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Research

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Summary

• The model brown alga *Ectocarpus* has a haploid-diploid life cycle, involving alternation between two independent multicellular generations, the gametophyte and the sporophyte. Recent work has shown that alternation of generations is not determined by ploidy but is rather under genetic control, involving at least one master regulatory locus, *OUROBOROS* (*ORO*).

• Using cell biology approaches combined with measurements of generation-specific transcript abundance we provide evidence that alternation of generations can also be regulated by non-cell autonomous mechanisms.

• The *Ectocarpus* sporophyte produces a diffusible factor that causes major developmental reprogramming in gametophyte cells. Cells become resistant to reprogramming when the cell wall is synthetized, suggesting that the cell wall may play a role in locking an individual into the developmental program that has been engaged. A functional *ORO* gene is necessary for the induction of the developmental switch.

• Our results highlight the role of the cell wall in maintaining the differentiated generation stage once the appropriate developmental program has been engaged and also indicate that ORO is a key member of the developmental pathway triggered by the sporophyte factor. Alternation between gametophyte and sporophyte generations in *Ectocarpus* is surprisingly labile, perhaps reflecting an adaptation to the variable seashore environment inhabited by this alga.

Introduction

Life cycle regulation is a fundamental developmental process because life cycle transitions involve major switches between distinct developmental programs. However, very little is known about how these transitions are mediated at the molecular level (Coelho *et al.*, 2007). The brown alga *Ectocarpus* represents an interesting system in which to investigate the regulation of life cycle transitions because its haploid-diploid life cycle involves alternation between two morphologically similar, multicellular organisms, the sporophyte and the gametophyte. This feature has allowed the isolation of homeotic mutations that cause switching between the two generations (Peters *et al.*, 2008; Coelho *et al.*, 2011).

The life cycle of the model brown alga *Ectocarpus* has been analyzed in detail in culture (Müller, 1967; Peters *et al.*, 2008; Bothwell *et al.*, 2010). The alternation between the sporophyte and gametophyte generations constitutes the sexual cycle (Supporting Information Fig. S1). The sporophyte produces meio-spores (meiosis-derived spores), which germinate to give the gametophyte generation. Male and female gametes produced by the dioecious gametophytes fuse to produce a zygote, which is the initial cell of the sporophyte generation. Several variations on this life cycle are possible (Fig. S1); for example, gametes that fail to fuse with a gamete of the opposite sex can germinate partheno-genetically to produce haploid partheno-sporophytes. Another interesting phenomenon was observed in *E. siliculosus* (Dillwyn) Lyngbye strains from Naples (Italy), where 16–43% of the meio-spores (depending on the strain used) adopted the sporophytic rather than the gametophytic developmental program (Müller, 1967). This phenomenon is called heteroblasty.

Recent work has shown that the alternation of generations in *Ectocarpus* is not determined by ploidy (Bothwell *et al.*, 2010) but is rather under genetic control, involving at least one master regulatory locus, *OUROBOROS* (*ORO*; Coelho *et al.*, 2011). Here we provide evidence that alternation of generations can also be regulated non-cell autonomously. We show that the *Ectocarpus* sporophyte produces a diffusible factor that acts in a similar manner to the *oro* mutation (Coelho *et al.*, 2011), causing major developmental reprogramming of cells that would

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normally develop as gametophytes so that they grow to become fully functional sporophytes. Moreover, the diffusible factor is only active on gametophyte cells that possess a functional copy of the *ORO* gene, suggesting that the ORO protein is part of the developmental pathway targeted by the sporophyte-derived factor. Our data indicate that the alternation between gametophyte and sporophyte generations in *Ectocarpus* is surprisingly labile, perhaps reflecting an adaptation to the highly variable seashore environment inhabited by this alga.

Materials and Methods

Biological material

Two of the *Ectocarpus* strains used in this study were derived from offspring of a field sporophyte collected in 1988 in San Juan de Marcona, Peru (Ec 17). These strains were Ec 32 (wild-type male) and a female sibling Ec 25. Ec 32 is the strain for which a complete genome sequence is available (Cock *et al.*, 2010). The *ouroboros* mutant strain (Ec 560) was a gametophyte obtained after a backcross of the original, *oro* mutant strain Ec 494 with Ec 25, resulting in sporophytes heterozygous for *oro* which in turn produced a generation of gametophytes that carried *oro* (Coelho *et al.*, 2011). These gametophytes should have had a reduced level of any additional mutations introduced during the mutagenesis procedure. The female and male strains used for test crosses (Coelho *et al.*, 2012a) were Ec 560 and Ec 561.

