Conformational Variability of Nucleo-cytoplasmic Transport Factors*

The transport of macromolecules between the nucleus and cytoplasm of eukaryotic cells is largely mediated by a single family of transport factors, the karyopherin or importin β-like family. Structural and biochemical evidence suggests conformational flexibility of these modular HEAT-repeat proteins is crucial for their regulation. Here we use small angle x-ray scattering to assess the extent of conformational variation within a set of nuclear import and export factors. The study reveals that importin β, transportin, and the exportin Xpo-t share a similar S-like superhelical conformation in their unbound state. There are no obvious differences in the overall structures that might generally distinguish nuclear export from nuclear import mediators. Two other members of the family, the exportins Cse1 and Xpo1, possess a significantly more globular conformation, indicating that the extended S-like architecture is not a hallmark of all karyopherins. Binding of RanGTP/cargo to importin β, transportin, and Xpo-t triggers distinct conformational responses, suggesting that even closely related karyopherins employ different mechanisms of conformational regulation and that cargo and nuclear pore-interacting surfaces of the different receptors may be unique.

Nucleo-cytoplasmic transport factors of the karyopherin β family shuttle through nuclear pore complexes spanning the nuclear envelope and mediate the nuclear import and/or export of specific protein or RNA cargoes (for review, see Refs. 1–4). Directionality is imparted by Ran, a small GTPase present in its GTP-bound form at high concentrations in the nucleus and at low concentrations in the cytosol. RanGTP regulates cargo loading to nuclear export mediators (exportins) and unloading from nuclear import mediators (importins). Exportins bind their cargoes in the presence of RanGTP in the nucleus, traverse nuclear pore complexes as ternary cargo-exportin-RanGTP complexes, and disassemble in the cytoplasm upon hydrolysis of Ran-bound GTP. Conversely, importins bind their cargo at low RanGTP levels in the cytoplasm, traverse nuclear pore complexes as cargo-importin complexes, and dissociate from their cargo upon binding RanGTP in the nucleus. After delivering cargoes, components of the transport machinery shuttle back to the original compartment.

The karyopherin β family includes 14 members in yeast and at least 22 in humans (for review, see Refs. 1, 3, 5, 6). The prototypic representative of the family is importin β, which mediates import of proteins containing classical NLSs (see reviews, Refs. 1–4). Importin β does not bind classical nuclear localization signals directly but requires the adaptor protein importin α to bridge the interaction. Despite being considered “classical,” this import pathway is unusual in that most karyopherins bind their cargoes directly rather than with an adapter. Transportin, for example, recognizes multiple import cargoes, such as the M9 signal of heterogeneous nuclear ribonucleoprotein A1 (7) and importin β itself, can also bind cargoes directly (8, 9). In the case of nuclear export, several mediators have been studied to date. They include the importin α export factors (human CAS and yeast Cse1) (10–12), tRNA export factors (human Xpo-t and yeast Los1) (13–15), and NES1 export factors (human Crm1 and yeast Xpo1) (16–19). The transport machinery is remarkably versatile, with individual karyopherins recognizing multiple cargoes and with individual cargoes binding to multiple karyopherins (see reviews, Refs. 1–4).

Karyopherin β proteins are large molecules with molecular mass of 95–145 kDa sharing low sequence homology (sequence identity <20%). The similarity is higher in the N-terminal half, which mediates RanGTP binding (20). A similar repetitive pattern with a loose consensus of hydrophobic residues can be identified in all the sequences. Each repeating unit is on average 40 residues long and has the fingerprint of HEAT motifs (21, 22). The x-ray structure of full-length importin β locked in its interaction with a cargo, the IBB domain of importin α, has a spiral conformation built by the packing of 19 contiguous HEAT repeats (23). The snail-like superhelix can be dissected into an N-terminal arch and a C-terminal arch, both characterized by a similar crescent shape. The structure of a different karyopherin, transportin, locked in its RanGTP-bound state reveals a twisted superhelix built by the repetitive stacking of 20 HEAT repeats (24). The transportin structure can also be dissected into two arches but has an open S-like rather than a closed snail-like conformation. The structure of RanGTP bound to the N-terminal arch of importin β (25) is known, but it is not possible to extrapolate which conformation is adopted by the full-length protein. Furthermore, no structural information is available on other members of the family, exportins in particular.

