CHAPTER FIVE

The *Ectocarpus* Genome and Brown Algal Genomics

The Ectocarpus Genome Consortium

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Contents

Abstract

Brown algae are important organisms both because of their key ecological roles in coastal ecosystems and because of the remarkable biological features that they have acquired during their unusual evolutionary history. The recent sequencing of the complete genome of the filamentous brown alga *Ectocarpus* has provided unprecedented access to the molecular processes that underlie brown algal biology. Analysis of the genome sequence, which exhibits several unusual structural features, identified genes that are predicted to play key roles in several aspects of brown algal metabolism, in the construction of the multicellular bodyplan and in resistance to biotic and abiotic stresses. Information from the genome sequence is currently being used in combination with other genomic, genetic and biochemical tools to further investigate these and other aspects of brown algal biology at the molecular level. Here, we review some of the major discoveries that emerged from the analysis of the *Ectocarpus* genome sequence, with a particular focus on the unusual genome structure, inferences about brown algal evolution and novel aspects of brown algal metabolism.

1. INTRODUCTION

1.1. The Brown Algae

Brown algae (or Phaeophyceae) are a group of multicellular algae that belong to the stramenopile lineage (also known as heterokonts). They occur

almost exclusively in marine environments, particularly rocky coastlines in temperate regions of the globe. Brown algae are often the main primary producers of such ecosystems and therefore play an important ecological role, creating habitats for a broad range of other marine organisms. As a consequence, there has been considerable interest in understanding the biology and ecology of the brown algae. These organisms have also attracted interest for a number of other reasons. The stramenopiles are very distantly related to well-studied groups such as the opisthokonts (animals and fungi) and the green lineage (which includes land plants); the common ancestor of these major lineages dating back to the crown radiation of the eukaryotes more than a billion years ago (Yoon, Hackett, Ciniglia, Pinto & Bhattacharya, 2004). During this long period of evolutionary time, the brown algae have evolved many unusual characteristics that are not found in the other groups, including a number of features that have exquisitely adapted these organisms for the harsh environment of the intertidal and subtidal zones. Brown algae exhibit novel features even at the basic level of their cell biology. For example, they acquired their plastid via a process of secondary endosymbiosis involving the capture of a red alga (Archibald, 2012, in this volume; Keeling, 2004), an event that had a major consequence both on the ultrastructure of the cell and on the composition of the nuclear genome (as a result of gene transfers from the endosymbiont). Brown algae are also remarkable in that they are one of only a small number of eukaryotic groups to have evolved complex multicellularity (Cock *et al.*, 2010).

1.2. *Ectocarpus*, a Model Organism For The Brown Algae

Over the past two decades, the adoption of genomic approaches such as genome sequencing and efficient methods to analyse gene function has allowed remarkable progress in our understanding of the biology of selected model organisms in the animal, plant and fungal lineages. During this time, it became clear that it would be necessary to select an analogous model organism for the brown algae if similar approaches were to be applied to this group. In 2004, several potential brown algal model species were compared, leading to a proposition to develop genomic and genetic tools and techniques for the filamentous brown alga *Ectocarpus* (Fig. 5.1; Peters, Marie, Scornet, Kloareg & Cock, 2004). *Ectocarpus* was selected because mature thalli are small, highly fertile and progress rapidly through the life cycle (Müller, Kapp & Knippers, 1998), characteristics that are essential for the application of genetic approaches. It had also been shown that basic genetic

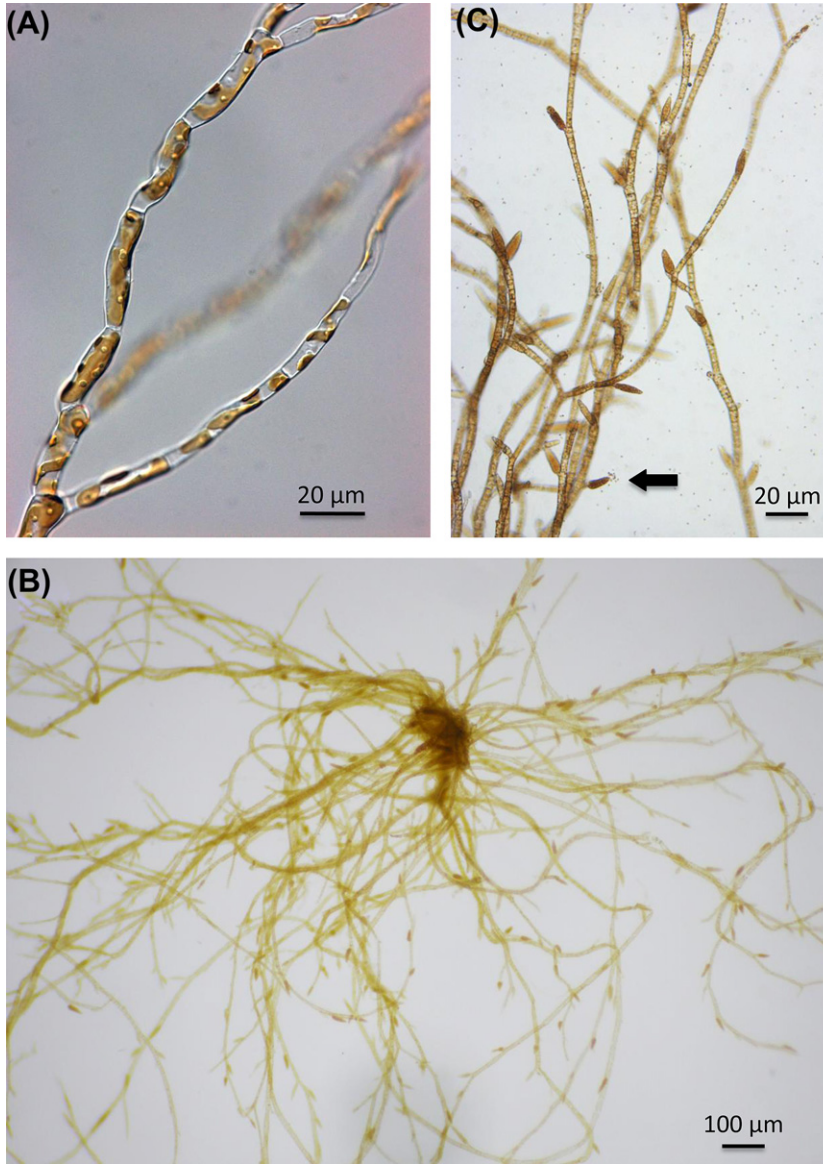

Figure 5.1 The filamentous brown alga *Ectocarpus*. Photographs are of the strain Ec32, which was used to obtain the complete genome sequence. (A) partheno–sporophyte filaments, (B) gametophyte filaments bearing plurilocular gametangia, (C) mature gametophyte filaments releasing gametes from plurilocular gametangia (arrow). See the colour plate.

methods such as crosses and segregation analysis could be used with this organism (Bräutigam, Klein, Knippers & Müller, 1995) and axenic cultures can be established (Müller, Gachon & Küpper, 2008). In addition, several aspects of the biology of *Ectocarpus* had been studied, including taxonomy, life cycle, different aspects of cell biology and metabolism and responses to biotic and abiotic stresses (Charrier *et al.*, 2008).

One of the key steps towards the emergence of *Ectocarpus* as a model organism was the development of a complete genome sequencing project for this organism. This project, which involved more than 30 laboratories, was initiated in 2006 and was completed with the publication of the genome sequence in 2010 (Cock *et al.*, 2010). The following sections describe the many interesting features of this genome and the insights into brown algal biology that analysis of the genome has afforded.

1.3. The *Ectocarpus* Genome Project

The *Ectocarpus* genome sequence was obtained using strain Ec32, which is a male meiotic offspring of a field sporophyte collected in 1988 in San Juan de Marcona, Peru (Peters *et al.*, 2008). Initially considered to belong to the species *E. siliculosus*, more recent phylogenetic analyses suggest that strain Ec32 belongs to a so far unnamed species of the same genus (Peters *et al.*, 2010a). The size of the genome in this strain had been estimated at 214 Mbp using flow cytometry (Peters *et al.*, 2004) and the length of the assembled genome sequence, which comprised 1561 supercontigs of greater than 2 kbp, was consistent with this estimation (Cock *et al.*, 2010). A sequence-anchored genetic map was used to assign 325 of the longest supercontigs (137 Mbp or 70% of the genome) to linkage groups and thereby produce a large-scale assembly of the genome sequence by concatenating supercontigs to produce pseudochromosomes (Heesch *et al.*, 2010). The number of linkage groups (26 major and 8 minor linkage groups) is consistent with previous estimates of chromosome number based on cytogenetic studies (Müller, 1966, 1967) if we assume that the eight minor linkage groups represent fragments that should be associated with the larger groups.

2. STRUCTURE OF THE *ECTOCARPUS* GENOME

2.1. Large-scale Structure

Analysis of gene and transposon density along the *Ectocarpus* pseudochromosome sequences did not reveal any obvious large-scale structures that could have represented centromeres or heterochromatic knobs, although it

is possible that such regions were lost during the assembly stage if they are very rich in repeated sequences. Linkage group 30 was particularly rich in transposons and poor in genes compared to the other major linkage groups. No evidence was found for large-scale duplication events such as genome duplications. This is unusual for an organism that belongs to a group that has evolved complex multicellularity.

