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How Do Giant Plant Cells Cope with Injury?—The Wound Response in Siphonous Green Algae

Review Article

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Contents

1. Introduction

Injury to a multicellular plant is generally not fatal, even if many of the cells are damaged and subsequently die. This is the result of wound-induced formation of protective and regenerative tissues adjacent to the wound (BUGGELN 1981, LIPETZ 1976). Repair of individual cells is, therefore, of minor importance. However, survival of an organism composed of just a few very large cells or, in the extreme, of a single giant cell, obviously depends on the integrity of the individual cell being maintained. It would, therefore, be anticipated that a different mechanism has evolved in such giant-celled organisms to cope with physical damage [1]. Several distinct phylogenetic branches of algae have evolved a giant-celled architecture; *e.g.*, the *Charophyta*, some orders in the *Chlorophyta*, several genera in the *Rhodophyta* and two genera in in the *Chrysophyta* (Tab. 1; for overview, see BOLD and WYNNE 1985, PICKETT-HEAPS 1975). The extreme situation of one large cell comprising the whole organism is found in the siphonocladalean alga *Ventricaria* OLSEN and WEST 1988, formerly *Valonia ventricosa*, EGEROD 1952) and in all of the siphonous green algae of the two orders, *Dasycladales* and *Caulerpales*. These examples demonstrate the relative evolutionary success of such a peculiar thallus architecture, seemingly prone to fatal injuries in comparison to multicellular algae. According to the fossil records, some siphonous green algae have

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[1] For a general account of the physiology of giant algal cells see HOPE and WALKER (1975).

Table 1. *Widespread occurrence of giant-celled architecture within the algae**

Thallus composed of indeterminate numbers of cells		
Charophyta		
Charales:	5 genera (208 species)	Wood 1965
Chlorophyta		
Chlorococcales:	Hydrodictyon (5 species)	Marchant & Pickett-Heaps 1970
	Protosiphon	Pocock 1960
	Characiosiphon	Thomas 1971
		Stewart 1971
Siphonocladales:	15 genera (70 species)	Olsen-Stojkovich 1986
Rhodophyta		
Ceramiales:	Griffithsia	Duffield et al. 1972
	Halurus	
	Monosporus	
Chrysophyta		
Vaucheriales:	Botrydium (6 species)	Vischer 1938
	Vaucheria (40 species)	Goetz 1897
Thallus composed of a single cell		
Chlorophyta		
Siphonocladales:	Ventricaria	Olsen & West 1988
Dasycladales:	10 genera (37 species)	Valet 1969
Caulerpales:	ca. 20 genera (>350 species)	Ducker 1967
		De Paula & West 1986
		Meinez 1980a,b; Neumann, 1974
		Olsen-Stojkovich 1985
		Silva & Womersley 1956
		Weber van Bosse 1898

*) Classification according to Bold & Wynne 1985
Estimation of species numbers based in part on Melchior & Werdermann 1954

maintained their ecological niches and remained essentially unchanged morphologically for 140 million years (Cretaceous era: DELOFFRE and GENOT 1982). Siphonous and giant-celled architecture reaches back well into the Silurian era (ca. 440 million years, BASSOULET *et al.* 1983, SCHOPF 1970, TAPPAN 1980).

The physical dimensions of giant-celled algae are in or somewhat below the millimeter range, but most siphonous green algae can go much beyond that, some (*e.g., Caulerpa* species) becoming truly gigantic (diameter > 1 cm, length > 1 meter). The siphonocladalean genus *Ventricaria* with a diameter up to 10 cm can certainly also be regarded as a giant cell in comparison to the rest (EGEROD 1952, OLSEN and WEST 1988).

Common to most giant-celled algae is an increase in the number of nuclei per cell, resulting in syncytia. Survival from injury such as loss of parts of the cell obviously requires nuclei to remain in the truncated cell after an accidental amputation. Interestingly, a few siphonous green algae remain uninucleate throughout much of their life cycle, *e.g., Acetabularia* (*Dasycladales*), and one wonders what additional factors have contributed to the guaranteed survival of such a genus.

