

Photosynthesis and the role of plastids (kleptoplastids) in Sacoglossa (Heterobranchia, Gastropoda): a short review

Fotosintesis dan peran plastida (kleptoplastids) pada Sacoglossa (Heterobranchia, Gastropoda): tinjauan singkat

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Abstract: In this manuscript I will give a short summary of our knowledge on photosynthesis in the enigmatic gastropod group Sacoglossa. Members of this group are able to sequester chloroplasts from their food algae (mainly Chlorophyta) and store them for weeks and months and it was assumed for a long time that they can use chloroplasts in a similar way as plants do. Only few sacoglossan species are able to perform photosynthesis for months, others are less effective or are not able at all. The processes involved are investigated now for a few years, but are still not clear. However we know now that many factors contribute to this enigmatic biological system. These include extrinsic (environment, origin and properties of the nutrition and the plastids) and intrinsic factors of slugs and algae (behaviour, physiological and anatomical properties). Plastids are not maintained by genes that might have originated by a horizontal gene transfer (HGT) from the algal genome into the slug genome, as was hypothesized for many years. We therefore have to focus our research now on other factors to understand what actually contributes to this unique metazoan phenomenon which is not yet understood. In this review, some of these new approaches are summarized.

Keywords: Mollusca; Heterobranchia; Sacoglossa; photosynthetic activity; kleptoplasty

Abstrak: Dalam tulisan ini saya akan memberikan ringkasan singkat tentang fotosintesis pada gastropoda kelompok misterius Sacoglossa. Organisme anggota dari kelompok ini mampu menyerap kloroplas dari alga makanan mereka (terutama Chlorophyta) dan menyimpannya selama berminggu-minggu, bahkan berbulan-bulan, sehingga telah diasumsikan bahwa mereka dapat menggunakan kloroplas dengan cara yang sama seperti tanaman. Hanya sedikit spesies sacoglossan dapat melakukan fotosintesis selama berbulan-bulan, yang lain kurang efektif atau tidak mampu sama sekali. Proses yang terlibat diselidiki sekarang selama beberapa tahun, namun masih belum jelas. Namun kita tahu sekarang bahwa banyak faktor yang berkontribusi terhadap sistem biologis misterius ini. Ini termasuk ekstrinsik (lingkungan, asal dan sifat gizi dan plastida) dan faktor intrinsik siput dan ganggang (perilaku, fisiologis dan sifat anatomis). Plastida tidak dikelola oleh gen yang mungkin berasal oleh transfer gen horizontal (HGT) dari genom alga ke dalam genom slug, seperti yang dihipotesiskan selama bertahun-tahun. Oleh karena itu kita harus fokus penelitian kami sekarang pada faktor-faktor lain untuk memahami apa yang sebenarnya memberikan kontribusi terhadap fenomena ini metazoan unik yang belum dipahami. Dalam ulasan ini, beberapa pendekatan baru dirangkum.

Kata-kata kunci: Moluska; Heterobranchia; Sacoglossa; aktivitas fotosintetik; kleptoplasty

INTRODUCTION

Opisthobranchia, nowadays not considered as monophyletic anymore, are known for their enigmatic biological features usually associated with the loss of a protective shell. This loss had to be compensated by other survival strategies, like becoming cryptic, or using chemical compounds from their food, producing spiny spicules or even incorporating cnidocysts from their cnidarian prey in order to use these so-called cleptocnides against

putative predators (see review Wägele and Klussmann Kolb, 2005). On the other hand loss of the shell allowed various life styles including the incorporation of photosynthetic organisms that might provide photosynthates, as this is known and demonstrated for many reef organisms (e.g., Venn *et al.*, 2008; Whitehead and Douglas, 2014). Incorporation of *Symbiodinium* and its mutualistic symbiotic relationship with sea slugs have been investigated in the last few decades (e.g., Rudman, 1991; Burghardt *et al.*, 2008a, b; Burghardt and

