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Protein-interacting C2H2-type zinc fingers: structural studies by NMR

Mariasina S.[1]*, Dukhalin S.[1], Balagurov K.[2], Efimov S.[3], Bocharov E.[4], Bonchuk A.[2], Polshakov V.[1]

[1] *Lomonosov Moscow State University, Moscow, Russia*

[2] *Institute of Gene Biology, Moscow, Russia*

[3] *Kazan Federal University, Kazan, Russia*

[4] *Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia*

* *sm1024sm@yandex.ru*

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Motivation and Aim: Transcription factors are proteins that regulate gene expression. One common type of domain found in transcription factors is the C2H2 zinc finger. This type of domain has two β-strands and an α-helix, which is stabilized by a zinc ion coordinated by two cysteine and two histidine residues. These domains are typically involved in specific DNA binding through the residues in the α-helix. However, there is increasing evidence that these domains may also play a role in protein-protein interactions.

In our study, we investigated the structural features and protein-protein interactions of two N-terminal C2H2 zinc finger domains from different *Drosophila* proteins. The first studied object was the Chromatin-Linked Adaptor for MSL Proteins (CLAMP). Previous research has shown that CLAMP directly interacts with the male-specific organizer of the complex (MSL2) [1]. MSL2 plays an important role in the specificity of binding to the X chromosome during the process of dosage compensation in *Drosophila*.

The second object is CG18262. Based on our preliminary investigation, it appears to be a member of a potentially new family of C2H2 zinc fingers. Unlike typical domains of this type, members of this new family have an additional β-strand at the N-terminus, which we hypothesize could be an effector domain responsible for dimerization.

Structural data are essential for understanding the physiological functions and interactions between proteins. This study aims to determine the three-dimensional solution structures of Zn finger protein domains and investigate their protein-protein interaction networks. The findings of this research will contribute to a better understanding of the processes involved in transcription regulation.

Methods: Heteronuclear NMR spectroscopy was used to obtain structural information. Unlabeled, ^{15}N-labeled, and ^{15}N/^{13}C-labeled protein samples were expressed in *E. coli*. 2D and 3D NMR spectra were recorded using NMR spectrometers with 600 and 700 MHz frequency, equipped with either a room temperature triple resonance probe or a cryoprobe.

Resonance assignments were made using a standard 3D heteronuclear NMR technique. Restrained molecular dynamics protocol was applied to obtain 3D structure of the proteins, using the distance restraints derived from nuclear Overhauser effects, torsion angle restraints obtained from the analysis of NMR chemical shifts, and hydrogen bond

restraints measured in H/D exchange NMR experiments. Protein-protein interactions were studied using the NMR titrations and isotope filtered experiments.
Results: We obtained the NMR solution structure of the N-terminal C2H2 Zn finger domain of the CLAMP protein (residues 87–153, 7.4 kDa). This domain has a classical C2H2 zinc finger fold with a rather unusual distribution of residues typically used for DNA recognition (PDB entry 7NF9, Fig. 1A).

