Structural analysis of transcription related complexes and operation of 200kV cryo-EM in KEK

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Transcription is fundamental process for withdrawing genetic information stored in the genome. In eukarya, multi-subunit complexes, known as RNA polymerase II, general transcription initiation factors –*i.e.* TFIIA, TFIIB, TBP/TFIID, TFIIE, TFIIF, TFIIH–, mediator, and chromatin factors, carry out this reaction. To elucidate the detailed mechanisms of the reaction, their tertiary structural information is indispensable. So far, we determined crystal structures of subunit/domain of TFIID [1,2] and examined molecular evolution of TBP and TFIIB [3,4]. We have also performed large-scale purification of eukaryotic transcription-related complexes for structural analysis [5]. Preliminary cryo-EM analysis showed that these complexes seemed to be disrupted due to the harsh condition during cryo-grid preparation. Further optimization for cryo-grid preparation is required.

March 2018, our institute, KEK, obtained 200kV cryo-EM (Talos Arctica with Falcon3EC) and prepared pipeline for solving high resolution structures of protein complexes. From October 2018, the cryo-EM facility in KEK is open to academic and industrial users for scientific research. Our mission is twofold: to provide cryo-EM machine time for external users, and to assist users in acquiring cryo-EM skills. Until now, we have provided machine time for 36 academic and 15 industrial users. We also have held an initial training for cryo-grid preparation and EPU operation 12 times and a RELION workshop for beginners 3 times. In the last two years, our facility obtained 25 cryo-EM maps whose resolution is higher than 5 Angstroem. Here we show two representative results: single particle analyses of 110kDa enzyme at 2.85 angstrom resolution and 860kDa enzymes at 2.24 angstrom resolution. These results suggest that we established a proper protocol for cryo-grid preparation, cryo-EM data collection, and single particle analysis. We would like to keep supporting external users and carry out cryo-EM analysis of transcription-related complexes.

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