RESEARCH HIGHLIGHT

A 'warhorse-shaped' topology adopted by the m^{6}A RNA writer complex

Wei Xie [1,2]✉ and Dinshaw J. Patel [3]✉

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The m^{6}A writer complex is a holoenzyme formed by catalytic MAC and regulatory MACOM subcomplexes that play a key role in the readout of this most prevalent epigenetic mark on RNA. Su et al. recently determined the cryo-EM structures of the MACOM complex at high resolution and the MAC–MACOM complex at medium resolution, thereby defining the composition and assembly of the core MACOM complex, as well as highlighting insights into the regulatory mechanism of m^{6}A RNA readout at the molecular level and its impact on function.

N^6-methyladenosine (m^{6}A) is the most abundant and widely distributed RNA epigenetic modification in mammals. The m^{6}A mark, whose distribution is controlled by readers, writers and erasers specific for this modification, is enriched in the vicinity of the stop codon, as well as within the 3′-UTR and long internal exons of mRNA, where it is involved in regulating various physiological and pathological processes.[1] The m^{6}A writer complex co-transcriptionally methylates mRNAs within the DRACH (D = A/G/U, R = A/G, H = A/C/U) motif.[2] A set of reader proteins in the nucleus (YTHDC family members) and the cytoplasm (YTHDF family members) recognize m^{6}A-mRNAs and thus alter their cell fate.[3] Given the reversible nature of this epigenetic mark, the m^{6}A modification can be removed by ALKBH5 and FTO proteins.[4] These reader and eraser factors, together with the writer component, have compiled a complicated regulatory network of m^{6}A deposition and its lifetime, with dysregulation of these readout processes implicated in tumorigenesis. Therefore, unveiling molecular mechanisms of m^{6}A regulatory factors and their readout should pave the way for developing new drug intervention strategies for m^{6}A-related diseases.[5,6]

The m^{6}A writer complex is a holoenzyme formed by the catalytic METTL3–METTL14 subcomplex (referred to as MAC) and its regulatory m^{6}A-METTL associated subcomplex (referred to as MACOM) counterpart. The MACOM subcomplex is comprised of multiple proteins that include WTAP (Wilms' tumor 1-associated protein), VIRMA (KIAA1429), HAKAI (E3 ubiquitin ligase CBLL1), RBM15/15 (RNA-binding motif protein 15/15 paralog), and ZC3H13 (zinc finger CCH domain-containing protein).[7–10] In the absence of MACOM, MAC alone showed relatively low m^{6}A writer activity in vitro and it has been suggested that MACOM is responsible for guiding the MAC core complex to specific regions on mRNA. Given that previous structural studies have primarily focused on the MAC subcomplex, there remains a lack of structural information about the regulatory MACOM subcomplex. This knowledge gap highlights the challenge associated with an improved understanding of the critical regulatory role of MACOM, both initially from the structural and eventually the functional perspective.

In a recent study published in *Cell Research*,[11] the groups of Jinbiao Ma and Kaiming Zhang conducted a comprehensive structural biology characterization on the human m^{6}A writer complex by solving structures of four distinct complexes containing different MACOM components. These efforts included cryo-EM structures of two core HWVZ (HAKAI–WTAP–VIRMA–ZC3H13) and HWV (HAKAI–WTAP–VIRMA) MACOM subcomplexes at 3.0 Å resolution (Fig. 1a, b), as well as the core MACOM bound to the MAC subcomplex at an overall resolution of 4.4 Å, though at lower resolution for its MAC component (Fig. 1c). Notably, both core MACOM complexes comprising HAKAI, WTAP, and VIRMA domains in the absence or presence of the ZC3H13 domain, form similar compact structures in which WTAP and VIRMA form the scaffold of MACOM, which in turn serves as a template for further recruitment of ZC3H13 and HAKAI.

Since there has been no previous structural information for any of the components of MACOM, the authors examined the structure of each subunit's fold and found many unique features. WTAP forms a 'saddle-shaped' homodimer through coiled-coil interactions, with the twisting of the two protomers around each other assisted by three linker regions. Interestingly, the two protomers show conformational heterogeneity as indicated by low root mean square deviation and low cross-correlation coefficient between their maps. VIRMA, the largest component of the MACOM subcomplex, adopts a horse-shaped conformation involving twenty armadillo modules. The overall structural scaffold of MACOM is composed of the WTAP homodimer that adopts a 'saddle-shaped' clamp anchored onto the VIRMA 'warhorse-shaped' scaffold through extensive interactions involving an overall interface above 6000 Å^2. Unlike WTAP and VIRMA, only a fragment of ZC3H13 can be traced in the final map, with this ZC3H13 highly conserved fragment strongly interacting with VIRMA and slightly stretching the MACOM conformation (Fig. 1a, b). Notably, the majority of the ZC3H13 subunit and the entire HAKAI subunit cannot be monitored due to their dynamic nature relative to the remainder of the core MACOM scaffold, though it is conceivable that missing components of these subunits could be stabilized through interactions with other macromolecules in vivo.

