5A,17A/2009G577,2011A1875

Sensing actin dynamics: Structural basis for G-actin-sensitive nuclear import of MAL

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1 Introduction

The coordination of cytoskeletal actin dynamics with gene expression reprogramming is emerging as a crucial mechanism to control diverse cellular processes, including cell migration, differentiation and neuronal circuit assembly. The actin-binding transcriptional coactivator MAL (also known as MRTF-A/MKL1/BSAC) senses G-actin concentration and transduces Rho GTPase signals to serum response factor (SRF) [1]. MAL rapidly shuttles between the cytoplasm and the nucleus in unstimulated cells but Rho-induced depletion of G-actin leads to MAL nuclear accumulation and activation of transcription of SRF:MAL-target genes. MAL is implicated in human diseases: MAL was originally discovered as a gene fusion in patients with acute megakaryocytic leukemia, and defective regulation of MAL contributes to cancer cell migration and invasion.

MAL has a multi-domain structure containing an N-terminal actin-binding RPEL domain, a central SRF-binding domain, and a C-terminal transcription activation domain. The N-terminal domain has three tandem repeats (each containing the RPxxxEL sequence motif) in the amino acid sequence. The N-terminal domain is necessary and sufficient for Rho-regulated nuclear accumulation in mouse fibroblast [2]. Herein we report a crystal structure of MAL N-terminal domain in complex with nuclear import receptor (Impα). We also determined a crystal structure of MAL N-terminal domain in complex with five molecules of G-actin. The structures reveal how nuclear import machinery interacts with MAL in a way that responds to changes in G-actin concentrations [3].

2 Experiment

Crystals of MAL-Impα complex and MAL-actin complex were grown by hanging drop vapour diffusion method. X-ray diffraction datasets were collected at Photon Factory and SPring-8 beamlines. The structures of MAL-Impα complex and MAL-actin complex were solved by molecular replacement at a resolution of 2.10 Å and 3.45 Å, respectively.

3 Results and Discussion

The structure of MAL-Impα complex was refined to R_{free} 20.80 % (R_{cryst} 16.60%). The structure shows that MAL has a classical bipartite nuclear localization signal (NLS) in the N-terminal 'RPEL' domain (Fig. 1a). The NLS residues of MAL adopts an extended conformation and bind along the surface groove of Impα, interacting with the major- and minor-NLS binding sites.

The MAL-actin complex was refined to R_{free} 27.33 % (R_{cryst} 22.44%). The structure shows that the N-terminal RPEL domain of MAL can bind to five molecules of G-actin (Fig. 1b). In this structure, the crank-shaped MAL meanders through five G-actins, and the NLS residues are surrounded by multiple actins. The folding of MAL in this pentavalent complex is entirely different from that in the MAL-Impα complex. The α-helical conformation of MAL NLS residues in complex with actins is distinct from the extended conformation of the NLS in the MAL-Impα complex, and so is incompatible with Impα-binding. Thus, the induced folding and occlusion of NLS by G-actins can explain why G-actins inhibit nuclear import of MAL.

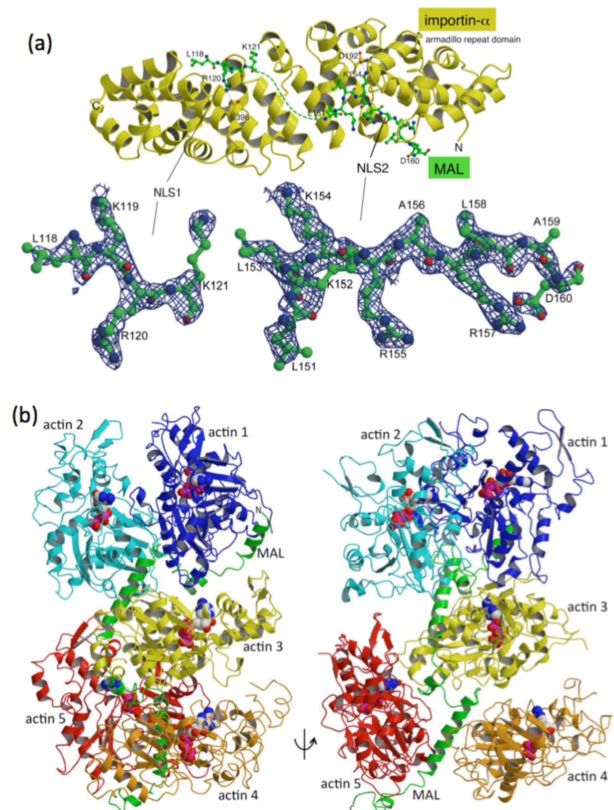

Fig. 1: Overview of the structures of the MAL complexes. (a) MAL-Impα complex. (b) MAL-actin complex.

References

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