

# Nuclear Import of the TATA-binding Protein: Mediation by the Karyopherin Kap114p and a Possible Mechanism for Intranuclear Targeting

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**Abstract.** Binding of the TATA-binding protein (TBP) to the promoter is the first and rate limiting step in the formation of transcriptional complexes. We show here that nuclear import of TBP is mediated by a new karyopherin (Kap) (importin) family member, Kap114p. Kap114p is localized to the cytoplasm and nucleus. A complex of Kap114p and TBP was detected in the cytosol and could be reconstituted using recombinant proteins, suggesting that the interaction was direct. Deletion of the *KAP114* gene led to specific mislocalization of TBP to the cytoplasm. We also describe two other potential minor import pathways for TBP. Consistent with other Kaps, the dissociation of TBP from Kap114p

is dependent on RanGTP. However, we could show that double stranded, TATA-containing DNA stimulates this RanGTP-mediated dissociation of TBP, and is necessary at lower RanGTP concentrations. This suggests a mechanism where, once in the nucleus, TBP is preferentially released from Kap114p at the promoter of genes to be transcribed. In this fashion Kap114p may play a role in the intranuclear targeting of TBP.

**Key words:** biological transport • transcription factors • nuclear localization signal • *Saccharomyces cerevisiae*

**T**RANSPORT between the nucleus and cytoplasm occurs through the nuclear pore complex (NPC).<sup>1</sup> The NPC also provides sites of interaction for the soluble components of the transport machinery (for review see Pemberton et al., 1998; Wozniak et al., 1998). Docking at particular sites on the NPC is believed to facilitate the transport of these proteins and their cargo across the nuclear envelope (Pemberton et al., 1998; Wozniak et al., 1998). The most well characterized soluble nuclear transport factor is the heterodimeric import receptor, comprised of karyopherin (Kap)  $\alpha$  and  $\beta$  (also known as importin  $\alpha$  and  $\beta$ ). Kap  $\alpha$  binds to the basic or classical nuclear localization sequence (NLS) present in many proteins to be imported into the nucleus and also interacts with Kap  $\beta$ . Kap  $\beta$  can interact with specific NPC proteins

and with the small GTPase Ran, which acts as a molecular switch for transport (for review see Moore, 1998). This function is thought to be brought about by the asymmetric localization of the regulators of Ran, which may result in RanGDP predominating in the cytoplasm and RanGTP predominating in the nucleus (Izaurralde et al., 1997; Moore, 1998). RanGTP is thought to bind all the  $\beta$  Kaps (from herein referred to as Kaps) and its presence in the nucleus is necessary for the dissociation of import substrates from the cognate Kap (Rexach and Blobel, 1995; Görlich et al., 1997; Chook and Blobel, 1999). Nuclear RanGTP is also necessary for the formation of a trimeric complex of export substrates and their cognate Kaps (Fornierod et al., 1997; Kutay et al., 1997), and appears to facilitate the docking of these complexes at the NPC while they exit the nucleus (Floer and Blobel, 1999).

Recent studies have shown that there is a large family of Kaps that function in either import or export (Pemberton et al., 1998; Wozniak et al., 1998). These Kaps appear to function without adaptor proteins (e.g., Kap  $\alpha$ ) and can bind directly to their substrates, the NPC, and to Ran (Pemberton et al., 1998; Wozniak et al., 1998). The yeast *Saccharomyces cerevisiae* has been an excellent organism for the study of the Kap family as its entire genome is known. It has been proposed that there are 14 putative Kaps in yeast (Görlich et al., 1997; see also Pemberton

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1. *Abbreviations used in this paper:* DAPI, 4',6-diamidino-2-phenylindole; ds, double stranded; GTF, general transcription factor; Kap, karyopherin; MS, mass spectrometry; NLS, nuclear localization sequence; NPC, nuclear pore complex; PIC, preinitiation complex; PrA, protein A; RNAP, RNA polymerase; TBP, TATA-binding protein; TBP-PrA, PrA-tagged copy of TBP.

et al., 1998; Wozniak et al., 1998 for all fourteen), and many of these have homologous genes or expressed sequence tags in metazoans, suggesting that the family is evolutionary conserved (Pemberton et al., 1998; Wozniak et al., 1998). To date, in yeast, eight Kaps have been shown to function in import into the nucleus and four Kaps have been shown to function in export, whether any will function in both is not known (Enenkel et al., 1995; Aitchison et al., 1996; Pemberton et 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; Kunzler and Hurt, 1998; Senger et al., 1998; Solsbacher et al., 1998; Yan et al., 1998; Titov, A., and G. Blobel, manuscript submitted for publication). Kaps have been shown to import several different classes of proteins into the nucleus such as transcription factors, RNA-binding proteins, ribosomal proteins, and signal transduction molecules (Pemberton et al., 1998; Wozniak et al., 1998). The fact that many of these nuclear proteins appear to be imported into the nucleus by different pathways suggests that their import must be coordinately regulated. In most cases the specific NLSs recognized by these Kaps have yet to be elucidated. It seems likely that structural or sequence motifs will form the basis for this recognition and Kap-substrate specificity.

Many factors have to be imported into the nucleus for the transcription of genes by all three RNA polymerases (RNAPs) to occur (for review see Orphanides et al., 1996; Roeder, 1996). One factor involved in transcription by all three RNAPs is the TATA-binding protein (TBP), which is the only sequence-specific DNA binding component of the transcription machinery (for review see Burley and Roeder, 1996; Roeder, 1996). For the transcription of protein encoding genes by RNAP II to occur, a multiprotein preinitiation complex (PIC) must be formed at the core promoter (Burley and Roeder, 1996; Roeder, 1996). This complex includes the general transcription factor (GTF) TFIID, which is comprised of TBP with its associated factors, and the other GTFs such as TFIIB, TFIIA, TFIIF, and TFIIE (Orphanides et al., 1996). TBP plays a crucial role in the recognition of the promoter and the assembly of the PIC. Recruitment of TBP to the promoter is the first and major rate limiting step of PIC formation (Burley and Roeder, 1996). This protein-DNA complex serves as a platform for the assembly of the other factors and ultimately the RNAPII holoenzyme (Burley and Roeder, 1996; Roeder, 1996). TBP and other GTFs are essential proteins that must be imported into the nucleus to carry out their function (Orphanides et al., 1996). Recent studies have shown that TFIIA and the transcription elongation factor TFIIS are imported into the nucleus by Pdr6p/Kap122p and Kap119p, respectively, suggesting that the import of the general transcriptional machinery may be coordinated along separate pathways (Albertini et al., 1998; Titov, A., and G. Blobel, manuscript submitted for publication). It is not known how TBP is imported into the nucleus and how it reaches its intranuclear point of function.

We show here that the major import pathway of yeast TBP into the nucleus is mediated by the new Kap family member, Kap114p. Kap114p is not an essential protein and we identify two additional Kaps that may potentially

participate in the import of TBP. We also identify a specific role for double-stranded TATA-containing DNA in the RanGTP-mediated dissociation of Kap114p from TBP and suggest that this may represent a mechanism for the targeted dissociation of TBP at its point of function, i.e., the promoter of genes to be transcribed.

## Materials and Methods

### Yeast Strains and Media

All yeast strains were derived from DF5 (Finley et al., 1987) and the procedures for yeast manipulation were as described (Aitchison et al., 1995). *KAP114* was deleted by integrative transformation of *HIS3*. Heterozygous diploids were sporulated and tetrads dissected to generate *kap114* haploid strains (Aitchison et al., 1995). *kap114/kap123* deletion strains were constructed by mating, sporulating, and dissecting the relevant strains. Diploid strains expressing the Kap114-protein A (PrA) and TBP-PrA fusion proteins were constructed by integrative transformation of the coding sequence of four and a half IgG binding repeats of *Staphylococcus aureus* PrA immediately upstream of the relevant stop codon as described (Aitchison et al., 1995). Haploid strains were generated by sporulation and dissection. Other strains were constructed by PrA integration downstream of TBP in the relevant haploid deletion strains.

### Immunofluorescence Microscopy

After fixation in 3.7% formaldehyde for 20 min, immunofluorescence microscopy on yeast spheroplasts was done as previously described (Pemberton et al., 1997). PrA tags were visualized using rabbit anti-mouse IgG (preadsorbed against formaldehyde-fixed wild-type yeast cells) followed by Cy3-conjugated donkey anti-rabbit IgG (Jackson Labs). GFP-expressing cells were applied to slides and briefly permeabilized in methanol, followed by acetone. All cells were mounted in a 4',6'-diamidino-2-phenylindole (DAPI)-containing medium. All images were viewed under the 63 $\times$  oil objective on a Zeiss Axiophot microscope, images were collected with a video imaging system, and manipulated in the computer program Adobe Photoshop.

