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PERSPECTIVE

Ulva: An emerging green seaweed model for systems biology

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Abstract

Green seaweeds exhibit a wide range of morphologies and occupy various ecological niches, spanning from freshwater to marine and terrestrial habitats. These organisms, which predominantly belong to the class Ulvophyceae, showcase a remarkable instance of parallel evolution toward complex multicellularity and macroscopic thalli in the Viridiplantae lineage. Within the green seaweeds, several *Ulva* species ("sea lettuce") are model organisms for studying carbon assimilation, interactions with bacteria, life cycle progression, and morphogenesis. *Ulva* species are also notorious for their fast growth and capacity to dominate nutrient-rich, anthropogenically disturbed coastal ecosystems during "green tide" blooms. From an economic perspective, *Ulva* has garnered increasing attention as a promising feedstock for the production of food, feed, and biobased products, also as a means of removing excess nutrients from the environment. We propose that *Ulva* is poised to further develop as a model in green seaweed research. In this perspective, we focus explicitly on *Ulva mutabilis*/*compressa* as a model species and highlight the molecular data and tools that are currently available or in development. We discuss several areas that will benefit from future research or where exciting new developments have been reported in other *Ulva* species.

KEYWORDS
algae, gene expression, genomics, model organism, phenomics, seaweed, systems biology

A MODEL SPECIES

Ulva mutabilis was originally sampled along the coasts of Olhão and Faro in South Portugal by Bjørn Føyn in 1952 (Figure 1). The wild-type form is a foliose thallus composed of three cell types: blade, stem, and rhizoid cells. The species was coined *U. mutabilis* since some original strains gave rise to multiple developmental mutants in the following five years (Føyn, 1958). Later observations suggested that the high rate of mutability probably resided in only one of the three original isolates. Whereas the original strain was lost in culture, the "mutabilis trait" survived in specific developmental mutants (Fjeld & Børresen, 1975). One of the earliest described spontaneous mutants is the tubular *slender*, which only develops blade and primary rhizoid cells (Fjeld, 1971; Spoerner et al., 2012). *Slender* is currently the most popular strain for developing genetic tools due to its fast growth and the ease of inducing gametogenesis (Blomme et al., 2021; Føyn, 1959; Oertel et al., 2015). While wild-type has a life cycle of 2–3 months, *slender* can reproduce twice as fast under optimal conditions (Føyn, 1959; Løvlie, 1964).

Strains of *Ulva mutabilis* can be maintained as haploid gametophytes via parthenogenetic development of gametes. Individuals grow well in synthetic growth medium (Stratmann et al., 1996), even with a minimal microbiome of the mutualistic bacteria *Roseovarius*

Abbreviations: APT, adenosine phosphoribosyl transferase; PEG, polyethylene glycol.

