Expression of nuclear-encoded proteins for photosynthesis in sea slug (*Elysia chlorotica*)

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Writer's Comment: *This paper was written as an assignment for UWP 102B instructed by Jared Haynes. The assignment asked that I write a research review on a biological topic of my choice using recent research articles. For my topic, I remembered hearing of a photosynthetic sea slug while in community college and chose to write about* Elysia chlorotica *so I could learn more about what I thought was an interesting subject. I collected information from the most recent relevant articles and tried presenting the information in a manner so my peers with a biological background could understand. To keep within the assignment's word limit, I had to omit certain details. I hope that what I presented here offers readers a sufficient introduction to current research efforts concerning the photosynthetic slug* E. chlorotica.

Instructor's Comment: *The major assignment in my UWP 102B class is the literature review—a document that attempts to bring the readers up to date on research in a particular field. It's a difficult assignment because the student must pick a topic, find recent research articles that are pertinent to the topic, and then read, understand, synthesize, and organize the material from the articles in a coherent presentation. Tim did a stellar job on the topic of the photosynthetic abilities of a sea slug. The introduction explains the topic in a way that even non-scientists can understand, and it narrows down the scope so that a reader has a clear idea of what the rest of the review will do. While Tim doesn't shy away from using technical terminology, the explanations are still easy to follow; that's a function of that strong introduction and also of his ability to lay out the material in an organized way.*

—Jared Haynes, University Writing Program

Introduction

Upon consuming its algal prey, *Elysia chlorotica* can retain and maintain active chloroplasts within its cells for photosynthesis (Bhattacharya et al. 2013, p 1843). This phenomenon, known as kleptoplasty, occurs in several elysiid sea slug species and allows these organisms to metabolize photoautotrophically (Bhattacharya et al. 2013, p 1843; Schwartz et al. 2010, p 29). While many species are able to maintain chloroplasts for a few days or months, *E. chlorotica* can maintain active chloroplasts for as long as nine months, which is among the longest retentions known among sea slugs (Schwartz et al. 2010, p 29). *E. chlorotica* is native to salt marshes along the east coast of North America, from Nova Scotia to Florida (Devine et al. 2012, p 139). Its primary algal food source, *Vaucheria litorea*, provides chloroplasts, which become incorporated into cells lining the slug's digestive tract (Soule and Rumpho 2012, p 337). Once *E. chlorotica* establishes a sufficient number of kleptoplasts, "stolen" chloroplasts, it no longer requires feeding on algae and may complete the rest of its lifecycle as a photoautotroph (Rumpho et al. 2009, p 1384). Within the cells of *E. chlorotica*, the kleptoplast will not divide and will not be transmitted to or inherited by future generations (Rumpho et al. 2009, p 1384; Soule and Rumpho 2012, p 373). Because *E. chlorotica* is able to maintain active chloroplasts and carry out photosynthesis for such a long period of time, an interesting implication arises. While many genes needed for photosynthesis are found within the chloroplast genome, the plastid remains dependent on proteins normally encoded within the nuclear genome for proper function and regulation (Soule and Rumpho 2012, p 373). Therefore, essential photosynthetic proteins typically supplied by *V. litorea* nuclear DNA to the chloroplast must be present within *E. chlorotica* (Rumpho et al. 2009, p 1384-1385). The slugs do not appear to retain the nucleus of their algal prey when incorporating the chloroplast into their cells (Bhattacharya et al. 2013, p 1843). Recent research has attempted to reveal the identity and location of genes typically encoded in the algal nuclear genome that are expressed by *E. chlorotica*.

Discovering nuclear-encoded enzymes for photosynthesis in *E. chlorotica*

Because nuclear-encoded proteins are essential for photosynthesis, *E. chlorotica* must provide at least some proteins to the kleptoplast that would normally be supplied by the nuclear genome of *V. litorea*.

