Chloroplasts in the animal organism: examples and possible applications

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Abstract. Chloroplasts are cellular organelles specific to plants, containing pigments, including chlorophyll, necessary for photosynthesis. Our short paper is a discussion of cases of chloroplast-animal cell endosymbiosis, as well as the potential applications of chloroplast transfer to animals, something that has not yet been accomplished by the research groups involved in this challenge.
Key words: autotrophic, chloroplasts, *Euglena,* gastropod, photosynthesis, practical applications.

Introduction. Man, in his creative endeavors, has always taken models and ideas from nature (Han et al 2021; Yin et al 2022). The existence of working examples in nature certifies functionality. The present paper presents the utility of an innovative idea in the context of the existence of similar functional models in nature.

What could *Euglena* tell us? There is an organism in the living world that lives a life both heterotrophic and autotrophic, doing photosynthesis (Yao et al 2022; Inwongwan et al 2023). This well-known organism is *Euglena*. In fact, it is a genus, which includes several species (Kim et al 1998). These species suggest that autotrophic and heterotrophic organisms descended from common ancestors in the distant past, and with luck, heterotrophy and autotrophy may still be compatible in some eukaryotes.

It seems that they are! A group of researchers studied marine gastropod molluscs of the species *Tridachia crispata, Placobranchus ianthobapsus* and *Tridachiella diomedea*. They showed that these molluscs possess free and functional chloroplasts within the cells of the digestive epitelium, as shown in some ultrastructural observations, in the results of pigment analyses, and in the results of experiments investigating photosynthetic function (Trench et al 1969) (see more species in Figure 1). In the light, the chloroplasts incorporate $H^{14}CO_3^-$ in situ (Trench et al 1969). According to this study, reduced radiocarbon is translocated to various animal tissues that do not possess chloroplasts. Slug species feed on various siphonaceous algae from which chloroplasts are derived (Trench et al 1969). In this research, pigments collected from the slug species and from known siphonaceous algae, when separated chromatographically and compared, showed similar components (Trench et al 1969). Absorption spectra of extracts of slugs and algae were very similar. The larvae of the slugs are pigment-free up to the post-veliger stage. This indicates that chloroplasts are acquired de novo with each new generation (Trench et al 1969).

Figure 1. Sacoglossan species illustrating habitus typical of major plakobranchacean clades (geographic location and approximate length - in parentheses). (A) *Cyerce nigricans* on *Chlorodesmis fastigiata* (Lizard Island, Great Barrier Reef; 4 cm), (B) *Polybranchia orientalis* (Lizard Island, Great Barrier Reef; 2 cm), (C) *E. ornata* (Lizard Island, Great Barrier Reef; 2 cm), (D) *E. crispata* on sediment (Dominican Republic; 4 cm), (E) *Plakobranchus ocellatus* (Lizard Island, Great Barrier Reef; 4 cm), (F) *E. pusilla* (Maldives; 1 cm), (G) *E. tomentosa* (Lizard Island, Great Barrier Reef; 1.5 cm) (H) *Thuridilla carlsoni* (Lizard Island, Great Barrier Reef; 2 cm). (I) *E. viridis* (Mediterranean Sea, mollusc placed on brown algae; 1,5 cm). (J) *T. hopei* (Mediterranean Sea, 2 cm) (the figure in its entirety is taken from Händeler et al 2009).

Another interesting case is that of *Codium fragile* chloroplasts in *Elysia viridis* (Trench et al 1973a,b; Gallop et al 1980). When *C. fragile* chloroplasts in cells of *E. viridis* were examined by electron microscopy, no structural damage or alteration was observed beyond a general change in shape from ellipsoid in the plant to more oval in the animal; outer chloroplast envelopes were always intact (Trench et al 1973a,b; Gallop et al 1980). Some chloroplasts in animal cells were enclosed by a membrane external to the envelope but others were not; the origin of this membrane is unclear (Trench et al 1973a,b).

“Neither the mechanism of entry nor the ultimate fate of chloroplasts in animal cells is fully understood”, stated Trench et al (1973b). Rates of photosynthetic carbon fixation by chloroplasts in *Elysia* and *Codium* were of a similar order (Trench et al 1973b). However, chloroplasts in *Elysia* are unlikely to be completely autonomous, concluded the authors (Trench et al 1973a,b).

Endosymbiotic chloroplasts within the cells of the sacoglossan slug species *E. chlorotica* synthesize a series of proteins including the large subunit of RuBisCO and the photosystem II protein D1 (Pierce et al 1996). Moreover, the effects of protein synthesis inhibitors indicate the fact that some chloroplast-associated proteins are synthesized in the animal cytosol and subsequently translocated into the chloroplasts (Pierce et al 1996). So, the plastids not only synthesize proteins during this long-lived association, but the host cell seems to play a role in plastid protein turnover, concluded Pierce et al (1996).

Later, the researchers defined this particular case of symbiosis "kleptoplasty" (Händeler et al 2009). The phenomenon is important for understanding the evolution of endosymbiosis (Greene & Muscatine 1972). Kleptoplasty is the intracellular maintenance of plastids in the slug's digestive epithelium (Händeler et al 2009). However, photosynthetic ability varies widely among sacoglossans; some species have no plastid retention while others survive for months solely on photosynthesis, observed Händeler et al (2009).

Practical applications of animals with chloroplasts. There is no publicly available information indicating that researchers have successfully transferred chloroplasts from plants to animals in an efficient manner. While there have been experiments attempting to introduce chloroplasts into animal cells, they have not led to authentic or sustainable functionality in terms of photosynthesis (Rumpho et al 2011). Transferring chloroplasts to animals, if it were successful, could potentially have several practical applications.

Photosynthetic capacity in heterotrophs. Most animals are heterotrophic, meaning they cannot produce their own food through photosynthesis. If they were equipped with functional chloroplasts, they could potentially generate their own energy from sunlight, reducing their dependence on external food sources.

Reduced environmental impact. Animals with chloroplasts might require less traditional food, potentially reducing the environmental impact associated with animal agriculture. This could lead to a more sustainable and efficient food production system.

Survival in low-nutrient environments. Animals with chloroplasts might be better equipped to survive in environments where traditional food sources are scarce. They could potentially use sunlight and carbon dioxide to sustain themselves in areas where plant-based food is limited.

Bioenergy production. Animals with functional chloroplasts could potentially serve as living bioenergy sources. They might be used to generate biomass or other biofuels through photosynthesis.

Medical applications. Chloroplasts contain various compounds that have potential medical applications, such as antioxidants and pigments. If incorporated into animals, these compounds might be produced internally and used for therapeutic purposes.

Bioremediation. Animals with chloroplasts might be engineered to help clean up polluted environments. They could potentially use photosynthesis to remove pollutants from the air or water.

Scientific research. Animals with incorporated chloroplasts could be valuable for scientific research, helping to understand fundamental processes in biology and ecology.

Ethical impediments. Successful transfer of chloroplasts to animals poses significant technical challenges and ethical considerations. Any such experiments would need to be conducted with careful consideration for the welfare of the animals involved and the potential ecological implications.

Conclusions. The solution to chloroplast transfer in some animals is far from being found. However, we have many models of chloroplast endosymbiosis inside animal cells of some species of slugs, which suggests that the compatibility of autotrophy with heterotrophy in animals is not out of the question and may someday be demonstrated experimentally.

Conflict of interest. The authors declare that there is no conflict of interest.

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