and ~20 kD (**) contained Kap142p and Ybr137wp by mass spectrometric analysis, respectively. h.c., heavy chain; l.c., light chain.

at ~55 kD was thought to be a mixture of Rpa2p-PrA and IgG heavy chain because the 57-kD Rpa2p-PrA most likely was not resolved from the IgG heavy chain. RPA subunits were pulled out simultaneously from the cytosol and were partially disassembled only at high $MgCl_2$ concentrations (see also Fig. 9 a), suggesting that its subunits existed in the cytosol as a stable trimeric complex.

An additional band that migrated in the mass range expected for Kaps was seen to bind to cytoplasmic Rpa2p-PrA. The band migrating at ~150 kD (Fig. 3, single asterisk) was excised and the eluted protein was analyzed by mass spectrometry. 12 peptides matched Kap142p, covering 16% of the protein, confirming that the band contained Kap142p. The band migrating at ~20 kD (Fig. 3, double asterisk) was also excised and analyzed by mass spectrometry. Seven peptides derived from this band covered 37% of Ybr137wp. From these results, we conclude that Rpa2p-PrA existed in the cytoplasm as a complex with Rpa1p and Rpa3p and bound to Kap142p and Ybr137wp.

Defective Nuclear Import of RPA in KAP142Δ Strain

As RPA is nuclear at steady state, RPA that was isolated as a cytoplasmic complex with Kap142p could have been intercepted en route to the nucleus. To examine whether RPA is indeed an import substrate for Kap142p, the localization of Rpa1p and Rpa2p was examined in both wild-type and *KAP142*Δ strains. Like a similar strain reported previously (Alepuz et al., 1999), our *KAP142*Δ haploid strain grew slowly at 35°C in rich medium compared with a wild-type haploid strain, whereas it did not show any growth phenotype at 30°C (Fig. 4 a). *RPA1-PrA* and *RPA2-PrA* strains did not show any growth phenotype in the presence or absence of *KAP142* at 30°C. There was no significant difference between *KAP142*Δ and *RPA1-PrA/KAP142*Δ strains at 35°C. Interestingly, *RPA2-PrA/KAP142*Δ was inviable at 35°C, providing additional evidence that Kap142p and Rpa2p functionally interacted. The expression levels of Rpa1p-PrA and Rpa2p-PrA at 30°C in total cell extracts were examined by immunoblotting and the levels were indistinguishable in both wild-type and *KAP142*Δ strains (data not shown). Immunofluorescence microscopy showed that Rpa1p-PrA and Rpa2p-PrA colocalized with DAPI-stained DNA in the nuclei of wild-

