2 Brown Algae
Ectocarpus *and* Saccharina *as Experimental Models for Developmental Biology*

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CONTENTS

2.1 INTRODUCTION

Brown algae (also named Phaeophyceae) are a group of eukaryotic multicellular organisms comprising ~2000 species. They are autotrophic organisms using photosynthesis to transform light into chemical energy (ATP through NADP reduction). Their evolutionary history is distinct from that of animals, fungi and plants. In the tree of life, molecular phylogeny and cytological characters position brown algae within the division of Stramenopiles (Heterokonta), diverging from the last common Stramenopile ancestor ~250 million years ago (Mya) (Kawai et al. 2015) (Figure 2.1a).

The Stramenopiles are characterized by reproductive cells that possess two flagella ("konta") of different size and structure (Derelle et al. 2016). Other photosynthetic stramenopiles

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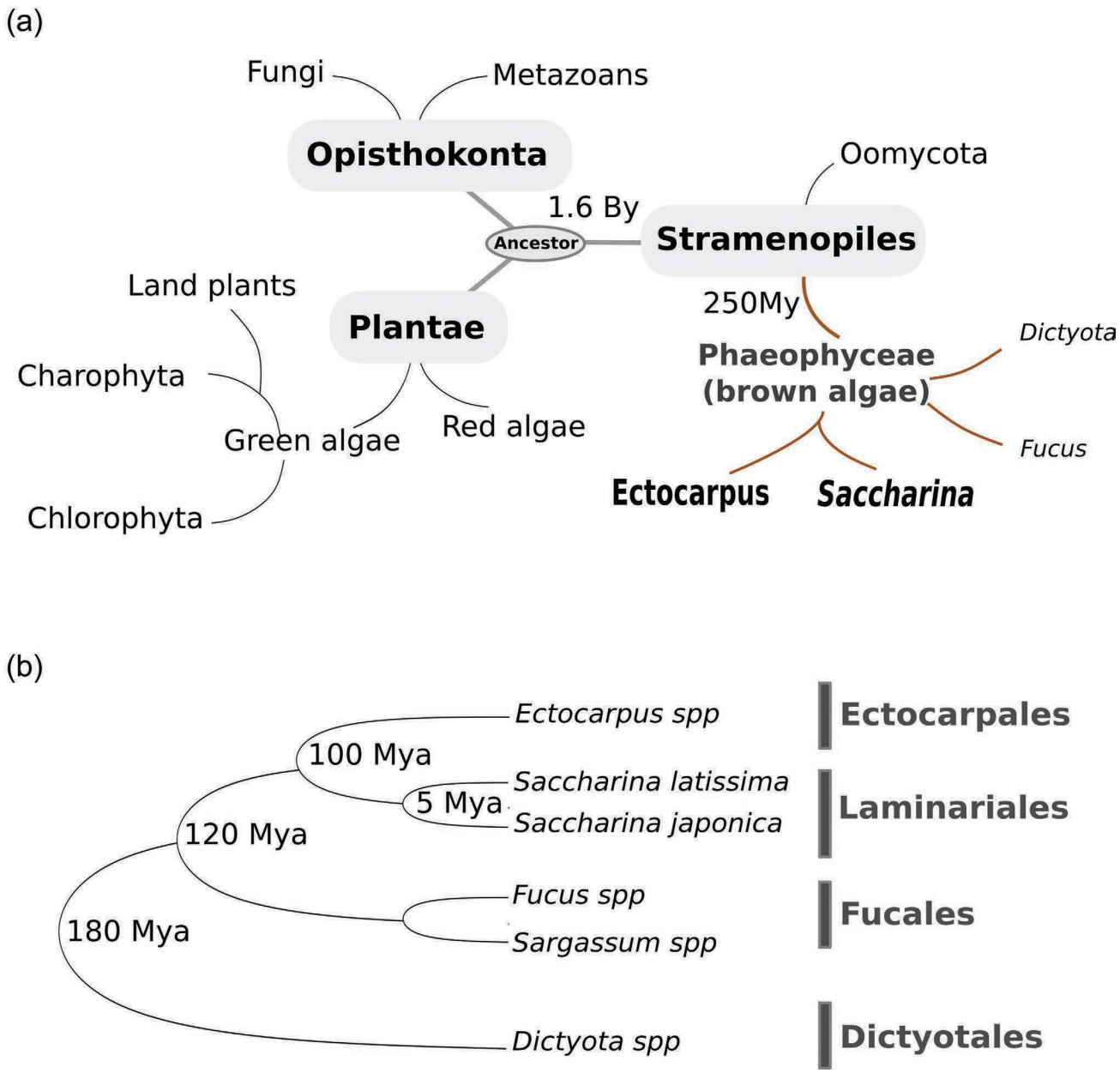

FIGURE 2.1 **Evolution of brown algae**. (a) Phylogenetic position of brown algae (Phaeophyceae) in the eukaryotic tree of life. Phaeophyceae diverged ~250 million years ago (Mya) from the last common stramenopile ancestor. Stramenopiles include multicellular organisms only (the syncitial oomycota are not considered true multicellular organisms). (b) Simplified phylogenetic tree of some brown algal genera and orders. *Ectocarpus* spp. and *Saccharina* spp. belong to closely related orders, the Ectocarpales and the Laminariales, which split ~75 Mya. Other brown algal models belonging to Fucales or Dictyotales are more distant phylogenetically (diverged 120 Mya and 180 Mya, respectively). ([a] Kawai et al. 2015; [b] Starko et al. 2019; Silberfeld et al. 2010; Kawai et al. 2015.)

(Ochrophyta) are diatoms and Xanthophyceae; however, brown algae are the only group presenting complex multicellularity. Brown algae exhibit a wide range of morphologies and a fairly high level of morphological complexity (Charrier et al. 2012). This group of algae is extremely diverse in size, ranging from just a few hundreds of micrometers to up to 40 m, for example, the kelp forests that provide shelter and feeding grounds for many marine animals. Their diversity in shape is also considerable, ranging from crusts to digitated blades, all growing attached to rocky surfaces or on other algae (epiphystism).

This chapter reports on research carried out on two very different brown algal species: the microscopic filamentous *Ectocarpus* sp., which entered the genomics and other -omics era 10 years ago, and the large laminate *Saccharina latissima*, which is currently raising increasing interest in Europe as a future source of food and derived agri-food and pharmaceutical products. These algae belong to the orders Ectocarpales and Laminariales, respectively, which diverged ~100 Mya (Silberfeld et al. 2010). Here, we present these two models in the context of studies focused primarily on development and growth.

2.2 *ECTOCARPUS* SP.

2.2.1 History of the Model and Geographical Location

Records of the occurrence of *Ectocarpus siliculosus* in the environment emerged about two centuries ago. This species was first described as *Conferva siliculosa* by Dillwyn in 1809 from material collected in England (Dillwyn 1809). Ten years later, Lyngbye recorded *Ectocarpus* sp. as *Conferva confervoides* from material collected in Denmark (Lyngbye 1819). As a result, this species is now named *Ectocarpus siliculosus* (Dillwyn) Lyngbye.

This species belongs to the order Ectocarpales, which includes most of the brown algae with a simple body architecture, mainly filamentous in habit. Due to these morphological features, *Ectocarpus* sp. was initially classified at the root of the brown algae phylogenetic tree with the Discosporangiales (e.g. *Choristocarpus* spp.), displaying similarly low morphological complexity. However, molecular markers identified in the 1980s led to more accurate phylogenetic analyses and classified the Ectocarpales as a sister group to the most morphologically complex family of brown algae, the Laminariales (kelps, see Section 2.3), far from the basal brown algal groups (Silberfeld et al. 2014) (Figure 2.1b).

Ectocarpus sp. is a tiny, filamentous brown alga, thriving in all temperate marine waters in both hemispheres. There is a recent geographical inventory of several species, together with their phylogenetic relationship (Montecinos et al. 2017). Although some *Ectocarpus* species are highly sensitive to salinity (Dittami et al. 2012; Rodriguez-Rojas et al. 2020), other species can also thrive in freshwater, particularly in rivers. The complexity of their associated microbiome may contribute to their adaptation to these environments (Dittami et al. 2016; Dittami et al. 2020). Interestingly, in contrast to other Phaeophyceae, *Ectocarpus* species have spread extensively around the world and are not confined to any specific geographical area. This wide distribution is likely due, for the most part, to the high capacity of *Ectocarpus* spp. to adhere to various artificial surfaces, such as boat hulls, ropes, and so on (biofouling), promoting their dispersal through maritime traffic (Montecinos et al. 2017).

2.2.2 Life Cycle

Ectocarpus spp. grow following a microscopic, haplodiplontic, dioicous life cycle (Figure 2.2). For some species, however, only a part of this complex life cycle can be observed in natural conditions, regardless of the ecological niche (Couceiro et al. 2015). The different stages of the life cycle and related mutants are described in Figure 2.2 and Section 2.2.6.

2.2.3 Embryogenesis and Early Development

Embryogenesis is not a term well adapted to *Ectocarpus* sp., because its early body lacks complex tissue organization and has only one growth axis. Instead, from the onset of zygote germination, *Ectocarpus* sp. develops a primary uniseriate filament along a proximo-distal axis, on which secondary filaments subsequently emerge serially (Le Bail et al. 2008). Successive and iterative branching continues and results in the development of a bushy organism of a few millimeters after 1–2 months. Interestingly, this low level of morphological complexity and slow growth (~3 μm.h-1; Rabillé et al. 2019a) endow Ectocarpus with the features of a convenient model for studying several fundamental cell growth and cell differentiation processes.

The development of the sporophyte (2n) is initiated by the emergence of a tip from the zygote (Figure 2.3a, b). The growth of this tip is indeterminate throughout the development of the organism, and it can be described by a simple and original biophysical model based on the control of the thickness of the algal cell wall in the tip area (Rabillé et al. 2019a). In this area, the cell wall is mainly composed of the two main polysaccharides identified in brown algal cell walls: alginates [combination of two types of residues: (1→4) α-L-guluronic acid (G residues) and (1→4) β-D-mannuronic acid (M residues); 40% of the cell wall] and fucans (polysaccharides containing α-L-fucosyl residues; 40%) (reviewed in Charrier et al. 2019). When sulfated, these fucans are called fucose-containing sulfated polysaccharides (FCSPs; Deniaud-Bouët et al. 2014). Although alginates may be necessary in particular for the growth of highly curved cell surfaces (Rabillé et al. 2019b), sulfated fucans may provide additional biophysical properties, for example, hygroscopy and high flexibility (Simeon et al. 2020).