Culture media and culture conditions

Standard culture conditions were 14°C, illumination at 12h day length with daylight-type white fluorescent tubes at 20 μmol photons m^{-2} s^{-1} (Coelho *et al.*, 2012c). The culture medium was half-strength Provasoli-enriched autoclaved natural sea water (PES; Starr & Zeikus, 1993). Gametophyte-conditioned and sporophyte-conditioned media consisted of PES recovered from Petri dishes where gametophytes or sporophytes had been growing at high density (10 individuals/100 ml) for 4 wk. This medium was filtered (0.2 μM), and half strength Provasoli solution was added. Cultures were started from filaments that had been submitted to an axenization protocol (Müller *et al.*, 2008). The sporophyte- and gametophyte-conditioned media had the same pH (10.5) and salinity (36.4%). To test the effect of the addition of sporophyte or gametophyte filaments to developing protoplasts, the fragments were separated from the developing protoplasts with a 45-μm mesh. Control dishes were set up without protoplasts to verify that the added fragments were not able to traverse the mesh.

Protoplast preparation

Protoplasts were prepared according to Coelho *et al.* (2012b). Briefly, *c.* 250 *Ectocarpus* individuals were pooled, finely chopped using a sterile razor blade and incubated in chelation medium (NaCl 700 mM, $MgCl_2$ 30 mM, $MgSO_4$ 30 mM, KCl 20 mM, and EGTA 20 mM) in the dark at 13°C. After 20 min, the chelation medium was replaced with digestion medium (NaCl 400 mM, $MgCl_2$ 130 mM, $MgSO_4$ 22 mM, KCl 160 mM, $CaCl_2$ 2 mM, MES 10 mM, 1% w/v cellulase and 3 U ml^{-1} alginate lyase) and the filaments incubated in the dark with gentle agitation for 5 h. Protoplasts were then filtered through a 40-μm filter to remove undigested filament material, and washed twice by centrifugation at 50–100 ***g*** for 15 min at 4°C before being re-suspended in either regeneration medium ($MgCl_2$ 150 mM, KCl 100 mM, $NaHCO_3$ 4 mM, KNO_3 2 mM and NaH_2PO_4 100 μM) or conditioned medium supplemented with osmoticum ($MgCL_2$ 150 mM and KCl 100 mM) and antibiotic solution. Protoplasts were cultivated at very low density in the dark at 13°C for 2 d, after which the high-osmolarity medium was slowly replaced by either PES or conditioned medium. Finally, the material was transferred to standard culture conditions (see the previous section). Three to five replicate Petri dishes were prepared for each experimental condition. At least 75 individuals were scored per experiment. Results are representative of five independent experiments.

Staining

Ectocarpus siliculosus filaments were snap-frozen in liquid nitrogen and immediately stained with fresh 6% Congo red solution for 10 min followed by five washes in seawater. For cell wall staining, calcofluor white was used at 0.5 mg ml^{-1} concentration in seawater. Filaments were incubated for 5–10 min in the dark at room temperature, followed by three washes (5 min each) in seawater. Calcofluor white fluorescence was observed using an upright Olympus microscope BX60 with a UV filter set.

RT-qPCR analysis of transcript abundance

Bulks of 10 individuals (three biological replicates) were used to extract total RNA using a protocol adapted from Peters *et al.* (2008). Contaminating genomic DNA was eliminated by DNase treatment using the Qiagen DNA-free kit (Qiagen). The concentration and quality of the RNA were determined by spectrophotometry and agarose gel electrophoresis. Between 0.2 and 2.0 μg of total RNA was reverse-transcribed to produce cDNA using the SuperScript® First-Strand Synthesis System for RT-PCR (Invitrogen).

qRT-PCR was carried out using the ABsolute™ QPCR SYBR® Green ROX Mix (ThermoScientific, Brebieres, France) in a Chromo4™ thermocycler (BioRad Laboratories) and data were analyzed with the Opticon monitor 3 software (BioRad Laboratories). Amplification specificity was checked using a dissociation curve. Amplification efficiency was tested using a genomic dilution series and was always between 90 and 110%. To allow quantification, a standard curve was established for each gene using a range of dilutions of *Ectocarpus* genomic DNA (between 80 and 199 600 copies) and expression level was normalized against the *EF1α* (Elongation factor 1 alpha) reference gene (Le Bail *et al.*, 2008). Two technical replicates were carried out for the standard curves and three technical replicates for the samples. The data shown correspond to the mean ± SE for three biological replicates. Statistical analysis (one-way ANOVA and

Tukey's multiple comparison test) was performed using XLSTAT (http://www.xlstat.com/fr/) and PRISM 5 (http://www.graphpad.com/scientific-software/prism/).

