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DISTAG/TBCCd1 IS REQUIRED FOR BASAL CELL FATE

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DETERMINATION IN *ECTOCARPUS*

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ABSTRACT

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Brown algae are one of the most developmentally complex groups within the eukaryotes. As in many land plants and animals, their main body axis is established early in development, when the initial cell gives rise to two daughter cells that have apical and basal identities, equivalent to shoot and root identities in land plants, respectively. We show here that mutations in the *Ectocarpus DISTAG* (*DIS*) gene lead to loss of basal structures during both the gametophyte and the sporophyte generations. Several

29 abnormalities were observed in the germinating initial cell in *dis* mutants including
30 increased cell size, disorganisation of the Golgi apparatus, disruption of the microtubule
31 network and aberrant positioning of the nucleus. *DIS* encodes a TBCCd1 protein, which
32 has a role in internal cell organization in animals, *Chlamydomonas* and trypanosomes.
33 Our study highlights the key role of subcellular events within the germinating initial cell
34 in the determination of apical/basal cell identities in a brown alga, and emphasizes the
35 remarkable functional conservation of TBCCd1 in regulating internal cell organization
36 across extremely distant eukaryotic groups.

37 SHORT TITLE

38 Basal cell fate determination in *Ectocarpus*.

39 ONE SENTENCE SUMMARY

40 Mutations in the *Ectocarpus* *DISTAG* gene, which encodes a TBCCd1 protein, disrupt
41 the subcellular architecture in the germinating initial cell leading to loss of all basal
42 structures from the developing alga.

43 INTRODUCTION

44 Events in the zygote leading up to and including the first cell division play a key role in
45 the developmental patterning of multicellular organisms. In many plants and animals,
46 asymmetric division of the zygote establishes the principal body axis of the early embryo,
47 representing the first major patterning event during embryogenesis (Gönczy and Rose,
48 2005; Ueda and Laux, 2012). In land plants, the first cell division usually coincides with
49 the establishment of apical and basal cell identities (Ueda and Laux, 2012) (but note that
50 some fern gametophytes have tripolar germination patterns, Schneider, 2013). In
51 *Arabidopsis* the asymmetrical division of the zygote is associated with a number of
52 cellular events including movement of the nucleus to the apex of the cell, repositioning of
53 other major organelles, formation of a large vacuole in the basal part of the cell and
54 reorganisation of the microtubules into transverse cortical arrays associated with cell

55 outgrowth in the apical direction (Jeong et al., 2011a; Kimata et al., 2016; Lau et al.,
56 2012). The establishment of the apical-basal axis in *Arabidopsis* has been shown to be
57 controlled by a complex genetic network involving both auxin-dependent and auxin-
58 independent pathways (Lau et al., 2012). The auxin-dependent network involves the
59 transcription factor MONOPTEROS/AUXIN RESPONSE FACTOR 5, the auxin
60 response inhibitor BODENLOS/INDOLE-3-ACETIC-ACID 12 and the auxin efflux
61 carrier PIN-FORMED 7, whereas the auxin-independent pathway involves EMBRYO
62 SURROUNDING FACTOR 1 (ESF1), the receptor-like cytosolic kinase SHORT
63 SUSPENSOR, the MAP kinase kinase YODA, the MAP kinases MPK3 and MPK6 and
64 several transcription factors including GROUNDED/RKD4 and WOX2 (Jeong et al.,
65 2011b; Waki et al., 2011; Costa et al., 2014; Lau et al., 2012; Rademacher et al., 2012).
66 However, it is not yet clear how these genetic networks implement the cellular events that
67 underlie the formation of the apical-basal axis and the establishment of apical and basal
68 cell identities.

69 The brown algae have evolved complex multicellularity independently of land plants
70 but the two lineages share several key developmental characteristics such as the
71 importance of the establishment of the apical/basal axis during development, extensive
72 post-embryonic developmental patterning and developmental programs constrained by
73 the absence of cell migration. The apical and basal structures of brown algae and land
74 plants can also be considered to be analogous, with the basal systems of brown algae
75 being functionally equivalent to rooting structures in land plants. In both lineages, the
76 basal systems are composed of tip-growing filamentous rhizoid cells that extend into the
77 substrate or air/water surrounding the plant and are used for anchoring (Jones and Dolan,
78 2012). In both brown algae and flowering plants apical/basal polarity is usually
79 determined very early, before the first cell division (Fritsch, 1935). However, this process
80 may be less complex in brown algae, because the development of initial cells (e.g. the
81 zygote) does not occur within maternal tissues. Consequently, axis formation is expected
82 to occur in a cell autonomous manner (but see (Whitaker, 1931) and not to be influenced
83 by signals emitted by nearby parental cells (Costa et al., 2014).

84 The brown alga *Fucus* has been used for many years as a model system to study apical-
85 basal axis formation during embryogenesis (e.g. Coelho et al., 2002; Brownlee and
86 Bouget, 1998; Kropf et al., 1988). As in land plants, the zygote cell divides
87 asymmetrically to establish the apical-basal axis (Bouget et al., 1998; Goodner and
88 Quatrano, 1993). The two products of this division go on to produce the apical and basal
89 systems of the alga, the thallus and the rhizoid, respectively (Brownlee and Bouget,
90 1998). The establishment of the apical-basal axis in *Fucus* has been studied in detail at
91 the cellular level and has been shown to involve position dependent information from the
92 cell wall (Berger et al., 1994). Apoplastic diffusible gradients appear also to be involved
93 in pattern formation in the multicellular *Fucus* embryo (Bouget et al., 1998).

94 Whilst the large zygote and the external fertilisation process (fusion of male and
95 female gametes released from the parent thallus) of *Fucus* makes it well adapted for the
96 study of the cell biology of axis formation, this genus is not amenable to genetic
97 approaches and, consequently, the genetic networks that control apical-basal axis
98 formation have not been characterized in the brown alga lineage. Recently, the
99 filamentous alga *Ectocarpus* sp. has emerged as a genetic model for the brown algae
100 (Coelho et al., 2012c; Cock et al., 2014). A high-quality genome sequence is available for
101 this species (Cock et al., 2010; Cormier et al., 2017), together with extensive
102 transcriptomic data (e.g. Lipinska et al., 2015; Luthringer et al., 2015) and genetic tools
103 including a dense genetic map (Heesch et al., 2010; Avia et al., 2017). Genetic
104 transformation is not yet possible but gene knockdown using RNA interference has been
105 demonstrated (Macaisne et al., 2017). These various tools were employed in a recent
106 study that was the first to identify a brown algal developmental gene using a forward
107 genetic approach (Macaisne et al., 2017).

108 *Ectocarpus* has a complex life cycle involving alternation between multicellular
109 sporophyte and gametophyte generations (Coelho et al., 2012c; Peters et al., 2008)
110 (Supplemental Figure 1). Unlike land plants, both the sporophyte and the gametophyte
111 are derived from single cells, which are released into the seawater medium (gametes and
112 meio-spores, respectively) greatly facilitating the analysis of early events during
113 development. This characteristic also means that any parental influence on developmental

114 processes is essentially limited to information provided to the propagules before their
115 release (but see also Arun et al., 2013).

116 Both the gametophyte and the sporophyte possess an apical-basal axis and clearly
117 defined apical and basal filamentous systems (Coelho et al., 2011; Peters et al., 2008). In
118 the gametophyte, the basal system consists of rhizoids and the apical system is composed
119 of upright filaments that bear the gametangia. In the sporophyte, the basal system is more
120 extensive, consisting of a network of firmly attached basal filaments, whereas the apical
121 system resembles that of the gametophyte, consisting of branched upright filaments that
122 bear the sexual structures. The general similarities between the gametophyte and
123 sporophyte generations, in terms of their size and overall morphologies, make *Ectocarpus*
124 an ideal system to investigate how two distinct developmental programs can be deployed
125 from the same genome.

126 In this study, we report the identification of the *DISTAG (DIS)* locus, which is required
127 for the formation of basal structures during both the sporophyte and gametophyte
128 generations of the *Ectocarpus* life cycle. *DIS* encodes a Tubulin Binding Co-factor C
129 (TBCC) domain protein of the TBCCd1 class. Mutations in the *Ectocarpus DIS* gene are
130 associated with several modifications at the initial cell stage: disorganisation of the Golgi
131 apparatus, increased cell size, disruption of the pattern of the microtubule network and
132 aberrant positioning of the nucleus. *dis* mutants therefore link subcellular events within
133 the initial cell with the acquisition of apical/basal cell identities. The phenotypes of *dis*
134 mutants also confirm that the basal filament system of the sporophyte is developmentally
135 equivalent to the rhizoid of the gametophyte generation, providing insight into the
136 evolutionary events that led to the emergence of the sporophyte and gametophyte
137 developmental programs in this species.

138 RESULTS

139 ***dis* mutants lack a basal system**

140 During the *Ectocarpus* gametophyte generation the apical/basal axis is established in
141 the initial cell, prior to the first cell division. The two cells derived from the division of

142 the initial cell grow, in the form of two germ tubes, to establish a rhizoid (basal, root-like
143 organ) and a filamentous thallus (apical, shoot-like organ) (Figure 1). A UV mutagenesis
144 screen identified two mutant strains (Ec722 and Ec799, Supplemental Table 1;
145 Supplemental Figure 2) that failed to develop any of the basal structures normally
146 observed in the wild type gametophyte generation, i.e., the initial cells of the mutants
147 immediately developed as apical upright filaments.