In the wild type, the apical cell of each filament is a very long cylindrical cell (length > 40 μm; diameter 7 μm), but in the mutant *etoile*, the apical cell is shorter and wider. In this mutant, tip growth stops shortly after it is initiated, and cells have a thicker cell wall and an extensive Golgi apparatus (Le Bail et al. 2011).

The expansion of the tip outward is accompanied by cell division (~1 every 12 h in standard lab conditions; Nehr et al. 2011). The first cell division separating the round zygotic cell and the growing elongated cell is asymmetrical (Le Bail et al. 2011; Figure 2.3b). Once the filament has grown a few cells on one end, the initial zygotic cell germinates on the opposite end, thereby producing a filament along the same axis as the initial filament. The two processes result in the formation of a multicellular uniseriate filament made up of a series of elongated cells aligned along a single growth axis.

These cylindrical cells progressively change shape and become round (Le Bail et al. 2008) (Figure 2.3). This rounding up from a cylindrical cell to a spherical cell is reminiscent of the cell rounding that takes place in highly polarized metazoan cells before mitosis, where this process has been shown to ensure proper spindle assembly (Lancaster and Baum 2014) and equal distribution of cellular materials. In *Ectocarpus* sp., the underlying mechanisms for this cell rounding differentiation process are still unknown, but modeling has shown that local cell–cell communication between neighboring cells is likely involved, not long-range diffusion of a signaling molecule (Billoud et al. 2008).

Branching takes place primarily on maturing polarized cells and to a lesser extent on already formed round cells

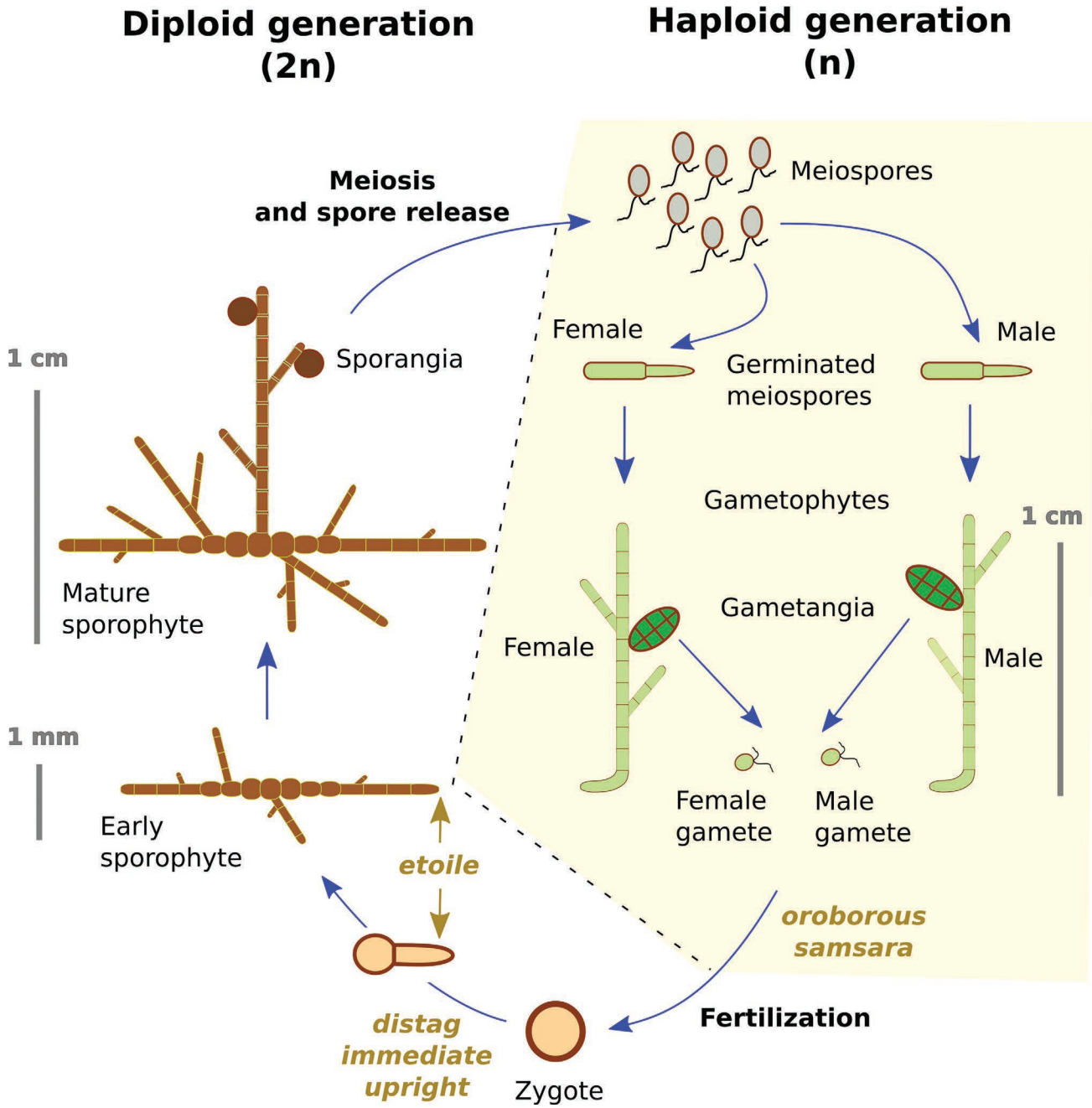

FIGURE 2.2 **Life cycle of *Ectocarpus* sp.** (summarized in Charrier et al. 2008). Diploid (brown) phase (left-hand side) is made of microscopic sporophytes composed of branched uniseriate filaments. Meiosis takes place in unilocular sporangia (dark brown circles) differentiating laterally on erect branches. Haploid (light green) phase (right-hand side, yellow shaded area) corresponds to the formation of gametophytes, which are erect branched uniseriate filaments growing from germinated meiospores (gray circles and light green cells). Male and female gametes are each released from male and female gametophytes (dioicous life cycle) and fuse freely in the external environment (seawater), producing a free zygote (orange circle). *Ectocarpus* sp. is therefore characterized by its small size, distinguishing it from most of the other brown algae (e.g. the kelp *Saccharina* sp.) (note the scale). Characterized mutants impaired in the different steps of the life cycle are indicated in light brown.

(Figure 2.3c). The detailed process is unknown. It does not seem to depend on actin filaments (although growth is) (Coudert et al. 2019) or microtubules (personal observations). A biophysical study based on the assumption that the cell wall is a poro-elastic material suggests that an increase in surface tension during the enlargement of rounding cells is sufficient to induce branching (Jia et al. 2017). Branching never occurs in the apical cell or twice in the same cell, suggesting the action of inhibitory mechanisms ensuring spacing between branches (Figure 2.3d). One potential contributor to inhibition is the phytohormone auxin, shown to accumulate at the tip of Ectocarpus filaments (Le Bail et al. 2010). Auxin may then establish a decreasing gradient along the linear filament, preventing the emergence of

