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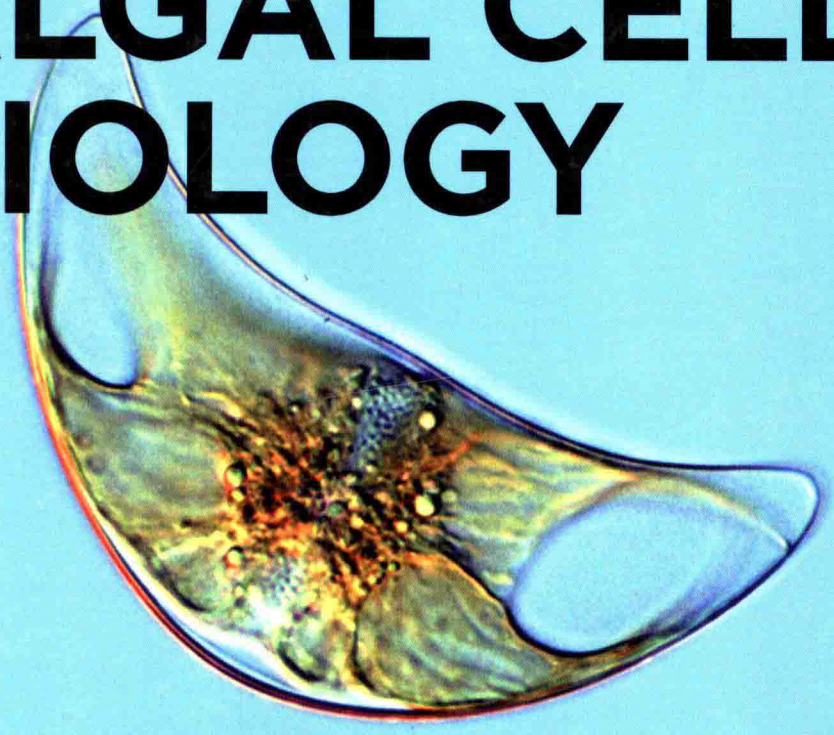
Kirsten Heimann,
Christos Katsaros (Eds.)

ADVANCES IN ALGAL CELL BIOLOGY



MARINE AND FRESHWATER BOTANY

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Advances in Algal Cell Biology

Edited by Kirsten Heimann
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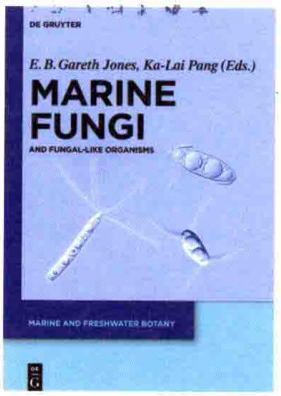

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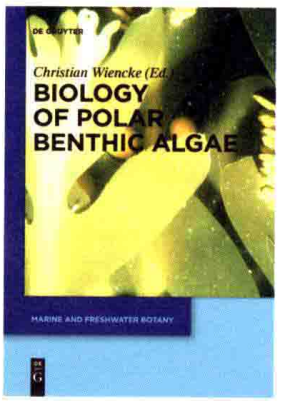

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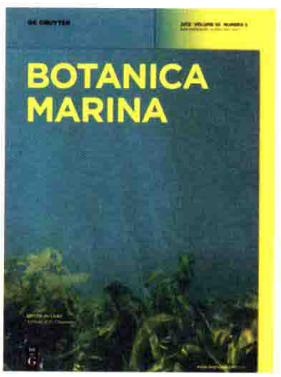

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Preface

Almost every algal textbook starts by underlining the fundamental importance of algae. It is true that they are key primary producers in marine and freshwater environments and represent a relatively untapped resource for food, bioenergy and biopharmaceuticals. Knowledge of algal cell biology is indeed the successful recipe for the current boom of biotechnological applications of micro- and macroalgae. Apart from these indisputable features, algae have attracted the interest of researchers since the first studies in the plant kingdom.