148 During the early development of the wild type sporophyte generation, establishment of
149 apical structures is delayed and an extensive system of prostrate filaments consisting of
150 round cells is formed before the apical thallus filaments develop (Peters et al., 2008;
151 Figure 1). Several observations indicate that this basal network of prostrate filaments is
152 equivalent to the basal system of the gametophyte generation, represented by the rhizoid:
153 1) both structures develop at the base of the alga (in contact with the substratum), 2) both
154 structures serve an anchoring function and 3) in sporophytes carrying the *immediate*
155 *upright* (*imm*) mutation the system of prostrate filaments is homeotically replaced by a
156 rhizoid similar to that of the gametophyte (Peters et al., 2008; Macaisne et al., 2017).

157 The sporophyte generations of the Ec722 and Ec799 mutants failed to produce a
158 network of prostrate filaments and therefore also lacked basal structures (Figure 1). The
159 establishment of reproductive structures on apical systems was unaffected in both
160 generations, and the mutants were fully fertile as both gametophytes and sporophytes
161 (Figure 1).

162 The absence of a basal system significantly affected the capacity of the mutants to
163 adhere to the substratum (Figure 2) and consequently Ec722 and Ec799 were named
164 *distag-1* (*dis-1*) and *distag-2* (*dis-2*), respectively ('distag' means detached in the Breton
165 language). The loss of basal systems during both the gametophyte and sporophyte
166 generation in *dis* mutants is consistent with these two systems (rhizoids and prostrate
167 filaments, respectively) being developmentally equivalent between the two generations.
168 Developmental equivalence between the basal systems of the two generations was further
169 supported by the observation that when *dis-1 imm* double mutants were constructed these
170 lacked the rhizoid that replaces the prostrate filaments in the *imm* mutant (Figure 2).

171 Analysis of a segregating family generated from a cross between *dis IMM* and *DIS imm*
172 parents indicated that *DIS* is epistatic to *IMM* (Supplemental Table 2).

173 In wild type *Ectocarpus*, secondary rhizoids are produced from the apical system cells
174 at a late stage of development (Peters et al., 2008) (Figure 1). These rhizoids can be
175 considered to be analogous to the adventitious roots produced from the stems of some
176 land plants (Atkinson et al., 2014). The *dis-1* and *dis-2* mutants failed to produce
177 secondary rhizoids during both the sporophyte and the gametophyte generations. Hence,
178 production of all basal structures, both primary and secondary, was blocked in these
179 mutants.

180 In fucoid brown algae, the apical system (the thallus), responds to damage by
181 producing rhizoids (e.g. (Bouget et al., 1998), and we observed a similar phenomenon in
182 wild type *Ectocarpus*. In contrast to the wild type, wounded apical filaments of the *dis*
183 mutants failed to regenerate rhizoids, and instead produced new cylindrical apical
184 filament cells (Figure 2; Supplemental Figure 3). Taken together, our results indicate that
185 basal system formation fails to occur, at all developmental stages, in the absence of a
186 functional *DIS* gene product.

187 One consequence of the loss of basal structures in the *dis* mutants is that the
188 sporophyte and gametophyte generations are more similar than in the wild type because
189 in both cases the vegetative thalli consist uniquely of upright filaments made up of
190 cylindrical cells. Despite this resemblance, the sporophyte and gametophyte generations
191 of the *dis* mutants retained generation-specific features. First, morphometric
192 measurements showed that the length and width of the filament cylindrical cells and the
193 angle at which secondary upright filaments branch off from primary filaments
194 statistically distinguished sporophyte from gametophyte thalli in both wild type and *dis*
195 mutant strains. In contrast, no significant differences were detected when these
196 parameters were compared between the same generation of wild type and mutant strains
197 (Supplemental Table 3). Second, at maturity *dis* gametophytes produced plurilocular
198 gametangia, which contained gametes that were able to fuse with gametes of the opposite
199 sex to produce zygotes. The sporophyte generation of the *dis* mutants, on the other hand,

200 produced plurilocular sporangia containing spores (incapable of fusion) and unilocular
201 sporangia with haploid, meiosis-derived meio-spores. Finally, the expression patterns of
202 generation-specific marker genes (Peters et al., 2008) were consistent with assigned life
203 cycle generations in both wild type and *dis* individuals (Supplemental Figure 4).

204 **Analysis of the *dis* transcriptome**

205 To further characterize the *Dis*⁻ phenotype, an RNA-seq approach was employed to
206 study gene expression in the *dis-1* mutant compared with wild type basal and apical
207 tissues (Supplemental Table 4). Consistent with the fact that *dis* has lost the ability to
208 produce a basal system and is composed exclusively of apical system cells, analysis of
209 *dis* transcriptional profiles indicated that they were more similar to apical than to basal
210 transcriptomes of the wild type strain (Figure 3A, 3B; Supplemental Figure 5).

211 Several analyses were carried out to characterize the set of genes that were
212 differentially expressed in the three tissues analysed. These included an analysis of the
213 putative functions of clusters of genes with shared patterns of regulation (Supplemental
214 Table 5 and 6), an analysis of the 200 most differentially expressed genes (100 most up-
215 and 100 most down-regulated) in these comparisons (Supplemental Table 7 and 8), an
216 analysis of the differentially regulated genes that coded for proteins with putative signal
217 peptides (representing putative secreted proteins, Supplemental Table 9 and 10) and a
218 KEGG pathway analysis (Supplemental Table 11). Global patterns in the changes in gene
219 expression were analysed using a set of manually assigned functional categories (based
220 on the functional categories used in Tarver et al. (2015). GO term enrichment analysis
221 (Blast2Go; Conesa and Götz, 2008) and KEGG analysis were then used to provide more
222 detailed information about the processes and pathways affected. The relative frequency of
223 the manually assigned functional categories was visualized using a word cloud (Figure
224 3C). Note that about half of the genes in the transcriptome datasets were of unknown
225 function, and these were excluded from our analysis.

226 **Putative basal and apical system effector genes.** The gene cluster analysis focused
227 on genes that were either up- or downregulated in both the *dis-1* mutant and wild type
228 apical tissues compared to wild type basal tissues (Figure 3A, 3B; Supplemental Table 5).

229 The aim of this approach was to identify potential basal and apical system effector genes.
230 The set of genes that was upregulated in *dis* and the wild type apical system compared to
231 the wild type basal system (potential apical system effectors) were enriched in the GO
232 categories ‘kinase activity’, ‘protein modification’, ‘cell differentiation’ and ‘cell
233 communication’ (Fisher’s exact test, $p < 0.05$) while the genes that were downregulated in
234 both *dis-1* and the apical system compared to the basal system (potential basal system
235 effectors) were enriched in categories related to ‘transcriptional activity’, ‘signal
236 transduction’ and ‘extracellular’ or ‘membrane-located processes’ (Supplemental Table
237 6). Accordingly, the assignment of basal and apical effector genes to manually curated
238 functional groups highlighted an important proportion of genes coding for proteins
239 putatively involved in membrane functions and transport, cell wall biosynthesis and
240 modification, vesicle trafficking, adhesion, cell regulation and signalling (Figure 3C
241 upper panel, Supplemental Table 12).

242 **Top-200 most differentially regulated genes.** GO term enrichment analysis of the
243 200 most differentially regulated genes in the *dis-1* mutant compared with wild type basal
244 and apical systems highlighted a range of GO term categories (Supplemental Table 8),
245 but there was a particular abundance of genes with functions related to ‘membrane’,
246 ‘oxidation-reduction process’ and ‘carbohydrate metabolic processes’. The cellular
247 components highlighted by the GO term analyses were ‘membrane’ and ‘extracellular
248 region’. Manual assignment to functional categories revealed that the common functional
249 groups represented within this set of genes included cell wall biosynthesis and
250 organization, membrane function and transport, cell regulation and signalling, adhesion,
251 vesicle transport and cytoskeleton (Figure 3C middle panel, Supplemental Table 12).

252 **Secreted proteins.** The set of differentially expressed genes that were predicted to
253 encode secreted proteins were analysed in detail because the phenotypes of the *dis*
254 mutants are associated with disruption of the Golgi apparatus. Interestingly, a
255 disproportionate number of genes coding for proteins with a putative signal peptide were
256 present in the pool of genes that were differentially regulated in *dis* versus wild type basal
257 system (Fisher test, $p\text{-value} = 0.00002877$ and $p\text{-value} = 0.01992$ for up and downregulated
258 genes, respectively; Supplementary Table 4). Analysis of the GO term associated with

259 these genes indicated enrichment for range of GO categories including carbohydrate
260 transport, transmembrane transport, extracellular components, integral components of
261 membrane, transmembrane signalling and Golgi associated vesicles (Supplemental Table
262 10). The most commonly represented manually annotated functional categories
263 represented within this dataset included, as above, cell wall, adhesion, extracellular
264 processes, membrane function and transporters, cytoskeleton, and cellular regulation and
265 signalling (Figure 3C lower panel).

266 **KEGG analysis.** Analysis of the assignment of the differentially expressed genes to
267 KEGG pathways (Xie et al., 2011) revealed a number of processes that were
268 differentially regulated in *dis* versus wild type samples. Pathways involved in interactions
269 with the extracellular-matrix, protein processing in the endoplasmic reticulum and protein
270 export were upregulated in the *dis-1* mutant compared with the wild type basal system,
271 whereas signalling pathways and protein processing in the endoplasmic reticulum were
272 downregulated (Supplemental Figure 7; Supplemental Table 11).

