



Importin β -type nuclear transport receptors have distinct binding affinities for Ran–GTP

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ARTICLE INFO

Article history:

Received 26 January 2011

Available online 15 February 2011

Keywords:

Exportin

Importin

Nucleocytoplasmic transport

Ran GTPase

Transport receptor

Surface plasmon resonance spectrometry

ABSTRACT

Cargos destined to enter or leave the cell nucleus are typically transported by receptors of the importin β family to pass the nuclear pore complex. The yeast *Saccharomyces cerevisiae* comprises 14 members of this protein family, which can be divided in importins and exportins. The Ran GTPase regulates the association and dissociation of receptors and cargos as well as the transport direction through the nuclear pore. All receptors bind to Ran exclusively in its GTP-bound state and this event is restricted to the nuclear compartment. We determined the Ran–GTP binding properties of all yeast transport receptors by biosensor measurements and observed that the affinity of importins for Ran–GTP differs significantly. The dissociation constants range from 230 pM to 270 nM, which is mostly based on a variability of the off-rate constants. The divergent affinity of importins for Ran–GTP suggests the existence of a novel mode of nucleocytoplasmic transport regulation. Furthermore, the cellular concentration of β -receptors and of other Ran-binding proteins was determined. We found that the number of β -receptors altogether about equals the amounts of yeast Ran, but Ran–GTP is not limiting in the nucleus. The implications of our results for nucleocytoplasmic transport mechanisms are discussed.

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1. Introduction

The nuclear envelope separates the cytoplasm from the nucleoplasm in eukaryotic cells. The transport of molecules occurs across nuclear pore complexes (NPC), which are imbedded in the double membrane of the nuclear envelope. Whereas smaller molecules can diffuse through the nuclear pore, macromolecules are usually transported by an energy-consuming and receptor-dependent mechanism. Proteins are regularly translocated by soluble transport receptors of the importin β superfamily, which are also called β -karyopherins and are divided into importins and exportins. Humans contain 20 β -receptors and in baker's yeast there are 14 family members, 10 importins and four exportins [1]. The translocation pathways are regulated by the Ran GTPase, an abundant and mostly nuclear Ras-like protein [2–5]. The differential localization of Ran modulators, the cytoplasmic GTPase activating protein (RanGAP in vertebrates, Rna1 in yeast) and the nuclear guanine exchange factor (GEF, RCC1 in vertebrates, Prp20 in yeast) establishes a gradient, where Ran–GTP is predominantly present in the nucleoplasm whereas Ran–GDP is essentially cytoplasmic [6]. Therefore,

Ran–GTP acts as a major signal for the nuclear compartment. By binding to importin β -like receptors in the nucleus, Ran–GTP dissociates molecular cargos from importins and concurrently brings forward cargo association by exportins.

Ran–GTP is exported complexed either to cargo-free importins or to cargo-bound exportins and is released from these complexes after GAP-catalyzed GTP hydrolysis in the cytoplasmic compartment. This process additionally requires the activity of Ran binding protein 1 (RanBP1 in vertebrates, Yrb1 in yeast), a co-activator of the GAP that releases Ran–GTP from export complexes, which are otherwise resistant to the GAP [7–10]. The Ran binding protein 1 contains a Ran–GTP binding domain different to that of β -receptors. In yeast, Yrb1 shares this domain with the nucleoporin Nup2 and with Yrb2, a cytoplasmic factor specifically involved in protein export [11]. After GTP hydrolysis, Ran undergoes a conformational change [5] and the GDP-bound form is released from β -receptors and RanBP1-type proteins. Ran–GDP is then re-imported into the nucleus by Ntf2 and subsequently converted to Ran–GTP by the GEF [12].

Whereas importins usually bind directly to their molecular cargo [13], importin β (Kap95 in yeast) can also bind to importin α , which is unable to pass the NPC by itself. Importin α (Srp1 in yeast) binds to proteins bearing a so called classical nuclear localization signal and functions as an adapter to access the importin β -dependent import pathway [14]. Ran–GTP is again necessary for import termination in the nucleus. In this study, we asked the question

Abbreviations: GTP, guanosine 5'-triphosphate; GST, glutathione-S-transferase; SPR, surface plasmon resonance.

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whether the binding affinity of Ran–GTP differs for the various β -receptors, which would have implications for the association and dissociation kinetics of transport complexes. Remarkably, we found that the affinities vary over a broad range of more than three orders of magnitude. We also determined the cellular concentration of most yeast Ran-binding proteins and found that their combined abundance is higher than that of total cellular Ran, but the amount of Ran about equals the amount of all β -receptors. However, the concentration of Ran–GTP was estimated to exceed that of Ran–GTP binding proteins in the nucleus indicating that Ran–GTP is not limiting under normal conditions.