2.2. Gene Structure and Gene Organization

Ectocarpus genes contain many long introns (seven per gene on average, with an average size of 704 bp) and, in consequence, introns make up an exceptionally large percentage of the genome (40.4%). Only 5.3% of the predicted genes lack introns completely; this is the smallest fraction for any eukaryotic genome reported to date. The 3' untranslated regions are also very long for a genome of this size (average size: 855 bp). Mouse genes have a comparable mean 3'UTR length, despite the fact that the mouse genome is more than 13 times larger. It is possible that the long *Ectocarpus* 3'UTRs contain regulatory elements in which case messenger RNA (mRNA)s bearing different 3' regulatory signals could be generated by the use of alternative polyadenylation sites. However, the frequency of the use of alternative polyadenylation sites did not appear to be particularly elevated in *Ectocarpus* (Cock *et al.*, 2010).

The features described in the previous paragraph are typical of large expanded genomes, but the *Ectocarpus* genome also exhibits a number of features more typical of small compact genomes. For example, a significant proportion (61.5%) of the 16,256 predicted protein-coding genes is arranged in an alternating manner along the chromosome, so that adjacent genes are on opposite strands (Fig. 5.2; Cock *et al.*, 2010). The proportion

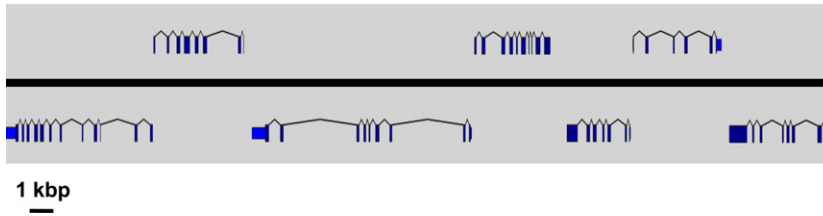

Figure 5.2 Diagram showing the alternating pattern of gene organization typical of many regions of the *Ectocarpus* genome. Coding exons are shown as dark blue bars and untranslated regions as light blue bars. Lines joining exons represent introns. Genes above the black line are transcribed from left to right and genes below the black line are transcribed from right to left. See the colour plate.

of alternating genes is comparable with that found in highly compact genomes such as those of *Ostreococcus tauri* (12.6 Mbp, 61.5% alternating genes) and *Phaeodactylum tricornutum* (27.4 Mbp, 64.2% alternating genes), while the percentage of alternating genes in larger genomes normally tends to decrease as a function of genome size (Cock *et al.*, 2010; note that 50% alternating genes is expected if the genes are randomly organized in the genome). One factor that may contribute to the high number of alternating genes is the relative rarity of tandem duplications in *Ectocarpus* because tandem duplications will tend to disorganize arrays of alternating genes. Only 823 of the 16,256 *Ectocarpus* genes are part of tandem duplications. It is not clear whether the organization of many *Ectocarpus* genes into alternating arrays is important for gene function. In yeast, adjacent genes tend to be co-regulated (Kruglyak & Tang, 2000) and this effect is more marked for divergently expressed genes than for either convergently expressed or same-strand gene pairs (Kensche, Oti, Dutilh, & Huynen, 2008; Trinklein *et al.*, 2004). An analysis of stress-response data indicated that adjacent genes also showed a greater degree of co-regulation than non-adjacent genes in *Ectocarpus*, but this effect was not dependent on the relative orientation of the genes (Cock *et al.*, 2010). This is consistent with the fact that there is almost exactly the same number of divergently and convergently transcribed gene pairs in the genome (4078 and 4076 pairs, respectively).

Another feature typical of compact genomes is that the intergenic regions between the 5' ends of divergently transcribed genes (i.e. genes on opposite strands with adjacent 5' ends) are often very short in *Ectocarpus* (29% are less than 400 bp long). This feature may help stabilize arrays of alternating genes by grouping the genes closely together (Hurst, Williams, & Pál, 2002). Another consequence of short intergenic regions is that either promoter regions must be very compact or regulatory elements must be located elsewhere in the genes, in introns, for example. Post-transcriptional processes, such as transcript degradation, for example, might also play a prominent role in gene regulation. *Ectocarpus* possesses all the components of the exosome (or PM/Scl) complex (Cock *et al.*, 2010), which is involved in the degradation not only of mRNA but also ribosomal RNA (rRNA) and many species of small RNA (Belostotsky, 2009). Gene expression may also be regulated at other steps, such as intron splicing (this may be particularly important given the intron-rich nature of the *Ectocarpus* genes) and mRNA translation. Detailed annotations of the genes involved in these processes in *Ectocarpus* have been carried out (Cock *et al.*, 2010).

The large number of introns in the *Ectocarpus* genome could potentially allow the production of multiple transcripts from individual genes. A genome-wide analysis of alternative transcripts based on 91,041 expressed sequence tag (EST) sequences indicated that only a small proportion of genes (less than 3%) produced alternative transcripts, but it is likely that this EST data set was insufficient to exhaustively describe this phenomenon in *Ectocarpus*, and deeper sequencing of the trancriptome is expected to allow the identification of additional alternative transcripts in the future.

2.3. Non-coding RNAs

In addition to mRNA transcripts, *Ectocarpus* cells produce many endogenous small RNAs. An analysis of more than seven million small RNA sequences from sporophyte and gametophyte tissues identified 24,132 unique small RNA sequences, which mapped to 1,031,522 loci in the *Ectocarpus* genome (Cock *et al.*, 2010). For small RNAs that mapped to intergenic regions, transposons, introns or exons, the largest size group in each case was 21 nucleotides, indicating that a proportion of these molecules may have specific functions that impose a size constraint. Small RNAs originating from rRNA and transfer RNA (tRNA), on the other hand, did not exhibit this size bias, suggesting that they may simply be degradation products of longer transcripts.

Micro RNAs (miRNAs) are small (21–25 nucleotide) RNAs generated by Dicer enzymes (ribonucleases of the RNAseIII family) by processing imperfect stem-loop structures in longer RNA transcripts. miRNAs are incorporated into silencing complexes, which include Argonaute proteins, allowing these complexes to target-specific nucleic acid sequences in the cell. miRNAs have been shown to have important regulatory roles in many cellular and developmental pathways in both green plants and animals (Carthew & Sontheimer, 2009; Voinnet, 2009). *Ectocarpus* also appears to employ this system. Using a set of stringent rules (Meyers *et al.*, 2008), 26 miRNA sequences were identified in the *Ectocarpus* genome (Cock *et al.*, 2010). In addition, *Ectocarpus* possesses both a Dicer and an Argonaute gene, and there are two RNA-dependent RNA polymerase (RdRP) homologues, which may have a role in the amplification of double-stranded RNA molecules. The small RNA machinery in *Ectocarpus* seems to be rather simple compared to other eukaryotes; many organisms possess multiple copies of Dicer, Argonaute and RdRP proteins (Carthew & Sontheimer, 2009; Voinnet, 2009). This diversification has been shown to be linked to

functional specialization in many cases, for example, between small interfering RNA and miRNA silencing. miRNAs regulate their targets in the cell by binding to regions of partial or complete base complementarity. A total of 71 potential target sequences were identified for 12 of the 26 *Ectocarpus* miRNAs. Surprisingly, three quarters of these targets contain leucine-rich repeat (LRR) domains, including 28 members of a large Ras of complex proteins (ROCO) GTPase family and 5 tetratricopeptide repeat (TPR) -containing proteins (see Table 5.1 for an updated list of predicted *Ectocarpus* miRNA targets). LRR proteins are involved in recognition and transduction events linked to immunity in both plants and animals (Kumar, Kawai, & Akira, 2011; Meyers, Kaushik, & Nandety, 2005) and the *Ectocarpus* LRR ROCO genes may be involved in similar processes, with exon shuffling allowing an adaptive immune response (Zambounis, Elias, Sterck, Maumus & Gachon, 2012). miRNAs may regulate this important class of molecule, which would be consistent with recent results showing that miRNAs act as master regulators of NB-LRR genes in land plants (Zhai *et al.*, 2011). The discovery of miRNAs in *Ectocarpus* taken together with the miRNAs previously described in animals, green plants and slime moulds (Hinas *et al.*, 2007), indicated an ancient origin for these important regulatory molecules. More recently, miRNAs have also been described in the diatom *P. tricornutum* (Huang, He & Wang, 2011).

A whole genome tiling array approach identified 8,741 expressed regions longer than 200 nucleotides located outside the 16,256 predicted genes. Many of these regions represent potential candidates for genes encoding non-coding RNAs (Cock *et al.*, 2010). A large proportion of the expressed regions correspond to repeated elements and the vast majority (8706) are not conserved in the *Thalassiosira pseudonana* genome, suggesting that they have originated since the divergence from diatoms.