Results

Complexity and variability of the *Ectocarpus* life cycle in culture

The *Ectocarpus* strain that was used for the genome sequencing project (Ec 32; Cock *et al.*, 2010) was derived from a sporophyte collected in Peru (Peters *et al.*, 2008). It is therefore distantly related to the strains from Naples, and in fact possibly corresponds to a separate species (Peters *et al.*, 2010). We examined whether the developing meio-spores of this strain exhibited the phenomenon of heteroblasty that had previously been observed with strains from Naples (Müller, 1967). Thirty-six unilocular sporangia were isolated from mature Ec 32 sporophytes and the development of the meio-spores released from these sporangia (*c.* 100 meio-spores per unilocular sporangium) was followed under the microscope. All of the meio-spores developed as gametophytes ($n = 2280$). To test whether variation of the culture conditions could induce switching between life cycle generations, meio-spores were cultivated at different temperatures (10, 14 or 20°C) and under different light intensities (20 or 100 μmol photons $m^{-2} s^{-1}$) and their development was followed for 2 wk. No effect of temperature or light intensity on the developmental fate of the meio-spores was detected; all of the meio-spores developed as gametophytes ($n = 20$ unilocular sporangia; > 1600 germlings; Fig. 1a).

Sporophyte-conditioned medium induces switching from the gametophyte to the sporophyte developmental pathway

The above experiments indicated that, unlike the Naples strains, the meio-spores of the Ec 32 strain do not undergo spontaneous switching from the sporophyte to the gametophyte developmental program in culture. However, when Ec 32 meio-spores were cultivated in the presence of sporophyte filaments or in sporophyte-conditioned medium (SCM) (from which all sporophyte tissue had been removed), 34% on average of the developing germlings exhibited morphological characteristics of sporophytes rather than gametophytes (Fig. 1b; 366/1064 germlings; $n = 22$ unilocular sporangia). These included an asymmetrical rather than a symmetrical first cell division, strong attachment to the substrate, and the production of a prostrate base composed of highly pigmented round cells with less pigmented, elongated cells at the ends of the filaments (Peters *et al.*, 2008). The sporophytic nature of these individuals was further confirmed by incubating tissue in the presence of Congo red, which stains the gametophyte but not the sporophyte generation and can be used to distinguish the two generations cytochemically (Fig. 1d,e; Coelho *et al.*, 2011).

The sporophyte-like germlings were isolated and grown to fertility. When test crosses with a female gametophyte were performed, no zygotes were produced, indicating that the sporophyte-like individuals were indeed functional sporophytes (i.e. produced spores and not gametes). Likewise, the sporophyte-like individuals produced unilocular sporangia, structures that are only observed during the sporophyte generation (Fig. 1f).

Analysis of six generation-specific marker genes (Peters *et al.*, 2008) showed that, overall, the sporophyte-like individuals accumulated transcripts that have been shown to be significantly more abundant in the sporophyte generation and, conversely, had reduced levels of transcripts that have been shown to be significantly more abundant in the gametophyte (Fig. 1g).

Taken together, these results strongly indicate that a proportion of the meio-spores incubated in the presence of SCM switched their developmental fate and became sporophytes. This effect was specific for the sporophyte because growth in gametophyte-conditioned medium (GCM) had no effect on the developmental destiny of the growing meio-spores; all meio-spores developed to form gametophytes (Fig. 1c). Note that the conditioned medium for these experiments was produced using axenic *Ectocarpus* cultures, effectively ruling out the possibility that the factor was produced by a contaminating organism growing with *Ectocarpus* in the cultures. Moreover, although the pH of the conditioned medium was higher than that of nonconditioned medium, the SCM and GCM had identical pH values and salinities (see the Materials and Methods section). Therefore, the difference in pH was not responsible for the observed reprogramming effect. When SCM was added to meio-spores 48 h after their release from unilocular sporangia, it failed to induce developmental reprogramming, and all meio-spores developed as gametophytes ($n = 669$; nine unilocular sporangia). The timing of the incubation in SCM appeared therefore to be essential for the reprogramming effect. Staining of developing meio-spores with the cellulose-binding dye calcofluor white indicated that these cells, which initially lack a cell wall, start secreting detectable amounts of cellulose fibers between 24 and 48 h after release (Fig. 2). Recalcitrance to developmental reprogramming induced by incubation in SCM was therefore temporarily correlated with the formation of a cell wall around the meio-spores.

Regenerating protoplasts reiterate the life cycle generation of the individual from which they were derived

To further investigate the role of the cell wall in life-cycle-related developmental processes, we determined the effect of removing this structure on a cell's developmental identity. Thalli of adult gametophytes or sporophytes were subjected to enzymatic digestion to remove the cell walls, allowing the isolation of individual, wall-less cells (protoplasts). These cells were then allowed to regenerate into adult individuals in PES. Developing sporophytes can be clearly distinguished from developing gametophytes, even during the early stages of development (Peters *et al.*, 2008). All gametophyte-derived protoplasts regenerated into individuals with gametophyte morphology ($n > 300$) and all sporophyte-derived protoplasts regenerated into individuals with sporophyte morphology ($n > 300$). One week after isolation, gametophyte-

derived protoplasts had divided to produce a rhizoid cell and a cylindrical cell typical of those found in upright filaments. Subsequent development closely resembled that of wild-type gametophytes in that the upright filament grew up into the medium (rather than attaching to the substratum) and branched profusely to produce highly ramified individuals that adhered weakly to the substratum (Fig. 3a,b). Occasionally, some abnormal early cell divisions could be observed in the regenerating protoplasts, but these did not affect later morphologies (not shown). To verify that these individuals were functional gametophytes, 15 individuals were crossed with a female tester line (Ec 560). All 15 crosses resulted in the formation of zygotes,

