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(別紙様式 2)
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論文の要旨
Summary (Abstract) of doctoral thesis contents

Our body consists of about 40 trillion cells, each of which contains 2 m of genomic DNA in a nucleus with a diameter of ~10 μm. The long strands of DNA are wrapped around core histone proteins to form nucleosomes and three-dimensionally organized in the cells as chromatin. The chromatin DNA must be read out (RNA transcription) for various cellular functions, and also be copied (DNA replication) for the next cell division while maintaining its integrity (DNA repair). These “DNA transaction” reactions are essential for cell viability. In this thesis, I performed two projects to investigate the interplay between the DNA transaction and chromatin environment using interdisciplinary approaches such as chemical biology, cell biology, biophysics, and computational biology.

At first I report interplay between DNA transaction and chromatin condensation induced by a platinum-based chemical reagent 5-H-Y, which was synthesized by Dr. Seiji Komeda. Platinum-based drugs, such as cisplatin, have been used extensively in cancer chemotherapy. It is well known that the drug–DNA interaction causes covalent DNA crosslinks and subsequent cytotoxicity. I showed that 5-H-Y has a great inhibition ability on human cell growth by arresting the cells in the S/G2 phase, and that 5-H-Y is effective against cisplatin-resistant cancer cells. To understand the cytotoxic mechanism of 5-H-Y, I took an interdisciplinary approach, and revealed that the cytotoxicity is caused by suppression of DNA replication and RNA transcription. Interestingly the cytotoxic mechanism of 5-H-Y is distinct from that of cisplatin: although cisplatin inhibits cell growth by inducing covalent crosslinks in DNA strands, 5-H-Y has a unique ability to tightly bind to DNA and induce chromatin condensation, instead of crosslinking DNA. Since DNA replication and RNA transcription might occur at opened chromatin at the surface or outside of compact chromatin domains, I propose that 5-H-Y can inhibit the opening of chromatin by condensation and subsequent initiation processes of the DNA transactions in the treated cells. While my study on 5-H-Y will contribute to expanding its clinical applications for cisplatin-insensitive cancers, my study implies that higher order chromatin organization itself is important to proper regulation of DNA transaction reactions for cell survival.

Next, I investigated the chromatin environment in living cells, especially condensed chromatin regions, and its relation to protein accessibility. It is known that chromatin can be roughly categorized into two types, euchromatin and heterochromatin, which correspond to sparse and dense chromatin regions, respectively. Less condensed euchromatin usually contains gene-rich, transcriptionally active regions. In contrast, the dense heterochromatin is gene-poor or transcriptionally silenced. DNA regions located in heterochromatin are generally replicated later in S-phase. Recently various heterochromatin features including heterochromatin-specific proteins and histone modifications were revealed. It seemed to me that the heterochromatin provides a unique opportunity to understand how DNA transaction reactions are regulated in

terms of chromatin condensation and its accessibility to proteins.

To look into the interplay between chromatin condensation and protein accessibility, I investigated euchromatin and heterochromatin environments in living cells, from the viewpoint of density, which can be important for chromatin accessibility of various proteins. For this purpose, I used orientation-independent differential interference contrast (OI-DIC) microscopy, which was developed by Dr. Michael Shribak and is capable of mapping optical path differences, to quantify the density of the total materials of samples. I examined condensed pericentric chromatin in live mouse NIH3T3 cells, a representative heterochromatin model. Although I expected that heterochromatin would be very dense, I found that the total density of heterochromatin (208 mg/ml) was only 1.53-fold higher than that of the surrounding euchromatic regions (136 mg/ml) while the DNA density of heterochromatin was 5.5- to 7.5-fold higher. A similar minor difference was obtained in another classical heterochromatin model, the inactive human X chromosomes of RPE1 cells, which one of the copies of the X chromosome is transcriptionally silenced and condensed. This surprisingly small difference may be due to that non-nucleosomal materials (proteins/RNAs) (~120 mg/ml) are dominant in both chromatin regions. Monte Carlo simulation suggested that non-nucleosomal materials contribute to creating a moderate access barrier to heterochromatin, allowing minimal protein access to regions of DNA transactions.

Finally, from my studies I emphasize the importance of understanding the physical chromatin environments in live cells, which both facilitate and constrain the diffusion of protein factors and their complexes and govern DNA transaction reactions.

