

# Chlorophyll *d*: the puzzle resolved

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Chlorophyll *a* (Chl *a*) has always been regarded as the sole chlorophyll with a role in photochemical conversion in oxygen-evolving phototrophs, whereas chlorophyll *d* (Chl *d*), discovered in small quantities in red algae in 1943, was often regarded as an artefact of isolation. Now, as a result of discoveries over the past year, it has become clear that Chl *d* is the major chlorophyll of a free-living and widely distributed cyanobacterium that lives in light environments depleted in visible light and enhanced in infrared radiation. Moreover, Chl *d* not only has a light-harvesting role but might also replace Chl *a* in the special pair of chlorophylls in both reaction centers of photosynthesis.

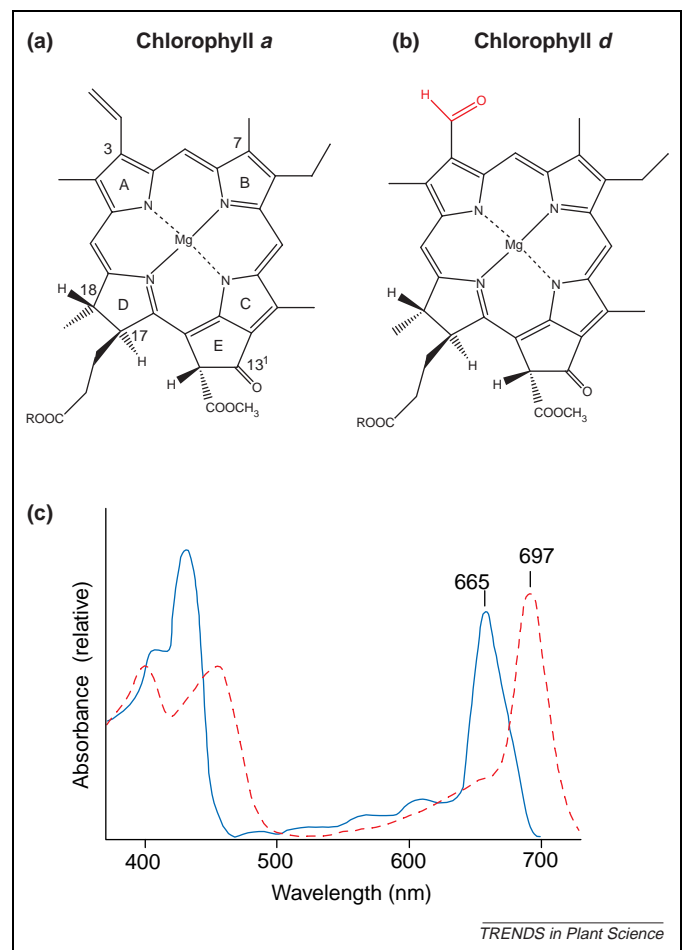
## Introduction

Chlorophyll *d* (Chl *d*) (Figure 1) was discovered in red algae (Rhodophyta) >60 years ago by Harold Strain and Winston Manning, two expert natural chemists [1]. It was the fourth type of chlorophyll to be discovered and hence derived its name following the earlier discovery of chlorophyll *c* (Chl *c*) by Strain and Manning [2]. Both chlorophyll *b* (Chl *b*), discovered in the 19th century, and Chl *c* were considered to be accessory chlorophylls, now better known as light-harvesting chlorophylls [i.e. they augment the light-harvesting properties of oxygenic photosynthetic organisms (cyanobacteria, algae and plants) by passing on light excitation to chlorophyll *a* (Chl *a*)].

Manning and Strain carefully characterized Chl *d*. They found that a divinyl group in ring A of Chl *a* is replaced by a formyl group in chlorophyll *d* (Figure 1). This is similar to the structure of Chl *b*, which differs from Chl *a* by the replacement of a methyl group with a formyl group in ring B. However, it differs markedly from all the other chlorophylls in having its  $Q_y$  peak (the major red or infrared peak of absorption in chlorophylls and bacteriochlorophylls) in the near infrared, rather than in the red, spectral region (peak at ~720 nm *in vivo*). Therefore, in its absorption spectrum, it is allied more with bacteriochlorophylls, which also have  $Q_y$  peaks in the infrared region. However, no bacteriochlorophyll (except bacteriochlorophyll *e*, which absorbs at 715–725 nm) has an absorption band in the same region (i.e. 700–730 nm); the spectral properties of Chl *d* are, therefore, unique. With hindsight this should have indicated how the occurrence of Chl *d* could be explained.