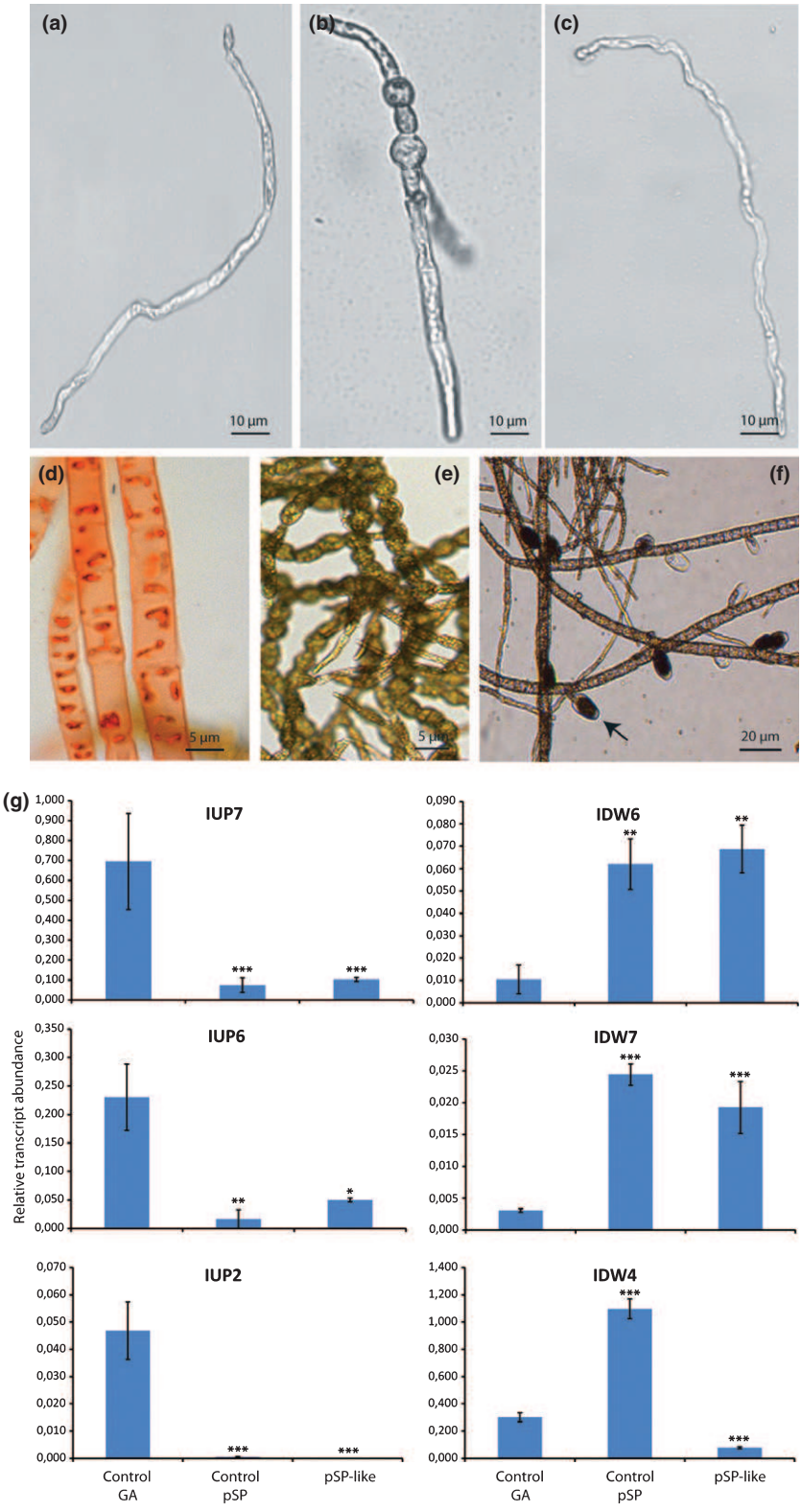

Fig. 1 Developmental fate of *Ectocarpus siliculosus* meiospores cultivated in different culture conditions. (a) Control gametophyte germling derived from an Ec 32 meio-spore grown in Provasoli enriched seawater (PES) (3 d after release). (b) Sporophyte-like germling derived from an Ec 32 meio-spore grown in sporophyte-conditioned medium (SCM) (3 d after release). (c) Gametophyte-like germling derived from an Ec 32 meio-spore grown in gametophyte-conditioned medium (GCM) (3 d after release). (d) Congo red staining of control gametophyte germlings derived from an Ec 32 meio-spore grown in PES. (e) Congo red staining of sporophyte-like germlings derived from Ec 32 meio-spores grown in SCM. (f) Unilocular sporangia on sporophyte-like germlings derived from Ec 32 meio-spores grown in SCM. (g) Quantitative PCR analysis of transcript abundance for six genes that have been shown to be differentially expressed in the sporophyte and gametophyte generations (Peters et al., 2008). The genes assayed are described in more detail in Supporting Information Table S1. GA, control gametophytes grown in PES; pSP, control partheno-sporophytes grown in PES; pSP-like, meio-spore-derived thalli grown in sporophyte-conditioned medium that exhibited sporophyte morphology. Data are means of three independent biological replicates ± SE. Asterisks indicate a significant difference between samples compared with control gametophyte. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

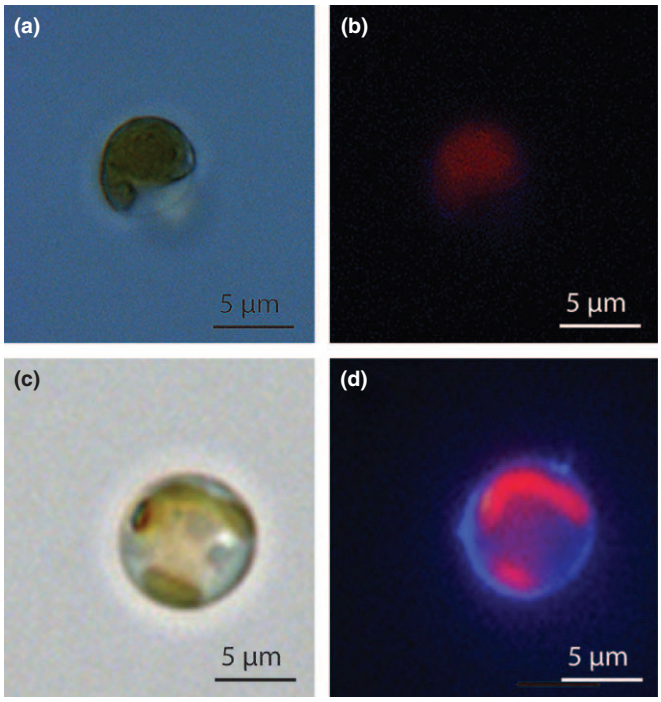

Fig. 2 Calcofluor white staining of developing *Ectocarpus siliculosus* meio-spores. Red, autofluorescence from chloroplasts. Blue, calcofluor white fluorescence. (a) Transmitted light image of a meio-spore 24 h after release; (b) epifluorescence image of a meio-spore 24 h after release; (c) transmitted light image of a meio-spore 48 h after release; (d) epifluorescence image of a meio-spore 48 h after release.

indicating that the protoplasts had regenerated into functional gametophytes.