2. Materials and methods

2.1. Plasmids and protein purification

The purification of GST–Gsp1Q71L, Yrb1, Xpo1, Pse1, Cse1 [15], Kap120, Kap95, Kap114, Yrb4, Kap104, and Nmd5 [16] was described before. The complete coding regions of *MTR10*, *MSN5*, *PDR6*, *LOS1*, and *SXM1* were PCR-amplified using Pwo polymerase (Roche) and inserted into plasmids pQE9 or pQE30 (Qiagen) for N-terminal 6His tagging yielding pQE9–*MTR10* (pGS883), pQE30–*MSN5* (pGS1097), pQE30–*PDR6* (pGS1126), pQE30–*LOS1* (pGS1131), and pQE30–*SXM1* (pGS1542). Protein purification of transport receptors from *Escherichia coli* lysates was performed by nickel–nitrotri-acetic agarose (Qiagen) and Mono Q (GE Healthcare) chromatography. Polyclonal antibodies were raised by injecting rabbits with full-length proteins and affinity purification of the respective antisera.

2.2. Quantification of transport factors

For Western blotting, yeast cells (8×10^6 units) from liquid cultures were pelleted, resuspended in 1 ml 0.25 M NaOH/1% 2-mercaptoethanol, incubated for 10 min on ice, and precipitated with 160 μ l cold 50% trichloroacetic acid. After 10 min, the samples were centrifuged for 5 min, washed with cold acetone, vacuum-dried, and resuspended in 190 μ l SDS sample buffer and 10 μ l 1 M Tris–HCl pH 8.0. The immunological detection after transfer to PVDF membranes using peroxidase-coupled secondary antibodies (Sigma) was performed with the Super Signal West Pico Substrate Kit (Pierce). The quantification of the protein bands by densitometry was carried out with the ImageQuant software (GE Healthcare). To exactly determine the concentration of the purified proteins, we combined the Bradford assay, absorption measurement at 280 nm, and comparative densitometry using bovine serum albumin standards. To quantify the cellular protein concentrations, we compared fixed amounts of cell lysates giving a chemiluminescent signal in the linear range with a calibration line of purified proteins of known concentration using densitometry.

2.3. Protein interaction assays

GST pulldown assays and SPR spectrometry were performed as described [17] using PBSKMT buffer (25 mM sodium phosphate, 150 mM NaCl, 3 mM KCl, 1 mM MgCl₂, and 0.1% Tween 20, pH 7.3) as running, incubation, and washing buffer. SPR measurements were carried out with a BIALITE biosensor and a GST kit for fusion capture using monoclonal anti-GST antibodies that were covalently coupled to CM5 chips (BIAcore). The reference flow chamber was loaded with GST alone. GST–Gsp1Q71L–GTP served as the ligand and was immobilized to record 200–400 resonance units. The Ran-binding proteins functioned as analytes and were diluted into PBSKMT buffer. After association of the analytes, the chips were washed and the data were examined with the BIAcore

X and BIAevaluation 3.1 software programs (BIAcore). The calculation of the binding constants based on the 1:1 Langmuir model or the 1:1 Langmuir mass transfer model.

3. Results and discussion

3.1. The affinity of β -receptors for Ran–GTP is widely different

To visualize the binding of β -receptors to Gsp1, the essential yeast homologue of Ran, we first prebound a purified GST fusion protein to Gsp1Q71L, a mutant defective in GTP hydrolysis [15] and therefore present predominantly in the GTP-bound state, to glutathione Sepharose and then incubated with purified receptors tagged with a N-terminal or C-terminal 6His-tag (Fig. 1). As expected, all importins bound well to the immobilized Gsp1–GTP, whereas the tRNA exportin Los1 did not. Likewise, the exportins Cse1 and Xpo1 did not associate with Gsp1–GTP (not shown). This is in agreement with earlier observations that these exportins do not significantly bind to Gsp1–GTP in the absence of cargo [15,18,19]. In contrast, the binding of Msn5, which acts mostly as an exportin but can also function as an importin [20], was detected well in Coomassie-stained SDS gels (Fig. 1).

The binding properties of the β -receptor/Gsp1–GTP complexes were characterized in real time by SPR spectrometry using a BIAcore device. The ligand GST–Gsp1Q71L was bound to the matrix of a sensor chip using crosslinked GST-specific antibodies, which allows full accessibility to the Gsp1 moiety. To reduce the recording of unspecific interactions, we employed GST covalently linked to a sensor chip of the reference flow chamber. The 14 yeast β -receptors served as analytes and were passed over the surface of the sensor chip at various concentrations (Fig. 2). The receptors were injected into the flow cells for 60–90 s (association phase) and then buffer alone was applied to initiate the dissociation phase. The difference in the resonance signals between the sample flow cell and the reference cell was recorded. The obtained curves served to calculate the binding constants using established BIAcore software programs.

