LETTER TO THE EDITOR

Cryo-EM structure of human SRCAP complex

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Dear Editor,

Eukaryotic genome packs into highly condensed chromatin, in which genomic DNA wraps around histone octamer formed by evolutionarily conserved histone proteins H2A, H2B, H3, and H4. The histone variant H2A.Z primarily exists within nucleosomes that flank gene promoters, centromeres, and chromatin boundaries. The H2A.Z-containing nucleosomes undergo rapid turnover, which is important for the regulation of gene expression, DNA repair, and chromosome stability¹. The deposition of H2A.Z into genome is uniquely dependent on the SWI/SNF (switch/sucrose non-fermentable)-related Swr1 complex (referred to as Swr1-C hereafter) in yeast and SNF2-Related CBP Activator Protein (SRCAP) complex (referred to as SRCAP-C hereafter) in human. Swr1-C/SRCAP-C incorporates H2A.Z/H2B dimer into canonical nucleosomes coupled with the eviction of H2A/H2B dimer in a stepwise exchange manner^{2–4}.

SRCAP complex is named for its scaffold protein, SRCAP, which is a member of ATP-dependent INO80 chromatin remodeler family that belongs to SNF2 family of ATPases². The representative SNF2 chromatin remodeler family members include INO80, SWI/SNF, ISWI (imitation SWI) and CHD (chromodomain helicase DNA-binding). These chromatin-remodeling complexes play critical roles in transcriptional regulation through carrying out histone exchange, sliding, and spacing of nucleosomes in an ATP-dependent manner.

SRCAP-C is an ~1 MDa complex consisting of 10 components including SRCAP, DMAP1, YL1, RUVBL1, RUVBL2, ACTL6A, ARP6, ACTIN, GAS41 and ZNHIT1 (Fig. 1a). The yeast homolog Swr1 complex consists of Swr1, Swc4, Swc2, Rvb1, Rvb2, Arp4, Arp6, Actin, Yaf9, Swc6, and four yeast specific subunits, Swc3, Swc5, Swc7 and Bdf1. Previous biochemical and structural studies indicate that SRCAP-C consists of a SRCAP as a scaffold, a head module formed by RUVBL1-RUVBL2 heterohexameric ring, a C module containing YL1, ARP6 and ZNHIT1, and an N module containing ACTL6A, ACTIN, DMAP1 and GAS41^{5,6} (Fig. 1a).

Although the functions of Swr1-C/SRCAP-C have been extensively studied, the structural studies have been limited by low-resolution EM structures of yeast Swr1 complex^{5,7,8}. Recently, cryo-EM structure of human INO80 complex bound to mononucleosome was reported⁹. However, the mechanisms for SRCAP-C assembly and histone exchange remain largely unknown.

We purified the human SRCAP-C to homogeneity for structural analysis (Supplementary information, Fig. S1). Interestingly, histone proteins H2A.Z and H2B were co-purified with the SRCAP-C in our experimental condition. The H2A.Z and H2B were derived from endogenous protein because no corresponding cDNAs were co-transfected. Although no such co-purification was reported in previous complex preparation^{2,5}, tandem affinity purification of H2A.Z could pull down Swr1 complex, supporting the interaction between H2A.Z and Swr1/SRCAP complex. Thus, the SRCAP-C used in this study is possibly in a priming state and is ready to deposit H2A.Z/H2B heterodimer upon nucleosome association.

The cryo-EM structure was determined using single-particle reconstruction with the full complex map refined to 8.5 Å resolution (Supplementary information, Fig. S2, 3 and Table S1). Comparison of SRCAP-C EM map with those of yeast Swr1 and Swr1-nucleosome complexes reveals a generally similar architecture and obviously distinct features⁵ (Supplementary information, Fig. S4). The difference likely results from the different complex composition: 14 subunits in yeast Swr1 complex and 10 subunits in human SRCAP complex bound to H2A.Z/H2B. The EM density extension out of the ATPase domain and the ARP6 are rather weak, possibly due to intrinsic flexibility of the corresponding regions.

