Structure
Article

Cse1p-Binding Dynamics Reveal a Binding Pattern for FG-Repeat Nucleoporins on Transport Receptors

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SUMMARY

Nuclear pore proteins with phenylalanine-glycine repeats are vital to the functional transport of molecules across the nuclear pore complex. The current study investigates the binding of these FG-nucleoporins to the Cse1p:Kap60p:RanGTP nuclear export complex. Fourteen binding spots for FG-nucleoporin peptides are revealed on the surface of Cse1p, and 5 are revealed on the Kap60p surface. Taken together, and along with binding data for two other transport receptors, the data suggest that the ability to bind FG-nucleoporins by itself is not enough to ensure viable nuclear transport. Rather, it is proposed that the density of binding spots on the transport receptor surface is key in determining transport viability. The number of binding spots on the transport receptor surface should be large enough to ensure multiple, simultaneous FG-repeat binding, and their arrangement should be close enough to ensure multiple binding from the same FG-nucleoporin.

INTRODUCTION

The exchange of material between the cell nucleus and cytoplasm takes place exclusively through nuclear pore complexes (NPCs), large protein assemblies embedded in the nuclear envelope (see Stewart, 2007; Lim et al., 2006a; Tran and Wente, 2006; Weis, 2003; Fried and Kutay, 2003; Macara, 2001; Rout et al., 2000 for a review). While the NPC allows for the free, unprotected exchange of small molecules (below ~40 kDa) via simple diffusion (Görlich and Kutay, 1999), it also provides a second means for the protected exchange of larger molecules between ~9 and 39 nm in diameter (Pante and Kann, 2002). This "active transport" is able to distinguish between macromolecules destined to cross the nuclear envelope and those which are not.

The overall structure of the NPC has been revealed mainly through electron microscopy (Beck et al., 2004; Stoffler et al., 2003; Kiseleva et al., 1998; Goldberg et al., 1997; Akey and Radermacher, 1993; Jarnik and Aebi, 1991; Aebi et al., 1990). The NPC has octagonal radial symmetry and pseudo two-fold symmetry across the nuclear envelope, which explains why it has such a large mass (~44 MDa in yeast [Rout et al., 2000], ~60 MDa in vertebrates [Cronshaw et al., 2002]) while containing a relatively small number (~30) of distinct proteins (Rout et al., 2000; Cronshaw et al., 2002). Larger mass estimates (Rout and Blobel, 1993; Yang et al., 1998) are likely upper limits due to the presence of NPC-associated proteins in the pore (Rout et al., 2000; Cronshaw et al., 2002).

The proteins that compose the NPC are termed nucleoporins (nups) and can be broadly separated into three categories: scaffolding, structural, and transport. Scaffolding nups contain transmembrane helices and anchor the NPC into the membranous nuclear envelope (Mansfeld et al., 2006; Schwartz, 2005). Structural nups provide the NPC its shape. They include a large subset of nups with two distinct fold types, α-solenoid and β-propellar (Devos et al., 2004, 2006). Transport nups are involved directly in the movement of complexes across the NPC. These nups are largely made up of proteins with a repeating sequence involving the amino acids phenylalanine and glycine. These FG-nups typically contain the repeating sequences FG, GLFG, or FxFG (where x is any amino acid, largely S) separated by a linker sequence of 10–20 charged or hydrophilic amino acids. Other less regularly occurring core sequences, such as SAFGxPSFG, SLFG, and SPFG, also exist (see Denning and Rexach, 2007 for a thorough analysis).

The active transport of macromolecules across the NPC involves several proteins apart from those that make up the NPC itself. A cargo macromolecule destined for transport cannot cross the NPC alone, but must associate with a chaperone transport receptor protein. These transport receptors are collectively members of the karyopherin-β family and are also known simply as karyopherins (see Pemberton and Paschal, 2005 and Mosammaparast and Pemberton, 2004 for a review). The transport complex then crosses the NPC and, once on the opposite side of the pore, dissociates, leaving the cargo in the proper compartment. The propensity of transport receptors for cargo binding or unbinding is determined by the state of Ran (GTP- or GDP-bound) on the nuclear and cytoplasmic sides of the NPC. RanGDP is present in high