### Protein Purification

Postnuclear, postribosomal cytosol was prepared from 4 liters of the TBP-PrA and Kap114-PrA strains and 1 liter of TBP-PrA/*kap114* and 160 ml of TBP-PrA/*kap114/kap123* strains grown to an OD<sub>600</sub> of 1.6 as described (Aitchison et al., 1996). Kap114-PrA or TBP-PrA and associated proteins were immunoprecipitated by overnight incubation of cytosol with rabbit IgG Sepharose (Cappel Laboratories) as described. After washing in TB (20 mM Hepes, pH 7.5, 110 mM KOAc, 2 mM MgCl<sub>2</sub>, 1 mM DTT, 0.1% Tween 20), proteins were eluted from the Sepharose with a step gradient of MgCl<sub>2</sub> and precipitated with methanol before analysis by SDS-PAGE. Coomassie staining bands were excised and prepared for analysis by MALDI-TOF mass spectrometry (MS) and by peptide sequencing (Fernandez et al., 1994; Gharahdaghi et al., 1996).

### Solution Binding Assay

The entire coding region of *KAP114* was amplified by PCR and ligated into the vector pGEX5X-1 (Pharmacia Biotech). The resulting GST-Kap114 fusion protein was purified from induced bacteria following the manufacturer's instructions. Purified GST was obtained from the manufacturer (Pharmacia) and recombinant TBP expressed in bacteria as a His<sub>6</sub> fusion protein was a gift of Dr. P.A. Weill (Vanderbilt University, Nashville, TN). Approximately 0.5  $\mu$ g or 1  $\mu$ g of GST-Kap114 or 2  $\mu$ g of GST was bound to 20  $\mu$ l of a 50% slurry of glutathione-Sepharose in TB/0.1% casamino acids. After washing, Sepharose was incubated with  $\sim$ 1  $\mu$ g of TBP for 30 min in the above buffer. The supernatant was collected and constituted the unbound fraction, the Sepharose was washed six times in TB, and the bound fraction was collected by boiling in the sample buffer.

### Dissociation Experiments

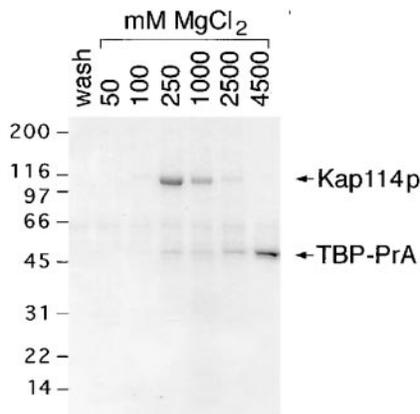
Ran (Gsp1p) was expressed in bacteria and purified and loaded with GTP or GDP as described (Floer and Blobel, 1996). Oligonucleotides (TATA,

5'-CTGTATGTATATAAAAACG-3'; M1, 5'-CTGTATGTAGAGAAA-ACG-3') were annealed by standard procedures and diluted in TB. Purified TFIIA was a gift from Dr. S. Hahn (Fred Hutchinson Cancer Research Center, Seattle, WA) (Ranish et al., 1992). Kap114-PrA and TBP were isolated in batch by incubation with IgG Sepharose overnight, ~1–1.5 ml of cytosol (equivalent to 33–50 ml of yeast grown to an OD<sub>600</sub> of 1.6) per dissociation was used. After washing in TB, the Sepharose was divided into separate microfuge tubes for each experiment, resulting in ~10–20  $\mu$ l of Sepharose per tube. Ran, DNA, and TFIIA were added as indicated to give a final volume of 100  $\mu$ l. 0.1 mM GTP was included, except when using RanGDP. Tubes were rotated for 50 min at room temperature. The entire contents of the tube were transferred to a 2-ml disposable column and the liquid was drained and collected. The Sepharose was washed with a further 100  $\mu$ l of TB, together these fractions constituted the eluted fraction. The columns were washed with 10 ml of TB and drained. Proteins bound to the Sepharose were eluted either with 200  $\mu$ l of 1 M MgCl<sub>2</sub> followed by 200  $\mu$ l of 4.5 M MgCl<sub>2</sub>, or directly with 4.5 M MgCl<sub>2</sub> to elute the Kap114-PrA and bound TBP from the Sepharose, this constituted the bound fraction. All fractions were precipitated and separated by SDS-PAGE and immunoblotted. Amido black-stained blots were observed to see the excess unbound Ran in the eluted fraction that acted as an internal standard for the protein loading in this fraction. Procedures for Western blotting and subsequent detection by enhanced chemiluminescence were as described (Amersham). An affinity-purified rabbit polyclonal antibody was used to detect TBP. This antibody also interacts with PrA, so the levels of Kap114-PrA were compared as an internal standard for the bound fractions. All dissociations were carried out several times and representative experiments are shown.

## Results

### *TBP Interacts with an Uncharacterized Kap Family Member in the Cytosol*

In yeast, TBP is encoded by the gene *SPT15* (Eisenmann et al., 1989). Analysis of the TBP amino acid sequence revealed no known NLSs. To find the Kaps that are responsible for the import of TBP, we sought to isolate proteins that interacted with the low abundance, cytosolic pool of TBP. This pool most likely represents newly synthesized TBP en route to the nucleus. We genomically tagged TBP with an in frame carboxy-terminal fusion of the IgG-binding domain of PrA. Therefore, haploid cells expressed a single PrA-tagged copy of TBP (TBP-PrA) from its endogenous promoter. The tagged version of TBP was functional as it complemented growth and was localized to the nucleus (see below). TBP-PrA and associated proteins were isolated by incubation of a postribosomal cytosol with IgG-Sepharose and elution with a step gradient ranging from 0.05 to 4.5 M MgCl<sub>2</sub>. The eluted fractions were analyzed by SDS-PAGE and Coomassie blue staining (Fig. 1). As expected, the TBP-PrA, which binds IgG with high affinity because of the PrA moiety, was eluted at high concentrations of MgCl<sub>2</sub>. A few faint bands were visible in every fraction suggesting that they may be nonspecific contaminants or keratin. A band of ~116 kD, visible mainly in the 0.25- and 1-M MgCl<sub>2</sub> eluates, was in the expected size range for an associated Kap (Pemberton et al., 1998; Wozniak et al., 1998). The band was excised and analyzed by MS. The band represented a protein encoded by the uncharacterized open reading frame YGL241W (also called Hrc1004). YGL241W is predicted to encode a protein of 1,004 amino acids with an expected molecular mass of 113.9 kD. Comparison of this protein sequence with the database showed strongest homology with Kap family members (15–17% identity), suggesting that this protein was, indeed, a member of the Kap superfamily (Görlich



**Figure 1.** Cytosolic TBP binds the previously uncharacterized Kap, Kap114p. TBP-PrA and associated proteins were isolated from cytosol by IgG-Sepharose and eluted by an MgCl<sub>2</sub> gradient, separated by SDS-PAGE, and visualized by Coomassie blue staining. The bands representing Kap114p and TBP-PrA are indicated. The positions of molecular mass standard in kilodaltons are shown.

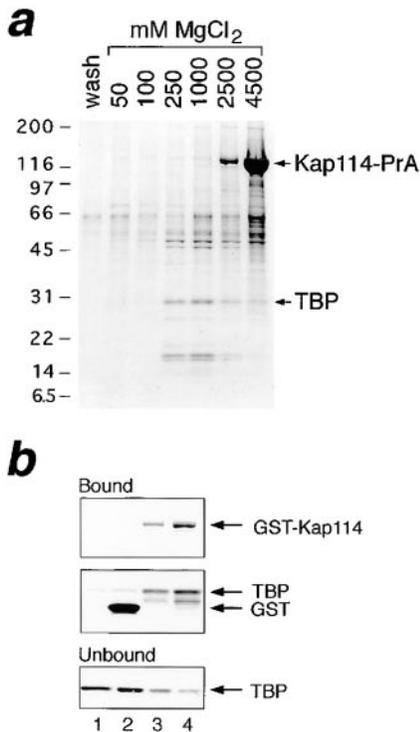
et al., 1997; Pemberton et al., 1998; Wozniak et al., 1998). Consistent with its role in nuclear transport (described below) and the standard nomenclature for other yeast Kaps, we will refer to this protein as Kap114p.

To verify that the interaction of Kap114p with TBP was specific, we tagged Kap114p as before with PrA (Kap114-PrA). Using cytosol prepared from the Kap114-PrA strain we purified by affinity chromatography those proteins that interacted with Kap114p. Kap114-PrA eluted at the high concentrations of MgCl<sub>2</sub>, whereas several bands, including one of ~27 kD, eluted at 0.25–1 M MgCl<sub>2</sub> (Fig. 2 a). The 27-kD band was excised and subjected to MS analysis and peptide sequencing which showed that this protein was indeed TBP. These data confirm that Kap114p and TBP interact in the cytosol, suggesting that TBP might represent an import substrate for Kap114p. Other bands detected in this experiment may also include Kap114p interacting proteins and, hence, potential import substrates for Kap114p.