Phosphoribulokinase (PRK) is a nuclear-encoded enzyme found to be synthesized by *E. chlorotica* (Pierce et al. 2012; Rumpho et al. 2009; Schwartz et al. 2010; Soule and Rumpho 2012). The *prk* gene is, with few exceptions, found within the nuclear DNA of all photosynthetic organisms, including *V. litorea* (Rumpho et al. 2009, p 1385). PRK is both essential and unique to the Calvin cycle of photosynthesis. Rumpho et al. (2009) found that *E. chlorotica* synthesized PRK even after being starved of its algal food source for five months, which supported the existence of a *prk* gene within cells of the sea slug. However, the researchers discovered that regulation of PRK activity was different between slugs and algae. Under dark conditions, PRK typically binds to the proteins GAPDH and CP12. The binding inactivates PRK and prevents futile cycling in the absence of light. While *V. litorea* appeared to have regulated PRK activity, *E. chlorotica* did not. Rumpho et al. (2009) hypothesized that PRK was not bound to GAPDH and CP12 in the slug under dark conditions. Given that CP12 is a nuclear-encoded protein, it is possible that this and other regulatory enzymes are not expressed in *E. chlorotica* (Rumpho et al. 2009).

Differences between *E. chlorotica* and *V. litorea* PRK expression have also been found at the transcriptional level (Soule and Rumpho 2012). Genes important for photosynthesis, including *prk*, in *V. litorea* were found to be transcriptionally regulated by light while the same genes in *E. chlorotica* were not (Soule and Rumpho 2012). It appeared that *E. chlorotica* failed to express genes involved in regulation of transcription. Those genes were believed to be part of a circadian pathway, including nuclear-encoded genes. Again, *E. chlorotica* may contain nuclear-encoded genes essential for photosynthesis, but seems to lack genes involved in regulation. So while certain essential nuclear-encoded genes are present in the sea slug, other regulatory enzymes of photosynthesis appear to be missing (Rumpho et al. 2009; Soule and Rumpho 2012).

PCR-based approaches have been useful for the discovery of many algal nuclear gene sequences within the *E. chlorotica* genome and/or transcriptome (Rumpho et al. 2009 and Schwartz et al. 2010). Among those genes, six were originally identified by Schwartz et al. (2010) using PCR. *V. litorea* mRNA was extracted, reverse-transcribed, and partially sequenced to generate ESTs. From the ESTs, primers were developed for PCR and used to identify and amplify any sequences within the genome of *E. chlorotica* that matched genes of *V. litorea*. From their results,

Schwartz et al. (2010) determined all six genes to function in aspects of photosynthesis such as light harvesting, pigment synthesis, and the Calvin cycle. Rumpho et al. (2009) also used PCR and discovered two partial sequences in *E. chlorotica* genomic DNA that exactly matched the algal nuclear-encoded gene for PRK. Such experiments indicate that the sea slugs possess DNA sequences corresponding to photosynthetic genes found in the nuclear genome of *V. litorea* (Rumpho et al. 2009; Schwartz et al. 2010).

Another approach in gene discovery has been to generate and compare genetic sequence data from *E. chlorotica* and *V. litorea* (Pierce et al. 2012; Bhattacharya et al. 2013). Pierce et al. (2012) used transcriptome analysis to identify nuclear-encoded genes for photosynthesis in the sea slug. The team generated transcriptome sequence information from both *V. litorea* and *E. chlorotica*. By comparing the sequence data from the two species, the team identified 52 nuclear-encoded genes from *V. litorea* within *E. chlorotica*. All genes shared in common were found to function in pigment synthesis, the Calvin cycle, or other parts of photosynthesis. Pierce et al. (2012) also confirmed the presence of a gene for PRK synthesis within the slug transcriptome, as well as other previously described genes. In all, *E. chlorotica* has been found to possess over 60 nuclear-encoded genes from its algal food source (Pierce et al. 2012).