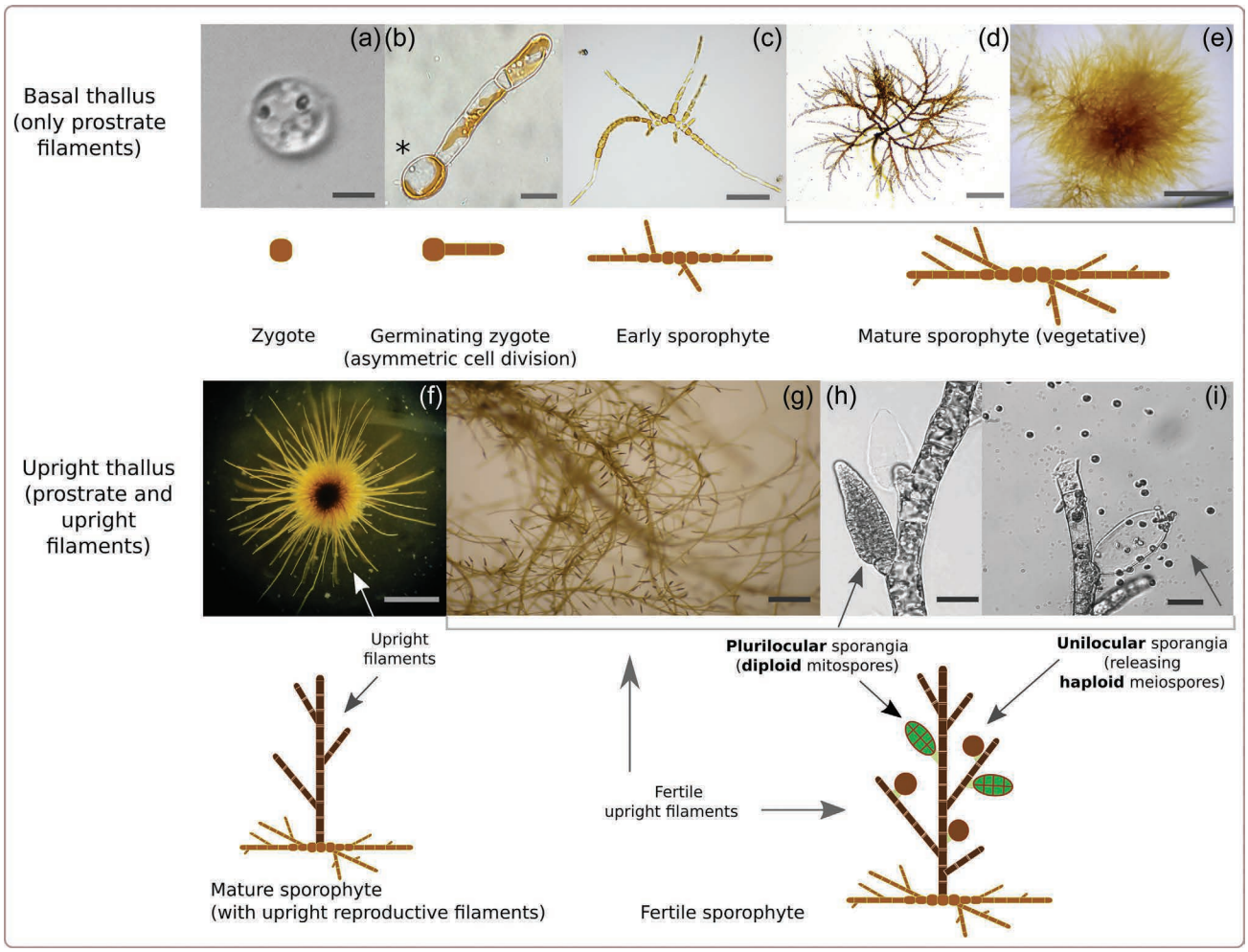

FIGURE 2.3 **Developmental stages of the *Ectocarpus* sp. sporophyte**. Photos and accompanying schematic representations of the different stages of sporophyte development. From top left to bottom right: the zygote (a) germinates, forming a tube, and then divides asymmetrically (b). (c) Filaments are formed by apical cell growth and cell division of the primary filament, followed by branching, leading first to a small tuft after ~20 days (d), then to a larger one after ~1 month (e). This makes up the prostrate part of the thallus (top). After ~1 month, upright filaments emerge (f, dark brown on the schematic representation), on which two kinds of reproductive organs differentiate: plurilocular sporangia (g) releasing mitospores (h, green in the schematic, not shown in Figure 2.2 for simplicity), which have the capacity to germinate as their parent, generating another sporophyte genetically and morphologically identical to its parent, and unilocular sporangia releasing meiospores after meiosis (i, brown in the schematic). These haploid spores germinate as female and male gametophytes in equal proportion (not shown). Scale bars (a, b) 5 µm, (c) 50 µm, (d, g) 100 µm, (e, f) 1 mm, (h, i) 20 µm. ([b] Le Bail et al. 2011; Billoud et al. 2008.)

branches in the most distal area of the filament and allowing branching in the more central regions. However, there must be additional mechanisms operating to explain the spacing between branches. Interestingly, during growth, growing filaments generally tend to avoid each other, following curved trajectories. This observation suggests the existence of lateral inhibition mechanisms through chemical diffusion in the environment. It is not known whether branching spacing relies on the diffusion of inhibitors in the external medium or is transported by the neighboring cells within the filaments (*Ectocarpus* sp. cells possess plasmodesmata, i.e. holes in the cell wall connecting the cytoplasms of neighboring cells; Charrier et al. 2008). Finally, branching may also be controlled by an internal clock pacing the branching process not in space but in time, ultimately resulting in an evenly spaced branching pattern in organisms growing at a regular pace (Nehr et al. 2011). Very interestingly, this cadence is maintained in the tip-growth mutant *etoile* (see previously), but the relative position of branches is not. In this mutant, branching continues at the same rate as in the wild type, but tip growth stops, leading to the formation of a compact bushy tuft (Nehr et al. 2011).

Branching results in branches with exactly the same morphology as the "parental" filament. Therefore, the reiteration of branching leads only to the addition of filaments identical to the very first one. Altogether and after ~1 month, the adult body looks like a tuft of filaments (Figure 2.3e).

Regarding the conservation of branching mechanisms on an evolutionary scale, the branching pattern observed in *Ectocarpus* sp. shares some morphological features with

mosses and fungi. However, the underlying mechanism seems to be different to some extent, thereby indicating that these lineages took different evolutionary paths to develop similar, low-complexity body architectures (Coudert et al. 2019).

dis mutants lack the basal, prostrate part of the sporophyte body and are impaired in microtubule and Golgi network organization (Godfroy et al. 2017). The *DISTAG* (*DIS*) gene codes for a protein containing a TBCC domain, whose function in internal cell organization is conserved throughout the tree of life.

2.2.4 Anatomy—Later Development

Beyond the early stages of sporophyte development, *Ectocarpus* sp. develops a second type of filament (Figure 2.3f). This filament grows upright, away from the substratum surface, and differentiates into different cell types: cells are chunky and lined up on top of each other, making a straight and stout filament, on which few branches emerge. However, these filaments remain uniseriate, like the earlier, prostrate ones. Therefore, the level of complexity of the overall morphology of the *Ectocarpus* sp. sporophyte remains low. After roughly two weeks, these upright filaments allow the differentiation of lateral reproductive organs (plurilocular sporangia and unilocular sporangia; see Charrier et al. [2008] for a review; Figure 2.3g–i). The mechanisms initiating the growth of these specific filaments, and those initiating the differentiation of the reproductive organs, are completely unknown to date.

2.2.4.1 Meiosis and the Gametophytic Phase

Meiosis takes place in the unilocular sporangia borne by the upright filaments of the sporophyte (see previously). They release roughly 100 meiospores in the seawater, and each meiospore germinates into a female or male gametophyte, making this second phase of the *Ectocarpus* sp. haplodiplontic life cycle dioicous (reviewed in Charrier et al. 2008).

The first cell division in the gametophyte leads to the formation of a rhizoid and an upright filament. Upright filaments keep developing, but the rhizoid remains inconspicuous. *Dis* mutants are characterized by their lack of basal, prostrate filaments in the sporophyte (see previously) and also lack rhizoids in the gametophyte phase (Godfroy et al. 2017), suggesting that the formation of the gametophyte rhizoid and of the sporophyte prostrate filaments are controlled by the same genetic determinism.

The upright filament continues growing and produces lateral branches morphologically similar to the upright sporophyte branches, except that they never carry unilocular sporangia, and they are more densely distributed with different branching angles (Godfroy et al. 2017).

Transcriptomics studies have shown that only 0.36% of the total number of transcripts are specific to the sporophyte phase (12% are biased by a fold change ratio of at least 2), while 7.5% are specific to the gametophyte phase (23% biased) (Lipinska et al. 2015; Lipinska et al. 2019). Therefore, more than 90% of the total transcriptome identified in *Ectocarpus* sp. is shared by both generations of the life cycle. This differential expression may account for the slight morphological differences between sporophytes and gametophytes (see previously), or, more likely, to the different reproductive organs and behavior. Nevertheless, genes related to carbohydrate metabolism and small GTPase signaling processes are expressed more abundantly in sporophytes, and expression of those related to signal transduction, protein–protein interactions and microtubule and flagellum movement are enriched in gametophytes.

Ultimately, lateral buds on these gametophyte filaments differentiate into pedunculate plurilocular gametangia. Each gametangium, either female or male, releases roughly 100 flagellated gametes in the external medium. Females secrete pheromones and mediate the attraction of male gametes (e.g. ectocarpene; Müller and Schmid 1988), and specific recognition of female and male gametes is based on a glycoprotein ligand-receptor interaction (Schmid 1993; reviewed in Charrier et al. 2008).

Some *Ectocarpus* species have both sexual and asexual life cycles (Couceiro et al. 2015). In an asexual cycle, an unfertilized gamete can germinate if it does not fuse with a sexual partner, resulting in a haploid parthenosporophyte with the same morphology as the diploid sporophyte. Like the diploid sporophyte, this parthenosporophyte bears unilocular sporangia in which meiosis takes place. Endoreduplication has been shown to take place very early during growth of the parthenosporophyte or just at the onset of the sporangium emergence (Bothwell et al. 2010). The gametes of the mutant *oroborous* (*oro*) do not grow as parthenosporophytes but instead develop as gametophytes (Coelho et al. 2011). The gene *oro* codes for a homeodomain (HD) protein, which, through an heterodimer formed with the other HD protein SAMSARA, controls the sporophyte-to-gametophyte transitions, as in basal members of the Archaeplastida (Plantae) (Arun et al. 2019).

2.2.4.2 Sex Determination

The gametophyte phase is represented by female and male haploid gametophytes. Sex in *Ectocarpus* sp. is based on the UV sexual system, where female (U) and male (V) sexual traits are expressed in the haploid phase (in contrast to the XY and ZW systems in which the sexual traits are expressed in the diploid phase). Similar sexual systems are also found in green algae (e.g. the charophyte *Volvox* sp.) and in the bryophytes *Ceratodon* sp. (moss) and *Marchantia* sp. (liverwort) (Umen and Coelho 2019). In *Ectocarpus sp.*, the sex determining regions (SDRs) are relatively small genomic areas of ~0.9 Mbp (representing ~0.5% of the total genome of 214 Mbp), of similar size in females and males and framed by pseudoautosomal regions (PARs) (Ahmed et al. 2014; Bringloe et al. 2020). The SDR contains a few coding genes (15 in the female and 17 in the male) that are expressed during the haploid phase; the PAR contains genes mainly expressed during the sporophyte phase. Noteworthily, most (11) of these genes are shared by both the female and the male SDR and have homologs elsewhere in the genome (either in the PAR region or in autosomes). Therefore, the

identity of the *Ectocarpus* sp. sex locus is weak compared with other species, both in the number and in the specificity of its genes. Nevertheless, these SDR loci control the expression of 753 female genes (with a -fold change [FC] > 2), representing 4.3% of the total transcripts (5.5% of the transcripts expressed in the female gametophyte), located in the rest of the genome during the haploid phase (Lipinska et al. 2015). In the male gametophyte, 1391 genes (7.9% total transcripts, 10% male-gametophyte-expressed genes) are specifically expressed with a FC > 2.

However, the role of these gametophyte genes in sex determination remains unclear, because the sexual dimorphism observed in this genus is nonexistent in vegetative gametophytes and subtle during the reproductive phase, during which male gametophytes produce more gametangia and slightly smaller gametes than female gametophytes (Lipinska et al. 2015; Luthringer et al. 2014). This slight dimorphism is reflected by the weak differential expression of sex-biased genes at these two stages of gametophyte development.