To determine whether the interaction between Kap114p and TBP was direct, a complex was formed between bacterially expressed, purified GST-Kap114 and bacterially expressed, purified His<sub>6</sub>-tagged TBP (Fig. 2 b). In control experiments very little TBP could be detected bound to glutathione-Sepharose alone (Fig. 2 b, lane 1) or to GST bound-Sepharose (Fig. 2 b, lane 2). However, a much greater amount of TBP bound to GST-Kap114 bound-Sepharose, suggesting that the binding was due to the presence of Kap114p (Fig. 2 b, lanes 3 and 4). TBP binding was also seen to increase with increasing amounts of GST-Kap114 immobilized on the Sepharose (Fig. 2 b, lanes 3 and 4). These results suggested that TBP and Kap114p interacted directly.

### *Kap114p Is Localized to the Nucleus and Cytoplasm and Is Not Essential for Cell Growth*

Using the Kap114-PrA-tagged strain it was possible to determine the localization of the Kap114p by visualization of

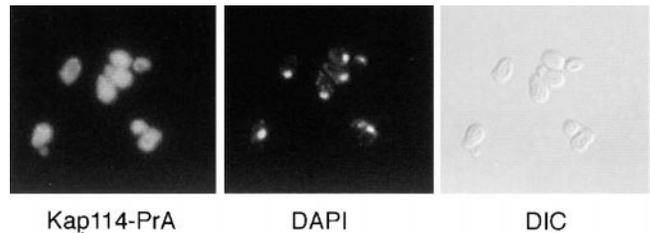


**Figure 2.** Kap114p binds directly to TBP suggesting TBP is a potential import substrate. (a) Kap114-PrA and associated proteins were isolated from cytosol by IgG-Sepharose and eluted by an  $MgCl_2$  gradient, separated by SDS-PAGE, and visualized by Coomassie blue staining. The bands representing Kap114-PrA and TBP are indicated. The positions of molecular mass standards in kilodaltons are shown. (b) Purified recombinant TBP was incubated with glutathione-Sepharose (lane 1), GST-bound Sepharose (lane 2), 0.5- $\mu$ g GST-Kap114-bound Sepharose (lane 3), and 1- $\mu$ g GST-Kap114-bound Sepharose (lane 4). The unbound and Sepharose-bound material was separated by SDS-PAGE and visualized by Coomassie blue staining.

the PrA tag. The Kap114-PrA was localized to the cytoplasm and nucleus, which would be consistent with its function as a nucleocytoplasmic shuttling protein (Fig. 3). To determine whether the gene encoding Kap114p was essential for cell growth the gene was replaced by genomic integration with *HIS3*. Viable haploid *HIS3* yeast strains deleted for *KAP114* ( $\Delta$ *kap114*) were obtained (data not shown), suggesting that the *KAP114* gene was not essential for cell growth. Deletion strains were also grown on rich medium at 37°C and 17°C, however, no growth defect was detected under these conditions (data not shown).

### Kap114p Imports TBP into the Nucleus

To ascertain whether TBP was imported into the nucleus by Kap114p in vivo, the localization of TBP-PrA in the *KAP114* deletion strain ( $\Delta$ *kap114*) was determined. As expected TBP-PrA appeared nuclear in wild-type cells, whereas in  $\Delta$ *kap114* cells a large proportion of the TBP-PrA was now localized to the cytoplasm (Fig. 4 a). These results were confirmed using an antibody to detect endogenous TBP in the  $\Delta$ *kap114* strain demonstrating that the mislocalization was not dependent on the PrA moiety



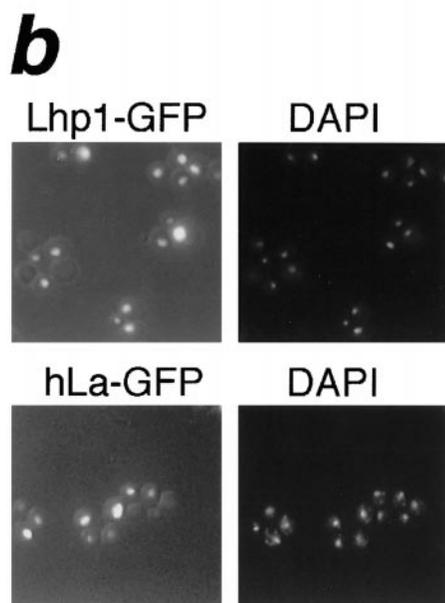
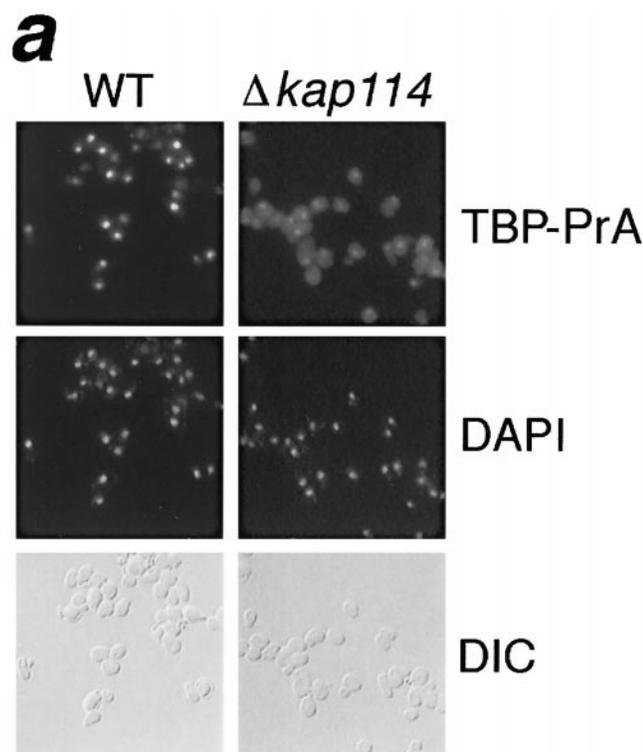
**Figure 3.** Kap114p is localized to the nucleus and cytoplasm. Kap114-PrA expressing cells were fixed and the PrA moiety was detected by indirect immunofluorescence. The coincident DAPI and Nomarski (DIC) images are also shown.

(data not shown). Thus the nuclear import of TBP appears to be partially abrogated in the absence of Kap114p, confirming that Kap114p imports TBP in vivo. The localization of Lhp1p-GFP and a human La-GFP reporter, which have been previously shown to be substrates for other Kap-mediated import pathways (Rosenblum et al., 1998), were also analyzed in the  $\Delta$ *kap114* strain. These proteins were correctly localized to the nucleus in this strain (Fig. 4 b), suggesting that we were not observing a general defect in nuclear transport and the effect on TBP localization was specific to deletion of *KAP114*.

### TBP Is Imported into the Nucleus by Overlapping Pathways

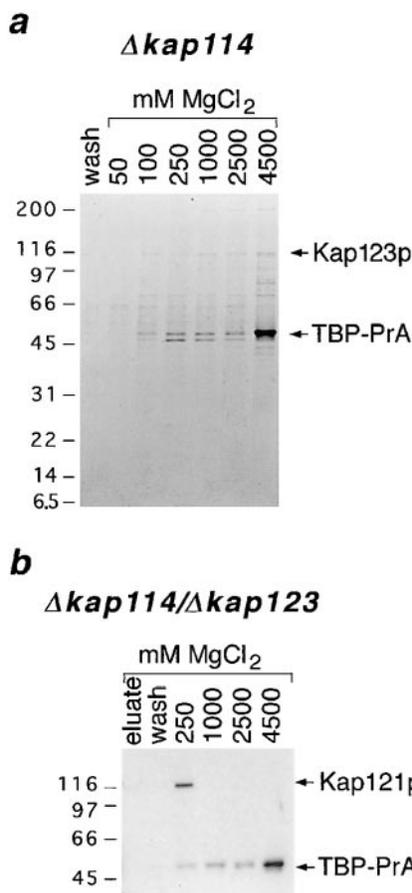
As strains deleted for *KAP114* appeared to have no growth defect under the described conditions and some of the TBP was nuclear in the absence of Kap114p (Fig. 4 a, as evidenced by the nuclear foci visible in some cells in the  $\Delta$ *kap114* strain), it seemed likely that a fraction of the TBP was imported into the nucleus via an alternative pathway. Cytosol was made from the TBP-PrA/ $\Delta$ *kap114* strain to determine which Kaps interacted with TBP in the absence of Kap114p. Coomassie blue staining of TBP-PrA-copurifying proteins revealed one in the correct molecular weight range for a Kap (Fig. 5 a). MS analysis revealed that this Kap was Kap123p, which has been previously shown to be involved in the import of ribosomal proteins (Rout et al., 1997; Schlenstedt et al., 1997). Kap123p is the most abundant Kap and is not encoded by an essential gene, although deletion strains grow very slowly at 37°C (Rout et al., 1997; Pemberton, L.F., unpublished data). TBP was PrA-tagged in the  $\Delta$ *kap123* strain and the localization of TBP-PrA was determined by immunofluorescence. TBP-PrA did not appear to be mislocalized in this strain at any temperature (Fig. 6). As Kap114p was still present in this strain we made a double deletion of *KAP114* and *KAP123* (to generate  $\Delta$ *kap114*/ $\Delta$ *kap123*), to determine whether they were synthetically lethal. This strain was also viable and phenotypically appeared similar to the single mutants, in that TBP was mislocalized to a similar degree as in  $\Delta$ *kap114* cells and that the cells grew poorly at 37°C as observed in the  $\Delta$ *kap123* strain (Fig. 6).