273 Taken together, comparison of the *dis-1* transcriptome with wild type basal and apical
274 tissues suggested that carbohydrate/cell wall-related processes, membrane transport,
275 cellular signalling and secretory activity may be affected in *dis* mutants.

276 Note that transcript abundance was also measured using an independent method (RT-
277 qPCR) for 12 of the differentially regulated genes to verify that the differential
278 expression patterns observed were robust (Supplemental Figure 6). Globally, the results
279 of the RT-qPCR analysis confirmed those obtained based on the RNA-seq data
280 (Spearman's correlation $R^2=0.619$, p-value = 0.0012).

281 **Loss of bipolar germination from the initial cell in *dis* mutants**

282 The vast majority of initial cells of both the sporophyte and gametophyte generations
283 of the *dis* mutants produced a single germ tube rather than the two germ tubes normally
284 observed with wild type strains (Peters et al., 2008). This phenotype was observed more
285 consistently with the *dis-2* mutant than with the *dis-1* mutant (85% and 95% for *dis-1* and
286 *dis-2* sporophytes respectively, n=591; 82% and 95% for *dis-1* and *dis-2* gametophytes

287 respectively, n=54) (Figure 2). We also noted that, whilst the *dis-2* mutant completely
288 failed to produce a second germ tube, a proportion of *dis-1* individuals (65%, n=235)
289 produced one or more enlarged and abnormally shaped cells at the end where the second
290 germ tube would normally emerge, possibly corresponding to an aborted basal system
291 (Figure 2).

292 **Genetic analysis and identification of the *DIS* gene**

293 Sporophytes can be propagated asexually through the production of mito-spores
294 (Figure 1). The Dis⁻ mutant phenotype was stable through 30 rounds of asexual
295 generations via mito-spores.

296 A male *dis-1* gametophyte (Ec722) was crossed with an outcrossing wild type female
297 gametophyte of the strain Ec568 (Coelho et al., 2011; Peters et al., 2008) (Supplemental
298 Table 1, Supplemental Figure 2). The resulting sporophyte (Ec653) exhibited a wild type
299 pattern of development indicating that the *dis-1* mutation was recessive. A segregating
300 population of 200 individuals derived from this cross consisted of 92 and 108
301 phenotypically wild type and mutant individuals, respectively, consistent with a 1:1
302 segregation ratio and Mendelian inheritance of a single-locus recessive mutation (Chi-
303 square test = 1.28, df = 1, p-value = 0.257). A female individual from this progeny that
304 carried the *dis-1* mutation was crossed with a male gametophyte of the *dis-2* strain
305 (Ec799). The resulting sporophytes (Ec808, Ec809) exhibited a Dis⁻ phenotype indicating
306 that the two mutations *dis-1* and *dis-2* were allelic.

307 A cloning-by-sequencing approach (Schneeberger et al., 2009) identified a candidate
308 locus on chromosome 05 for the location of the mutation in the *dis-1* mutant (Figure 4A)
309 and sequencing of this genomic region identified a single nucleotide mutation at position
310 3330523 that was present in the *dis-1* mutant (Ec722) but absent from the Ec32 and
311 Ec568 wild type strains (Figure 4B). This mutation was located in intron 15 of the gene
312 Ec-05_001860. A cleaved amplified polymorphic sequence (CAPS) marker, developed
313 based on the candidate causal mutation, showed absolute co-segregation of this mutation
314 with the Dis⁻ phenotype in 265 individuals of the segregating family derived from
315 sporophyte Ec653. Re-sequencing of the Ec-05_001860 gene in the *dis-2* mutant

316 identified a point mutation in exon 13 that results in the introduction of a stop codon into
317 the coding region of the gene (Figure 4B). Taken together, these analyses provide strong
318 evidence that Ec-05_001860 corresponded to the *DIS* gene.

319 A protocol for genetic transformation does not exist for *Ectocarpus* (or for any other
320 brown alga), but injection of double stranded RNA into zygotes of the brown alga *Fucus*
321 has been shown to induce an RNA interference (RNAi) response, leading to knockdown
322 of target gene expression (Farnham et al., 2013). We have recently adapted this *Fucus*
323 RNAi protocol for *Ectocarpus*, using synthetic siRNA molecules instead of long double
324 stranded RNA molecules and a lipofectant specifically adapted for dsRNA delivery to
325 introduce the siRNAs into the cell rather than the microinjection procedure used for
326 *Fucus* (Macaisne et al., 2017).

327 Following simultaneous introduction of three siRNA molecules targeting the *DIS* gene,
328 a small proportion (about 2.5%) of the parthenogenetic gametes exhibited a pattern of
329 development that closely resembled the phenotypes of the *dis* mutants (Figure 4C); the
330 gametes emitted a germ tube in a unipolar manner. Germination gave rise to an upright
331 filament consisting of the cylindrical cells typical of the apical system. The gametes that
332 showed unipolar germination occasionally produced an aborted second germ tube with an
333 abnormally shaped cell similar to the enlarged and abnormally shaped cells that are
334 sometimes produced by *dis-1* mutants. This phenotype was not observed in the control
335 treatments, where initial cells were incubated with siRNA molecules directed against
336 another *Ectocarpus* sp. gene (Ec-13_001890). These observations indicated that RNA-
337 interference-induced knockdown of *DIS* gene expression had the same developmental
338 consequences as the *dis* mutation in a proportion of the treated individuals, further
339 confirming that Ec-05_001860 corresponds to the *DIS* gene.

340 **Expression pattern of the *DIS* gene during the life cycle**

341 RT-qPCR analysis indicated that, as expected, *DIS* transcript abundance was
342 significantly reduced in both *dis-1* and *dis-2* mutants (Supplemental Figure 8).

343 We used RNA-seq to assay the abundance of the *DIS* transcript throughout the life
344 cycle of *Ectocarpus*. Specifically, we compared the expression of *DIS* at several stages of
345 the ontogeny of the gametophyte and in several tissues of the sporophyte generation
346 (Supplemental Figure 1B). This analysis indicated that transcription of *DIS* was
347 upregulated in the basal system compared with the apical upright system during the
348 sporophyte generation (pairwise analysis between apical system and basal system,
349 $FC=1.9$; $p_{adj}= 7.66E^{-06}$). The *DIS* transcript was detected at low abundance during the
350 gametophyte generation (which consists predominantly of apical filament cells), similar
351 to the level detected in the apical system of the sporophyte (Supplemental Figure 8).
352 Taken together, the expression pattern of *DIS* during the life cycle of *Ectocarpus* is
353 consistent with a role in the regulation of the basal system, both during the gametophyte
354 and the sporophyte generations.

355 ***DIS* encodes the conserved protein TBCCd1**

356 The *DIS* gene is predicted to encode a 774-amino acid protein with a tubulin binding
357 cofactor C (TBCC) domain (accession PF07986 in Pfam database; Supplemental Table
358 13). Three classes of TBCC proteins have been described in other eukaryotic lineages:
359 canonical TBCC, retinitis pigmentosa protein 2 (RP2) and TBCCd1. Phylogenetic
360 analysis indicated that *DIS* encodes a TBCCd1 protein (Figure 4D; Supplemental Figure
361 9; Supplemental Table 14). *DIS* is the only TBCCd1-encoding gene in the *Ectocarpus*
362 genome. Note that the stop codon in *dis-2* is located within the region encoding the
363 TBCC domain (Figure 4B).

364 Canonical TBCC functions together with several other tubulin cofactors/chaperones in
365 $\alpha\beta$ -tubulin assembly and therefore plays a key role in microtubule dynamics (Tian et al.,
366 1996; Nithianantham et al., 2015). RP2 is also thought to be involved in microtubule
367 assembly but the role TBCCd1 is less clear. Phenotypic analyses have shown that
368 TBCCd1 plays an important role in positioning organelles within the cell in diverse
369 organisms (Feldman and Marshall, 2009; André et al., 2013) but the molecular
370 mechanism through which this protein acts is unclear and may not involve direct effects

371 on microtubule dynamics (Goncalves et al., 2010). These previous studies prompted us to
372 compare intracellular features of germinating *dis* initial cells with those of the wild type.

373 **No defects in flagella structure and function were detected in *dis* mutants**

374 *Chlamydomonas* mutants affected in the *ASQ2* gene (which encodes a TBCCd1
375 protein) have a variable number of flagella (Feldman and Marshall, 2009). In contrast, we
376 observed no variation in flagella number in *dis* mutant individuals and both the posterior
377 and the anterior flagella of *dis* gametes were morphologically similar to the wild type
378 equivalents and were positioned normally, being inserted asymmetrically on the “ventral”
379 side, close to the eyespot (Maier, 1997; Supplemental Table 15). *dis* gametes exhibited no
380 abnormal swimming behaviour that might have been indicative of loss of flagella
381 functionality; both wild type and *dis* gametes were positively phototactic (Supplemental
382 Table 15). Moreover, male gametes of *dis* and wild type strains showed no difference in
383 fertilization success, evidence that the former can swim towards female gametes in
384 response to pheromone release as efficiently as wild type gametes (Supplemental Table
385 15). In summary, we did not find any evidence that DIS is required for flagella
386 functionality.