In summary, *Ectocarpus* sp. is characterized by a low level of morphological complexity: cells are aligned, and growth is one dimensional, followed by reiterated branching events producing filaments similar to the "mother" filament. The life cycle is virtually isomorphic: sporophyte and gametophyte are both filamentous, mainly made up of upright filaments, and gender traits are absent.

2.2.5 Genomic Data

The nuclear genome of *Ectocarpus* sp. (accession CCAP 1310/4) has been estimated to contain 214 Mbp, and genome sequence annotation identified 17,418 genes (Cormier et al. 2017). As the first sequence known for a brown alga at that time, it revealed unusual features. With a high GC content, genes are composed of, on average, 8 × 300 bp exons, separated by seven introns of 740 bp. Alternative splicing takes place with a frequency leading to 1.6 transcript per gene (Cormier et al. 2017), comparable to alternative splicing in metazoans and plants. Promoters have not been characterized to date, and 3'-UTR regions are particularly long (~900 bp), in contrast to most other organisms of similar genome size but similar to mammalian genomes. From this genome, several families of transposable elements, of which retrotransposons and retroposons are the most abundant, cover ~20% of the genome (Cock et al. 2010), as well as 23 microRNAs identified from a genome-based approach and whose expression has been quantified by q-RT-PCR (Billoud et al. 2014). This inventory also includes a set of 63 miR candidates identified from an RNA-seq-based approach, limited by the extent of range and level of gene expression (Tarver et al. 2015).

Interestingly, a significant proportion of *Ectocarpus* sp. genes are organized on alternating DNA strands along the chromosome, a feature specific to compact genomes.

A preliminary genetic map built with microsatellite markers was proposed in 2010 (Heesch et al. 2010), since supplemented with single nucleotide polymorphism (SNP) markers, facilitating the identification of mutated loci (Billoud et al. 2015; Cormier et al. 2017). All together, based on genetic linkage and flow cytometry data (although from another species), *Ectocarpus* sp. does not appear to have more than 28 chromosomes (Cormier et al. 2017).

2.2.6 Functional Approaches: Tools for Molecular and Cellular Analyses

Based on a solid knowledge of its biology and life cycle (reviewed in Charrier et al. 2008), *Ectocarpus* sp. was chosen as a genetic model for brown algae in the 2000s (Peters et al. 2004). Its genome was sequenced in 2010, which was a major breakthrough as the first genomic sequence for a brown alga and, what's more, the first multicellular macroalga (Cock et al. 2010). This breakthrough was accompanied by the development of a full palette of technical tools. Only techniques related to cell biology, cultivation and genetics are considered in the following.

2.2.6.1 Cultivation in the Laboratory

Ectocarpus sp. is easily grown in laboratory conditions (Le Bail and Charrier 2013). Growth speed, morphology and fertility induction depend on (white) light intensity (usually dim, <30 $\mu E.s^{-1}.m^{-2}$), photoperiod (long day or equal day: night cycle) and temperature (13–14°C). Due to its small size, optical microscopes and stereo microscopes are required to follow the different stages of the life cycle. Micromanipulation (using tweezers) is often necessary to separate the different organs of the *Ectocarpus* sp. body, such as sporangia. The adult organism is a few centimeters long, meaning that the whole life cycle can be carried out in a small recipient such as a Petri dish. Altogether, the cultivation of *Ectocarpus* sp. is amenable to rudimentary laboratory conditions and equipment. To avoid contamination with either bacteria or protozoa, *Ectocarpus* sp. is preferably handled under a sterile laminar hood.

2.2.6.2 Cell Biology and Biophysical Techniques

Transmission and scanning electronic microscopy techniques have both been used to observe *Ectocarpus* sp. cells and filaments (e.g. Le Bail et al. 2011; Tsirigoti et al. 2015), facilitated by the filamentous shape of this organism, exposing all cells to observation. However, because the cells are small (filament cell diameter, 7 µm), observation of a specific cell orientation may be difficult to handle. However, exploiting the fact that *Ectocarpus* sp. grows on surface, it is possible to make serial sections of apical filament cells in longitudinal and transversal axes, as illustrated in Rabillé et al. (2019), who measured the thickness of the cell wall along the meridional axis of the cell.

Protocols for immunocytochemistry (ICC, or immunolocalization) of cytoskeleton components have been developed in the past 20 years, inspired by protocols developed on other brown algae (reviewed in Katsaros et al. 2006). Microtubules (Coelho et al. 2012; Katsaros et al. 1992), actin filaments (Rabillé et al. 2018b) and centrin (Katsaros et al. 1991; Godfroy et al. 2017) can now be visualized in *Ectocarpus* sp. cells. These ICC protocols rely on the high conservation of these molecules, allowing the use of commercial primary antibodies

raised against animal homologs. ICC using antibodies specific to *Ectocarpus* sp. has not been reported yet. However, monoclonal antibodies raised against polysaccharide components of the brown algal cell wall have been produced (Torode et al. 2016, 2015) and are now used to map specific blocks of alginates (Rabillé et al. 2019b) and fucans (Simeon et al. 2020).

A recent study on *Ectocarpus* sp. using mRNA *in situ* hybridization after an attack by a pathogen (Badstöber et al. 2020) showed mRNA in subcellular locations within the infected cell. The development of filament-wide *in situ* mRNA labeling is needed to monitor responses or cell-fate programs at the level of the whole organism.

Additional techniques, previously developed in other organisms, have been transferred to *Ectocarpus sp.* Growth of the cell surface can be monitored by loading sticky fluorescent beads on the filament surface. Recording the position of the beads as the cell expands (either during growth or in response to a stimulus) makes it possible to measure the propensity for deformation of specific cell areas. This measurement provides information on cell mechanical properties (Rabillé et al. 2018a). Mechanical properties can also be studied using atomic force microscopy, a biophysical technique that records how deep a cantilever can plunge into a cell surface and retract, according to the cell wall stiffness and adhesion (Gaboriaud and Dufrêne 2007). *Ectocarpus* sp. is particularly amenable to such approaches, because its cells are directly exposed to the cantilever (Tesson and Charrier 2014). This technique helped show that the cells along the sporophyte filament display different degrees of surface stiffness (Rabillé et al. 2019b).

2.2.6.3 Modification of Gene Expression

Attempts to genetically transform *Ectocarpus* sp. have been numerous and so far unsuccessful. Agrotransformation, electroporation, PEG-mediated protoplast or gamete transformation and micro-injection have all been tested and shown to be inefficient. A major issue is that there is little to no information on *Ectocarpus* sp. gene promoters, and heterologous promoters tested so far (e.g. diatom, Ulva, Maize or Plant virus CaMV35S) have not been shown to be functional (personal communication).

Therefore, "ready-to-use" molecules that can alter the expression of host genes without relying on the host transcription and translation machinery currently appear to be a more promising approach. Morpholinos and RNA interference have not proven to be efficient enough for routine transient knock-down experiments (personal communication; Macaisne et al. 2017).

Efforts are currently being put into the development of the CRISPR-Cas9 technology (Lino et al. 2018), shown to be a powerful tool to stably modify the genome of several marine organisms, including echinoderms (sea urchins; Lin et al. 2019) and tunicates (*Phallusia* sp.; McDougall et al. 2021). Because the expression of the guided RNA and the Cas9 protein from the host genome remains challenging, the use of pre-assembled guide RNA-Cas9 protein complex, as illustrated in Brassicaceae plants (Murovec et al. 2018), is currently considered the most promising strategy.

Several morphogenetic mutants have been generated by UV irradiation, among which some have been genetically characterized. These mutants are impaired in tip growth (Le Bail et al. 2011), cell differentiation (Godfroy et al. 2017; Macaisne et al. 2017; Le Bail et al. 2010), branching and reproductive phase change (Le Bail et al. 2010). In most mutants, several morphogenetic processes are affected, reflecting the low level of complexity of *Ectocarpus* sp. morphogenesis and suggesting an overlap in genetic functions (see transcriptomic results previously). Others are impaired in the alternation of the sporophyte and gametophyte generations (life cycle mutants: Coelho et al. 2011; Arun et al. 2019) (Figure 2.2).

2.3 *SACCHARINA LATISSIMI*

2.3.1 Nomenclature History, Evolution, Geographical Distribution and Uses

2.3.1.1 History of Its Nomenclature

Saccharina latissima (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders 2006 is a marine photosynthetic eukaryotic organism with many different common names, including sugar kelp, sea-belt, kombu, sugar tang, poor man's weather glass and so on. Originally, in 1753, Linnaeus considered it an *Ulva* species, *Ulva latissima*, due to its sheet-like blade, common in the genus *Ulva* (Linnaeus and Salvius 1753). In 1813, Lamouroux reclassified it as *Laminaria saccharina* (Lamouroux 1813), despite its original genus name *Saccharina* given by the botanist J. Stackhouse in 1809. This genus name was resurrected in 2006 when molecular phylogenetics made it apparent that the order Laminariales should be split into two clades or families (Lane et al. 2006), which diverged ~25 Mya (Starko et al. 2019). Now, *Laminaria* spp. are assigned to the Laminariaceae family, and *Saccharina* spp. are part of the Arthrothamnaceae family (Jackson et al. 2017).

2.3.1.2 Evolution and Diversification

Classic taxonomy using morphological or physiological characteristics is useful for identifying species in the field; however, in the absence of a genetic approach, they can lead to long-lasting species confusions.

Among the brown algae, kelps are thought to have emerged ~75 Mya (Starko et al. 2019). Within the kelps (order Laminariales), *S. latissima* belongs to the so-called "complex kelps" (Starko et al. 2019) and thus shows close genetic similarity with various genera, allegedly resulting from an important upsurge in speciation beginning 31 Mya, concomitant to a massive marine species extinction due to the cooling of the Pacific Ocean during the Eocene–Oligocene boundary.

2.3.1.3 Geographical Distribution

Kelps are now almost cosmopolitan species, their presence ranging from temperate to cold waters on both sides of the Atlantic and Pacific Oceans (Bartsch et al. 2008). *Saccharina* genus appears to have initially emerged in the Northwest Pacific (North Japan, Russia) (Bolton 2010;

Luttikhuizen et al. 2018; Starko et al. 2019) and then spread further to three or four distinct regions of the globe where different lineages of *S. latissima* can be traced: in temperate to cold-temperate (sub-Arctic) waters of the Northeast Pacific, where the early diversification of Laminariales ancestors took place, and in the Northeast and Northwest Atlantic (Neiva et al. 2018; Starko et al. 2019). *S. latissima* is absent from the southern hemisphere (Bolton 2010).