These results suggested that the essential protein TBP may in fact have several routes into the nucleus and to find further import pathways for TBP, TBP-PrA-copurifying proteins were isolated from the  $\Delta$ *kap114*/ $\Delta$ *kap123* strain



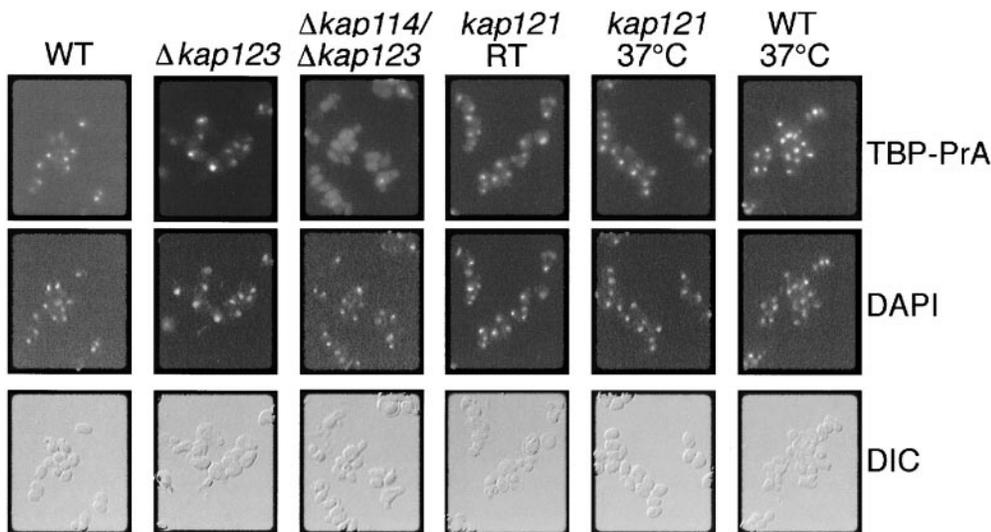
**Figure 4.** The specific import of TBP is abrogated in the absence of Kap114p. (a) Wild-type and  $\Delta kap114$  strains expressing TBP-PrA were fixed and the PrA moiety was detected by indirect immunofluorescence. The coincident DAPI and Nomarski (DIC) images are also shown. (b)  $\Delta kap114$  strains expressing either an Lhp1-GFP or hLa-GFP reporter (2- $\mu$ m plasmid, TPI promoter) were fixed, permeabilized, and mounted in DAPI-containing medium. The GFP moiety was detected by direct fluorescent imaging with coincident Nomarski optics. The coincident DAPI images are also shown.

as before. A very faint band in the correct molecular weight range for a Kap was detected by Coomassie blue staining of the proteins after separation by SDS-PAGE (data not shown), however, there was insufficient material



**Figure 5.** TBP interacts with Kap123p and Kap121p. (a) TBP-PrA was expressed in a  $\Delta kap123$  strain. TBP-PrA and associated proteins were isolated from cytosol by IgG-Sepharose and eluted by a  $MgCl_2$  gradient, separated by SDS-PAGE, and visualized by Coomassie blue staining. The bands representing Kap123p and TBP-PrA are indicated. (b) TBP-PrA was expressed in a  $\Delta kap114/\Delta kap123$  strain, TBP-PrA and associated proteins were isolated and eluted as before. Eluate is a fraction of the cytosol supernatant after removal of IgG-Sepharose. After separation by SDS-PAGE, proteins were immunoblotted with an antibody to Kap121p. Kap121p (and TBP-PrA) are indicated. The positions of molecular mass standards in kilodaltons are shown.

for MS analysis. Another Kap, Kap121p, has been previously shown to have overlapping functions with Kap123p and to compensate for a lack of Kap123p in the import of ribosomal proteins (Rout et al., 1997; Schlenstedt et al., 1997). We determined whether the TBP-PrA-interacting band of the  $\Delta kap114/\Delta kap123$  strain represented Kap121p by probing the eluted fractions, after transfer to nitrocellulose, with a polyclonal antibody to Kap121p (Marelli et al., 1998). This analysis showed that this band indeed contained Kap121p (Fig. 5 b). Kap121p is encoded by an essential gene and a temperature sensitive strain, *pse1-1*, has been described (Seedorf and Silver, 1997). A *pse1-1* strain expressing TBP-PrA was grown at the restrictive temperature for 3 h to determine the effect on TBP localization. After this treatment, TBP-PrA was still localized to the nucleus (Fig. 6). This suggests that although Kap121p and Kap123p may play a role in the import of TBP, this role is likely to be redundant with Kap114p. Interestingly,



**Figure 6.** TBP is mislocalized to the cytoplasm only in the absence of Kap114p. TBP-PrA-expressing strains were fixed and the PrA moiety detected by indirect immunofluorescence. The coincident DAPI and Nomarski (DIC) images are also shown. The strains used are shown above the frames. *kap121* was the temperature sensitive *psel-1* strain. Cells were grown at 30°C unless another temperature is indicated. These were grown at room temperature (RT) and shifted to 37°C for 3 h before fixation as indicated.

Kap121p could also be detected by immunoblot in TBP-PrA isolation experiments in the presence of Kap114p (data not shown). These data suggest that Kap114p mediates the bulk of TBP nuclear import, as evidenced by the fact that only a *kap114* deletion leads to mislocalization of TBP, even in the presence of an otherwise wild-type complement of Kaps.

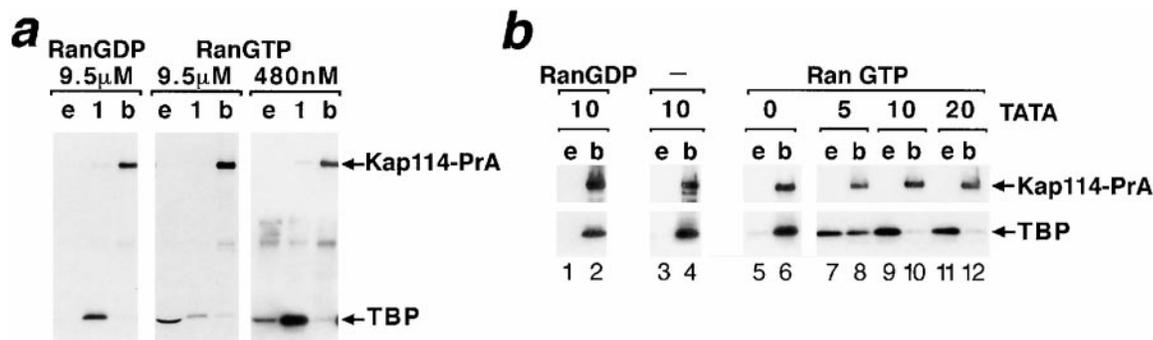
#### **The Dissociation of TBP from Kap114p Is RanGTP-dependent**

In the nucleus, the dissociation of Kaps from their cognate import substrates is thought to be achieved by RanGTP and not RanGDP (Rexach and Blobel, 1995). To determine whether this is also the case for the Kap114p-TBP complex, we incubated the IgG-Sepharose bound Kap114-PrA-TBP complex, isolated from yeast cytosol, with RanGTP or RanGDP. The material eluted after the incubation with Ran was collected (constituting the unbound fraction) and the IgG-Sepharose bound material was eluted with 1 M MgCl<sub>2</sub> to dissociate the remaining TBP,

followed by 4.5 M MgCl<sub>2</sub> to dissociate the Kap114-PrA (both fractions constituting the bound fraction). After separation by SDS-PAGE and transfer to nitrocellulose, TBP and Kap114-PrA were detected by immunoblotting with an anti-TBP polyclonal antibody. This antibody not only recognizes TBP but also Kap114-PrA, by virtue of its PrA tag. TBP was not eluted at high (9.5 μM, Fig. 7 a) or low concentrations of RanGDP (data not shown). A high concentration of RanGTP (9.5 μM) led to dissociation of more than half the TBP from the complex (Fig. 7 a). However at lower concentrations of RanGTP (480 nM) only a small amount of TBP was dissociated (Fig. 7 a). These results suggested that RanGTP rather than RanGDP was required to effect dissociation of TBP from Kap114p and the extent of dissociation depended on the concentration of RanGTP.

