Structure of the Light-Harvesting Bacteriochlorophyll *c* Assembly in Chlorosomes from *Chlorobium limicola* Determined by Solid-State NMR

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We have determined the atomic structure of the bacteriochlorophyll *c* (BChl *c*) assembly in a huge light-harvesting antenna, the chlorosome of green photosynthetic bacteria, for the first time by solid-state nuclear magnetic resonance (NMR). Previous electron microscopic and spectroscopic studies indicated that chlorosomes have a cylindrical architecture with a diameter of about 10 nm consisting of layered BChl molecules. Assembly structures in the huge noncrystalline chlorosomes have been proposed mainly from structure-dependent chemical shifts and a few distances acquired by solid-state NMR, but these studies did not provide a definite structure. Our approach is based on the ^{13}C dipolar spin-diffusion solid-state NMR of uniformly ^{13}C-labeled chlorosomes under magic-angle spinning (Fig. 1). About 90 intermolecular C–C distances were obtained by the simultaneous assignment of distance correlations and structure optimization preceded by the polarization-transfer matrix analysis. These distances revealed that BChl *c* molecules form piggyback-dimer based parallel layers in the antenna (Fig. 2). This result rules out the well-known monomer-based structures. We have built a molecular model of the cylinder in the chlorosomes with the parallel layers in reference to the electron micrograms (Fig. 3A and B). This antenna structure provided insights into the mechanisms of efficient light harvesting and excitation transfer to the reaction centers (Fig. 3C). This work constitutes an important advance in the structure determination of huge intact systems that cannot be crystallized.

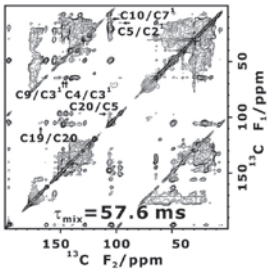

Fig. 1. 2D proton-driven spin-diffusion ^{13}C–^{13}C dipolar correlation NMR spectra of uniformly ^{13}C-labeled chlorosomes under magic-angle sample spinning at 12 kHz.

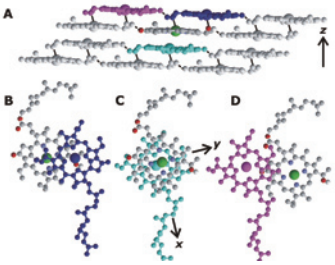

Fig. 2. The structure of the BChl *c* assembly determined under ^{13}C–^{13}C distance constraints. (A) A side view of the structure of the BChl *c* assembly. Solid and broken lines represent coordination and hydrogen bonds, respectively. Oxygen atoms are colored red. (B) A top view of the piggyback dimer. (C) A top view of the inter-dimer full stacking at the center of E. (D) A top view of the inter-column contact between the neighboring dimers.

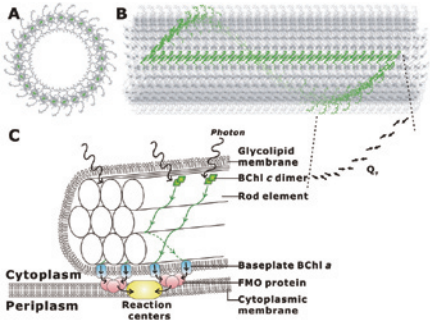

Fig. 3. A rod element structure built with the parallel dimer layers shown in Fig. 2. (A) A top view. Magnesium atoms are colored dark green. Columns perpendicular to the page are arranged on the circumference. (B) A side view. A single spiral layer and a single column are colored green. The arrows represent the Q_y transition dipole moments of BChls. (C) Schematic representation of the excitation energy transfer in a chlorosome. The green lines with arrows indicate the paths of the excitation transfer along the spiral layers shown in B to the baseplate.

Genome-wide Identification of Replication Origins in Fission Yeast

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DNA replication is a fundamental biological phenomenon, by which genetic information of living organisms is propagated to their daughters. DNA replication of eukaryotic chromosomes initiates at a number of discrete loci, called replication origins. Distribution and regulation of origins are important for complete duplication of the chromosomes. However, precise genome-wide localization of origins has not been determined in most eukaryotes including fission yeast, *Schizosaccharomyces pombe*.

The process of initiation of replication is composed of two major steps, licensing of replication origins in G1 phase and activation of the origins in the following S phase. The licensing is done by forming pre-RC (pre-Replicative Complex) composed of ORC and MCM on each origin. Subsequently, some of the pre-RC are activated and replication initiates. Thus, we mapped ORC and MCM localization sites in G1 phase as well as nascent replicated DNA in S phase by using high-resolution DNA micro-array covering almost the entire genome of fission yeast (Fig. 1). By comparing these genome-wide information, we have successfully identified 460 pre-RC sites, some of which have initiation activity in the early S phase (called early origins) while the others do not (called late origins) (Fig. 2). Early and late origins tend to distribute separately in large chromosome regions, implying that activation of pre-RCs may be regulated in large chromosome regions.

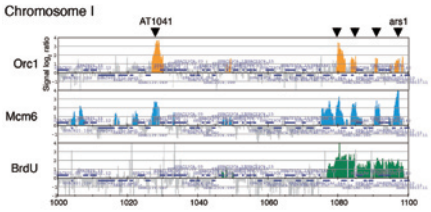

Fig. 1. A part of the micro-array results, showing localization of ORC (Orc1) and MCM (Mcm6) in G1 phase as well as newly replicated DNA (BrdU) in S phase.

As replication is likely to be coupled with various chromatin functions such as sister chromatid cohesion, condensation, DNA repair, checkpoint and chromatin structures, the data on replication machinery assembly sites and its activity will help to identify locations and movement of relevant proteins.

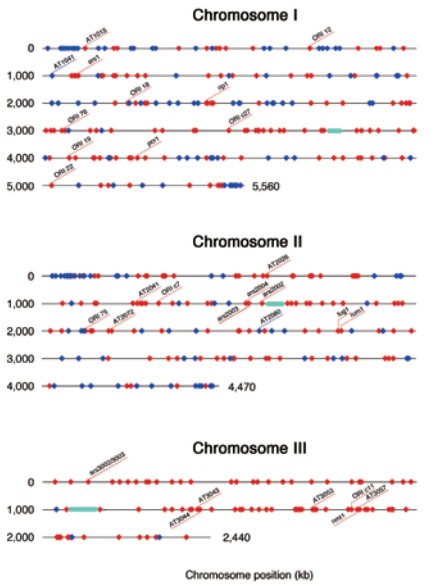

Fig. 2. A genome-wide map of early origins (red diamonds) and late origins (blue diamonds) on fission yeast chromosomes I, II and III.