Structural Basis for Substrate Recognition and Dissociation by Human Transportin 1

The transport of macromolecules between the nucleus and the cytoplasm through nuclear pore complexes (NPCs) is mediated via several transport pathways by transport receptors that are most commonly members of the importin-β family [1]. Transport receptors form complexes with their transport substrates ("cargoes") through cognate nuclear localization signals (NLSs) for import substrates or nuclear export signals (NESs) for export substrates, and target substrates to NPC components. Transportin 1 (Trn1) is a transport receptor that belongs to the importin-β family, and was first identified as the receptor of heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) [2]. Other substrates whose transport is mediated by Trn1 have since been identified. NLSs of these transport substrates have little sequence similarity, but recent mutational analyses have shown the importance of two successive proline and tyrosine residues (called a PY motif) conserved in the NLSs of hnRNP D, TAP, JKTBP, hnRNP A1, and hnRNP M for recognition by Trn1 [2,3]. Here, we show four crystal structures of human full-length wild-type Trn1 corresponding to Trn1 in a substrate-free form and bound to the following three NLS peptides, which all have a PY motif: hnRNP D NLS (residues 332-355), TAP NLS (residues 53-82), and JKTBP NLS (residues 396-420). We propose a mechanism for NLS recognition and dissociation from Trn1 [4]. Diffraction data for NLS-free Trn1 and Trn1 bound to hnRNP D NLS and JKTBP NLS were collected using an ADSC Quantum 315 CCD detector on **BL41XU** at SPring-8 and those for Trn1 bound to TAP NLS were collected using an ADSC Quantum 210 CCD detector on NW-12 at Photon Factory Advanced Ring (PF-AR).

Trn1 is a superhelical S-like molecule formed by two overlapping arches (N- and C-terminal arches), and is constructed by helical stacking of 20 HEAT repeats (H1-H20) and a long disordered H8 loop (Fig. 1(a)). The structure of the N-terminal arch (HEAT repeats 1-13) is almost the same in the four Trn1 structures, whereas that of the C-terminal arch (HEAT repeats 8-20) changes depending on the NLS to which Trnl is bound (Fig. 1(b)). The overlapping region of the N- and C-terminal arches (HEAT repeats 8-13) show no conformational change. Comparison of the four structures with the structure of the Trn1-RanGTP complex [5] demonstrates that RanGTP binding to the N-terminal arch produces substantial conformational changes in both the N- and C-terminal arches (Fig. 1(b)). Electron density appearance of the three NLSs (Fig. 1(c)) correlates with the dissociation constant for the NLS interaction with Trn1. A NLS with a PY motif is recognized by Trn1 at two sites, Site A (HEAT repeats 8-13) with high affinity and Site B (HEAT repeats 14-18) with low affinity (Fig. 2(a)). Furthermore, three consensus residues (H/R, P, Y) in hnRNP D NLS and TAP NLS and one hydrophobic residue (V) in hnRNP D NLS are recognized at Sites A

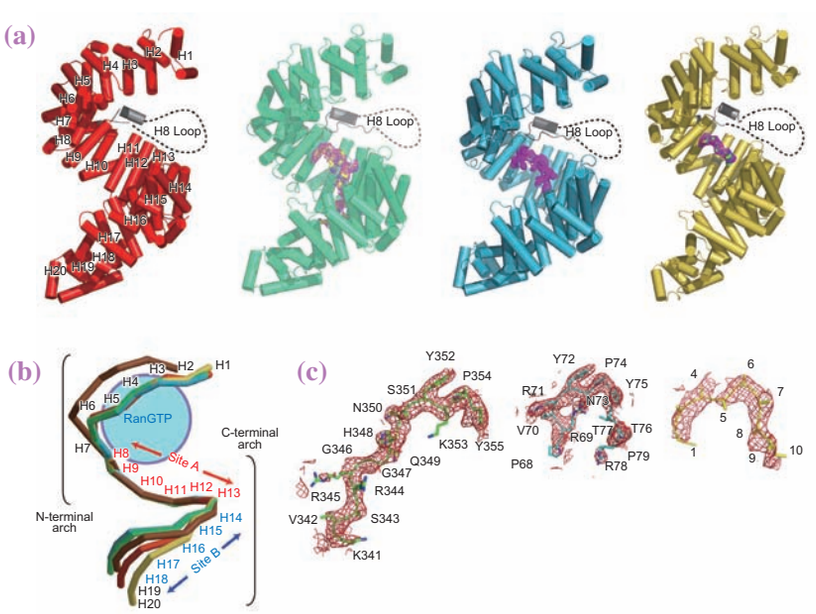

Fig. 1. Structures of Trn1. **(a)** Overall structures of NLS (substrate)-free Trn1 (red) and Trn1 bound to hnRNP D NLS (green), TAP NLS (blue), and JKTBP NLS (yellow), where α helices are represented by cylinders. Twenty consecutive HEAT repeats (H1-H20), each of which is composed of two antiparallel helices, are labeled on the NLS-free Trn1 structure. The H8 loop is represented by a black cylinder (α helix) and a black dotted line (disordered region). **(b)** Trn1 structure showing conformational changes upon either RanGTP or NLS binding. The structures of NLS-free Trn1 (red), Trn1 bound to hnRNP D NLS (green), Trn1 bound to TAP NLS (blue), Trn1 bound to JKTBP NLS (yellow), and Trn1-RanGTP complex (brown) were superimposed in the overlapping region of the N- and C-terminal arches (HEAT repeats 8-13). The 20 consecutive HEAT repeats in each Trn1 molecule are represented by straight lines. The structure of the Trn1-Ran complex was drawn using the refined coordinates deposited in the Protein Data Bank (accession code: 1QBK). **(c)** The CNS composite simulated annealing omit map of the NLS region bound to Trn1. The map was calculated with coefficient $2F_o$- F_c and contoured at 1.0 σ. NLSs of hnRNP D, TAP, and JKTBP are shown as stick models in green, blue, and yellow, respectively.