Even though these populations seem to be considered as a single species (assumption supported by crosses), barcoding studies (based on the cytochrome c oxidase gene, used for) indicate high divergence between regions (Neiva et al. 2018). In combination with their morphological divergence and history of glacial vicariance (Neiva et al. 2018), these regional groups of *S. latissima* are clearly differentiating into separate species.

2.3.1.4 Uses

Individual kelp can become enormous: *S. latissima* blades can grow up to 45 m in length (Kanda 1936). As such, kelps constitute the largest coastal biomass and one of the main primary producers of the oceans. According to the FAO (2018), kelps in general, and *S. latissima* in particular, are cultivated and consumed mainly in Asia for human sustenance as well as for their alginate and iodine contents. In comparison, European consumption and production are considerably lower, and wild populations are used for various applications, mainly food and feed (Rebours et al. 2014; Barbier et al. 2019). Recent innovations aim to combine *S. latissima* cultivation with salmon aquaculture (integrated multi-trophic aquaculture) to reduce the impact of fish farms in Norway (Fossberg et al. 2018). *S. latissima* has been proposed as a source of bioethanol (Adams et al. 2008; Kraan 2016), and substances such as the sulfated polysaccharides fucoidans, laminarin and other extracts have demonstrated antitumoral effects along with anti-inflammatory and anticoagulant pharmacological properties (Cumashi et al. 2007; Mohibbullah et al. 2019; Han et al. 2019; Long et al. 2019). The number of clinical studies on mice for testing the positive effects of kelp extracts keeps increasing, as attested by a simple search in the PubMed scientific literature search engine.

2.3.2 Life Cycle

S. latissima is characterized by a highly heteromorphic haplodiplontic life cycle (Figure 2.4). Meiosis leads to the haploid gametophytic stage (or generation) of the life cycle, which, upon fertilization, gives rise to a diploid sporophyte stage. In *S. latissima*, and generally in Laminariales, the sporophyte generation is considerably different morphologically from the gametophyte generation (Kanda 1936; Fritsch 1945), in contrast to the isomorphic haplodiplontic life cycle of Ectocarpales, as seen in the previous section (Figure 2.2). The gametophyte (haploid) is microscopic and slowly grows into a prostrate filamentous thallus, and the sporophyte (diploid) is large and conspicuous. Upon favorable conditions, reproduction is initiated by a gradual differentiation of the cells of the gametophytic filaments into reproductive cells, the gametangia—antheridia (male) or oogonia (female)—a process induced by blue light (Lüning and Dring 1972). Interestingly, this induction is accompanied by changes in gene expression that are by and large common to female and male gametophytes, suggesting that the initiation of germline differentiation follows similar general mechanisms independently of gender (Pearson et al. 2019). That is, transcriptomics studies have revealed enhanced transcriptional and translational activities as well as metabolic activities (carbohydrate biosynthesis and nitrogen uptake), suggesting that gametogenesis is accompanied by an intensification of primary cellular and metabolic functions. This intensification is surprising when weighed against the fact that only one single gamete is produced by each gametangium. Yet there are differences between female and male gametogenesis proper. A small set of genes display gender-dependent induction of their expression, seemingly faster in females than in males (Pearson et al. 2019). Genes involved in basic cellular function (protein modification, nucleoplasmic transport, intron splicing), energy production and metabolic pathways and more specifically in oogenesis (reactive oxidative species metabolism) are overexpressed during female gametogenesis, in addition to prostaglandin-biosynthesis genes (Pearson et al. 2019; Monteiro et al. 2019). In turn, and as expected, male gametogenesis is accompanied mainly by the over-expression of "high mobility group" (HMG) genes, a conserved marker of male gender determination in animals, fungi and brown algae (Ahmed et al. 2014), which suppresses the development of female gender, hereby considered as set by default (Pearson et al. 2019).

In relatively high temperature conditions (20°C), the male and female gametophytes show more similar transcriptomic patterns, probably indicating a change of focus from gametogenesis-related genes to resistance to heat stress, amplified in females (Monteiro et al. 2019).

The oogonium releases an egg, leaving behind an empty apoplast, a process that is subject to the circadian rhythm and, in contrast to the formation of gametangia, is inhibited by blue light (Lüning 1981). Male gametes swim to the egg in response to female pheromones (e.g. lamoxiren; Hertweck and Boland 1997), demonstrating conspicuous chemotaxis (Maier and Müller 1986; Maier and Muller 1990; Boland 1995; Maier et al. 2001; Kinoshita et al. 2017). Upon fertilization, the early sporophyte develops as a planar embryo. In *Saccharina japonica*, the ratio of genes specific to sporophytes or gametophytes is more balanced than in *Ectocarpus* sp., with ~4% (about 700 genes) of the total number of transcripts being specifically expressed in both phase organisms (Lipinska et al. 2019). This difference in transcripts can be interpreted as a reflection of the conspicuous morphological differences between these two life cycle generations in *S. latissima*, contrasting with the near isomorphy in *Ectocarpus* sp.

The developing diploid sporophyte requires several months before reaching sexual maturity (Andersen et al. 2011; Forbord et al. 2018, 2019). Then, sori, groups of sacs (sporangia) of meiospores (swimming spores that are the

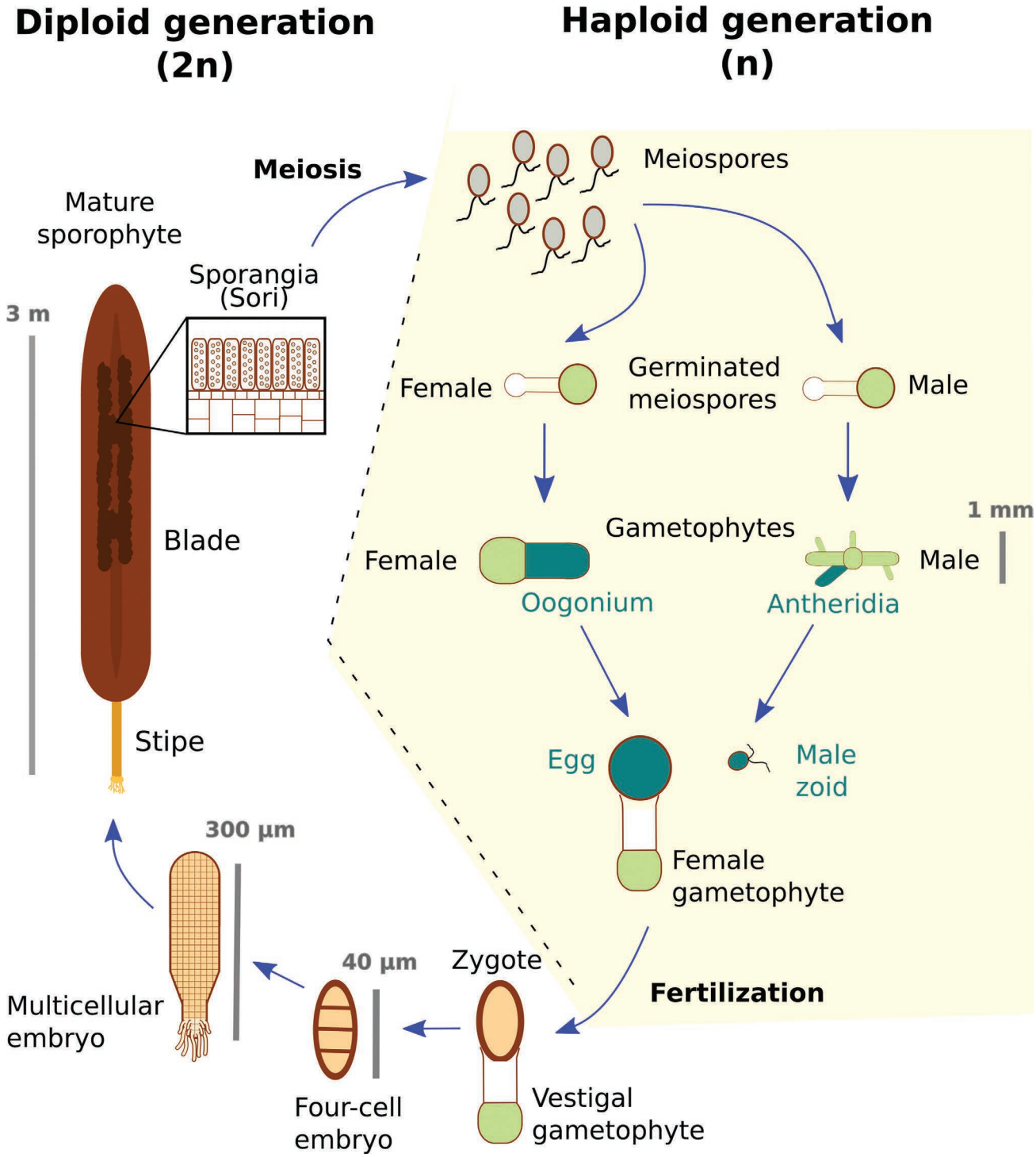

FIGURE 2.4 **The heteromorphic halplodiplontic life cycle of *Saccharina latissima*.** The large fertile sporophyte develops sporangia (located in sori) around and on the soft midrib of the blade. The released haploid meiospores germinate to female or male gametophytes. If conditions are optimal, the one- to two-celled female gametophytes and the few-celled male gametophytes produce gametes. The female gamete (egg) is retained on the empty female gametangium. Only one gamete per gametangium is produced in each sex. After fertilization, the diploid sporophyte begins to develop. After some months, a conspicuous juvenile sporophyte emerges and requires at least four to five additional months to become fertile and produce meiospores. Note the scale of the different generations and life stages.

product of meiosis) abundantly differentiate on the surface of the blade, usually on and near the midrib and far from the basal part of the blade (Drew 1910), suggesting an inhibitory control in this part of the body. In the related species *S. japonica*, the two phytohormones auxin and abscissic acid have opposite effects in the induction of sorus formation; it was hypothesized that auxin is synthesized in the basal meristem, allowing sorus differentiation only in the more

distal, apical areas of the blade (reviewed in Bartsch et al. 2008). Meiospores produced from these sporangia germinate into male or female gametophytes depending on the UV sex determination type inherited from meiosis (Lipinska et al. 2017; Zhang et al. 2019). Comparison of the genome of *S. japonica* and *Ectocarpus* sp., together with other brown algal genomes, shows that the sex determining region has evolved rapidly through gene loss and gene gain, similar to organisms with an XY or ZW sex determining system (Lipinska et al. 2017).