## Discovery of *Acaryochloris marina*

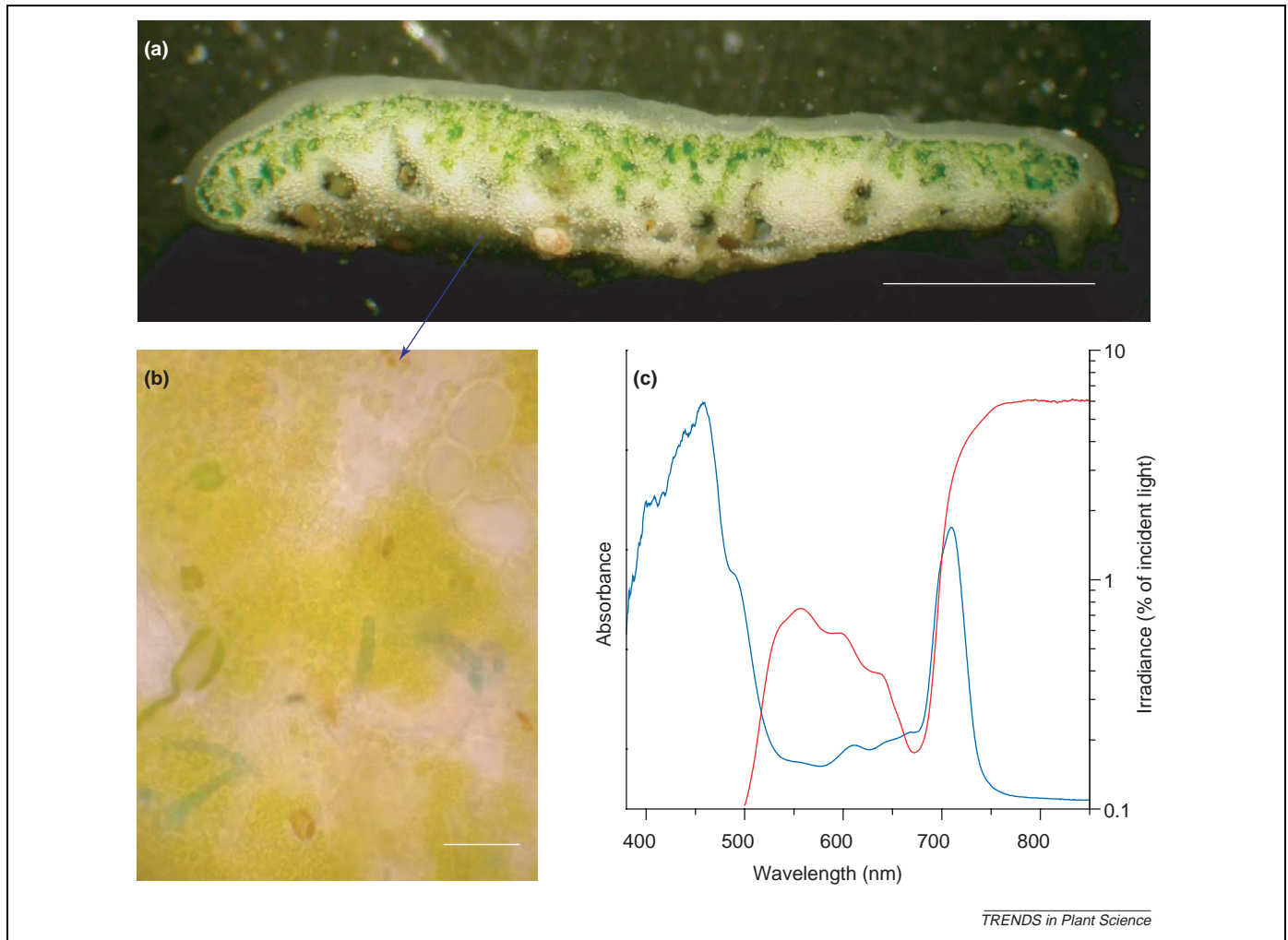
The real problem with the initial discovery was that (i) other workers could not always find Chl *d* in red algae, (ii) there were no reports of Chl *d* being found in freshwater alga, and (iii) there was a possibility that it might be produced as a breakdown product during pigment extraction [3]. Thus, the subject languished for 50 years until the dramatic report in 1996 [4] of a cyanobacterium that possessed >90% Chl *d* (the remainder as Chl *a*). This cyanobacterium, *Acaryochloris marina* [5], was discovered in isolates from didemnid ascidians from coral reefs. Shigeto Miyachi's group made this chance discovery during an attempt to grow the already-known symbiont of these ascidians, *Prochloron didemni*.



**Figure 1.** Structure of (a) chlorophyll *a* and (b) chlorophyll *d*. A divinyl group in ring A of chlorophyll *a* is replaced by a formyl group in chlorophyll *d* (highlighted in red). (c) A comparison of the *in vitro* absorption spectra of chlorophyll *a* (blue solid line) and chlorophyll *d* (red broken line).

