CellPress

Plastid survival in the cytosol of animal cells

Jan de Vries[1], Gregor Christa[1,2], and Sven B. Gould[1]

[1] Institute of Molecular Evolution, Heinrich-Heine-University Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany
[2] Zoologisches Forschungsmuseum Alexander Koenig, Adenauerallee 160, 53113 Bonn, Germany

Some marine slugs sequester plastids from their algae food, which can remain photosynthetically functional in the animal's digestive gland cells in the absence of algal nuclei. The sequestered plastids (kleptoplasts) appear to maintain functional photosystems through a greater autonomy than land plant plastids. If so, kleptoplast robustness is a plastid-intrinsic property, and it depends on the animal to manage an alien organelle on the loose in order to maintain it long term.

Photoheterotroph through kleptoplasty

Heritable plastids that harness light energy to fix CO_2 and generate reduced carbon compounds occur only in algae and plants. Some marine slugs are special, because they appear to profit from photosynthesis not through symbiosis but by retaining only the source of photosynthesis – the plastids – from their algal food [1]. This process (kleptoplasty) has been described for ~75 sacoglossan species, who tap the cell walls of siphonaceous algae and feed by sucking out the algal protoplasm including organelles. Based on pulse amplitude modulation (PAM) measurements that record photosystem II activity, the vast majority of slugs are either non-retention (NR) or short-term retention (StR) forms, that is, the kleptoplasts lose their photosynthetic capacity either immediately or within the first 2 weeks, respectively [2]. Current research focuses on long-term retention (LtR) species, six of which have so far been identified: *Elysia chlorotica*, *Elysia timida*, *Elysia crispata*, *Elysia clarki*, *Plakobranchus ocellatus*, and *Costasiella ocellifera* (Figure 1). All six can retain functional kleptoplasts for at least a month during starvation. The morphology of animals, greenish colouring and ability to house photosynthetically active kleptoplasts is why the scientific and popular press often refer to these animals as 'leaves that crawl' or 'solar-powered slugs'. The system offers the unique chance to study photobiology from the perspective of an animal host, in plastids that 'live' in the cytosol of an animal cell. Recent results are changing the way we view kleptoplasty in sacoglossan slugs. New findings show that kleptoplast longevity occurs without the support of lateral gene transfer events, suggesting that observed plastid robustness is a trait the organelle itself brings along.

'Crawling leaves' and photoautotrophy

Despite occasional claims to the contrary, sacoglossan slugs are not photoautotrophic; they are first and foremost heterotrophic. Because plastids are not transmitted vertically (i.e., from one slug generation to another via eggs), juveniles need to feed on algae for several days upon hatching before the first stable kleptoplasts are assimilated, a phase known as 'transient kleptoplasty' [3]. Adult slugs continue to feed as long as food is available too, but during starvation some species can survive for weeks or months whereas others cannot [1]. How, and in particular for how long, the plastids and slugs survive is less well understood than the literature might suggest. We recently pointed out that a few reports regarding the photosynthetic endurance of kleptoplasts during starvation are problematic [4]. In our hands, CO_2 fixation in slugs does not allow continued growth of the animals during starvation, and all species analysed so far have been shown to shrink and experience substantial weight loss – in some cases up to 93% – during starvation [5,6]. Undoubtedly, some slugs fix CO_2 in a light-dependent manner [6], but the question is for how long after the animals are deprived of their food, and is the amount of CO_2 fixation sufficient to support continued growth of the slugs?

The 'light-dependent reaction' measured through PAM fluorometry is currently the only method that has constantly provided evidence for a perpetuation of photosynthesis in several different slug species. Although F_v/F_m (maximum quantum yield) values provide a good indication about the general retention of functional photosystems in kleptoplasts [2], it is important to remember these values are relative and tell us nothing about what happens downstream of the photosystems. After weeks of starvation, a few remaining percent of plastids with functional photosystems might return high PAM values (which are ratios), but it is questionable whether the plastids can support the host with an amount of fixed carbon sufficient to be considered relevant. These details warrant attention, and the contribution of kleptoplasts in sacoglossan slugs is more complicated than one might think, especially when the behaviour of StR species is considered (Figure 2).

What mediates kleptoplast robustness?

Photosynthesis accompanies a constant high turnover of a subset of involved proteins, in particular the D1 protein of photosystem II [7]. Its maintenance requires accessory factors that are largely nuclear encoded. Algal nuclei, however, are rapidly digested after feeding [8]. In 1996, it was suggested that lateral gene transfer (LGT) from alga to animal might account for kleptoplast longevity [9]. This hypothesis became very popular, but has since been

Corresponding author: Gould, S.B. (gould@hhu.de).
Keywords: Sacoglossa; kleptoplasty; photoautotrophy; plastid maintenance; photosystem; carbon fixation.