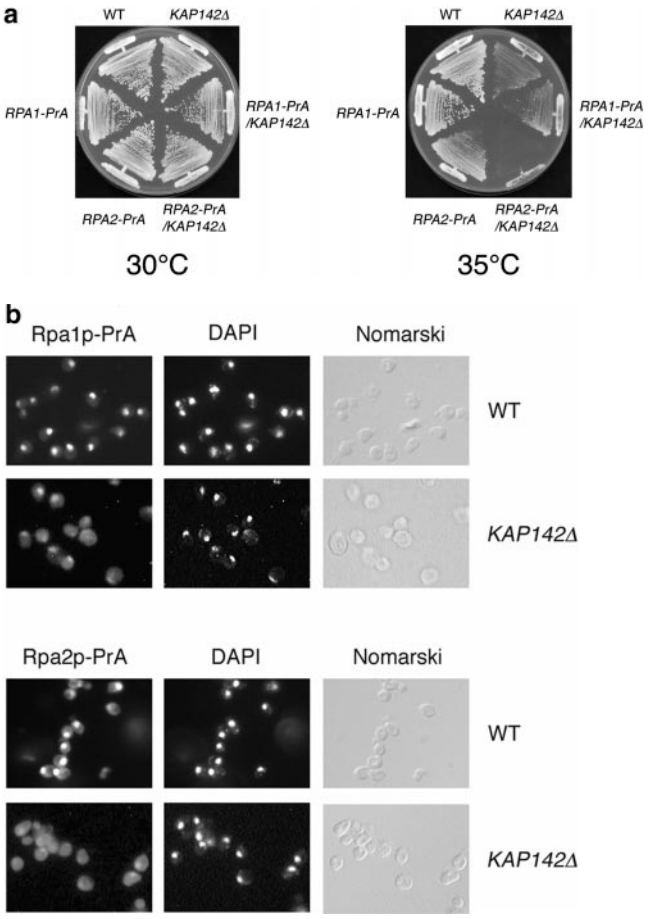


Figure 4. Deletion of *KAP142* leads to temperature-sensitive phenotype and mislocalization of Rpa1p-PrA and Rpa2p-PrA. (a) Strains, as indicated, were streaked on yeast extract/peptone/dextrose plates and incubated at 30°C (left) and at 35°C (right). (b) Wild-type (WT) or *KAP142*Δ strain whose endogenous Rpa1p or Rpa2p was tagged with PrA was grown at 30°C and examined by indirect immunofluorescence of the PrA tag (left). DNA was stained with DAPI (middle) and whole cells were visualized by Nomarski optics (right).

type cells at 30°C (Fig. 4 b, top). In contrast, in corresponding *KAP142*Δ strains, Rpa1p-PrA and Rpa2p-PrA were localized both to the cytoplasm and the nucleus at 30°C (Fig. 4 b, bottom). The partially deficient nuclear localization of Rpa1p-PrA and Rpa2p-PrA in the *KAP142*Δ strain is consistent with a role for Kap142p in the import of RPA.

Other Nucleocytoplasmic Pathways Are Not Affected by Deletion of KAP142

To determine whether deletion of *KAP142* would affect the localization of other transport substrates, several proteins whose transport pathways have been characterized previously were examined in both wild-type and *KAP142*Δ strains. For this experiment, GFP-tagged human and yeast La proteins, which are imported into the nucleus in yeast cells by Kap60p/Kap95p and Kap108p, respectively, were used as import substrates (Rosenblum et al., 1998). In both wild-type and *KAP142* deletion strains, these substrates showed nuclear localization, indicating that deletion of Kap142p did not affect at least two of the

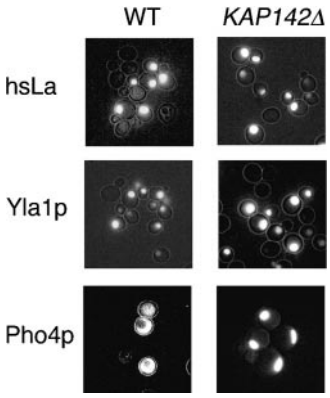


Figure 5. Deletion of *KAP142* leads to mislocalization of Pho4p-GFP, but not to a general transport defect. Wild-type (WT) haploid strain (left) and *KAP142*Δ strain (right) harboring plasmid-encoded GFP-tagged hsLa (top), Yla1p (middle), and Pho4p (bottom) were visualized by direct fluorescence.

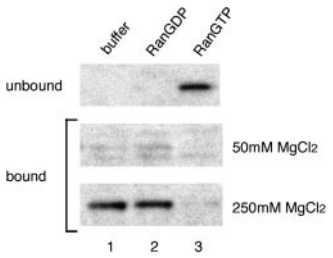


Figure 6. Dissociation of endogenous Rpa1p from Kap142p-PrA by RanGTP, but not RanGDP. The cytosol of a haploid *KAP142-PrA* strain was incubated with IgG-Sepharose. After washing, the proteins bound to IgG-Sepharose were incubated with buffer alone (lane 1), RanGDP (lane 2), or RanGTP (lane 3). After incubation, released proteins (unbound) were collected (top) and the remaining proteins (bound) were eluted with 50 mM (middle) and 250 mM $MgCl_2$ (bottom). Proteins were separated by SDS-PAGE and immunoblotted with anti-Rpa1p antibody.

other import pathways (Fig. 5, top and middle). Transcription factor Pho4p, which is exported from the nucleus in high phosphate conditions and imported into the nucleus in low phosphate conditions, was recently shown to be exported by Kap142p (Kaffman et al., 1998a). The localization of Pho4p-GFP was also examined in both the wild-type and *KAP142*Δ strains (Fig. 5, bottom). As reported previously, Pho4p-GFP was localized to the cytoplasm in the wild-type strain when it was grown in high phosphate medium, whereas under these conditions Pho4p-GFP was mislocalized to the nucleus in our *KAP142*Δ strain. Our *KAP142*Δ strain had a similar export phenotype to those generated in other laboratories (Kaffman et al., 1998a) and had no general import defect.