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**Figure 2.** (a) A cross-section of the didemnid ascidian *Trididemnum paracyclops*, hosting the green exosymbiont *Prochloron didemni* in its internal cavities. Scale bar = 5 mm. (b) Yellow-greenish colonies of *Acaryochloris marina*-like cyanobacteria grow below dense layers of other phototrophs in a biofilm covering the underside of the ascidian. Scale bar = 10 μm. (c) Absorbance spectrum of *Acaryochloris marina* (blue line) and spectral composition of light penetrating through the ascidian to *A. marina* (red line).

*P. didemni* was first reported independently in 1975 by Eldon Newcomb and Tom Pugh and by Ralph Lewin, and named by Lewin [6]. *P. didemni* attracted much interest at the time because it was the first cyanobacterium to be shown to possess Chl *b*, in addition to Chl *a*. Since then, two other groups of cyanobacteria have been shown to possess Chl *b*: the filamentous *Prochlorothrix hollandica* [7] and the unicellular *Prochlorococcus marinus* [8]. *P. marinus* is one of the smallest and most important primary producers in the world's oceans [9].

In showing that *A. marina* could be cultured from isolates of ascidians it was assumed by the authors and others (including ourselves [10]) that it was also symbiotic within the ascidians, possibly co-symbiotic with *Prochloron didemni*, which is an exosymbiont in the exhalant canals of these colonial animals. This raised puzzling questions about why *A. marina* could be cultured and *P. didemni* could not, and why the major  $Q_y$  band of *A. marina* is in the near infrared region (when the light impinging on the ascidians showed maximal energies in the green and orange regions of the spectrum)?

### **Acaryochloris is a widespread organism**

This question was further highlighted last year when Mamoru Mimuro's group reported that they had discovered a free-living cyanobacterium with a similar small subunit rRNA and Chl *d* characteristic of *A. marina* growing as an epiphyte on the stipes of certain red algae in Japanese waters [11]. This *Acaryochloris*-like organism is free-living and exploits a habitat beneath the fronds of the red algae that is depleted of visible light and enhanced mainly in near infrared radiation (NIR). Although this finding resolved the 60-year puzzle about the occurrence of Chl *d* in small amounts in red algae, it highlighted a doubt about the original observation of *A. marina* as a symbiont in didemnid ascidians. Was it possible that two so similar organisms could be living under such different nutritional and light habitats? An additional part of the puzzle was added recently when Michelle Wood's group [12] isolated an *Acaryochloris*-like organism in their enrichments from an artificial, eutrophic, saline lake in California (USA), which further indicates the widespread and free-living nature of *Acaryochloris*.

The situation now seems to have been resolved more fully with the recent discovery [13] of *Acaryochloris*-like

organisms growing on the undersides of didemnid ascidians on a coral reef (Heron Island reef) of the Great Barrier Reef. This report has shown that small colonies of the cells occur together with a rich community of other epiphytes (other cyanobacteria, diatoms and green algae) on the undersides of these ascidians (Figure 2). This environment is highly enriched in NIR and depleted in visible radiation. Pulse amplitude modulated (PAM) fluorescence imaging has shown that these algae are highly active in photosynthesis, which is mainly driven by NIR penetrating efficiently through the dense layers of *Prochloron* and other phototrophs above. Is this type of habitat the real home of *A. marina*? The answer is probably 'yes', although it is possible that bioturbation in the restricted environment of the ascidians can lead to some ingestion of *A. marina* by the filter-feeding ascidians.

It now appears that *A. marina* is a widespread, free-living organism that exploits light environments depleted of visible radiation and enhanced in NIR. Careful analysis of these types of habitats will probably reveal this group of organisms in many localities and sites around the world. Furthermore, Chl *d* not only acts as a light-harvesting pigment in *A. marina* but has also replaced Chl *a* in Photosystem I [14]; the possibility that it has also replaced Chl *a* in Photosystem II is currently a subject of investigation in several laboratories around the world, so *A. marina* and similar organisms will continue to attract much interest for some time to come. In the meantime, we seem to have a convincing solution to the puzzle of how Chl *d* came to be associated with red algae.

#### Acknowledgements

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# A versatile vector system for multiple gene expression in plants

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Today, cloning vectors that have been specifically designed to facilitate the fusion, overexpression or down-regulation of a variety of genes in plant cells are available from various sources. In most cases, their basic design allows the cloning of a single target gene, typically under a specific promoter, in parallel with the expression of selection and/or marker genes from the same vector.

However, new and versatile systems now exist that expand the user's choice to a large number of promoters and terminators, and various autofluorescent tags confer the ability to express multiple genes from a single transformation vector.

#### Introduction

The ability to stably and transiently express specific genes in cells, tissues and whole plants is a fundamental aspect

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