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# Purification, crystallization and preliminary X-ray diffraction analysis of yeast nucleosome assembly factor Cia1p

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## Introduction

The eukaryotic genomic DNA forms the nucleosome structure with histones, through which the DNA-mediated reactions such as gene expression are controlled. Although formation and disruption of nucleosome structure are important to control the function of the genome, the mechanism has been unclear. Since the structural modification of nucleosome is modulated by various factors, such as (1) nucleosome assembly factor, (2) nucleosome remodeling enzyme, and (3) histone modification enzyme, elucidation of the mechanism requires not only the conventional biochemical analysis, but also structural analysis of these factors.

CIA (yeast Cia1p and human CIA) possesses nucleosome assembly activity [1, 2]. CIA was identified as a human CCG1-interacting factor and it is highly conserved from yeast to human [1]. Previous studies have shown that the region of H3 responsible for CIA binding is thought to be (i) required for formation of $(H3/H4)_2$ tetramer, and (ii) the start and terminal points of interaction between histones and DNA in a nucleosome core particle. These results indicate that Cia1p plays a fundamental and central role in various chromatin-related reactions through alteration of nucleosome structure [1]. In fact, Cia1p participates in several reactions such as gene silencing [2], DNA replication, DNA repair, cell cycle and cell death [3]. Moreover, Cia1p regulates transcriptional activity in association with the general transcription initiation factor TFIID [4]. These findings indicate that elucidation of the mechanism for the action of Cia1p is necessary to establish the mechanisms involved in the chromatin structure and function.

Since the X-ray crystal structure of a nucleosome, which is the target of Cia1p, is known, determination of tertiary structure of Cia1p will provide further inference in molecular mechanism of nucleosome assembly. Hence, to better understand the mechanism of nucleosome assembly carried out by histone chaperone Cia1p, we have initiated to determine tertiary structure of Cia1p.

## Methods

The Cia1p was purified as described earlier [5]. Micro crystals were initially appeared within 2 - 3 days in a precipitant solution containing 0.1 M Tris-HCl (pH 7.5 at 293 K), 35% PEG 8000 and 0.2 M ammonium sulfate (Hampton Research). Systematic screening of buffer pH, PEG's (4000, 6000 and 8000) and different salts did not improve crystal size and its morphology. Hence, streak seeding method was employed to improve the growth of these microcrystals. Clusters of thin plate crystals (0.1 - 0.15 mm) were appeared in 7 - 10 days, with 30 % PEG 8000, 0.18 M ammonium sulfate and 0.1 M MES (pH6.5).

Diffraction data were collected from a single crystal on the beam line BL18B using the ADSC Quantum-4 CCD detector at the Photon Factory, Tsukuba, Japan. All data were processed and scaled using the programs DPS/MOSFLM and SCALA from the CCP4 package.

## Results

The Cia1p from *S.Cerevisiae* was crystallized. Crystals of better quality with dimension of approximately 0.15 x 0.1 x 0.03 mm were obtained by streak seeding method.

Crystals of yeast Cia1p were diffracted beyond to 2.95 Å resolution (Fig. 1). They belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a$ = 106.70, $b$ = 46.92, $c$ = 40.60 Å. A total of 17,216 measured reflections were merged into 4,606 unique reflections with an $R_{merge}$ of 8.5 %. The merged data set is 99.9 % complete to 2.95 Å resolution. A value for the Matthews coefficient of 2.54 $Å^3$ $Da^{-1}$ with solvent content of 51 % was obtained assuming one molecule in the asymmetric unit and a molecular weight of 19,125 Da. A search for heavy-atom derivatives for phase determination is in progress.

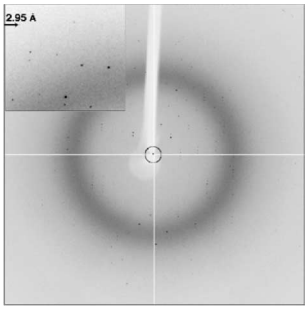


Fig. 1 An X-ray diffraction pattern of *S. cerevisiae* Cia1p crystal.

## References

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