Wägele, 2014; Wägele *et al.*, 2010a; Ziegler *et al.*, 2014), but biology is not very well studied compared to the extensive studies on *Symbiodinium* in association with Anthozoa. A unique system is described for the sea slug group Sacoglossa (Heterobranchia, Gastropoda) which usually feed on siphonaceous green algae (Chlorophyta) (e.g., Jensen, 1980, 1981, 1997; Händeler and Wägele, 2007). Members of this taxon are able to maintain plastids from the consumed algae in an active photosynthetic state, leading to the general opinion that slugs grow on CO₂ and light (e.g., Rumpho *et al.*, 2000, 2001, 2006; Wägele *et al.*, 2010a). This unique feature has attracted many scientists and several reviews with various emphases are now available (Rumpho *et al.*, 2011; Garrote-Moreno, 2011; Wägele and Martin, 2013; Cruz *et al.*, 2013). The slugs pierce the algal cell wall with one (the leading) tooth of the radula, digest most parts of the algae in the digestive gland, but incorporate the plastids into these digestive gland cells. Hence the plastids in the slugs are called kleptoplasts. They usually render the slugs bright green. It was shown by starvation experiments and measuring in vivo photosynthetic activity via a Pulse Amplitude Modulated Fluorometer that these kleptoplasts can remain photosynthetically active for several months in some species (e.g., Händeler *et al.*, 2009; Evertsen *et al.*, 2007). This has led to the opinion, that only a horizontal gene transfer from the algal nucleus genome into the slugs' nucleus genome can explain the long survival of the plastids in the slugs' cells (e.g., Pierce *et al.*, 1996, 2007; Rumpho *et al.*, 2000, 2008). This has to be seen under the aspect that usually plastids do not survive a long time, when isolated from the cytosol of the plant cell.

Only about 300 species of Sacoglossa are described, but a recent survey on subtidal algal flats around Guam revealed more than 20 undescribed and new species (unpublished data). Undescribed species in North Sulawesi/Indonesia were listed in Burghardt *et al.* (2006) and were also recently detected around Bunaken Island (North Sulawesi). Thus, diversity of this enigmatic group is probably highly underestimated as is the case for probably all opisthobranch groups in Indonesia (Jensen, 2013).

Here I want to summarize recent results and literature data on the peculiar association of sacoglossan sea slugs and the role of the incorporated plastids.

REVIEW

Following species are discussed as so called long term retention forms that show a prolonged working of Photosystem II for many weeks up to months, a fact that usually then is interpreted as the slugs performing active photosynthesis: *Elysia crispata* (Mørch, 1863) and *E. clarki* Pierce, Curtis, Massey, Bass, Karl, Finneay, 2006 from the Caribbean Sea, *E. timida* (Risso, 1818) from the Mediterranean Sea, *E. chlorotica* Gould, 1870 from the North Atlantic and *Plakobranthus ocellatus* van Hasselt, 1824 from the Indopacific. They all belong to the major group Plakobranchoidea. Most recently, Christa *et al.* (2014) confirmed preliminary results on limapontioidean species (e.g., Clark *et al.*, 1981) as a long term retention form: *Costasiella ocellifera*. This species is a member of the Limapontioidea, a sacoglossan group in general considered as not having any retention forms (Händeler *et al.*, 2009). No members of the third sacoglossan group, the Oxynoacea, are known to house any species which are able to maintain plastids even for a very short time.

Of all species investigated so far, *Elysia chlorotica* survives for more than one year of starvation in culture (Rumpho *et al.*, 2000). Händeler *et al.* (2009) reported retention for nearly three months in *Plakobranthus ocellatus*, however, unpublished data (H.W. and Valerie Schmitt) show retention for several months. *E. timida* retains chloroplasts for at least 50 days (Wägele *et al.*, 2011) and *E. crispata* for about 40 days (Händeler *et al.*, 2009). *E. clarki* also shows a survival of several weeks under starving conditions (unpublished data). *E. asbecki* Wägele, Stemmer, Burghardt, Händeler, 2010 exhibits a similar photosynthetic activity in the first 10 days, as observed in *E. timida* and *E. crispata* (Wägele *et al.*, 2010b). Therefore, it is possible that this species might be the second long-term retention form known from the Pacific Ocean. *Costasiella ocellifera* showed photosynthetic activity at least for 30 days and survived in the experiments for more than 50 days (Christa *et al.*, 2014a).