A review of the physiological parameters controlling the whole life cycle of Laminariales can be found in Bartsch et al. (2008).

2.3.3 Embryogenesis

The development of kelps was reported in some detail in the beginning of the 20th century. Since then, the developmental and cellular data amassed during the past decades pale in comparison with the ecophysiological and biochemical studies on kelps or the bioassays on the positive effects of their extracts. Especially for *S. latissima*, the majority of our knowledge on its development and histology is restricted to studies from the 19th century. Although detailed in histology and anatomy, information regarding the development of the blade and the stipe (schematized in Figure 2.4) is scarce, particularly for the earlier stages.

The early embryo has a distinct phylloid shape shared by most kelp species (Drew 1910; Yendo 1911; Fritsch 1945). Initially, there is no visible differentiation into stipe or blade, and the embryos are made of a flat layer of cells (Figure 2.5a–f). However, the proximal ends of these phylloids are narrower in width than the rest of the flat thallus (Figure 2.5e, f). Nevertheless, cellular divisions occur throughout the phylloid tissue without any hint of a pending superficial or intercalary meristem. At a certain point, probably related to the size of the thallus, the cells of the future stipe (Figure 2.5g, red arrow) divide internally, forming the first four layers. An increased rate of anticlinal divisions of the two outer layers and slow growth of the inner layers promote the formation of a cylindrical tissue (Fritsch 1945). The peripheral layer of cells, which are considerably smaller and more actively dividing than the internal cells, defines the meristoderm. The central cells surrounded by the cortex give rise to the first medullary elements, gradually becoming thinner and elongated, while their cell walls become enriched in mucilage (Killian 1911; Smith 1939; Fritsch 1945) (schematized in Figure 2.5i). At some point, a transition zone between the lamina and the stipe becomes visible, with the former being flat (Drew 1910; Yendo 1911).

The lamina becomes progressively polystromatic (several layers of cells in width), starting first in the vicinity of the transition zone and propagating toward the more distal, apical parts of the lamina (Figure 2.5g). Therefore, gradual polystromatization is basipetal. In parallel, specific organs and tissues are formed. In the longitudinal axis, blade, stipe and haptera differentiate, resulting in a clear apico-basal, asymmetrical axis; meanwhile, in the medio-lateral axis, specific tissues differentiate, mainly in the stipe and blade (meristoderm, cortex, medulla) (Figure 2.5h, i).

2.3.4 Anatomy

The female and male gametophytes develop microscopic filamentous bodies. Only the anatomy of the sporophyte will be described here. The mature thallus of *S. latissima* is composed of three main parts, the lamina, the stipe and the holdfast (Figure 2.4). The lamina, or blade, is unserrated, flat or bullate with a potential for growth of up to several meters (~40 m, according to Kanda 1936). Damage to the lamina may be irreversible if it exceeds a certain length. Otherwise, the lamina regenerates and continues growing (Parke 1948). This process seems to be age and season dependent, with lower potential for survival and development of a new blade after the first year of growth (reviewed in Bartsch et al. 2008). The stipe is cylindrical, with a flattened zone at the top corresponding to a transition zone between the stipe and the blade (Parke 1948) (Figure 2.5g). At the opposite end, an intricate structure appears with thick branched and intermingled protrusions called haptera (pl.) (hapteron [sg.]), which progressively form the holdfast, an organ anchoring the thallus to a solid substratum of the seabed (e.g. rocks). Histological observations show high secretory activity of adhesive material coming from the epidermal meristem of the haptera (Davies et al. 1973).

Histologically, the blade and the stipe are not very different (Fritsch 1945) (Figure 2.5i). However, the blade shows a more compressed lateral arrangement of the different tissues, and the borders of the most internal tissues seem obscured: the inner cortex is often not distinguishable from the outer cortex, making the transition to the medulla sudden (Figure 2.5h).

On the surface, an epidermal tissue covers the thallus of *S. latissima*, consisting of a few layers of small isodiametric cells (Sykes 1908; Smith 1939; Fritsch 1945) (Figure 2.5h, i). This tissue demonstrates high division activity, being responsible for the thickening of the stipe and of the blade to some extent, especially in the vicinity of the transition zone. This tissue is defined as the meristoderm, as it is essentially an epidermal meristem. According to Smith (1939), the blade's superficial tissue resembles an epidermis more than a meristoderm, implying the absence of meristematic activity. In contrast, Fritsch (1945) suggests that cell divisions still occur from the meristoderm, mostly along the anticlinal plane, thereby widening the blade. However, its division ceases in distal and mature regions away above the transition zone.

At the center of the thallus is found the medulla, an intricate network of elongated filamentous cells immersed in mucilage (Figure 2.5i). This tissue raised high interest in algal histology in the past (Sykes 1908; Schmitz et al. 1972; Lüning et al. 1973; Sideman and Scheirer 1977; Schmitz and Kühn 1982), most likely because of its intriguing structure but also because of its important physiological role: it offers structural resistance and is the main transporting tissue for

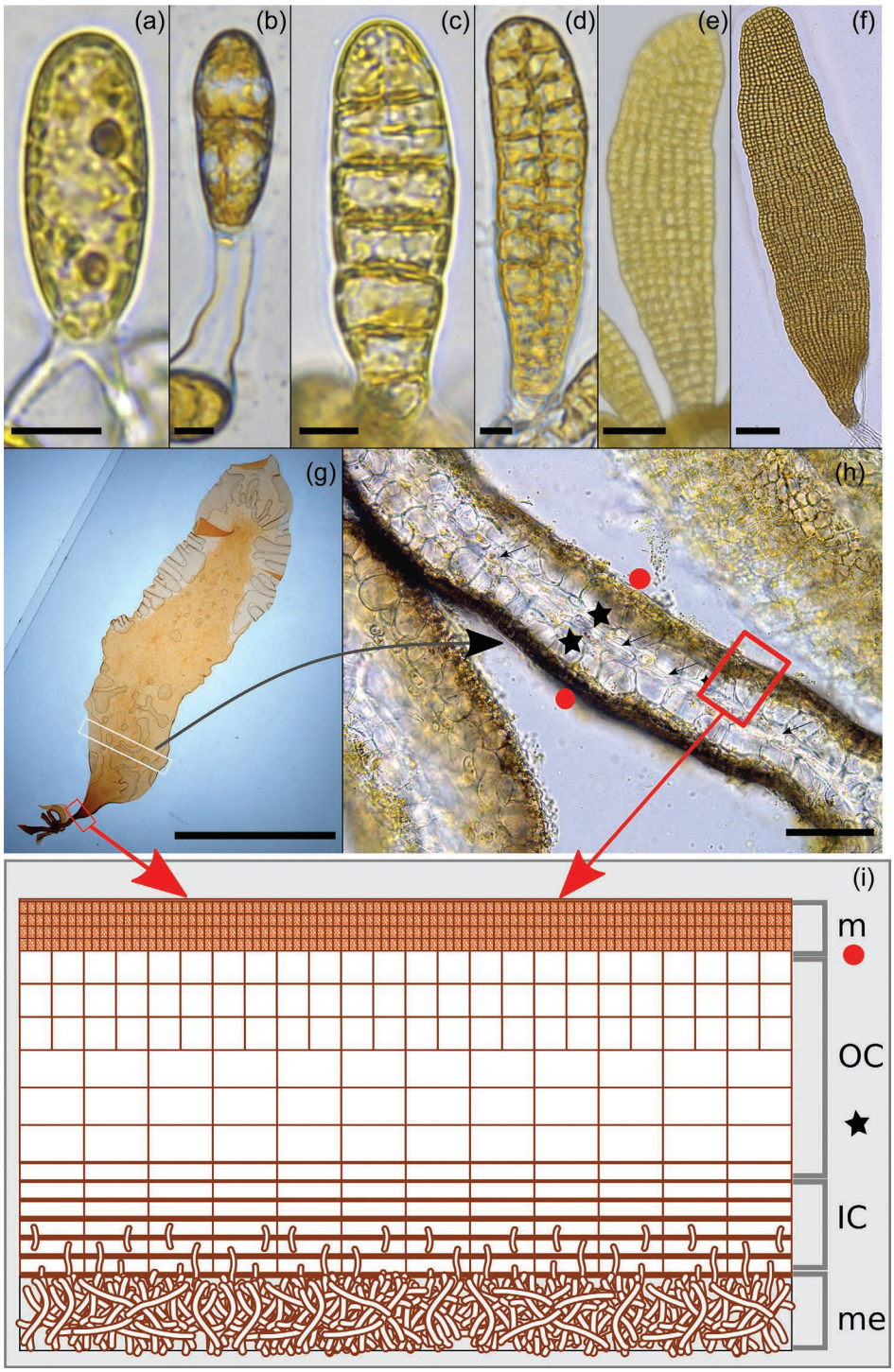

FIGURE 2.5 **Developmental stages and cross-sectional histology of a *Saccharina latissima* blade**. (a) A polarized zygote; (b) one- to two-day-old two-cell dividing embryo; (c) three-day-old embryo; (d) average projection from a z-stack of a four-day-old embryo; (e) average projection from a z-stack of a ten-day-old embryo; (f) focused projection from a z-stack of a three-week-old embryo; (g) a two-month-old juvenile; (h) cross-section from the middle part of the blade on (g). Red circles: meristodermal layer; black stars: cortical layer; arrows: medullary elements (hyphae-like cell protrusions); (i) schematic of the structure (cross-section) of a stipe or a blade at mature stages [older than in (g) and (h)]. Peripherally, the cylindrical stipe consists of a thin outer layer of mucilage and several layers of photosynthetic and actively dividing cells, the meristoderm (m). Inside, layers from large, opaque and highly vacuolated cells constitute the outer cortex (OC). In the inner cortex (IC), cells are thinner and elongated. The cell wall gradually thickens toward the center of the stipe; however, this is probably the result of gradual deposition of mucilage that relaxes the cell connections leading to the medulla (me). Protrusions from the innermost layers of IC already occupy the relaxed and filamentous medulla. Bars: (a-f) 10 µm, (e) 50 µm, (f,h) 100 µm, (g) 1 cm.

photoassimilates and nutrients. In recent studies on other kelp species, the medullary cells seem to have the capacity to generate turgor through the elastic properties of their cell walls (as illustrated in the kelp *Nereocystis* by Knoblauch et al. 2016a), possibly controlling the flow of the transported solutions. This and the alginate-rich extracellular matrix of the medulla make the sieve elements of kelps a study model for fluid mechanics in transport systems of plant organisms, since they are easily manipulable (Knoblauch et al. 2016b).