2.4. Repeated Sequences

Repeated sequences make up a significant proportion of the *Ectocarpus* genome (22.7%), dominated by transposable elements (TEs) and unclassified repeats that represent about 12.5 and 9.9% of the genome, respectively (Table 5.2). The TEs are mainly retrotransposons, including LTR retrotransposons (such as Ty1/copia, Ty3/gypsy and DIRS/Ngaro-like elements), TRIM/LARD-like elements and non-LTR retrotransposons, as well as DNA transposons of both subclass I (such as Harbinger, JERKY and POGO-like elements) and subclass II (Helitrons) (Cock *et al.*, 2010). The

Table 5.1 *Ectocrpus* Genes Predicted to be MicroRNA Targets

miRBase miRNA ID Number*	Target Gene Locus ID	Target Gene Domains	Target Gene Description
3463	Esi0041_0132	Kinesin	AGAP010519-PA
3453	Esi0010_0186	WSC	Conserved hypothetical protein
3453	Esi0057_0062		Conserved hypothetical protein
3457	Esi0012_0076	WSC	Conserved hypothetical protein
3468	Esi0380_0014		Conserved hypothetical protein
3454a, 3454b, 3454c, 3454e	Esi0062_0049		Conserved hypothetical protein
3454a, 3454b, 3454c	Esi0033_0024		Conserved hypothetical protein
3454d, 3454f	Esi0046_0125		Hypothetical aspartate carbamoyltransferase
3452	Esi0269_0025	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c	Esi0106_0041	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c	Esi0106_0045	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c	Esi0106_0087	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c	Esi0165_0070	LRR	Hypothetical LRR Protein
3454a, 3454b, 3454c	Esi0191_0069	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c	Esi0450_0002	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c, 3454d, 3454e	Esi0106_0084	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c, 3454d, 3454e, 3454f	Esi0015_0089	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c, 3454d, 3454e, 3454f	Esi0015_0097	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c, 3454d, 3454f	Esi0015_0090	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c, 3454d, 3454f	Esi0191_0070	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c, 3454e	Esi0055_0135	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c, 3454e	Esi0191_0006	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c, 3454e	Esi0191_0017	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c, 3454e	Esi0236_0019	LRR	Hypothetical LRR protein

(*Continued*)

Table 5.1 *Ectocrpus* Genes Predicted to be MicroRNA Targets—cont'd

miRBase miRNA ID Number*	Target Gene Locus ID	Target Gene Domains	Target Gene Description
3454d, 3454f	Esi0328_0036	LRR	Hypothetical LRR protein
3454e	Esi0055_0077	LRR	Hypothetical LRR protein
3454e	Esi0106_0039	LRR	Hypothetical LRR Protein
3454e	Esi0200_0010	LRR	Hypothetical LRR protein
3454g	Esi0029_0096	LRR	Hypothetical LRR protein
3454e	Esi0085_0019	LRR	Hypothetical LRR protein
3454a, 3454b, 3454c, 3454e	Esi0144_0013		Hypothetical potassium transporter
3457	Esi0198_0016		Hypothetical protein
3452	Esi0269_0037		Likely pseudogene
3452	Esi0269_0028	LRR	LRR protein
3454d, 3454f	Esi0088_0028	LRR	LRR protein
3452	Esi0269_0017	LRR	LRR protein
3454a, 3454b, 3454c, 3454d, 3454e, 3454f	Esi0011_0207	LRR	LRR-GTPase of the ROCO family
3454a, 3454b, 3454c, 3454d, 3454e, 3454f	Esi0138_0012	LRR	LRR-GTPase of the ROCO family
3454a, 3454b, 3454c, 3454d, 3454e, 3454f	Esi0141_0028	LRR	LRR-GTPase of the ROCO family
3454a, 3454b, 3454c, 3454d, 3454f	Esi0031_0015	LRR	LRR-GTPase of the ROCO family
3454a, 3454b, 3454c, 3454d, 3454f	Esi0032_0115	LRR	LRR-GTPase of the ROCO family
3454a, 3454b, 3454c, 3454e	Esi0164_0034	LRR	LRR-GTPase of the ROCO family
3454a, 3454b, 3454c, 3454e	Esi0264_0029	LRR	LRR-GTPase of the ROCO family
3454a, 3454b, 3454c, 3454e	Esi0265_0008	LRR	LRR-GTPase of the ROCO family
3454d, 3454f	Esi0032_0138	LRR	LRR-GTPase of the ROCO family
3454a, 3454b, 3454c	Esi0026_0057	LRR	LRR-GTPase of the ROCO family, putative pseudogene

3454a, 3454b, 3454c	Esi0041_0085	LRR	LRR-GTPase of the ROCO family, putative pseudogene
3454a, 3454b, 3454c	Esi0054_0138	LRR	LRR-GTPase of the ROCO family, putative pseudogene
3454a, 3454b, 3454c	Esi0138_0081	LRR	LRR-GTPase of the ROCO family, putative pseudogene
3454a, 3454b, 3454c, 3454d, 3454e, 3454f	Esi0014_0084	LRR	LRR-GTPase of the ROCO family, putative pseudogene
3454a, 3454b, 3454c, 3454e	Esi0416_0018	LRR	LRR-GTPase of the ROCO family, putative pseudogene
3454d, 3454f	Esi0027_0032	LRR	LRR-GTPase of the ROCO family, putative pseudogene
3454d, 3454f	Esi0112_0069	LRR	LRR-GTPase of the ROCO family, putative pseudogene
3454e	Esi0562_0010	LRR	LRR-GTPase of the ROCO family, putative pseudogene
3457	Esi0008_0050	TPR	NB-ARC and TPR repeat-containig protein
3457	Esi0008_0175	TPR	NB-ARC and TPR repeat-containing protein—likely pseudogene
3457	Esi0380_0021	TPR	NB-ARC and TPR repeat-containing protein—likely pseudogene
3453	Esi0007_0219		Ran-GTPase activating protein
3454a, 3454b, 3454c	Esi0141_0085	LRR	ROCO gene-associated coding fragment
3454a, 3454b, 3454c, 3454d, 3454e, 3454f	Esi0032_0177	LRR	ROCO gene-associated coding fragment
3454a, 3454b, 3454c, 3454d, 3454e, 3454f	Esi0047_0155	LRR	ROCO gene-associated coding fragment
3454a, 3454b, 3454c, 3454d, 3454e, 3454f	Esi0264_0036	LRR	ROCO gene-associated coding fragment
3454a, 3454b, 3454c, 3454d, 3454f	Esi0011_0242	LRR	ROCO gene-associated coding fragment

(*Continued*)

Table 5.1 *Ectocrpus* Genes Predicted to be MicroRNA Targets—cont'd

miRBase miRNA ID Number*	Target Gene Locus ID	Target Gene Domains	Target Gene Description
3454a, 3454b, 3454c, 3454d, 3454f	Esi0138_0092	LRR	ROCO gene-associated coding fragment
3454a, 3454b, 3454c, 3454e	Esi0032_0139	LRR	ROCO gene-associated coding fragment
3454a, 3454b, 3454c, 3454e	Esi0281_0054	LRR	ROCO gene-associated coding fragment
3454d, 3454f	Esi0014_0085	LRR	ROCO gene-associated coding fragment
3454e	Esi0041_0084	LRR	ROCO gene-associated coding fragment
3463	Esi0036_0127		Similar to CG3714-PA, isoform A
3457	Esi0126_0047	TPR	TPR repeat-containing protein
3457	Esi0274_0033	TPR	TPR repeat-containing protein, putative pseudogene

LRR, leucine-rich repeat; TPR, tetratricopeptide repeat; ROCO, Ras of complex proteins; WSC, Wall and stress response component.
* Add esi-MIR in front of the four digit number to construct the complete miRBase ID number.

Table 5.2 Abundance of Different Classes of Repeated Element in the *Ectocarpus* Genome

Class	Subclass	Category	Coverage (bp)	Genome Coverage (%)
Class 1	LTR-retrotransposon	Ty1/copia	4817138	2.40
		Ty3/gypsy	4348141	2.17
		DIRS/Ngaro	2918206	1.45
		TRIM/LARD	2600089	1.30
	Non LTR-retrotransposon	LINE	4074762	2.03
Class 2	Subclass 1	TIR	2042086	1.02
		TIR putative	228020	0.11
		non-autonomous TIR	2445103	1.22
	Subclass 2	Helitron	891778	0.44
Tandem repeat			721010	0.36
Unclassified repeat			19817249	9.88

LTR; long terminal repeats; TRIM, terminal-repeat retrotransposon in miniature; LARD; large retrotransposon derivative.

most abundant unclassified repeat in the *Ectocarpus* genome is a 676 nucleotide element dubbed Sower that accounts for about 1.5% of the genome.

Analysis of EST data indicated that several TE sequences (particularly Ty1/copia elements) were expressed at unexpectedly high levels in unstressed tissues grown under laboratory conditions (Cock *et al.*, 2010). In green plants and animals, TE silencing is mediated by mechanisms related to RNA interference, which usually involve methylation of DNA in the silenced regions of the genome (Malone & Hannon, 2009). High-performance liquid chromatography (HPLC) analysis of *Ectocarpus* genomic DNA did not detect any methylcytosine (mC) and several TE families were shown to be resistant to digestion with the mC-specific endonuclease McrBC, again indicating that these elements are not methylated (Cock *et al.*, 2010). It is therefore possible that the observed expression of TE sequences in *Ectocarpus* is a result of these elements being only weakly silenced in the absence of a DNA methylation system. Interestingly, genome-wide analysis of the small RNA sequences described above indicated that these sequences were derived preferentially from TE-rich regions of the genome, suggesting that these molecules play a role in TE silencing despite the lack of DNA methylation. In other organisms that lack DNA methylation, such as *Caenorhabditis elegans*, silencing is thought to be mediated by alternative chromatin signals such as histone modifications (Vastenhouw & Plasterk, 2004). It is possible that similar mechanisms operate in *Ectocarpus*.

Recently, an increase in the abundance of transcripts corresponding to several different TEs (especially long interspersed element (LINEs)) was detected during infection by the pathogen *Eurychasma dicksonii*, hinting at mechanisms that regulate TE expression (Grenville-Briggs *et al.*, 2011). This observation suggests that TE-mediated generation of genetic diversity may occur in response to biotic stress. It also confirms that most TEs are repressed under normal laboratory conditions.