#### **TBP Dissociation Is Stimulated by the Addition of TATA-containing Double Stranded (ds) DNA**

Many previously published Kap import substrate dissocia-



**Figure 7.** The dissociation of TBP from Kap114p is RanGTP-dependent and stimulated by TATA-containing double-stranded DNA. The Kap114-PrA-TBP complex was isolated from cytosol using IgG-Sepharose. After dissociation under defined conditions, fractions were separated by SDS-PAGE and immunoblotted with an antibody to TBP. This antibody also recognizes Kap114-PrA by virtue of its PrA tag. (a) The dissociation reactions included 9.5 μM RanGDP, 9.5 μM RanGTP, or 480 nM RanGTP. Eluted proteins (e) and bound proteins subsequently eluted with 1 M MgCl<sub>2</sub> (1) and 4.5 M MgCl<sub>2</sub> (b) are indicated. (b) Dissociation reactions were carried out with either 4.8 μM RanGDP, or in the absence of RanGTP (-), or with 380 nM RanGTP as indicated. TATA-containing ds DNA was added at the concentrations (ng/μl) shown. Eluted fraction (e) and bound fraction (b) that was released from the IgG-Sepharose by 4.5 M MgCl<sub>2</sub> are shown.

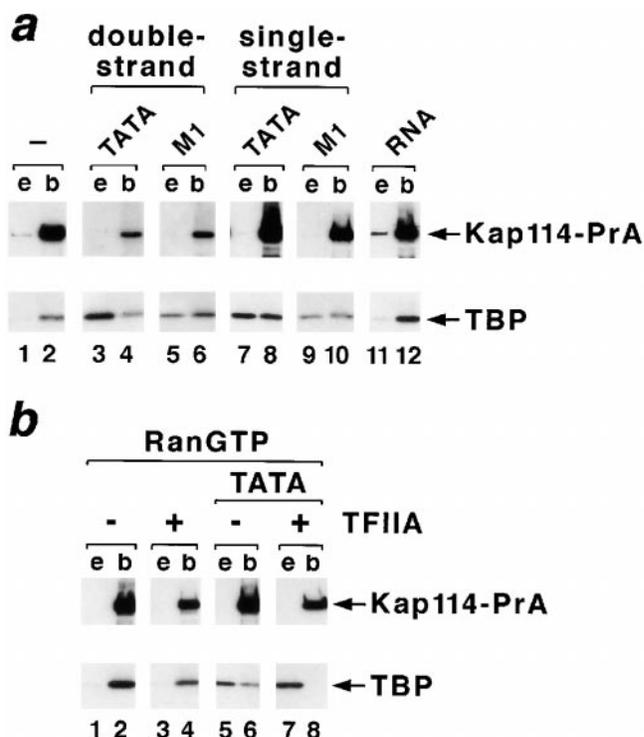
tion experiments have been carried out at RanGTP concentrations in the 5–10- $\mu$ M range (Schlenstedt et al., 1997; Albertini et al., 1998; Jäkel and Görlich, 1998; Kaffman et al., 1998b; Senger et al., 1998) and even at these concentrations many of the dissociation experiments appeared to be fairly inefficient. The concentration of unbound RanGTP in the nucleus is unknown, however, a number of RanGTP-binding proteins such as the 14 Kaps, RanBP1, and the RanGEF, present in the nucleus, will compete for binding to RanGTP. Additional factors might, therefore, be necessary to stimulate RanGTP-dependent dissociation of import substrates from their cognate Kaps. To investigate this possibility we reduced the RanGTP in our experiments to a level where we saw virtually no dissociation of TBP from Kap114p. As TBP functions by binding the TATA box at the core promoter, we first determined whether an 18-bp ds oligonucleotide containing the *CYC1* TATA box might stimulate the dissociation of TBP from Kap114p. This particular ds oligonucleotide was also used in the cocrystallization of TBP bound to DNA (Y. Kim et al., 1993; Geiger et al., 1996). Isolated Kap114-PrA-TBP complex was incubated with 380 nM RanGTP, and very little dissociation of TBP from the Kap was observed (Fig. 7 b, lanes 5 and 6). In these experiments the dissociated TBP fraction was collected and the bound fraction containing Kap114-PrA and bound TBP was eluted as one fraction with 4.5 M MgCl<sub>2</sub>. Surprisingly, the addition of increasing concentrations of TATA-containing DNA, in the presence of RanGTP, led to the dissociation of most of the TBP from Kap114p (Fig. 7 b, lanes 9–12). Even with a low concentration of DNA (5 ng/ $\mu$ l or 430 nM) at least half the TBP was released from Kap114p (Fig. 7 b, lanes 7 and 8). In contrast, when the complex was incubated with a high concentration of DNA but no RanGTP, no dissociation was observed, demonstrating that DNA alone could not release TBP from Kap114p (Fig. 7 b, lanes 3 and 4). The DNA-mediated dissociation was dependent on RanGTP rather than RanGDP, as high concentrations of RanGDP (4.8  $\mu$ M) and DNA (10 ng/ $\mu$ l) together caused no dissociation (Fig. 7 b, lanes 1 and 2). Using biotinylated ds TATA oligonucleotides captured on streptavidin beads, it was also possible to show that the released TBP was bound to DNA (data not shown). These experiments show that at this concentration of RanGTP, TBP is released from Kap114p only in the presence of DNA and suggests a possible mechanism for the specific release of TBP at its DNA target.

To determine whether this effect was specific for ds TATA-containing DNA, several controls were carried out using mutant TATA-containing ds DNA, single stranded DNA, and total yeast RNA in the presence of 290 nM RanGTP (Fig. 8 a). Total yeast RNA did not stimulate dissociation, suggesting that we were not observing a nonspecific effect of large negatively charged molecules (Fig. 8 a, lanes 11 and 12). We saw a spectrum of activity using other DNA controls, some double and single stranded templates led to low levels of dissociation (data not shown), suggesting that they would not stimulate the release of TBP from the Kap. Other DNA controls including the ds oligonucleotide, M1, that was identical to the TATA oligonucleotide but included two mutations in the TATA box (Fig. 8 a, lanes 9 and 10) stimulated dissociation quite efficiently.

Single stranded TATA DNA (Fig. 8 a, lanes 7 and 8) and single stranded M1 DNA (Fig. 8 a, lanes 9 and 10) stimulated some dissociation of the TBP from the Kap. However, most of the controls did not stimulate dissociation as well as the original TATA-containing ds oligonucleotides (compare all with Fig. 8 a, lanes 1 and 2).

### *The TBP-interacting Protein TFIIA Stimulates Dissociation Only in the Presence of DNA*

As DNA stimulated the dissociation of TBP in the presence of RanGTP, it was possible that a TBP-interacting protein could also function in this way. We examined TFIIA as it has been shown to bind TBP alone and complexed with DNA (Ranish et al., 1992; Geiger et al., 1996; Tan et al., 1996). At the RanGTP concentrations (290 nM) used in this experiment, almost no dissociation of TBP from Kap114p was observed (Fig. 8 b, lanes 1 and 2) and the addition of ds TATA-containing DNA (2 ng/ $\mu$ l) with RanGTP resulted in the dissociation of more than half of



**Figure 8.** TFIIA and RNA do not stimulate the RanGTP-mediated dissociation of TBP from Kap114p in the absence of DNA. The Kap114-PrA-TBP complex was isolated from cytosol using IgG-Sepharose. After dissociation under defined conditions, eluted and bound fractions were separated by SDS-PAGE and immunoblotted with an antibody to TBP as before. (a) All dissociation reactions included 290 nM RanGTP and either no additions (–; lanes 1 and 2), or 4 ng/ $\mu$ l ds TATA DNA (lanes 3 and 4), 4 ng/ $\mu$ l ds M1 DNA (lanes 5 and 6), 10 ng/ $\mu$ l single stranded TATA DNA (lanes 7 and 8), 10 ng/ $\mu$ l single stranded M1 DNA (lanes 9 and 10), total RNA 10 ng/ $\mu$ l (lanes 11 and 12) as indicated. (b) All dissociation reactions were carried out in the presence of 290 nM RanGTP. 2 ng/ml of TATA-containing DNA or 65 nM TFIIA were added where indicated. Eluted fraction (e) and bound fraction (b) that were released from the IgG-Sepharose by 4.5 M MgCl<sub>2</sub> are shown.

the TBP (Fig. 8 b, lanes 5 and 6). The addition of TFIIA (65 nM) together with RanGTP, however, did not result in the dissociation of any TBP (Fig. 8 b, lanes 3 and 4). At this concentration, analysis of amido black-stained blots suggested that TFIIA was in excess of TBP (data not shown). RanGTP-dependent dissociation was also not observed when a higher concentration of TFIIA (200 nM) was used (data not shown). This suggested that TFIIA could not stimulate the RanGTP-dependent dissociation of TBP from the Kap in the absence of ds DNA, raising the possibility that Kap114p may prevent TBP from associating with TFIIA before it has bound DNA. When TFIIA was added together with RanGTP and DNA, as expected most of the TBP was dissociated from Kap114p in a similar manner to that seen with RanGTP and DNA alone (Fig. 8 b, lanes 7 and 8). However, the addition of TFIIA appeared to stimulate the dissociation seen with RanGTP and DNA. This may be due to the fact that TFIIA stabilizes the TBP-DNA interaction (Imbalzano et al., 1994).