Plastids have a reduced number of genes having transferred important genes into the nucleus (Martin and Herrmann, 1998; Timmis *et al.*, 2004). In order to investigate the possible role of algal nuclear genes in maintaining plastids alive in sea slugs and to reveal any possible horizontal gene transfer (HGT) from the algal nucleus genome into the slugs nucleus genome, Wägele *et al.* (2011) investigated transcriptomes of two long term retention forms, *Elysia timida* and *Plakobranthus ocellatus*. They

could not find even traces of a HGT. This was also shown for *E. chlorotica* (Pelletreau *et al.*, 2011). Thus the major assumption to explain longevity of plastids in the sea slugs had to be rejected, leading to the necessity to find other traits that might explain long term survival of plastids in the slugs' cells. These are now assumed to include a reduction in photo-damage of the plastids during exposure to irradiance, as well as intrinsic properties of the plastids themselves or even the slugs.

Pelletreau *et al.* (2012) and Schmitt *et al.* (2014) could show in experiments that the juveniles of *E. chlorotica* and *E. timida* respectively need to feed until they have reached a certain age (maturity), before they are able to maintain plastids in their digestive tract. Schmitt *et al.* (2014) also showed that *E. timida* was able to feed and regenerate after several long starvation periods, indicating the benefits for survival when deprived of food for some time. Efficiency of PSII decreased quicker when animals were kept in higher temperature, indicating a higher metabolism of the slugs or the plastids. Schmitt *et al.* (2014) therefore concluded that survival of plastids is also temperature related - a fact that was seldom addressed (e.g., Hinde and Smith, 1972; Stirts and Clark, 1980; Clark *et al.*, 1981). In a former study, Schmitt & Wägele (2011) investigated the behaviour of *E. timida*. This species has lateral parapodia that can be used to cover the dorsal body parts and therefore shade the underlying plastids in the digestive gland. The authors showed a reduction of fluorescence when parapodia were covering the body thus assuming that less light also penetrated then into the body and therefore reduced photodamage of plastids. Reduction of irradiance certainly leads to a lower photodamage and thus increases longevity of plastids. This was shown in several experiments, in which slugs were opposed to high light conditions, low light conditions or even darkness. PSII activity maintained on a much higher level when slugs were kept in darker conditions (Christa *et al.*, 2013, 2014a). It can be concluded that specific behaviour in natural environment therefore leads to a lower photo-damage and longer photosynthetic activity of incorporated plastids. This behaviour might involve daily activities in natural low light situations, like in the morning or afternoon, and active search for shade during high noon, even burrowing in the sand, as is typical for *Plakobranchus ocellatus* during part of the day. It has to be emphasized here that this behaviour is probably not directed towards reducing photo-damage in the first place, but may

have facilitated the survival of chloroplasts just by chance.

Sacoglossan sea slugs are very specific in their food choice. Their preferences might even be limited to only one algal species. Recent food analyses using molecular barcoding, combined with literature data on feeding observations allow us nowadays to narrow down those food items that might be involved in long maintenance in sea slugs. Curtis *et al.* (2006), Händeler *et al.* (2010), Maeda *et al.* (2012) and Christa *et al.* (2014b) enlarged our knowledge on sacoglossan food by applying the chloroplast markers *tufA* and/or *rbcL* on whole slugs' DNA extractions. Christa *et al.* (2014b) was then able to pin down the most successful chloroplasts and food items in long term retention slugs. This was partly supported by investigating those chloroplasts that were maintained in the slugs after several weeks of starvation. It seems that plastids from the Dasycladales *Acetabularia*, the Bryopsidales *Halimeda*, *Avrainvillea*, *Caulerpa* and *Penicillus*, as well as the heterokontophyte *Vaucheria* are candidates for long term maintenance in sacoglossan sea slugs. Recently de Vries *et al.* (2014) pointed out a special gene, *ftsH*, which is important for repairing photo-damaged PSII. Nearly nothing is known about the presence of this gene in plastids of algae. But it is of course interesting, that algae with putative longevity in the slugs, like *Acetabularia* and *Vaucheria* are mentioned here to have this gene, whereas *Bryopsis*, a species that does not provide long term maintained plastids, lacks this gene.