Algal research passed from different stages, reflecting not only the interest of the scientists, but also the dynamics and the facilities available in each of these time periods. External morphology was completed by (light and electron) microscopy, chemistry by biochemistry and finally molecular biology. The tremendous progress of biological research during the last decades of the 20th century, which has made biology the most important science of the 21st century, has been extended to algal research by giving the tools for specialized studies which provided deep insights into algal structural and functional organization. In this way, the application of modern techniques and sophisticated tools contributed drastically not only to the study of algal cell metabolism but also to algal evolution, the latter, in turn, contributing to species evolution in general.

These approaches were used not only to study the physiological mechanisms functioning during the life cycles of algae, but also to clarify the taxonomic and phylogenetic relationships between them.

However, despite the vast of information revealed from these studies and published in many scientific journals, there is a considerable lack of a book dealing with the structure and molecular biology of algae.

The publication of this book was the physical continuation of the publication of the Botanica Marina special issue entitled "Advances in algal cell biology and genomics". The high quality of the articles included in this issue, revealed the tremendous progress in the field of the biology of algal cells.

Having the above accumulated information in hands and considering the necessity of a book in which scientists (students, phycologists, etc.) would find answers to questions and/or triggers for further research, we proceeded to this publication.

Apoptosis or programmed cell death is a fundamental mechanism for the development and repair of tissues. Indeed the process of apoptosis has even been realised in cyanobacteria where if functions in bloom control. Given the importance of programmed cell death, this book starts out with a review on programmed cell death in multicellular algae. This chapter investigates the implication of programmed cell death for algal development, such as spore germination, hair development, the development of reticulate thallus structures, cell surface cleaning mechanisms, reactions to parasites, senescence and abscission. These developmental patterns are compared to analogous processes in terrestrial plants. It can be concluded that programmed cell death is yet another unifying concept in biology.

Algal biodiversity is extremely high compared to other groups of organisms. Hence the second chapter reviews the mechanism by which this diversity was generated.

Current knowledge of endosymbiosis giving rise to the highly diverse plastids in the algae is placed into context with gene transfer and algal evolution.

The third chapter pays tribute to the unusual pennate diatom, *Phaeodactylum tricorunutum*. It summarises knowledge regarding factors and mechanisms involved in the polymorphism of this organism. It also investigates possible drivers for the conversion of one morphotype into the other and mechanisms that make such tremendous morphological changes possible.

The fourth chapter reviews cytological and cytochemical aspects of carrageenophytes, a group of red algae that are growing steadily in commercial applications.

The fifth chapter presents the findings of a desktop study using a molecular approach to unravel algal protein trafficking, specifically vacuolar protein sorting and provide strong evidence that such investigations can assist in the assembly of a holistic picture of protist evolution.

The sixth chapter presents data on the function of contractile vacuoles in green algae and places these into context with protists used as models for studies on contractile vacuole function and mechanisms, such as ciliates, slime moulds and the parasitic trypanosomes.

Chapter seven reviews advances in our understanding of the mechanisms and structures required for cytokinesis in brown algae. Particular focus has been given to the role of the cytoskeleton in cell wall morphogenesis, the deposition of wall materials, the role of the centrosome in the determination of the division site, and the formation of plasmodesmata, The techniques used in these studies include not only conventional microscopy, but also immunofluorescence and TEM as well as cryofixation – freeze-substitution and electron tomography.

Chapter eight provides new insight in the function of the cytoskeleton for sperm release in *Chara*. This study uses cytoskeletal drugs to modulate cytoskeletal function and demonstrates, using scanning laser confocal immunofluorescence microscopy, that sperm release in *Chara* is a highly dynamic process.

Chapter nine presents findings on the involvement of the cytoskeleton for the regulation of an important marine phenomenon – bioluminescence. Using cytoskeleton modulating drugs, evidence is presented that the cytoskeleton is involved in the reciprocal movement of chloroplasts and bioluminescent organelles at the transition of photoperiods in the marine dinoflagellate, *Pyrocystis lunula*.

Lastly, chapter 10 explores how the bioluminescent system of *Pyrocystis lunula* and specific signal modulators can be used to unravel potential signal transduction cascades required for eliciting the touch-induced bioluminescent response. It also provides insights into potential mechanisms involved in the reduction of bioluminescence when exposed to heavy metals and explores the use of the herbicide oxyfluorfen, which inhibits chlorophyll biosynthesis, for determining the biosynthetic origin of the bioluminescent substrate luciferin.

