Structure of the Nuclear Transport Receptor Importin β Bound to the IBB Domain of Importin α

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Not only does x-ray crystallography provide structures of individual biological macromolecules, but also it can show in detail how these molecules act in concert to fulfill specific functions in a living cell. Using beamlines at the ESRF the atomic structure of a nuclear transport receptor complex has recently been determined [1]. The complex structure leads to a deeper understanding of how molecules are transported in and out of the cell nucleus.

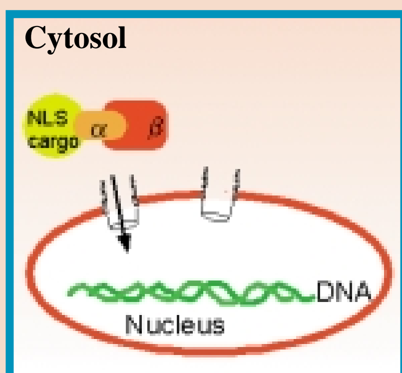
A defining feature of eukaryotic cells is the separation between the cell nucleus, where genetic information is stored and where transcription of DNA into RNA occurs, and the surrounding cytosol where messenger RNA molecules are translated into proteins. This spatial separation requires intensive traffic between the nucleus and the cytosol but also offers many possibilities for regulation. Exchange between the two cellular compartments occurs through the nuclear pore complex (NPC), a ~ 125 MDa structure containing approximately 100 different polypeptides embedded in the nuclear envelope. The nuclear pores allow passage by passive diffusion of molecules up to 40 kDa, but most molecules are actively transported bound to nuclear receptors [2].

Targeting of many proteins to the nucleus is determined by the presence of a nuclear localization sequence (NLS), a short sequence containing one or two clusters of basic amino acid residues. An NLS-bearing substrate, often termed the "cargo", is delivered to the nucleus by association with the heterodimer formed by importin α and importin β (also called karyopherin-α and -β). Importin α recognizes the NLS while importin β is responsible for docking to the NPC and translocation through the pore (Figure 1). Dissociation of the NLS-cargo:importin α:importin β trimer is triggered by the association of importin β with the protein Ran, a Ras-related GTPase. In the nucleus Ran is predominantly bound to GTP (a small molecule related to the cellular energy carrier ATP) while in the cytoplasm it is bound to its lower-energy analogue GDP. This uneven distribution leads to the correct spatial coordination of cargo release because importin β is recognized by RanGTP but not by RanGDP.

GLOSSARY

Eukaryotic cells: contain a cell nucleus and organelles. A eukaryotic cell can be a single cell organism or a part of a multicellular organism such as man.

Cell nucleus: contains the genetic information of the cell which is stored as a long linear DNA molecule. The nucleus is surrounded by a membrane, the nuclear envelope.
In the nucleus, DNA is constantly transcribed into RNA molecules which are exported to the cytosol.



Proteins: are chains of amino acid residues, which usually adopt well defined structures. Proteins are made in the cytosol where a cellular machine, the ribosome, translates messenger-RNA into proteins.

Nuclear pore complex (NPC): are large pores embedded in the nuclear envelope which allow the passage of proteins and RNA molecules between the nucleus and the cytosol.

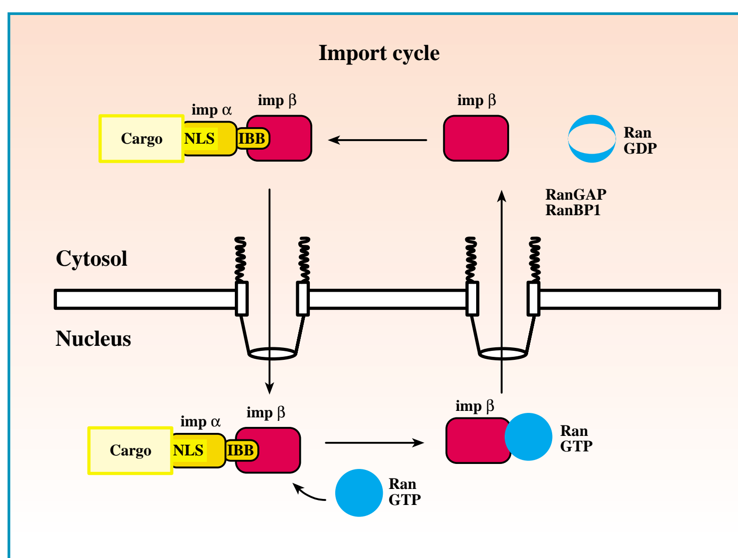
Nuclear localization sequence (NLS): are short peptide sequences which serve to direct proteins containing them into the nucleus.

Importin α, β: are nuclear transport receptor molecules. Proteins containing NLS bind to importin α, which in turn interacts via its N-terminal importin ß-binding (IBB) domain with importin ß. The trimeric import complex is translocated through the nuclear pore.