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論文の要旨
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Chloroplasts in algae and plants were established by endosymbiotic events in which a cyanobacterium or unicellular eukaryotic alga was integrated into previously non-photosynthetic eukaryotes. It is believed that chloroplasts were established through multiple independent occurrences of predation, temporary retention, or permanent retention of a photosynthetic prey/endosymbiont by eukaryotic host cells. Photosynthesis in the chloroplast converts light to chemical energy and supports the life of algae and plants by providing photosynthetic products. However, photosystems also generate reactive oxygen species (ROS) which damage the cell. Thus, algae and plants have developed various mechanisms to reduce ROS generation, quench ROS, and repair biomolecules damaged by the oxidative stress, which are prerequisites for eukaryotic cells to perform photosynthesis. Although it has not been verified experimentally, when unicellular transparent organisms feed on phototrophs in the daytime, light reaches the photosystems of the engulfed prey. In addition, unregulated photosynthetic electron flow and excitation of chlorophyll molecules detached from photosystems probably occur during digestion, which in turn produce higher levels of ROS inside the predator cells. On the basis of this assumption, I attempted to understand whether feeding on phototrophs under illumination exposes unicellular predators to oxidative stress, and how the predators cope with the stress if they are exposed to oxidative stress. These studies will yield important insights that would help in gaining a better understanding of the evolutionary course in the establishment of photosynthetic eukaryotes as well as the impacts of photosynthesis in microbial communities in ecosystems.

I established a co-cultivation system of herbivorous predators and photosynthetic or non-photosynthetic bacterial prey to examine the effects of photosynthetic traits of prey on predators. I isolated three species of predatory amoebae (*Naegleria* sp., *Acanthamoeba* sp., and *Vannella* sp.) that fed on both photosynthetic and non-photosynthetic bacterial prey from a sunny, shallow marsh. I chose the cyanobacterium *Synechococcus elongatus* as the prey. *S. elongatus* produced ample photosynthetic pigments (green prey) under normal conditions and had decreased photosynthetic pigments (pale prey) when reared under nitrogen-depleted conditions.

When the *Naegleria* sp. was illuminated (500 μE m^{-2} s^{-1}) when feeding on the green prey, about 30% of the amoeba cells burst but not the pale prey. Transcriptome analyses showed that genes related to oxidative stress responses, DNA repair, and carotenoid synthesis were upregulated upon illumination (200 μE m^{-2} s^{-1}) in all three amoeba species feeding on the green prey. Furthermore, most of the changes that occurred in the transcriptome upon illumination also occurred when the three amoeba species were treated with exogenous ROS. These results suggest that feeding on photosynthetic prey under illumination exposes the unicellular predators to photosynthetic oxidative stress.

The transcriptome analyses also indicated that genes related to phagocytosis, including actin and myosin, were downregulated upon illumination in the three amoeba species feeding on

the green prey. Consistent with this result, a reduction of phagocytic activity upon illumination was observed in *Naegleria* sp. feeding on the green prey but not the pale prey. In contrast, digestion of already engulfed prey was accelerated upon illumination in *Naegleria* sp. feeding on the green prey. Both of these responses resulted in a reduction in the amount of photosynthetic prey in the amoeba cells, which may have caused a reduction of photosynthetic oxidative stress under light conditions.

In addition to these responses, several other changes, such as upregulation of genes that are related to respiration, genes encoding several monooxygenases, and genes encoding components of v-ATPase, were observed in the transcriptome upon illumination in all the three amoeba species feeding on the green prey. These changes in transcriptome presumably resulted in the reduction of oxygen that is generated by photosystems of prey and a consequent reduction of ROS generation in amoeba cells. Acidification of phagosomes by v-ATPase is likely related to the acceleration of digestion of the green prey upon illumination. All the above mentioned changes in mRNA levels were shared by the three amoeba species which are distantly related to one another in terms of evolution, suggesting that these responses are probably prerequisites for unicellular amoebae to feed on phototrophs.

With regard to the low uptake and rapid digestion of the green prey by amoebae upon illumination, digestion/expulsion of facultative algal endosymbionts has been observed in other eukaryotes upon elevation of oxidative stress. Thus, reducing the number of phototrophs in the cells is probably a common strategy to reduce oxidative stress in eukaryotes accommodating/feeding on phototrophs. In contrast, it is known that algae and sessile land plants, which permanently possess chloroplasts, escape from high light and relocate their chloroplasts in the cells, respectively, to minimize light absorption under high light conditions. Such changes could possibly be prerequisites for eukaryotes to permanently possess chloroplasts.