2.5. An Integrated Viral Genome

One particularly interesting feature of the *Ectocarpus* nuclear genome sequence was the presence of a single copy of a large viral genome (more than 310 kbp) that had inserted into one of the algal chromosomes (Cock *et al.*, 2010). The inserted viral genome is closely related to the *Ectocarpus* phaeovirus EsV-1, a member of the Phycodnaviridae family, which are icosahedral viruses with internal lipid membranes and large double-stranded DNA genomes (Müller *et al.*, 1998; Wilson, Van Etten, &

Allen, 2009; see also Grimsley *et al.*, 2012, in this volume). Phaeoviruses are pandemic in several brown algal species (Müller *et al.*, 1998) and about half of the individuals in natural *Ectocarpus* populations show symptoms of viral infection (Dixon, Leadbeater, & Wood, 2000; Müller *et al.*, 2000). EsV-1 infects free-swimming zoids (spores or gametes, which lack a cell wall) and the 313 kbp viral genome then integrates into the cellular genome with the result that there is a copy in all the cells of the host as it develops (Bräutigam *et al.*, 1995; Delaroque, Maier, Knippers, & Müller, 1999; Müller, 1991). The virus remains latent in vegetative cells and viral particles are only produced in the reproductive organs (the sporangia and gametangia; Fig. 5.3) following a stimulus such as a change in light, seawater composition or temperature (Müller, Lindauer, Brüderlein & Schmitt, 1990; Müller *et al.*, 1998). Infected algae exhibit no obvious growth or developmental defects other than the partial or total inhibition of reproduction caused by the replacement of zoids by viral particles (Del Campo, Ramazanov, Garcia-Reina & Müller, 1997).

A single copy of the viral genome was found in the *Ectocarpus* genome, almost all located at a single locus on linkage group 16 (although a second, much smaller fragment was found on linkage group 24, possibly due to a translocation event that occurred after viral integration). This is consistent with previous studies in which the integrated EsV-1 was shown to behave as

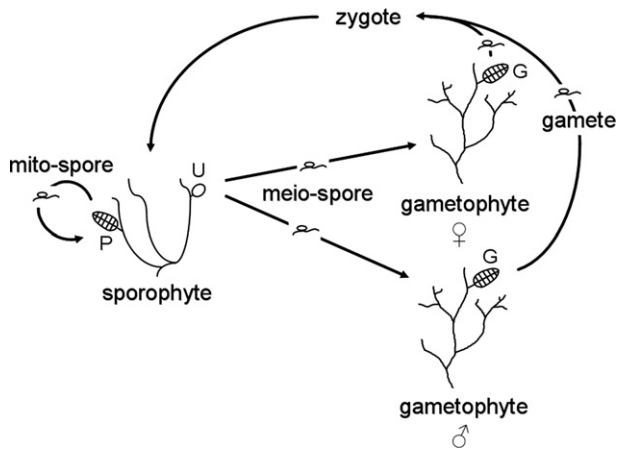

Figure 5.3 Diagram showing the alternation between the sporophyte and gametophyte generations during the life cycle of *Ectocarpus*. U, unilocular sporangium; P, plurilocular sporangium and G, plurilocular gametangium. Meiosis occurs in the unilocular sporangia to produce haploid meiospores, male and female gametes are produced in the plurilocular gametangia on the dioecious gametophytes.

a single, Mendelian locus (Bräutigam *et al.*, 1995; Delaroque *et al.*, 1999; Müller, 1991). The virus appears to have integrated as a circle, as the terminal repeat regions are adjacent in the algal chromosome. Again, this is consistent with previous reports indicating that the ends of the linear viral genome can associate to form a circle (Delaroque *et al.*, 2001).

The integration of such a large segment of foreign DNA, representing more than 1% of the total gene content of the genome, could potentially have had a significant influence on genome function. However, expression analysis showed that almost all the viral genes were silent (Cock *et al.*, 2010). The viral genes remained silent following several different stress treatments (hyperosmotic, hypoosmotic and oxidative stress) and in fertile gametophytes (where viral particles are normally produced in EsV-1-infected strains), indicating that the integrated viral genome is either defective or silenced by the host. These observations were consistent with the fact that strain Ec32 had never been observed to produce virus particles. Interestingly, small RNAs did not map preferentially to the inserted viral sequence (as had been observed for the TE-rich regions of the genome, see above), suggesting that the viral genome had been silenced by a mechanism different to that used to silence transposons. Apart from the inserted viral genome, the *Ectocarpus* genome contained very few genes that were predicted to be of viral origin, which was surprising given the pandemic levels of viral infection observed in the field (Dixon *et al.*, 2000; Müller *et al.*, 2000). *Ectocarpus* therefore may possess a mechanism that limits gene acquisition via this route.

3. EVOLUTIONARY HISTORY OF THE *ECTOCARPUS* GENOME

3.1. Evolution of Genome Gene Content

Several approaches have been used to compare the inventory of proteins encoded by the *Ectocarpus* genome (the proteome) with those of other organisms in an effort to understand the evolutionary history of the genome (Cock *et al.*, 2010). These have included comparisons with complete proteomes from other stramenopiles, blast comparisons with the National Center for Biotechnology Information database and a reconstruction of gene family loss and gain during evolution using Dollo logic (Cock *et al.*, 2010). These analyses have highlighted how different the *Ectocarpus* genome is from previously sequenced genomes. For example, more than a third (35.8%) of the *Ectocarpus* proteins matched none of the proteins in the NCBI nr_prot database, indicating that these are either

entirely novel sequences or that they have diverged considerably from homologues in other species. This result underlines not only the potential of the genome as a source of new bioactivities but also the challenges associated with investigating gene function in this organism.

Analysis of gene family expansions can also provide insights into evolutionary forces that have shaped genome content. Expansions in the *Ectocarpus* genome have been particularly marked for genes involved in the cytoskeleton, flagella function, protein degradation and protein phosphorylation/dephosphorylation (http://bioinformatics.psb.ugent.be/dollo_analysis). One specificity of the *Ectocarpus* genome is the expansion of a glycosyl hydrolase family and a fucoxanthin chlorophyll-*a*/*c*-binding protein subfamily underlining the importance of carbohydrate metabolism and photosynthesis in this organism. A similar result was obtained from an analysis of the frequency of protein domains in the *Ectocarpus* genome. Domains involved in carbohydrate binding (IPR002889) and photosynthesis (IPR001344) were particularly abundant. Notch (IPR000800) and ankyrin (IPR002110 and PTHR18958) domain proteins were also significantly overrepresented. These proteins may have important roles in intercellular communication.

3.2. Endosymbiosis and Organellar Genomes

The plastids of almost all eukaryotic algae are thought to be ultimately derived from an endosymbiotic event that occurred early in the evolution of the green (Archaeplastida) lineage before the separation of the glaucophytes from the red and green algae (Reyes-Prieto, Weber & Bhattacharya, 2007; see also De Clerck, Bogaert & Leliaert, 2012, Chapter II of this volume). This event involved the capture of a cyanobacterium by a eukaryotic host cell and enslavement of the former to produce a plastid. The plastids of algae in other major eukaryotic lineages, including the chromalveolate group (to which *Ectocarpus* belongs), were then acquired by secondary, or even tertiary, endosymbiotic events involving the capture of plastid-containing algae (see Archibald, 2012, Chapter III of this volume). Both primary and secondary endosymbiosis events involved large-scale transfer of genes from the endosymbiont to the host nucleus resulting in the acquisition of a broad range of novel functions (Baurain *et al.*, 2010; Bowler *et al.*, 2008; Dorrell & Smith, 2011; Keeling, 2004). *Ectocarpus* genes that were acquired during secondary endosymbiosis can be identified by their phylogenetic affinity with red algal sequences because brown algal plastids were derived from

a captured red alga. Phylogenetic analysis identified 611 such red-alga-derived (or 'red') genes in the *Ectocarpus* genome (Cock *et al.*, 2010). These genes are predicted to carry out a broad range of cellular functions indicating that the secondary endosymbiosis event not only influenced photosynthetic processes (31% of the 'red' genes were predicted to be involved in plastid function) but also represented an opportunity to optimize other cellular processes. For example, 34 mitochondrial proteins are encoded by 'red' genes (5.5% of the 'red' genes and 9.5% of proteins predicted to be targeted to the mitochondria in *Ectocarpus*), suggesting that advantageous features of the two ancestral mitochondrial proteomes may have been combined during evolution. Similarly, the 'red' genes included a glutamate/ornithine acetyltransferase and an acetylornithine aminotransferase, suggesting that the urea cycle in brown algae is partly derived from the red algal lineage.

Little is known about organelle function in brown algae at the molecular level, although the genome project has provided tools to address this aspect of brown algal biology. Both the plastid and mitochondrial genomes have been described in *Ectocarpus* (Cock *et al.*, 2010; Le Corguillé *et al.*, 2009). The mitochondrial genome is relatively small (37 kbp) and encodes only a small fraction of the mitochondrial components (3 ribosomal RNA genes, 24 tRNA genes, 35 conserved protein-coding genes and 5 putative (open reading frame (ORFs)). The majority of the mitochondrial proteins are encoded in the nucleus and transported into the mitochondria. The predicted components of this transport machinery have been analysed recently, providing several insights into the evolution of this system during the emergence of the eukaryotes (Delage *et al.*, 2011). The Hectar algorithm (Gschloessl, Guermeur & Cock, 2008) predicts that 605 proteins are encoded in the nucleus and enter the mitochondria via the protein transport system. One particularly interesting protein is the nicotinamide adenine dinucleotide (NADH) dehydrogenase (complex I) iron–sulfur protein subunit encoded by the *nad11* gene. In many protists (including oomycetes and some diatoms), the *nad11* gene is located on the mitochondrial genome, whereas in green plants, animals and fungi, the gene is nuclear. *Ectocarpus* and some other stramenopiles (*Cafeteria, Traustochytrium* and other brown algae; Oudot-Le Secq, Loiseaux-de Goër Stam, & Olsen *et al.*, 2006) possess a truncated version of this gene in the mitochondrion. A nuclear gene encoding the C-terminal part of the nad11 subunit (with a mitochondrial-targeting sequence) was found in the *Ectocarpus* genome, suggesting that the *nad11* gene was split during evolution and one part of the coding region transferred to the nucleus. The diatom *P. tricornutum* may represent an intermediate step in this process as its *nad11* gene has been split into

two loci but both components are still present in the mitochondrial genome (Oudot-Le Secq & Green, 2011). The red alga *Cyanidioschyzon merolae* possesses two nuclear genes corresponding to the N-terminal and C-terminal regions of nad11. Taken together, these observations suggest that the transfer of *nad11* from the mitochondrial to the nuclear genome occurred several times during evolution, in some cases involving the sequential transfer of fragments of the gene to create two individual nuclear loci.