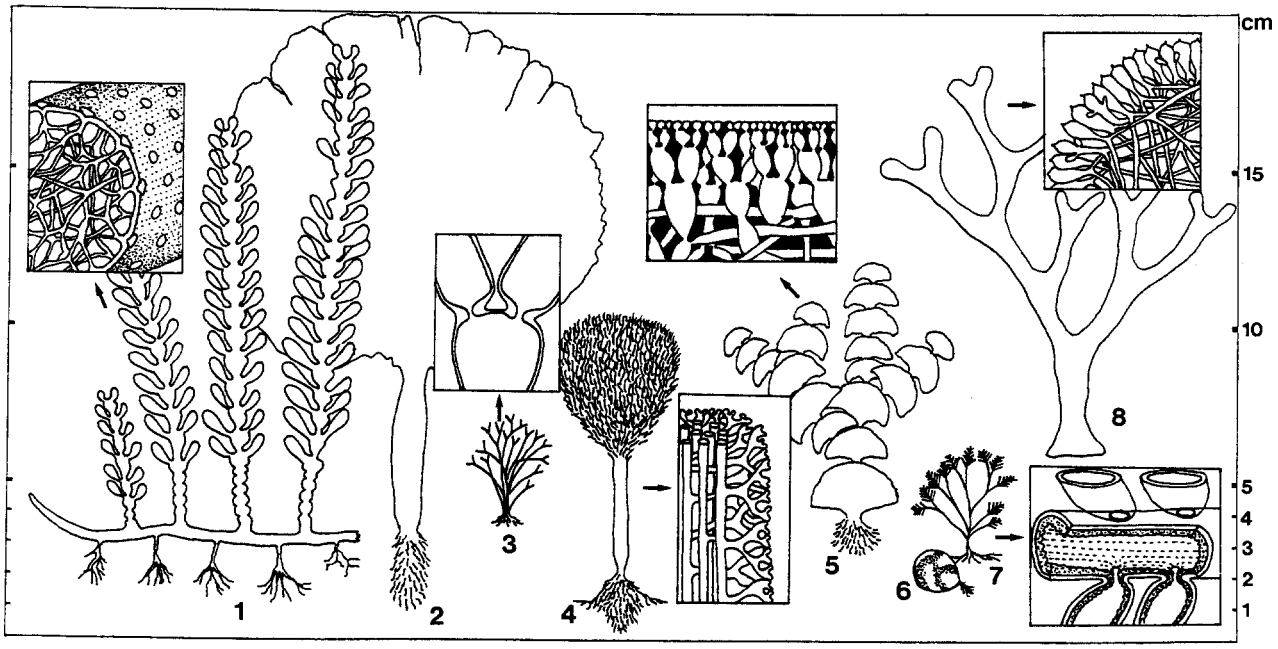

Fig. 1. Different strategies of thaluus reinforcement in the siphonous green algae. Species of some selected genera are schematically drawn to scale to demonstrate the giant dimensions of some of the coenocytic thalli. In the insets the principle of thallus construction is depicted. The examples are: *1 Caulerpa cactoides*, the inset shows a view into the cut open frond displaying the network of intracellular cell wall trabeculae. *2 Avrainvillea gardineri* (after OLSEN-STOJKOVICH 1985), tightly interwoven network of siphons differentiated into medulla and cortex build this giant, blade like thallus. *3 Chlorodesmis sp.*, bush of regularly, dichotomously branching siphons; inset shows constrictions in a dichotomous branch point. *4 Penicillus capitus* (after FRIEDMANN and ROTH 1977), siphons form a pseudotissue in the stem as shown in the inset, but separate from each other to form the bushy head. *5 Halimeda tuna*, segmented, heavily calcified thallus. The inset shows part of the cut open cortex with utricles and medullary siphons. Spaces between the siphons are filled with calcium carbonate. *6 Halicystis ovalis*, a single ovoid cell depicted in the state when the layer of cytoplasm differentiates into internal gametangial reticulum. *7 Bryopsis plumosa*, gametophyte. Inset shows simple cellular architecture. When side branches differentiate into gametangia, each constriction will be closed by a gametangial plug. *8 Codium fragile*, uncalcified multiaxial system of siphons. In contrast to *Penicillus*, one thallus is usually not a single coenocyte but originates from several codifferentiating zygotes (FRIEDMANN and ROTH 1977). Inset shows part of a cross-section through the thallus. Medullary siphons are frequently constricted and plugged

Physical threats to the integrity of the giant celled algae are widespread in the maritime environment. Among them are water currents and wave actions, sand abrasion, grazing by fishes, gastropods, echinoderms and crustaceans. Additional factors include invasions by pathogenic fungi, bacteria, algae and protozoa (discussed in NORRIS and FENICAL 1982). Many of the interactions between grazers and dietary alga are highly specific (JENSEN 1980, 1981) and may be regarded as another example of co-evolution between animals and plants.

What constitutes the arsenal of defense mechanisms that has enabled giant-celled algae to survive? In the following account, special emphasis will be laid on the siphonous green algae[2], where the lack of cross walls combined with gigantic cell dimensions, makes extreme demands on the reliability of the defense mechanisms. An answer to this question is sought by first studying the principles of thallus construction in this group of algae and then by examining the various responses that can be observed after an injury has occurred.

[2] Here, the *Dasycladales* and *Caulerpales* are, collectively, referred to as siphonous green algae (formerly *Siphonales*). Other orders within the coenocytic green algae are the *Acrosiphonales, Cladophorales* and *Siphonocladales*. The latter two may be merged into one group: the "*Siphonocladales-Cladophorales* complex" (OLSEN-STOJKOVICH 1986). Experimental data on the wound response in the *Acrosiphonales* are not yet available but some selected species within the S/C-complex have been studied in the recent past and will be discussed in chapter 3.2.3. It is not the intention of this review to contribute to the dispute on phylogenetic relationships within the coenocytic green algae. However, it appears to be relevant to the topic of wound healing to note that the three orders *Acrosiphonales*, *Cladophorales* and *Siphonocladales* differ from the siphonous green algae by their thallus construction, cell growth, cytoplasmic architecture and by the lack of cytoplasmic streaming. Most of these aspects are excellently summarized in OLSEN-STOJKOVICH (1986).

2. Basic Thallus Construction of the Siphonous Green Algae

Despite the diverse macroscopic morphologies in the siphonous green algae (Fig. 1), the basic structural element of the thallus is the siphon, a continuous tube lacking cross walls. It consists of a cylindrical cell wall lined on the inside by a thin layer of cytoplasm with the central vacuole occupying most of the cell's volume. Organelles within the cytoplasmic layer are commonly transported over long distances throughout the cell. The siphon grows at the tip by polar growth and branches according to species-specific patterns. The base can differentiate likewise to form rhizoids and holdfasts (for overview, see BOLD and WYNNE 1985).

This basic construction allows species to grow to dimensions of 0.3–0.5 mm in diameter and several centimeters in length. Good examples for this type are the genera *Bryopsis* (Fig. 1.3; NEUMANN 1974) and *Chlorodesmis* (Fig. 1.7; DUCKER 1967).

Reinforcement of mechanical integrity by addition of further cell wall layers combined with a swelling of the cylinder to the form of a sphere results in species which can reach a few centimeters in diameter. One example is *Halicystis* (Fig. 1.6), whose remarkable stability and elasticity has been attested by HOLLENBERG (1935): "... the alga will bounce like a rubber ball, when thrown against a hard surface." A second type of reinforcement is found in the *Codiaceae*, where the dimensions of the siphons remain below the millimeter range but become highly branched and tightly interwoven to form a multiaxial thallus (Fig. 1.8; SILVA and WOMERSLEY 1956). Additional strength is provided to such a thallus if the spaces between the branches become encrusted with calcium carbonate as in several genera of the *Udoteaceae* (Fig. 1.5; BOROWITZKA and LARKUM 1977, MEINEZ 1980 a, WILBUR *et al.* 1969). Finally, another peculiar way to allow for giant growth has been adopted by the *Caulerpaceae*. The siphon in this family is modified by the formation of interior cell wall extensions called trabeculae, which protrude into the lumen of the cell, where they branch and interconnect to form an intricate internal skeleton. Species in this family grow to truly gigantic dimensions (an example is given in Fig. 1.1; MEINEZ 1980 b, MENZEL and GRANT 1981, WEBER VAN BOSSE 1898).

These principal characteristics of thallus construction in the siphonous green algae set the physical limits for growth and strategies of protection against damage.

3. The Wound Response

When a siphon is severed, a complex, multistep response is triggered which encompasses an almost instant repair of damage to the cytoplasm followed by a transient sealing of the wound by a plug and the subsequent formation of a new cell wall. At least 6 distinct repair steps can be recognized in principle. The length and intensity of each step as well as the plug precursor materials involved, may vary greatly between families.

These steps are:

1. Repair of membrane damage.
2. Contraction of the cut edge of cytoplasm.
3. Extrusion of plug precursor material stored in the vacuole.
4. Restoration of turgor after the cytoplasmic aperture has closed.
5. Formation of a wound plug by crosslinking of plug precursor material followed by compaction and hardening of the plug.
6. Formation of a new cell wall underneath the internal face of the wound plug.

3.1. Repair of Membrane Damage

The cytoplasmic layer in the siphon is delimited inwardly by the tonoplast and outwardly by the plasma membrane. In some families (*Caulerpaceae, Udoteaceae*), the tonoplast is extensively invaginated forming a lacunal system. When the cell wall is ruptured both membrane systems are instantly affected and the cytoplasmic integrity challenged. For simplicity consider a straight, transverse cut through a siphon. In this type of injury an open-ended cylinder is created. The central vacuole is then in continuity with the external medium and the cytoplasmic layer will undoubtedly be damaged along the cut edge (Fig. 2 *a*). Unfortunately, current observational techniques are not sensitive enough to capture these changes in terms of the biophysical parameters or of the fine structural changes involved. Hence, it is not known how the damaged membranes actually seal. One possibility is that the tonoplast and plasmalemma never rupture, but deform and instantaneously fuse maintaining continuous covering. The cytoplasm at the cut edge, for instance in *Bryopsis* (*Caulerpales*) and *Acetabularia* (*Dasycladales*), appears sealed by membranes only seconds after wounding as judged from electron microscopy (unpublished data). Such a rapid plasma membrane repair has been documented in the slime mold *Physarum*, another giant, coenocytic organism (WOHLFARTH-BOTTERMANN and STOCKEM 1970). On the other hand, it has also been reported that in *Caulerpa simpliciuscula* (*Caulerpales*) damage to the cytoplasm close to the wound can result