To understand the assembly of the m^{6}A writer holoenzyme complex, the authors further analyzed the 4.4 Å structure of the

[1]College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, Zhejiang, China. [2]Department of Infectious Diseases, The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang, China. [3]Structural Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA. ✉email: xie_wei@zju.edu.cn; pateld@mskcc.org

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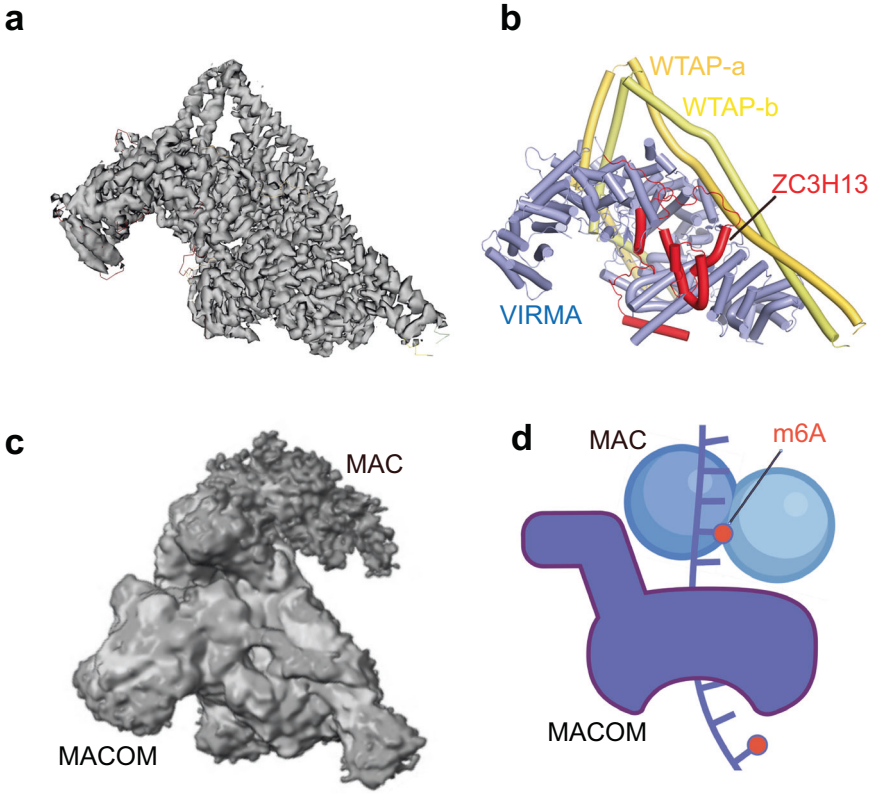

Fig. 1 Structural insights into the core MACOM subcomplex at 3.0 Å resolution and the MAC–MACOM complex at lower resolution. **a**, **b** The overall structure of the core MACOM complex composed of VIRMA, WTAP and ZC3H13 shown in density (**a**) and ribbon (**b**) representations (PDB code: 7VF2). **c** The overall structure of the MAC–MACOM complex shown in a density representation at a low threshold, given the lower resolution for the MAC component of the complex (modified from[11]). **d** Schematic drawing of the m^{6}A writer holocomplex emphasizing the 'saddle-shaped' WTAP and 'warhorse-shaped' VIRMA components of the scaffold.

MAC–MACOM complex (Fig. 1c). Although the density of the MAC component is less traceable, the authors found an extra α-helix density in the L2 and H segments of WTAP. Cross-linking mass spectrometry and pull-down experiments indicated that the additional density might be the Leader Helix at the N-terminus of METTL3, an interpretation consistent with a previous report.[12] Therefore, it appears that the recognition is mediated by interactions between the WTAP subunit of MACOM and the METTL3 subunit of MAC. Based on the above structural information, together with protein–RNA cross-linking data and m^{6}A methyltransferase enzymatic activity assays, the authors proposed a plausible model for how MACOM forms a complex with MAC to enhance m^{6}A modification (Fig. 1d).

The reported cryo-EM structures of the MACOM subcomplex by Su et al. provide the molecular details of the relative alignment and interactions between WTAP, VIRMA and ZC3H13 components of this subcomplex. Detailed insights into the alignment and intermolecular contacts between the MAC and MACOM subcomplexes on formation of the MAC–MACOM complex must await higher resolution structural data, perhaps achievable upon addition of substrate RNA, as part of an effort to stabilize flexible components of the complex. If successful, such an effort would improve our current understanding of the assembly mode adopted by the m^{6}A writer holoenzyme and its complex with substrate RNA. The current study has set the stage for addressing the next set of interesting challenges in the field. Can structural studies elucidate the trajectory of the bound RNA and address whether conformational changes are triggered in the MAC–MACOM complex upon RNA substrate binding? How does the structural platform formed by the MAC–MACOM complex facilitate interactions with other regulators? Moreover, can structural information unveil new drug targets within the MAC–MACOM scaffold associated with m^{6}A-mediated diseases, thereby guiding the rational design of potential therapeutics?

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ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Wei Xie or Dinshaw J. Patel.

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