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Crystal structure of the Xpo1p nuclear export complex bound to the SxFG/PxFG repeats of the nucleoporin Nup42p

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1 Introduction

Xpo1p (yeast CRM1) is the major nuclear export receptor that carries a plethora of proteins and ribonucleoproteins from the nucleus to cytoplasm. Previous structural studies on Xpo1p (CRM1) have provided rich insights into the molecular mechanism of how cargo is loaded onto Xpo1p (CRM1) in the nucleus and how cargo is unloaded in the cytoplasm [1]. Although it is well established that the passage of the Xpo1p (CRM1) nuclear export complex through nuclear pore complexes (NPCs) is facilitated by interactions with nucleoporins containing extensive repeats of phenylalanine-glycine (FG repeats), the precise role of each nucleoporin in the nuclear export reaction remains incompletely understood. Here we report a crystal structure of the Xpo1p nuclear export complex bound to the SxFG/PxFG repeats of Nup42p, a nucleoporin localized at the cytoplasmic face of yeast NPCs [2].

2 Experiment

Crystals of Xpo1p-PKI-Gsp1p-GTP complex were grown by hanging drop vapor diffusion method [3]. Crystals of Xpo1p-PKI-Nup42p-Gsp1p-GTP complex were formed by soaking the crystals of Xpo1p-PKI-Gsp1p-GTP complex in a stabilizing solution containing Nup42p (residues 88-122). Preliminary X-ray diffraction experiments were carried out at Photon Factory and the data set used for final structure determination was collected at SPring-8.

3 Results and Discussion

We determined the structure of Xpo1p-PKI-Nup42p-Gsp1p-GTP complex by molecular replacement at 2.2 Å resolution (Fig. 1). The structure identified three binding sites for the SxFG/PxFG repeats on the outer surface of Xpo1p (Fig. 2). The structure showed atomic detail of how the serines and prolines in the SxFG/PxFG sequence repeat motif, together with the key phenylalanines, interact directly with Xpo1p. Mutational analyses of Nup42p provided evidence that the conserved serines and prolines in the SxFG/PxFG repeats contribute to Xpo1p-Nup42p binding. Our structural and biochemical data suggest that the SxFG/PxFG-repeat containing nucleoporins at the cytoplasmic face of NPCs provide high-affinity docking site for the Xpo1p nuclear export complex in the terminal stage of NPC passage. The structure of the Xpo1p-PKI-Nup42p-Gsp1p-GTP complex also provided insights into evolutionary conservation of the FG repeat binding sites on Xpo1p (CRM1) across species.

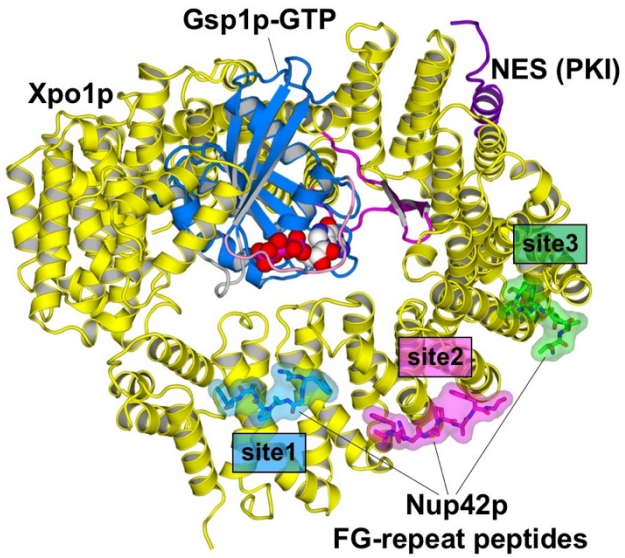

Fig. 1: Crystal structure of Xpo1p-PKI-Nup42p-Gsp1p-GTP complex (PDB code, 5XOJ).

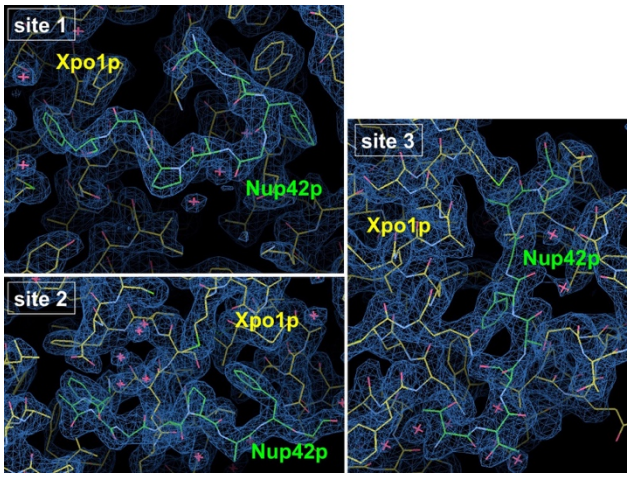

Fig. 2: Electron density map at the Nup42p binding sites.

References

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