Dissociation of Endogenous RPA from Kap142p by RanGTP

Import substrates form stable complexes with their cognate Kaps in the cytoplasm and are dissociated by RanGTP in the nucleus. To examine whether this was also the case for the RPA–Kap142p complex, IgG-Sepharose–bound RPA–Kap142p-PrA was prepared from cytosol of a *KAP142-PrA* strain. This complex was incubated with RanGTP, RanGDP, or buffer alone, and proteins released from the IgG-Sepharose–bound Kap were collected. Remaining proteins were then eluted with $MgCl_2$. Proteins were separated by SDS-PAGE and immunoblotted with an antibody that specifically recognizes Rpa1p (Fig. 6). This showed that incubation with RanGTP released Rpa1p from Kap142p-PrA, whereas incubation with RanGDP or buffer alone did not release Rpa1p from Kap142p-PrA. Since the three RPA subunits exist as a stable complex in the cytoplasm, as shown in Fig. 3 and Fig. 9 a, this result indicated that an endogenous complex of RPA–Kap142p-PrA was sensitive to dissociation by RanGTP but not RanGDP, which was shown similarly for other import substrate–Kap complexes. This observation provided further evidence that RPA was indeed an import substrate for Kap142p.

Cells Lacking KAP142 Are Sensitive to BLM and Not to 4NQO

RPA was partially mislocalized to the cytoplasm (Fig. 4 b) in a *KAP142*Δ strain, suggesting that deletion of *KAP142* might lead to a defect in RPA-mediated DNA metabolism. Our strain lacking *KAP142* grew comparably to the wild-type strain at 30°C, but grew slowly at 35°C (Fig. 4 a). Although the reason for this temperature sensitivity remains unclear, it might be possible that more RPA in the nucleus could be required for DNA replication and cell growth under stress conditions than under normal growth conditions. In the process of eukaryotic DNA metabolism, RPA plays essential roles not only in DNA replication but also in DNA repair and recombination. The radiomimetic antibiotic BLM induces DNA double-strand breaks (DSBs) in cells (Mages et al., 1996) and, in *S. cerevisiae*, the repair of DSBs is carried out predominantly by mechanisms that promote homologous recombination and require RPA (for review see Pâques and Haber, 1999). To investigate the physiological role of Kap142p in RPA-mediated DNA metabolism in vivo, we tested for defects in DNA DSB repair using BLM in cells lacking, or with mutated, Kaps.

We tested for BLM sensitivity with wild-type and the following strains: *KAP104*Δ, *KAP108*Δ, *KAP111*Δ, *KAP114*Δ, *KAP119*Δ, *pse1-1*, *KAP122*Δ, *KAP123*Δ, *xpo1-1*, *KAP142*Δ, and *KAP120*Δ. Although *KAP111*Δ, *KAP119*Δ, *pse1-1*, *xpo1-1*, and *KAP120*Δ strains grew more slowly than the wild-type strain at 30°C, they did not show any additional growth phenotype in the presence of BLM (data not shown). We found that *KAP104*Δ, *KAP122*Δ, and *KAP142*Δ strains were sensitive to BLM. The sensitivity of *KAP104*Δ to BLM became apparent only at high BLM concentration. In contrast, the *KAP122*Δ and *KAP142*Δ strains showed high sensitivity to 1 μg/ml BLM (Fig. 7 a). Strikingly, at 2 μg/ml BLM, *KAP142*Δ cells survived at <1% of the rate of *KAP122*Δ cells, which themselves survived at a rate of only 1.7% of wild-type cells (Fig. 7 b). High sensitivity of the *KAP142*Δ to BLM provided additional independent evidence that the import of RPA, which plays crucial roles in the repair of DNA DSB, was disturbed in the absence of Kap142p.

To study the physiological role of Kap142p on RPA-mediated DNA metabolism further, we tested for 4NQO sensitivity with wild-type and *KAP142*Δ strains. 4NQO causes DNA damage that is primarily repaired by way of nucleotide excision repair (NER) where RPA plays a role with other repair proteins. In contrast to the effect of BLM on *KAP142*Δ strain, 1 μM 4NQO did not affect the growth of *KAP142*Δ compared with the growth of the wild-type strain (Fig. 7 c). Although the direct reason for insensitivity of *KAP142*Δ strain to 4NQO is unknown, NER might require less nuclear RPA than DNA DSB repair (see also Discussion).