The recorded sensorgrams are shown in Fig. 2. Table 1 shows for each individual Gsp1-binding protein/Gsp1–GTP (analyte/ligand) pair the calculated dissociation constant K_d , the association constant K_a , the rate constant for dissociation k_d (off-rate constant), the rate constant for association k_a (on-rate constant), and the χ^2 value, an indicator of the mean deviation of the experimental data from the calculated curves. In order to compare the biochemical properties of different types of Gsp1-binding proteins, we first ana-

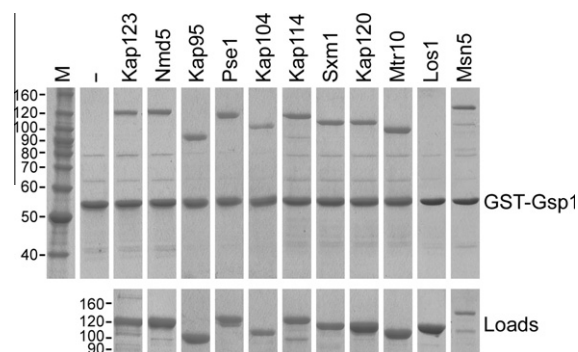


Fig. 1. Binding of β -receptors to Gsp1–GTP. A bacterially expressed GST fusion protein to Gsp1Q71L (10 μ g) loaded primarily with GTP was immobilized for 30 min at 4 °C on glutathione Sepharose and then incubated for 30 min at 4 °C with PBSKMT buffer or 12 μ g of the indicated β -receptors purified from *E. coli*. The bound proteins were washed three times and analyzed by SDS PAGE and Coomassie blue staining together with 20% of the loads (lower panel). Molecular weight markers (M) are given in kDa.