### Discussion

Here we show that the major import pathway for TBP is mediated by the newly characterized Kap, Kap114p. TBP is also imported into the nucleus by Kap114p-independent pathways and we suggest that these pathways may be mediated by Kap123p and Kap121p. As expected for a Kap family member, the dissociation of TBP and Kap114p is RanGTP-dependent. However, we show here that this dissociation is greatly stimulated by the addition of TATA-containing ds DNA. This suggests a mechanism for the targeted dissociation of TBP at the promoter of genes to be transcribed.

The isolation of a cytosolic Kap114p-TBP complex suggested that Kap114p mediated the import of TBP into the nucleus and analysis of the localization of TBP in the absence of Kap114p confirmed this. Our ability to reconstitute the Kap114p-TBP complex using recombinant proteins suggested that they interact directly. In addition, the Kap114p-TBP complex, isolated from yeast cytosol, was resistant to incubation with S7 nuclease and DNase, suggesting that the complex was not mediated by nucleic acid (data not shown). Strains lacking Kap114p are viable, in contrast to strains lacking TBP, which is encoded by an essential gene. In common with the Pdr6p/Kap122p-mediated import pathway for TFIIA (Titov, A., and G. Blobel, manuscript submitted for publication) and the import of ribosomal proteins by Kap123p proteins (Rout et al., 1997; Schlenstedt et al., 1997), this represents another example of a nonessential Kap importing an essential substrate. This led us to search for additional pathways into the nucleus for TBP. The isolation of TBP-interacting Kaps, Kap123p and Kap121p, suggested that they too might mediate import of TBP. In both a Kap123p deletion strain and in a Kap121p temperature sensitive strain, however, we saw no mislocalization of TBP. The fact that we could detect no other Kaps in a complex with TBP-PrA, and that these two Kaps were not isolated in analogous coprecipitation experiments with Lhp1p-PrA (Rosenblum, S., and G. Blobel, unpublished data) were indicative of a potential role for Kap123p and Kap121p in TBP import. The demonstration of direct binding and of an effect on nuclear im-

port will be needed, however, to conclusively demonstrate the role of Kap123p and Kap121p in TBP import. It is likely that Kap114p mediates the bulk of TBP nuclear import, as evidenced by the fact that among the TBP-interacting Kaps, only a *kap114* deletion leads to mislocalization of TBP, even in the presence of an otherwise wild-type complement of Kaps.

We do not know if these are the only Kaps responsible for the import of TBP into the nucleus. Kap121p is encoded by an essential gene (Chow et al., 1992), and we were, therefore, not able to test for synthetic lethality by deletion of all three genes in one strain. It is possible that there are additional Kap-mediated pathways for the import of TBP as Kap121p was only detected by immunoblotting, indicating that other Kaps could be interacting below the limits of detection by Coomassie blue staining. In strains lacking both Kap114p and Kap123p only very small amounts of TBP-copurifying Kap were detected by our assay. As some TBP was being imported into the nucleus to support normal transcription and cell growth (discussed below), it is possible that we are not able to detect very nonabundant Kaps in this way. The fact that we could not detect them may suggest that these Kaps interact with their substrates with lower affinity or the interaction is less stable. It is also possible that some TBP is entering the nucleus by a Kap-independent pathway or by diffusion. Diffusion is unlikely, however, as TBP is believed to dimerize in the cytoplasm, increasing its molecular weight to beyond the exclusion limit of the NPC (Coleman et al., 1995).

Like mammalian ribosomal proteins, this is an example of a single substrate that appears to have multiple secondary pathways into the nucleus (Jakel and Görlich, 1998). It may prove to be a general phenomenon, whereas one Kap plays a major role in the import of a given substrate, this function is redundant with several other Kaps. Such a multiply redundant, hierarchical import pathway also suggests that the coordinate regulation of the Kap family is likely to be very complex. This may help explain why there are so many nonessential or redundant Kaps. Of the fourteen  $\beta$  Kaps present in the yeast genome, only four are encoded by essential genes (Kap95p, Kap121p, Crm1p, and Cse1p), whereas deletion of the others lead to varying effects on cell growth (Chow et al., 1992; Toda et al., 1992; Shen et al., 1993; Xiao et al., 1993; Enenkel et al., 1995; Aitchison et al., 1996; Akada et al., 1996; Pemberton et al., 1997; Rosenblum et al., 1997; Rout et al., 1997; Schlenstedt et al., 1997; Seedorf and Silver, 1997; Albertini et al., 1998; Ferrigno et al., 1998; Chaves, S., and G. Blobel, unpublished data; Titov, A., and G. Blobel, manuscript submitted for publication). It is possible that the essential Kaps transport essential proteins, or they may play a more general role and can backup many pathways. Alternatively, they may carry out the bulk of nuclear transport, although their relative abundance would suggest that this is not the case (Rout et al., 1997). Kap121p has been shown to functionally overlap with Kap123p and can participate in the import of ribosomal proteins (Rout et al., 1997; Schlenstedt et al., 1997). If Kap123p and Kap121p also play a role in the import of TBP, it raises the possibility that the Kap family may be divided into functional subgroups. Future identification of additional substrates for these three Kaps will

determine whether this subgroup coordinates the import of other proteins.

Kap123p copurified with TBP-PrA in the absence of Kap114p, whereas Kap121p was detected in small quantities in both the presence and absence of Kap114p. As Kap114p would be present in a wild-type yeast cell, it is possible that some TBP import is constitutively mediated by other Kaps or that other Kaps participate in TBP import only under specific conditions. These conditions could be dictated by the available carbon source, stress, or phase of the cell cycle amongst others. Several Kaps have been shown to have more than one substrate, and the fact that there are many more nuclear proteins than Kaps suggests that this will be a general phenomenon (Aitchison et al., 1996; Rosenblum et al., 1997; Rout et al., 1997; Schlenstedt et al., 1997; Albertini et al., 1998; Ferrigno et al., 1998; Kaffman et al., 1998b). We observed several Kap114-PrA copurifying bands in addition to TBP, suggesting that it also may have other import substrates and future characterization should determine the identity of these proteins.

The Kaps may be coordinated in a complex network of overlapping substrate specificities, raising the problem of how several Kaps can recognize the same substrates while distinguishing between others. The recognition of a substrate by a Kap is determined by the NLS contained in the substrate. The crystal structure of TBP has been elucidated both alone and complexed with DNA, TFIIA, and TFIIB (Nikolov et al., 1992, 1995, 1996; J.L. Kim et al., 1993; Y. Kim et al., 1993; Geiger et al., 1996; Tan et al., 1996). This has allowed the precise binding sites of these molecules within TBP to be determined. It is not yet known whether the TBP NLS recognized by Kap114p overlaps with one of these sites, such as the DNA-binding domain, or whether the Kap recognizes another domain of TBP. It is possible that other Kaps, for example Kap121p and Kap123p, may recognize the same NLS in TBP as Kap114p. However, comparison of TBP with known Kap123p and Kap121p substrates has not yet revealed any similarities at the amino acid level that may represent a consensus NLS.

To function in transcription, TBP in the nucleus must be first dissociated from Kap114p and then interact with DNA and other components of the transcriptional machinery. All the Kaps are believed to bind RanGTP with their homologous amino-terminal domain as shown in the structure of mammalian Kap  $\beta$ 2 complexed with RanGTP (Chook and Blobel, 1999). As expected RanGTP, which is believed to be the predominant form of Ran in the nucleus, is necessary for the dissociation of TBP from Kap114p. RanGDP does not appear to be able to function in this process. However, we observed *in vitro* that not all the TBP was dissociated from Kap114p, even at high (10  $\mu$ M) RanGTP concentrations. Previously published Kap import substrate dissociations have been carried out at RanGTP concentrations in the 5–10- $\mu$ M range, however, many of these dissociation experiments appeared similarly inefficient (Schlenstedt et al., 1997; Albertini et al., 1998; Jakel and Gorlich, 1998; Kaffman et al., 1998b; Senger et al., 1998). Although the total Ran concentration in the whole cell has been suggested to be as high as 5–10  $\mu$ M, it is difficult to predict the concentration of unbound

RanGTP in the nucleus (Bischoff and Ponstingl, 1991; Gygi et al., 1999). As there are at least 16 RanGTP binding partners present in the nucleus, which bind RanGTP with dissociation constants in the micromolar to nanomolar range, the concentration of unbound RanGTP may be much lower than this (Görlich et al., 1997; Kutay et al., 1997; Schlenstedt et al., 1997; Senger et al., 1998; Floer and Blobel, 1999). At lower (190–380 nM) concentrations of RanGTP, we observed almost no dissociation of TBP from Kap114p that led us to search for stimulatory factors. We identified ds TATA-containing DNA as one such factor. As nonnucleosomal ds TATA-containing DNA would be the available form of DNA at genes about to undergo transcription, this suggests a mechanism whereby TBP may only be dissociated from the Kap at its point of function.