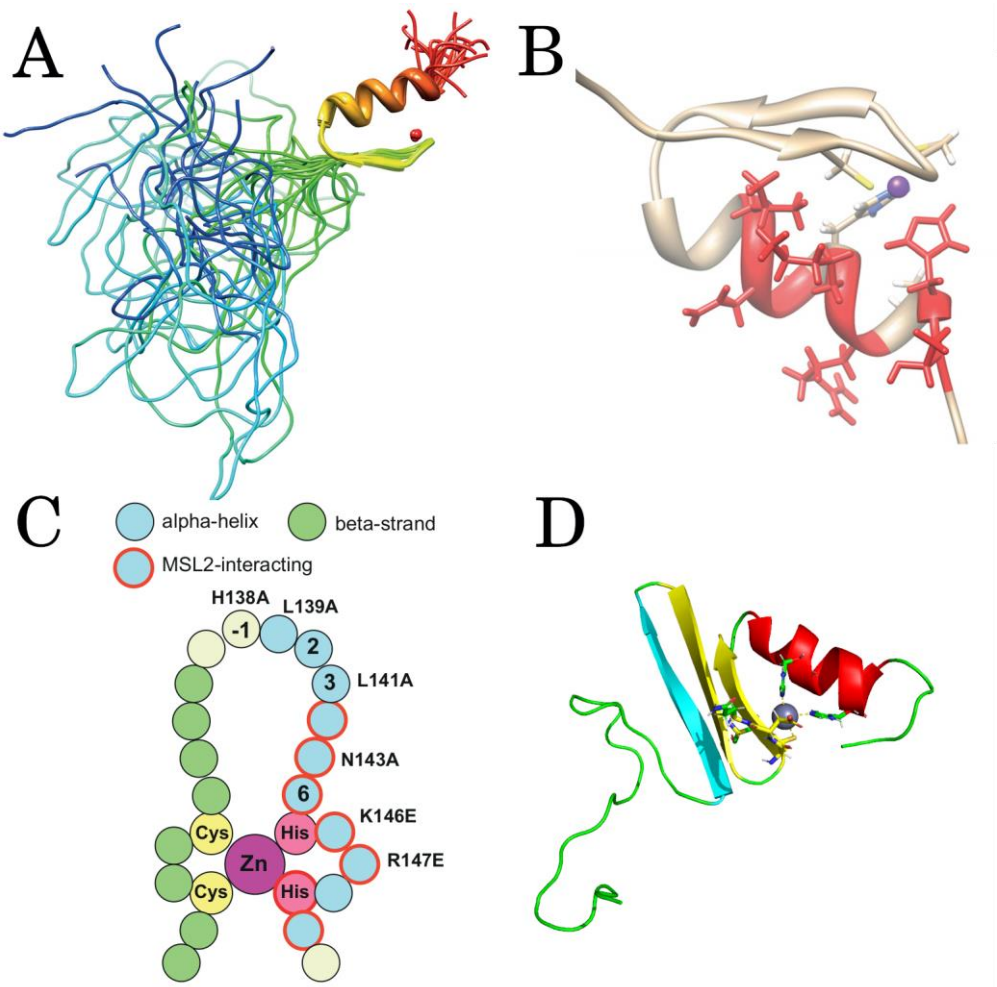

Fig. 1. Structural studies of N-terminal zinc finger domain of CLAMP and CG18262. **A**. Family of NMR solution structures of CLAMP$^{87\text{-}153}$ (PDB id 7NF9). **B**. 3D structure of CLAMP$^{87\text{-}153}$ with residues interacting with MSL2 shown in red. **C**. Schematic representation of the secondary structure of the zinc finger domain of CLAMP, with α-helical residues shown in blue and β-strand residues in green. are represented by sticks. The MSL2-binding residues, which were the subject of mutagenesis, are surrounded by red circles. **D**. Cartoon representation of the NMR solution structure of CG18262$^{1\text{-}62}$. The β-strands from the canonical C2H2 zinc finger are shown in yellow, while residues 25-32, which form an additional β-strand responsible for dimerization, are shown in cyan

To identify the critical residues for MSL2 binding pattern within CLAMP, we carried out NMR titration experiments using ^{15}N-labeled CLAMP$^{40–153}$ or CLAMP$^{1–153}$ in the presence of an excess of unlabeled MSL2$^{618–655}$. These experiments showed that only residues within the α-helix of C2H2 Zn finger are involved in interactions with MSL2 (Fig. 1B and C). The roles of these residues were confirmed by mutagenesis experiments. The dissociation constant (K_d) for the CLAMP–MSL2 complex was determined to be relatively weak (0.2 ± 0.1 mM at 25 °C), but the interactions were highly specific [2]. We also obtained the structure of the N-terminal domain of CG18262 (residues 1-62, Fig. 1D). As expected, it appears to be a typical C2H2 zinc finger, but it is stabilized by an additional β-strand at the N-terminus.

Consistent with our model predictions, the N-terminal domain of CG18262$^{1-62}$ has dimerization activity, which was confirmed by NMR experiments using mixtures of ^{15}N,^{13}C-labbeled and unlabeled protein. The dimerizing interface involves residues 25-32 attributed to the additional β-strand in the C2H2 zinc finger.

Conclusion: In this study, structural data on two protein-interacting N-terminal domains of the C2H2 zinc fingers from *Drosophila* have been obtained. CLAMP$^{87-153}$ is a classical C2H2 zinc finger with an unusual ability to interact with its protein partner MSL2 via set of residues on its α-helix. The second protein, CG18262, has an unusual structure for a canonical C2H2 zinc finger, with an additional β-strand at the N-terminus that represents an effector domain responsible for dimerization.

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