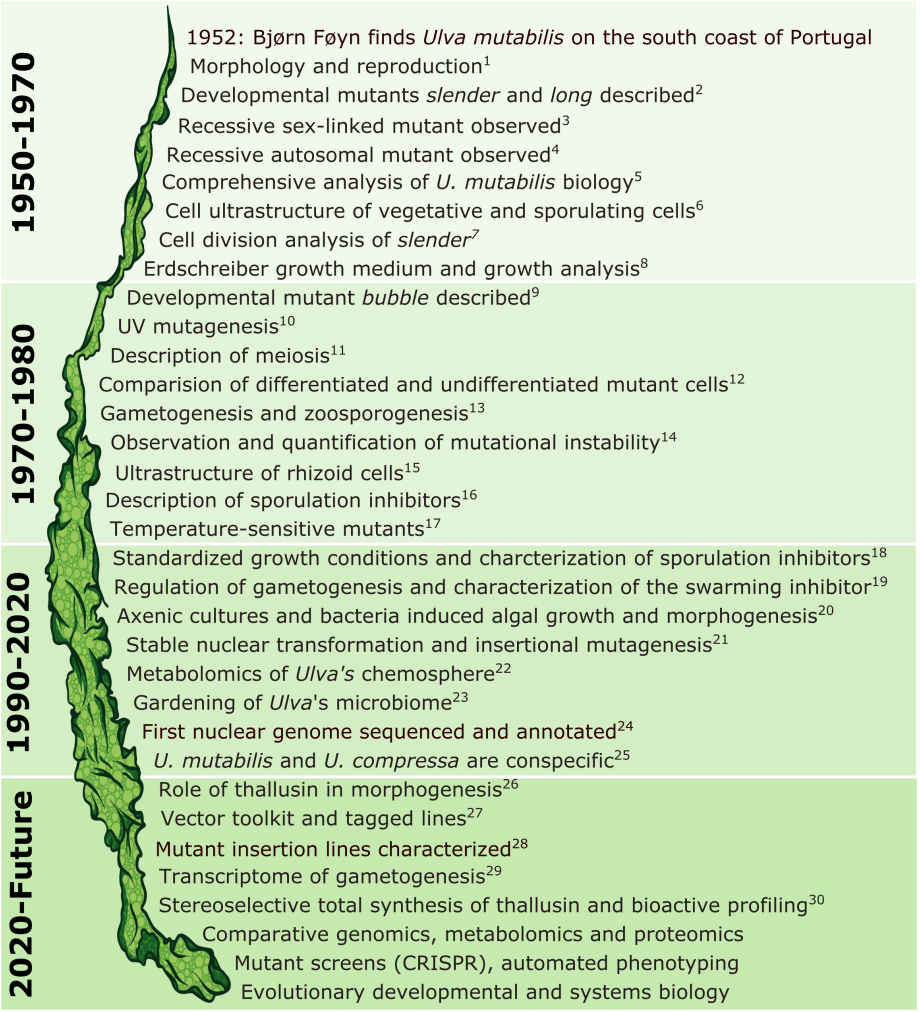

FIGURE 1 Timeline of 60 years of *Ulva mutabilis* research summarized in 30 major contributions to the development as a model organism. Image depicts the morphology of a *slender* individual. Numbers relate to the following references: 1: Føyn (1958), 2: Føyn (1959), 3: Føyn (1960), 4: Føyn (1961), 5: Løvlie (1964), 6: Bråten & Løvlie (1966), 7: Løvlie & Bråten (1968), 8: Løvlie (1969), 9: Fjeld (1970), 10: Fjeld (1971), 11: Nordby & Hoxmark (1972), 12: Bryhni (1974), 13: Nordby (1974), 14: Fjeld & Børresen (1975), 15: Bråten (1975), 16: Nilsen & Nordby (1975), 17: Løvlie (1978), 18: Stratmann et al. (1996), 19: Wichard & Oertel (2010), 20: Spoerner et al. (2012), 21: Oertel et al. (2015), 22: Alsufyani et al. (2017); Kessler et al. (2017), 23: Kessler et al. (2018), 24: De Clerck et al. (2018), 25: Steinhagen et al. (2018), 26: Alsufyani et al. (2020), 27: Blomme et al. (2021), 28: Kwantes & Wichard (2022), 29: Liu et al. (2022), and 30: Dhiman et al. (2022). [Color figure can be viewed at wileyonlinelibrary.com]

sp. MS2 and *Maribacter* sp. MS6 forming a tripartite community (Ghaderiardakani et al., 2017; Spoerner et al., 2012). Crossing strains with different mating types is well-described (Føyn, 1959, 1960; Hoxmark, 1976). Such crossing experiments have demonstrated that *U. mutabilis* and *U. compressa* are fully interfertile (Steinhagen et al., 2018), with the latter being a morphologically variable species that has a global distribution and is involved in green tide formation (Steinhagen et al., 2018, 2019). To remain consistent with the existing literature and avoid confusion with older literature in which natural isolates were solely identified based on morphological characteristics, we will keep the distinction between *U. mutabilis* (lab strains) and *U. compressa* (natural populations) throughout this perspective.

GENOMIC RESOURCES

The availability of a reference genome sequence is an important feature of any model species. The wild-type genome (mt(–); strain 1–41) was the first whole-genome sequence of a green seaweed (De Clerck et al., 2018). More recently, the genome of a Chilean *Ulva compressa* isolate has been reported (Osorio et al., 2022). Whereas the genomes have not been completed at a chromosomal level, comparison of both genomes reveal substantial differences in genome size (98.5 vs. 80.8 Mb), protein-coding genes (12,924 vs. 19,207), and repetitive elements (35% vs. 19%; Osorio et al., 2022). Flow cytometry-based genome size estimates from French (120 Mb) and Japanese (135 Mb) *U. compressa* strains further suggest significant

genome size variation (Kagami et al., 2005; Le Gall et al., 1993). Seven chloroplast and five mitochondrial genomes are currently available. Similar to the nuclear genome, intraspecific differences in organelle genome size due to gain or loss of group I/II introns and the integration of foreign DNA fragments and non-coding intergenic spacer regions have been observed, which is remarkable because most sequenced strains originate from the same geographic area (Yellow Sea; Cai et al., 2018, 2021; Liu et al., 2020; Liu & Melton, 2021; Xia, He, et al., 2021; Xia, Qin, et al., 2021).

