2P-272 蛍光タンパク質を用いた核膜透過性の検討

Diffusion of Large Molecules into Assembling Nuclei Revealed Using an Optical Highlighting Technique

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The nuclear envelope (NE) defines the nuclear compartment, and nuclear pore complexes (NPCs) on the NE form aqueous passages through which small water-soluble molecules can passively diffuse. It is well known that proteins smaller than 50 kDa can diffuse through NPCs, whereas proteins larger than 60 kDa rarely enter by passive diffusion. Little, however, is known about how this size cut-off develops as the NE reassembles and the nucleus expands. In 1987, a well-known study by Swanson and McNeil identified an efficient mechanism by which large diffusing proteins (>60 kDa) were excluded from the reassembling nucleus after mitosis. Since then, it has been generally accepted that after mitosis, newly formed nuclei completely exclude all proteins except those initially bound to the mitotic chromosomes and those that are selectively imported through NPCs. Here, the tetrameric complex of the photoconvertible fluorescent protein KikGR (~103 kDa) was optically highlighted in the cytoplasm and followed to examine its entry into nuclei. Remarkably, highlighted complexes efficiently entered newly assembled nuclei during an approximately 20-min period after the completion of cytokinesis. Because KikGR contains no known nuclear-localization or chromosome-binding sequences, our results indicate the diffusion barrier is less restrictive during nuclear reassembly.

2P-273 テトラアンミン錯体とアポフェリチンを用いた新規化合物半導体ナノ粒子の作製

Synthesis of new semiconductor nano-particles by a tetraammine complex and cage-shaped protein, apoferritin

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Nano-particle (NP) is one of the most important elements in the Nano-technology. A lot of methods to produce NPs have been studied. We fabricated many kinds of nano-particles in the spherical bio-template, apoferritin from horse spleen (HsAFr). HsAFr, the cellular iron-storage proteins, is a spherical hollow shell composed of 24 polypeptide subunits. The inner and outer diameters of the apoferritin are about 7 nm and 12 nm respectively. Many kinds of metal oxide NPs such as Fe, Co, Ni, In, Zn were efficiently fabricated in the bio-templates with small size dispersion. From the point of view of nano-electronic device applications, a small and uniform quantum dots are more preferable. Recently, we designed a slow chemical reaction system (SCRY) and a two-step synthesis protocol (TSSP) to synthesize compound semiconductor NPs in the cavity of apoferritins. Uniform semiconductor NPs (CdSe, ZnSe, CdS, ZnS, Au₂S) were successfully fabricated in the apoferritin by the combination of the SCRY and TSSP. In this contribution, we report a successful synthesis of new semiconductor NPs in HsAFr. An optimization of CuS NPs synthesis was performed and the results suggested that the concentration of sulfide ion in the reaction solution is important to the synthesis of CuS NPs. The optimized condition efficiently produced CuS NPs with 5 nm at a diameter. The obtained CuS NP was analyzed by XRD and EDS, resulting that the NP showed a hexagonal CuS crystal. The plausible biomineralization mechanism of CuS NPs and various semiconductor NPs in the SCRY are also discussed. This work was partially supported by KAKENHI (20710089).

2P-274 細胞観察用 tip-scan 型高速原子間力顕微鏡の開発

Development of tip-scan type of high-speed AFM for cell imaging

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In the past decade, the performance of the atomic force microscope (AFM) has been improved significantly in the imaging rate and low invasiveness. As a result, it is now possible to capture moving biomolecules on video at 30-60 ms/frame for a scan range of ~250 nm, without disturbing their physiological functions. We can directly visualize dynamic structural changes of proteins and their dynamic interactions with other molecules.

However, at present, the high-speed AFM cannot be applied to the observation of dynamic molecular events occurring on live cell membranes. This infeasibility arises from that the scan area is limited to 2-4 μm^2 , a small sample stage can only be scanned quickly, and live cell membranes are soft compared with a cantilever. To overcome the difficulties, here we completely change the design of our high-speed AFM. (1) A high-speed AFM is combined with an optical microscope; (2) a cantilever tip is scanned instead of scanning a sample stage. By the first change, cells to be observed would be found quickly, and by the second a large sample stage could be used. However, for the tip-scan a laser beam of an optical detector for cantilever deflection has to track the small-cantilever movement in the x- and y-directions. We employed a method of scanning a small

focusing lens synchronously with the tip-scan and succeeded in the tracking. With the progress of other devices in the new design, high-speed AFM for cell imaging will be materialized.

2P-275 自己集合核酸からなる拡張可能なナノトラuss構造体：そのデザインと階層的な構築

Expandable nano-truss-structures made of self-assembled nucleic acids: Their designing and hierarchical construction

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We are aiming at the establishment of a novel design system for self-assembly of double-stranded DNA-DNA- or DNA-RNA-framed nanostructures, which can be hierarchically assembled. We present software to generate sequences for hierarchical building of three-dimensional nano-truss-structures, which are oligomers of octahedron units. The software T-GUSP, written in C, outputs a set of strands, which can stably pair only between intended complementary segments. We incorporated dynamic programming algorithm to avoid formation of unintended structures by exhaustively evaluating the free energy of every pairs of involved strands. The structures were designed so that many short DNA strands bind at their proper positions on a long single-stranded DNA/RNA, which runs through all edges of the oligomer of octahedrons. The designed DNAs/RNAs were synthesized, and assembled in one-pot reaction, simply by mixing and keeping at an appropriate temperature. Successful constructions were confirmed by agarose gel electrophoresis and thermal melting profiles. They will be further confirmed by AFM/TEM imagings.

2P-276 相同組換えタンパク質 RecA を用いた新規オプティカルマッピング法の開発

Development of novel DNA optical mapping method using homologous recombination protein, RecA

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see 2TA5-06

2P-277 異種ビーズを隣接させた状態で観察可能なダイナミックマイクロアレイ

Dynamic Microarray Technology for observation of adjacent different beads

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see 2TA5-08

2P-278 bistable な DNA で実装する温度バンドパスフィルタ

Thermal band pass filter implemented with a bistable DNA

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see 2TA5-10

3P-001 PCNA G178S 変異体の構造解析

Structural analysis of PCNA with G178S mutation

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The G178S mutation in yeast PCNA was found in a screen for mutants deficient for UV-induced reversion. The mutation abolished translesion synthesis (TLS), which is one strategy to avoid replication arrest at damaged template. PCNA forms a ring-shaped homotrimer encircling DNA helix, which slides freely along the DNA axis and functions as the processivity factor of DNA polymerase. PCNA interacts with lesion-specific DNA polymerases (TLS polymerases) as well and stimulates their activity. We previously showed that the G178S mutation in human PCNA reduced stability of the trimer structure, suggesting that instability of the trimer-ring could abolish interaction with DNA and recruitment of TLS