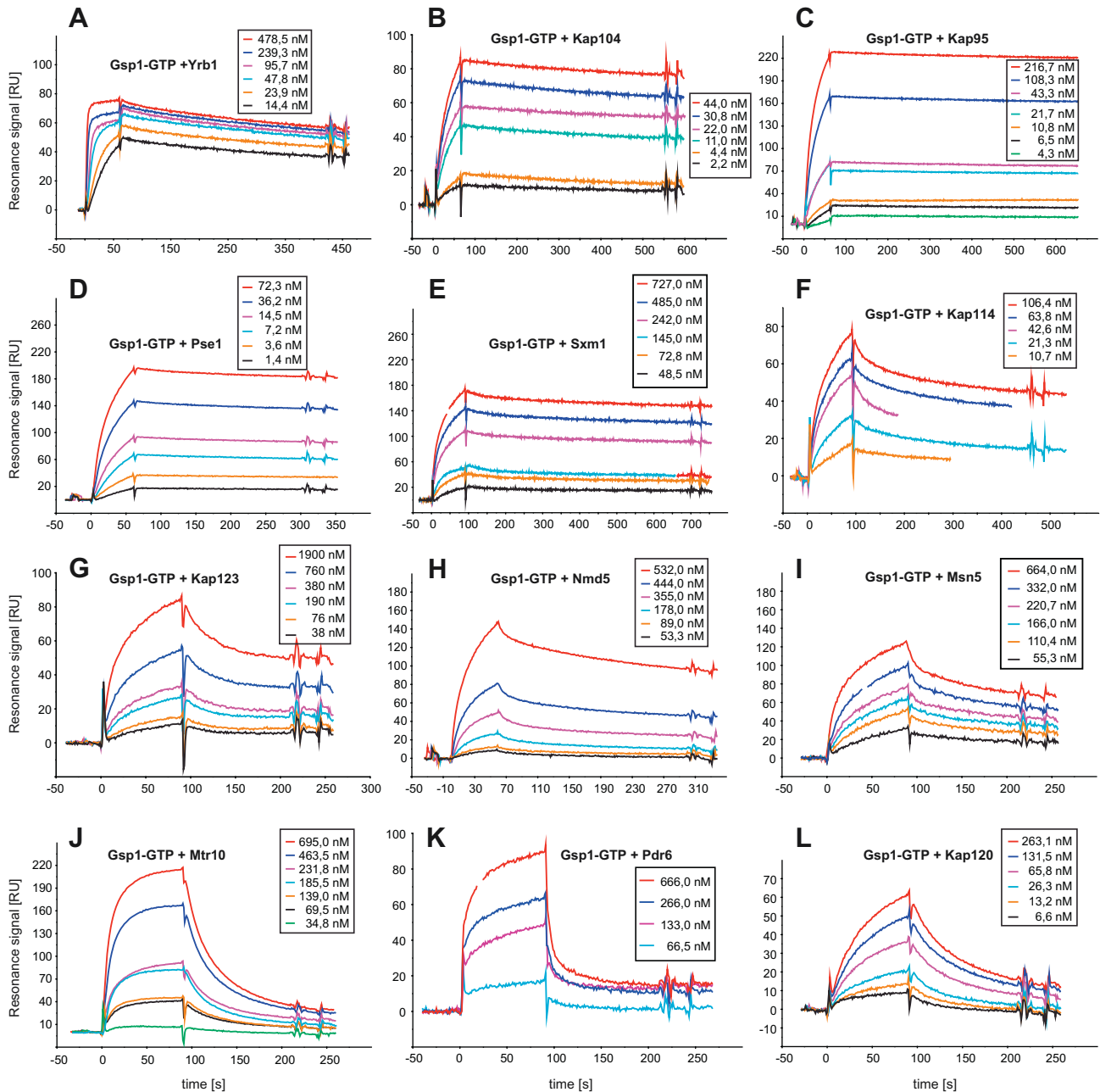


Fig. 2. Sensorgrams of the interaction of Gsp1-binding proteins with Gsp1-GTP. Each sensorgram contains all measurement cycles for a specific ligand-analyte pair. Monoclonal anti-GST antibodies were covalently linked to a Biacore CM5 chip. A fusion protein consisting of GST and full-length Gsp1Q71L loaded with GTP was bound to the CM5 chip of the sample flow chamber. Then, purified Yrb1, Kap104, Kap95, Pse1, Sxm1, Kap114, Kap123, Nmd5, Msn5, Mtr10, Pdr6, or Kap120 (A–L) at the concentrations indicated were injected into the sample flow chamber. After association, the samples were washed with buffer. The sensorgrams show the difference of surface plasmon resonance signals (in resonance units, RU) between the sample flow chamber and the reference flow chamber. The reference flow chamber contained only coupled GST.

lyzed the binding of Yrb1 to Gsp1-GTP. We determined a dissociation constant of 0.45 nM for the Yrb1/Gsp1-GTP interaction. This value is in agreement with previous measurements. An EDTA-induced guanine nucleotide exchange assay was used before to determine a K_d value of 0.7 nM [9]. Similarly, the dissociation constant for the interaction of the mammalian RanBP1/Ran-GTP complex was reported to be 0.6 nM by fluorescence spectrometry, 1.2 nM by SPR spectrometry [21], or 0.13 nM by GTP exchange inhibition assays [22]. It is evident from Fig. 1A and Table 1 that Yrb1 binds to Gsp1-GTP with very fast kinetics, the on-rate constant $k_a = 1.8 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ is the highest of all investigated Gsp1-binding proteins, while the dissociation of the once formed Yrb1/Gsp1-GTP complex is slow ($k_d = 8.2 \times 10^{-4} \text{ s}^{-1}$). This is in

good agreement with a function of Yrb1 as a cytoplasmic dissociation factor for export complexes. The high on-rate constant ensures a fast binding to receptor-bound Gsp1-GTP, which is followed by Rna1-catalyzed GTP hydrolysis resulting in dissociation of the export complex.

Fig. 2B–L and Table 1 show the sensorgrams and binding constants for the β -receptor/Gsp1-GTP interactions. For Kap95, we calculated a K_d value of 0.23 nM, which is similar to the measured dissociation constant of 0.3 nM for the human importin β complexed to human Ran-GTP determined by EDTA- and RCC1-induced nucleotide exchange or GTPase protection assays [22]. Remarkably, the dissociation constants of the various yeast importin/Gsp1-GTP complexes differ considerably ranging from 230 pM

Table 1
Binding constants for the interaction of Gsp1-binding proteins with Gsp1-GTP and cellular concentrations of transport factors. The ascertained amounts of Gsp1 itself and of yeast importin α are included as well. For comparison, the concentration of protein A-tagged proteins according to Ghaemmighami et al. (2003) [31] is listed. The mammalian homologues of the yeast transport factors [1] are given in the last column. Abbreviations: und. undetectable, n.d. not determined, Imp importin, Trn transportin, Exp exportin.