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Contents

1 Programmed cell death in multicellular algae

David J. Garbary, Moira E. Galway, Christina E. Lord and Arunika N. Gunawardena

Introduction

Growth and differentiation of multicellular organisms typically involves the addition of new cells through cell division; unicellular organisms may undergo cell enlargement to accomplish similar ends. In addition, many aspects of morphogenesis and differentiation are associated with cell death. While regularized patterns of cell death have been recognized in the animals and plants, such cell death has rarely been the focus of developmental studies or cell biology in multicellular algae. Cell death resulting from trauma, severe injury or acute physiological stress has been classified as necrosis (Reape et al. 2008; Palavan-Unsal et al. 2005, but see discussion in Kroemer et al. 2009 and in the introduction to Noodén 2004). When localized and endogenously induced death of cells occurs, it may be considered under the general rubric of Programmed Cell Death (PCD) or Apoptosis (APO). The literature on PCD in plant and animal systems is extensive, and there is considerable controversy in defining the various forms of cell death based on processes of ultrastructural and biochemical changes (e.g., Morgan and Drew 2004; Noodén 2004; Reape et al. 2008; Kroemer et al. 2009). While APO and PCD were considered synonymous in much earlier literature, there is a general consensus that APO is a specialized case of PCD from which Apoptosis-Like (APL) phenomena and autophagy (AUT) also need to be distinguished (Reape et al. 2008; Reape and McCabe 2010). As a result of this state of flux, the umbrella term of programmed cell death or PCD will be used hereafter.

In plant (i.e., non-algal) and animal systems, cell death is also a basic feature of development (e.g., Noodén 2004; Bishop et al. 2011). In plants, PCD can be divided into two broad categories: environmentally induced or developmentally regulated (Greenberg 1996; Pennell and Lamb 1997; Palavan-Unsal et al. 2005; Gunawardena 2008; Reape et al. 2008; Williams and Dickman 2008). Environmentally induced PCD is an outcome of external biotic or abiotic factors. Examples of environmentally induced PCD include, but are not limited to, the hypoxia-triggered development of internal gas-filled spaces (lysigenous aerenchyma) (Gunawardena et al. 2001; Morgan and Drew 2004), and the hypersensitive response (HR) triggered by pathogen invasion (Heath 2000; Palavan-Unsal 2005; Khurana et al. 2005; Williams and Dickman 2008). The latter is an example of PCD for which an analogous process has been identified among multicellular algae (Wang et al. 2004; Weinberger 2007). Conversely, developmentally regulated PCD is a predictable event that occurs in response to internal signals. Developmentally regulated PCD typically removes cells to produce spaces (such as in xylem elements for water

transport, or the perforations in leaves of certain plants), or it removes mature cells, tissues and organs that have fulfilled their functions (Greenberg 1996; Pennell and Lam 1997; Palavan-Unsal et al. 2005; Gunwardena and Dengler 2006; Williams and Dickman 2008). Plant developmental processes that involve PCD for which analogous processes can be identified amongst the multicellular algae include the death and usually shedding of cells derived from root caps, root epidermis and trichomes (Greenberg 1996; Wang et al. 1996; Pennell and Lamb 1997; McCully 1999; Enstone et al. 2003; Palavan-Unsal et al. 2005; Hamamoto et al. 2006; Papini et al. 2010), leaf perforation formation (Gunawardena and Dengler 2006; Gunawardena et al. 2004, 2005), senescence and abscission (Greenberg 1996; Pennell and Lamb 1997; Taylor and Whitelaw 2001; Palavan-Unsal 2005; Lim et al. 2007).

With the exception of certain examples (for example, xylogenesis) in which PCD can be studied *in vitro* under controlled conditions, it is striking how little is actually known about developmentally regulated PCD in plants. Molecular details of plant PCD have been primarily obtained from cultured plant cells due to the difficulty in accessing and assessing cells in tissues of intact plants (Reape et al. 2008; Palavan-Unsal et al. 2005).