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1 The abbreviations used are: NES, nuclear export signal; IBB, importin β binding domain; SAXS, small angle x-ray scattering; DR, dummy residue; H.s., Homo sapiens; S.p., S. pombe; NLS, nuclear localization signal.
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**EXPERIMENTAL PROCEDURES**

**Purification of Recombinant Proteins—**All proteins were expressed in *Escherichia coli* as His-tag fusions. Cells were disrupted with a homogenizer (Avestin), and the resulting crude extracts were cleared by centrifugation at 15,000 × *g* to remove insoluble material. Soluble fractions were purified using a Talon resin and subsequently by ion exchange chromatography and gel filtration.

The complexes were formed by adding a 4-fold excess of the smaller components (Ran loaded with GTP, tRNA) and separated by gel filtration. Yeast Phe tRNA was purchased from Sigma. The complexes were purified using a Talon resin and subsequently by ion exchange chromatography and gel filtration.

**Small Angle Scattering Data Collection and Analysis—**Synchrotron x-ray scattering data were collected at the EMBL X33 beamline (27) (DESY, Hamburg) at multiple protein concentrations between 3 and 20 mg/ml in 15 successive 1-min frames to check for radiation damage. The data were processed using the programs SAPOKO and PRIMUS (28). The overall parameters were computed using the Guinier and Porod approximations and the programs ORTGNOM (29) and GNOM (30). The latter program also permits diminishing of the effect of possible unspecific aggregation by discarding the scattering at very low angles, which is mostly influenced by the aggregation. The molecular mass of the solute was evaluated by comparison with the scattering from bovine serum albumin. Theoretical scattering curves and *Rg* from the coordinates of crystallographic structures and structure-based models were computed using the program CRYOSOL (31).

**SAXS ab Initio Reconstructions—**Two *ab initio* approaches were employed to reconstruct low resolution shapes. The program DAMMIN (32) represents the particle shape as an ensemble of densely packed beads inside a spherical search volume with diameter *Dmax*. Starting from a random distribution of beads, simulated annealing is employed to find a compact configuration minimizing the discrepancy χ between the experimental and calculated scattering curves. This shape analysis is performed using data in the range up to *s* = 2.5 nm⁻¹, discarding the scattering at higher angles dominated by the internal structure of the particle. In a more versatile approach implemented in the program GASBOR (33), the protein is represented as an assembly of dummy residues (DR). Starting from randomly positioned residues, a chain-compatible spatial distribution of DRs inside the same search volume is found by simulated annealing. For the ternary complex Xpo-Ran-tRNA containing an RNA molecule only DAMMIN was used. Because the *ab initio* methods do not provide unique three-dimensional models, 10 independent reconstructions were performed for each protein or complex. The models were aligned using the program SUPCOMB (34), which also provides a quantitative measure of dissimilarity of two (low or high) resolution models. After alignment, the program DAMAVER (35) was used to construct the average model representing at a lower resolution the common structural features of all reconstructions and to select the most probable model (giving the smallest dissimilarity with the average).

**RESULTS AND DISCUSSION**

**Overall Parameters and Benchmarking—**All the samples were purified to homogeneity, their dilute solutions were exposed to synchrotron x-rays, and the data processed were described under "Experimental Procedures." The sample homogeneity was monitored by evaluating the apparent molecular mass of the solute and the excluded volume (*V*) of the hydrated particle. Most solutions were found to be monodisperse, which was a necessary prerequisite for the shape analysis. Table I presents the overall parameters computed directly from the scattering data for all the samples, including the radius of gyration (*Rg*) and maximum particle dimension (*Dmax*).