It is still not known what actually happens when chloroplasts are sequestered and endocytosed. Literature on this subject was recently reviewed by Wägele and Martin (2013). Usually sequestered material is surrounded by a special membrane that is necessary for digestion of nutritive material (e.g., Evertsen and Johnsen, 2009). According to literature, sequestered plastids in slugs belonging to the long term retention forms do not show this membrane, which then would explain the lack of digestion. But the few results published on the various species are contradictory. Our experiments (unpublished) have shown the necessity of the slugs to actually digest plastids first after a starvation period, when fed again. Since usually no details of the slugs condition in the experimental design are provided (how they were kept before preservation and processing for electron microscopic investigations), all published data have to be interpreted cautiously. Especially, when animals are taken from their natural habitat to a laboratory, some days may pass before experiments start. When

feeding them again after these few days of starvation, they probably always digest. This would explain the peculiar findings of Martin *et al.* (2012), where adult *Elysia timida* were preserved directly after feeding to investigate plastid incorporation. They found even naked thylakoids in the lumen of the digestive gland and also within the cytosol. Surrounding membranes were ruptured. These slugs were probably in the need of digestion and not in the state of putative incorporation.

Christa *et al.* (2014c) confirmed former investigations that incorporated plastids in deed fix CO₂ in light, but do not in darkness. They also applied a photosynthesis blocker, Monolinuron, and showed that photosynthesis is nearly not existent in *Plakobranthus ocellatus* when slugs are exposed to this chemical. Nevertheless, when comparing weight loss in animals kept in light, kept in darkness or kept in light with Monolinuron, the authors could show that weight loss in light is much higher than when kept in darkness or in Monolinuron. Loss of weight and a shrinking process was already observed in former times (e.g., Klochkova *et al.* 2013). These findings indicate, that chloroplasts do not provide energy in the same amount as it is used up in the daily activity of the slugs. The fact that animals kept in darkness lose less weight can be interpreted with lower activity (thus lower metabolic rate) in darkness and the same might be true for those animals exposed to the chemical.

These findings need to be corroborated now on a genetic base to get more insight into the intrinsic factors of the slugs as well as of the algae. Additionally the role of the pigments has to be investigated in more details as was done e.g. by Cruz and Serodio (2008) for diatoms, Jesus *et al.* (2010) for *Elysia timida*, and as was outlined as an important factor by Cruz *et al.* (2013). We have found many evidences that slugs' behaviour and physiology are adapted to housing plastids from their food. Or vice versa, pre adaptations with regard to behaviour and physiology support longer survival of plastids in the slug. We still do not know how much the plastids finally contribute when they degrade. Former investigations on *Elysia timida* indicated less than 10% (Marin and Ros, 1992; Cruz *et al.*, 2013). There degradation has been shown in many investigations, measuring PSII and documenting the slow decrease of photosynthetic ability. Plastids of the chlorophyte *Acetabularia* still produce starch, when this alga is enucleated (Vettermann, 1973). Why not also in the slug, when sitting undigested and unharmed in the cytosol of the slug and still fixing CO₂? Although the loss of weight indicates that contribution does not totally

compensate for an active life style, it still might contribute to a longer survival of starvation periods a factor that still needs to be investigated much further. Thus slugs probably use the chloroplasts only as an additional nutritional depot when stored while functioning (Christa *et al.* 2014c).

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