PCD has rarely been considered for multicellular algae. This is in spite of the occurrence of complex morphologies in which there may be very strict cell and tissue differentiation, and considerable cell death. Even in syntagmatic (i.e., pseudoparenchymatous) algal anatomies, with their fundamentally filamentous structure, cell differentiation is extensive. Thus multiple cell types occur that are specialized for photosynthesis, structural integrity and reproduction (e.g., Bold and Wynne 1985; Gabrielson and Garbary 1986). Development of these systems is often accompanied by cell death.

The purpose of this review is to demonstrate how multicellular (and unicellular – but functionally multicellular) algae provide a rich assemblage of developmental phenomena that would be appropriate as model systems for studies of PCD. While the modes of PCD in the sense of animal or terrestrial plant systems have been largely unstudied in algae, these developmental phenomena provide models that should be useful to cell biologists. Hence, the focus here is on endogenous, localized cell death that is associated with clearly defined morphogenetic patterns. We will consider these processes in the general context of PCD, and point out where additional evidence may suggest more specialized forms of PCD (e.g., APO or AUT). The relevant evidence to distinguish among the various forms of PCD include nuclear DNA fragmentation and laddering, occurrence of metacaspases and caspase-like enzyme activity, calcium ion flux, production of reactive oxygen species, specific changes in mitochondrial function and permeability, in organelle number and morphology and in cell vacuolation, as well as tonoplast rupture, plasmolysis and cell wall modification (Gunawardena et al. 2004, 2007; Morgan and Drew 2004; Reape et al. 2008; Reape and McCabe 2010). Since there are only two studies on macroalgae that considered even some of these syndromes (i.e., Garbary and Clarke 2001; Wang et al. 2004), we will refer to all of the algal developmental processes described here as simply PCD pending further study. Illustrations of the organisms, their authorities and many of the phenomena are available in the cited literature, and also on AlgaeBase (Guiry and Guiry 2011).

There is a literature on PCD and APO in diverse unicellular lineages. These include cyanobacteria (e.g., *Microcystis*, Ross et al. 2006), and various unicellular algae and protists (e.g., Gordeeva et al. 2004; Zuppini et al. 2007; Darehshouri et al. 2008; Affenzeller

et al. 2009). PCD has been considered an underlying regulatory process in phytoplankton populations (Franklin et al. 2006; Veldhuis and Brusard 2006). The cytology of cell death in these systems may be equivalent to those in multicellular organisms, and many of the same gene products and pathways may be involved. However, we largely exclude unicellular organisms from this review having rejected the analogy that a single free-living cell in a population is the equivalent of a single cell in a multicellular organism. Since PCD and APO were first identified and are best understood in multicellular organisms, evidence for these phenomena is best sought among analogous developmental processes in multicellular algae.

Thus this review deals with multicellular and macroscopic algae. Rather than being exhaustive, we provide selected examples of developmentally regulated cell death across the three primary assemblages of multicellular eukaryotic algae, i.e. Chlorophyta, Phaeophyceae and Rhodophyta. Unicellular forms such as *Acetabularia* will be considered only when differentiation produces structures that can be considered as clearly cell-like (e.g. hairs). Where possible, we will examine these developmental phenomena in the context of analogous features of plant systems (i.e., the terrestrial plant clade from bryophytes to flowering plants). Because of space constraints we have limited the discussion largely to vegetative processes and omit reproductive development. Where the plant systems have no apparent anatomical analogy in the algae (e.g., xylogenesis) we have not discussed them. Our review will provide a useful starting point for algal cell biologists to begin more definitive studies on these important and intriguing developmental patterns.

Spore germination

Spore germination has attracted the interest of phycologists because of its inherent importance in morphogenesis. While early 20th century phycologists lacked the media and technical expertise to complete the life histories of seaweeds, it became obvious that a variety of different ontogenies were present that could characterize different groups at a variety of taxonomic levels (e.g., Sauvageau 1918; Chemin 1937; Fritsch 1935, 1945). Thus various algae showed patterns of unipolar and bipolar germination as well as ontogenies in which cell walls were formed inside the original spore wall, the latter typically leading to a basal disc from which upright axes were formed. Of particular interest to this discussion are those forms with unipolar germination in which a single axis (typically a filament) is formed, and the original spore is left empty of cytoplasm, or if it retains cytoplasm, dies early in development.