	Yeast transport factor	K_d [nM]	K_a [1/M]	k_d [1/s]	k_a [1/(M×s)]	χ^2	Cellular conc. [μ M]	Molecules per cell	Molecules per cell (Ref. [31])	Mammalian homologue
Type I	Kap95	0.23	4.3×10^9	6.3×10^{-5}	2.7×10^5	0.6	0.30	12,650	51,700	Imp β
	Kap104	0.23	4.4×10^9	2.7×10^{-4}	1.2×10^6	0.5	0.06	2500	2130	Trn (Kap β 2)
	Pse1 (Kap121)	0.32	3.1×10^9	2.6×10^{-4}	8.1×10^5	1.2	0.41	17,300	15,500	Imp5, Imp6
	Sxm1 (Kap108)	2.60	3.7×10^8	2.0×10^{-4}	7.7×10^4	2.5	1.40	59,000	16,300	/
Type II	Kap114	7.00	1.4×10^8	1.6×10^{-3}	2.4×10^5	0.8	0.29	12,200	2940	Imp9
	Kap123 (Yrb4)	8.30	1.2×10^8	4.0×10^{-3}	4.8×10^5	1.4	6.00	250,000	38,300	Imp4
	Nmd5 (Kap119)	16.20	6.2×10^7	1.5×10^{-3}	9.3×10^4	1.8	2.10	88,500	13500	Imp7, Imp8
	Msn5 (Kap142)	52.00	1.9×10^7	3.0×10^{-3}	5.8×10^4	1.6	n.d.	n.d.	3,500	Exp5
Type III	Mtr10 (Kap111)	130.00	7.8×10^6	1.1×10^{-2}	8.5×10^4	2.8	n.d.	n.d.	6340	TrnSR, Imp13
	Pdr6 (Kap122)	231.00	4.3×10^6	7.3×10^{-3}	3.1×10^4	2.2	n.d.	n.d.	9760	/
	Kap120	270.00	3.7×10^6	1.3×10^{-2}	4.8×10^4	1.1	1.40	46,400	6300	Imp11
Type IV	Cse1			und.	und.		0.80	33,700	23,500	CAS
	Los1			und.	und.		n.d.	n.d.	3490	Exp-t
	Xpo1 (Crm1)			und.	und.		0.14	5900	7080	CRM1 (Exp1)
	Yrb1	0.45	2.2×10^9	8.2×10^{-4}	1.8×10^6	0.5	8.00	337,200	n.d.	RanBP1
	Yrb2			n.d.	n.d.		4.40	185,500	3620	RanBP3
	Prp20 (Srm1)			n.d.	n.d.		4.10	172,800	12,100	RCC1
	Srp1 (Kap60)						7.00	295,000	2790	Imp α
	Gsp1						16.00	674,500	n.d.	Ran

(Kap95 and Kap104) to 270 nM (Kap120). This large difference in affinities for Gsp1-GTP is expected to reflect life span variability among importin/cargo complexes after passage of the NPC. The importins exhibiting a low affinity for Gsp1-GTP might prevent misfolding of their respective cargo molecule and escort it to the intranuclear site of function. On any account, the different importin/Ran-GTP affinities hint to the presence of a new mode of nucleocytoplasmic transport regulation.

We classified the β -receptors into four groups. Type 1–3 receptors represent strong, medium, and weak Gsp1-GTP binders, respectively, whereas binding to Gsp1 of type 4 receptors was undetectable under our experimental conditions. Even the highest possible (micromolar) concentrations of type 4 receptors did not yield a SPR response (data not shown). The type 4 proteins Xpo1, Cse1, and Los1 represent those receptors exclusively transporting export cargo. Our results are in accordance with previous observations that these exportins do not detectably interact with Gsp1-GTP in the absence of their cargo [15,18,19].

Until now three dissociation constants for yeast importin/Gsp1-GTP complexes have been reported. Our determined K_d values are consistent with previous results obtained by GTP exchange inhibition and GTPase protection assays. Dissociation constants of 2 nM for Kap123 and Pse1 [9] as well as of roughly 200 nM for Mtr10 [23] were found. For mammalian importin/Ran-GTP complexes, the K_d values were 2-fold for transportin [7], 5-fold for Imp5 [24], 6-fold for Imp8 [25], and 30-fold for Imp7 [25] higher compared to Imp β , respectively. These values considerably match to those of the yeast orthologues Kap104, Pse1, and Nmd5 (Table 1). Hence different affinities apply to mammalian importins as well.

Whereas GTP exchange inhibition and GTPase protection assays solely provide estimates of dissociation constants, SPR spectrometry allows to follow the binding characteristics in real time and, moreover, on-rate and off-rate constants can be calculated. Remarkably, the on-rate constants for importin/Gsp1-GTP complex formation differ only roughly 20-fold, whereas the off-rate constants vary by a factor of approximately 500 indicating that binding of Gsp1-GTP to all importins occurs relatively fast but the dissociation kinetics is distinguishing for individual importins (Table 1).