1360-1385/
 http://dx.doi.org/10.1016/j.tplants.2014.03.010

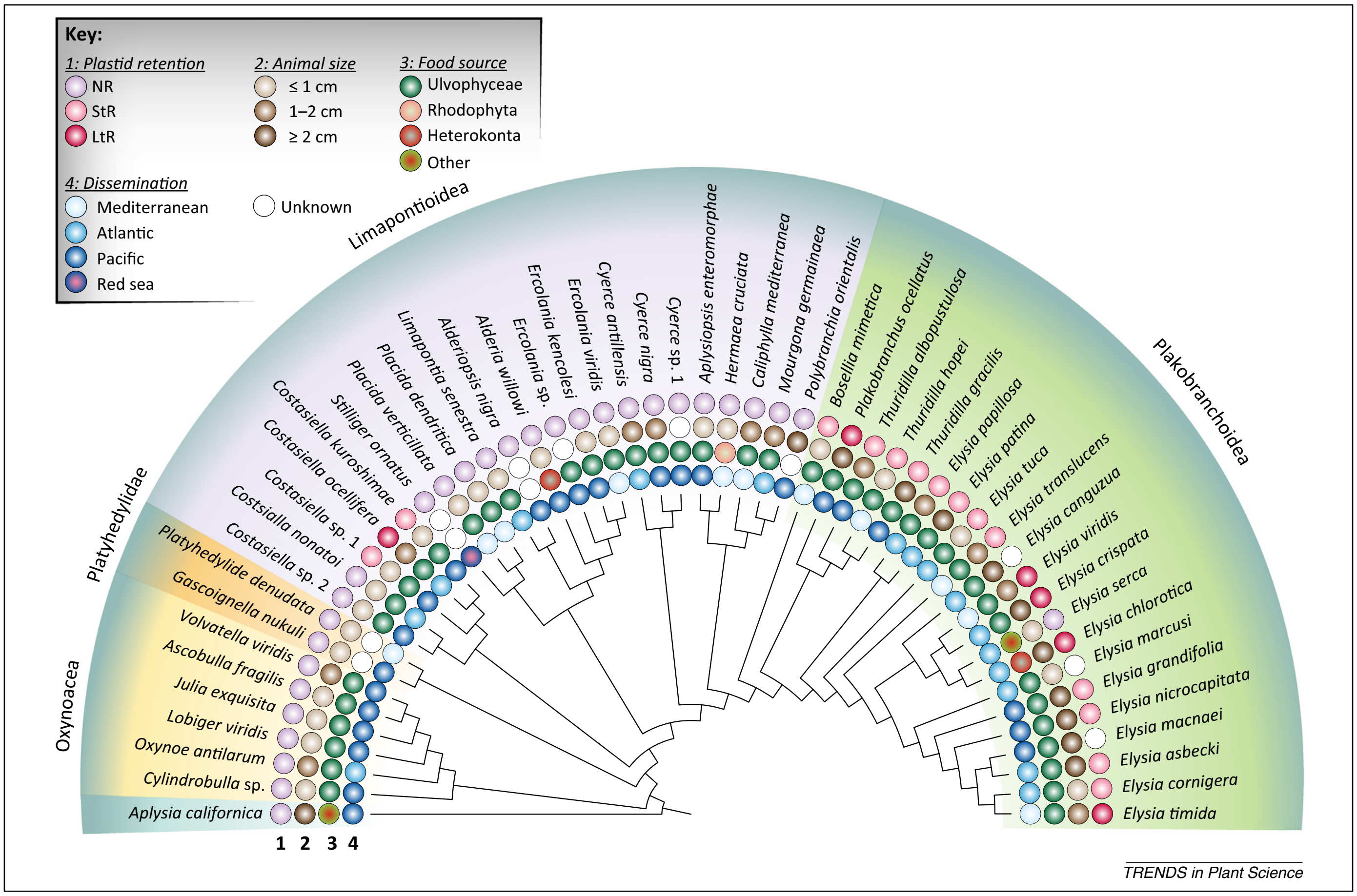

Figure 1. Phylogenetic relationship of the Sacoglossa. The shelled Oxynoacea are the most basal Sacoglossa. The sister taxon, the Plakobranchacea, unites the Limapontioidea and the Plakobranchoidea that are morphologically characterised by having either cerata (lateral appendices of the dorsal body) or parapodia (lateral appendices of the foot), respectively. The most basal Plakobranchacea are the worm-like Platyhedylidae that are, however, assigned to the Plakobranchoidea. Besides the genus *Costasiella*, neither the shelled Oxynoacea nor the Limapontioidea exhibit functional retention of kleptoplasts, which is common to nearly all Plakobranchoidea. Only six species (highlighted by dark red circles) are long-term retention (LtR) forms that are always larger than their corresponding sister taxa, and that are either non-retention (NR) or short-term retention (StR) forms. Besides some very specialised species, the majority of Sacoglossa feeds on ulvophycean algae, independent of whether being able to retain functional kleptoplasts or not. The cladogram is based on a four-gene Bayesian analysis using the anaspidean *Aplysia californica* (California sea hare) as the outgroup.

refuted [10,11] and the ability to service damaged photosystem II appears an intrinsic property of the plastids sequestered. Through sequencing the plastid genome of the ulvophycean alga *Acetabularia acetabulum* (food algae of the LtR species *E. timida*) and comparing it with other plastid genomes, we recently noticed that genomes of sequestered algae plastids encode, among other potentially crucial factors, FtsH [12]. This quality control protease specifically removes photodamaged D1, which is essential, because it stops the further generation of reactive oxygen species and allows the integration of *de novo* synthesised D1 into photosystem II [7]. Potentially, sequestered algal plastids bring along their own molecular toolkit for photosystem repair.

