

## Structural insights into how Yrb2p accelerates the assembly of the Xpo1p nuclear export complex

Masako Koyama<sup>1</sup>, Natsuki Shirai<sup>1</sup> and Yoshiyuki Matsuura<sup>\*1,2</sup>

<sup>1</sup>Division of Biological Science and <sup>2</sup>Structural Biology Research Center, Graduate School of Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8602, Japan

### 1 Introduction

Rapid exchange of macromolecules between the cytoplasm and the nucleus is a crucial cellular function that regulates many physiological processes in eukaryotes. Nuclear transport occurs through nuclear pore complexes and is mediated in most cases by soluble transport receptors that belong to the karyopherin- $\beta$  superfamily. In *Saccharomyces cerevisiae*, Xpo1p (CRM1 in vertebrates) is the most versatile nuclear export receptor that carries a broad range of cargo proteins and ribonucleoproteins from the nucleus to the cytoplasm. The majority of cargoes of Xpo1p contain the so-called leucine-rich nuclear export signal (NES) that typically harbor four or five characteristically spaced hydrophobic residues.

Yrb2p (RanBP3 in vertebrates) is a primarily nuclear Gsp1p (yeast Ran)-binding protein that has a multi-domain structure based on an N-terminal domain containing a nuclear localization signal, a central domain containing FG-repeats that bind Xpo1p specifically, and a C-terminal Gsp1p-binding domain (RanBD) that binds Gsp1p-GTP weakly. Although it has been known for more than a decade that efficient export of NES-cargoes requires Yrb2p (RanBP3), the mechanism of action of Yrb2p (RanBP3) remains poorly understood. To dissect the mechanism of action of Yrb2p (RanBP3), we performed detailed structural and functional characterization of the interactions between Xpo1p, Yrb2p, Gsp1p, NES, and FG-nucleoporins in this study [1].

### 2 Experiment

Crystals of Xpo1p-Yrb2p-Gsp1p-GTP complex and Xpo1p-PKI-Gsp1p-GTP complex were grown by hanging drop vapor diffusion method. Preliminary X-ray diffraction experiments were carried out at Photon Factory and the datasets used for final structure determination were collected at SPring-8.

### 3 Results and Discussion

FRET-based kinetic measurements showed that Yrb2p remarkably accelerates association of Gsp1p-GTP and NES to Xpo1p. To understand the structural basis for how Yrb2p accelerates the assembly of the Xpo1p nuclear export complex, we determined the structure of a key assembly intermediate (Xpo1p-Yrb2p-Gsp1p-GTP complex) by molecular replacement at 2.22 Å resolution (Fig. 1; PDB code, 3WYF). To enable a complete comparison to be made between different conformations of Xpo1p, we also determined the structure of Xpo1p

bound to PKI (a representative NES-cargo) and Gsp1p-GTP at 2.15 Å resolution (PDB code, 3WYG).

The NES-binding cleft, formed between the outer helices of HEAT repeats 11 and 12 of Xpo1p, is closed in the crystal structure of Xpo1p-Yrb2p-Gsp1p-GTP complex. However, biochemical data suggested that preloading of Gsp1p-GTP onto Xpo1p by Yrb2p, conformational flexibility of Xpo1p, and the low affinity of RanBD enable active displacement of Yrb2p RanBD by NES to occur effectively.

The structure of Xpo1p-Yrb2p-Gsp1p-GTP complex also revealed the major FG-repeat binding sites on Xpo1p.

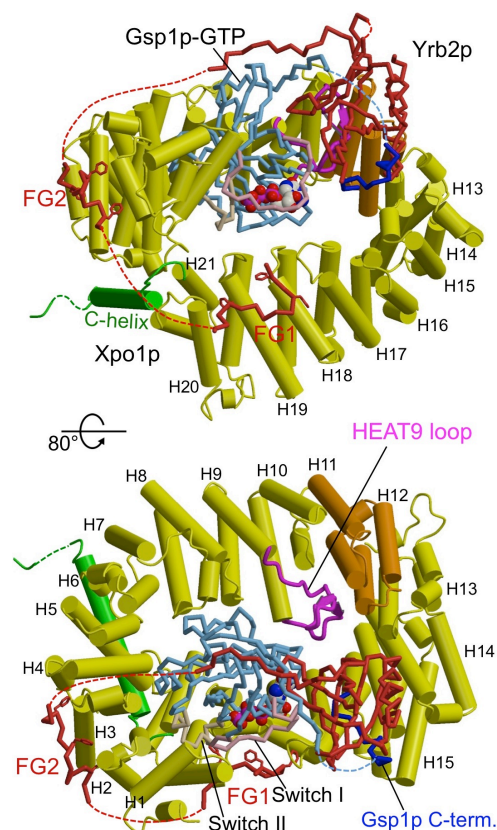


Fig. 1: Crystal structure of Xpo1p-Yrb2p-Gsp1p-GTP complex (PDB code, 3WYF).

### References

- [1] M. Koyama, N. Shirai, & Y. Matsuura, *Cell Reports* **9**, 983-995 (2014).

\* matsuura.yoshiyuki@d.mbox.nagoya-u.ac.jp