Dual targeting of the same protein into both the plastid and the mitochondrion has been described in land plants but the situation is more complicated in stramenopiles because a bipartite N-terminal-targeting sequence (including a signal peptide and a plastid transit peptide) is required for uptake via the four membranes of the plastid. However, at least one potential example of a dual-targeted *Ectocarpus* protein has been described, a GTPase orthologous to the bacterial MnmE (TrmE) involved in tRNA modification (Cock *et al.*, 2010). Sequence analysis suggests that dual targeting may in this case occur by the use of alternative translation initiation sites, but this will need to be confirmed experimentally.

4. INSIGHTS INTO BROWN ALGAL METABOLISM

4.1. Photosynthesis and Photosynthetic Pigments

The *Ectocarpus* light reaction and electron transport system gene complements are very similar to those of green plants, except for the lack of plastocyanin, which is usually replaced by cytochrome c_6 in chromist algae. More chlorophyll-binding protein (CBP) genes were found in the *Ectocarpus* genome than in any green plant genome studied to date (Dittami, Michel, Collen, Boyen & Tonon, 2010), suggesting that *Ectocarpus* employs a complex repertoire of CBP genes in order to cope with the exceptionally dynamic environment of the intertidal and upper subtidal zones. The 53 CBP *Ectocarpus* genes include a family of stress-induced proteins similar to the light-harvesting complex stress-related (LHCSR or LI818) CBPs. LHCSR proteins have been shown to have a photoprotective role and appear to be involved in the xanthophyll cycle-related dissipation of excess energy (Bailleul *et al.*, 2010; Gundermann & Büchel, 2007; Peers *et al.*, 2009; Zhu & Green, 2010). Interestingly, the phylogeny of LHCSR proteins suggests that they may have originated in a chlorophyll-*a*/*c*-containing organism and then been transferred horizontally to the green lineage (Dittami *et al.*, 2010).

Brown algal cells are highly pigmented, with most of the pigments playing a role in photosynthetic processes. The major brown algal pigments are chlorophyll *a*, chlorophylls c_1 and c_2, fucoxanthin, violaxanthin and β-carotene (Bjørnland & Liaaen-Jensen, 1989; Jeffrey, 1976). Some of these molecules, such as the c_1 and c_2 chlorophylls, are only found in chromalveolates and the enzymes that synthesize these pigments are unknown. Other pigments are also found in the green lineage and their biosynthesis has been studied in more detail. The *Ectocarpus* genome contains orthologues of all the genes that have been shown to be involved in the biosynthesis of chlorophyll *a*, β-carotene and violaxanthin in vascular plants and green algae (Cock *et al.*, 2010). Interestingly, the *Ectocarpus* gene encoding subunit CHL27 of the magnesium-protoporphyrin IX monomethyl ester cyclase (*acsF*) is located in the plastid. This has also recently been shown to be the case for the raphidophyte *Heterosigma akashiwo*, the xanthophyte *Vaucheria litorea* and the phaeophyte *Fucus vesiculosus* (Le Corguillé *et al.*, 2009). *CHL27* is located in the nuclear genomes of seed plants and green algae but is absent from both the nuclear and the plastid genomes of diatoms, suggesting that independent loss in the diatom lineage relative to other stramenopiles.

Light-independent (dark) protochlorophyllide oxido reductase (DPOR) allows efficient synthesis of chlorophyll in the dark or under dim light conditions (Shui *et al.*, 2009). The *Ectocarpus* plastid contains three genes (*chlB*, *chlL* and *chlN*) that encode subunits of this complex (Le Corguillé *et al.*, 2009). This is consistent with earlier observations, which indicated that species of the closely related Laminariales synthesize chlorophyll in the dark, allowing arctic species to grow during the winter (Lüning, 1990).

The carotenoid biosynthesis pathway is responsible for the production of several important molecules in chromist algae, including fucoxanthin, which is involved in the harvesting of blue light for photosynthesis and which gives these organisms their brown colouration. Diatoms and haptophytes possess two xanthophyll-based systems, the violaxanthin cycle and the diadinoxanthin cycle, which have a role in dissipating excess light energy in the plastid (Lohr & Wilhelm, 1999). These cycles are part of a biosynthetic pathway that produces fucoxanthin and both cycles are catalysed by the activity of two opposing enzymes, zeaxanthin epoxidase and violaxanthin de-epoxidase (Wilhelm *et al.*, 2006). Brown algae, in contrast, only possess the violaxanthin cycle and this was correlated with the presence of only one zeaxanthin epoxidase gene rather than the two or three copies that are usually found in diatom and haptophyte genomes (Frommolt *et al.*, 2008).

This suggested that the additional zeaxanthin epoxidase enzymes in diatoms and haptophytes might be involved in the diadinoxanthin cycle.

4.2. Carbon Metabolism

Genes for most of the enzymes involved in photosynthetic inorganic carbon fixation were found in the *Ectocarpus* genome (Cock *et al.*, 2010). The genome potentially encodes the enzymes necessary for C4 photosynthesis, consistent with the suggestion that brown algae are able to use C4 or CAM metabolism (Axelsson, 1988; Kremer & Küppers, 1977). However, the encoded proteins are not predicted to be targeted to the cellular location expected for a C4 system (Cock *et al.*, 2010). *Phosphoenolpyruvate carboxylase*, for example, is predicted to be located in the mitochondria. This is nonetheless interesting because photosynthetic activity decreases in brown algae when mitochondrial respiration is inhibited (Carr, 2005) and large numbers of mitochondria have been detected close to the cell wall in fucoid algae (Axelsson, 1988). Hence, mitochondria might play an important role in inorganic carbon uptake in the brown algae, with the initial steps of inorganic carbon fixation being partly located in the mitochondria. In addition, a recent study that combined gene analysis with extensive metabolite profiling did not provide clear support for the occurrence of an alanine/aspartate-based inducible C4-like metabolism in *Ectocarpus* (Gravot *et al.*, 2010) and suggested the presence of a classical glycolate-based photorespiration pathway in this brown alga rather than the malate synthase pathway found in diatoms.

In brown algae, excess assimilated carbon is stored as laminarin (a β-1,3-glucan with occasional β-1,6-linked branches; Read, Currie & Bacic, 1996) and the alcohol sugar mannitol (Percival & Ross, 1951). These two molecules are thought to fulfil essentially the same functions as starch and sucrose in flowering plants (Yamaguchi, Ikawa, & Nisizawa, 1966). Several potential components of the laminarin biosynthetic pathway have been identified, including putative glucose-6-phosphate isomerases, phosphoglucomutases, uridine-diphosphate (UDP)-glucose-pyrophosphorylases, β-1,3-glucan synthases and KRE6-like proteins (Michel, Tonon, Scornet, Cock & Kloareg, 2010a). Similarly, genes with predicted roles in mannitol biosynthesis and catabolism have been identified in *Ectocarpus* (Cock *et al.*, 2010) and the biochemical function of an enzyme that catalyses the first step of mannitol biosynthesis, a mannitol-1-phosphate dehydrogenase, has been investigated (Rousvoal *et al.*, 2011). The β-1,3-glucan biosynthetic pathway is thought to have been inherited vertically from the last eukaryotic

common ancestor, whereas the mannitol biosynthetic pathway appears to have been acquired via a horizontal gene transfer from an actinobacterium (Michel *et al.*, 2010a).

Another unusual feature of brown algae is their cell walls, which contain large amounts of the anionic polysaccharides alginates and fucoidans (Kloareg & Quatrano, 1988). These molecules play an important role in providing the cell wall with the strength and flexibility necessary to resist the physical stresses characteristic of shoreline environments. Cell wall biosynthesis pathways, like the carbon storage pathways described above, appear to have had a complex evolutionary history (Michel, Tonon, Scornet, Cock & Kloareg, 2010b). For example, phylogenetic analysis indicates that the terminal steps of the alginate biosynthesis pathway were acquired from an actinobacterium via a horizontal gene transfer event. In contrast, the cellulose synthesis pathway appears to have been inherited from a red alga, probably via the secondary endosymbiosis event that led to the acquisition of a plastid by an ancient ancestor of the brown algae (Reyes-Prieto *et al.*, 2007). Note that, as with the carbon storage pathways, cell wall biosynthesis enzymes are starting to be characterized at the biochemical level (Tenhaken, Voglas, Cock, Neu & Hiuber, 2011).

