Structural and functional insights into transcriptional co-repressor TRIM28 complex

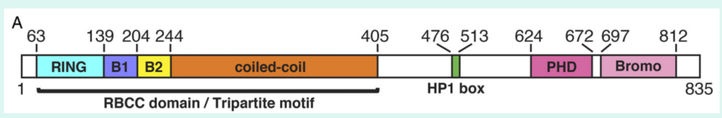
Abstract

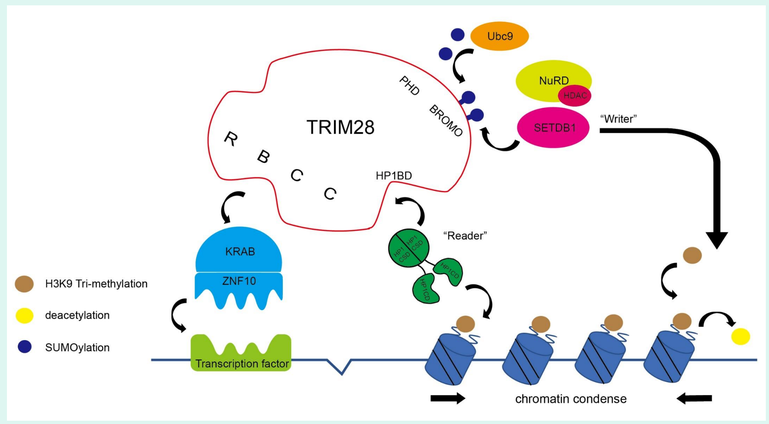
TRIM28 is now known to participate in many aspects of cellular biology, either promoting cell proliferation or mediating anti-proliferative activities. KRAB domain-containing zinc finger proteins(KRAB-ZFPs) recognize specific retrotransposon sequences and recruit TRIM28, inducing the assembly of an epigenetic silencing complex, with chromatin remodeling activities that repress transcription of the targeted retrotransposon and adjacent genes. Although the crystal structure of RBCC domain of N-terminus TRIM28 had been resolved, our work aim to show the biophysical and structural data of TRIM28- transcription repress complex through cryogenic electron microscopy, and further illustrate the molecular ratio of triple-complex. Relying on liquid-liquid phase separation assay, we would like to demonstrate the detailed mechanism of H3K9-methylation mediated transcriptional repression and heterochromatin formation.

Introduction

TRIM28 protein, a large multi-domain protein (110 kDa), which is a member of a family of almost 60 human Tripartite motif-containing (TRIM) proteins, is also known as KAP1. As a transcriptional co-repressor, TRIM28 protein is essential for KRAB-ZNF proteins to unleash their repressive potential. Molecular mechanism of KRAB-ZNF-mediated transcriptional regulation depends on the interaction with chromatin-remodeling factors through the TRIM28 protein. Briefly, KRAB-ZNF proteins bound to specific DNA recognition motifs (transcription factor binding site, TFBS) through their zinc finger domains recruit TRIM28 protein which acts as a scaffold for various heterochromatin-inducing factors. This enrollment is dependent upon the specific interaction of the TRIM28 N-terminal RBCC domain with a conserved KRAB repression domain. Next, PHD-mediated SUMOylation of bromodomain and resulting recruitment of SETDB1 and NuRD complex proteins lead to the creation of the H3K9me3 mark on nearby nucleosomes together with deacetylation of histone proteins. Further HP1 protein binding to TRIM28 at the PxVxL motif and to the H3K9me3 mark, subsequently stabilize the TRIM28-containing complex bound to the KRAB-ZNF.