Type 1 receptor complexes with Gsp1-GTP are very stable (Fig. 2B–E). The extraordinary stability of the Kap95/Gsp1-GTP

complex with an off-rate constant of $6.3 \times 10^{-5} \text{ s}^{-1}$ explains why besides Ran-binding protein 1 and the GAP also importin α is required for efficient disassembly of this complex [7]. Type 2 receptor/Gsp1-GTP complexes display a moderate stability (Fig. 2F–I). Surprisingly, Msn5 belongs to this group and its affinity for Gsp1-GTP is higher than that of some importins. Thus despite Msn5 functions primarily as an exportin, it behaves biochemically as an importin. Type 3 receptor/Gsp1-GTP complexes are comparably unstable (Fig. 2J–L). The predicted consequences of this low affinity regarding the transport mechanism are first, a reduced export efficiency through the NPC due to premature dissociation of Gsp1-GTP before the cytoplasmic compartment is reached. Indeed Mtr10 and Kap120, the type 3 receptors with the highest off-rate constants ($\sim 10^{-2} \text{ s}^{-1}$) are the only importins with a predominant nuclear localization [23,26], whereas the other importins are usually equally distributed between the nucleoplasm and the cytoplasm. Second, Yrb1 might be dispensable for the dissociation of type 3 receptors bound to Gsp1 after export. It was observed for many β -receptor export complexes that the Ran GTPase activation is blocked as long as the receptor is bound to Ran-GTP. Ran binding protein 1 releases this GTPase inhibition by forming a disassembly intermediate and thereby stimulating the off-rate of export complexes by more than two orders of magnitude [7]. Because the off-rate constants of type 3 receptor/Gsp1-GTP complexes are intrinsically low, Yrb1 may not be required for efficient disassembly of these export complexes. Third, the affinity of Gsp1-GTP for the receptor is expected to be insufficient for proper cargo release after import. Remarkably, this was again observed for Mtr10 and Kap120 in *in vitro* cargo dissociation experiments [23,26]. In these cases, other nuclear factors become necessary to assist in cargo release. Interestingly, Npl3 and Rpf1, the respective cargos of Mtr10 and Kap120, are both RNA-binding proteins and RNA is required for Mtr10 import complex disassembly [23]. A similar situation exists for Kap114-mediated import of DNA-binding cargos, the TATA-binding protein and histones [16,27]. Thus a low affinity for Gsp1-GTP explains a prevention of rapid import cargo dissociation in the nucleus. In these cases a function of importins in intranuclear targeting might be favorable because they act as chaperones and deliver their cargo to the site of action where it is transferred to other binding partners.

3.2. The cellular concentration of Gsp1 is not limiting for transport processes

To answer the question whether Gsp1–GTP is a limiting factor for nucleocytoplasmic transport, we quantified the cellular amounts of Gsp1 and Gsp1–GTP binding proteins. We used polyclonal affinity-purified antisera raised against full-length polypeptides and purified recombinant proteins in quantitative immunoblotting experiments as described in “Materials and methods” (data not shown). This allowed to determine the cellular concentration and the number of molecules per cell based on earlier calculations that an average haploid cell comprises a volume of $70 \mu\text{m}^3$ [28]. The data are summarized in Table 1.

For Gsp1 we found a cellular concentration of $16 \mu\text{M}$ corresponding to 674,500 molecules per cell, which confirms that Gsp1 is a very abundant protein. *Saccharomyces cerevisiae* contains two Ran genes, the essential *GSP1* and the dispensable *GSP2*, the latter being at least 10-fold less expressed than *GSP1* [29]. It can be expected that our polyclonal antibody will comparably recognize the two proteins because both Gsp proteins exhibit a sequence identity of more than 95%. We also determined the cellular amounts of yeast importin α . Surprisingly, the concentration of Srp1 is ~ 23 times higher than that of Kap95. Therefore, the majority of yeast importin α is not complexed to importin β . The abundance of Srp1 is explained by its specific role in snRNA biogenesis [30].

We confirmed that Kap123, a protein involved in ribosomal protein import, is the most abundant β -receptor [9]. The ten quantified β -receptors combined sum up to roughly 530,000 molecules per cell. The individual numbers often differ from values obtained by a genome-wide study, where open reading frames were fused to the TAP tag to quantify the protein amounts [31]. Because we analyzed untagged endogenous proteins, our interpretation is that fusion to the TAP tag can affect the protein stability. The four receptors not quantified by us because antibodies were not available seem not to be abundant proteins (Table 1). It can be estimated that the cellular concentration of the 14 β -receptors altogether approximately equals that of Gsp1. Gsp1 is localized predominantly in the nucleus. Likewise, the exportins as well as Mtr10 and Kap120 are also mostly nuclear. However, the majority of importins, like the highly abundant Kap123, are usually equally distributed in the cytoplasm with a slightly higher concentration in the nucleus [9]. Therefore the concentration of Gsp1–GTP will exceed that of the β -receptors in the nucleus. Given that the nuclear volume represents about 7% of the cellular volume [32] and assuming that all exportins plus Mtr10 and Kap120 are exclusively nuclear whereas the concentration of the other importins is twofold higher in the nucleus than in the cytoplasm, one can estimate that a threefold excess of Gsp1–GTP over all β -receptors is available in the nucleus.