Ran: is a small GTP-hydrolyzing enzyme. It is found in a complex with GTP, the higher energy form of triphosphate nucleotide, in the nucleus and in a complex with GDP in the cytosol. In the nucleus, binding of RAN to GTP leads to the dissociation of the trimeric import complex.

HEAT motifs: are sequence repeats found in several eukaryotic proteins. X-ray structures show that HEAT motifs consist of two α-helices, A and B, which are connected by a short turn.

Fig. 1: Schematic representation of the import cycle. NLS-containing cargo molecules are bound via the adapter molecule importin α to importin β. Following transport through the nuclear pore, binding of RanGTP to importin β dissociates the trimeric complexes. Importin β / RanGTP leaves the nucleus and dissociates in the cytosol after hydrolysis of RanGTP to RanGDP.



Importin α is a 60 kDa protein composed of a small N-terminal domain and a large NLS-binding domain connected by a flexible linker. The NLS-binding domain of importin α forms an elongated right-handed superhelix with a shallow groove where the NLS peptide is bound. The N-terminal domain, termed importin β binding (IBB) domain, is a basic stretch of approximately 40 highly conserved residues which are minimally required for binding importin β and for efficient nuclear entry. The IBB domain is thus the nuclear targeting signal of importin α.

Importin β is a 97 kDa protein and is the best characterized member of a superfamily of homologous nuclear transport receptors. Members of this superfamily vary between 90 and 130 kDa in size and are characterized by an N-terminal Ran-binding region and by NPC-binding activity. Sequence comparisons predicted importin β to contain a number of tandem HEAT motifs, sequence repeats of roughly 40 residues found in a variety of eukaryotic proteins. Apart from the classical protein import pathway, importin β is also involved in the nuclear import of ribosomal, cell-cycle-controlling and viral proteins (including HIV-I proteins), which bind directly to importin β without using the adapter molecule importin α.

STRUCTURE DETERMINATION

Co-crystallization experiments of importin β overproduced in bacteria together with a chemically synthesized IBB-domain peptide gave rise to crystals which diffracted beyond 2.5 Å resolution. Native data sets were collected from two crystal forms at beamlines ID14-3 and ID2 to 2.5 Å and 2.3 Å resolution, respectively. Solving the structure by conventional heavy-atom soaks turned out to be difficult because of significant non-isomorphism between native and derivative crystals. Therefore selenomethionine substituted importin β was produced, and the structure of the complex was solved by the multiwavelength anomalous diffraction (MAD) method at the "MAD-beamline" BM14 of the ESRF. This powerful technique, which uses the signal of anomalous scatterers in the crystal, is nowadays routinely used to solve the "phase problem". Its application is further facilitated through the automated "MAD and MIR structure solution" program SOLVE, which in our case successfully located 21 out of the possible 24 selenium atoms. 38 polyalanine helices were placed into the first experimental electron density at 3.3 Å resolution. Recombination of MAD phases with those calculated from the model yielded a map allowing much of the sequence to be traced. The chain tracing of importin β was greatly expedited by the selenium peaks in an anomalous difference Fourier map. The final refined model at 2.5 Å resolution has an R_{cryst} = 22.1% and R_{free} = 27.3%, and consists of IBB domain residues α11- α54, all 876 importin β residues, and 44 water molecules.

OVERVIEW OF THE COMPLEX

The structure of the complex is shown in Figure 2. The complex takes

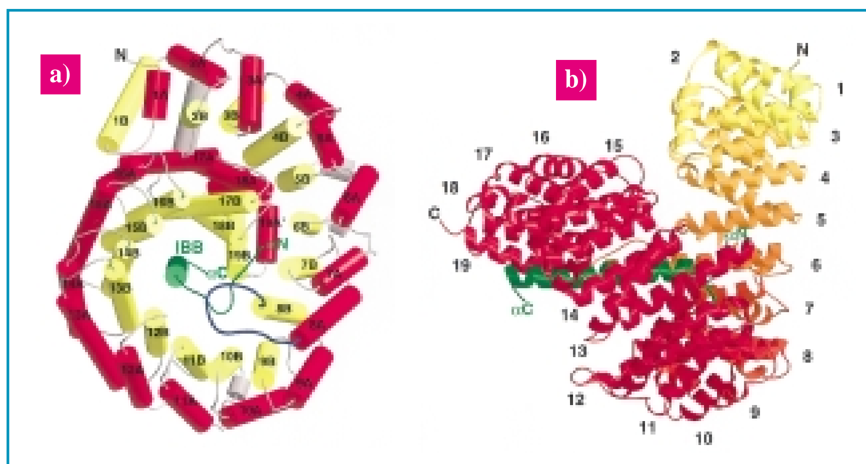


Fig. 2: Structure of importin β bound to the IBB domain of importin α. a) View down the superhelical axis. A and B helices are shown in red and yellow, respectively with connecting residues in grey. The loop in HEAT repeat 8 containing mostly acidic residues is depicted in blue, the IBB domain in green. b) orthogonal view with respect to a. The ribbon representing importin β is colored progressively from yellow to red as the chain proceeds from N- to C-terminus.