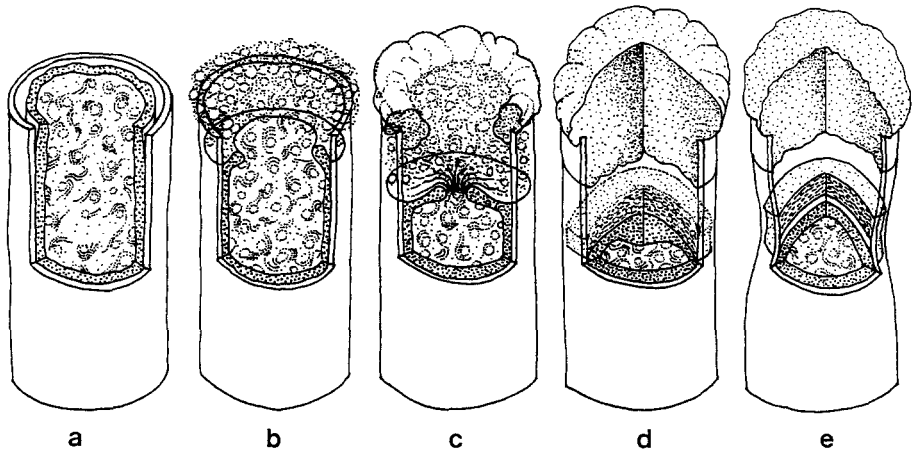

Fig. 2. Schematic sequence showing the principle events following wounding in *Bryopsis*. *a* Cut open siphon depicting the single layer of cytoplasm and plug precursor material filling the central vacuole. Not drawn to scale. *b* 15–30 seconds postwounding. Beginning of retraction and concentric closure of cytoplasmic layer. During contraction, plug precursor is expelled, swells and adheres to the edge of the cut cell wall. *c* About 1 minute postwounding. Contraction is nearing completion, plug precursor coagulates, and wound plug begins to form. *d* 5–10 minutes postwounding. A contraction nodule remains in the cytoplasm, where the aperture had closed, and may be visible for several minutes postwounding. Wound plug develops internal and external layers; stage of intense cross-linking and hardening. *e* > 60 minutes postwounding. New cell wall has formed underneath the internal wound plug and starts expanding. Cell wall which is not in close contact with cytoplasm will degenerate.

in massive fragmentation producing a large number of vesicles, which are assumed to secondarily fuse, thus restoring cytoplasmic integrity (DREHER *et al.* 1978). A detailed *in vivo* analysis of this process by using video enhanced microscopy techniques has not yet been attempted. *De novo* synthesis of plasma membrane has also been suggested as a mechanism of cytoplasmic repair in the *Udoteaceae* (MARIANI-COLOMBO *et al.* 1980).

Obviously, particular membrane properties in some species of siphonous green algae are important for the wound response. The tonoplast in the giant internodal cells of characean algae spontaneously disintegrates when cells are cut open and perfused with EGTA-containing media (EGTA = ethyleneglycol-bis-(β-aminoethyl ether) − N,N,N',N'-tetraacetic acid; TAZAWA *et al.* 1976). In *Acetabularia* (*Dasycladales*), a similar experimental procedure does not produce this effect (KOOP 1981, MENZEL 1987 b). Instead, the cytoplasmic layer remains intact and functional, as judged from persistent cytoplasmic streaming and contractility. This suggests that completely different membrane properties characterize these two algae, but what these properties are remains to be investigated.

3.2. Cytoplasmic Contraction

3.2.1. Contraction Localized to the Wound

While sealing of the membrane at the cytoplasmic cut edge cannot be observed directly, the subsequent reaction of the cytoplasm adjacent to the cut edge can easily be observed. Three species, *Acetabularia* (MENZEL 1987 b), *Bryopsis*, and *Caulerpa* (MENZEL 1982, 1987 b), representative of three distinct families of siphonous green algae, are presently under intense study with respect to wound contraction. Some earlier observations on *Halicystis* (MENZEL 1979 a), *Caulerpa* (GODDARD and DAWES 1983) and *Bryopsis* (BURR and WEST 1970) are also available. A coherent picture is emerging from these studies with respect to the mechanism involved in contraction.

Returning to the example of the transversely cut siphon, the first detectable event after the cut is a localized contraction at the cut edge, visible as a swelling and retraction away from the wound. Shortly thereafter, the contracting edge symmetrically detaches from the wall and forms a contraction ring that concentrically closes like an iris aperture (Figs. 2 *b* and *c*). During this process more cytoplasm pulls away centripetally from the cell wall with the result that the entire protoplast retracts from the wound. Following closure of the aperture a contraction nodule will remain in the center for several minutes (Fig. 2 *d*). The process of cytoplasmic wound closure takes only 30 to 60 seconds in *Acetabularia* or *Bryopsis* (MENZEL 1987 b), and up to several minutes in *Caulerpa* (GODDARD and DAWES 1983).

In experiments using the cap ray chambers of *Acetabularia (Polyphysa) cliftonii* (MENZEL 1987 b), prelim-

inary evidence suggests that external calcium stimulates contraction. Cap rays of *Polyphysa* can be equilibrated in calcium-free artifical sea water medium and then induced to contract by adding calcium to a concentration of 1 μM [free calcium] or higher. The cut edge is the region of the cytoplasm where entry of calcium is facilitated. Calcium-mediated signaling is sensitive to calmodulin inhibitors. Wound contraction is generally insensitive to microtubule inhibitors (see also MARIANI-COLOMBO 1984) but is blocked by cytochalasins, indicating an involvement of actin but not micotubules in the mechanism of contraction. With the recently adopted immunofluorescence technique for the visualization of cytoskeletal elements in siphonous green algae (MENZEL 1986, MENZEL and SCHLIWA 1986), it is now possible to show that actin filaments form conspicuous foci in contraction centers during wound healing (MENZEL 1986, 1987 b). These preliminary observations are very promising and indicate that the motility observed after wounding is a consequence of dynamic reorganisation of the actin cytoskeleton. The actual mechanism of force production remains unknown.

3.2.2. Contraction Leading to Protoplast Formation

Although the transverse cut, considered here for simplicity, can occure in nature as a consequence of grazing by fish with sharp mouth parts, less specifically directed recurring physical damage (*e.g.*, sand abrasion) might be a frequent cause of injury. This is potentially devastating for those species of siphonous green algae with a more delicate thallus construction such as *Derbesia* and *Bryopsis*. However, *Bryopsis* has a remarkable ability to regenerate even from small droplets of protoplasm liberated after extreme physical damage. Japanese workers have shown that protoplasts of a few micrometers in diameter containing at least one nucleus along with other essential organelles can form a cell wall and regenerate complete algae (TATEWAKI and NAGATA 1970). This feature offers an easy way to produce axenic cultures of *Bryopsis* (MIZUKAMI and WADA 1981). External calcium has also been identified as the signal that triggers actin based-contraction and formation of protoplasmic spheres (MENZEL and SCHLIWA 1986).

In contrast, *Acetabularia* cytoplasts derived by gentle extrusion from the cell will not regenerate complete algae, because a nucleus is missing. They can, however, be kept alive for some time and will display lively cytoplasmic motility (GIBOR 1965).

