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Plastid survival in the cytosol of animal cells

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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.

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'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

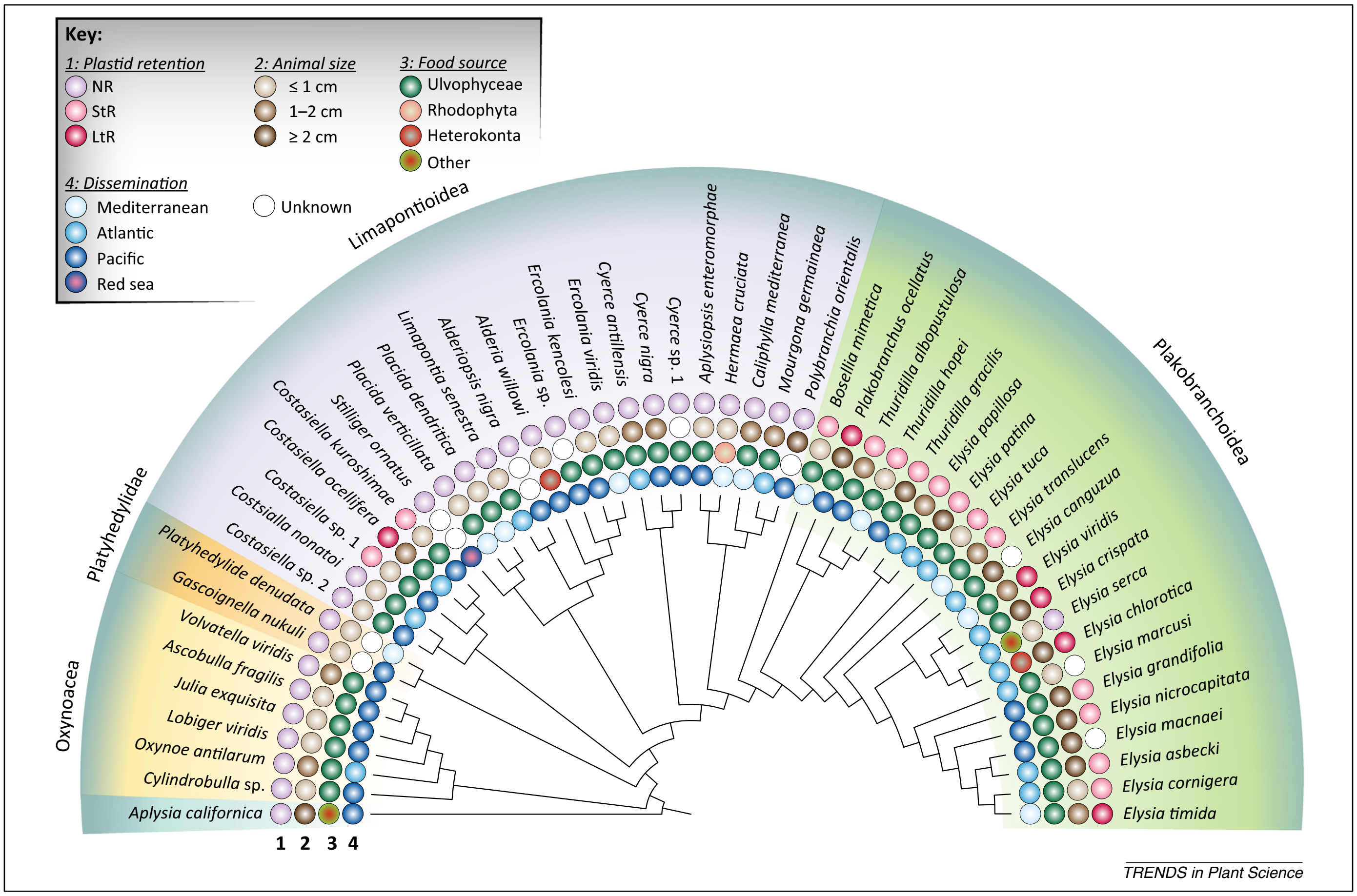

Figure 1. Phylogenetic relationship of the Sacoglossa. The shelled Oxynoacea are the most basal Sacoglossa. The sister taxon, the Plakobranchacea, unites the Limapontioidea and the Plakobranchoidea that are morphologically characterised by having either cerata (lateral appendices of the dorsal body) 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