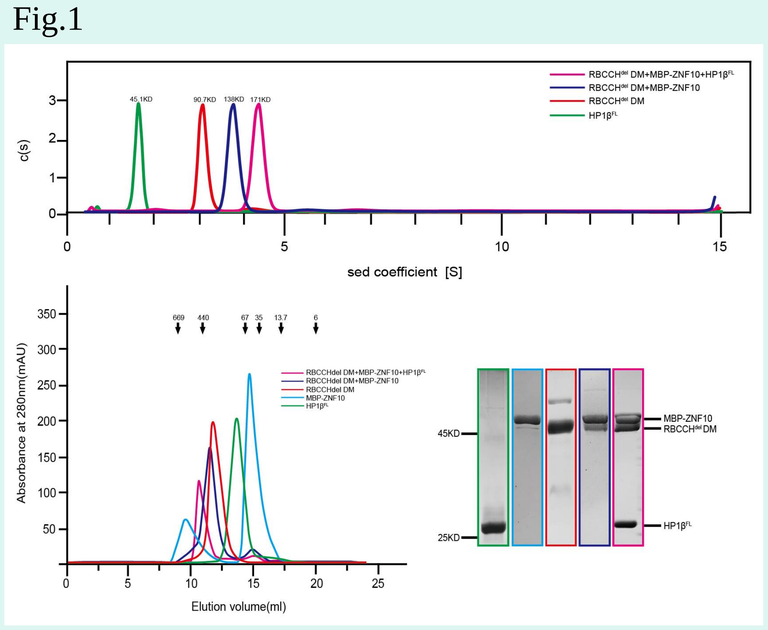
Human-TRIM28^{FL}





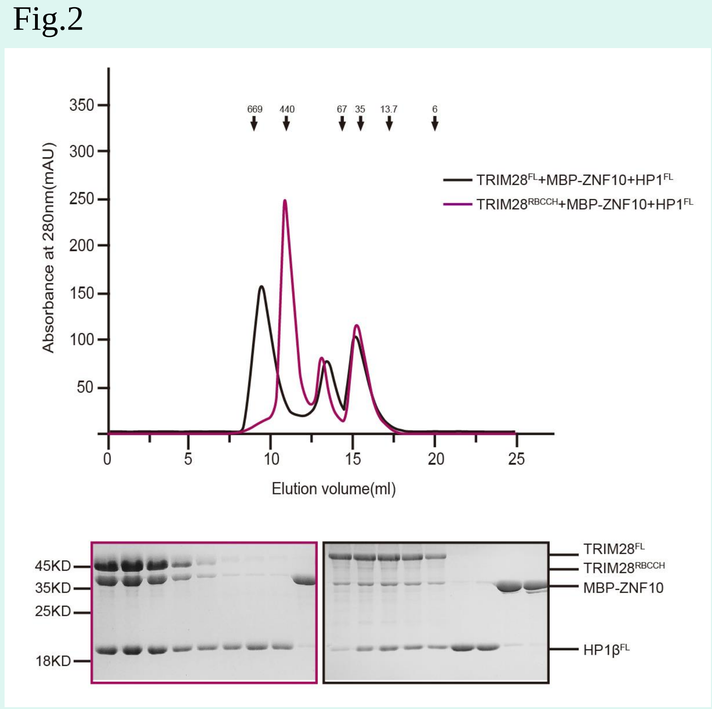
Results

Fig.1

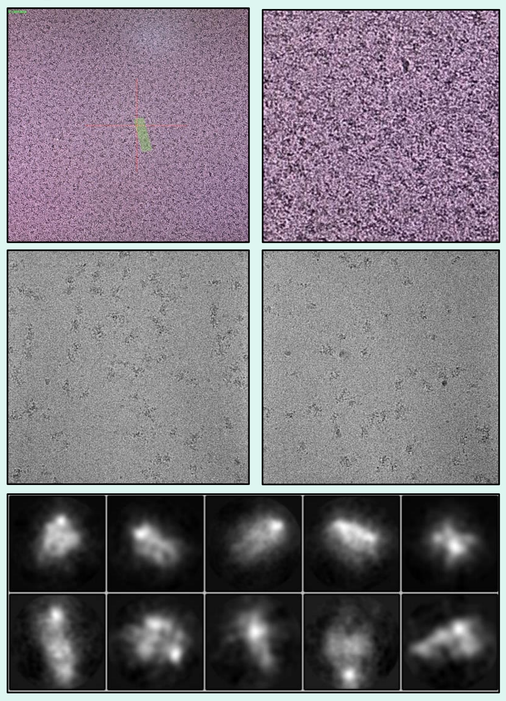


Analysis of ultracentrifugation and gel filtration results suggested that the stoichiometric ratio of TRIM28^{RBCCH},HP1^{FL} and MBP-ZNF10 is 2:2:1.

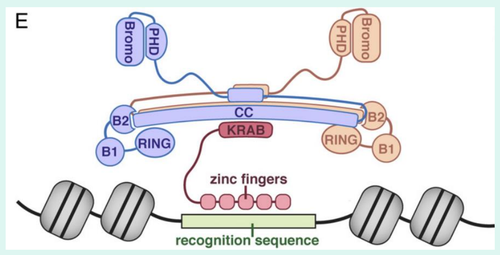
Fig.2



The TRIM28-complex for cryo-EM



The structure of TRIM28-complex analysis is in progress.



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How TRIM28-complex assembles and specifically recognizes DNA sequences and further regulates gene transcription through the formation of heterochromatin needs to be clarified.