387 **Initial cells of *dis* mutants exhibit increased cell size, modified Golgi architecture,**
388 **abnormal positioning of the nucleus and modification of the microtubule network**

389 The germinating initial cells of *dis* mutants were significantly larger than wild type
390 initial cells (mean areas of 53.9 μm^2 , 54.1 μm^2 and 32.0 μm^2 for *dis-1*, *dis-2* and wild
391 type, respectively; Supplemental Table 15; Figure 5D) and analysis of transmission
392 electron microscopy (TEM) images indicated an unusually abundant trans Golgi network
393 in the initial cells of *dis* mutants, suggesting a perturbation of secretory activity (Figure
394 5A-F). Moreover, Golgi cisternae of *dis-1* and *dis-2* cells were significantly shorter than
395 those of the wild type (Wilcoxon test, p-value=1.22e⁻¹³; Figure 5H). A similar phenotype
396 has been observed in TBCCd1-depleted human cells, which are enlarged and exhibit a
397 disorganized Golgi (Goncalves et al., 2010). The cellular phenotype of the *dis* mutants is
398 therefore consistent with TBCCd1 having a conserved role in maintaining the structural

399 integrity of the Golgi during early development across extremely distant eukaryotic
400 groups. Note that depletion of the tubulin binding co-factor TBCE also causes
401 disorganisation and fragmentation of the Golgi (Haase and Rabouille, 2015).

402 The microtubule network plays an important role in the positioning of cellular
403 organelles and has been implicated in the organisation and positioning of the Golgi
404 apparatus close to the nuclei (reviewed in Rios and Bornens, 2003). We therefore
405 investigated whether the microtubule network was altered in *dis* mutants. In wild type
406 initial cells, a well organised interphase microtubule network was visible during initial
407 cell germination. Microtubules nucleated from the pole opposite the first germination
408 tube and microtubule bundles were gently curved and oriented parallel to the germination
409 axis towards the germination pole (Figure 6A, B; Supplemental Figure 10). In *dis* mutant
410 cells, microtubule bundles were more abundant compared with the wild type (Figure 6;
411 Wilcoxon test, $p=0.000156$ and $p=0.0069$ for wild type versus *dis-1* and wild type versus
412 *dis-2* comparisons, respectively) and the architecture of the microtubule network was
413 disturbed. *dis* mutant microtubules were organised in wavy bundles with a criss-cross
414 pattern (Figure 6A, B; Supplemental Figure 10). The perturbations of the microtubule
415 architecture were only observed in the germinating initial cell, before the first cell
416 division. No abnormalities of the microtubule network were detected in *dis* mutant
417 filament cells at or after the two-cell stage, compared with wild type cells (Figure 6C).
418 Cells of wild type and *dis* 15 day old germlings exhibited similar microtubule networks
419 with bundles of a variety of thicknesses.

420 In addition to the above-mentioned flagellar phenotype, the *Chlamydomonas asq2*
421 mutant exhibits defects in centriole positioning and number (Feldman and Marshall,
422 2009). We examined two aspects of centriole positioning in the *Ectocarpus dis* mutants:
423 the degree with which centrioles were associated with the nucleus and the positioning of
424 the associated centriole and nucleus with respect to the germination axis. Regarding the
425 first aspect, centrioles were positioned close to the nuclei in both wild type and *dis*
426 mutant cells and there was no evidence of detachment of centrioles from the nuclear
427 envelope in *dis* mutants (Figure 5A-C). In contrast, we detected marked differences
428 between wild type and *dis* mutant cells with respect to the position of the centrioles in

429 relation to the germination pole. In wild type cells, centrioles (which co-localise with
430 microtubule nucleating sites, Figure 6E) were located close to the nucleus on the side
431 opposite the germination pole ('distal'), whereas in *dis* mutant cells, we observed
432 microtubule nucleation both laterally and on the proximate side of the nucleus (Figure
433 6F).

434 More than 200 *dis* mutant germlings were inspected at the two-cell stage to determine
435 whether the aberrant positioning of microtubule nucleation sites in germinating initial
436 cells leads to defects in the plane of cell division when these cells divides. This analysis
437 failed to detect any individuals showing signs of aberrant patterns of cell division, i.e.
438 there were no individuals in which the new septum was laid down obliquely, in a division
439 plane that was not perpendicular to the growth axis.

440 We also analysed the position of the nucleus within the germinating cell. In the
441 majority of wild type germinating initial cells the nucleus was positioned near the pole
442 opposite the first germination tube (Figure 6E). In contrast, the majority of the nuclei in
443 *dis-1* and *dis-2* initial cells were positioned centrally and in up to 30% of the cells the
444 nucleus was located at the germinating end, a configuration that was only observed very
445 rarely (about 2%) in wild type germinating initial cells (Figure 6E, F). Analysis of
446 individuals from the two-cell stage onwards did not detect any aberrations in the
447 positioning of the nuclei in *dis* mutants compared to the wild type (Wilcoxon test, p-
448 value=0.837 and P=0.207 for wild type vs *dis-1* and wild type vs *dis-2* respectively),
449 indicating that the phenotype observed in initial cells is limited to that stage of
450 development (Figure 6E).

451 Taken together, these analyses indicate that loss of the DIS protein leads to several
452 defects in subcellular architecture of the initial cell and that these defects are associated
453 with alterations in the microtubule network.

454 To further examine the role of microtubules during early development of *Ectocarpus*
455 initial cells, we analysed the effect of drugs that disrupt both microtubule polymerisation
456 and depolymerisation (although note that the increased number of microtubule bundles in
457 *dis* mutants is more consistent with a depolymerisation defect). Oryzalin, which induces

458 microtubule depolymerisation, significantly inhibited elongation of wild type initial cells
459 and delayed the first cell division but did not result in perturbations of the microtubule
460 network resembling those observed in the *dis* mutants (Supplemental Figure 11;
461 Supplemental Table 16). Furthermore, oryzalin treatment did not lead to aberrant
462 positioning of the nuclei, compared with wild type untreated control cells (Wilcoxon test,
463 $W= 101.5$, $p\text{-value} = 0.3818$, Supplemental Figure 11). Similarly, treatment of wild type
464 initial cells with taxol, a microtubule stabilizing drug, inhibited germination and cell
465 division but did not produce a cellular phenotype similar to that of the *dis* mutants. Taxol
466 treated cells had thick microtubule bundles that were mostly oriented parallel to the cell
467 membrane (Supplemental Figure 10, 11). This pattern differed from those of both
468 untreated wild type cells and *dis* cells. Taxol treatment did not have any significant effect
469 on the position of the nucleus in germinating cells (Wilcoxon test, $W= 95$, $p\text{-}$
470 $\text{value}=0.1978$, Supplemental Figure 11D).

471 The lack of any detectable effect of pharmacological perturbation of the microtubule
472 network on the positioning of the nucleus within the initial cell suggests that alternative
473 cytoskeletal components, such as actin filaments, may be involved in positioning the
474 nucleus, as observed in plant root hair cells (Ketelaar et al., 2002).

475 Brefeldin A (BFA) has been shown to disrupt the Golgi and to inhibit polarisation of
476 the germinating *Fucus* embryo (Bisgrove and Kropf, 2001; Hable and Kropf, 1998).
477 Treatment of *Ectocarpus* sp. initial cells for 24h with BFA weakened the cell wall
478 (leading to the release of cytosolic material) and inhibited cell elongation, but did not
479 reproduce the *Dis*⁻ phenotype (Supplemental Table 16, Supplemental Figure 11).

480 Taken together, the results of these pharmacological experiments suggest that the
481 phenotypes associated with the *dis* mutations are not simply due to defects in microtubule
482 polymerisation or depolymerisation nor to an overall deficiency in secretion.

483 DISCUSSION

484 ***DIS* is required for the establishment of basal structures in *Ectocarpus***

485 Analysis of the phenotypes of the two *dis* mutants described in this study indicated that
486 the *DIS* gene is required for the development of basal structures throughout the
487 *Ectocarpus* life cycle. In wild type *Ectocarpus* gametophytes, the initial cell divides
488 asymmetrically to produce two cells with apical and basal identities. Further divisions in
489 opposite directions of each of these cell types generate an apical thallus and a basal
490 rhizoid, respectively. In the gametophytes of *dis* mutants the basal germ tube that gives
491 rise to the rhizoid is not produced. The consequent unipolar germination gives rise to
492 individuals with an apical system but no basal structures. We suggest that the basal
493 system is not produced because the *dis* mutants fail to deploy a putative basal determinant
494 factor ('B') in the initial cell and, as a result, the first division produces an apical cell and
495 a quiescent undifferentiated cell (Figure 7).

496 In the wild type sporophyte, the first division of the initial cell is symmetrical and the
497 two germ tubes form the two ends of a basal filament, which is strongly attached to the
498 substratum. In the absence of the *DIS* protein, the initial cell produces just one germ tube,
499 which gives rise to the apical system. We interpret the failure to initiate a second germ
500 tube as being a consequence of the failure to establish basal cell identity in the
501 sporophyte of this mutant. We suggest that, in the absence of the basal determinant factor
502 'B', the initial cell divides to produce an apical cell and a quiescent undifferentiated cell in
503 the same manner as observed in the gametophyte generation.