We first benchmarked the feasibility of SAXS models for proteins of the karyopherin β family by analyzing the transportin-RanGTP complex, for which a crystallographic structure is available (24). Some aggregation effects observed in the scattering from transportin-RanGTP preparation (Fig. 2A) could be eliminated by discarding the innermost scattering points followed by data processing using the program GNOM (30). The particle shape was reconstructed by two *ab initio* simulated annealing methods, and several independent reconstructions were carried out starting from random initial approximations to compute the average and the most probable models (see "Experimental Procedures"). The bead models and the average of the DR models (Fig. 2B, blue and gray, respectively) display elongated shapes compatible with the crystallographic model of transportin-RanGTP, whereas the most probable DR model yields the best agreement with the high resolution structure (Fig. 2B, red). Below, the most probable DR models will be discussed for all the samples.

**Transportin Assumes an Extended S-like Conformation in Its Unbound, M9-bound, and RanGTP-bound States—**Transportin imports heterogeneous nuclear ribonucleoprotein A1 by binding to its M9 nuclear localization signal and releasing it in the nucleus upon RanGTP binding (7). The scattering pattern of unbound transportin (Fig. 3A) displays a pronounced shoulder at *s* = 1 nm⁻¹, probably reflecting the pitch of the transportin superhelix (about 6 nm). This shoulder is absent in both experimental and calculated curves from the transportin-RanGTP complex (24) (Fig. 2A). Removal of RanGTP from this crystallographic model makes the shoulder appear in the cal-
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**TABLE I**

| Model                  | MMth | MMexp | Volume | \( R_g \) | \( D_{\text{max}} \) | \( \chi_{\text{cryst}} \) | \( \chi_{\text{ab initio}} \) |
|------------------------|------|-------|--------|-----------|----------------|----------------|----------------|
| Transportin-Ran\(^*\)  | 126  | 130 ± 30 | 300 ± 30 | 4.6 ± 0.2 | 15 ± 2 | 1.6 | 0.84 |
| Transportin            | 102  | 130 ± 40 | 290 ± 20 | 4.3 ± 0.1 | 15 ± 1 | 1.8 | 1.1 |
| Transportin-M9         | 105  | 140 ± 30 | 280 ± 20 | 4.4 ± 0.2 | 16 ± 1 | 1.6 | 0.81 |
| Importin\(\beta\)-RanGTP | 123  | 130 ± 20 | 205 ± 15 | 3.6 ± 0.1 | 11 ± 1 | 1.4 | 0.80 |
| Xpo-4 H.s.\(^*\)       | 110  | 105 ± 20 | 270 ± 30 | 4.8 ± 0.2 | 16 ± 2 | 0.92 |
| Xpo-4 S.p.             | 110  | 100 ± 15 | 260 ± 10 | 4.7 ± 0.1 | 15 ± 1 | 0.79 |
| Los1 S.c.              | 126  | 110 ± 10 | 260 ± 20 | 5.1 ± 0.1 | 17 ± 2 | 1.7 |
| Xpo-4 S.p.-RanGTP-tRNA | 157  | 220 ± 10 | 190 ± 15 | 3.6 ± 0.1 | 11 ± 1 | 1.1 |
| Cse1 S.c.              | 110  | 110 ± 15 | 210 ± 10 | 3.9 ± 0.1 | 11 ± 1 | 0.73 |
| Xpo1 S.c.              | 125  | 110 ± 15 | 210 ± 10 | 3.9 ± 0.1 | 11 ± 1 | 1.1 |

_mm\(\text{th}\) and \(\text{Mexp}\) are the molecular masses predicted from sequence and evaluated from the scattering data, respectively. Volume is the Porod volume of hydrated particle. \( D_{\text{max}} \) and \( R_g \) are calculated using programs ORTOGNOM (29) and GNOM (30); \( \chi_{\text{cryst}} \) and \( \chi_{\text{ab initio}} \) are discrepancies provided by high resolution models (atomic coordinates based on crystal structures) and \(\text{ab initio}\) models, respectively. The asterisk (*) represents parameters computed by GNOM, discarding the low angle region up to \( s = 0.4 \text{ nm}^{-1} \) to eliminate aggregation effects.