Between the medulla and the meristoderm resides the cortex (Figure 2.5h, i). It is divided into two parts: the outer cortex and the inner cortex. The outer cortex is easily distinguished from the meristoderm due to its sizable isodiametric opaque cells with pointy corners. The inner cortex is closer to the medulla and has elongated, thick-walled cells (closer to the medulla) with straight edges. At the transition zone and young parts of the stipe, the outer cortex cells widen and lengthen following the enlargement of the organ. A gradual change toward the more elongated cells of the inner cortex is visible. The innermost cells close to the medulla have protrusions on their most internal (proximal) longitudinal cell walls that may overlap each other, gradually resembling the shape and size of the medulla cells, as they progressively occupy this intricate mesh. At the transition zone, both the abundant mucilage deposits and the elongation of the innermost cells in combination with their growing septate protrusions "relax" the inner cortex tissue, which gradually differentiates into medullary cells (Killian 1911; Fritsch 1945). The inner cortex is supplied with cells from the outer cortex, which themselves originate from the actively dividing meristoderm.

In summary, growth of the blade and the stipe in the longitudinal axis is ensured by the transition zone, which furnishes the blade and the stipe with new tissues (Smith 1939; Fritsch 1945; Parke 1948; Steinbiss and Schmitz 1974). Therefore, the transition zone is characterized by both cell division activity in the longitudinal axis, which provides the cells for the lamina and stipe tissues, and active cell division in the peripheral meristoderm, whose role is to renew and keep providing cells to the transition zone. In this area, cell division and cell differentiation take place centripetally. Recently, transcriptomics studies confirmed an increasing meristematic activity in this location through the upregulation of ribosomal proteins and *immediate upright* genes a in the basal part of the blade (Ye et al. 2015), as in juvenile sporophytes (Shao et al. 2019).

As soon as the blade and the stipe can be identified, haptera start differentiating in the very basal part of the *S. latissima* thallus. These are outgrowths that originate from the lower end of the stipe, where a disc-like structure initially forms on top of the rhizoids (Drew 1910; Yendo 1911). Above this structure, the first haptera start developing.

While it shares the cortex and meristoderm with the stipe, the medulla of the stipe does not extend into the haptera (Yendo 1911; Smith 1939; Fritsch 1945; Davies et al. 1973). Haptera growth seems to be apical, but there is no extensive research on that matter. Haptera cells contribute to carbon fixation through photosynthesis, except when sheltered from light, resulting in cells of the haptera meristoderm displaying underdeveloped plastids with a rudimentary thylakoid membrane system (Davies et al. 1973). In addition, their endomembrane system is very well developed, with hypertrophied dictyosomes containing cell wall polysaccharides and alginate acid.

2.3.5 Genomics

The *S. latissima* genome sequence is expected to be released in 2021 (Project "Phaeoexplorer", led by FranceGenomics and the Roscoff Marine Station, www.france-genomique.org/projet/phaeoexplorer/). In the meantime, a draft genome sequence was published in 2015 for the close relative *S. japonica* (Ye et al. 2015), which diverged from *S. latissima* only ~5 Mya (Starko et al. 2019). It was enhanced by recent genome assembly work (Liu et al. 2019), leading to a genome of 580 Mbp for >35,000 genes.

The *S. japonica* genome is 2.7 times bigger than that of *Ectocarpus* sp. (Cock et al. 2010), and it contains twice as many genes; as expected, gene length is similar in the two species (Liu et al. 2019). Average exon lengths (~250 bp) are similar, but introns are less abundant (only 4.6 per gene on average in *S. japonica* vs. 7 in *Ectocarpus* sp.). Oddly, because introns are longer (1200 bp vs. 700 in *Ectocarpus* sp.), the overall exon:intron ratio per gene remains similar in *Saccharina* sp. and *Ectocarpus* sp. However, a significant difference lies in the presence of repeated sequences (46% in *S. japonica* vs. 22% in *Ectocarpus* sp.), mainly composed of class I and class II transposons and microsatellite sequences (Liu et al. 2019).

A large proportion of the gene content (85%) is distributed in gene families found in *Ectocarpus* sp. Nevertheless, detailed analysis shows interesting differences, in line with the biology of the organisms. In particular, the high capacity of *S. japonica* to accumulate iodine is reflected in the composition of its genome, which displays a very rich group of vanadium-dependent haloperoxidases (vHPOs), most likely resulting from gene expansion (Ye et al. 2015; Liu et al. 2019). Gene expansion may also have led to a significant increase in cell wall biosynthesis proteins (especially those involved in the synthesis of alginates), protein kinases and membrane-spanning receptor kinases. All together, in comparison with the *Ectocarpus* sp. genome, gene expansion would have been the genetic basis for the diversification of body plans and more generally of the complex multicellularity of Laminariales (Liu et al. 2019), which, together with the increased bioaccumulation of iodine, are the main characteristics differentiating Laminariales from Ectocarpales.

Interestingly, compared with other genomes, *Ectocarpus* sp. and *S. japonica* genomes display a significant increase in gene families (~1200) counterbalanced with a limited loss (~300), whose functions involve enzyme hydrolysis and cupin-like proteins (Ye et al. 2015). Although the functions of the gained gene families are largely unknown due to the lack of sequence conservation with other organisms, protein kinase and helix-extended-loop-helix super family domains have been identified as enriched domains in this group, suggesting a role in cell signaling and cell differentiation.

2.3.6 Functional Approaches: Tools for Molecular and Cellular Analyses

Cultivating macroalgae in laboratory conditions usually requires extensive experience and skills, because algae can be extremely sensitive to water and light parameters.

2.3.6.1 Culture Methods

2.3.6.1.1 Cultures of Gametophytes

Cultures of gametophytes can be initiated simply from fragments from an older laboratory culture or from material collected in the wild. This approach can be used for most kelps: collecting a healthy sporophyte with dark spots (sori) on the blade (schematized in Figure 2.4). Fertile blades can generally be found on the coast during the cold months. For example, in Roscoff and specifically on Perharidy beach (48°43'33.5"N 4°00'16.7"W), mature sporophytes with fully developed sori can be found from October to late April. Alternatively, fragments of large sporophytes from the intercalary meristem can be kept in short-day conditions in tanks for at least ten days to induce sporogenesis (Pang and Lüning 2004). Then, gametophytes will emerge from the released, germinated spores (Figure 2.4). More details on collecting and isolating gametophytes, as well as culture maintenance, can be found in Bartsch (2018). Care should be taken to ensure adequate temperature and light conditions while keeping the cultures under red light (Lüning and Dring 1972; Lüning 1980; Bolton and Lüning 1982; Li et al. 2020), as well as in a low concentration of chelated iron to maintain the gametophytes in a vegetative state (Lewis et al. 2013). Spontaneous gametogenesis can still be observed; however, its rate of occurrence is low and negligible. Sufficient amounts of biomass should be secured before beginning any experiments, but because *S. latissima* is a slow-growing alga, this can require several months to one year.

2.3.6.1.2 Gametogenesis

The simplest way to induce gametogenesis is to transfer the gametophytes into normal light conditions (Bartsch 2018; Forbord et al. 2018). However, if there is a high density of biomass, this may lead to reduced vegetative growth (Yabu 1965) and reproduction efficiency (Ebbing et al. 2020). Therefore, gametogenesis may be facilitated by reducing gametophytic density before transferring the cultures to normal light.

2.3.6.2 Immunochemistry and Ultrastructure Protocols

Several older studies that have examined the ultrastructure of *S. latissima* sporophytes (Davies et al. 1973; Sideman and Scheirer 1977; Schmitz and Kühn 1982), and others have employed immunochemistry on other *Saccharina* species (Motomura 1990; Motomura 1991; Klochkova et al. 2019). These studies have contributed to a better understanding of the general structure of the life cycle and histology of *Saccharina* spp. and kelps in general. However, there are no recent works focusing on the development or cytology of *S. latissima* despite its high economic and environmental interest.