504 The *dis-2* mutation creates a premature stop codon within the region that encodes the
505 TBCC domain and is therefore likely to be a null mutation. The *dis-1* mutation, on the
506 other hand, is located in an intron (Figure 3). This mutation causes a reduction in
507 transcript abundance but does not modify the open reading frame of the transcript.
508 Analysis of the RNA-seq data did not detect any evidence that the mutation affects
509 splicing of the transcript. Therefore, *dis-1* may not represent a null mutation. If *dis-1* is
510 not a null allele, then under the model shown in Figure 7, the abortive structures
511 produced instead of the second germ tube in *dis-1* mutant sporophytes can be interpreted
512 as arising due to incomplete penetrance of the *dis-1* mutation, allowing a small amount of
513 the putative basal determinant 'B' to accumulate, and the production of an abortive basal
514 system.

515 The model proposes that the observed phenotypes are due to variations in
516 concentration, rather than the subcellular distribution, of the putative 'B' factor. One
517 possible scenario would be that the abnormal Golgi apparatus of the initial cell is unable
518 to produce sufficient amounts either of the putative 'B' factor itself or of a protein that
519 generates the 'B' factor enzymatically. Interestingly, comparison of the *dis-1*
520 transcriptome with those of wild type apical and basal tissues identified an enrichment in
521 genes coding for secreted proteins and highlighted a putative role for *DIS* in, among other
522 processes, cell wall modification processes, secretion, transport and vesicle trafficking.
523 Secretion and diffusion of cell fate determinants, particularly from the cell wall, have
524 been shown to play a crucial role in pattern formation in brown algae (Bouget et al.,
525 1998; Arun et al., 2013). Interestingly, mutant analysis indicates that cell wall synthesis,
526 cell wall integrity sensing, and vesicle trafficking are also important processes during the
527 development of *Marchantia polymorpha* rhizoids and *Arabidopsis* root hairs (Honkanen
528 et al., 2016).

529 In *Ectocarpus*, the apical and basal developmental programs appear to be independent,
530 in the sense that removal of the basal system in the *dis* mutants had no visible effect on
531 patterning of the apical system. In contrast, *Arabidopsis* mutations that result in the loss
532 of basal structures, such as *monopteros* and *bodenlos* for example, also tend to cause
533 more or less subtle modifications to the apical structures (Hardtke and Berleth, 1998;
534 Hamann et al., 1999). This difference may reflect the simpler developmental program of
535 *Ectocarpus* compared to *Arabidopsis* but may also be related to the fact that the primary
536 function of brown algal basal systems is to act as a holdfast, whereas the aerial tissues of
537 land plants are dependent on the root system not only for anchorage but also for water
538 and nutrient supply. In land plants, therefore, there may be more of a tendency for the
539 developmental programs of the apical and basal systems to be interdependent.

540 ***DIS* encodes a TBCC protein with a conserved role in intracellular organisation**

541 The *DIS* gene encodes a TBCCd1 protein. TBCCd1 is a member of the TBC group of
542 proteins, which also includes the retinitis pigmentosa protein 2 (RP2) and TBCC, a
543 component of the chaperone complex (TBCA to TBCE) that catalyses the formation of

544 α,β -tubulin heterodimers (Tian and Cowan, 2013; Steinborn et al., 2002). All three
545 TBCC proteins have been strongly conserved across the eukaryotic tree (Figure 4D,
546 Supplemental Table 13) suggesting that each has a specific function in the cell. However,
547 whilst the role of TBCC has been described in detail, the exact cellular and molecular
548 function of TBCCd1 remains unclear.

549 The role of TBCC in the α,β -tubulin heterodimerisation pathway is to trigger GTP
550 hydrolysis catalysing the release of newly formed $\alpha\beta$ -tubulin heterodimers. Importantly,
551 this pathway is reversible so that TBCC plays a role in both microtubule assembly and
552 disassembly (Nithianantham et al., 2015). Both TBCC and RP2 have GAP activities,
553 mediated by their TBCC domains, and RP2 can complement a TBCC mutant (Schwahn
554 et al., 1998; Schwarz et al., 2012). In contrast, the TBCC domain of TBCCd1 lacks
555 several conserved residues including a critical arginine residue and TBCCd1 is thought
556 not to be a GAP protein (Bartolini et al., 2002; Scheffzek et al., 1998), although it has
557 been suggested that a nearby conserved arginine residue may substitute for the lost
558 arginine residue (Feldman and Marshall, 2009) (Supplemental Figure 9). Therefore,
559 although TBCCd1 probably interacts with the cytoskeleton in some way to mediate its
560 cellular functions, it is currently unclear whether this specifically involves a direct role in
561 microtubule polymerisation/depolymerisation.

562 TBCCd1 has been localised to the centrosome and the Golgi in organisms as diverse as
563 humans, *Chlamydomonas* and trypanosomes and loss-of-function experiments have
564 indicated a conserved and important role for TBCCd1 in various aspects of the
565 organisation of cellular architecture in these diverse eukaryote groups (André et al., 2013;
566 Feldman and Marshall, 2009; Goncalves et al., 2010). Loss or knockdown of TBCCd1
567 has severe consequences for cell morphology, for example leading to alterations in the
568 shape and motility of human cells (Goncalves et al., 2010), mitotic defects in
569 trypanosomes (André et al., 2013) and defects both in flagella number and functioning
570 and during cell division due to misorientation of the mitotic spindle in *Chlamydomonas*
571 (Feldman and Marshall, 2009). Although we did not detect any defects in flagella
572 structure or centriole positioning in *Ectocarpus dis* mutants, the initial cells of these
573 mutants did show defects in the positioning of the nucleus, and an atypical microtubule

574 network during germination. Moreover, *dis* initial cells were markedly larger than the
575 wild type equivalents and their Golgi network was more abundant and fragmented
576 (shorter cisternae). Increased cell size and disorganisation of the Golgi apparatus have
577 been observed in TBCCd1-depleted human and trypanosome cells (Goncalves et al.,
578 2010; André et al., 2013). The role of TBCCd1 in regulating organelle positioning,
579 microtubule and Golgi architecture, highlighted by our study, can therefore be traced
580 back to the crown divergence of the major eukaryotic groups, more than a billion years
581 ago (Eme et al., 2014).

582 The Golgi defects in the *dis* mutants are particularly interesting with regard to the
583 model described in Figure 7, which proposes that the Dis⁻ phenotype is due to failure to
584 deploy a hypothetical basal-cell-fate-determining factor 'B' during the first cell division.
585 In animals, the Golgi is critical for cellular differentiation and morphogenesis because it
586 spatially constrains developmental pathways and it is required for the establishment of
587 cell polarity and normal subcellular organization (Copeland et al., 2016; Vinogradova et
588 al., 2009; Zhong, 2011). Moreover, in the embryo of the brown alga *Fucus*, targeted
589 secretion from the Golgi to the cell wall has been implicated in axis fixation and
590 establishment of the basal versus the apical system (Shaw and Quatrano, 1996).

591 In *Arabidopsis*, asymmetrical division of the zygote is preceded by reorganisation of
592 the cytoskeleton and re-positioning of the nucleus from a central position to the apical
593 region of the cell (Kimata et al., 2016; Ueda et al., 2011). Mutation of the zinc finger
594 transcription factor gene *WRKY2* not only disrupts the asymmetrical division of the
595 zygote cell but also leads to partial loss of basal identity in the basal cell lineage (Ueda et
596 al., 2011). Interestingly, the *Ectocarpus dis* mutants not only fail to produce a basal
597 system but also exhibit disrupted organisation of the microtubule cytoskeleton and
598 defects in nucleus positioning in the initial cell. This suggests that, as in flowering plants,
599 the establishment of the basal cell lineage in brown algae depends critically on events
600 leading up to and including the first division of the initial cell.

601 It is not clear how loss of the TBCCd1 protein leads to disorganisation of the
602 microtubule cytoskeleton in the *dis* mutants (whether this is a direct or an indirect effect

603 for example) but this phenotype does not appear to be simply due to defects in
604 microtubule polymerisation or depolymerisation because treatment with oryzalin or taxol
605 did not result in a phenotype that resembled that of the *dis* mutants. The observed
606 modifications to the microtubule architecture in *dis* mutants may be due to more subtle
607 changes in nucleating activity, rates of assembly and disassembly of bundles,
608 modifications of microtubule dynamic instability and/or tubulin or microtubule
609 posttranslational modifications (Mitchison and Kirschner, 1984; Song and Brady, 2015).

610 Based on the phenotypes associated with TBCCd1 loss in other eukaryotes and on the
611 phenotypes of the *dis* mutants, we propose that acquisition of basal cell identity in
612 *Ectocarpus* depends critically on features of the initial cell architecture, that are disrupted
613 in the absence of a functional DIS/TBCCd1 protein. Further work is required to
614 understand the exact cellular role of TBCCd1 in *Ectocarpus* but the identification of this
615 component will allow new insights into the cellular mechanisms involved in establishing
616 apical/basal identity.

617 ***DIS* and the evolution of sporophyte and gametophyte developmental programs**

618 The pattern of early development of the *Ectocarpus* sporophyte, which involves
619 deployment of an extensive basal system before the establishment of the apical/basal
620 axis, is unusual because most brown algal sporophytes exhibit early establishment of the
621 apical/basal axis leading directly to the production of a thallus cell and a rhizoid,
622 respectively (Fritsch, 1935). In the *Ectocarpus immediate upright (imm)* mutant the
623 extensive basal system of the sporophyte fails to form and this structure is replaced by a
624 rhizoid (Peters et al., 2008). Based on the phenotype of the *imm* mutant, we have
625 suggested that the pattern of early sporophyte development in extant *Ectocarpus* evolved
626 from a simpler, ancestral developmental program that more closely represented that of
627 the gametophyte, i.e. involving an asymmetrical initial cell division that gave rise directly
628 to apical and basal organs (Macaisne et al., 2017). The *dis* mutants provide further
629 support for this hypothesis because they exhibit complete loss both of the rhizoid during
630 the gametophyte generation and of the extensive network of prostrate filaments during
631 the sporophyte generation, suggesting that the two structures are indeed equivalent.