Besides with transport receptors, Gsp1–GTP also interacts with other proteins, among them with the abundant and mostly cytoplasmic Yrb1, which shuttles between the cytoplasm and the nucleus [15], with the RanGEF, and with miscellaneous factors [33–35]. Taken together, Gsp1–GTP is apparently still not limiting within the nucleus, but it will not be present in large excess over its various interactors under normal conditions, which explains why perturbations of the Ran GTPase cycle rapidly affect nucleocytoplasmic transport and nuclear integrity.

Acknowledgments

We thank Karsten Mayr and Silke Guthörl for expert technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft and by the Homburger Forschungsförderung (HOMFOR).

References

- [1] Y. Quan, Z.-L. Ji, X. Wang, A.M. Tartakoff, T. Tao, Evolutionary and transcriptional analysis of karyopherin β superfamily proteins, *Mol. Cell. Proteomics* 7 (2008) 1254–1269.
- [2] M. Dasso, The Ran GTPase: theme and variations, *Curr. Biol.* 12 (2002) R502–R508.
- [3] L.F. Pemberton, B.M. Paschal, Mechanisms of receptor-mediated nuclear import and nuclear export, *Traffic* 6 (2005) 187–198.
- [4] H. Fried, U. Kutay, Nucleocytoplasmic transport: taking an inventory, *Cell. Mol. Life Sci.* 60 (2003) 1659–1688.
- [5] A. Cook, F. Bono, M. Jinek, E. Conti, Structural biology of nucleocytoplasmic transport, *Annu. Rev. Biochem.* 76 (2007) 647–671.
- [6] P. Kalab, K. Weis, R. Heald, Visualization of a Ran–GTP gradient in interphase and mitotic *Xenopus* egg extracts, *Science* 295 (2002) 2452–2456.
- [7] F.R. Bischoff, D. Görlich, RanBP1 is crucial for the release of RanGTP from importin β -related nuclear transport factors, *FEBS Lett.* 419 (1997) 249–254.
- [8] F.R. Bischoff, H. Krebber, E. Smirnova, W. Dong, H. Ponstingl, Co-activation of RanGTPase and inhibition of GTP dissociation by Ran–GTP binding protein RanBP1, *EMBO J.* 14 (1995) 705–715.
- [9] G. Schlenstedt, E. Smirnova, R. Deane, J. Solsbacher, U. Kutay, D. Görlich, H. Ponstingl, F.R. Bischoff, Yrb4p, a yeast Ran–GTP-binding protein involved in import of ribosomal protein L25 into the nucleus, *EMBO J.* 16 (1997) 6237–6249.
- [10] G. Schlenstedt, D.H. Wong, D.M. Koepp, P.A. Silver, Mutants in a yeast Ran binding protein are defective in nuclear transport, *EMBO J.* 14 (1995) 5367–5378.
- [11] T. Taura, H. Krebber, P.A. Silver, A member of the Ran-binding protein family, Yrb2p is involved in nuclear protein export, *Proc. Natl. Acad. Sci. USA* 95 (1998) 7427–7432.
- [12] K. Ribbeck, G. Lipowsky, H.M. Kent, M. Stewart, D. Görlich, NTF2 mediates nuclear import of Ran, *EMBO J.* 17 (1998) 6587–6598.
- [13] N. Mosammaparast, L.F. Pemberton, Karyopherins: from nuclear-transport mediators to nuclear-function regulators, *Trends Cell Biol.* 14 (2004) 547–556.
- [14] D.S. Goldfarb, A.H. Corbett, D.A. Mason, M.T. Harreman, S.A. Adam, Importin α : a multipurpose nuclear-transport receptor, *Trends Cell Biol.* 14 (2004) 505–514.
- [15] P. Maurer, M. Redd, J. Solsbacher, F.R. Bischoff, M. Greiner, A.V. Podtelejnikov, M. Mann, K. Stade, K. Weis, G. Schlenstedt, The nuclear export receptor Xpo1p forms distinct complexes with NES transport substrates and the yeast Ran binding protein 1 (Yrb1p), *Mol. Biol. Cell* 12 (2001) 539–549.
- [16] M. Greiner, S. Caesar, G. Schlenstedt, The histones H2A/H2B and H3/H4 are imported into the yeast nucleus by different mechanisms, *Eur. J. Cell Biol.* 83 (2004) 511–520.
- [17] S. Hahn, P. Maurer, S. Caesar, G. Schlenstedt, Classical NLS proteins from *Saccharomyces cerevisiae*, *J. Mol. Biol.* 379 (2008) 678–694.
- [18] K. Hellmuth, D.M. Lau, F.R. Bischoff, M. Künzler, E. Hurt, G. Simos, Yeast Los1p has properties of an exportin-like nucleocytoplasmic transport factor for tRNA, *Mol. Cell. Biol.* 18 (1998) 6374–6386.
- [19] J. Solsbacher, P. Maurer, F.R. Bischoff, G. Schlenstedt, Cse1p is involved in export of yeast importin α from the nucleus, *Mol. Cell. Biol.* 18 (1998) 6805–6815.
- [20] K. Yoshida, G. Blobel, The karyopherin Kap142p/Msn5p mediates nuclear import and nuclear export of different cargo proteins, *J. Cell Biol.* 152 (2001) 729–740.
- [21] J. Kuhlmann, I. Macara, A. Wittinghofer, Dynamic and equilibrium studies on the interaction of Ran with its effector, RanBP1, *Biochemistry* 36 (1997) 12027–12035.
- [22] D. Görlich, N. Panté, U. Kutay, U. Aebi, F.R. Bischoff, Identification of different roles for RanGDP and RanGTP in nuclear protein import, *EMBO J.* 15 (1996) 5584–5594.
- [23] B. Senger, G. Simos, F.R. Bischoff, A. Podtelejnikov, M. Mann, E. Hurt, Mtr10p functions as a nuclear import receptor for the mRNA-binding protein Npl3p, *EMBO J.* 17 (1998) 2196–2207.
- [24] R. Deane, W. Schäfer, H.P. Zimmermann, L. Müller, D. Görlich, S. Prehn, H. Ponstingl, F.R. Bischoff, Ran-binding protein 5 (RanBP5) is related to the nuclear transport factor importin- β but interacts differently with RanBP1, *Mol. Cell. Biol.* 17 (1997) 5087–5096.
- [25] D. Görlich, M. Dabrowski, F.R. Bischoff, U. Kutay, P. Bork, E. Hartmann, S. Prehn, E. Izaurralde, A novel class of RanGTP binding proteins, *J. Cell Biol.* 138 (1997) 65–80.
- [26] S. Caesar, M. Greiner, G. Schlenstedt, Kap120 functions as a nuclear import receptor for ribosome assembly factor Rpf1 in yeast, *Mol. Cell. Biol.* 26 (2006) 3170–3180.
- [27] L.F. Pemberton, J.S. Rosenblum, G. Blobel, Nuclear import of the TATA-binding protein: mediation by the karyopherin Kap114p and a possible mechanism for intranuclear targeting, *J. Cell Biol.* 145 (1999) 1407–1417.
- [28] G. Guthrie, G.R. Fink (Eds.), *Guide to Yeast Genetics and Molecular Biology*, Academic Press, Methods in Enzymology, 1991.
- [29] P. Belhumeur, A. Lee, R. Tam, T. DiPaolo, N. Fortin, M.W. Clark, GSP1 and GSP2, genetic suppressors of the prp20-1 mutant in *Saccharomyces cerevisiae*: GTP-binding proteins involved in the maintenance of nuclear organization, *Mol. Cell. Biol.* 13 (1993) 2152–2161.