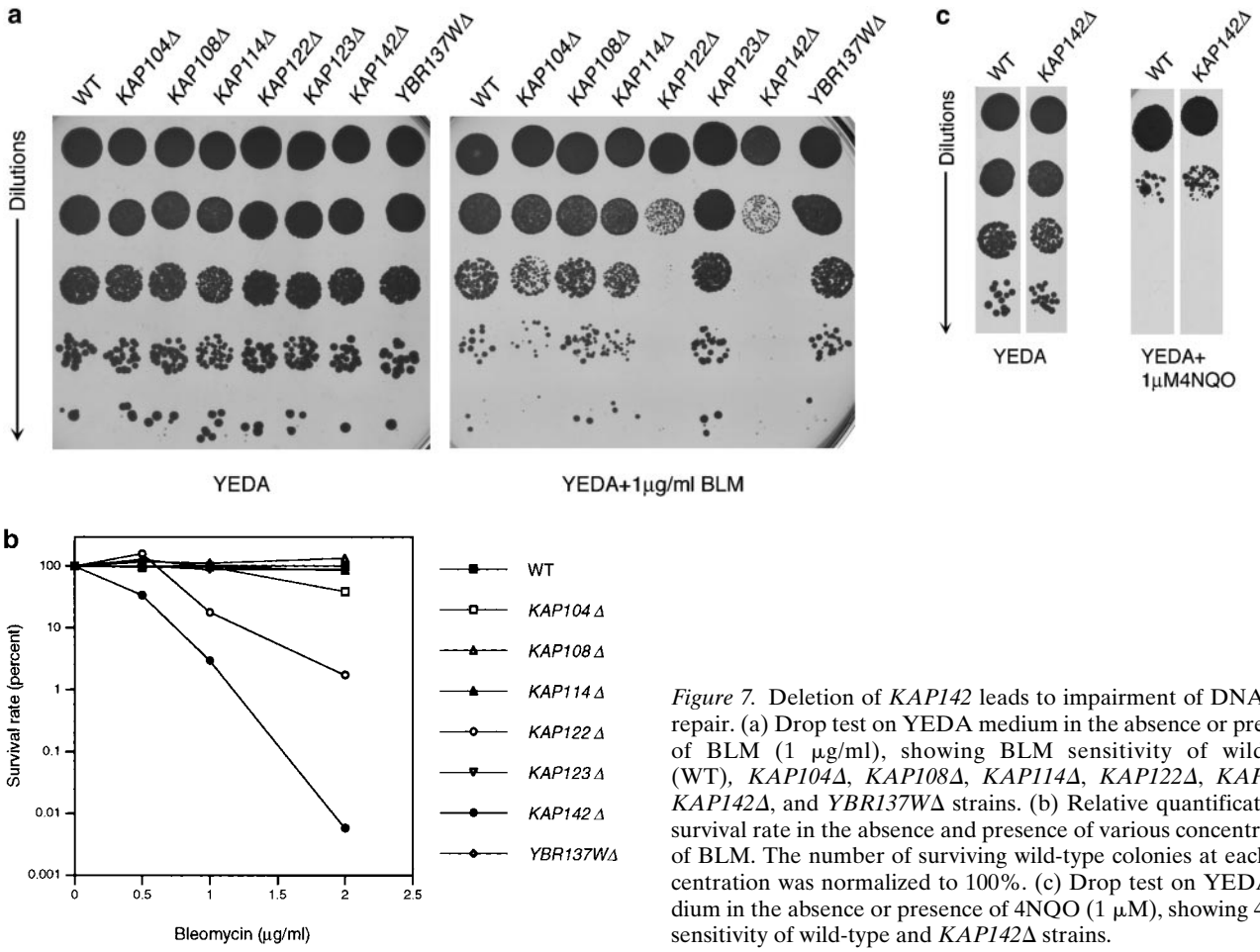


Figure 7. Deletion of *KAP142* leads to impairment of DNA DSB repair. (a) Drop test on YEDA medium in the absence or presence of BLM (1 μg/ml), showing BLM sensitivity of wild-type (WT), *KAP104Δ*, *KAP108Δ*, *KAP114Δ*, *KAP122Δ*, *KAP123Δ*, *KAP142Δ*, and *YBR137W*Δ strains. (b) Relative quantification of survival rate in the absence and presence of various concentrations of BLM. The number of surviving wild-type colonies at each concentration was normalized to 100%. (c) Drop test on YEDA medium in the absence or presence of 4NQO (1 μM), showing 4NQO sensitivity of wild-type and *KAP142*Δ strains.