3.2.3. Cytoplasmic Contraction in Siphonocladalean Green Algae

Many giant-celled members of the *Siphonocladales* also seem to have envolved contractile mechanisms as an integral part in the wound response. In a survey of 12 species LA CLAIRE (1982 b) identifies several different types of cytoplasmic contraction. Contractile behavior in one group (*Boodlea, Chamaedoris, Ernodesmis,* 3 species of *Struvea*) resembles somewhat the type described above for siphonous green algae: The injured cytoplasm retracts away from the wound site for variable distances and the open-ended cytoplasmic cylinder closes centripetally. But this reaction is very slow, on the order of 30 to 60 min, whereas wound contraction in siphonous green algae (of similar cell diameter) takes only seconds. Even in the large cap rays of *Polyphysa* (> 1 mm diameter) wound closure takes less than 3 minutes. In another group of siphonocladalean species (*Boergesenia, Chladophoropsis, Siphonocladus, Valonia macrophysa, Ventricaria*) wound induced contraction is not restricted to the cut edge but involves variable parts of the entire cytoplasm. This produces quite heterogeneous effects ranging from the formation of a few separately contracting units (*Chladophoropsis*) to reticulation and subsequent collapse of the entire cytoplasm (*Siphonocladus*) or to the formation of a multitude of small spheres (*e.g., Boergesenia,* see also ENOMOTO *et al.* 1972, ISHIZAWA *et al.* 1979, O'NEIL and LA CLAIRE 1984). Independent of the size, the contracted cytoplasmic fragments remain within the injured "mother"-plant, recover to form turgescent cytoplasmic spheres and eventually build new cell walls around themselves. This phenomenon is also part of the developmental sequence leading to new daughter cells, referred to as segregative division *sensu strictu* (OLSEN-STOJKOVICH 1986).

Some species of *Siphonocladales* obviously do not recover from injury, such as *Valonia aegagropila* (LA CLAIRE 1982 b). The significance of this apparent heterogeneity in the wound response is not known. It will be necessary to extend the survey of wound contraction behavior through the remaining species of the *Siphonoclades* and it will be important to include the *Acrosiphonales* and *Cladophorales* before an assessment of this phenomenon can be attempted.

Tremendous progress has been made in the study of the contractile mechanism: Wound contraction in siphonocladalean algae is also triggered by calcium influx and calcium signaling seems to be mediated by calmodulin (LA CLAIRE 1983). The mechanism of con-

Table 2. *Plug precursor material in four major families of siphonous green algae*

family	vacuolar inclusions	chemical composition	reference
Caulerpaceae	1. osmiophilic globules	lipids?	1
	2. dense granular bodies	phenolics?	1
	3. network of fine fibers	ß-glucanes	2
		carbohydrate	3
		proteoglycanes	4
		sulfated poly-saccharides	1,5,6,7
		inorganic salt	1,3,5
Udoteaceae	1.osmiophilic globules	phenolic ?	8
	2.fibrous, spherical bodies (SBs) ⌀ 2-5µm	sulfated poly-saccharides	8,9,10,11 12, 13
	3.tubules (⌀ 9-12nm) in paracrystalline arrays	proteinaceaous	8
	4.mineral crystals	Ca-oxalate	14
Derbesiaceae	1.osmiophilic globules	phenolic ?	15
	2.tubules (⌀ 35nm) in paracrystalline arrays	proteinaceous	15,16
	3.fibrous spheres ⌀ 20-100 µm	proteinaceous	15,17
	4.disperse fibrous material	polysaccharide	15
Dasycladaceae	1. osmiophilic material of various forms	phenolic ?	15,18,19
	2. cubic crystals	proteinaceous indole-derivatives	15,20
	3. disperse, granular-fibrillar material	protein plus polysaccharide	15,18

References

1 Lohr 1975
2: Howard et al. 1976
3: Dawes & Goddard 1978
4: Dreher et al. 1978
5: Goddard & Dawes 1983
6: Dreher et al. 1982
7: Hawthorne et al. 1981
8: Menzel 1987a
9: Borowitzka & Larkum 1977
10: Wilbur et al. 1969
11: Turner & Friedmann 1974
12: Mariani et al. 1980
13: Mariani & Postai 1978
14: Friedmann et al. 1972
15: Menzel 1982
16: Burr & West 1971
17: Burr & Evert 1972
18: Menzel 1979c
19: Menzel et al. 1983
20: Tandler 1962

traction was further probed by using a detergent permeabilized cell model of *Ernodesmis*. In these experiments, LA CLAIRE (1984) conclusively demonstrated that the cytoplasm undergoes contraction if the level of calcium is elevated to greater than 1 µM in the presence of ATP at millimolar concentration. Recent immunofluorescence studies further indicate that contractile movement is associated with the formation of actin bundles (LA CLAIRE, personal communication). Despite important differences, it can be concluded that many of the giant-celled *Siphonocladales* and the siphonous green algae share an essential cytoplasmic survival mechanism, *i.e.*, actin-based contraction. This is in marked contrast to some other giant-celled algae such as the *Charophytes* (KAMIYA 1986) or the *Rhodophyte Griffithsia* (WAALAND and CLELAND 1974), whose cytoplasm simply collapses after major injury, eventually leading to cell death.

3.3. Plug Precursor Material

Upon closure of the wound aperture, the surface of the cytoplasm is still exposed and unprotected against the environment. Under favorable conditions and given some time, a new wall may eventually form and provide protection. However, most siphonous green algae have adopted means to transiently protect the cytoplasm by forming a wound plug, before a new cell wall is deposited.

3.3.1. Biogenesis and Storage of Plug Precursor Material

For nearly a century, scientists have recognized striking cytoplasmic motility in siphonous green algae and the presence of structured material in the lumen of the vacuole that was expelled when a siphon was cut (ERNST 1904, FIGDOR 1910, HABERLANDT 1929, KLEMM 1894, KÜSTER 1899, 1925, NOLL 1899). This material was originally thought to consist of storage products, but later it was hypothesized that some of the mysterious material was specifically synthesized and stored as "patch" material. Analogous to platelet "soft clot" of blood it was suspected that the plug substratum was preassembled to ensure a rapid clotting response upon discharge.

Since the first discovery of this plug material (NOLL 1899), a large body of experimental work has accumulated that deals with its various structural and chemical aspects covering all the major families in the siphonous green algae except the *Phyllosiphonaceae* and *Dichotomosiphonaceae*. In the majority of the studies, fine structural and histochemical methods have been employed (BOROWITZKA and LARKUM 1977, BURR and WEST 1970, BURR and WEST 1971, BURR and EVERT 1972, DREHER *et al.* 1978, FRIEDMANN *et al.* 1972, FRIEDMANN and ROTH 1977, MARIANI-COLOMBO *et al.* 1980, MARIANI-COLOMBO and POSTAI 1978, MENZEL 1979 b, 1980 a, b, 1982, 1987 a). A more rigorous biochemical characterization has been attempted only in the case of *Caulerpa* (DREHER *et al.* 1982, DAWES and GODDARD 1978, HAWTHORNE *et al.* 1981, HOWARD *et al.* 1976, LOHR 1975). It is remarkable, in this context, that a report on the presence of lectins in *Bryopsis* and other siphonous green algae (ROGERS *et al.* 1980) has remained without impact on the various attempts to analyze the plug precursor material. Nevertheless, the major conception evolving from these studies is that the fine structural nature of the plug precursor material varies considerably among families but is well-conserved within a family. The composition of these various materials is heterogeneous including at least three major chemical classes: phenolic compounds, polysaccharides, and proteins (Tab. 2).