- [30] D. Görlich, R. Kraft, S. Kostka, F. Vogel, E. Hartmann, R.A. Laskey, I.W. Mattaj, E. Izaurralde, Importin provides a link between nuclear protein import and U snRNA export, *Cell* 87 (1996) 21–32.
- [31] S. Ghaemmaghami, W.K. Huh, K. Bower, R.W. Howson, A. Belle, N. Dephoure, E.K. O'Shea, J.S. Weissman, Global analysis of protein expression in yeast, *Nature* 425 (2003) 737–741.
- [32] P. Jorgensen, N.P. Edgington, B.L. Schneider, I. Rupeš, M. Tyers, B. Futcher, The size of the nucleus increases as yeast cells grow, *Mol. Biol. Cell* 18 (2007) 3523–3532.
- [33] A. Braunwarth, M. Fromont-Racine, P. Legrain, F.R. Bischoff, T. Gerstberger, E. Hurt, M. Künzler, Identification and characterization of a novel RanGTP-binding protein in the yeast *Saccharomyces cerevisiae*, *J. Biol. Chem.* 278 (2003) 15397–15405.
- [34] M. Oki, T. Nishimoto, A protein required for nuclear-protein import, Mog1p, directly interacts with GTP-Gsp1p The *Saccharomyces cerevisiae* Ran homologue, *Proc. Natl. Acad. Sci. USA* 95 (1998) 15388–15393.
- [35] B. Goodman, Y. Zheng, Mitotic spindle morphogenesis: Ran on the microtubule cytoskeleton and beyond, *Biochem. Soc. Trans.* 34 (2006) 716–721.