The dissociation caused by TATA-containing DNA was most efficient, however, other DNAs could also stimulate dissociation of TBP from Kap114p to varying extents. This lack of specificity *in vitro* may be because TBP can bind all DNA with some affinity (Chen and Struhl, 1988; Coleman and Pugh, 1995). *In vivo*, other GTFs such as TFIIA and TFIIB would be present and there is evidence to suggest that they increase the specificity of TBP for TATA sequences (Imbalzano et al., 1994). Alternatively, *in vivo* the initial dissociation at the promoter may be TATA-independent; once dissociated, TBP could then move to the TATA box itself to form a high affinity interaction (Coleman and Pugh, 1995). In this way, the dissociation would still most likely occur at the promoter as only a small proportion of DNA in the nucleus is actually available for TBP binding. Most DNA is incorporated into nucleosomes, and naked, nonnucleosomal DNA is only likely to be found at promoters of genes that are about to be transcribed or after the replication fork (Sogo et al., 1986).

The TBP-interacting protein TFIIA did not stimulate the dissociation of TBP in the absence of TATA-containing DNA. It is possible that association of TBP with Kap114p prevents TBP from prematurely interacting with other PIC components before reaching the promoter. TFIIA did stimulate dissociation in the presence of DNA, which may be due to its ability to stabilize TBP-TATA interactions (Imbalzano et al., 1994). It is not yet known whether other TBP-interacting proteins such as TBP-associated factors, TFIIB, negative regulators of TBP, and components of the SAGA complex can stimulate the dissociation of TBP from Kap114p, either with or without DNA. Future experiments will be needed to determine the sequence of events of PIC complex formation *in vivo*. There is believed to be enough TBP present in the cell to participate in transcription from all 6,000 plus promoters at one time (Lee and Young, 1998). There are at least three mechanisms by which the availability of TBP and its ability to function at specific promoters is negatively regulated. These include the proteins or protein complexes Mot1p, NC2, and Nots (Lee and Young, 1998). It is likely that much of the cellular TBP is negatively regulated by these factors, which may act by sequestering TBP (Lee and Young, 1998). Our *in vitro* dissociation experiments would argue that the Kap participates in this regulation, by only releasing the TBP it has imported, at its point of function.

By reducing the amount of RanGTP we observed a dissociation requirement for further factors that may prove to be a general phenomenon in nuclear transport. Previous studies using Kap111p/Mtr10p have shown that the addition of total cellular RNA also stimulated the RanGTP-dependent dissociation of the mRNA-binding protein Npl3p, from Kap111p (Senger et al., 1998). The DNA-stimulated, RanGTP-mediated targeted dissociation of TBP from Kap114p we observe in vitro suggests that in vivo dissociation might be effected at much lower concentrations of RanGTP than previously thought. For targeted dissociation to occur, Kap114p would be predicted to travel with TBP from the nucleoplasmic face of the NPC to the promoter, and this raises the possibility that this Kap may also be functioning in intranuclear delivery of TBP. Very little is known about the movement of molecules within the nuclear interior and it is possible that Kaps function not only in nucleocytoplasmic transport but may also mediate the efficient transport of proteins within the nucleus.

It was surprising that although much of the TBP was mislocalized in the  $\Delta kap114$  deletion strain, we could detect no growth defect. It is possible that the small amount of TBP that was imported into the nucleus was sufficient to support transcription. Whether this small amount of TBP is now no longer subject to the negative regulation discussed above, and is all functioning in transcription, remains to be determined. In the absence of Kap114p, TBP imported by other Kaps such as Kap121p and Kap123p, may also be dissociated in a DNA-stimulated, RanGTP-dependent fashion at its point of function and it will be interesting to determine whether this will be a generalized theme in nuclear transport. The experiments presented here for TBP and previously published data for TFIIA suggest that at least two components of the PIC are imported by separate pathways (Titov, A., and G. Blobel, manuscript submitted for publication). It remains unclear how other components of the PIC are imported into the nucleus and whether their import by separate pathways plays a role in their regulation.

In summary, we have identified a new pathway for protein import into the nucleus, mediated by the novel transport factor Kap114p. This pathway constitutes the major import pathway for TBP, however, other Kaps also appear to participate in TBP import. The import of TBP is one of the first examples in yeast of a substrate having multiply redundant, hierarchical import pathways and suggests that the Kaps may be coordinated in a complex network of overlapping substrate specificities. We also present data suggesting that TBP is only released by the Kap at its point of function, raising the possibility that the Kaps may play a role in the intranuclear targeting of their substrates as well as nucleocytoplasmic transport.

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## References

- Aitchison, J.D., M.P. Rout, M. Marelli, G. Blobel, and R.W. Wozniak. 1995. Two novel related yeast nucleoporins Nup170p and Nup157p: complementation with the vertebrate homologue Nup155p and functional interactions with the yeast nuclear pore-membrane protein Pom152p. *J. Cell Biol.* 131: 1133-1148.
- Aitchison, J.D., G. Blobel, and M.P. Rout. 1996. Kap104p: a karyopherin involved in the nuclear transport of messenger RNA binding proteins. *Science*. 274:624-627.
- Akada, R., L. Kallal, D.I. Johnson, and J. Kurjan. 1996. Genetic relationships between the G protein beta gamma complex, Ste5p, Ste20p, and Cdc42p: investigation of effector roles in the yeast pheromone response pathway. *Genetics*. 143:103-117.
- Albertini, M., L.F. Pemberton, J.S. Rosenblum, and G. Blobel. 1998. A novel nuclear import pathway for the transcription factor TFIIIS. *J. Cell Biol.* 143: 1447-1455.
- Bischoff, F.R., and H. Ponstingl. 1991. Mitotic regulator protein RCC1 is complexed with a nuclear ras-related polypeptide. *Proc. Natl. Acad. Sci. USA*. 88:10830-10834.
- Burley, S.K., and R.G. Roeder. 1996. Biochemistry and structural biology of transcription factor IID (TFIID). *Annu. Rev. Biochem.* 65:769-799.
- Chen, W., and K. Struhl. 1988. Saturation mutagenesis of a yeast his3 "TATA element": genetic evidence for a specific TATA-binding protein. *Proc. Natl. Acad. Sci. USA*. 85:2691-2695.
- Chook, Y., and G. Blobel. 1999. Structure of the nuclear transport complex karyopherin- $\beta$ -Ran-GppNHp. *Nature*. 399:230-237.
- Chow, T.Y., J.J. Ash, D. Dignard, and D.Y. Thomas. 1992. Screening and identification of a gene, PSE-1, that affects protein secretion in *Saccharomyces cerevisiae*. *J. Cell Sci.* 101:709-719.
- Coleman, R.A., and B.F. Pugh. 1995. Evidence for functional binding and stable sliding of the TATA binding protein on nonspecific DNA. *J. Biol. Chem.* 270:13850-13859.
- Coleman, R.A., A.K. Taggart, L.R. Benjamin, and B.F. Pugh. 1995. Dimerization of the TATA binding protein. *J. Biol. Chem.* 270:13842-13849.
- Eisenmann, D.M., C. Dollard, and F. Winston. 1989. SPT15, the gene encoding the yeast TATA binding factor TFIID, is required for normal transcription initiation in vivo. *Cell*. 58:1183-1191.
- Enenkel, C., G. Blobel, and M. Rexach. 1995. Identification of a yeast karyopherin heterodimer that targets import substrate to mammalian nuclear pore complexes. *J. Biol. Chem.* 270:16499-16502.
- Fernandez, J., L. Andrews, and S.M. Mische. 1994. An improved procedure for enzymatic digestion of polyvinylidene difluoride-bound proteins for internal sequence analysis. *Anal. Biochem.* 218:112-117.
- Ferrigno, P., F. Posas, D. Koepp, H. Saito, and P.A. Silver. 1998. Regulated nucleocytoplasmic exchange of HOG1 MAPK requires the importin beta homologs NMD5 and XPO1. *EMBO (Eur. Mol. Biol. Organ.) J.* 17:5606-5614.
- Finley, D., E. Ozkaynak, and A. Varshavsky. 1987. The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation, and other stresses. *Cell*. 48:1035-1046.
- Floer, M., and G. Blobel. 1996. The nuclear transport factor karyopherin  $\beta$  binds stoichiometrically to Ran-GTP and inhibits the Ran GTPase activating protein. *J. Biol. Chem.* 271:5313-5316.
- Floer, M., and G. Blobel. 1999. Putative reaction intermediates in Crm1-mediated nuclear protein export. *J. Biol. Chem.* 274:16279-16286.
- Fornerod, M., M. Ohno, M. Yoshida, and I.W. Mattaj. 1997. CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell*. 90:1051-1060.
- Geiger, J.H., S. Hahn, S. Lee, and P.B. Sigler. 1996. Crystal structure of the yeast TFIIA/TBP/DNA complex. *Science*. 272:830-836.
- Gharahdaghi, F., M. Kirchner, J. Fernandez, and S.M. Mische. 1996. Peptide-mass profiles of polyvinylidene difluoride-bound proteins by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in the presence of nonionic detergents. *Anal. Biochem.* 233:94-99.
- Görlich, D., M. Dabrowski, F.R. Bischoff, U. Kutay, P. Bork, E. Hartmann, S. Prehn, and E. Izaurralde. 1997. A novel class of RanGTP binding proteins. *J. Cell Biol.* 138:65-80.
- Gygi, S.P., Y. Rochon, B.R. Franza, and R. Aebersold. 1999. Correlation between protein and mRNA abundance in yeast. *Mol. Cell Biol.* 19:1720-1730.
- Hellmuth, K., D.M. Lau, F.R. Bischoff, M. Kunzler, E. Hurt, and G. Simos. 1998. Yeast Los1p has properties of an exportin-like nucleocytoplasmic transport factor for tRNA. *Mol. Cell Biol.* 18:6374-6386.
- Hood, J.K., and P.A. Silver. 1998. Cse1p is required for export of Srp1p/importin-alpha from the nucleus in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 273: 35142-35146.
- Imbalzano, A.N., K.S. Zaret, and R.E. Kingston. 1994. Transcription factor (TF) IIB and TFIIA can independently increase the affinity of the TATA-binding protein for DNA. *J. Biol. Chem.* 269:8280-8286.
- Izaurralde, E., U. Kutay, C. von Kobbe, I.W. Mattaj, and D. Görlich. 1997. The asymmetric distribution of the constituents of the Ran system is essential for