Could a single protein such as FtsH be that molecular toolkit and explain kleptoplast longevity? Apart from the contribution of slugs, it is likely that a combination of several mechanisms mediates kleptoplast robustness and allows ongoing photosynthesis in the cytosol of an animal's cell. These could include, for instance, non-photochemical quenching through a xanthophyll cycle and a generally slower turnover of plastid proteins. Interestingly, a first analysis suggests that the physiological photoregulation mechanism of kleptoplasts in slugs is surprisingly similar to that of the corresponding plastids in the algae [13]. The greater autonomy of some plastids sequestered by slugs, in comparison to land plant plastids, is a prerequisite for functional kleptoplasty, but it needs to be matched by the physiology of the slug in order to profit.

The origin of kleptoplasty and long-term retention in sacoglossans

The ability to survive prolonged starvation is rare among sacoglossans. Of the more than 300 described species, only six have been identified to retain functional kleptoplasts and withstand food deprivation for many months [2]. Five of the six species described belong to the Plakobranchoidea, one to the Limapontioidea [14], suggesting that this ability has evolved several times independently (Figure 1). The majority of sacoglossan sea slugs can feed on several algal species simultaneously, including the LtR forms *P. ocellatus*, *E. clarki*, and *E. crispata*, whereas some, including the LtR forms *E. timida*, *E. chlorotica*, and *C. ocellifera*, specialize on a single algal food source [2,14]. It is currently not known whether reliance on one source of kleptoplasts is more advantageous than a polyphagous life style. It is