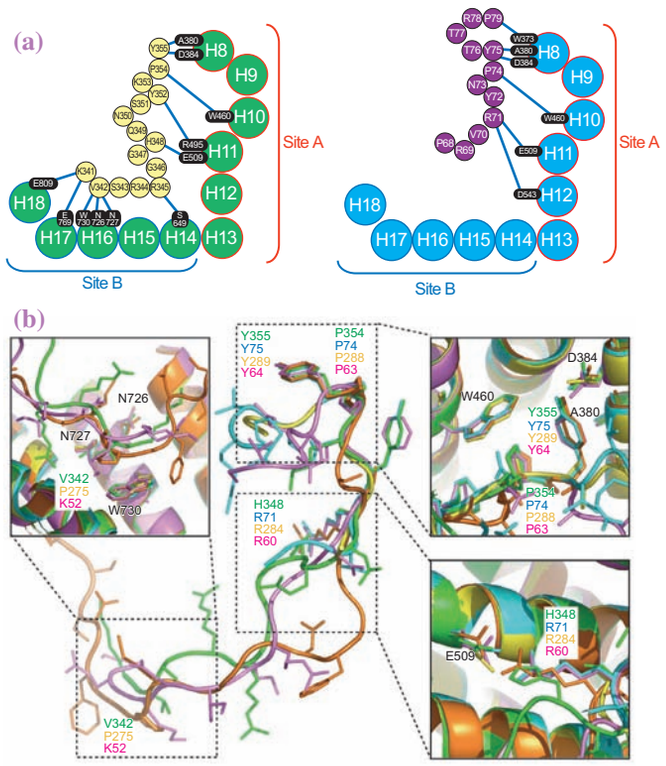

Fig. 2. NLS recognition by Trn1. **(a)** Schematic illustrations of Trn1 interactions with hnRNP D NLS (left) and TAP NLS (right). Trn1-NLS contacts less than 3.8 Å are shown. **(b)** Structures of hnRNP D NLS (green), TAP NLS (blue), hnRNP A1 NLS (orange), and hnRNP M NLS (purple) bound to Trn1. Two close-up views of structures on the right side and one close-up view on the left side show the interactions with Trn1 at Sites A and B, respectively. Structures of hnRNP A1 NLS and hnRNP M NLS bound to a H8 loop-truncated Trn1 mutant were drawn using the refined coordinates deposited in the Protein Data Bank (accession codes: 2H4M and 2OT8).

and B, respectively, in the same manner as that for hnRNP A1 NLS and hnRNP M NLS (Fig. 2(b)).

Figure 3 shows a proposed mechanism for NLS recognition and dissociation from Trn1. In the cytoplasm, Trn1 recognizes the NLS H/R-$X_{(2\text{-}5)}$-P-Y motif (X: any residue) at Site A (pre-loaded state). In some cases, Trn1 changes its conformation to make hydrophobic interaction between Site B and the hydrophobic motif of NLS (loaded state). The Trn1-NLS complex is transported through NPC into the nucleus. In the nucleus, RanGTP binding to the N-terminal arch in NLS-bound Trn1 induces a competing interaction from the H8 loop against Site B (pre-unloaded state), resulting in displacement of NLS from Site B. This displacement from Site B is critical for NLSs such as hnRNP D, which interact strongly with Site B. This displacement, however, is not critical for NLSs with weak or no interaction at all with Site B (such as TAP NLS and JKTBP). After the release from Site B, NLS is displaced from Site A by the spatial overlap of the H8 loop with the part of the NLS at Site A, resulting in a complete dissociation from Trn1.

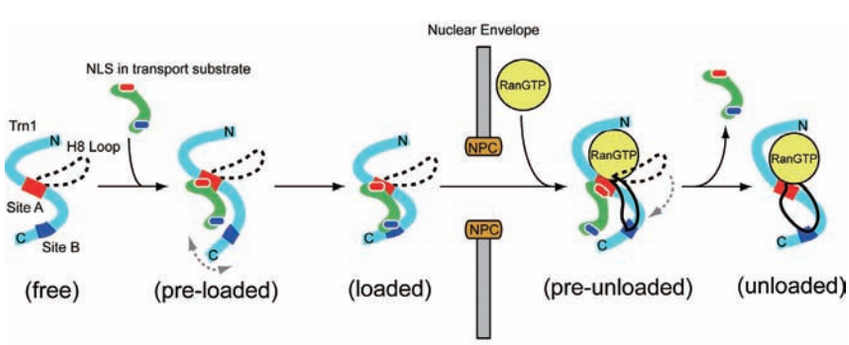

Fig. 3. Proposed mechanism for nuclear import pathway mediated by Trn1. The Trn1 molecule is represented by S-like cyan ribbon labeled with N- and C-termini. NPC is the nuclear pore complex. Red and blue ellipsoids on NLS in the transport substrate show the three consensus residues (red ellipsoid) and one hydrophobic residue (blue ellipsoid).

T. Imasaki, T. Shimizu, H. Hashimoto, M. Yamada and M. Sato*

Field of Supramolecular Biology,
Yokohama City University

*E-mail: msato@tsurumi.yokohama-cu.ac.jp

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