By contrast, the sporophyte-derived protoplasts underwent a symmetrical initial cell division to produce basal filaments consisting of the two cell types, round and elongated, typical of this sporophytic structure (Fig. 3c). After 2 wk in culture, these individuals produced plurilocular sporangia containing spores, and some individuals produced unilocular sporangia (structures that are produced only during the sporophyte generation), indicating they were functional sporophytes (Fig. 3d). Individuals regenerated from gametophyte-derived protoplasts stained with Congo red but individuals regenerated from sporophyte-derived protoplasts did not (Fig. 3e,f).

Similar results were obtained when protoplasts of each generation were allowed to regenerate at different temperatures (10–18°C) and light intensities (20 or 100 µmol photons m^{-2} s^{-1}; not shown). Taken together, these results indicate that, despite being individualized cells lacking a cell wall, protoplasts retained the generation identity of the individual from which they were derived.

Developmental reprogramming of protoplasts in the presence of sporophyte-conditioned medium

When gametophyte-derived protoplasts were allowed to regenerate in the presence of a fragment of a sporophyte or in SCM, 49% of the regenerated individuals closely resembled sporophytes ($n = 202$). The first cell division was symmetrical, rather than being asymmetrical, and a prostrate basal system was produced (Fig. 4a,b). The plurilocular reproductive organs that formed after 1–2 wk in culture resembled sporangia rather than gametangia, lacking the elongated aspect of gametangia and occurring both on the prostrate base and on the upright filaments (Fig. 4c). The zoids within these structures behaved as spores; test crosses ($n = 10$) with a reference strain of the opposite sex failed to result in the production of zygotes. Moreover, the regenerated individuals did not stain with Congo red (Fig. 4d) and occasionally produced unilocular sporangia. Identical results were obtained using either male or female gametophytes as the starting material for protoplast production. By contrast, sporophyte-derived protoplasts incubated in the presence of gametophyte filaments or in GCM did not regenerate as gametophytes but retained sporophyte identity ($n = 88$). Note also that incubation of gametophyte-derived protoplasts in GCM or sporophyte-derived protoplasts in SCM had no observable effect on development compared with the seawater (PES) controls.