Function of Ybr137w Protein Remains Unclear

Since Ybr137wp was isolated in a complex with Kap142p-PrA, Ybr137wp may be an import substrate of Kap142p or an adapter protein between Kap142p and RPA. To examine these possibilities, *YBR137W* was genomically tagged with *PrA* and the resultant *YBR137W-PrA* strain was examined for the localization of Ybr137wp. Unlike Kap142p and RPA, Ybr137wp was localized diffusely throughout the cell and in a cytoplasmic focus both in wild-type (Fig. 8 a) and *KAP142*Δ strains (data not shown), making it unlikely that Ybr137wp was an import substrate for Kap142p. If Ybr137wp were an adapter protein between Kap142p and RPA, the localization of RPA would require the presence of Ybr137wp. To examine the effect of Ybr137wp on nuclear import of RPA, the localization of Rpa2p-PrA was examined in wild-type and *YBR137W* deletion (*YBR137W*Δ) strains. *YBR137W* was not encoded by an essential gene and the *YBR137W*Δ strain did not show any growth phenotype in rich media at any temperature. Immunofluorescence microscopy showed that there was no apparent difference in localization of Rpa2p-PrA between an isogenic wild-type strain (Fig. 8 b, top) and *YBR137W*Δ (Fig. 8 b, bottom). To further investigate a possible role of Ybr137wp in import of RPA in the nucleus, we examined the sensitivity of *YBR137W*Δ to BLM. We found that deletion of *YBR137W* did not lead to a decrease in DNA DSB repair efficiency (Fig. 7, a and b), whereas deletion of *KAP142* lead to a marked decrease, suggesting that Ybr137wp was not required for import of RPA into the nucleus. From these observations, Ybr137wp seemed to be neither an import substrate of Kap142p nor an adapter protein between Kap142p and RPA. The function of Ybr137wp remains unclear.

RPA Is Imported into the Nucleus by Overlapping Pathways

Unlike each of three *RPA* genes, *KAP142* is not essential for viability. Further, some nuclear accumulation of RPA was seen in some of the *KAP142*Δ cells (Fig. 4 b). RPA that localizes to the nucleus must be imported via active transport, as its mass exceeds 110 kD. Taken together, these observations indicate that in the absence of Kap142p, RPA is imported into the nucleus via alternative Kap-dependent pathways. To explore this issue, we purified Rpa2p-PrA from cytosol deleted for *KAP142* (Fig. 9 a). As expected from their high affinity for Rpa2p, Rpa1p and Rpa3p were eluted from Rpa2p-PrA only at very high $MgCl_2$ concentrations. In addition to RPA subunits, a prominent band in the molecular mass range for Kaps was eluted between 250 and 1,000 mM $MgCl_2$ (Fig. 9 a, single asterisk), which is a typical range of $MgCl_2$ concentrations to dissociate Kap–substrate complexes (Fig. 2 a). Mass spectrometry revealed that this band was Kap95p. 13 matching peptides covered 14% of the protein. This result suggests that in the absence of Kap142p, RPA may be imported into the nucleus in a Kap95p-dependent manner.