Clockwise, models of transportin-RanGTP in two different orientations: From left to right, DAMMIN model (blue), averaged model from all DR reconstructions (gray), and most probable DR model superimposed with the crystal structure (red, with Ca-trace in black).

Fig. 2. Comparison of SAXS and crystallographic structural analysis of transportin bound to RanGTP. A, scattering curves of transportin-RanGTP. Shown are the experimental x-ray scattering curve (1) and calculated scattering from the corresponding crystallographic model (2) (24). Scattering was calculated from the \(\text{ab initio}\) model reconstructed by the program DAMMIN (32) (3) and by GASBOR (33) (4). The logarithm of scattering intensity is displayed as a function of momentum transfer (\( s = 4\pi \sin(\theta)/\lambda \)), where \( \theta \) is the scattering angle, and \( \lambda = 0.15 \text{ nm} \) is the x-ray wavelength up to \( s = 4 \text{ nm}^{-1} \) (i.e., a resolution of 1.6 nm). B, models of transportin-RanGTP in two different orientations: From left to right, DAMMIN model (blue), averaged model from all DR reconstructions (gray), and most probable DR model superimposed with the crystal structure (red, with Ca-trace in black).

Conformations in Its Free and Bound States—The scattering pattern of unbound importin \(\beta\) displays a shoulder at \( s \approx 1 \text{ nm}^{-1} \) similar to that of unbound transportin and superposes reasonably well with the curve computed from the crystallographic model of the transportin-RanGTP complex (Fig. 3B, upper curves). The most probable \(\text{ab initio}\) model of free importin \(\beta\) has the S-like appearance similar to the \(\text{ab initio}\) model of free transportin (Fig. 4B). The overall shape of unbound importin \(\beta\) is much less compact than that observed in the crystal structure of importin \(\beta\) bound to the IBB cargo (23), in agreement with biochemical experiments showing an increased susceptibility of importin \(\beta\) to proteases when in the free rather than the IBB-bound state (36).

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The scattering pattern of the importin \(\beta\)-RanGTP complex differs significantly from that of unbound importin \(\beta\) (Fig. 3B, lower curves) and also from the scattering curve of the transportin-RanGTP complex. In comparison to transportin-RanGTP, the \( R_g \) of importin \(\beta\)-RanGTP is 1 nm smaller, and its \( D_{\text{max}} \) is decreased by 4 nm (Table I), suggesting a rather compact shape. \(\text{Ab initio}\) reconstructions yield a compact model...
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FIG. 5. SAXS patterns of nuclear-export mediators. A, experimental scattering data from human Xpo-t (1), S. pombe Xpo-t (2), their S. cerevisiae orthologue Los1 (3), and importin β shown for comparison (4). Lower group, experimental scattering of human Xpo-t (5) and S. pombe Xpo-t (6) in their ternary export complexes with RanGTP and tRNA. Fits from ab initio reconstructions of S. pombe Xpo-t (upper group), Los1 (middle group), and the S. pombe tertiary complex (lower group) are shown in black (7). B, upper group, experimental scattering from yeast Cse1 (1) and importin β-RanGTP (2) for comparison and the calculated pattern from the importin β-IBB crystal structure (23) (3). Middle group, scattering from unbound Cse1 (4). Lower group, scattering from yeast Xpo1 (5). The fits by ab initio models of Cse1 and Xpo1 are shown in black (6).