It is well established that all these materials are stored in the vacuole, but their biogenesis is often unclear. The most detailed developmental sequence has been presented by BURR and WEST (1971) for the formation of the plug precursor in *Bryopsis hypnoides*. This material originates in the cytoplasm in compartments of the endoplasmic reticulum which swell up into large vesicles with an amorphous content. The amorphous material is then discharged into the vacuole where it undergoes considerable structural modifications involving a transitory paracrystalline form. The end product is a granular-fibrillar mass.

Similar observations have been made in other *Bryopsis* species (MENZEL 1982). A pathway from the cytoplasm into the vacuole, involving some steps of reassembly, is also indicated for plug precursors in *Udotea* (MARIANI-COLOMBO *et al.* 1980) and *Chlorodesmis* (MENZEL 1987 a). However, it must be remembered that at present we know very little about the other siphonous green algae in this respect. Biosynthetic pathways, assembly steps involving the formation of plug precursor components, and the significance of structural and chemical differences in the various plug precursor components are poorly understood among the different families.

3.3.2. Extrusion of Plug Precursor Material

Plug precursor material is commonly present in abundance throughout the length of the siphon so that each part of the thallus will have sufficient reserves if wounding occurs. Two successive phases of extrusion may be distinguished. In the first phase high turgor results in explosive extrusion at the moment of injury. Although large amounts of plug precursor material are released, most is lost by dilution in the surrounding medium. In the second phase, a more controlled extrusion is observed. Slow accumulation of plug precursor at the cut cell wall edge is driven by cytoplasmic contraction, which stops when the hole in the cytoplasm is closed. Extrusion of vacuolar material in a stream of vacuolar fluid may serve three separate functions. First, it transports plug precursor to the wound site. Second, it prevents accidental penetration of microorganisms[3] into the lumen of the cell. Third, it may prevent, or at least minimize, the influx of seawater into the vacuole. However, a considerable perturbation of ion balance at the wound site and of membrane potentials throughout the cell inevitably occurs during wounding and it can be anticipated that ion transport mechanisms are involved in restoring the cell's original ionic equilibrium.

[3] In this context it may be worthwhile to note, that many siphonous green algae contain bacteria in the vacuolar compartment (discussed in MENZEL 1987 a). These microorganisms are commonly referred to as endosymbiontic bacteria although the metabolic relationship between the bacteria and the algae is unknown. It is conceivable that over time bacteria and other microorganisms may have escaped the defense mechanisms of the algae and may have been able to establish a relationship of mutual tolerance, even symbiosis.

3.4. Restoration of Turgor

Siphonous and giant-celled green algae have proven very useful as model organism in the study of membrane properties, and in the elucidation of ion transport mechanisms in plant cells (GRADMANN and MUMMERT 1980). Siphonous green algae, *Acetabularia* in particular, maintain an unusually high potential difference across the plasmalemma by an electrogenic chloride pump (GRADMANN *et al.* 1982, GRADMANN 1984). Moreover, regular hyperpolarizations of the plasma membrane at the cell's apex create rhythmic action potentials that propagate basipetally (GRADMANN and MUMMERT 1980).

These action potentials are indicative of the establishment of polarity in the cells. Upon wounding, spatial polarity of the membrane potential is affected and action potentials are interrupted (NOVAK and BENTRUP 1972, NOVAK and SIRONVAL 1975). It can be predicted that regulation of other ion fluxes (ALLEN and BOWLES 1985) is also affected.

An immediate problem for the cell will be a massive influx of calcium into the vacuole and cytoplasm around the wound site. This increase serves the important function of triggering a quick cytoplasmic response and is hypothesized to be important for coagulation of plug precursors (see below). However, in order to terminate cytoplasmic contraction, calcium ions must be pumped out again before cytoplasmic functions can return to normal levels. This may be accomplished by outwardly directed calcium-pumps (MACKLON 1984, LA CLAIRE 1982 a).

Recovery of normal ion balance is indicated by restoration of turgor, which usually takes only 10–30 seconds. In *Acetabularia* as well as *Bryopsis* (BURR and WEST 1971), increase in turgor results in hyperextension of the protoplast which may bulge from the wound site. In *Acetabularia*, it may even extend beyond the siphon to form a protoplasmic bubble (GIBOR 1965). The protoplast eventually bursts and this in turn triggers a new wound contraction. This process can go back and forth several times and, each time, more plug precursor leaves the vacuole and contributes to the formation of a wound plug (BURR and WEST 1971, MENZEL 1982, 1987 b). Repetitive expulsion of plug precursor thus seems to be a major component in the mechanism of wound repair in some siphonous green algae. To date, it has not been described in the *Udoteaceae* (MENZEL 1987 a) or *Caulerpaceae* (DREHER *et al.* 1982).

Increase in turgor is not considered a specific injury response. WENDLER *et al.* 1983 have conclusively shown that turgor in *Acetabularia* is rhythmically regulated by bursts of chloride efflux. Turgor pressure fluctuations between 2.2 and 2.6 bar (WENDLER *et al.* 1983) cause the protoplast to repeatedly burst after wounding, until enough plug material has been deposited at the wound site to counteract further expansion. In other families of siphonous green algae, turgor either does not increase so dramatically, or the plug forms more quickly and prevents further expansion of the protoplast before it can burst.

3.5. Wound Plug Formation

3.5.1. Phenomenology of Plug Formation and Structural Aspects

The formation of wound plugs is well understood in a descriptive sense. Appearance of variously structured layers and the probable type of precursor material to be incorporated is well documented (BURR and WEST 1971, DREHER *et al.* 1978, GODDARD and DAWES 1983, LOHR 1975, MARIANI-COLOMBO *et al.* 1980, MENZEL 1979 a, 1980 b, 1982, 1987 a, WHEELER and PAGE 1974). Unfortunately, the availability of fine structural and histochemical data does not significantly contribute to our understanding of the various functional components of plug formation.

Despite differences in plug precursor composition three general processes seem to be involved: Expansion/dispersal, adhesion/cross-linking and impregnation/hardening of precursor material.

During extrusion the storage form of the plug precursor is assumed to convert to an active form, which entails swelling to a variable degree. This phenomenon is illustrated in three examples.

(i) In all species of the *Caulerpaceae* the turbid, highly viscous plug precursor material extrudes instantly to form a bulging globule over the cut opening and it quickly jells (DREHER *et al.* 1978). The driving force for extrusion is mainly derived from expansion of the plug precursor and not from the contracting protoplast. This is easily demonstrated by cutting a cell longitudinally rather than transversally. In this case the protoplast is completely opened and cannot force the vacuolar content out by contraction. Still the plug precursor material expands much beyond the diameter of the cell and gels (DREHER *et al.* 1982, MENZEL unpublished data).

(ii) In the *Udoteaceae*, the spherical bodies (see Tab. 2) expand to several times their original volume during wound plug formation (MENZEL 1987 a).