632 Moreover, in a *dis-1 imm* double mutant sporophyte the rhizoid that normally replaces the
633 prostrate filament system fails to develop, providing further evidence that the rhizoid that
634 forms in the *imm* mutant sporophyte is developmentally equivalent to the basal prostrate
635 filament system of the wild type sporophyte.

636 In land plants, which also have haploid-diploid life cycles, there has been considerable
637 interest in the evolutionary origins of the sporophyte and gametophyte developmental
638 programs; specifically whether each generation has independently evolved its own
639 developmental pathways or, alternatively, whether there has been recruitment of
640 developmental programs from one generation to the other during evolution (Dolan, 2009;
641 Pires and Dolan, 2012; Shaw et al., 2011). Current evidence indicates that the
642 developmental networks that implement land plant sporophyte programs were mainly
643 recruited from the gametophyte generation, which was initially the dominant generation
644 in the land plant lineage (Dolan, 2009; Niklas and Kutschera, 2010) but there is also
645 evidence that there have been sporophyte-specific innovations (Szovenyi et al., 2011;
646 Sano et al., 2005). Analyses of the *imm* (Macaisne et al., 2017) and *dis* (this study)
647 mutants suggest that the evolution of developmental systems in the brown algae also
648 involved both co-opting of programs from one generation to the other and generation-
649 specific developmental innovations. The phenotypes of the *dis* mutants suggest that,
650 despite their clear morphological differences, the basal systems of the sporophyte and
651 gametophyte generations share underlying mechanistic features because the TBCCd1
652 protein is necessary for the deployment of both types of basal structure. In this respect,
653 therefore, the sporophyte and gametophyte basal structures appear to be homologous. On
654 the other hand, it is only in the sporophyte that the rhizoid has been modified to produce
655 an extensive system of prostrate filaments (note that gametophytes carrying the *imm*
656 mutation are indistinguishable from wild type gametophytes; Peters et al., 2008). The
657 developmental program that depends on the IMM protein therefore appears to have been
658 a sporophyte-specific innovation, which was presumably built onto a more ancient
659 program shared with the gametophyte generation that requires the action of the *DIS* gene.

660 MATERIAL AND METHODS

661 **UV mutagenesis and isolation of mutant strains**

662 Strain cultivation, genetic crosses, raising of sporophytes from zygotes and isolation of
663 meiotic families were carried out as described in (Coelho et al., 2012a, 2012d). Gametes
664 of *Ectocarpus* are able to develop parthenogenetically to produce haploid partheno-
665 sporophytes, which are identical morphologically to the sporophytes that develop from
666 diploid zygotes (Peters et al., 2008; Coelho et al., 2011). This phenomenon was exploited
667 to screen directly, in a haploid population, for mutants affected in early sporophyte
668 development. UV mutagenesis of gametes was carried out as described previously
669 (Godfroy et al., 2015) and mutant partheno-sporophytes lacking basal structures were
670 identified by visual screening under a light microscope.

671 **Genetic mapping of the *DIS* locus**

672 To obtain an approximate map position for the *DIS* gene, the *dis-1* mutant (Ec722) was
673 crossed with the outcrossing line Ec568 to generate a mapping population of 265
674 individuals. Each of the 265 individuals was derived from a different unilocular
675 sporangium (each unilocular sporangium contains 50-100 meiospores, derived from a
676 single meiosis followed by at least 5 mitotic divisions). The meio-spores germinated to
677 produced gametophytes, which were isolated and allowed to germinate
678 parthenogenically. The resulting partheno-sporophytes were then observed under a light
679 microscope to determine whether they exhibited the Dis^- phenotype. The *dis-1* mutation
680 was then approximately mapped using 21 Dis^- individuals from this segregating
681 population and 74 microsatellites markers from the *Ectocarpus* microsatellite-based
682 genetic map (Heesch et al., 2010). Using this approach, we localised the *dis-1* mutation to
683 chromosome 05 at 9.5 cM from the M_114 marker.

684 A cloning-by-sequencing strategy (using the SHOREmap approach,(Schneeberger et
685 al., 2009) was then used to precisely localise the *dis-1* mutation. Genomic DNA was
686 extracted from 87 wild type and 96 *dis* individuals from the mapping population using

687 NucleoSpin® 96 Plant II (MACHEREY-NAGEL, Düren, Germany) and pooled into two
688 wild type and Dis⁻ pools. An Illumina HiSeq2500 platform (Fasteris, Genève, Suisse) was
689 the used to generate 44.9 Gb of sequence data, corresponding to 117 and 104 million 2x
690 100 bp paired-end reads for the wild type and Dis⁻ pools, respectively (SRA accession
691 numbers SRR3710253 and SRR3710254, respectively). The same DNA extraction
692 protocol and sequencing platform were employed to sequence the *dis-2* mutant strain
693 Ec799, producing 49 million 2x125 paired-end reads (SRA accession number
694 SRR3714421). Reads were cleaned to remove nucleotides with quality scores of less than
695 20 from both ends using Prinseq (Schmieder and Edwards, 2011). Reads were then only
696 retained if they were longer than 50 nucleotides, had a mean quality of at least 25 and no
697 non-determined nucleotides. About 3.6% and 4.3% of the raw wild type and *dis* reads
698 were removed, respectively. Bowtie2 (Langmead and Salzberg, 2012) was used to map
699 71.9% and 74.7% of the wild-type and *dis* cleaned reads, respectively, onto the Ec32
700 reference genome. The read mapping was then improved by read realignment and base
701 quality recalibration using the GATK software suite. Variant calling was carried out
702 using the “consensus” tool of the SHORE pipeline in order to analyse allele frequency.
703 Since we had only one reference genome sequence for *Ectocarpus*, corresponding to the
704 wild type strain that was mutagenised to generate the *dis* mutants, we were not able to
705 apply the strategy described in the SHOREmap publication to generate the list of markers
706 (association of SHOREmap extract and create). We therefore selected genetic markers by
707 comparing SNPs called in wild type and Dis⁻ pools and extracted SNPs that segregated as
708 expected, i.e., common SNPs for which the sum of the wild type and *dis-1* allele
709 frequencies was between 0.8 and 1.2. In order to prevent loss of markers close to the
710 mutation, we also included SNPs that were specific to one of each of the pools with a
711 frequency of more than 0.9 and less than 0.1. Using this marker list, and crossing
712 SHOREmap annotation results with manual selection of mutations from SHORE
713 consensus, SHORE qVar and samtools/VarScan (Koboldt et al., 2013; Schneeberger et
714 al., 2009) identified a unique SNP specific to the *dis-1* pool at position 50914 of scaffold
715 sctg_184 (position 3330523 on chromosome 5). This A to G transition mutation was
716 located in intron 14 of the *DIS* gene 15 base pairs after the end of exon 14.

717 A cleaved amplified polymorphic sequence (CAPS) marker (Supplemental Table 2),
718 developed based on the single nucleotide polymorphism at position 3330523 of
719 chromosome 05, was used to genotype the 265 individuals of the mapping population
720 (which included 90 wild type and 175 *dis-1* individuals). This analysis confirmed that the
721 presence of the A to G transition was 100% correlated with the Dis⁻ phenotype in this
722 large family. In addition, PCR amplification and Sanger sequencing of the genomic
723 region containing the mutation confirmed its presence in the *dis-1* mutant (Ec722) and its
724 absence in the Ec32 and Ec568 strains (the original strain used for the mutagenesis and
725 the outcross female line used to generate the segregating population, respectively).

726 Resequencing of the *DIS* gene in the *dis-2* mutant identified a point mutation (a C to T
727 transition) in exon 13 that results in the introduction of a stop codon into the coding
728 region of the gene (chr05, position 3332908).

729 **Genetic interaction between *DIS* and *IMM***

730 An *imm* mutant female (Ec602) was crossed with a *dis-1* male (Ec722) and the
731 resulting sporophyte (Ec649) gave rise to a 40-gametophyte progeny, all derived from
732 independent meiotic events. The phenotypes of the progeny were scored under an
733 inverted microscope, and genotypes were assessed using CAPS markers for *IMM* and
734 *DIS* loci (Supplemental Table 2).

