Reconstitution and Crystallization of Sir 3/4 in a Complex with Nucleosome

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Packaging of eukaryotic DNA into chromatin is critical for organizing the genome.1 Chromatin is composed of arrays of nucleosome, which consists of 146 base pairs of DNA wrapped around a histone octamer.2 Depending on the state of compaction, chromatin can be classified into loose euchromatin regions which are associated with active transcription and compact heterochromatin regions which are related with gene silencing. Chromatin compaction by several proteins, such as polycomb group proteins and Sir complex, block the access of transcription factors leading to gene silencing at chromatin level.3,4

In Saccharomyces cerevisiae (yeast), Sir complex consists of three subunits, Sir2p, Sir3p, and Sir4p, and regulates gene expression by forming heterochromatin-like structure at subtelomeric region, mating type loci HML and HMR, and the rDNA locus.5 According to the proposed mechanism of heterochromatin-like structure formation by the Sir complex, chromatin compaction is initiated by nucleating the target sequence through recruiting Sir2p and Sir4p, which then recruits Sir3p to induce silencing the chromatin. Sir3/4 complex directly interacts with the nucleosome and histone tails through the BAH domain of Sir3p in a manner sensitive to acetylation and methylation of H4K16 and H3K79, respectively. The AAA+ ATPase domain of Sir3p also interacts with the nucleosome and binds to the C-terminal coiled-coil domain of Sir4p. Sir3/4 complex is important to interact with nucleosome, leading chromatin compaction and heterochromatin-like structure formation.6,7 Although there have been numerous studies on the Sir complex, the molecular mechanism of the Sir3/4 complex regulating heterochromatin formation still remains elusive. Here, we have reconstructed a stable complex of Sir3/4 with the nucleosome and generated crystals for X-ray crystallographic analysis. Our data set a foundation to further investigate the structure of Sir complex, which will enlighten our understanding of details in the mechanism of heterochromatin formation by the Sir complex.

In order to elucidate the detailed mechanism of nucleosome recognition by the Sir complex in the process of regulating the heterochromatin formation, we first generated a complex encompassing the Sir3/4 complex and the nucleosome. We reconstituted nucleosome with recombinant Xenopus Histones and 146 bp α-satellites DNA in a high purity and further purified by prep cell (BIO-RAD, Berkeley, CA, USA) according to Luger’s protocol.8 The homogeneity was analyzed by 0.2X Tris/Borate/EDTA (TBE) 4% Native polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulfate (SDS) PAGE (Figure 1(a) and (b)). S. cerevisiae Sir3 protein (residue 1–845) with D205 mutation for tightly interacting with the nucleosome9 and Sir4 (residue 1242–1358) protein were co-expressed in BL21 RILP and purified through ion exchange (Hitrap SP-HP, GE healthcare, Milwaukee, WI, USA) and size exclusion chromatography (Superdex200 26/60, GE healthcare) in a buffer containing 300 mM NaCl and 50 mM Tris pH 8.0 (Figure 1(c)).

To examine the binding property of Sir3/4 complex with the nucleosome, we incubate nucleosome with Sir3/4 complex by increasing amount of Sir3/4, and the nucleosome-Sir3/4 complexes were visualized by 0.2X TBE 4% native PAGE (Lane 2 in Figure 1(d)). As increasing the amount of Sir3/4, nucleosome-Sir3/4 forms a stable complex containing several Sir3/4 complexes. Furthermore, upon adding more Sir3/4, the stable complex seems to form a multimeric supra complex (Lane 4 in Figure 1(d)). These data might reflect nucleosome compaction upon Sir3/4 binding.

We then decided to analyze the structure of nucleosome-Sir3/4 complex by X-ray crystallography. For this purpose, the stable nucleosome-Sir3/4 complex was further purified by size exclusion chromatography (Superose 6 10/300, GE healthcare) in a buffer containing 50 mM NaCl, 50 mM Tris pH 7.5. The purity and homogeneity was confirmed by 0.2X TBE 4% native PAGE and SDS-PAGE, which is shown in Figure 1(e) and (f). We then crystallized the purified nucleosome-Sir3/4 complex by vapor diffusion hanging drop method at 20 °C, in a solution containing hexane diol as a major precipitant (Figure 2(a)). To confirm that the crystal obtained contains nucleosome and Sir3/4 complex, we took and washed the crystals, and analyzed by SDS-PAGE (Figure 2(b)). Unexpectedly, we were not able to detect any band at 93 kDa which corresponds to Sir3.

Instead, two bands appeared at approximately 23 and 40 kDa raising a possibility that Sir3 is degraded during
crystallization. To verify the identities of these bands, we subjected these bands to mass spectrometric analysis (Liquid chromatography–mass spectrometry) showing that bands contain Sir3 protein (data not shown). We then performed X-ray diffraction experiment with these crystals at Pohang Accelerator Laboratory (PAL) to obtain diffraction data. However, unfortunately, we were not able to collect any data as the crystals did not diffract well.

In this study, we have successfully reconstituted and analyzed sir3/4 binding to nucleosome. In addition, we generated a stable nucleosome-Sir3/4 complex and produced crystals of nucleosome-Sir3/4 complex. This work set a platform for further structural analysis on nucleosome-Sir3/4 complex to enlighten our understanding of heterochromatin compaction through the Sir complex.

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References