4.3. Nitrogen Metabolism

Based on analysis of the genome sequence, *Ectocarpus* appears to be able to take up nitrogen in three different forms: ammonium, nitrate and urea (Cock *et al.*, 2010). Nitrate is presumably assimilated via nitrate reductase and nitrite reductase. Interestingly, like diatoms, *Ectocarpus* possesses both a ferredoxin and an NAD(P)H nitrite reductase. Production of a NAD(P)H nitrite reductase may allow these organisms to reduce nitrite under conditions where reduced ferredoxin is limiting, such as low light conditions. Another interesting feature that *Ectocarpus* shares with diatoms is the presence of a complete urea cycle (Allen *et al.*, 2011; Armbrust *et al.*, 2004; Cock *et al.*, 2010).

4.4. Halogen Metabolism

Some algae can accumulate halides (iodide and/or bromide) to high levels; *Laminaria digitata*, for example, can concentrate iodine to 30,000-fold the level found in the surrounding seawater. These accumulated halides have anti-oxidant activity and can also be used to produce volatile halocarbons some of which are thought to have anti-microbial activity and which may also have a significant impact on the chemistry of the atmosphere (Küpper

et al., 2008). *Ectocarpus* does not accumulate iodine to the same levels as *Laminaria* (0.08 mg/g dry weight of *Ectocarpus* filaments corresponding to a 1000-fold concentration compared with seawater). This difference between the two brown algae is reflected in the complement of halide metabolism genes in their genomes. Vanadium-dependent haloperoxidases (vHPO) are thought to play a central role in brown algal halogen metabolism, both with regard to halide uptake and to the production of halogenated compounds (La Barre, Potin, Leblanc & Delage, 2010). *L. digitata* possesses two large multigenic families of vanadium-dependent bromoperoxidases (vBPOs) and vanadium-dependent iodoperoxidases (Colin *et al.*, 2003, 2005), whereas *Ectocarpus* possesses only one vHPO (predicted to be an apoplastic vBPO), which is expressed at a low level during the sporophyte generation (0.1% of the *Ectocarpus* ESTs compared to the 4% vBPO sequences in *L. digitata* sporophyte ESTs). vHPOs also potentially catalyze oxidative cross-linking of cell wall polymers, an activity that is consistent with a role in spore and gamete adhesion and in cell wall strengthening (Potin & Leblanc, 2006).

In *L. digitata* sporophytes, defence responses appear to involve tightly coordinated regulation of the two distinct haloperoxidase gene families, which have probably evolved from an ancestral gene duplication (Colin *et al.*, 2005; Cosse, Potin & Leblanc, 2009). Hence, there is a marked difference between the closely related Ectocarpales and Laminariales in that a highly developed iodine-based defence metabolism has evolved in the macroscopic parenchymatous sporophytes of kelps, but this system is not present, or at least not to the same degree, in the smaller thalli of the Ectocarpales.

Interestingly, other halogen-related enzymes have been identified in the *Ectocarpus* genome. These include at least three different families (21 loci) of haloacid dehalogenase (HAD) and two haloalkane dehalogenases. The HADs belong to a large superfamily of hydrolases with diverse substrate specificity, including phosphatases and ATPases. The dehalogenase enzymes may serve to defend *Ectocarpus* against halogen-containing compounds produced as defence metabolites by kelps (Küpper *et al.*, 2008) allowing it to successfully grow as an epiphyte or endophyte on kelp thalli (Russell, 1983a, 1983b).

4.5. Uptake and Storage of Iron

Iron is an important cofactor for a broad range of enzymes involved in photosynthesis, respiration and general redox reactions. In general, iron is scarce in the marine environment, particularly in the open ocean (Bruland,

Donat, & Hutchins, 1991; Martin & Fitzwater, 1988; Wu & Luther, 1994). Analysis of the *Ectocarpus* genome has provided evidence (Cock *et al.*, 2010) that it has an iron uptake system that resembles that of strategy I plants (Moog & Bruggemann, 1994). Homologues of both FRO2, an iron chelate reductase, and natural resistance-associated macrophage proteins (NRAMP), a M^{2+}-H^{+} symporter with a preference for Fe(II) (Bauer & Bereczky, 2003; Curie & Briat, 2003; Morrissey & Guerinot, 2009), have been identified in *Ectocarpus* (Cock *et al.*, 2010). The *Ectocarpus* NRAMP homologues may be important for iron release or mobilization but no homologues of the iron carrier CCC1p were detected. Physiological studies using the bathophenanthroline disulphonic acid assay (Eckhardt & Buckhout, 1998) support the involvement of an iron chelate reductase. The iron uptake system in *Ectocarpus* would therefore appear to be similar to that of the pennate diatom *P. tricornutum* and hence different from the reductive–oxidative pathway found in the centric diatom *T. pseudonana* (Kustka, Allen, & Morel, 2007). *Ectocarpus* appears to lack homologues of any of the common iron regulatory genes including *ide*, *dtxR*, *fur* or *irr* (Bauer & Bereczky, 2003; Curie & Briat, 2003; Morrissey & Guerinot, 2009). Furthermore, while some coastal diatoms such as *P. tricornutum* have ferritin genes, ferritins have not been detected in other open-ocean taxa such as *T. pseudonana* (Marchetti *et al.*, 2009); *Ectocarpus* has no homologues of any of these proteins making it similar to *Thalassiosira* in this respect. More recently, an alternative method of iron storage in vacuoles has been elucidated in yeast and several other eukaryotes including the halotolerant alga *Dunaliella salina* (Martinoia, Maeshima, & Neuhaus, 2007; Paz, Shimoni, Weiss & Pick, 2007). Thus, at present, there is no genetically identifiable iron storage system in *Ectocarpus*. In line with this, iron K-edge x-ray absorbance spectroscopy (XAS) and Mössbauer spectroscopic analysis of *Ectocarpus* tissue showed that most of the Fe pool is present as Fe(III), most of which is coordinated in FeS clusters and FeO mineral species but, as expected not to ferritin (Lars H. Böttger, Eric P. Miller, Christian Andresen, Berthold F. Matzanke, Frithjof C. Küpper, and Carl J. Carrano, J. Exp. Bot., in press, 2012).

4.6. Lipid Metabolism

One of the interesting features of brown algal metabolism is that it includes pathways for the biosynthesis of both C18 and C20 polyunsaturated fatty acids (PUFAs), which are typical of green plant and animal lipid metabolisms, respectively. Brown algal PUFAs are probably important precursors both of oxylipins involved in defence and stress responses and of sexual

pheromones (Müller, Jaenicke, Donike & Akintobi, 1971; Pohnert & Boland, 2002). In *Ectocarpus*, PUFAs appear to be synthesized in the cytoplasm from malonyl-CoA, which is itself derived from acetyl-CoA in the plastid. The most abundant PUFAs are 18:2$n-6$, 18:3$n-6$ and 20:4$n-6$ for the omega 6 series, and 18:3$n-3$ and 20:5$n-3$ for the omega 3 series. *Ectocarpus* appears to lack docosahexaenoic acid (Schmid, Müller, & Eichenberger, 1994). A number of genes with potential roles in oxylipin biosynthesis have been identified in *Ectocarpus* (Cock *et al.*, 2010), but it was not possible to assign genes definitively to the C18 or C20 pathways based on sequence information. Sphingolipids act both as structural components of membranes and as signalling molecules in plants and mammals, and most of the genes required for the biosynthesis of these molecules have been identified in *Ectocarpus*. As far as fatty acid degradation is concerned, *Ectocarpus*, like diatoms (Armbrust *et al.*, 2004), possesses two beta-oxidation pathways, one localized in mitochondria and the other in peroxisomes.

4.7. Secondary Metabolism

Brown algae produce many phenolic compounds through the acetate–malonate pathway, and these molecules have important roles as ultraviolet (UV) protectants, adhesives, cell wall strengtheners and defence molecules (Emiliani, Fondi, Fani, & Gribaldo, 2009). Phloroglucinol is the precursor of brown algal tannins. Synthesis of this molecule in *Ectocarpus* is predicted to involve three type III polyketide synthases, enzymes that appear to be absent from oomycete or diatom genomes (Cock *et al.*, 2010). Cytochrome P450s are known to oxidatively tailor polyketide products in other organisms as seen in aflatoxin biosynthesis. *Ectocarpus* has 11 cytrochrome P450 genes and 1 pseudogene, of which only 3 have putative functions (CYP51C1 is a sterol 14-alpha demethylase, CYP97E3 and CYP97F4 are presumed carotenoid hydroxylases based on the role of the highly conserved CYP97 family in green plants). The eight remaining P450s are potential modifiers of polyketide structures or other secondary metabolites.

The shikimate pathway appears to be present in *Ectocarpus*, but some of the derivatives of this pathway that are found in terrestrial plants, such as phenylpropanoids and salicylic acid, are predicted to be absent (Cock *et al.*, 2010). *Ectocarpus* also possesses a flavonoid metabolism, a feature shared with a broad range of photosynthetic organisms including other stramenopiles, but lacks a phenylalanine ammonia-lyase (*PAL*) gene. Given that terrestrial plants appear to have acquired *PAL* following a horizontal gene transfer

from a bacterial genome (Emiliani *et al.*, 2009), this suggests that the PAL enzyme was integrated into a pre-existing flavonoid metabolism in the green lineage.