735 **RNAi knockdown of *DIS* expression**

736 Small interfering RNAs (siRNAs) directed against the *DIS* gene transcript were
737 designed using version 3.2 of E-RNAi (Horn and Boutros, 2010). The specificity of the
738 designed siRNAs was determined by comparing the sequence (Blastn) with complete
739 genome and transcriptome sequences. Candidates that matched, even partially, genomic
740 regions or transcripts in addition to *DIS* were rejected. Three siRNAs with predicted high
741 specificity corresponding to different positions along the *DIS* transcript were selected
742 (Supplemental Table 17). The control siRNA was directed against the *Ectocarpus* sp.
743 gene Ec-13_001890 which is located within the sex-determining region of the male sex
744 chromosome and therefore not an essential gene for sporophyte function. siRNAs were

745 introduced into *Ectocarpus* sp. strain Ec32 gametes using the transfection reagent
746 HiPerFect (Qiagen, Valencia, USA). One microlitre each of 0.5 µg/µl solutions of
747 siRNAs in 1X Universal siMAX® siRNA Buffer (MWG Eurofins, Ebersberg, Germany)
748 was mixed with 12 µl of HiPerFect transfection reagent in a final volume of 100 µl of
749 natural seawater, vortexed to mix and incubated for 10 min at room temperature before
750 being added dropwise to 100 µl of freshly released gametes in natural seawater in a Petri
751 dish. After rotating gently to mix, the Petri dish was incubated overnight at 13°C. The
752 following day, 10 ml of culture medium was added (Coelho et al., 2012c) and incubation
753 continued at 13°C. RNAi-induced phenotypes were observed under a light microscope
754 and the number of individuals that resembled the *dis* mutant were scored in at least 400
755 individuals for three experimental replicates. Control treatments were carried out in the
756 same manner using an siRNAs directed against the endogenous gene Ec-13-001890.

757 Unfortunately, it is currently not possible to determine whether the siRNA treatment
758 reduces *DIS* transcript abundance because, based on the observed phenotypes, gene
759 expression is affected in only a small percentage of the treated cells. However, the use of
760 an siRNAs directed against the endogenous gene Ec-13-001890 showed that the effect
761 was sequence-specific. It would therefore seem highly unlikely that the observed
762 phenotypes were due to processes that did not involve interference with the expression of
763 the *DIS* gene.

764 **Comparative transcriptome analyses**

765 RNA-seq analysis was carried out to compare the abundances of gene transcripts in the
766 *dis-1* mutant with those in basal and upright filaments of the wild type sporophyte
767 generation. Duplicate synchronous cultures (with more than 100 individuals each) were
768 prepared for each sample under standard conditions (Coelho et al., 2012c). Upright
769 filaments of adult (4-week old) wild type partheno-sporophytes were dissected from
770 prostrate, basal filaments using the sharp end of a Pasteur pipette under a binocular
771 microscope. Visual inspection under the microscope ensured the absence of
772 contaminating basal filaments. Wild type basal filaments were obtained by harvesting
773 immature individuals before the emergence of the upright filaments, two weeks after

774 initial cell release from plurilocular gametangia. The *dis-1* mutant was grown under the
775 same conditions as the wild type tissue and was similarly used at two weeks after release
776 of initial cells from plurilocular gametangia. Tissue samples were rapidly frozen in liquid
777 nitrogen and processed for RNA extraction. Total RNA was extracted from each sample
778 using the Qiagen Mini kit (<http://www.qiagen.com>) as previously described (Lipinska et
779 al., 2015). For each replicate, cDNA was produced by oligo-dT priming, fragmented and
780 prepared for 2x 100 bp paired end sequencing on an Illumina Hiseq 2000 platform by
781 FASTERIS (CH-1228 Plan-les-Ouates, Switzerland). TopHat (v2.0.8) was used to map the
782 RNA-seq reads to the reference genome. Supplemental Table 18 provides details of the
783 sequencing, mapping statistics and accession numbers. Pairwise differential expression
784 analysis between *dis-1*, basal and upright filaments was performed with the DESeq2
785 package (Bioconductor) using an adjusted p-value cutoff of 0.1 and a minimal fold-
786 change of 2.

787 **Analysis of predicted gene functions**

788 InterProScan (Zdobnov and Apweiler, 2001) and Blast2GO (Conesa and Götz, 2008)
789 were used to recover functional annotations for *Ectocarpus* proteins. For Blast2GO, a
790 Fisher exact test with a p-value cutoff of 0.05 was used to detect enrichment of specific
791 GO-terms in the various groups of genes. KEGG pathway enrichment was analysed using
792 the KOBAS 2.0 platform (Xie et al., 2011). Signal peptides were predicted using Hectar
793 (Gschloessl et al., 2008) implemented through the Galaxy platform ([http://webtools.sb-
794 roscoff.fr/](http://webtools.sb-roscoff.fr/)). A gene-by-gene manual curation was used to associate each gene in the
795 differential transcriptomic datasets to a functional category. Enrichment in specific
796 functional categories in relation to the *Ectocarpus* sp. genome was carried out by
797 statistical analysis using Chi-squared test ($P < 0.05$).

798 **Phylogenetic analysis of TBCC proteins**

799 A multiple alignment of the TBCC domains of diverse TBCC, RP2 and TBCCd1
800 proteins was generated with Muscle (<http://www.ebi.ac.uk/Tools/msa/muscle/>). Based on
801 this alignment, an unrooted maximum likelihood phylogenetic tree was built with the

802 LG+G model using MEGA7 (<http://www.megasoftware.net/mega.php>) and 1000
803 bootstrap replicates. Model testing was carried out in MEGA7. The coordinates of the
804 TBCC domains within the protein sequences were determined using the NCBI Conserved
805 domains tool (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). Accession numbers
806 and domain coordinates are provided in Supplemental Table 14.

807 **Microdissection and regeneration of *dis* mutants and wild type filaments**

808 Microdissection was performed with the sharp end of a Pasteur pipette under an
809 inverted microscope. After dissection, the material was kept at 13°C under standard
810 culture conditions (Coelho et al., 2012e). Wild type and *dis* sporophyte apical filament
811 cells were isolated by micro-dissection 7 days after the emergence of the first upright
812 filaments (20 days after gamete release); gametophyte apical filament cells were isolated
813 by micro-dissection 15 days after meio-spore release.

814 **Morphometric analysis of germinating partheno-sporophytes**

815 Germinating initial cells of *dis-1*, *dis-2* and wild type partheno-sporophytes 36h after
816 release from gametangia were photographed under an upright microscope (BX41,
817 Olympus, Tokyo, Japan). Cell areas were measured for a total of 57 cells using ImageJ
818 (<http://imagej.nih.gov/ij/>). Morphological measurements and analysis of images of adult
819 filaments were also performed under an inverted microscope (CKX41, Olympus, Tokyo,
820 Japan) using ImageJ (<http://imagej.nih.gov/ij/>, 1997-2016). Between 25 and 100 adult
821 filament cells were measured in each of eight different wild type or *dis-1* and *dis-2*
822 individuals. The statistical analysis was performed in R.

823 **Electron microscopy analysis of cellular ultrastructure**

824 Medium containing mature *Ectocarpus dis* partheno-sporophytes was pipetted onto a
825 plastic film (gel support films, ATTO CO., Tokyo, Japan). The film was cut into <1 cm
826 side triangles, and these were attached to Petri dishes by adhesive tape. Two days after
827 the release of mito-spores from plurilocular sporangia, the resulting germlings, which
828 were attached to the triangles, were rapidly immersed in liquid propane cooled to -180°C

829 by liquid nitrogen, and immediately transferred into liquid nitrogen. The samples were
830 submerged in substitution solution containing 2% osmium tetroxide with acetone at -
831 80°C for two days, at -40°C for 2 h and at 4°C for 2 h. Finally, the temperature of the
832 samples was gradually allowed to rise to room temperature, and they were then washed
833 with acetone several times. The gel support films were infiltrated and embedded in
834 Spurr's low-viscosity resin (Polysciences Inc., Warrington, PA, USA) on aluminium foil
835 dishes. The films with the samples were turned inside out on the upper surface of the
836 resin. Serial sections were cut with a diamond knife on an ULTRACUT ultramicrotome
837 (Reichert-Jung, Depew, NY, USA) and mounted on formvar-coated slot grids. Sections
838 were stained with TI blue (Nisshin EM Co. Ltd, Tokyo, Japan) and lead citrate, and
839 observed using an electron microscope (JEM-1011, JEOL Ltd., Tokyo, Japan). The
840 images shown in Figure 5 are representative of 14 initial cells.

841 Golgi fragmentation was quantified using a method adapted from (Tang et al., 2011)).
842 Transmission electron microscope photographs were used to measure the lengths of
843 cisternae. Measurements were performed on 14 different individuals, and a total of 113
844 cisternae from *dis-1*, *dis-2* and wild type individuals were analysed. Data from *dis-1* and
845 *dis-2* were pooled. Cisternae were defined as long membranous structures with a length
846 greater than twice their width, the latter not exceeding 60 nm. Normal cisternae ranged
847 from 20 to 30 nm in width and were longer than 400 nm. Statistical significance was
848 assessed by a Wilcoxon test, implemented in R.

849 **Quantitative reverse transcriptase PCR analysis**

850 The abundance of gene transcripts during the *Ectocarpus* life cycle was assessed using
851 RT-qPCR. RT-qPCR was performed as previously described (Coelho et al., 2011;
852 Lipinska et al., 2013). Primer pairs were designed to amplify regions of the 3'UTR or the
853 most 3' exon of the gene to be analysed (Supplemental Table 19). *In silico* virtual PCR
854 amplifications were carried out using the e-PCR program (Rotmistrovsky et al., 2004)
855 and the male Ec32 genome sequence to check the specificity of oligonucleotide pairs.