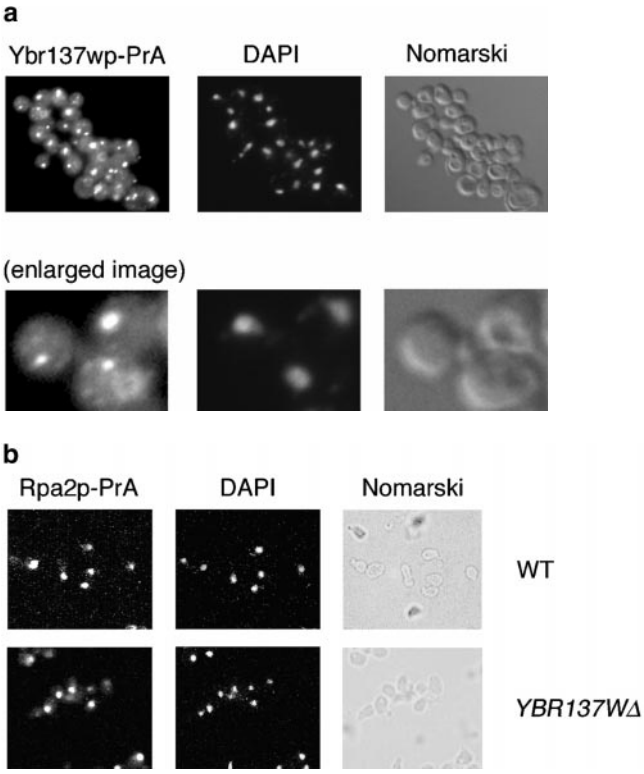


Figure 8. (a) Ybr137wp-PrA was localized throughout the cell and in a cytoplasmic focus. Haploid cells whose endogenous Ybr137wp was tagged with PrA were examined by indirect immunofluorescence of the PrA tag (left), DNA was stained with DAPI (middle), and whole cells were visualized by Nomarski optics (right). Images in the bottom row were enlarged using Adobe Photoshop®. (b) Deletion of *YBR137W* does not lead to mislocalization of Rpa2p-PrA. Wild-type (WT) haploid strain or *YBR137W*Δ strain (bottom) whose endogenous Rpa2p was tagged with PrA was examined by indirect immunofluorescence of the PrA tag (left), DNA was stained with DAPI (middle), and whole cells were visualized by Nomarski optics (right).

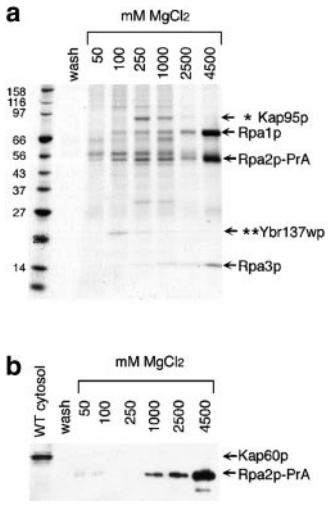


Figure 9. In the absence of Kap142p, RPA interacts with Kap95p and Ybr137wp. (a) Rpa2p-PrA was expressed in a *KAP142*Δ strain. Rpa2p-PrA and associated proteins were isolated as in the legend to Fig. 2. The bands migrating at ~90 kD (*) and ~20 kD (**) were identified by mass spectrometry as Kap95p and Ybr137wp, respectively. (b) Cytosol from wild-type (WT) strain and Rpa2p-PrA–associated proteins from Fig. 9 a were immunoblotted with anti-Kap60p antibody.

To examine whether import of RPA into the nucleus in the absence of Kap142p is mediated by the Kap60p–Kap95p complex, we immunoblotted the Rpa2p-PrA–associated proteins with an antibody that specifically recognizes Kap60p (Fig. 9 b). We found no specific signal that corresponded to Kap60p, indicating that RPA was imported into the nucleus in the absence of Kap142p by a Kap95p-mediated pathway that was independent of Kap60p. The ~20-kD band (Fig. 9 a, double asterisk) eluting between 100 and 1,000 mM $MgCl_2$ was identified as Ybr137wp by mass spectrometry. The five matched peptides covered 25% of the protein. Since Ybr137wp was purified in complex with Rpa2p-PrA from a strain lacking Kap142p, Ybr137wp appeared to specifically bind RPA.

Discussion

It has been shown previously that Kap142p is the export receptor for several proteins. Here, we show that Kap142p also serves as the import receptor for RPA. This is the first demonstration, to our knowledge, of a Kap that imports one set of proteins and exports a different set of proteins.

Although *KAP142* was first identified in a genetic screen as a multicopy suppressor of mutants defective in the *SNF1* protein kinase, based on its sequence similarity, the product of *KAP142* of *S. cerevisiae* was recently suggested to be a member of the Kap transport factor family (Görlich et al., 1997). The members of the Kap family have so far been characterized only as import or export receptors. Kap142p was shown to be an export Kap for various proteins, including the transcription factors, Pho4p, Mig1p, inhibitor of G1 cyclin–Cdk complex, Far1p, and a mitogen-activated protein kinase cascade scaffold protein, Ste5p (Kaffman et al., 1998a; Blondel et al., 1999; DeVit and Johnston, 1999; Mahanty et al., 1999). In this paper, we report that the product of the *KAP142* gene functions not only as an export Kap, but also as an import Kap for RPA.

We found that Kap142p was localized both to the cytoplasm and the nucleus when tagged at its COOH terminus with PrA. However, it has been reported that Kap142p localizes predominantly to the nucleus when it is tagged in different ways (Alepuz et al., 1999; Blondel et al., 1999). The reason that there is a discrepancy of localization of Kap142p remains unclear, although it has precedent in other Kaps, but this may be due to the orientation of the tag (NH_2 terminus or COOH terminus), what kind of moiety is attached (PrA, Myc, or GFP), how it is studied (in living or fixed cells), or how it is expressed (under its own promoter or overexpressed). The sensitivity of nucleocytoplasmic localization of Kap142p to tags or expression is consistent with its function as a Kap shuttling between the nucleus and the cytoplasm.

