氏　　名	宇塚 明洋
学位(専攻分野)	博士(理学)
学 位 記 番 号	総研大甲第 2006 号
学位授与の日付	平成 30 年 3 月 23 日
学位授与の要件	生命科学研究科 遺伝学専攻 学位規則第6条第1項該当
学 位 論 文 題 目	Responses of herbivorous unicellular organisms to photosynthetic oxidative stress
論 文 審 査 委 員	主　査　教授 角谷 徹仁 教授 岩里 琢治 教授 平田 たつみ 教授 中村 保一 教授 柏山 祐一郎 福井工業大学 環境情報学部

(別紙様式 2)
(Separate Form 2)

論文の要旨
Summary (Abstract) of doctoral thesis contents

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.

(別紙様式 3)
(Separate Form 3)

博士論文審査結果の要旨

Summary of the results of the doctoral thesis screening

葉緑体は、細胞内共生をするシアノバクテリアや真核藻類から進化したと考えられるが、その成立過程では、宿主細胞が藻類を捕食する段階や未消化の藻類やその葉緑体を宿主細胞内に維持する段階などが想定される。一方、捕食された藻類による光合成では様々な活性酸素種（ROS）が生成されるため、宿主はそれらに対処する機構を獲得する必要があったと考えられる。

宇塚君は、葉緑体の共生現象の進化を理解するために、光合成生物を捕食する単細胞真核生物の活性酸素種ストレスに対処する機構の研究を行った。まず、野外で採取したサンプルから、シアノバクテリアを捕食して増殖しうるアメーバを３種類系統化し、研究材料とした。シアノバクテリアを餌として暗条件で培養したアメーバは、強光条件に移すと個体数が減少した。次に、シアノバクテリアを捕食した個体を光照射することにより特異的に発現変化する遺伝子をRNA-seqによって探索した。さらに、コントロールとして、窒素飢餓によって光合成能を低下させたシアノバクテリア、あるいは、クロロフィルを細胞表面に付着させた大腸菌や付着させていない大腸菌を餌として用いることで、クロロフィルを含む餌を捕食した場合に特異的な応答を探索した。その結果、酸化還元酵素、DNA修復酵素、呼吸関連の遺伝子群、液胞酸性化関連の遺伝子群などの発現上昇とともに、アクトミオシン系など、運動関連の遺伝子群の発現低下がみられた。これらの遺伝子発現変化の多くが、進化的に離れた３種類のアメーバで共通して観察されたことから、これらの応答はこれらの生物群に普遍的なものと示唆された。

これらの遺伝子の多くが、H2O2や一重項酸素（光増感剤Rose bengalを作用）でアメーバを処理した場合にも同様の発現変化を示したことから、これらの応答は活性酸素種に仲介されていると推察された。また、シアノバクテリアを捕食しているアメーバに光照射すると、捕食速度を減少させるとともに、細胞内に取り込んだ餌の消化を加速させた。これらの応答は上記の発現変化で説明でき、光条件下における活性酸素生成に対処するための応答と考えられる。

これらの研究は、環境中における光合成藻類とその捕食者の相互作用を光合成由来の活性酸素種という新しい切り口で説明を試みたものであり，さらには，細胞内共生や盗葉緑体現象，あるいは葉緑体獲得進化の過程における宿主の応答について新たな知見をもたらすものである。宇塚君は、目的にあった実験系統を確立すると共に、良く考えたアッセイ系を設定し、これらの重要な結果を得た。審査員全員で審査した結果、本大学院における学位授与の水準を十分に満たす論文であると判断した。