5. CELLULAR PROCESSES

5.1. Receptors, Ion Channels and Signal Transduction Pathways

The *Ectocarpus* genome provided evidence for several types of sensor molecule, including molecules located both on the cell membrane and within the cytoplasm (Cock *et al.*, 2010). These included molecules that have been found in other stramenopiles, such as G-protein-coupled receptors and their associated heterotrimeric G-proteins, and molecules that have so-far appeared to be absent from chromalveolates, such as membrane-located histidine kinases. The *Ectocarpus* genome encodes three membrane-located histidine kinases, with N-terminal Mase or Chase sensor domains. Interestingly, one of these proteins is predicted to have seven trans-membrane domains and seems to be a G-protein-coupled receptor (GPCR)-histidine kinase fusion protein. One of the most remarkable discoveries in the genome was a family of 11 receptor kinases (Cock *et al.*, 2010). Both land plants and animals possess large families of similar receptor kinases and it has been suggested that the acquisition of these membrane-located signalling molecules was a key step towards the evolution of complex multicellularity in each lineage. Interestingly, animal and plant receptor kinases appear to have evolved independently and phylogenetic analysis indicated that this was also the case for the brown algal receptor kinases. Hence, the independent evolution of complex multicellularity in the plant, animal and brown algal lineages can, in each case, be correlated with the evolution of receptor kinase families.

Ectocarpus possesses at least six different types of membrane-localized ion channel, including members that are predicted to play an important role in sensing and responding to changes in the extracellular environment. For example, the ion channel proteins include a large family of transient receptor potential channels, which have been shown, in other organisms, to respond to a broad range of stimuli including temperature, light, chemicals and mechanical stress (Venkatachalam & Montell, 2007). Other families of ion channels represented in the *Ectocarpus* genome include bacterial-type, small conductance mechanosensitive channels, ionotropic glutamate receptors

(which are absent from other stramenopile genomes) and an inositol triphosphate (IP3)/ryanodine-type receptor (IP3R/RyR). The presence of the latter is consistent with the observation that IP3 induces Ca^{2+} release in *Fucus serratus* embryos (Coelho *et al.*, 2002; Goddard, Manison, Tomos & Brownlee, 2000).

In animals, integrins are membrane-localized proteins involved in the transmission of mechanical signals perceived at the cell surface to the cytoskeleton (Arnaout, Goodman, & Xiong, 2007). *Ectocarpus* has three proteins that share similarity with integrin alpha subunits. Moreover, although there are no homologues of animal extracellular interacting proteins such as collagen, fibronectin and vitronectin, *Ectocarpus* does have homologues of talin and α-actinin, which are intracellular integrin partners that interact with actin microfilaments (Ziegler, Gingras, Critchley, & Emsely, 2008).

In addition to these membrane-localized molecules, *Ectocarpus* is predicted to possess the following cytosolic photoreceptors: three phytochromes, three cryptochromes (including a (6-4) family 'animal type' cryptochrome and two Cry-DASH genes) and five aureochromes (Cock *et al.*, 2010). Aureochromes are thought to be the stramenopile equivalents of phototropin blue-light receptors (Ishikawa *et al.*, 2009), explaining the absence of genes encoding the latter class of receptor in brown algal genomes.

A number of *Ectocarpus* pathogens have been described, including viruses, an oomycete (*Eurychasma dicksonii)*, a chytrid (*Chytridium polysiphoniae)*, a hyphochytrid (*Anisolpidium ectocarpii)* and a plasmodiophorid (*Maullinia ectocarpii*) (Charrier *et al.*, 2008; Gachon, Sime-Ngando, Strittmatter, Chambouvet and Kim, 2010). *Ectocarpus* presumably possesses sensor systems that allow it to detect the presence of these pathogens. A search for protein domains commonly found in components of land plant pathogen recognition systems failed to identify any caspase activation and recruitment domain (CARD), domain in apoptosis and interferon response (DAPIN) or Toll/Interleukin-1 receptor (TIR) domain-containing proteins. However, the genome does encode a large family of more than 250 LRR-domain-containing proteins and 15 proteins with nucleotide-binding adaptor shared by APAF-1, R proteins and CED-4 (NB-ARC) domains. A role in pathogen-specific immune responses is particularly likely for the NB-ARC-TPR proteins and a subset of about 60 of the LRR-domain loci, which encode GTPases of the ROCO family (including about 20 apparent pseudogenes). Both families exhibit evidence of rapid evolution of their ligand-binding (LRR and TPR) domains

via exon shuffling (Fig. 5.4; Zambounis *et al.*, 2012). The LRR and NB-ARC domain genes are often grouped into small clusters of closely related genes and associated with probable pseudogenes in a similar fashion to the fast-evolving clusters of disease-resistance genes found in land plants (Meyers *et al.*, 2005). The predicted regulation of *Ectocarpus* LRR domain genes by microRNAs (Table 5.1) has also been observed to be a feature of land plant disease-resistant genes (Li *et al.*, 2012; Zhai *et al.*, 2011).

Ectocarpus also possesses homologues of many of the pathogenesis-related proteins, which are induced following infection or attack in land plants (Antoniw, Ritter, Pierpoint, & Van Loon, 1980; Van Loon, Rep, & Pieterse, 2006), and potential components of a programmed cell death pathway, such as metacaspases (Cock *et al.*, 2010).

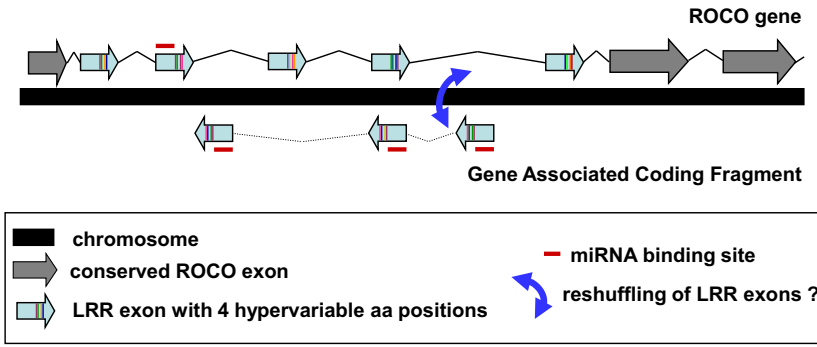

Figure 5.4 Gene structure and hypothetical post-transcriptional regulation features of the ROCO family. *Ectocarpus* ROCO proteins contain LRR domains in the N-terminal region. Each LRR motive is specified by a precisely delineated, 24-codon exon (blue arrows). Each LRR exon contains four hypervariable codons corresponding to amino acids that are exposed at the protein surface and are believed to dictate the ligand-binding specificity of the LRR domain (multicolour vertical bars in each LRR exon). These amino acid residues evolve under positive selection, suggesting a potential role of ROCO proteins in pathogen detection or immune reactions. Sequences resembling the LRR-encoding exons are also present in the introns but located on the opposite strand of the DNA. It is possible that these sequences are integrated into ROCO gene sequences as new exons following intragenic recombination events (curved blue arrow). Phylogenetic analysis also suggests that recombination occurs between members of the ROCO family, leading to swapping of LRR exons (not shown here). Moreover, the more conserved N-terminal regions of some LRR exons are predicted to be targets of miRNAs (red bars), suggesting that shuffling of LRR exons might also modulate post-transcriptional regulation. It is not yet known whether the LRR-encoding sequences on the opposite strand are transcribed but if so they could serve as alternative targets for ROCO-directed miRNAs and hence potentially modulate ROCO gene expression. See the colour plate.

Cytoplasmic signal transduction presumably involves members of the eukaryotic protein kinase (ePK) superfamily (Manning, Whyte, Martinez, Hunter, & Sudarsanam, 2002). The ePK family in *Ectocarpus* includes 258 predicted proteins representing a broad range of subfamilies (Cock *et al.*, 2010). Cytoplasmic protein kinase-mediated pathways in *Ectocarpus* also include the broadly conserved target of rapamycin (TOR) signalling pathway regulating cell growth (Durán & Hall, 2012; van Dam, Zwartkruis, Bos, & Snel, 2011). Exceptionally among stramenopiles, *Ectocarpus* lacks the central regulator of TOR kinase complexes, the small GTPase Rheb (Cock *et al.*, 2010), but this appears to have been lost several times independently in the eukaryotic evolution (van Dam *et al.*, 2011); it would be interesting to investigate whether the independent loss of Rheb leads to similar or different compensatory modifications in the TOR regulation.

A proportion of the signal transduction pathways are expected to modulate gene transcription. The *Ectocarpus* genome is estimated to encode 401 transcription-associated proteins (this includes both the transcription factors that bind directly to the DNA and the transcriptional regulators that act indirectly on transcription). *Ectocarpus* possesses eight RWP-RK domain (or nodule-inception(NIN)-like) transcription factors. Members of this family, which is completely absent from the diatom genomes studied to date, have been implicated in both nitrate signalling (Fernandez & Galvan, 2008) and gametogenesis (Lin & Goodenough, 2007) in other species.

5.2. Cell Cycle, Endoreduplication and Meiosis

Cell cycle regulation by cyclins and cyclin-dependent kinases (CDK) appears to be a universal feature across the eukaryotic tree (Cross, Buchler, Skotheim, 2011). The complement of cell cycle regulatory genes in *Ectocarpus* is more similar to that of green plants than that of animals (Cock *et al.*, 2010). This is true both for the core cyclin/CDK complex and regulators of this complex.