A significantly lower percentage of protoplasts regenerated as sporophytes if incubation in SCM began 6 d after protoplast isolation (29%; $n = 34$). Calcofluor white staining indicated that day 4 corresponded to the time when the protoplasts start regenerating their cell walls (Fig. 5). It is likely that the gametophyte-derived protoplasts that regenerated as sporophytes when SCM was added after 6 d corresponded to cells that had not yet produced a cell wall.

ORO is necessary for developmental reprogramming

A genetic lesion at the *ORO* locus has recently been shown to induce switching to the gametophyte generation at life cycle stages when the sporophyte generation would normally be expected (Peters *et al.*, 2008). To investigate further the mode of action of the sporophyte-derived reprogramming factor, we determined whether it was active on strains carrying the *oro* mutation. The *oro* mutant repeatedly reiterates the gametophyte generation and hence does not produce meio-spores. We therefore used the protoplast regeneration approach to test the effect of SCM on this strain. Protoplasts prepared from gametophytes carrying the *oro* mutation were insensitive to either SCM or added sporophyte filaments, and regenerated as gametophytes ($n = 228$; Fig. S2). Note that incubation of *oro* protoplasts in GCM also had no observable effect compared with the PES controls (not shown).

The gametophytic nature of the thalli that regenerated from *oro* protoplasts grown in both control and conditioned medium was verified by test crosses ($n = 7$). Zygotes were observed in all the crosses, indicating that the regenerated *oro* protoplasts produced gametes at maturity. Taken together, these data suggest that the SCM had no effect on the regeneration of protoplasts derived from the *oro* mutant.

Discussion

Ectocarpus is an interesting model with which to study life cycle transitions because its haploid-diploid life cycle involves alternation between two similar, multicellular organisms (the