We next focused on the relatively stable core complex and obtained the EM map locally refined to 4.0 Å resolution (Supplementary information, Fig. S2 and 3). The structures of RUVBL1-RUVBL2 (PDB ID: 5OAF), ARP5, and ATPase domain of human INO80-nucleosome complex (PDB ID: 6ETX)⁹ were used as templates for model building. The structural model was built by rigid-body docking, flexible fitting, homology modeling, and manually building into the 4.0-Å resolution EM density.

The SRCAP-C structure reveals a fold containing a ring-shaped “head” with two separate arms. The head consists of a single hexameric ring formed by three copies of RUVBL1-RUVBL2 heterodimer (Fig. 1b). The right arm is an ATPase domain formed by two split lobes (Lobe 1 and Lobe 2) of SRCAP protein with Lobe 2 associating with the bottom edge of the head module. The left arm is primarily formed by ARP6 and some unassigned regions, which bind the head module and is located proximal to the ATPase domain. The SRCAP insert (residues 910–2036) form a ring fold that extensively associates with the bottom of RUVBL1-RUVBL2 ring, and two parallel α-helices that link the head module and ARP6 (Fig. 1b).

Comparison of the structural models of SRCAP-C and nucleosome-bound INO80 complex⁹ reveals that the two core complexes share a similar overall architecture (Fig. 1c, d). This observation is also consistent with sequence similarity of INO80 protein and SRCAP protein on their split ATPase domains and part of their inserts (Supplementary information, Fig. S5).

The characteristic inserts of SRCAP and INO80 both extensively associate with the head modules (Fig. 1d). Although the sequences and lengths of the two inserts are quite different, the peripheral regions of the two inserts take similar paths to interact with the inner surface of RUVBL1-RUVBL2 hexameric ring, suggesting a relatively conserved surface for assembly of RUVBL1-RUVBL2 hexamer into SRCAP-C/INO80-C. The insert of SRCAP protein has two parallel α-helices, which protrude out of the bottom of the RUVBL1-RUVBL2 ring (Fig. 1b). The two helices make contacts with ARP6 and serve as a bridge to buttress the interaction between the head module and ARP6. This unique feature was not observed in INO80 structure, suggesting that the longer insert (compared to that of INO80) of SRCAP may extend out of the head module and be involved in complex assembly or functional regulation yet to be discovered. Consistently, we

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Fig. 1 Structure of human SRCAP complex. **a** Structural organization and color-coded domain structure of human SRCAP complex components. The colors used for RUVBL1/2 hexamer are shown in a hexagon (right) with similar relative position in the structure. The same color scheme is used in all structure figures. The SRCAP protein serves as a scaffold. The three modules are indicated in boxes with grey background. N module, H2A.Z/H2B, and part of C module are invisible in the EM map and are indicated in dashed boxes. **b** Ribbon representations of SRCAP core complex in two distinct views. The insert of SRCAP is shown in mesh and indicated. The two parallel α -helices (right panel) are from the insert of SRCAP protein. **c** Structural comparison of SRCAP complex (left panel) and INO80-nucleosome complex (right panel) in a similar view. The structures are shown in ribbon representations. The predicted binding position of nucleosome in SRCAP-C is indicated. The histone octamer and nucleosomal DNA in INO80-nucleosome structure are colored in grey and yellow, respectively. The inserts of SRCAP and INO80 are shown in surface representations for clarity. **d** Isolated inserts of SRCAP and INO80 shown in a similar view with surrounding RUVBL1-RUVBL2 parts shown in ribbon representation. **e** Isosurface representations of cryo-EM reconstruction of SRCAP complex with the EM density at 8.5 Å resolution shown in low threshold. **f** Electrostatic potential surfaces of ARP6 are shown in the context of core complex structure. The surface colored in blue reveals potential DNA-binding surface

observed unassigned density that binds ARP6 and is physically close to the two α -helices (Fig. 1b, c).