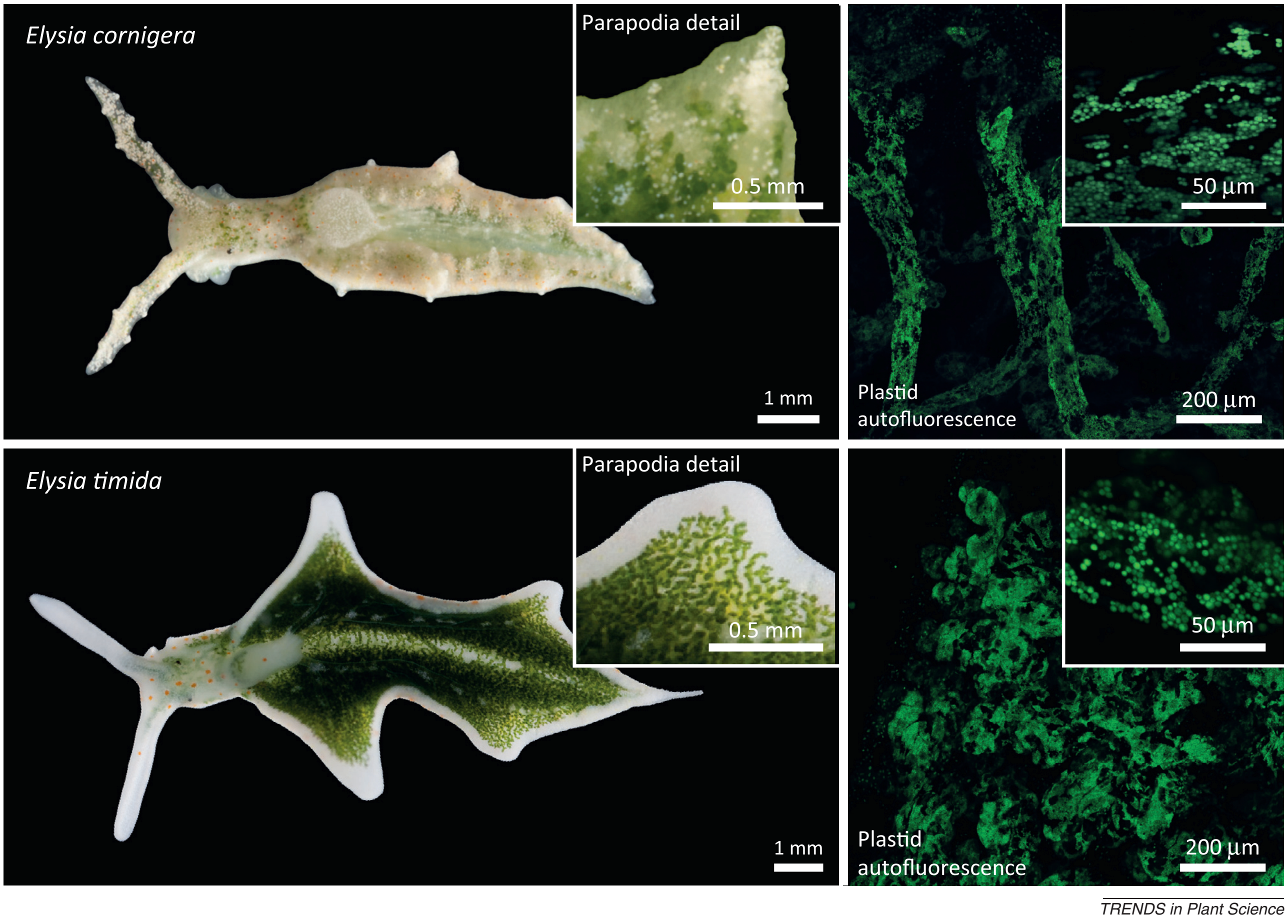

Figure 2. Kleptoplasts are biochemical lucky bags with a drawback. Pictured are the two phylogenetically close sacoglossans, *Elysia timida* and *Elysia cornigera*. Both slugs embed vast quantities of functional plastids they sequester from the ulvophycean alga *Acetabularia acetabulum* (plastid autofluorescence shown in green) and embed them within epithelial cells of digestive tubules that pervade the parapodia of slugs. Plastids are biochemical all-rounders and their abilities stretch beyond those of light-driven carbon fixation. Owing to their evolutionary legacy they can further synthesise, for example, certain amino acids, fatty acids, iron–sulphur clusters, haem, isoprenoid precursors, and certain cofactors. It has not been explored to what degree the slugs benefit from the biochemistry of kleptoplasts other than photosynthesis, but the vast amount of organelles stored reflects the rich nutritious depot from which the slugs could profit during starvation. Photosynthesis also accompanies the generation of toxic byproducts, such as reactive oxygen species or glycolates, for example. Their elimination is often complex and can require the interplay with other compartments. Intriguingly, when *E. cornigera* dies, the digestive tubules are still loaded with a quantity of kleptoplasts that visually appear similar to the quantity observed in *E. timida*, which however survives for many more weeks to come. LtR slugs might have developed strategies to better cope with self-contained, robust kleptoplast.

tempting to speculate that specializing on a single species as a food source is a prerequisite of evolution of stable kleptoplasty. Further work should address such issues.

Importantly, the ability of slugs to survive starvation is not strictly dependent upon the ability to retain functional plastids. Some NR species that have been analysed survive without food for many weeks to months too [5,14]. The LtR species *C. ocellifera*, *E. timida*, and *P. ocellatus* were shown to survive in the dark and with chemically blocked photosynthesis, all while not losing weight faster than the control group [4,14]. There is, furthermore, currently no evidence that the animals die when they are deprived of their food due to a lack of kleptoplasts and nutrition. In dying individuals of *E. chlorotica*, numerous kleptoplasts were still identified [15]. We observed that the StR species *Elysia cornigera* dies with digestive gland cells that are filled with a quantity of kleptoplasts that is similar to those of the phylogenetically closely related LtR species *E. timida*, whose starvation was commenced at the same time (Figure 2). Both species had been fed solely on the ulvophycean alga *A. acetabulum*, demonstrating that the food source alone cannot account for the differences observed in terms of how long an individual species survives food deprivation. Could there be another reason for slugs to die rather than a lack of nutrition? Research has largely focused on trying to understand how the slugs keep kleptoplasts functional in the absence of hundreds of algal nuclear genes, which are apparently not required. What if a more substantial part of slug–kleptoplast evolution is driven by the need to evolve mechanisms to cope with plastid-derived factors (possibly toxins generated through photosynthesis) rather than servicing the foreign organelle? In any case, some sacoglossan species can house functional plastids for months during starvation, but the exact nature of their contribution to slug biology is still not explored.