(iii) In *Bryopsis*, the highly structured precursor (Tab. 2) can be seen to gradually disperse into a granular fibrillar matrix during plug formation (BURR and WEST 1971), which takes up a much greater volume than the condensed form.

Plug precursor material is believed to possess adhesive properties. BURR and WEST (1971) noted that plug precursor material in *Bryopsis* moved from the interior of the vacuole, close to a wound site, through the closing cytoplasmic aperture to its final position in the plug (Fig. 2). Upon physical contact with the cut edge of the cell wall, this material "stuck" and quickly accumulated. An initial ring pad was formed on which more and more material accreted until the wound aperture was closed (Fig. 2 *c*). Probing the forming plug with a dissection needle resulted in the plug precursor firmly sticking to the surface of the needle. Adhesive properties have also been inferred from fine structural observations in some species of the *Udoteaceae*, where the spherical bodies tend to aggregate even before wounding MARIANI-COLOMBO and POSTAI 1978, MENZEL 1987 a). Unfortunately, conclusive *in vitro* experiments with isolated components of plug precursor material have not been performed that specifically address this question.

3.5.2. Coagulation and Hardening of Plugs

The chemical basis of coagulation is still poorly understood. In the absence of experimental evidence, comparisons have been made with blood clotting and with gelling of sieve tube exudate. Although such comparisons are analogous only, they provide useful models and will help guiding future experimental approaches aimed at elucidating the mechanisms involved.

The fact that the components of plug precursors differ between families may point to more than one mechanism of coagulation. Depending on the chemical nature of the precursor material (see Tab. 2), one such mechanism might be based on protein-protein interactions, a second mechanism may involve interactions between polysaccharides, and yet a third may involve proteins and polysaccharides. In all cases it would be desirable, if the putative mechanism permits clot stabilization by cross-linking.

Two hypotheses have been put forward regarding the chemical mediation of plug formation. The first suggests a nonenzymatical self assembly sequence and the second an enzymatically controlled process. The nonenzymatical mechanism has been explored in *Caulerpa* by exposing the thalli to several chemically defined media during plug formation. DREHER *et al.* (1982) found that gelling took place even under mild protein denaturing conditions. Release of sulfate groups, which is expected during enzyme catalysed cleavage of alkalilabile sulfate from sugar residues, was also not observed during gelling of *Caulerpa* wound plugs (LAWSON and REES 1970). Gelling by complexation of divalent cations, as in the sulfated polysaccharides of other green algae (HANG 1976, MCCANDLESS 1981, PERCIVAL 1978) or by disulfide bonding as in gelling of phloem exudates (KOLLMANN 1980) has also been ruled out. On the other hand, the fact that isolated fractions of sulfated polysaccharides from *Caulerpa* cannot be induced to form gels (DREHER *et al.* 1982) only demonstrates that gelling is not an inherent physicochemical property of this purified polysaccharide fraction. We are therefore left with the meager conclusion that rather specific components are necessary to induce gel formation *in vivo*. These components would have to be stored separately from the polysaccharide matrix present in the vacuole and would be released as a result of wounding (GODDARD and DAWES 1983). So, although enzyme mediation does not seem to apply, the possibility cannot yet be ruled out.

Since sulfated polysaccharides are also the major plug precursor component in the *Udoteaceae* the same problems apply to this family as well. In contrast to *Caulerpa*, however, the plug precursor remains condensed (as spherical bodies, see Tab. 2), suggesting differences in the mechanisms of coagulation. With the exception of the *Caulerpaceae*, cross-linkage of polymeric substrates by low molecular weight enzyme-mediated cross-linkers has been hypothesized as a possible mechanism of plug formation and hardening in the siphonous green algae. This hypothesis is based on observations that in the majority of siphonous green algae, either the plug precursors or the plugs, or both, contain peroxidase (EC 1.11.1.7, MENZEL 1979 a, b, c, 1980, 1982, MENZEL and GRANT 1982, MENZEL *et al.* 1983, see Tab. 3). This enzyme has also been partially purified from several dasycladalean and caulerpalean species and its range of substrate specifity and isoenzyme composition examined (MENZEL and GRANT 1982).

The suggested function of peroxidase offers the possibility for a unifying mechanism of clotting and cross-linking in the siphonous green algae which is independent of the chemical nature of the plug precursor matrix. There are many examples describing a peroxidase/phenylpropane interaction leading to cross-linking which involves protein (HALL 1978, LA BELLA *et al.* 1968, SCHMELL and GULYAS 1980, WHITMORE 1978 a)

Table 3. *Survey of peroxidase occurrence in siphonous green algae detected by histochemical techniques*[$]

Family	Species	Plug precursor	Wound plug	Septal plug
Derbesiaceae				
	Bryopsis plumosa	+++	+++	+++
	Derbesia tenuissima	/	+++	+++
	Halicystis parvula	/	+++	+++
Codiaceae				
	Codium fragile	/	+++	+++
Udoteaceae				
	Halimeda cylindrica	++	+	++ 1)
	Halimeda tuna	–	–	– 1)
	Halimeda opuntia	–	–	– 1)
	Udotea flabellum	++	++	++ 1)
	Chlorodesmis fastigiata	++	++	++ 1)
Caulerpaceae				
	Caulerpa simpliciuscula *)	–	–	– 2)
	Caulerpa racemosa	–	–	– 3)
Dasycladaceae				
	Dasycladus clavaeformis	/	+++	+++
	Cymopolia barbata	/	++	++
	Batophora oerstedii	/	++	++
	Bornetella oligospora	/	–	+
Acetabulariaceae				
	Acetabularia acetabulum #)	/	+++	+++
	Polyphysa cliftonii	/	+++	+++

Relative amount of peroxidase as demonstrated by o-Tolouidin/H_2O_2 reaction product is indicated as

/ = not tested
– = non
+ = weak
++ = medium
+++ = strong.

$) data from Menzel 1982
*) for survey of peroxidase in 17 Caulerpa species see Menzel & Grant (1982).
#) essentially same pattern in 4 other species of this genus

1) septal plugs form only after wounding, see section 5
2) trabeculae positive (++)
3) trabeculae negative

or polysaccharides (FRY 1979, HARTLEY and JONES 1976, PAINTER and NEUKOM 1968, WHITMORE 1978 b). The assumption that a similar mechanism may also be operating in siphonous green algae has become especially tempting after the discovery of coumarin derivatives in the dasycladalean algae (MENZEL *et al.* 1983). Phenylpropane derivatives can easily be converted to coumarines and both can be used as a substrate for peroxidase (PAINTER and NEUKOM 1968, for review see GASPAR *et al.* 1982, HARBORNE 1980). Intermediate reaction products of peroxidase with phenolic compounds would include highly reactive oxygen radicals. These could covalently bind to protein or polysaccharides. They could also form covalent dimeric cross-bridges or cross-bridges mediated by calcium complex bonding (PAINTER and NEUKOM 1986). Finally, if phenolic substrate concentration is high enough, polymerization may occur in the presence of peroxidase, resulting in an impregnation of the plug matrix analogous to lignification of plant cell walls or artificial polysaccharide substrates (STAFFORD 1964).