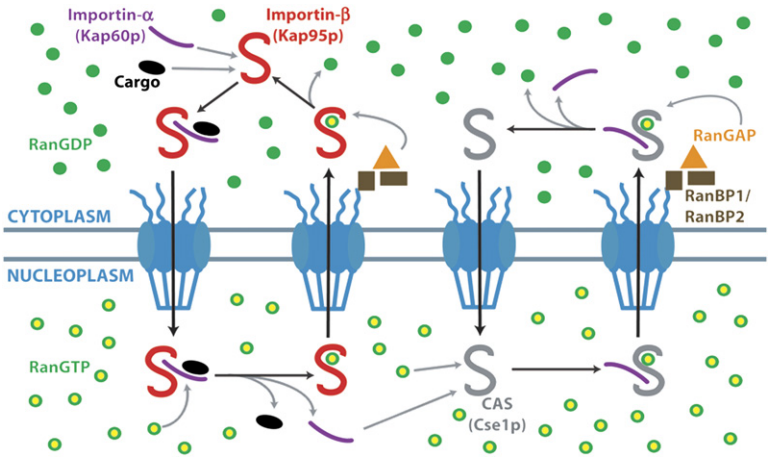

Figure 1. A Representation of Nuclear Import and Export

In order to be imported into the nucleus, a cargo macromolecule (black) must first associate in a transport complex with importin-β (red) (or another transport receptor). Protein names in yeast are shown in parentheses. This complex can then cross the nuclear pore complex into the nucleus, where the binding of RanGTP (green and yellow) causes dissociation, resulting in cargo import. Importin-β and RanGTP are then recycled to the cytoplasm, where the hydrolysis of GTP causes complex dissociation, leaving importin-β free for another round of import. The presence of the enzyme RanGTPase-activating protein, RanGAP (orange), and its accessory factors Ran-binding proteins RanBP1 and RanBP2 (brown) in the cytoplasm, causes the hydrolysis of GTP at rates much higher than the intrinsic rate. Nuclear export occurs in an analogous manner. The export of importin-α is shown. Importin-α (purple) must first associate in a transport complex with both its export receptor CAS (gray; Cse1p in yeast) and RanGTP. This complex can then navigate the nuclear pore complex and reach the cytoplasm, where the hydrolysis of GTP causes the complex to dissociate, resulting in the net export of importin-α. CAS is recycled alone to the nucleus. (The balance of Ran is reestablished by the Ran importer, NTF2, which is not shown.)

concentrations in the cytoplasm due to the presence of RanGTPase-activating protein (RanGAP), which, along with accessory factor Ran-binding proteins (RanBP1 and RanBP2), causes the hydrolysis of RanGTP at rates much higher than intrinsic (Bischoff et al., 1994, 1995a, 1995b). RanGTP is present in high concentrations in the nucleus due to the presence of the Ran guanine nucleotide exchange factor (RanGEF) RCC1, which promotes the exchange of GDP for GTP. Import receptors bind their cargo in the cytoplasm and, upon entering the nucleoplasm, release it when they bind RanGTP. Export receptors, on the other hand, only bind cargo along with RanGTP, and the complex dissociates in the cytoplasm upon hydrolysis. The restriction of RanGAP to the cytoplasm (Saitoh et al., 1998; Mahajan et al., 1997; Matunis et al., 1996; Hopper et al., 1990) and RanGEF to the nuclear chromatin (Nemergut et al., 2001; Ohtsubo et al., 1987, 1989) maintains the concentration gradients of Ran across the nuclear envelope, which is required for nucleocytoplasmic transport (Izaurralde et al., 1997; Richards et al., 1997) and imposes directionality on transport (Nachury and Weis, 1999) (see Moore, 1998 and Fried and Kutay, 2003 for a review). Furthermore, evidence exists for the role of nups in formation of intermediate states in the disassembly of transport complexes (Matsuura and Stewart, 2005; Gilchrist and Rexach, 2003). A schematic of nuclear import and export is shown in Figure 1.

Whereas much experimental data exist for the overall nuclear import and export cycle, the details of transit through the NPC are a relative mystery. However, several key pieces of information that implicate FG-nups as being vital to transport are known. Electron microscopy studies with gold-labeled antibodies have revealed that most FG-nups are localized toward the center of the NPC (Rout et al., 2000; Grote et al., 1995). Furthermore, it has also been shown that FG-nups exhibit properties similar to those of natively unfolded proteins (Denning et al., 2003), their flexibility being demonstrated directly with atomic force microscopy (Lim et al., 2006b). Moreover, FG-nups have been shown to interact with several transport receptors in vivo, in vitro, and in silico (Isgro and Schulten, 2005, 2007; Lim et al., 2007; Liu and Stewart, 2005; Cushman et al., 2004; Rodriguez et al., 2004; Morrison et al., 2003; Bednenko et al., 2003; Bayliss et al., 1999, 2000, 2002a, 2002b; Braun et al., 2002; Strawn et al., 2001; Quimby et al., 2001; Lane et al., 2000; Chaillan-Huntington et al., 2000; Damelin and Silver, 2000; Kose et al., 1999; Seedorf et al., 1999; Kehlenbach et al., 1999; Shah et al., 1998; Chi and Adam, 1997; Hu et al., 1997; Fornerod et al., 1997; Clarkson et al., 1996; Iovine et al., 1995; Paschal and Gerace, 1995; Radu et al., 1995a, 1995b; Rexach and Blobel, 1995). Another extensive study (Strawn et al., 2004) revealed that the deletion of different combinations of FG-nups rendered yeast cells inviable. While up to half of the FG-repeat mass could be deleted while still retaining a functioning NPC, the deletion of specific FG-repeat combinations was lethal. Deletion of the FG-repeat region from Nup116p alone was lethal. See Tran and Wente (2006) for a review of FG-nups and the manner in which they facilitate transport. Several theories have been proposed to explain the mechanism of nucleocytoplasmic transport through the NPC (Peters, 2005; Rout et al., 2000, 2003; Shulga and Goldfarb, 2003; Ribbeck and Görlich, 2001; Ben-Efriam and Gerace, 2001; Macara, 2001).

While a large body of evidence detailing the interactions of several transport receptors with FG-nups has been uncovered, not much study has been devoted to the yeast transport receptor Cse1p (CAS in vertebrates), whose crystal structure is available in complex with Kap60p (importin-α) and RanGTP (Matsuura and Stewart, 2004) and in cargo-free form (Cook et al., 2005). Cse1p functions as the Kap60p exporter (Solsbacher et al., 1998; Hood