upper curves), suggesting similar structures for the two orthologues. The ab initio model of S.p. Xpo-t reveals an extended superhelical shape similar to that of free transportin and importin β (Fig. 6). In contrast, yeast Los1 has a larger $R_g$ and $D_{\text{max}}$ as compared with the S. pombe and human orthologues (Table 1). The scattering curve of Los1 differs from that of H.s. and S.p. Xpo-t, lacking the characteristic shoulder at $s = 1 \text{ nm}^{-1}$ (Fig. 5A, center set of curves). The ab initio model of Los1 (Fig. 6) displays an elongated shape without superhelical twist. The closer similarity in SAXS data of human Xpo-t with the S. pombe rather than with the S. cerevisiae orthologue correlates with their relative similarity in sequence (25 and 19% sequence identity, respectively) and in molecular weight (about 110 kDa for H.s. and S.p. Xpo-t and 126 kDa for Los1).

The scattering patterns of H.s. and S.p. Xpo-t in a ternary complex with tRNA and Ran coincide with each other (Fig. 5A, lower curves) but differ significantly from the scattering of unbound Xpo-t. Upon formation of the ternary complex, the molecular weight of the particle increases by almost 50 kDa, but its $R_g$ decreases by 1 nm and also $D_{\text{max}}$ is 4 nm smaller (Table 1), indicating a more compact conformation than that of the unbound exportin. Because the complex contains tRNA, its model cannot be adequately reconstructed using GASBOR (for which the search model is represented by dummy residues). The average low resolution shape reconstructed with DAMMIN reveals a globular envelope that superposes reasonably well with the tight snail-like conformation of the importin β-IBB complex (Fig. 6). Thus, Xpo-t appears to undergo a large global conformational change between its unbound and bound states. In the free state this exportin has an open S-like twisted structure similar to the two import mediators importin β and transportin. Upon binding RanGTP and cargo, Xpo-t closes up to a compact conformation reminiscent of the conformational changes observed in the case of importin β.

The Extended S-like Architecture Is Not a Common Feature of Karyopherins—The similarity in overall extended superhelical structure of unbound importin β, transportin, and exportin-t raises the question of whether this S-like conformation is a

reminiscent of the closed importin β-IBB conformation (Fig. 4B). Indeed, the experimental data of importin β-RanGTP compare well with the scattering curve calculated from a model where the atomic coordinates of the importin β-IBB complex (23) have been modified to remove the IBB and to add RanGTP as observed in the complex with the N-terminal half of importin β (25) (Fig. 3B, lower curves). In this model composite of two crystal structures, the C-terminal arch of importin β clashes against Ran. A model obtained by applying a relatively small rigid-body movement to the C-terminal arch to avoid steric clashes with the GTPase provides a very good fit to the experimental data (Table 1).

These results indicate that the conformation of importin β is ligand dependent (Fig. 4B). Association of the IBB cargo to free importin β involves a large global conformational change with considerable flexing of the two arches. Dissociation of the IBB cargo by RanGTP binding instead appears to involve only a limited deformation of the snail-like superhelix. The importin β superhelix thus adopts two globally different conformations dependently on its ligand bound/unbound state.

The Export Mediator Xpo-t and the Import Mediator Importin β Have Similar Global Structural Features—To assess the overall shape and conformational changes of exportins, we targeted the tRNA export factor Xpo-t in its unbound state and export competent RanGTP- and tRNA-bound state. The scattering patterns were recorded on human Xpo-t (H.s. Xpo-t) and also on its orthologues from Schizosaccharomyces pombe (S.p. Xpo-t) and Saccharomyces cerevisiae (Los1) (Fig. 5A). All proteins are monomeric in solution (Table 1) with the exception of free H.s. Xpo-t, whose scattering pattern displays aggregation effects at low angles (Fig. 5A). At higher angles (beyond $s = 0.5 \text{ nm}^{-1}$, i.e. resolution of 10 nm), the scattering curve of H.s. Xpo-t virtually coincides with that of S.p.Xpo-t (Fig. 5A,
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Fig. 6. Molecular shapes of exportins. SAXS ab initio reconstructions shown in two orthogonal views. From left to right shown are yeast tRNA export factor Los1 (light green), S. pombe tRNA export factor Xpo-t unbound and in a ternary complex with tRNA and RanGTP (green), yeast NES-export factor Xpo1 (magenta), and yeast export factor Cse1 (yellow). Shown superposed in black is the crystal structure of S-like transportin (24) to unbound Xpo-t and the snail-like structure of importin β-IBB complex (23) to the Xpo-t ternary complex.