The existing resources would benifit greatly from high-quality assemblies of different laboratory strains such as *Ulva mutabilis* (*slender*), but also with natural strains. *Ulva compressa* is a cosmopolitan species that has a remarkable intraspecific variation in morphology. Individuals can form blades, tubes, or branched thalli (Figure 2a). Populations thrive in broad irradiance, temperature, or salinity gradients (Steinhagen et al., 2019; Taylor et al., 2001) and show high resistance to both heavy metal contamination (Ratkevicius et al., 2003) and organic micro-pollutants (Hardegen et al., 2023). The power of population genomics should therefore be harnessed to explore genomic diversity using a pan-genome to associate genetic regions to specific phenotypes. In addition, genome-wide association study (GWAS) approaches can identify associations between measured phenotypes and genotypes (Bayer et al., 2020; Savolainen et al., 2013). So far, mapping populations, like those available for brown and red seaweeds (e.g., Avia et al., 2017; Huang & Yan, 2019; Wang et al., 2018) to statistically link genetic and phenotypic variation, have not been generated in *Ulva* yet.

Several *Ulva mutabilis*/*compressa* transcriptomes have been reported to complement the genomic resources. The transcriptional responses of copper exposure and hyposalinity as well as the interaction of temperature and light intensity on gene expression have been measured (Dong et al., 2022; Laporte et al., 2016, 2020; Rodríguez et al., 2018; Xing et al., 2021) as have sex-dependent expression (PRJDB3466) and differential gene expression during gametogenesis (Liu et al., 2022). Furthermore, the transcriptome under standard conditions was compared to that of other *Ulva* species in order to understand the mechanisms

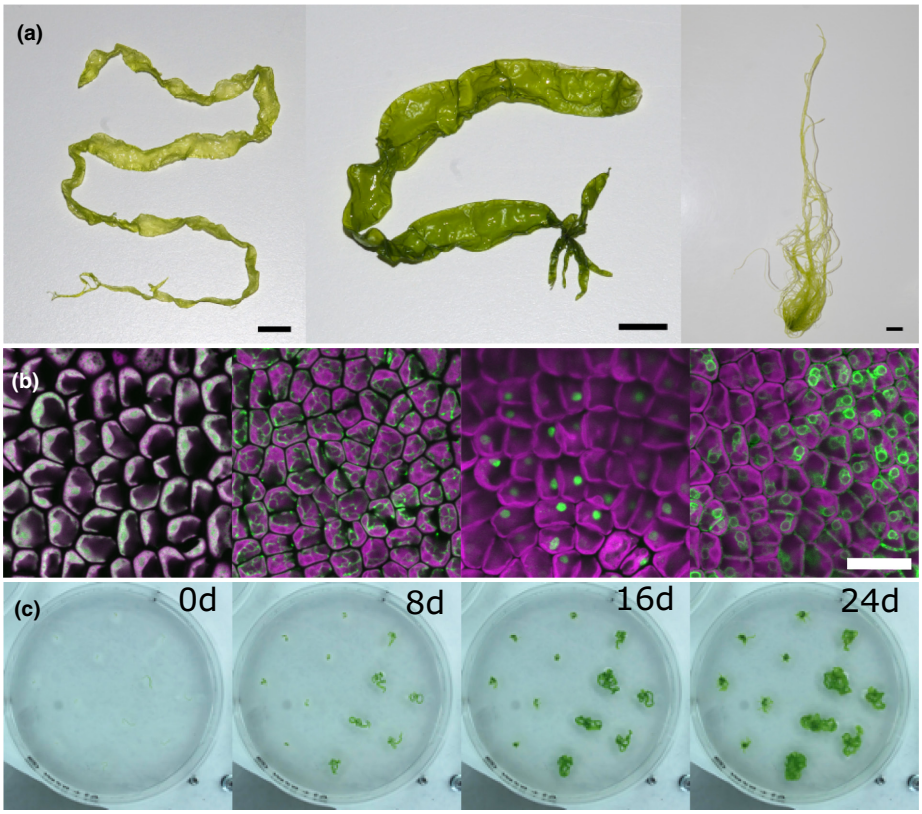

FIGURE 2 Overview of selected *Ulva mutabilis*/*compressa* characteristics of a model seaweed. (a) Illustration of intraspecific variability in morphology (from left to right): tubular slender, blade-forming wild-type and tubular-branched *Ulva compressa* isolate. Scale: 1 cm. (b) Availability of genetic tools, illustrated by four transgenic marker lines expressing endogenous *Ulva* genes tagged with YFP targeted to different intracellular locations (from left to right): chloroplast (*UM120_0017*), mitochondria (*UM013_0128*), nucleus (*UM001_0379*), and secretory pathway (*UM080_0043*). Green indicates the YFP signal and magenta represents chlorophyll autofluorescence. Scale: 20 μM. (c) Control of life cycle and development, illustrated by time-course growth of wild-type and slender on artificial medium containing agar. [Color figure can be viewed at wileyonlinelibrary.com]