Spo11 creates the double-strand DNA breaks that are required to initiate recombination during meiosis (Keeney, Giroux, & Kleckner, 1997; Krogh & Symington, 2004; Lichten, 2001). Land plants possess three Spo11 homologues, Spo11-1 and Spo11-2, which are specifically required for meiosis, and Spo11-3/Top6A, which functions with Top6B as a topoisomerase and is required for endoreduplication (Hartung *et al.*, 2007; Sugimoto-Shirasu, Stacey, Corsar, Roberts, & McCann, 2002; Yin *et al.*,

2002). *Ectocarpus* is predicted to possess both a meiotic Spo11 and a Top6A homologue (together with its Top6B partner). The presence of the latter is particularly interesting, given the recent demonstration that a proportion of haploid partheno–sporophytes (produced by parthenogenetic germination of gametes) undergo endoreduplication very early in development to produce diploid individuals. As a result, these individuals can produce haploid meiospores via a normal meiotic division, allowing progression to the gametophyte generation of the life cycle (Bothwell, Marie, Peters, Cock & Coelho, 2010).

In addition to *Spo11*, *Ectocarpus* possesses most of the core meiotic genes. One notable feature of this set of genes is that, compared to other stramenopiles, *Ectocarpus* has a very complete set of *Rad51* genes, possessing at least six of the seven known eukaryotic members of the *Rad51* family (Lin, Kong, Nei & Ma, 2006), including a likely *DMC1* homologue. Completeness of the *Rad51* family has been linked to multicellularity, so this may be another factor that was important for the evolution of complex multicellularity in the brown algae.

Ectocarpus has a homologue of the DNA helicase *Mer3,* but no clear homologues of *Mms4* and *Mus81* were found (although *ERCC4*-like genes are present). This suggests that *Ectocarpus* may only have the class I, interference-sensitive crossover pathway, although it is possible that the class II, interference-insensitive pathway is present but mediated by highly divergent proteins.

5.3. Cytoskeleton, Flagella and Vesicle Trafficking

One of the remarkable features of cytokinesis in brown algae is that it has characteristics typical of both green plant and animal systems; centrosomes function as microtubule organizing centres (as in animal cells), but during cytokinesis, a structure resembling a cell plate is formed, and this extends out from the centre of the cell towards the plasma membrane (Nagasato & Motomura, 2002). This latter feature resembles cytokinesis in green plants, but no specialized phragmoplast is formed in brown algae and therefore, as expected, *Ectocarpus* does not possess genes encoding dynamin-related phragmoplastins. *Ectocarpus* does, however, possess the centrosome-associated tubulins δ and ε in addition to α-, β- and γ-tubulin.

Microtubules also play an important structural role in the flagella. Brown algal zoids have the two heteromorphic flagella typical of stramenopiles. Comparison of the *Ectocarpus* radial spoke proteins (RSP) with those of *Chlamydomonas reinhardtii*, *Thalassiosira pseudonana*, *Micromonas pusilla* and

Homo sapiens, indicated that that the complement of RSPs can be very variable (Cock *et al.*, 2010), probably depending on the mechanism of flagella bending in different species. Several flagellum- or basal body-associated proteins are encoded by Bardet–Biedl syndrome (BBS) genes, which are of considerable interest because of their implication in human disease. Seven of them are widely conserved in eukaryotes with flagella and were suggested to represent a functional module consisting of a regulatory small GTPase (BBS3 = ARL6) and its putative effector, the multisubunit complex BBSome, which probably serves as a membrane coat for trafficking to the flagellum (Jin *et al.*, 2010). The *Ectocarpus* genome encodes all the known BBS proteins that have been detected in *Chlamydomonas* and may therefore be an interesting system to study the function of these proteins. Other flagellar components encoded by the *Ectocarpus* genome include intraflagellar transport proteins and SF-assemblin (a component of system I fibers, which run parallel to flagellar root microtubules from the basal bodies; Lechtreck & Melkonian, 1991) and orthologues of the mastigoneme components Ocm1, Ocm2, Ocm3 and Ocm4 (Honda *et al.*, 2007; Yamagishi, Motomura, Nagasato, & Kawai, 2009; Yamagishi, Motomura, Nagasato, Kato, & Kawai, 2007).

In brown algae, the cortical actin network plays a crucial role both in determining the polarity of apical and axillary cell divisions and in cell wall morphogenesis (Katsaros, Karyophyllis & Galatis, 2006). *Ectocarpus* possesses a single actin gene, plus actin-associated proteins such as formin, profilin, villin, fimbrin and myosin. *Ectocarpus* also has all seven subunits of the ARP2/3-complex, which plays an important role in the reorganization of the actin cytoskeleton at the cell cortex during processes such as vesicular trafficking. There is evidence that the ARP2/3 complex has a role in mediating polarized growth in brown algae (Hable & Kropf, 2005). The Rho family of small GTPases is known to play a central role in mediating signalling directed towards the actin cytoskeleton (Etienne-Manneville & Hall, 2002). In contrast to other multicellular (and even many unicellular) eukaryotes (Brembu, Winge, Bones, & Yang, 2006), no lineage-specific expansion of the Rho family has occurred in *Ectocarpus,* which possesses only a single *Rho* gene (Cock *et al.*, 2010).

Based on ultra-structure studies, the intracellular trafficking system appears to be very active in vegetative *Ectocarpus* cells (Bouck, 1965; Oliveira & Bisalputra, 1973). It was therefore not surprising to find that the gene families associated with this process are quite complex, including, for example, 20 SNARE proteins and significant numbers of coat protein

complex proteins (Cock *et al.*, 2010). However, the set of Rab GTPases (which play a key role in determining the specificity of vesicle trafficking between the diverse compartments of the endomembrane system) is not markedly expanded in *Ectocarpus* compared to unicellular relatives (diatoms and *Aureococcus anophagefferens*). This contrasts with the situation in metazoans, where there has been a significant expansion of this family, but more closely resembles the situation in green plants, where the Rab family did not undergo a marked expansion during the transition to multicellularity (Elias, Brighouse, Gabernet-Castello, Field, & Dacks, 2012). Another group of GTPases important for the function of the endomembrane system are atlastins, which are responsible for homotypic fusion of ER membranes and the generation of the tubular endoplasmic reticulum (ER) network (Hu *et al.*, 2009). Atlastins had been thought to be restricted to Metazoa, but putative orthologs have been found in the genomes of *Ectocarpus* and other stramenopile algae (Cock *et al.*, 2010).

6. FUTURE DIRECTIONS

Analysis of the *Ectocarpus* genome sequence has provided a large amount of information about the probable molecular systems that underlie a broad range of processes in brown algae. However, this information is based principally on parallels with other organisms (sequence homology) and an important challenge for the future will be to develop methods of directly investigating gene function in *Ectocarpus*. These methods need to include not only ways of disrupting gene function but also tools to analyse spatiotemporal patterns of gene expression and interactions between gene products and to assay protein activities in heterologous or *in vitro* systems. Several genetic tools are already available including protocols for UV and chemical mutagenesis, phenotypic screening methods, genetic crosses, methods for handling large populations, a large number of genetic markers, defined strains for genetic mapping and a microsatellite-based genetic map (Cock *et al.*, 2011; Coelho *et al.*, 2012; Heesch *et al.*, 2010; Peters *et al.*, 2008). At present, however, there is no reliable method to knock out or knock down gene expression in *Ectocarpus*, although a considerable effort is being invested in the development of genetic transformation and RNA interference approaches, and a targeting-induced local lesions in genomes (TILLING) population (Comai & Henikoff, 2006) is being developed for reverse genetic analysis.

As far as analysis of gene expression is concerned, an EST-based expression microarray has been developed (Dittami *et al.*, 2009), and a considerable amount of cDNA and small RNA sequence data are available (Cock *et al.*, 2010). The latter is currently being significantly expanded using new generation RNA-seq methodology. In addition, protocols have been developed for proteome and metabolome analysis (Dittami *et al.*, 2011; Ritter *et al.*, 2010).

The genome sequence data are currently being used in combination with the tools described above to explore several different aspects of brown algal biology. These include life cycle regulation (Bothwell *et al.*, 2010; Coelho *et al.*, 2011), morphogenesis (Le Bail, Billoud, Le Panse, Chenivesse, & Charrier, 2011), metabolic processes (Dittami *et al.*, 2011), interactions with the environment and with brown algal pathogens (Grenville-Briggs *et al.*, 2011; Ritter *et al.*, 2010; Zambounis *et al.*, 2012) and taxonomy (Peters *et al.*, 2010a, 2010b). In several of these studies (Coelho *et al.*, 2011; Le Bail *et al.*, 2011; Peters *et al.*, 2008), major regulatory loci have been identified through the isolation of mutants and an important aim for the future is to identify the genes affected by these genetic lesions. Phylogenetic analyses have shown that the genus *Ectocarpus* is a complex of several (cryptic) species inhabiting different geographic regions and ecological environments and which we only begin to understand (Peters *et al.*, 2010a, 2010b). Reproductive isolation between species is incomplete because interspecific crosses can be performed, although vegetative development or formation of meiospores may be impaired in the hybrids (reviewed in Stache-Crain, Müller, & Goff, 1997). Genome data from the different species of *Ectocarpus* would help to better understand the mechanisms and processes determining their interfertility, ecological niches, as well as (historical) biogeography.

Another important future objective for brown algal genomics will be to extend knowledge gained using *Ectocarpus* as a model system to other brown algae. This process should include extension of the sequencing effort to additional brown algal genomes and transcriptomes. In this respect, two approaches would be particularly interesting. First, as a group, the Ectocarpales exhibit a considerable amount of variation in terms of their life cycles, sexual systems, morphology and cell biology (Silberfeld *et al.*, 2010) and yet are expected to share a significant level of genome synteny, facilitating the use of comparative genomic approaches to investigate the genetic basis of these biological variation. Second, compared to the kelps, the Ectocarpales exhibit only a limited level of developmental complexity. It would be of considerable

interest to analyse a kelp genome sequence, particularly with respect to the emergence of complex multicellularity in the brown algae.

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