Phaeophyceae

The spores of many groups of brown algae apparently undergo a process of empty spore germination (e.g. Sauvageau 1918; Fritsch 1945). In this process the spores settle onto the substratum, form a bulge on the side of the spore that develops into a germ tube into which cytoplasmic contents of the spore are extruded. This typically forms the first cell in a prostrate filament and, when all of the cytoplasm has been extruded into the germ tube, a septum is formed that cuts off the original spore wall from the initial filament (e.g., Hubbard et al. 2004). Accounts of spore germination in various taxa suggest that this germination and the formation of two cells may occur in the absence of mitosis.

In many species, the empty spore germination is associated with complete evacuation of the original spore. Even when complete evacuation of the spore cytoplasm does not occur (e.g., Toth 1976), the long-term survival of the original spore is doubtful.

Rhodophyta

Many red algae in diverse lineages have a developmental pattern in which spore germination proceeds by unipolar germination to form a filament (Chemin 1937). In some taxa all of the cytoplasm evacuates the original spore and forms the apical cell of the primary axis. This leaves behind an empty wall that usually breaks down over time. In other cases a mitotic division may occur and the spore is cut off from the developing filament. Here the original spore may or may not be long-lived, and often undergoes degeneration (e.g, Chemin 1937; Dixon 1973; Bouzon et al. 2005). Variation in the extent to which the original spore is evacuated is common at the infraspecific level, and cytoplasmic remnants may include a nucleus and some chloroplasts (e.g., Guiry et al. 1987).

Chlorophyta

The genus *Blidingia* shows several different zoospore germination patterns including empty spore germination in *B. minima* (Bliding 1963; Kornmann and Sahling 1978). In one form of *Blidingia minima*, i.e., *B. minima var. stolonifera* the empty spore development may be continued for several cells into the developing prostrate axis (Garbary and Tam 1989). The terminal cell can repeat the empty spore process several times to form a green terminal cell at the end of several 'empty' cells, or the apical cell may form a disc of cells. The later may generate one to several cells from the margin that grow along the substratum and produce further empty cells. These 'empty' cells have not been studied ultrastructurally, and it is unclear if there is any remaining cytoplasm in them when they are cut off, or if all of the cytoplasm is collected at the apical end prior to cytokinesis. Regardless, the formation of these anucleate cell wall remnants can be considered a form of PCD which may be unique to algae. This process can be interpreted as an ecological adaptation allowing the germinating spore to occupy a large basal area prior to the development of the erect axes (Garbary and Tam 1989).

Hairs

Algal hairs are extremely variable: they may be present or absent, unicellular or multicellular, secretory or absorptive, uninucleate, multinucleate or anucleate, photosynthetic or non-photosynthetic, produced once or many times from subtending cells, and they may be associated with either vegetative or reproductive development (Rosenvinge 1911; Feldmann-Mazoyer 1940; Fritsch 1945; Duckett et al. 1974; Whitton 1988; Pueschel 1990; Oates and Cole 1994; Delivopoulos 2002). Except for some specialized cases in which hairs have thick walls, hairs are typically short-lived and deciduous; hence they should provide excellent examples of developmental PCD. While unicellular hairs typically have tip growth like plant root hairs, multicellular hairs (e.g., trichoblasts in Rhodophyta) may grow by means of an apical cell or basal meristem (e.g., multicellular brown algal hairs); in the latter case the terminal cell is the oldest

in the hair. Multicellular hairs with a basal or intercalary meristem are typical of Phaeophyceae, and they are often associated with trichothallic growth in which vegetative tissues of fronds are added based on cell divisions at the base of the hairs (Graham and Wilcox 2000; Lee 2008). Terminal cells are often dead and this suggests that developmentally they are undergoing PCD. Because of their position at the ends or periphery of thalli, hairs are relatively easy to visualize and should provide simple model systems for study of algal cell death. A discussion of PCD in plant systems then follows the presentation of algal examples to provide a deeper context in cell biology.