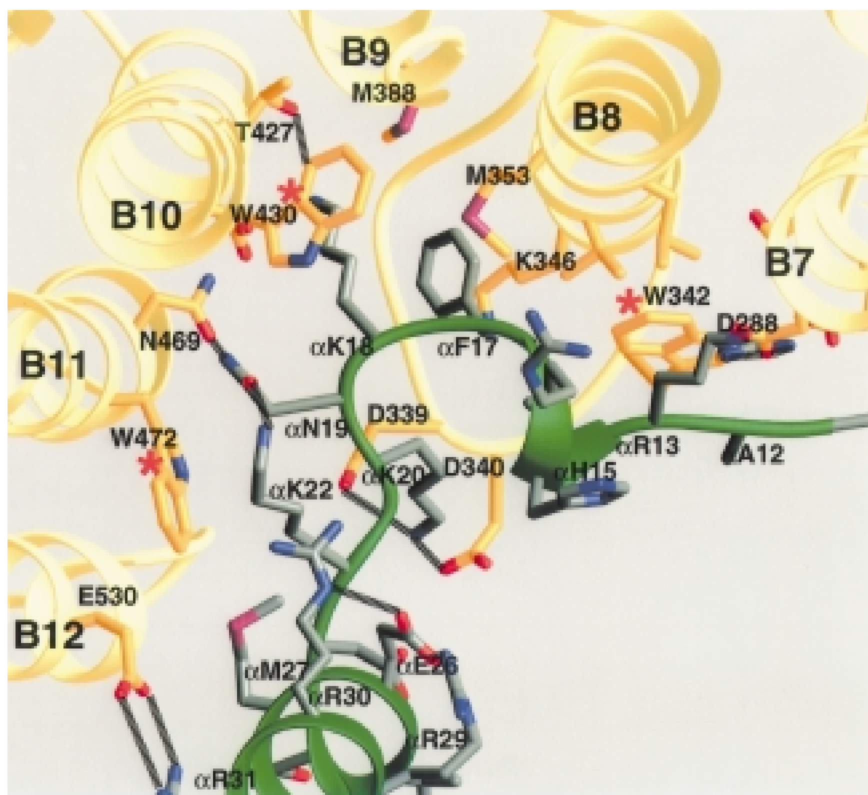


Fig. 3: Recognition of the IBB domain by importin β. Depicted are interactions involving the N-terminal moiety of the IBB-domain. Polar interactions are indicated by dashed lines. Tryptophan residues W342, W430 and W472 are marked with red asterisks.

on a snail-shaped appearance with the IBB domain at the center and importin β wrapped around the outside. The complex adopts a highly compact, essentially globular shape with a diameter of 85 Å. Importin β is an all helical protein composed of 19 tandem HEAT repeats, arranged in a right-handed superhelix with a pitch and mean diameter both equal to approximately 50 Å. The relatively short pitch results in N- and C-terminal HEAT repeats nearly touching each other, giving the molecule a rather "closed" appearance. Each HEAT repeat is composed of an A and a B helix connected by a short turn of residues and varies between 32 and 61 residues in size. Repeats are arranged within the molecule so as to yield an outer layer of A helices defining the convex surface, and an inner layer of B helices defining the concave surface. Repeats 7 to 19 are responsible for the recognition of the IBB domain, while HEAT repeats 1 to 6 are not involved in the IBB binding but interact with RanGTP.

The IBB domain is intimately bound on the inner surface of importin β and adopts an L-shaped conformation. An N-terminal moiety (residues α11- α23) and a C-terminal helix (residues α24-α51) run in mutually perpendicular directions, with the C-terminal helix roughly coinciding with the importin β superhelix. Basic residues account for nearly 40% of the IBB domain, creating an electrostatic surface potential complementary to that of the inner surface of importin β, which is lined with acidic residues. The intermolecular interactions are intimate, involving 42% of the surface area of the IBB domain. Nearly every residue in the IBB N-terminal moiety interacts with a B helix in HEAT repeats 7 to 11 and with one prominent loop containing mostly acidic residues in HEAT repeat 8 (Figure 3), while the IBB helix packs against helices from HEAT repeats 12 to 19.

CONFORMATIONAL CHANGES OF TRANSPORT RECEPTORS

The closed conformation of importin β and the eight-HEAT "embrace" of the IBB helix suggests that a significant conformational change may occur upon binding or release of the IBB domain. Probably importin β adopts a more open conformation when not bound to the IBB domain of importin α. This is corroborated by the structure of a homologous transport receptor (transportin or karyopherin β-2) bound to Ran:GppNHp which was published at the same time as our complex [3]. In that structure the cargo has been released upon the binding of Ran:GTP and the C-terminal part of transportin adopts a much more open conformation than in the importin β:IBB domain complex. While these two complementary complex structures do not reveal everything we wanted to know about nuclear transport, they nevertheless provide a good starting point for asking even better questions. ■

References

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ACKNOWLEDGEMENTS

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