of green tide formation (Wang et al., 2019). These transcriptomes remain correlative to date and rely heavily on gene functions experimentally verified in, e.g., land plants, but they can provide a good basis for gene characterization studies. Summarizing data on differential gene expression using a user-friendly portal can assist in predicting the role of a gene in a certain life stage or environment. Preliminary investigations of epigenetic variations in protoplast-derived germlings of *U. reticulata* (Gupta et al., 2012) have been reported, but more investigations into the epigenetic control of gene expression are needed.

To complete normal morphogenesis, *Ulva mutabilis*/*compressa* requires associated bacteria in a mutualistic relationship exchanging infochemicals (Kessler et al., 2018; Spoerner et al., 2012). The diverse *Ulva* microbiome changes in natural populations in relation to environmental parameters or bloom formation (Ghaderiardakani et al., 2017, 2020; van der Loos et al., 2022). Currently, few published genome sequences of *Ulva*-associated bacteria are available, but these resources are expected to add an extra layer of complexity and to shed light on the molecular functions shared by these microbiome partners (Alsufyani et al., 2020; Morales-Reyes et al., 2022).

GENETIC TRANSFORMATION

The first reported transient transgene expression in *Ulva* demonstrated the pyrenoid localization of the N-terminal region of the Rubisco small subunit fused to GFP (Suzuki et al., 2014). Discharged *Ulva* gametes do not contain a cell wall and can be transformed like land plant protoplasts using the chemical polyethylene glycol (PEG). Since every vegetative cell can theoretically differentiate into 16 gametes during gametogenesis (Stratmann et al., 1996), it is straightforward to obtain a sufficient amount of transformable cells despite the relatively low transformation efficiency (1/5,000 gametes; Boesger et al., 2018; Oertel et al., 2015). Stable transformation of *U. mutabilis* was established by selecting transformants using a Bleomycin resistance cassette (Oertel et al., 2015). Inclusion of the *Ulva* small subunit Rubisco promoter, intron, and terminator sequences to control the codon-optimized *ble* resistance marker has corroborated earlier observations in *Chlamydomonas* that endogenous regulatory sequences positively affect transgene expression (Oertel et al., 2015).

Crucially, efficient stable transgene expression is reported in about 75% of transformed individuals (Blomme et al., 2021). To facilitate the generation of transgene constructs, a flexible and modular Golden Gate-based cloning toolkit was designed, including 125 entry vectors, 26 destination vectors, and 107 functionally validated expression vectors, a size that exceeds similar efforts for the green algae *Chlamydomonas* (Blomme, et al., 2021; Crozet et al., 2018). A transformation experiment with up to 30 different vectors can be readily performed and even has the potential to be scaled up further.

FUNCTIONAL GENOMICS

Ulva mutabilis is currently the only seaweed where both gain- and loss-of-function lines can be generated.

Expressing (tagged) transgenes is instrumental in functional genomics. The molecular toolkit allows the efficient generation of transgenic lines but is still limited to constitutive expression (Figure 2b; Blomme et al., 2021). No conditional promoters have been described, although there are some candidates in *Ulva prolifera* (Guo et al., 2017; Wu et al., 2019). Transformant selection solely relies on the bleomycin resistance cassette. *Ulva* is resistant to various antibiotics (Spoerner et al., 2012), including hygromycin, which is used in *Chlamydomonas* (Berthold et al., 2002), and the symbiotic bacteria must be resistant to the selection agent (Oertel et al., 2015), complicating the use of additional selectable markers. Furthermore, the expression of large non-endogenous transgenes needs to be explored and might require the insertion of endogenous introns or codon optimization to allow recombinant gene expression (Baier et al., 2018). Given the relatively low transformation and mutation frequencies, *Ulva*-specific optimizations might be required to allow future large-scale genetic screens. The maintenance of many (transgenic) strains cannot be underestimated and could represent a future challenge. Current long-term storage solutions supplemented with cryopreservation (Gao et al., 2017; Lee & Nam, 2016) are expected to prevent the loss of biological material.