The two lobes of INO80 ATPase domain grasp the nucleosomal DNA and adopt a stable conformation relative to the core complex (Fig. 1f). In contrast, without association with DNA, the two lobes of SRCAP ATPase domain adopt a relatively flexible conformation. The Lobe 2 (residues 841–909 and 2036–2190) associates with the head module and could be traced in the 4.0 Å-resolution EM density, whereas the Lobe 1 (706–840) is barely observed in the EM density with low threshold (Fig. 1e). The flexibility of the two lobes is consistent with their function in mediating ATP-dependent conformational switch and the translocation of nucleosomal DNA.

Previously reported cryo-EM structures of mononucleosome-bound Swr1 complex suggest that the nucleosome is primarily recognized by Swr1 protein⁵. However, the CHIP-exo data indicate a similar nucleosome binding mode of the Swr1 and INO80

complexes¹. The structural comparison of INO80-nucleosome and SRCAP-C strongly support the later model that SRCAP-C recognizes nucleosome in a manner similar to INO80-C (Fig. 1c). Consistent with the potential nucleosomal DNA-binding property, a stretch of the ARP6 surface (faces toward the ATPase domain) is rich in positively charged residues (Fig. 1f). This surface is formed by evolutionarily conserved residues (Supplementary information, Fig. S6), supporting its functional importance. Thus, Swr1/SRCAP and INO80 complexes may use similar core complexes to recognize nucleosome and carry out histone exchange through SRCAP complex- and INO80 complex-specific components, respectively. For example, YL1 has been shown to be SRCAP complex-specific component that binds H2A.Z/H2B dimer and is essential for histone exchange function¹⁰ (Fig. 1a).

The EM map indicates that the unassigned subunits/regions protrude out of the ARP6 and ATPase domain and would have steric hindrance if SRCAP-C binds nucleosome (Fig. 1e and

Supplementary information, Fig. S4). Thus, SRCAP-C will undergo a drastic conformational change upon binding to nucleosome. The Swr1-C/SRCAP-C has epigenetic reader modules, such as YEATS domain-containing Yaf9, which specifically recognizes histone lysine acetylation. These modules may facilitate conformational changes of Swr1/SRCAP to favor the recognition of nucleosome.

We here proposed mechanism for SRCAP complex-mediated histone exchange. The nucleosome is primarily recognized by SRCAP-C on two binding surfaces. The ATPase domain of SRCAP protein binds DNA for translocation. The nucleosome is further stabilized by ARP6 and possibly other SRCAP-C components. The ATPase domain serves as a motor to push DNA to the ARP6, leading to a partial unwrapping of nucleosomal DNA and loss of contacts between H2A/H2B dimers within the histone octamer, and thus facilitating histone exchange. YL1 may specifically bind to H2A.Z/H2B dimer and be involved in the histone exchange process.

Intriguingly, INO80 complex possesses histone exchange activity and nucleosome sliding activity, but Swr1-C/SRCAP-C has no sliding activity. A possible explanation is that ARP6 and some other SRCAP-C-specific components stably bind nucleosome and prevent translocation of DNA with respect to histone octamer. Thus, although the ATPase domain can translocate nucleosomal DNA, the relative position of DNA and histone octamer is fixed to avoid nucleosome sliding. The complex-specific components, ARP5-IES6 in INO80 and ARP6-YL1 in SRCAP, may contribute to this functional difference.

In summary, our study reveals the structure of SRCAP complex. We proposed a model for nucleosome recognition and histone exchange mediated by SRCAP complex. The structure provides a framework for further investigating SRCAP complex assembly and regulatory mechanism of histone exchange.

ACCESSION CODES

The electron density maps and corresponding atomic coordinates have been deposited in the Protein Data Bank (<http://www.rcsb.org/pdb>) with code: 6IGM, and EMDB (<http://www.ebi.ac.uk/pdbe/emdb/>) with codes: EMD-9668 (core complex) and EMD-9669 (full complex).

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AUTHOR CONTRIBUTIONS

Y.F., Y.T. and Y.X. designed the experiments. Y.F. purified the proteins. Y.T. prepared the cryo-EM sample, collected the data and determined the structure. Z.W. helped with structure determination. Y.F., Y.T. and Y.X. analyzed the data and wrote the manuscript. Y.X. supervised the project.

ADDITIONAL INFORMATION

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Competing interests: The authors declare no competing interests.

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