Future directions

An accumulating amount of data suggests that kleptoplast longevity is first and foremost an intrinsic property of the sequestered organelles, and a prerequisite for functional kleptoplasty. If these plastids are able to maintain functional photosynthesis more autonomously than their relatives from higher land plants, this has two main implications for future research: (i) the slugs highlight those algae, whose photobiology is the most valuable to study; and (ii) we should begin comparing the biology of StR and LtR slugs to understand what it is that allows LtR animals to better match with kleptoplast robustness so as to profit from maintaining the organelles long term.

Acknowledgements

S.B.G. would like to acknowledge the funding of this project through the 'Deutsche Forschungsgemeinschaft'. We thank CAi HHU Düsseldorf (Steffen Köhler) for help with imaging.

References

1 Cruz, S. *et al.* (2013) Crawling leaves: photosynthesis in sacoglossan sea slugs. *J. Exp. Bot.* 64, 3999–4009
2 Händeler, K. *et al.* (2009) Functional chloroplasts in metazoan cells – a unique evolutionary strategy in animal life. *Front. Zool.* 6, 28
3 Pelletreau, K.N. *et al.* (2012) Laboratory culturing of *Elysia chlorotica* reveals a shift from transient to permanent kleptoplasty. *Symbiosis* 58, 221–232
4 Christa, G. *et al.* (2014) Switching off photosynthesis: the dark side of sacoglossan slugs. *Commun. Integr. Biol.* http://dx.doi.org/10.4161/cib.28029
5 Klochkova, T.A. *et al.* (2013) Morphology, molecular phylogeny and photosynthetic activity of the sacoglossan mollusc, *Elysia nigrocapitata*, from Korea. *Mar. Biol.* 160, 155–168
6 Christa, G. *et al.* (2014) Plastid-bearing sea slugs fix CO_2 in the light but do not require photosynthesis to survive. *Proc. R. Soc. B* 281, 20132493
7 Nickelsen, J. and Rengstl, B. (2013) Photosystem II assembly: from cyanobacteria to plants. *Annu. Rev. Plant Biol.* 64, 609–635
8 Green, B.J. *et al.* (2000) Mollusc–algal chloroplast endosymbiosis. Photosynthesis, thylakoid protein maintenance, and chloroplast gene expression continue for many months in the absence of the algal nucleus. *Plant Physiol.* 124, 331–342
9 Mujer, C.V. *et al.* (1996) Chloroplast genes are expressed during intracellular symbiotic association of *Vaucheria litorea* plastids with the sea slug *Elysia chlorotica*. *Proc. Natl. Acad. Sci. U.S.A.* 93, 12333–12338
10 Wägele, H. *et al.* (2011) Transcriptomic evidence that longevity of acquired plastids in the photosynthetic slugs *Elysia timida* and *Plakobranchus ocellatus* does not entail lateral transfer of algal nuclear genes. *Mol. Biol. Evol.* 28, 699–706
11 Bhattacharya, D. *et al.* (2013) Genome analysis of *Elysia chlorotica* egg DNA provides no evidence for horizontal gene transfer into the germ line of this kleptoplastic mollusc. *Mol. Biol. Evol.* 30, 1843–1852
12 de Vries, J. *et al.* (2013) Is *ftsH* the key to plastid longevity in sacoglossan slugs? *Genome Biol. Evol.* 5, 2540–2548
13 Jesus, B. *et al.* (2010) Behaviour and a functional xanthophyll cycle enhance photo-regulation mechanisms in the solar-powered sea slug *Elysia timida* (Risso, 1818). *J. Exp. Mar. Biol. Ecol.* 395, 98–105
14 Christa, G. *et al.* (2014) Functional kleptoplasty in a limapontioidean genus: phylogeny, food preferences and photosynthesis in *Costasiella* with focus on *C. ocellifera* (Gastropoda, Sacoglossa). *J. Mollus. Stud.* http://dx.doi.org/10.1093/mollus/eyu026
15 Mondy, W.L. and Pierce, S.K. (2003) Apoptotic-like morphology is associated with annual synchronized death in kleptoplastic sea slugs (*Elysia chlorotica*). *Invert. Biol.* 122, 126–137