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Structural basis for cell-cycle-dependent nuclear import mediated by Kap121p

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1 Introduction

Precise regulation of macromolecular exchange between the cytoplasm and the nucleus is an essential aspect of many cellular processes. Nuclear transport occurs through nuclear pore complexes (NPCs) and is mediated in most cases by soluble transport receptors that belong to the karyopherin-β superfamily. Kap121p (also known as Pse1p) is an essential nuclear import receptor that mediates nuclear import of a broad range of cargoes including transcription factors, cell cycle regulators and ribosomal proteins in *Saccharomyces cerevisiae*.

Altering the structure of NPCs is an emerging mechanism to control nuclear transport. It has been proposed that the cell-cycle-dependent molecular rearrangement of yeast NPC exposes the Kap121p-binding domain of the yeast nucleoporin Nup53p and thereby arrests Kap121p-mediated nuclear import at metaphase, while leaving import mediated by other nuclear transport receptors unaffected [1]. The Kap121p-specific import inhibition is required for normal progression through mitosis [1].

To understand the structural basis for Kap121p-mediated nuclear import and its unique regulatory mechanism during mitosis, we determined X-ray crystal structures of Kap121p in isolation and also in complex with either its import cargoes, Nup53p or RanGTP [2].

2 Experiment

Crystals of unliganded Kap121p, Kap121p-cargo complexes, Kap121p-Nup53p complex and Kap121p-RanGTP complex were grown by hanging drop vapor diffusion method. X-ray diffraction datasets were collected at Photon Factory and SPring-8. The structures were solved by selenomethionine (SeMet) single-wavelength anomalous dispersion (SAD) phasing and molecular replacement.

3 Results and Discussion

Kap121p is constructed from 24 tandem HEAT repeats arranged into a right-handed superhelical solenoid (Fig. 1). The nuclear localization signals (NLSs) of cargoes (Ste12p and Pho4p) bound in an extended conformation to line the concave surface of the central region of Kap121p (HEAT repeats 7-12) (Fig. 1). These structures established the structural basis for recognition of a novel NLS that has a consensus sequence of KV/IxK$x_{1\text{-}2}$K/H/R, where x can be any amino acid.

The structure of Kap121p-Nup53p complex showed that cargo and Nup53p compete for the same binding site. Surface plasmon resonance showed that the Kap121p-specific NLSs and Nup53p bind to Kap121p with comparable affinity (in 10 nanomolar range), suggesting that the binding of Nup53p is strong enough to displace cargoes when the pseudo-NLS sequence of Nup53p is exposed during mitosis.

The binding of RanGTP to Kap121p in the nucleus is important to terminate nuclear import. The structure of Kap121p-RanGTP complex showed that RanGTP binds to the inner surface of Kap121p. The RanGTP-binding site partially overlaps with the NLS-binding site, and the RanGTP-binding is associated with conformational changes in the NLS-binding site. Thus, the structure explains how RanGTP forces Kap121p into a conformation that is incompatible with NLS-binding.

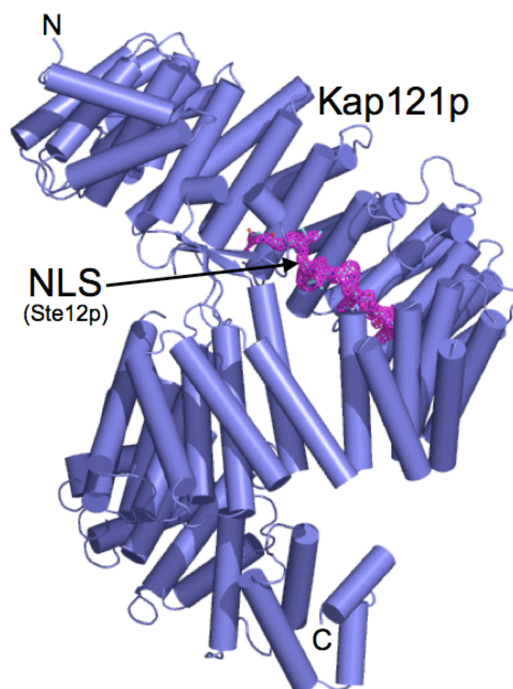

Fig. 1: Crystal structure of Kap121p bound to Ste12p (PDB code, 3W3W).

References
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