Phaeophyceae

There are numerous examples of multicellular hairs in Phaeophyceae associated with the vegetative structure and morphogenesis of thalli from microscopic filaments (e.g. *Streblonema* species) to large fronds (e.g., *Desmarestia* species) (Fritsch 1945). In all cases, these hairs are deciduous and undergo PCD. While hairs in Phaeophyceae in general, and those in mature fronds of fucoids may function in nutrient uptake (e.g. Hurd et al. 1993; Steen 2003), here, we limit our discussion to the well-known case of hairs formed in fucoid embryogenesis. The formation of these apical hairs and their subsequent degeneration provides key landmarks in fucoid embryogenesis.

Following zygote germination to form a rhizoid cell and a thallus cell, the latter typically undergoes a series of cell divisions in which cells are undifferentiated and the embryo is merely a club-shaped mass of cells with basal rhizoids (McLachlan et al. 1971). After four to six days, a series of hairs have formed in an apical groove or pit. The hairs are multicellular and have basal meristems. Above the meristematic region, the hair cells undergo considerable elongation. After maturation, the entire hair is shed, although it is unclear if some or all or the cells in the hairs are already dead (about 10–15 days after zygote germination). Before being shed, one or more of these hairs are associated with an apical cell at the base of the apical groove. This indicates the completion of the meristematic differentiation and the end of embryogenesis. This process occurs in all fucoids, but has been described in numerous papers associated with differentiation of *Fucus*, *Ascophyllum*, *Himanthalia* and other genera (Moss 1969, 1970). Little is known about the cell biology of these hairs. Hair formation, development and abundance can be readily manipulated and modified based on a wide range of environmental characters, e.g., light, temperature, and medium composition (McLachlan 1974; McLachlan 1977; McLachlan and Bidwell 1983), indicating that they are a useful model system to study PCD.

Rhodophyta

In a comprehensive review of red algal hairs, Oates and Cole (1994) compare hair morphology and development across a wide range of red algae. They emphasize that these hairs are short-lived and deciduous. The single observation that might indicate PCD concerns the formation of a "large granular structure containing numerous longitudinally oriented striations" that form at the base of the hair in many species. The authors interpreted this structure as representing "degenerating cytoplasm" when the hair is no longer functional.

Hair morphogenesis in the filamentous red alga, *Audouinella hermannii* (Hymes and Cole 1983) is one of the best descriptions for this process. These uninucleate hairs are apoplastidic and have a large central vacuole with most of the cytoplasm, including the nucleus, near the hair tip. These thin-walled cells have an extensive endomembrane system with several layers of smooth and rough ER surrounding the nucleus. While the possibility of PCD was not specifically addressed, the altered morphology and staining of the nucleus in the most mature hairs is consistent with some reports of PCD in plant systems (Palavan-Unsal et al. 2005; Gunawardena et al. 2004).

Judson and Pueschel (2002) described the ontogeny of hairs and their associated cells (a trichocyte complex) in the coralline red alga, *Jania rubens*. This paper clarified the hair ontogeny in relation to the surface structure and anatomy described by Garbary and Johansen (1982) and Pueschel et al. (2002). Not only are the hairs deciduous in *J. rubens*, but during their ontogeny a specialized crown cell is formed at the thallus surface. The developing hair then grows through the crown cell and its remnants remain in place during subsequent hair formation from the underlying cortical cell. Judson and Pueschel (2002) do not describe the state of the nucleus in these cells although they contain abundant endoplasmic reticulum or were filled with "amorphous material". It is unclear whether these cells die before they are penetrated by the growing hair or as a consequence of that penetration.