Concluding Remarks—SAXS is very sensitive to the macro-molecular shapes, and despite the relatively low resolution (about 1.6 nm) of the ab initio models, the reported structural differences between karyophorins are clearly and reproducibly observable. The reliability of SAXS-derived models has been documented in numerous studies (for review, see Refs. 26) and is well suited to obtain the shape in solution of macromolecules such as karyophorins with masses of ~100 kDa, too large for NMR and too small for cryo-EM.

Nuclear import and export mediators of the karyopherin β family share sequence and functional similarities. Nevertheless, different mechanisms of conformational regulation are used even for closely related importins. The SAXS results we report here indicate that importin β and transportin have a similar open S-like superhelical structure in their unbound state. In the context of the different overall conformations observed in the importin β-IBB and transportin-RanGTP crystal structures (23, 24), this similarity is unexpected. Detailed structural comparison reveals that, despite the different relative orientation of the N- and C-terminal arches, more than 50% of the residues between HEAT 2 and HEAT 10 of importin β and transportin have a close α-carbon position (root mean square deviation of less than 3.5 Å) and, similarly, more than 60% of the residues between HEAT 12 and HEAT 17 (37). With hindsight, what is surprising is not that the two importins assume a similar S-like structure in their unbound form but that they respond with different conformational changes upon ligand binding and release.

SAXS data together with the available crystallographic and biochemical data on transportin (24, 38) yield a scenario where M9 cargo association and dissociation does not involve global rearrangements of the karyopherin S-like superhelix but, instead, a local conformational change of a long internal loop in the hinge region connecting the two arches. For importin β, the two arches are pulled together in a closed conformation in the presence of either an IBB cargo or RanGTP. SAXS and crystallographic results (8, 22, 25) suggest that dissociation of the IBB by RanGTP involves displacement at overlapping binding sites at the hinge region and only a minor conformational change of the snail-like superhelix. Altogether, it appears that the different conformational regulation of these two closely related importins might arise from differences at the hinge region.

The SAXS results on karyophorins involved in three different nuclear export pathways yield the first structural view of exportins. The tRNA export factor Xpo-t is structurally similar to importin β in that it cycles between an open S-like confor-
mation in the unbound form and a closed compact conformation when bound to Ran and tRNA. Thus, there are no intrinsically different conformations that might be characteristic of importins or of exportins. Unexpectedly, human and fission yeast Xpo-t are structurally more similar to metazoan importin β than they are to their budding yeast orthologue Los1, suggesting that we should expect variation between functional homologues from different species. Another major result of this study is that not all karyopherins have an extended conformation in their unbound state. The exportins Cse1 and Xpo1 show more compact and distinct shapes, which are yet difficult to rationalize in terms of HEAT-repeat arrangement based on currently available high resolution structures. These results are striking, considering that the relative sequence similarity of Cse1 and Xpo1 with one another or in comparison with Xpo-t, importin β, and transportin provides no evidence for primary structure differences that would explain the dramatic variations in shape. The conformational variability we observe within the set of karyopherins and ligands examined in this study suggests a great variety in the mechanisms of conformational regulation employed by this family of HEAT-repeat transport factors. It reinforces the impression that each of this family of proteins, although having evolved from a common ancestor, has developed an idiosyncratic set of interactions with its partner proteins.

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