These studies clearly demonstrate that *S. latissima*, as well as other brown algae, are amenable to fixation in paraformaldehyde or glutaraldehyde of various concentrations in seawater or other buffer solutions, such as microtubule stabilization buffer (Motomura 1991; Katsaros and Galatis 1992). The next step for immunochemistry is the digestion of the cell wall, which does not seem very challenging for *Saccharina angustata* when using abalone acetone powder. Because this powder has been discontinued, it has become necessary to test different cell wall digestion mixes, as shown for filamentous brown algal species (Tsirigoti et al. 2014) and green algal species (*Ulva mutabilis*) (Katsaros et al. 2011; Katsaros et al. 2017). Cell wall digestion is followed by extraction to remove most of the chlorophyll and other pigments from the cells. Triton is most commonly used, but in some cases, DMSO can be added for more efficient extraction (Rabillé et al. 2018b). This extraction step is carried out to reduce autofluorescence but also to perforate the cellular membrane to allow for the penetration of fluorescent probes. Motomura (1991) did not use an extraction step on *S. angustata* zygotes and parthenospores but noted increased autofluorescence, which can be reduced using a combination of filters during observation. The fluorescent probes, being chemical or primary and secondary antibodies, are added after the extraction step. This step can also be optimized, according to the species, because concentrations and washing steps may depend on the species and on the extraction step. The whole process can take two days of work, including observation. An antifade mounting medium, such as Vectashield or CitiFluor, can preserve the fluorescence of the samples and protect them from photobleaching. For transmission electron microscopy (TEM), there are several studies on *S. latissima* (Davies et al. 1973; Sideman and Scheirer 1977; Schmitz and Kühn 1982) that illustrate the general ultrastructure of the different cell types. In general, depending on the application, different fixatives can be chosen, and there are no cell wall digestion or extraction steps. After fixation, the specimen is post-fixed in osmium tetroxide and then dehydrated. Depending on the embedding resin, dehydration can be effected with ethanol or acetone. After embedding and polymerization of the resin, the blocks with the samples should be sectioned using an ultramicrotome. More information on the general considerations to take for TEM as well as the different protocol variations to use according to the desired application can be found in the aforementioned articles or in Raimundo et al. (2018) for a general protocol for seaweeds.

2.3.6.3 Modification of Gene Expression

To date, no genetic transformation protocol is available for *S. latissima*, but one was published for its relative *S. japonica* (formerly *Laminaria japonica*) using a biolistic approach on mature blades, showing transient expression of the GUS reporter gene (Li et al. 2009). Since then, despite demands from industry (Lin and Qin 2014; Qin et al. 2005), no additional studies have built on this technical breakthrough. Several genetic variants have been produced (reviewed in Qin et al. 2005).

2.4 CHALLENGING QUESTIONS IN BASIC AND APPLIED RESEARCH

2.4.1 Why Study Brown Algae?

2.4.1.1 Advancing Knowledge on Their Developmental Mechanisms

Brown algae make up a specific phylum of multicellular organisms. Their phylogenetic position in the eukaryotic tree (Baldauf 2008), distant from other multicellular organisms, makes them a key taxon for understanding the evolution of complex multicellularity and specific metabolic pathways. The literature abounds with biological questions and research topics positioning these organisms as essential ones to consider in future studies, and, more specifically related to this chapter, brown algae offer a wealth of candidate species to study the evolution of the formation of different body shapes. Furthermore, in contrast to the red and green algae, there is no representative unicellular species for brown algae, making the evolutionary scenario of the emergence of their diverse shapes even more intriguing.

However, the knowledge in the fields of evolution and development is very scarce compared with that on metazoans and land plants. In the following, two examples pertaining to kelp features illustrate the potential brown algae hold for leading to knowledge breakthroughs in developmental biology.

First, despite the similarities between brown algal tissues and complex histological structures in land plants, brown algal body architecture and shape remain fairly simple. Even kelps—the most complex brown algae at the morphological level—develop only a few different organs (blade, stipe and holdfast), with a limited number of specific tissues and cell types (i.e. epidermis, cortex, medulla, meristoderm, sorus [this chapter] and pneumatocysts, receptacles and conceptacles in other brown algae [reviewed in Charrier et al. 2012]). This relative simplicity provides a useful opportunity to study basic developmental mechanisms based on simple geometrical rules or morphogen gradients. Although auxin, the long-standing leading morphogen for land plants, is present in brown algae and affects morphogenesis of several morphologically simple brown algae, such as *Ectocarpus* sp. and *Dictyota* sp. (Dictyotales) (Le Bail et al. 2010; Bogaert et al. 2019), it has no conspicuous effect, nor is it specifically localized in the apex of *Sargassum* sp. (Fucales), a brown alga with relatively high morphological complexity, including the presence of an apical meristem (stem cell tissue) (Linardić and Braybrook 2017). This result casts doubt on the consistency of morphogen-mediated control mechanisms in brown algae and presages the identification of new, alternative growth control mechanisms.

The second example relates to one of the stunning characteristics of some brown algae: their size. How do cells communicate with each other over such a long distance, when it comes to organisms among the tallest on earth: kelps? The transport system in kelps is reminiscent of the vascular systems of land plants, except that the extracellular matrix (alginates) has a specific organization and distribution and contributes to the flow of photoassimilated products (Knoblauch et al. 2016a, 2016b). Cells connect with each other through pit structures where the plasmodesmata (channels or pore connecting two adjacent cells) are concentrated. These plasmodesmata are structurally similar to those in land plants (Terauchi et al. 2015), except for the absence of desmotubules and the lack of the ability to control the size of molecules transferred symplastically (Bouget et al. 1998; Terauchi et al. 2015). Although some kelps (e.g. *Macrocystis* spp.) adjust the size of their vascular tissues to the needs for photoassimilate distribution to "sink" organs (i.e. meristerm, storage tissues, sori) as land plants do, others do not, suggesting again different control mechanisms in the management of this important function (Drobnitch et al. 2015). One explanation is that larger kelp rely more heavily on an efficient transport system, especially when source and sink tissues are physically distant. Relying on a transport system would call for a regulated developmental process, as in land plants (Drobnitch et al. 2015).

2.4.1.2 Improving Aquaculture

Over the past several decades, *S. japonica* (known as "kombu") aquaculture in Asia has undergone many improvements at many different levels, because this alga has been cultivated for human consumption for several centuries. One improvement lever is breeding, and—beyond empirical approaches used in the past—genomics can now assist and speed up breeding programs (Wang et al. 2020), along with new knowledge on the control of the life cycle, reproduction and early growth steps (e.g. substrate adhesion, sensitivity to high density) (reviewed in Charrier et al. 2017). Regarding more specifically *S. latissima* cultivated in Europe, its genome has not yet been sequenced and, other than concerns on the ecological impact of seaweed aquaculture, the current bottlenecks are mainly technical and focused on scaling up production and reducing cultivation costs (reviewed in Barbier et al. 2019).

2.4.2 Biological Models: *Ectocarpus* sp., *S. Latissima* or Another Brown Alga?

Because *Ectocarpus* sp. is a morphologically and sexually simple organism, it is a convenient model for cellular and molecular studies requiring microscopy, and this asset is enhanced by the availability of many additional cell biology tools (e.g. protocols for immunolocalization of the cell wall and the cytoskeleton, laser capture microdissection, *in situ* hybridization, etc.). Therefore, as illustrated in this chapter, its amenability to laboratory experimentation and its short life cycle have made it a convenient organism to explore. However, its low biomass is an impediment for biochemical research, in addition to its simple morphology, which precludes the study of complex multicellular mechanisms.

This is how *S. latissima* landed on the roadmap: based on the wealth of cultivation practice-based knowledge from applied phycology and aquaculture R&D laboratories,

TABLE 2.1

Characteristics of the Two Brown Algal Models *Ectocarpus* sp. and *Saccharina latissima* and Suitability for Lab Experiments

	***Ectocarpus* sp.**	**Saccharina latissima**
Life cycle	Short, haplodiplontic, dioecious, slightly anisogamous.	Long, haplodiplontic, dioecious, strongly anisogamous/ oogamous.
Amenability to lab conditions	Good.	Good, time consuming to establish a stock culture (several months). Life cycle only partially completed *in vitro*?
Size	Microscopic (100 µm–1 cm) (both sporophyte and gametophyte).	Microscopic (gametophyte: 1 mm)–macroscopic (sporophyte: up to 3 m).
Growth rate	Rapid: Spore to fertile gametophyte: two to three weeks. Zygote to fertile sporophyte: three to four weeks.	Gametophyte: extremely slow. Sporophyte: zygote → fertile sporophyte: five to six months.
Amenable to research topics in	Cell biology, developmental biology, genetics, primary and secondary metabolisms, microbiome interaction, cell wall biosynthesis.	Same. Sex determinism.
Sexual dimorphism (gametophyte phase)	Extremely low; absent in the vegetative stage; subtle on fertile organisms (gametophytes).	Significantly conspicuous in the vegetative and reproductive phases (gametophytes).
Genome	214 Mbp, ~17,000 genes, <=28 chromosomes.	Not known. In *S. japonica:* 580.5 Mbp, 35,725 encoding genes.
Genetic modification	Characterized mutants (UV irradiated). Genetic transformation: ☐ Stable: No. ☐ CRISPR: No.	Genetic transformation: ☐ Stable: No. ☐ Transient in *S. japonica* (biolistic). ☐ CRISPR: No.
Cell biology techniques	Immunocytochemistry. *In situ* hybridization.	Immunocytochemistry.
Phylogenetic studies	Key position, as a stramenopile, distant from metazoans and land plants.	Same + presenting complex multicellularity.
Summary	**Good for genetics and cytology, not good for biomass production**.	**Good for biomass production, cytology and all kinds of experimentation taking place at an early developmental stage (~5 cm long)**.

fundamental research on *S. latissima* ramped up in the 2010s. The advent of high-throughput sequencing techniques (mainly RNA-seq) put the spotlight on this model, leading to the possibility to address biological questions specific to kelps with a new angle. Although few labs in the world work on *Ectocarpus* spp., those working on *Saccharina* spp. are numerous, driven by the potential economic benefit. However, more efforts are necessary before this model is amenable to the full range of technical tools required for comprehensive studies. Table 2.1 summarizes the main features of these two brown algal models for laboratory research.

Parallel to these avenues of research, studies have also been carried out on alternative pathways. *Dictyota* sp. (Dictyotales) has proved an excellent model for the study of early embryogenesis (Bogaert et al. 2016; Bogaert et al. 2017) and thallus dichotomy (reviewed in Bogaert et al. 2020), *Sargassum* spp. for the establishment of shoot phyllotaxis (Linardić and Braybrook 2017) and *Fucus* spp. for abundant embryogenetic studies (Brownlee et al. 2001; Corellou et al. 2001). However, these latter brown algae are relatively difficult to cultivate in the laboratory, making it impossible to address biological processes taking place later in development.

Most likely, the choice of models will continue to grow, depending on the biological features inherent to each model and on the biological question to be addressed. In the end, it is the species the most amenable to genetic transformation that will dominate the field and become the favored model.

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