Red algae in the family Rhodomelaceae often have multicellular, branched, hair-like structures termed trichoblasts. These branch systems (i.e. trichoblasts) are typically colourless, or at least poorly pigmented, and they are usually fragile structures that are deciduous. Like the unicellular hairs described above, trichoblasts have not been well studied. The most comprehensive ultrastructual study by Delivopoulos (2002) gives an account of morphogenesis in *Osmundea spectabilis*, although this account stops at trichoblast maturity and does not attempt to deal with degeneration or PCD. The only study of trichoblasts that examined these structures in the context of PCD is in *Polysiphonia harvei* (Garbary and Clarke 2001). Here the trichoblasts are formed as lateral systems just below the apex of each vegetative branch. They undergo rapid cell divisions to form all of the cells in the trichoblast. As the vegetative branch grows, cells in the trichoblast elongate. Thus there is a gradation of trichoblast age and developmental state in relation to a vegetative branch apex. Garbary and Clarke (2001) demonstrated that trichoblast cells were undergoing PCD. Following the mitotic and cytokinetic events that formed these cells close to the branch apex, the nuclear DNA was in fact degrading while the cells were undergoing enlargement. Indeed, in the largest cells, staining with DAPI (4′,6-diamidino-2-phenylindole) was unable to show that nuclei were even present. This paper used terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) to show that DNA fragmentation was in fact taking place.

Chlorophyta

Species of *Acetabularia* have been widely studied by developmental biologists, indeed whole volumes have been written on morphogenesis and cell biology of the genus (Puiseux-Dao 1970). *Acetabularia* is a unicellular alga (e.g. Bold and Wynne 1985; Mandoli 1998; Dumais et al. 2000; Graham and Wilcox 2000; Berger and Liddle 2003). This interpretation is based on the coenocytic nature of the stalk that makes up the vast proportion of the cytoplasmic contents, and the presence of a single nucleus located at the base prior to reproductive development. Such an interpretation ignores the

development of the whorls of hairs that are successively formed at the stalk apex and then shed, leaving scars on the thallus surface (Solms-Laubach 1895; Gibor 1973; Ngo et al. 2005). Thus the vegetative state of *Acetabularia* might be better characterized as being uninucleate and multicellular. *Acetabularia* hair growth and development has been extensively studied. The hairs are initially cytoplasmic extensions of the stalk apex but become separated by an incomplete wall septum which must become completely occluded prior to hair shedding (Ngo et al. 2005). The extent to which cytoplasmic contents remain in the hairs when they are shed is unclear. The formation and shedding of these anucleate hairs of *Acetabularia* and other dasycladalean algae, like the anucleate cell remnants in germinating zoospores of *Blidingia minima* var. *stolonifera,* involves the subdivision of a nucleated cell, essentially via cytokinesis in the absence of mitosis. As previously indicated, this may be a form of PCD unique to the algae.

Unlike the anucleate hairs of *Acetabularia* spp., the hairs of *Sporocladopsis jackii* are multicellular, unbranched and clearly nucleate, at least when first formed (Garbary et al. 2005a). The nuclei in the hairs are not apparent in the mature structures and they appear to degrade. The lifespan of the hairs is not known and while dehiscence has not been observed, this is their likely end state.

Plant hairs

Like algae, plants also have hair-like structures known as trichomes. Trichomes are outgrowths or extensions of the epidermis and are generally found on leaves, stems and roots, where they are referred to as root hairs (Pennel and Lamb 1997; Evert 2006). Trichomes on shoots may be living or dead at maturity (Greenberg 1996; Evert 2006). Dead trichomes may protect plants from high intensity light or reduce water loss (Greenberg 1996). Trichome death may also be environmentally induced, for example by a pathogen (Wang et al. 2009). Papini et al. (2010) have investigated the ultrastructural development of *Tillandsia* spp. (Bromeliaceae) of the multicellular shoot epidermal trichomes. These are commonly used for the absorption of atmospheric water, minerals and organic nutrients. Water coming from outside can pass through the distal trichome cells via a symplastic route and subsequently reach mesophyll cells. Within the last stage of trichome ontogeny, when the hair is reaching maturity, the distal trichome cells die via what appears to be PCD.

In contrast to shoot trichomes, all root hairs are considered to be short-lived under natural growing conditions (Evert 2006), although there is surprisingly little data on root hair death, apart from the presumed death of hairs in roots in which there is developmental shedding of the epidermis (see section on epidermal shedding). One exception is a report of developmental PCD in root hairs and root cap cells found in the determinate primary roots of certain cacti using the TUNEL assay for DNA fragmentation (Shiskova and Dubrovsky 2005).

Perforations

Perforation formation in plants

The development of complex leaf shapes during leaf morphogenesis includes the formation of holes or perforations, and this forms a rare and unique type of developmentally