We found that cytosolic Kap142p exists as a complex with RPA. Previous findings showed that Kaps that function in nuclear import, in general, form stable complexes with their cognate import substrate in the cytoplasm where RanGTP is low. After translocation into the nucleus where RanGTP is high, this complex is dissociated via a mechanism that includes binding of RanGTP to the Kap. Therefore, formation of the Kap142p–RPA complex in the cytoplasm (Fig. 2 a) and dissociation of the complex by RanGTP (Fig. 6) suggested that Kap142p might function as an import Kap for RPA. Our ability to show partial mislocalization of RPA subunits at 30°C in a *KAP142*Δ strain (Fig. 4 b) confirmed that at least some portion of

RPA was imported into the nucleus by Kap142p. The *KAP142* gene is not essential for viability in yeast, whereas each *RPA* gene is essential. Including all of the identified transport substrates that are mediated by Kap142p, RPA is the only essential one characterized so far. Our cells lacking *KAP142* showed slow growth at 35°C (Fig. 4 a) like a similar strain reported previously (Alepuz et al., 1999). The direct cause of growth inhibition in the *KAP142*Δ strain might be that the essential RPA is not imported into the nucleus efficiently in the absence of Kap142p at 35°C and therefore DNA replication would be impaired. Nevertheless, we cannot exclude the effect of nuclear export substrates on cell growth.

If RPA is imported into the nucleus mainly by Kap142p, the cells lacking *KAP142* should have defects in RPA-mediated DNA metabolism. To look for such defects, we examined the sensitivity of Kap mutants to BLM. BLM induces DNA DSB in cells and the damage is repaired predominantly by a mechanism that promotes recombination, wherein RPA plays a crucial role (for reviews see Pâques and Haber, 1999; Flores-Rozas and Kolodner, 2000; Haber, 2000). Once DNA DSB occurs, the ends of the DSB are restricted by a 5′ to 3′ exonuclease or by a helicase coupled to an endonuclease to produce long, frequently >1 kb, 3′-ended ssDNA tails that can invade a homologous template. RPA binds to the ssDNA tails that are created by the nucleases and is suggested to have a role in sensing DNA damage (Lee et al., 1998). The repair of DSB by homologous recombination is dependent on a Rad52p-mediated mechanism in which Rad52p stimulates DNA strand exchange through specific protein–protein interaction between Rad52p, Rad51p, and RPA (New et al., 1998). Among the Kap mutants we examined, the *KAP142*Δ strain was most sensitive to BLM (Fig. 7, a and b). This can be explained, taken together with the direct binding of Kap142p with RPA in vitro (Fig. 2 a), partial mislocalization of RPA in *KAP142*Δ strain in vivo (Fig. 4 b) and the crucial roles RPA plays in DNA DSB repair, by a defect in RPA-mediated DNA repair in the *KAP142*Δ strain due to insufficient import of RPA into the nucleus in the absence of Kap142p.

To examine RPA-mediated DNA metabolism further, we studied the sensitivity of the *KAP142*Δ strain to 4NQO. 4NQO forms bulky adducts with nucleobases and this DNA damage is primarily targeted for NER. In the process of NER, DNA lesions are excised in the form of a DNA fragment ~25–30 nucleotides long and leaves ssDNA where RPA binds with other NER factors (for reviews see Araujo and Wood, 1999; Prakash and Prakash, 2000). The occluded binding sites for yeast RPA have been shown to be ~20–40 nucleotides (Sugiyama et al., 1997; Sibenaller et al., 1998). We found that 1 μM 4NQO did not affect the growth of the *KAP142*Δ (Fig. 7 c). Although the direct cause of insensitivity of the *KAP142*Δ strain to 4NQO remains to be elucidated, it could be that ssDNA generated in the process of NER is short enough that the amount of RPA imported into the nucleus in the absence Kap142p is sufficient, whereas ssDNA generated in the process of DNA DSB repair requires more RPA than is imported into the nucleus in the absence of Kap142p. Therefore, this differential drug sensitivity could arise because ssDNA generated in DNA DSB is >1,000 nucleotides long at each end of the break, whereas that generated in NER is 25–30 nucleotides long. As such, DNA DSB repair could require 80-fold more nuclear RPA than NER per lesion.