A unique feature of *U. mutabilis* is the rich history of developmental mutant research. The “mutabilis” trait resulted in strains with a tubular “slender” or “long,” a hollow spherical “bubble,” a disorganized “lumpy,” or a globular “globose” thallus phenotype that triggered studies on cell division and vegetative development (Bryhni, 1974; Føyn, 1959, 1961). More recently, similar and new developmental mutants (e.g., callus, filamentous branched, forked, and serrated) were generated by insertional mutagenesis, and genes are being functionally characterized (Kwantes & Wichard, 2022; Oertel et al., 2015; Wichard, 2023). Such forward-genetics methods have been employed to generate large mutant libraries in *Chlamydomonas,* but reaching a stage wherein every single gene is mutated is difficult (Li et al., 2016). The development of CRISPR/Cas-mediated genome engineering that allows targeted mutation of one or more genes simultaneously

would be a valuable asset. Once established, CRISPR/Cas can be scaled up to target gene families, molecular pathways, or whole genomes (Doench, 2017) and adapted to enable specific base changes (base or prime editing; Anzalone et al., 2019; Komor et al., 2016; Gaudelli et al., 2017). Alternatively, CRISPR/Cas can mediate the insertion of exogenous DNA sequences at a target site using homology-directed repair, as demonstrated in *Chlamydomonas* (Akella et al., 2021; Greiner et al., 2017; Picariello et al., 2020). While CRISPR/Cas was recently described in *U. prolifera*, mutation efficiency is currently low (approx. 1/1,000), and no target beyond the selectable marker gene *adenosine phosphoribosyl transferase* (*APT*) has been reported (Ichihara et al., 2021).

PROTEOMICS, METABOLOMICS, AND PHENOMICS TOOLKIT FOR SURVEY IN SYSTEMS BIOLOGY

Integration of omics approaches such as metabolomics and proteomics accompanied with the careful descriptions of phenotypes have proven to be especially successful in elucidating algal-bacterial cross-talk mechanisms, the effect of abiotic and biotic stimuli, and life cycle transitions (Alsufyani et al., 2017; Fan et al., 2022; Fort et al., 2019; Ghaderiardakani et al., 2022; Gu et al., 2022; He et al., 2019; Kessler et al., 2017; Liu et al., 2022).

Most famously, efforts have been made to determine algal growth and morphogenesis-promoting factors like thallusin released by bacteria into the culture medium (reviewed in Wichard, 2023). The algae differentiation factor (–)-thallusin and its derivatives are available through an advanced organic stereoselective synthesis for bioactivity profiling (Dhiman et al., 2022). Thallusin receptors and downsteam players in the presumed signaling pathway leading to proper rhizoid and cell wall formation, however, remain to be identified. Large-scale mutant screens, the like of the Chlamydomonas Library Project (CLiP), in combination with high-throughput phenotyping tools will be necessary to identify the respective genes.

In the short term, high-throughput tools need to be developed to screen and analyze (growth) phenotypes in a (semi-)automated way. *Ulva* is often phenotyped by cutting a tissue disc from an individual and measuring the expansion over time (Fort et al., 2019). This proxy is useful for large blade-forming species, but does not consider early vegetative development of individuals. Moving forward, the effect of different (a)biotic conditions and mutations on growth should be measured for the complete life cycle in a quantitative way (Figure 2c), supported by microscopic imaging techniques for screens at the cellular level (Dhondt et al., 2013).

INTEGRATION—OUTLOOK

Following a rich history of developmental biology and physiology (Figure 1), *Ulva mutabilis*/*compressa* holds promise for blooming into a systems biology model (Figure 2). To achieve this status, more data, molecular tools, and biological material need to be generated, and at some point, a centralized repository will be necessary. Large-scale algae-focused projects like Chlamydomonas Resource Center (https://www.chlamycollection.org), DiatOmicBse (https://www.diatomicsbase.bio.ens.psl.eu), and NanDeSyn (Gong et al., 2020) provide excellent models. Although several challenges lie ahead, we hope this perspective highlights the steps that are needed to further develop *Ulva* as a seaweed model organism in the genomics era.

AUTHOR CONTRIBUTIONS

Jonas Blomme: Conceptualization (lead); writing – original draft (lead). **Thomas B. Wichard:** Conceptualization (supporting); writing – review and editing (lead). **Thomas Jacobs:** Conceptualization (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Olivier De Clerck:** Conceptualization (supporting); writing – review and editing (supporting).

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