Phenolic compounds in other families of siphonous green algae have not been characterized by chemical analysis as components of the plug precursor. But it

should be recalled that almost all siphonous green algae studied thus far, contain large numbers of osmiophilic globules in the vacuole (Tab. 2) which are incorporated into the plug. Their possible relationship with phenolic compounds has been discussed (MENZEL 1987 a). Lipids, on the other hand, which also occur as electron dense deposits in plant cells do not significantly accumulate in the wound plug (DAWES and GODDARD 1978).

3.6. Formation of New Cell Wall and Restoration of Polarity

There is little argument that wound plug formation is the first line of defense at the wound site. Once in place, the relatively slow formation of new cell wall can proceed undisturbed (BURR and WEST 1971, DREHER *et al.* 1982, GODDARD and DAWES 1983, MARIANI-COLOMBO *et al.* 1980, MENZEL 1987). Usually the new cell wall is laid down along the innermost plug layer. Only in the most apical siphons of *Udotea* is new cell wall sometimes formed deeper in the siphon (MARIANI-COLOMBO and DE CARLI 1980).

The first layer of the new cell wall, the wound wall, is sometimes different from the parental cell walls in fine structure (BURR and WEST 1971) and composition. It usually has a higher content of electron dense components or of acidic polysaccharides (MARIANI-COLOMBO and POSTAI 1978). However, it is not known if this is due to different mechanisms of cell wall formation or whether additional components may simply be mixed in with the cell wall matrix. After new cell wall is laid down, parts of the old cell wall degenerate (*e.g.*, GODDARD and DAWES 1983), indicating that the wall provides maximal physical resistance and protection to the cell only when in contact with the cytoplasm.

The capacity of the cell to regenerate after the actual wound response has long been recognized (DOSTAL 1929, FIGDOR 1910, HABERLANDT 1929, STEINECKE 1925, WINKLER 1900) and these observations have prompted further studies on the mechanisms of maintaining and expressing polarity in siphonous green algae (JACOBS 1970, MARIANI-COLOMBO and DE CARLI 1980, NOVAK and SIRONVAL 1975). These studies have shown that thallus regeneration proceeds in most cases by the initiation of new tips some distance behind the wound plug. Original polarity of the injured thallus area is retained. *Acetabularia* is unique in that the wound cell wall itself becomes the new tip and will grow directly through the wound plug, thus restoring the original uniaxial morphology (HÄMMERLING 1943).

It is worth noting that the capacity to tolerate wounding and restoration of uniaxial morphology has made possible the development of grafting techniques. This has been exploited in the study of thallus polarity and to lay the foundation for cell biology studies on *Acetabularia* (SCHWEIGER and BERGER 1979) with emphasis on the relationship between the nucleus and the cytoplasm (NEUHAUS and SCHWEIGER 1987).

4. Chemical Defense

Phenolic compounds may be used not only to aid wound plug coagulation and impregnation. There is substantial evidence that phenolic derivatives (including terpenoids and possibly substances of nonphenolic composition) provide a chemical defense against herbivores and microbial disease. It is well-known that peroxidase enhances the antimicrobial potency of a given phenolic substrate and is thus regarded as a functional component in chemical defense mechanisms of animals and plants (GASPAR *et al.* 1982). Antimicrobial, antifeedant and growth inhibiting substances have been isolated from a great number of species in the siphonous green algae, among them are the caulerpins, cymopols, coumarins, halimedatrials and rhipocephalins (see Tab. 4).

In the case of *Dasycladus*, massive release of coumarins into the surrounding medium is an integral part of the wound response although small quantities are continuously released at low levels during normal development (MENZEL *et al.* 1983). A similar situation is likely in other species (*e.g., Batophora* and *Cymopolia*), which suggests that these algae have developed an additional strategy to protect the wounded cell during the most vulnerable phase of wound closure and plug formation.

5. Septal Plugs

Most siphonous green algae have adopted some form of developmentally regulated senescence which leads to the shedding of side branches. Before shedding, a plug is slowly assembled in the basal portion of the side branch. This process resembles the formation of a miniature wound plug with species specific characteristics (MENZEL 1980 a, Tab. 2). Also, gametangia or sporangia will be plugged off before discharge of the gametes or spores respectively (MENZEL 1982). Again these plugs are very similar to the wound plugs. In the *Udoteaceae*, septal plugs are preferentially formed as a response to wounding in addition to the plug at the actual wound site. In the highly branched system of siphons inside the multiaxial thallus in this family, con-

Table 4. *Biologically active compunds in the siphonous green algae*

Species	compound	effect	ref.
Caulerpa racemosa	?	fungicidal	1
Caulerpa taxifolia	?	bactericidal	2
sertularioides	?	"	2
prolifera	?	"	2
Caulerpa cupressoides	caulerpin	ichthyotoxic + neurotoxic	3, 4
racemosa	"	"	3
serrulata	"	"	3
sertularioides	"	"	3
taxifolia	"	"	3
verticillata	"	"	3
Rhipocephalus phoenix	rhipocephanal + rhipocephalin	ichthyotoxic + antimicrobial	5
Penicillus capitus	?	bactericidal	2
Udotea sp.	"	ichthyotoxic	3
Halimeda opuntia	?	bactericidal	6
		antimicrobial	2
Halimeda copiosa	halimedatrial	ichthyotoxic + cytostatic + antimicrobial	7
incrassata	"	"	7
opuntia	"	"	7
scabra	"	"	7
simulans	"	"	7
tuna	"	"	7
Codium sp.	?	bactericidal	8
Codium fragile	?	bactericidal	9,10
Codium tomentosum	?	bactericidal	9
Cymopolia barbata	phenolic	antimicrobial + fungicidal	11,2
Cymopolia barbata	cymopols	?	12
Dasycladus clavaeformis	coumarins	bactericidal	13
Derbesia sp.	?	bactericidal	14

caulerpin = dimeric indole derivative; rhipocephanal = sesquiterpene dialdehyde; rhipocephalin = sesquiterpene triacetate; halimedatrial = bicyclic diterpene trialdehyde; cymopols = several brominated hydroquinone derivatives; coumarins = trihydroxicoumarins and glycosidic derivatives.

References:
1: Welch 1961, 2: Burkholder et al. 1960, 3: Norris & Fenical 1982, 4: Santos & Doty 1971, 5: Sun & Fenical 1979, 6: Almodovar 1963, 7: Paul & Fenical 1983, 8: Kamimoto 1955, 9: Hornsey & Hide 1976, 10: Glombitza 1969, 11: Martinez Nadal et al. 1966, 12: Högberg & Thomson 1976, 13: Menzel et al. 1983, 14: Allen & Dawson 1960.

strictions are present at each branch point (Figs. 1.3, 1.4, and 1.5). As a consequence of explosive turgor discharge upon wounding, vacuolar material rapidly streams for a moment through the siphons. This in turn causes the cytoplasm to rupture at the constrictions which quickly become blocked by aggregates of spherical bodies (MENZEL 1987 a). This sequence of events reveals a close relationship between siphon morphology and the presence of preassembled plug precursor material, an aspect that has been ignored in current research on wound response. Plugging of constrictions obviously blocks communication between branches within the system of siphons, and this should have an impact on the pattern of regeneration and development, but as yet, this question has not been specifically addressed.