As we purified Ybr137wp bound to Kap142p-PrA, Ybr137wp could be either an import substrate for Kap142p or an adapter protein between Kap142p and RPA. However, Ybr137wp localized to the cytoplasm diffusively and the localization of Rpa2p-PrA was unchanged in the absence of Ybr137wp. A *YBR137W*Δ strain was not sensitive to BLM in contrast to *KAP142*Δ, suggesting that import of RPA into the nucleus was independent of Ybr137wp. In addition, Ybr137wp was purified as a binding protein to RPA from cytosol lacking Kap142p. From these results, we conclude that Ybr137wp binds to RPA specifically. The function of Ybr137wp remains to be elucidated.

Pho4p has been shown to be a nuclear export substrate of Kap142p under phosphate-rich conditions (Kaffman et al., 1998a). We also showed that Pho4p-GFP was localized to the cytoplasm when cells were grown in phosphate-rich medium and that it was mislocalized to the nucleus in the absence of Kap142p even if the cells were grown in phosphate-rich medium. However, proteins that bound to Kap142p-PrA in the cytoplasm did not include Pho4p by Coomassie blue staining. This might be explained as follows. Phosphorylated Pho4p forms a complex with Kap142p and RanGTP in the nucleus but as soon as this complex is exported into the cytoplasm, RanGTP is converted to RanGDP or phosphorylated Pho4p is dephosphorylated by phosphatase and this modification leads to dissociation of the complex faster than we are able to purify it with our method.

In common with the Kap114p-mediated import pathway for the TATA box-binding protein, Kap122p-mediated import pathway for transcription factor IIA, Kap123p-mediated import of ribosomal proteins, and Los1p-mediated export of tRNA, this Kap142p-mediated import pathway for RPA represents another example of a nonessential Kap importing an essential substrate (Rout et al., 1997; Hellmuth et al., 1998; Pemberton et al., 1999; Titov and Blobel, 1999). RPA does not pass through the NPC by free diffusion, because RPA forms a complex in the cytoplasm whose mass exceeds 110 kD. This led us to search for alternative pathways into the nucleus for RPA. We could identify Kap95p as a protein that forms a complex with Rpa2p-PrA in a strain lacking Kap142p. This case was similar to those for TATA box-binding protein, which binds Kap123p and/or Kap121p in the absence of Kap114p, and for ribosomal proteins, which bind Kap121p in the absence of Kap123p (Rout et al., 1997; Pemberton et al., 1999).

In addition to basic NLS (bNLS)-containing proteins, which interact with Kapα, vertebrate Kapβ1 can contact and import several proteins either via other adapters or directly (Palacios et al., 1997; Jäkel et al., 1999; Moore et al., 1999; Palmeri and Malim, 1999; Truant and Cullen, 1999). Kap95p, the yeast orthologue of Kapβ1, has so far only been shown to interact with bNLSs via Kap60p (Rexach and Blobel, 1995). Recently, RPA from *Xenopus* egg extract was demonstrated to be imported into the nucleus by Kapβ1 in combination with XRIPα as an adapter protein. However, XRIPα orthologues were found only in humans

and in *Drosophila*, but not in yeast (Jullien et al., 1999). The absence of XRIPα in yeast suggested that the import pathway of yeast RPA might be different from that of *Xenopus* RPA. The interaction of Kap95p with RPA in the absence of Kap142p may be indicative of a cryptic role for Kap95p in RPA import and may be evolutionarily relevant to involvement of orthologues of Kap95 in import of RPA orthologues. In this fashion, the divergence in nuclear import of RPA is similar to that seen in the La proteins (Rosenblum et al., 1998). Yla1p was shown to be imported into the nucleus by Kap108p in yeast cells, whereas the human orthologue of Yla1p is imported into the nucleus by the Kapα–Kapβ1 complex in human cells. The nuclear import of RPA, a highly conserved complex, appears to be the second example of an evolutionary divergence in the import of orthologous proteins.

In summary, we have identified a new pathway for protein import into the nucleus, mediated by the Kap, Kap142p. Although Kap142p has been previously established as a nuclear export receptor for various proteins, we have now shown that it is also able to import RPA into the nucleus. We also present data suggesting that Kap95p is involved in the inefficient nuclear import of RPA that takes place in the absence of Kap142p. Interestingly, the mechanism of nuclear import RPA has diverged between yeast and *Xenopus.* The import of RPA into the nucleus by Kap142p is the first example for one transport receptor functioning as both an import and an export receptor for completely different proteins. It remains to be seen if this is a general feature of Kaps.

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