- transport into and out of the nucleus. *EMBO (Eur. Mol. Biol. Organ.) J.* 16: 6535–6547.
- Jakel, S., and D. Görlich. 1998. Importin beta, transportin, RanBP5, and RanBP7 mediate nuclear import of ribosomal proteins in mammalian cells. *EMBO (Eur. Mol. Biol. Organ.) J.* 17:4491–4502.
- Kaffman, A., N.M. Rank, E.M. O'Neill, L.S. Huang, and E.K. O'Shea. 1998a. The receptor Msn5 exports the phosphorylated transcription factor Pho4 out of the nucleus. *Nature.* 396:482–486.
- Kaffman, A., N.M. Rank, and E.K. O'Shea. 1998b. Phosphorylation regulates association of the transcription factor Pho4 with its import receptor Pse1/Kap121. *Genes Dev.* 12:2673–2683.
- Kim, J.L., D.B. Nikolov, and S.K. Burley. 1993. Co-crystal structure of TBP recognizing the minor groove of a TATA element. *Nature.* 365:520–527.
- Kim, Y., J.H. Geiger, S. Hahn, and P.B. Sigler. 1993. Crystal structure of a yeast TBP/TATA-box complex. *Nature.* 365:512–520.
- Kunzler, M., and E.C. Hurt. 1998. Cse1p functions as the nuclear export receptor for importin alpha in yeast. *FEBS (Fed. Eur. Biochem Soc.) Lett.* 433: 185–190.
- Kutay, U., F.R. Bischoff, S. Kostka, R. Kraft, and D. Görlich. 1997. Export of importin alpha from the nucleus is mediated by a specific nuclear transport factor. *Cell.* 90:1061–1071.
- Lee, T.L., and R.A. Young. 1998. Regulation of gene expression by TBP-associated proteins. *Genes Dev.* 12:1398–1408.
- Marelli, M., J.D. Aitchison, and R.W. Wozniak. 1998. Specific binding of the karyopherin Kap121p to a subunit of the nuclear pore complex containing Nup53p, Nup59p, and Nup170p. *J. Cell Biol.* 143:1813–1830.
- Moore, M.S. 1998. Ran and nuclear transport. *J. Biol. Chem.* 273:22857–22860.
- Nikolov, D.B., S.H. Hu, J. Lin, A. Gasch, A. Hoffmann, M. Horikoshi, N.H. Chua, R.G. Roeder, and S.K. Burley. 1992. Crystal structure of TFIID TATA-box binding protein. *Nature.* 360:40–46.
- Nikolov, D.B., H. Chen, E.D. Halay, A.A. Usheva, K. Hisatake, D.K. Lee, R.G. Roeder, and S.K. Burley. 1995. Crystal structure of a TFIIB-TBP-TATA-element ternary complex. *Nature.* 377:119–128.
- Nikolov, D.B., H. Chen, E.D. Halay, A. Hoffman, R.G. Roeder, and S.K. Burley. 1996. Crystal structure of a human TATA box-binding protein/TATA element complex. *Proc. Natl. Acad. Sci. USA.* 93:4862–4867.
- Orphanides, G., T. Lagrange, and D. Reinberg. 1996. The general transcription factors of RNA polymerase II. *Genes Dev.* 10:2657–2683.
- Pemberton, L.F., J.S. Rosenblum, and G. Blobel. 1997. A distinct and parallel pathway for the nuclear import of an mRNA-binding protein. *J. Cell Biol.* 139:1645–1653.
- Pemberton, L.F., G. Blobel, and J.S. Rosenblum. 1998. Transport routes through the nuclear pore complex. *Curr. Opin. Cell Biol.* 10:392–399.
- Ranish, J.A., W.S. Lane, and S. Hahn. 1992. Isolation of two genes that encode subunits of the yeast transcription factor IIA. *Science.* 255:1127–1129.
- Rexach, M., and G. Blobel. 1995. Protein import into nuclei: association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. *Cell.* 83:683–692.
- Roeder, R.G. 1996. The role of general initiation factors in transcription by RNA polymerase II. *Trends Biochem. Sci.* 21:327–335.
- Rosenblum, J.S., L.F. Pemberton, and G. Blobel. 1997. A nuclear import pathway for a protein involved in tRNA maturation. *J. Cell Biol.* 139:1655–1661.
- Rosenblum, J.S., L.F. Pemberton, N. Bonifaci, and G. Blobel. 1998. Nuclear import and the evolution of a multifunctional RNA-binding protein. *J. Cell Biol.* 143:887–899.
- Rout, M.P., G. Blobel, and J.D. Aitchison. 1997. A distinct nuclear import pathway used by ribosomal proteins. *Cell.* 89:715–725.
- Schlenstedt, G., E. Smirnova, R. Deane, J. Solsbacher, U. Kutay, D. Görlich, H. Ponstingl, and F.R. Bischoff. 1997. Yrb4p, a yeast ran-GTP-binding protein involved in import of ribosomal protein L25 into the nucleus. *EMBO (Eur. Mol. Biol. Organ.) J.* 16:6237–6249.
- Seedorf, M., and P.A. Silver. 1997. Importin/karyopherin protein family members required for mRNA export from the nucleus. *Proc. Natl. Acad. Sci. USA.* 94:8590–8595.
- Senger, B., G. Simos, F.R. Bischoff, A. Podtelejnikov, M. Mann, and E. Hurt. 1998. Mtr10p functions as a nuclear import receptor for the mRNA-binding protein Npl3p. *EMBO (Eur. Mol. Biol. Organ.) J.* 17:2196–2207.
- Shen, W.C., D. Selvakumar, D.R. Stanford, and A.K. Hopper. 1993. The *Saccharomyces cerevisiae* LOS1 gene involved in pre-tRNA splicing encodes a nuclear protein that behaves as a component of the nuclear matrix. *J. Biol. Chem.* 268:19436–19444.
- Sogo, J.M., H. Stahl, T. Koller, and R. Knippers. 1986. Structure of replicating simian virus 40 minichromosomes. The replication fork, core histone segregation and terminal structures. *J. Mol. Biol.* 189:189–204.
- Solsbacher, J., P. Maurer, F.R. Bischoff, and G. Schlenstedt. 1998. Cse1p is involved in export of yeast importin alpha from the nucleus. *Mol. Cell Biol.* 18: 6805–6815.
- Stade, K., C.S. Ford, C. Guthrie, and K. Weis. 1997. Exportin 1 (Crm1) is an essential nuclear export factor. *Cell.* 90:1041–1050.
- Tan, S., Y. Hunziker, D.F. Sargent, and T.J. Richmond. 1996. Crystal structure of a yeast TFIIA/TBP/DNA complex. *Nature.* 381:127–151.
- Toda, T., M. Shimanuki, Y. Saka, H. Yamano, Y. Adachi, M. Shirakawa, Y. Kyogoku, and M. Yanagida. 1992. Fission yeast pap1-dependent transcription is negatively regulated by an essential nuclear protein, crm1. *Mol. Cell Biol.* 12:5474–5484.
- Wozniak, R.W., M.P. Rout, and J.D. Aitchison. 1998. Karyopherins and kissing cousins. *Trends Cell Biol.* 8:184–188.
- Xiao, Z., J.T. McGrew, A.J. Schroeder, and M. Fitzgerald-Hayes. 1993. CSE1 and CSE2, two new genes required for accurate mitotic chromosome segregation in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 13:4691–4702.
- Yan, C., L.H. Lee, and L.I. Davis. 1998. Crm1p mediates regulated nuclear export of a yeast AP-1-like transcription factor. *EMBO (Eur. Mol. Biol. Organ.) J.* 17:7416–7429.