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X-ray crystallographic study on the human nucleosome core particles.

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The packaging of DNA in the eukaryotic nucleus is achieved by a hierarchical scheme of folding and compaction into protein-DNA assemblies, collectively called chromatin. Two copies of each histone protein, H2A, H2B, H3, H4, are assembled into an octamer that has 145-147 base pairs of DNA wrapped around it to form a nucleosome core. Depending on the structural context, chromatin can both promote and impede transcription, replication, recombination and DNA repair. Thus, chromatin plays a central role in the regulation of these vital processes. Histone proteins are highly conserved among eukaryotic organisms. However, fundamental differences between the human genome and that of other higher organisms suggest that chromatin might be organized in a different manner in human. Therefore, we aim to determine the crystal structure of the human nucleosome core particle, and compare this structure with that of the *Xenopus laevis* or yeast nucleosome core particle, which has already been determined.

The human nucleosome core particles were prepared as described previously [Luger, K., *et al.* (1999) *Methods Enzymol.* **304,** 3-19]. The crystals were grown under a similar condition to the previous one [Luger, K., *et al.* (1997) *Nature* **389,** 251-260]. The X-ray diffraction was recorded on the CCD detedctor with the synchrotron radiation (wavelength: 1.0000Å) at the beamline BL41XU, SPring-8. The images were indexed and reduced with the program HKL, which provided the R_{merge} of 0.078 within 50-2.5 Å resolution range. The crystallographic data (space group: $P2_12_12_1$, unit cell: a=99.6, b=108.4, c=169.4) is similar to those for the known crystal structures. Thus, the human nucleosome core particle is likely to form a similar structure to those from the other eucaryotic sources. The further investigation is in progress.

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Structure determination of Atg3, an E2-like protein essential for autophagy

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Introduction

Autophagy is a dynamic membrane generation process for bulk protein degradation in the lysosome/vacuole. Cytoplasmic components of the cell are enclosed by double-membrane structures known as autophagosomes for delivery to lysosomes/vacuole. This process is crucial for survival during starvation and cell differentiation. So far, two ubiquitin-like systems, the Atg12 conjugation system and the Atg8 conjugation system, have been discovered in autophagy. The Atg8 conjugation system comprises of Atg3, Atg4, Atg7 and Atg8. The ubiquitin-like protein, Atg8 is cleaved at its C-terminal arginine by Atg4, and the exposed carboxyl-terminal glycine of Atg8 is activated by Atg7, an E1-like protein, in an ATP-dependent manner, and then is transferred to Atg3, an E2-like protein. Finally, Atg8 is conjugated to phosphatidyl-ethanolamine (PE). Generation of Atg8-PE conjugate is reported to be essential for autophagy although its function remains to be determined. In order to elucidate the molecular mechanism of Atg8-PE conjugation reactions, crystallographic analysis of Atg3 was performed.

Experiments

For data collection, crystals were mounted in nylon loops after soaking in a cryoprotectant solution and then flash-frozen in a nitrogen stream at 100 K. Diffraction data were collected at the BL41XU beamline in SPring-8 using wavelength 1.0000 Å. Diffraction intensity was measured using ADSC CCD detector in 1 sec exposure time. Diffraction data were collected using the oscillation method, and individual frames consisted of 1.0° oscillation steps over a range of 90°. All data sets were processed and scaled using HKL 2000 program package. The crystal of Atg3 belongs to tetragonal space group $P4_1$ or $P4_3$, with unit-cell parameters of a = 59.3, c = 115.2 Å, and diffracted up to 2.5 Å resolution. Phasing experiments are now in progress.