

Crystal structures of *Saccharomyces cerevisiae* N-myristoyltransferase with bound myristoyl-CoA and inhibitors reveal the functional roles of the N-terminal region

Jian Wu^{1,2}, Meilan Zhang¹, and Jianping Ding^{1,*}

¹State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, and ²Graduate School of Chinese Academy of Sciences, 320 Yue-Yang Road, Shanghai 200031, China

Introduction

Protein N-myristoylation catalyzed by myristoyl-CoA:protein N-myristoyltransferase (NMT) plays an important role in a variety of critical cellular processes and thus is an attractive target for development of antifungal drugs. In previous reports about the X-ray structures of NMTs, the N-terminal region was either unordered and not refined or truncated to facilitate cloning and expression. To understand the functional roles of the N-terminal region and the detailed information of inhibitor-enzyme interactions, the crystal structures of full-length *Saccharomyces cerevisiae* NMT (ScNMT) with bound myristoyl-CoA and inhibitors have been determined at 2.9 Å, 3.1 Å, and 3.0 Å, respectively. Analysis of these structures provides valuable information for further research.

Method and Results

The ScNMT full length protein (455 amino acids) with N-terminal His₆-tag was obtained through Ni affinity chromatography and then ion-exchange chromatography methods. Sparse-matrix crystallization screening with Grid (ammonium sulfate) kits (Hampton Research) was performed using the hanging-drop vapor diffusion method at 4 °C. Crystals of ScNMT in complex with myristoyl-CoA (and inhibitors) were mounted on a cryo-loop and flash-frozen in liquid nitrogen. Data collection was carried out using the ADSC CCD detector of BL6A at PF.

In the ScNMT:myristoyl-CoA binary complex, an ordered N-terminal region could be clearly traced, revealing an α -helix (α B'), and a loop (B'A' loop) region. Together our structural and kinetics data indicate that the N-terminal region of NMT plays an important role in the binding of both myristoyl-CoA and peptide substrate, but not in subsequent steps of the catalytic mechanism (Fig. 1). The two inhibitors are found occupying the peptide substrate binding site and interact with the protein through primarily hydrophobic contacts. Analyses of the inhibitor-enzyme interactions provide valuable information for further improvement of antifungal inhibitors targeting NMT (Fig. 2).

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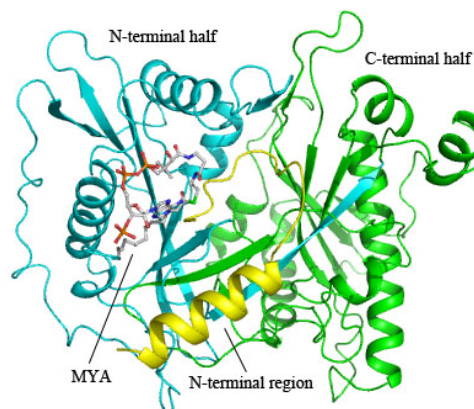


Fig. 1. Overall structure of the ScNMT binary complex.

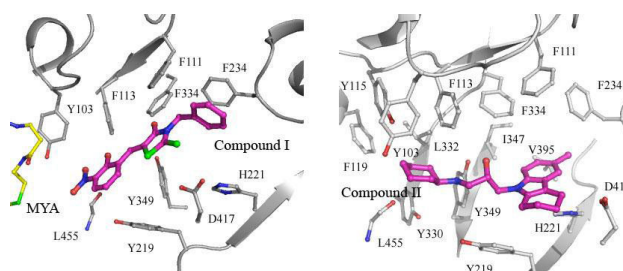


Fig. 2. Interactions of ScNMT and the inhibitors.

References

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* jpding@sibs.ac.cn