856 The Plant RNeasy extraction kit (Qiagen, Courtaboeuf, France) was used to extract
857 total RNA from at least three biological replicates for each of the stages of the life cycle:

858 gametes, young sporophytes, immature gametophytes, mature gametophytes, partheno-
859 sporophytes and diploid heterozygous sporophytes. The RNA was treated with RNase-
860 free DNase-I according to the manufacturer's instructions (Qiagen) to remove any
861 contaminating DNA and stored at -80°C. The concentration and integrity of the RNA was
862 checked using a NanoDrop 2000 spectrophotometer (ThermoScientific,
863 www.thermofisher.com) and by agarose gel electrophoresis. A control PCR without
864 reverse transcriptase was performed to ensure absence of contaminating DNA. For each
865 sample, up to 1 µg of RNA was reverse-transcribed to cDNA using oligo-dT and the
866 Superscript II RT kit (Life Technologies, Gaithersburg, MD, USA) according to the
867 manufacturer's instructions and the cDNA was diluted with water to 1.2 ng equivalent
868 RNA.µL-1. cDNAs were amplified using the IQ Sybrgreen supermix (Biorad
869 Laboratories, Hercules, CA, USA) on a Chromo4 System thermocycler (BioRad
870 Laboratories). The amplification efficiency was tested using a genomic dilution series
871 and was always at least 80%. The specificity of amplification was checked with a
872 dissociation curve. The EF1α gene (Ec-21_002980) or the Arp2/3 gene (Ec-09_003710)
873 were chosen as a constitutively expressed controls based on (Le Bail et al., 2008) and
874 their constitutive expression was validated by the RNAseq datasets. The normalised data
875 were expressed as the mean±s.d. calculated from three independent biological
876 experiments.

877 **Inhibitor treatments**

878 Stock solutions of inhibitors were prepared in DMSO (oryzalin at 100 mM, taxol
879 (paclitaxel) at 5 mg/ml, and brefeldin A at 25 mg/ml) and working solutions were diluted
880 in half strength Provasoli-enriched seawater (PES)(Coelho et al., 2012d). Freshly
881 released *Ectocarpus* sp. gametes were settled in Petri dishes and treated for 24h with
882 0.01-1 µM oryzalin, 5-20 µM paclitaxel or 2.5-25 µg/ml brefeldin A (BFA). Controls
883 were treated with 0.002% DMSO. Cells were allowed to develop after removal of the
884 inhibitor and observations were performed four and seven days later, using an inverted
885 Olympus CKX41 microscope. For each experiment, between 19 and 310 individuals

886 were counted in triplicate dishes (529, 1071 and 2142 cells scored in total in each of the
887 oryzalin, paclitaxel and brefeldine A treatments, respectively; Supplemental Table 16).

888 **Immunostaining**

889 *Ectocarpus* samples were processed as described by (Coelho et al., 2012b) using an
890 adapted protocol from (Bisgrove and Kropf, 1998). Briefly, *Ectocarpus* cells were settled
891 on coverslips and at appropriate times after settlement were rapidly frozen in liquid
892 nitrogen and fixed in 2.5% glutaraldehyde and 3.2% paraformaldehyde for 1h, then
893 washed in PBS and treated with 5% Triton overnight. Samples were then rinsed in PBS
894 and 100 mM NaBH₄ was added for 4h. Cell walls were degraded with cellulase (1% w/v)
895 and hemi-cellulase (4% w/v) for 1h, and the preparation was then rinsed with PBS and
896 blocked overnight in 2.5% non-fat dry milk in PBS. Samples were treated with anti-
897 tubulin antibody (1/200th, DM1A, Sigma-Aldrich) at 20°C overnight, and then treated
898 with the secondary antibody (AlexaFluor 488-conjugated goat anti-mouse IgG, Sigma-
899 Aldrich; 1:1000 in PBS) at 20°C overnight. The preparation was rinsed with PBS and
900 blocked overnight in 2.5% non-fat dry milk in PBS and then treated with anti-centrin
901 antibody (1/1000th anti-centrin 1 ab11257, Abcam) at 20°C overnight, followed by the
902 secondary antibody (1/1000th AlexaFluor 555-conjugated goat anti-rabbit IgG, Sigma
903 Aldrich) for 8h. Samples were stained with 4', 6-diamido-2-phenylindole (DAPI, 0.5
904 µg/ml in PBS) for 10 min at room temperature, and finally mounted in ProLong Gold
905 (Invitrogen).

906 **Confocal microscopy**

907 Confocal microscopy was conducted using an inverted SP8 laser scanning confocal
908 microscope (Leica Microsystem) equipped with a compact supply unit which integrates a
909 LIAchroic scan head, several laser lines (405 nm, 488 nm) and standard photomultiplier
910 tube detectors (PMT). We used the oil immersion lens HC PL APO 63x/1.40 OIL CS2.
911 The scanning speed was set at 400 Hz unidirectional. The pinhole was adjusted to one
912 Airy unit for all channels. The spatial sampling rate was optimized according to Niquist
913 criteria, generating a 0.058x0.058x0.299 µm voxel size (xyz). The Z-stack height fitted

914 the specimen thickness. A two-step sequential acquisition was designed to collect the
915 signal from three or four channels. The first step recorded the anti-tubulin fluorescence
916 signal (excitation 488 nm/emission 530 nm), and the transmitted light. The second step
917 acquired the DAPI fluorescence signal (excitation 405 nm/emission 415-480 nm) and the
918 anti-centrin signal (excitation 552 nm/emission 560-590 nm). Signal intensity was
919 averaged three times. The Fiji software was used to optimize the raw images, including
920 maximum intensity projection (MIP), and denoising (3*3 median filter). For any given
921 data, both wild-type and mutant images were analysed simultaneously with similar
922 settings. The Fiji software was also used to estimate the number of microtubule bundles
923 in each cell, before the first cell division. Tracking of bundles was performed on
924 maximum intensity projections of z-planes covering the whole thickness of the cells. We
925 drew three lines transversely, perpendicular to the growth axis of the cell: one in the
926 centre of the cell, and other two half way between the centre and the cell extremities (see
927 diagram in Figure 6D). Peaks corresponding to the microtubule bundles were then
928 identified in plots of intensity profiles at each of the three positions in the cell and
929 counted. The three values were averaged for each cell to derive an estimation of the
930 number of microtubule bundles in each cell. Note that in the *dis* mutants, due to the
931 disorganised nature of the microtubule network, average bundle numbers may be
932 somewhat underestimated. This is because this method is well adapted for tracking
933 microtubule bundles oriented with their long axis parallel to the image plane, but we may
934 have missed bundles that were perpendicular to the plane of the transection.

935 **Measurement of the positions of nuclei and microtubule nucleation sites**

936 Nuclei were stained *in vivo* with Hoechst 33342 (Thermofisher), imaged with an
937 Olympus BX microscope with a x40 objective, and distances were measured using Fiji
938 software. In germinating initial cells, the position of the nucleus was calculated by
939 measuring the distance from the nucleus to the germinating pole (i.e. the end
940 corresponding to the emerging germ tube) and the distance from the nucleus to the
941 opposite pole of the cell and dividing the latter by the former. Nuclei were scored as
942 being anterior (located near the emerging germ tube), central or posterior (located nearer

943 the pole opposite the germination pole). At the 2-cell stage, the positions of nuclei were
944 similarly measured in a directional manner by measuring the distance from the nucleus to
945 the cell boundary at the germinating end of the filament and the distance from the nucleus
946 to the opposite pole of the cell and dividing the latter by the former. For cells in
947 multicellular filaments at a later stage of development, it was not possible to assign a
948 direction of growth. Filaments were therefore orientated arbitrarily and the position of
949 each nucleus was calculated by dividing the distance from one end of the cell (x) by the
950 distance from the other end of the cell (y) as illustrated in Figure 6H.

951 Microtubule nucleation site positions were measured by dividing each cell into four
952 diagonal quadrants and scoring the position of the nucleating sites with respect to these
953 quadrants: proximal (located on the same pole as the emerging germ tube), lateral or
954 distal (located nearer the pole opposite the germination pole) as illustrated in Figure 6F.

955 **Accession numbers**

956 Accession numbers are provided in Supplemental Table 18.

957 [Supplemental Data](#)

958 Supplemental Data Set – Supplemental Tables S1 to S19

959 Supplemental Figure 1. The haploid-diploid life cycle of *Ectocarpus*.

960 Supplemental Figure 2. Pedigree of the strains used in this study.

961 Supplemental Figure 3. Cell regeneration in *dis* versus wild type filaments.

962 Supplemental Figure 4. Expression patterns, measured using RT-qPCR, of generation-
963 specific marker genes (Peters et al., 2008) during the gametophyte and sporophyte
964 generation of *dis-1* individuals compared with the wild type.

965 Supplemental Figure 5. Venn diagram comparison of the set of genes expressed in *dis-*
966 *1* with those expressed in wild type basal (Basal) and apical (Apical) filaments (TPM>1).

967 Supplemental Figure 6. Correlation between RNA-seq and RT-qPCR results.

968 Supplemental Figure 7. KEGG pathway analysis.

969 Supplemental Figure 8. Patterns of expression of *DIS*.

970 Supplemental Figure 9. Alignment of the TBCC domains of diverse TBCCd1 (green),
971 TBCC (orange) and RP2 (blue) proteins and predicted secondary structure representation
972 of the overall fold of the TBCC domain of the *Ectocarpus* sp. DIS/TBCCd1 protein.

973 Supplemental Figure 10. Additional representative confocal maximum z-projections of
974 untreated wild type cells, untreated *dis-1* and *dis-2* mutant cells and wild type cells treated
975 for 4h with 10 μ M taxol at several stages of early development of the initial cell.

976 Supplemental Figure 11. Effects of inhibitors treatment on the microtubule
977 cytoskeleton and early development.

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