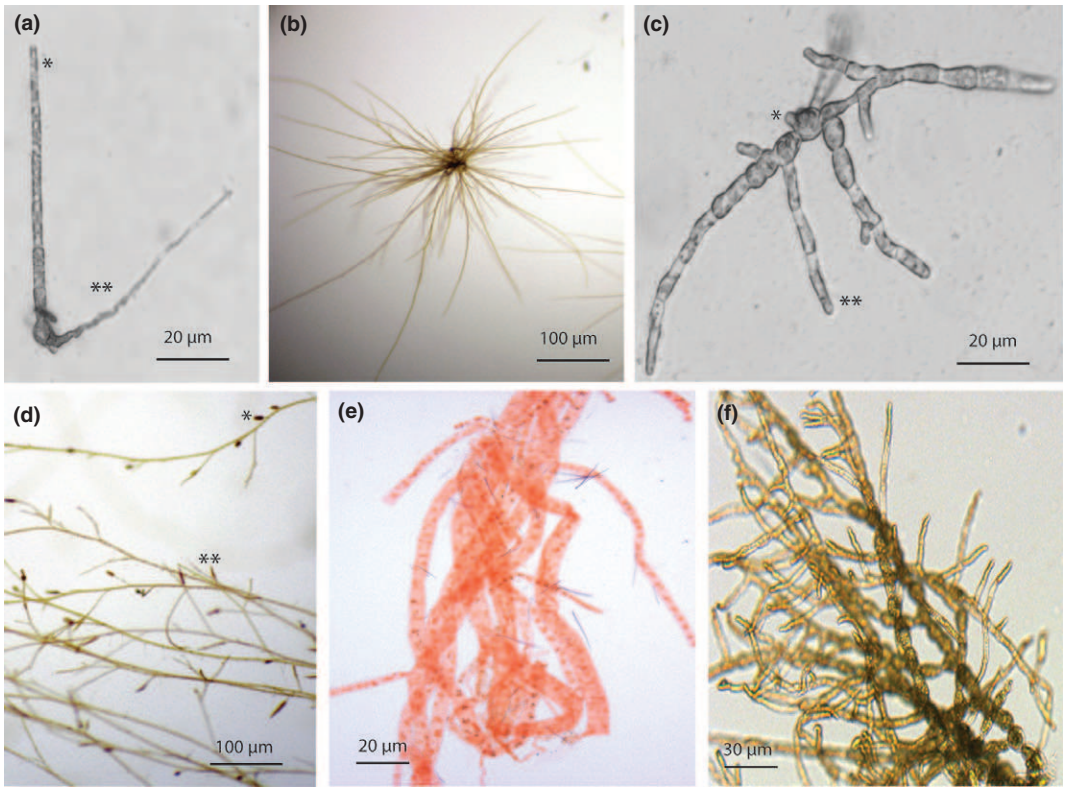

Fig. 3 Regeneration of gametophyte and sporophyte *Ectocarpus siliculosus* protoplasts. (a) Regeneration of a gametophyte-derived protoplast, 15 d after protoplast isolation. *, upright filament; **, rhizoid cell. (b) Regeneration of a gametophyte-derived protoplast, 30 d after protoplast isolation. (c) Regeneration of a partheno-sporophyte-derived protoplast, 15 d after protoplast isolation. Round cells (*) and elongated cells (**) are indicated. (d) Upright filaments bearing unilocular (*) and plurilocular (**) sporangia in an individual regenerated from a partheno-sporophyte-derived protoplast, 30 d after protoplast isolation. (e) Congo red staining of individuals regenerated from gametophyte-derived protoplasts (30 d after protoplast isolation) in Provasoli enriched seawater (PES). (f) Congo red staining of individuals regenerated from partheno-sporophyte-derived protoplasts (30 d after protoplast isolation) in PES.

sporophyte and the gametophyte), which develop almost completely independently, that is, from single initial cells that are released into the surrounding seawater. The similarity between the two generations has allowed the isolation of mutants that cause homeotic switching between the developmental programs of the two life cycle generations (Peters *et al.*, 2008; Coelho *et al.*, 2011).

Here we show that a diffusible factor, produced by the sporophyte generation, can induce cells that would normally express the gametophyte developmental pathway to become sporophytes. The action of this factor requires the presence of a functional *ORO* gene, indicating that the sporophyte factor and ORO are part of the same pathway, with ORO acting downstream of the diffusible factor. Interestingly, we found no evidence that gametophytes produce a similar factor that is able to induce switching to the gametophyte developmental program. One possible interpretation of this observation is that gametophyte development represents the default pathway and that an induction mechanism is required to switch to the sporophyte developmental pathway.

The sporophyte factor was only effective if present during the first 48 h following meio-spore release and loss of the ability to respond to the factor coincided with production of the cell wall. The diffusible factor had no effect if added later than 48 h but cells of mature gametophytes regained sensitivity to the diffusible factor if their cell walls were removed by digestion. These experiments suggest a role for the cell wall in modulating sensitivity to the diffusible factor. One possibility is that the presence of a cell wall prevents the diffusible factor from attaining its target in the cell. This hypothesis would only be valid if the cell walls of the factor producing sporophyte and the target cell differ in some way, so that the factor can be excreted by the former but be blocked from penetrating into the latter. An alternative, and perhaps more likely hypothesis is that the cell wall has an indirect effect, making the target cell recalcitrant to the factor, for example by signaling back to the cytoplasm and locking the cell into its gametophyte fate. The cell wall has been shown to influence another developmental process in the brown alga *Fucus spiralis*, where contact with the wall was sufficient to induce switching from thallus to rhizoid cell fate (Berger *et al.*, 1994; Bouget *et al.*, 1998). There is also evidence that the cell wall can influence developmental process such as morphogenesis in terrestrial plants (Hamant *et al.*, 2010).

Note that, while there is evidence that the cell wall has a role in preventing gametophyte cells from switching to sporophyte cell fate, it does not appear to be necessary for the maintenance of gametophyte fate. Protoplasts derived from a gametophyte maintained their gametophyte cell identities despite the absence of cell