Finding the location of nuclear-encoded genes for photosynthesis within *E. chlorotica*

While it is clear *E. chlorotica* produces proteins targeted to the kleptoplast for photosynthesis, it is less clear as to where the genes coding for those proteins are located. Horizontal gene transfer (HGT) is frequently discussed as a possible explanation as to why algal nuclear genes appear within the sea slug genome (Pierce et al. 2012; Schwartz et al. 2010; Rumpho et al. 2009; Bhattacharya et al. 2013). With the HGT hypothesis, researchers believe that some *V. litorea* nuclear-encoded genes have integrated into the nuclear genome of *E. chlorotica*. Using a PCR-based approach, Rumpho et al. (2009) discovered two *prk* sequences within the sea slug genome that matched the *prk* gene of *V. litorea* exactly. The researchers believed it possible that the gene was somehow incorporated into the slug genome from algae via HGT. However, the two sequences were not complete. If the sequences were related to algae *prk*, they had become pseudogenized over time and their

function lost. The researchers failed to find full-length sequences within the slug genome that may transcribe functional mRNA for PRK.

Schwartz et al. (2010) later confirmed the presence of *prk* sequences within *E. chlorotica*. The group also discovered several other sequences in *E. chlorotica* matching *V. litorea* nuclear genes. For their experiment, sea slug egg genomic DNA was analyzed using PCR methodology. Because the eggs would not have been physically able to consume algae, there was little possibility that results could have been contaminated by algal nuclear DNA. The presence of *V. litorea* sequences within the egg genome meant that those genes must have been inherited by the egg from the parent slugs. Schwartz et al. (2010) concluded that *V. litorea* nuclear-encoded genes had integrated into the sea slug germ line by horizontal gene transfer and were being inherited by offspring.

Transcriptome sequence data from *V. litorea* and *E. chlorotica* has yielded many photosynthetic genes thought to be present within the sea slug genome (Pierce et al. 2012). Pierce et al. (2012) reported the existence of 52 nuclear-encoded genes of *V. litorea* in *E. chlorotica*. Unlike previous methods that utilized PCR-based testing, the team used mRNA transcript data from algae and algae-starved sea slugs. Since the researchers discovered algal transcripts within the starved *E. chlorotica* transcriptome, they suggested that the slugs may have incorporated algal nuclear genes through HGT. Furthermore, 23 of the identified nuclear-encoded genes of *E. chlorotica* differed from matching *V. litorea* genes by 1-6bp, which may indicate that some gene transfer events occurred early enough for sequences to diverge over time.

While some researchers have hypothesized that HGT had occurred between *V. litorea* and *E. chlorotica*, a recent investigation by Bhattacharya et al. (2013) found no evidence for HGT between sea slugs and algae. In direct contrast to previous studies, no matches to algal nuclear DNA were found within genomic DNA of slug eggs. However, evidence for algal nuclear-encoded gene expression was found in adult slugs. Bhattacharya et al. (2013) concluded that *E. chlorotica* may retain algal nuclear-encoded genes in an extrachromosomal position after consuming its algal prey. The team explained that previous PCR-based studies pooled animals together, which would mean that any genes found would appear to come from all animals in the pool. Bhattacharya et al. (2013) conducted PCR analysis of individual adult slugs and revealed that there is variation in the presence or absence of algal nuclear genes and that the presence of a gene

did not always mean detection of a transcript, perhaps due to low copy numbers. Soule and Rumpho (2012) had also noticed that expression of *prk* was low, much lower than levels being expressed by algae. It is then possible that the sea slugs maintain algal nuclear genes in an unidentified extrachromosomal location (Bhattacharya et al 2013; Soule and Rumpho 2012).

Conclusion

To maintain photosynthetic activity, *E. chlorotica* must be able to synthesize proteins that are usually located within the nuclear genome of photosynthetic organisms. Current findings reveal that *E. chlorotica* is able to provide the kleptoplast with proteins that are normally provided by the algal cell from which the plastid was harvested. However, it is far less clear as to where the nuclear-encoded genetic information is stored within *E. chlorotica* cells and how it is obtained. Current research indicated two major possibilities. Nuclear-encoded information may be horizontally transferred and incorporated into the nuclear genome of the sea slug. Or nuclear-encoded information from the algae may be stored in an extrachromosomal location as yet unknown. Complete genome sequencing of *E. chlorotica* should prove very useful in answering the question of possible horizontal gene transfer.

Cited References

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