6. Plug Formation in Other Plant Cells

6.1. Wound Plugs in Other Giant-celled Algae

In contrast to the siphonous green algae, other giant-celled algae do not necessarily restore the cell's integrity after large-scale wounding, but wounding on a smaller scale, such as puncturing, is usually survived by the formation of a wound plug. This has originally been described by NICHOLS (1922) for *Nitella* (*Charophyta, Charales*) and for several genera in the *Siphonocladales* and *Cladophorales*. Unfortunately, the fine structure and composition of these plugs are unknown. There is only one recent study on *Nitella* (FOISSNER 1987) which reveals the involvement of so-called echinate bodies in the repair of puncture wounds. It remains to be seen whether these bodies resemble any of the plug precursors of the siphonous green algae which are also stored in the central vacuole.

Some interesting, alternative responses to wounding have been described in giant-celled algae. For example, cells of the siphonocladalean species *Boergesenia* (ISHIZAWA *et al.* 1979, O'NEIL and LA CLAIRE 1984) form hundreds of protoplasmic spheres upon massive wounding, which develop into new daughter cells (see section 3.2.3.). Another interesting repair mechanism is encountered in the red alga *Griffithsia* (*Ceramiales*). Puncturing of a cell results in destruction of the protoplast, but the neighbouring cells of the linear cell filament divide and form a repair shoot and repair rhizoid cell, which grow into the empty cell and fuse. Eventually, when the repair cell has laterally expanded, the dead cell has been replaced by a living cell and the integrity of the filament is restored (WAALAND 1980).

6.2. Septal Plugs in Algae, Fungi, and Lower Plants

The capacity to form plugs in the course of development is not unique to siphonous green algae. Septal plugs are a widespread characteristic in red algae (PUESCHEL and COLE 1982) and fungi (GULL 1978). However, our present knowledge suggests that these plugs are not phylogenetically related to those in the siphonous green algae. The same is probably true with respect to ultrastructure, histochemistry and, perhaps, function (*e.g.*, BRAWLEY and WETHERBEE 1981). Septal plugs have recently been discovered in a green alga (*Smithsoniella earleae, Chlorophyceae*, BRAWLEY and SEARS 1982), but the structure and composition of these plugs also distinctly differ from those in the siphonous green algae, showing greater affinities to red algae. Other types of developmental plugs have been reported in algae. Some examples include those found in the oogonial walls of the green alga *Oedogonium* (HOFFMAN 1973), the pore plugs in some chrysophyte statospore walls (MILBERT 1977). Perhaps, septal strucutes in the xanthophycean alga *Vaucheria* (HANSTEIN 1873, OTT and BROWN 1974 b) and spore aperture plugs in certain mosses (BROWN and LEMMON 1981) may also be included. These examples, however, seem to be quite isolated and it is difficult to see how they could be molded into a more general plug model for the siphonous green algae.

6.3. Plugs and Wound-induced Protective Structures in Higher Plant Cells

Early accounts of wound induced pathological changes in plant cells (KÜSTER 1925) have already suggested that higher plant cells have retained a general capacity to cope with injury by repair mechanisms on the level of the individual cell. However, this mechanism may have become partly replaced by more effective repair on the tissue level (LIPETZ 1976). An integrated part of the wound response in higher plants is the deposition of callose (KAUSS 1985). It is most prominent in the cells around the wound, where it is found in pit fields and sieve plates (CLARKE and MCCULLY 1985, GALWAY and MCCULLY 1987). Microwounds inflicted to single cells by microinjection become plugged with callose containing material (NIMES *et al.* 1967). Furthermore, deposition of callose is involved in resistance against parasitic fungi (AIST 1976, 1977). There is little doubt that these callose deposits serve a protective function. Callose is also the major polysaccharide in septal plugs of pollen tubes (CRESTI and VAN WENT 1976, KROH and KNUIMAN 1982). Wound induced plugging on a large scale occurs in resin ducts (CURRIER 1957) involving callose deposition and in sieve tubes of vascular plants (for review see BEHNKE 1983) involving p-protein mediated gelling (KOLLMANN 1980) as well as the formation of callose layers. Superficial resemblance of some of these examples with plug formation in siphonous green algae is quite striking, although at present, there is no good evidence that the mechanisms involved are related.

7. Conclusions

Research on the wound response in the siphonous green algae from the turn of the century to the present has contributed to our understanding of the intricate network of defense that has evolved in this group of algae to protect the coenocytic cell body against environmental threats. On the basis of the accumulated data, several layers of defense can be identified. These are:

1. At the level of thallus architecture: several principles of thallus construction provide sufficient reinforcement and flexibility for protection and support growth of giant cells.
2. At the level of the environment: chemical defense discourages potential herbivores and controls microbial attack.
3. At the level of cytoplasmic organization: a highly active and motile cytoskeleton has evolved a mechanism for quick repair of damage to the cytoplasm.
4. At the level of biosynthetic pathways: a high capacity biosynthetic machinery and targeting mechanism produces and distributes biopolymers, monomeric secondary metabolites and enzymes for use in the event of wounding. These levels integrate at the moment of wounding to provide maximum protection and quick regeneration.

In summary, it is concluded that the relative wealth of information available at present describes the wound response in the siphonous green algae quite accurately on the phenomenological level, but the underlying mechanisms remain obscure. Only a few pioneering experiments (*e.g.*, DREHER *et al.* 1982) have been aimed at elucidating functional aspects by studying *in vitro* behavior of plug precursor material. Obviously, we need to know; (i) how many different proteins and polysaccharides are present in the plug precursor material; (ii) what are the chemical properties of these components; (iii) how do these properties differ between species and families; (iv) which of the components are essential for the coagulation reaction; and (v) how do they interact to form plugs. One important prerequisite to experimentally approach these questions will be sufficient quantities of these algae in a reasonable homogeneous condition. Although it may be possible in exceptional cases, to harvest kilogram quantities of certain species from the natural habitat, it will be indispensable to establish reliable, large scale laboratory cultures of a few key species selected from the major families (listed in Tab. 2) to permit purification of proteins, polysaccharides and generation of antibodies. Decades of experiences made with culturing *Acetabularia* (SCHWEIGER *et al.* 1977) and some general guidelines summarized by ZIEGLER-PAGE (1973) may provide a foundation for setting up such cultures.

Provided the availability of sufficient plant material, three experimental approaches appear particularly promising for future studies on the mechanism of wound plug formation. The first approach should focus on the isolated components, involving extraction, purification and detailed biochemical characterization of plug precursor material. It should further prove profitable to prepare monoclonal antibodies agaist these isolated components and their macromolecular fragments to develop detection assays based on antibody binding. This would allow to screen other species, including field collected material, for the presence of specific components in order to reevaluate relationships between the siphonous green algae established on the basis of structural and histochemical data.

In the second approach, the production of antibodies against components of the plug precursor should allow one to specifically localize these components within the cell. Using immunocytochemical techniques on the light and electron microscope level it should be possible to study the origin, redistribution, assembly and turnover of plug precursor components.

The third approach should be aimed at the behavior of native plug precursor material isolated in the unreacted form in order to study the mechanisms of its dispersal, swelling and coagulation by using controlled experimental conditions. It should be especially interesting to study the effects of subcellular fractions or purified biochemical components, added to such native plug precursor material. This would allow to test the hypothesis of the involvement of compartmentalized cofactors, released *in vivo* as a result of injury, in triggering the coagulation mechanism.

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