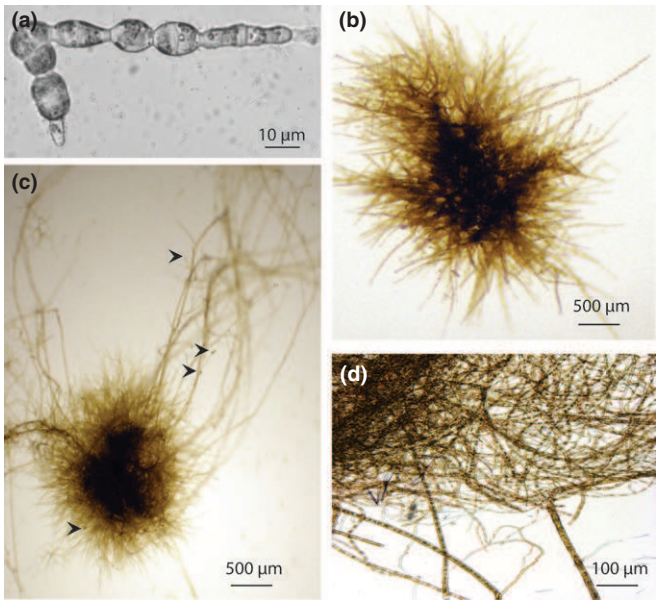

Fig. 4 *Ectocarpus siliculosus* protoplast regeneration in the presence of conditioned media. (a) Regeneration of a gametophyte-derived protoplast in the presence of sporophyte-conditioned medium (SCM), 15 d after protoplast isolation. (b) Regeneration of a gametophyte-derived protoplast in the presence of SCM, 30 d after protoplast isolation. (c) Gametophyte-derived protoplast regenerated in the presence of SCM showing the formation of upright filaments and plurilocular sporangia (arrow heads) after *c*. 45 d in culture. (d) Congo red staining of a regenerated gametophyte-derived protoplast incubated in the presence of SCM for 5 wk.

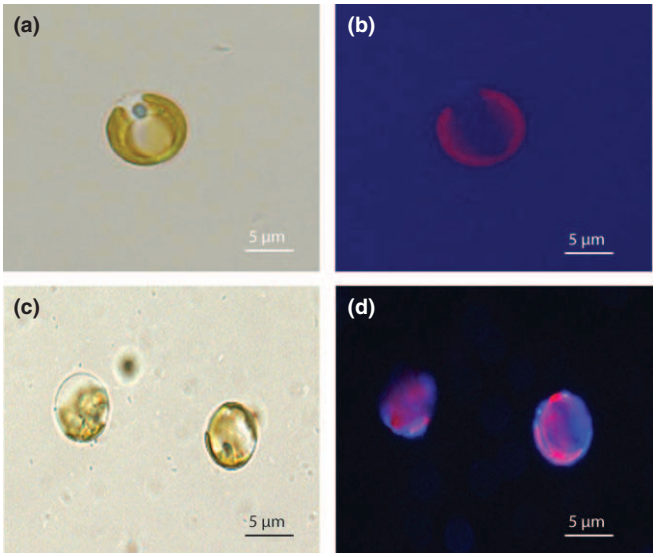

Fig. 5 Transmitted light (a, c) and epifluorescent (b, d) images of regenerating, partheno-sporophyte-derived *Ectocarpus siliculosus* protoplasts. Red, autofluorescence from chloroplasts. Cell wall (blue, calcofluor white staining) starts to be produced after 3–4 d in culture. Similar results were obtained for gametophyte-derived protoplasts (not shown). (a, b) 12 h after protoplast isolation; (c, d) 96 h after protoplast isolation.

wall for a period of several hours and regenerated as gametophytes. Sporophyte cells also retained their sporophyte identity when their cell wall was removed. Taken together, these observations indicate that removal of the cell wall does not provoke complete dedifferentiation of the cell, at least as far as life cycle generation identity is concerned.

Additional work will be required to elucidate the biochemical nature of the diffusible factor. Another important question is the role of this factor under natural conditions. One of the hypotheses that has been put forward to explain the stability of haploid-diploid life cycles is that each generation is able to exploit a different ecological niche and that this confers an advantage in a variable environment (in response to seasonal changes, for example; reviewed in Coelho *et al.*, 2007). Work on *Ectocarpus* has provided some support for this hypothesis in as far as transcriptome analysis has indicated significant metabolic differences between the sporophyte and gametophyte generations (Peters *et al.*, 2008). There may therefore be an advantage for the alga to be able to induce switching from the gametophyte to the sporophyte generation under some conditions. In the field, presumably this would involve released meio-spores perceiving the diffusible signal in the vicinity of mature sporophytes. The marine environment is conducive to signaling systems involving diffusible molecules and analogous systems have been described in both coastal and open water environments, including diffusible brown algal sex pheromones (e.g. Müller *et al.*, 1971) and several other infochemicals (e.g. Joint *et al.*, 2002; Pohnert *et al.*, 2007; Brownlee, 2008; Bidle & Vardi, 2011).

In conclusion, this study has identified a sporophyte-derived factor that acts in a non-cell autonomous manner to induce developmental reprogramming in wall-less gametophyte cells, causing them to become fully functional sporophytes. The study not only identifies a surprising developmental plasticity in *Ectocarpus*, but also emphasizes the role of the cell wall in maintaining the developmental program associated with each generation once these programs have been engaged. The insensitivity of the *oro* mutant to the sporophyte-factor indicates that ORO is a key member of the developmental cascade downstream of the sporophyte-conditioned factor. While more work will be necessary to identify its nature, the diffusible signal produced by the sporophyte clearly represents a powerful tool with which to dissect the molecular mechanism underlying life cycle transitions.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 *Ectocarpus siliculosus* life cycle in culture.

Fig. S2 Effect of conditioned media on the regeneration of protoplasts prepared from the life cycle mutant *oro*.

Table S1 Generation marker genes (Peters *et al.*, 2008) used for the RT-qPCR analysis

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