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GETTING A GRIP ON GENETIC MODIFICATION IN BROWN ALGAE

Brown algae (Phaeophyceae) are remarkable organisms that, independently of parallel developments in green algae and in land plants, evolved multicellularity and organs that look like their "counterparts" in land plants and are accordingly termed rhizoids (resembling root), cauloids (resembling stem), and phylloids (resembling leaf). Similar to trees on land, large brown algae (kelp) can form large underwater forests. While some brown algae show a remarkable difference between size and appearance of gametophytes and sporophytes, these generations of the brown algal life cycle can only be distinguished on the microscopic level in other brown algae (like the homothallic *Ectocarpus*). The genetic processes steering these complex developmental patterns are still largely unknown. The development and origin of specific structures of brown algal organs began to attract the attention of biologists in the early 1900s. For instance in 1904, when studying archegonia in different phyla, Davis investigated and compared the sporangia and gametangia in Phaeophyceae like *Ectocarpus* and *Dictyota* (Davis 1903). Since then, the scientific interest in brown algae increased at the taxonomic level, but also with respect to the characterization of the life cycle (Müller 1964), the discovery of pheromones (Müller et al. 1979) and various cellular processes including the polarization of *Fucus* zygotes (Brownlee and Wood 1986). As macroalgae are also considered a potential source of food, gelling agents, biofuels and new bioactive compounds, research on these algae has escalated in recent years. It is unfortunate that for this scientifically and economically interesting group of organisms, a useful set of critical genetic tools, including ways to suppress the activity of specific genes and express heterologous genes, has not been established. To my knowledge, there is only a single report on transient expression of a reporter gene in *Laminaria* gametophytes (Qin et al. 1994), but for unknown reasons this technique has not been widely used by the research community. Of course there are limitations regarding the use of genetically modified brown algae, and common sense dictates that they should not be released into the oceans. However, the ability to perform genetic manipulations of model brown algal systems under controlled laboratory conditions could strongly increase our understanding of cellular and developmental processes in multicellular algae. Genetic manipulation of other multicellular photoautotrophic organisms like mosses (*Physcomitrella patens*; Schaefer et al. 1991), land plants (*Arabidopsis thaliana*; Bent 2000), and green algae (*Volvox* sp.; Schiedlmeier et al. 1994) are much more advanced. The recent publication of the genome sequence of the brown alga *Ectocarpus siliculosus* by Cock et al. (2010) has raised expectations that genetic manipulation in brown algae might be feasible sooner or later. However, although different research groups around the globe have tried, a method for the stable genetic transformation of brown algae has yet to be developed.

In this issue of the *Journal of Phycology*, Brownlee et al. report on the first approach to silence genes in the brown alga *Fucus serratus* (Farnham et al. 2013). As a proof of concept, they decided to study cytoskeleton formation in *Fucus* zygotes (Fig. 1) as a phenotypic readout of altered gene expression. This was a splendid focus for 'knockdown' experiments since the altered cytoskeletal features in the suppressed strains can be readily observed using fluorescence microscopy (Corellou et al. 2005). The authors microinjected double-stranded RNA to induce the degradation of endogenous mRNAs (see Fig. 2), which resulted in reduced expression of the encoded proteins and an observable phenotype. More specifically, they injected fragments of double stranded RNA complementary to genes encoding α-tubulin and β-actin. Similar to earlier pharmacological experiments in which microtubule development was disrupted in *Fucus*, the authors observed growth arrest and disruption of cell division. The efficacy of the gene silencing approach is suggested by the findings that all zygotes that had been injected with the β-actin fragment failed to develop properly, while 80% of the zygotes injected with control DNA showed a normal developmental progression.

The classical way to achieve down-regulation of a gene is by inducing mutations that modify or

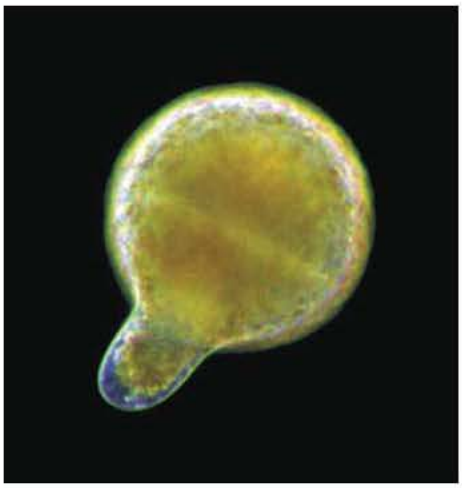

FIG. 1. 24 h old embryo of *Fucus serratus*. The size of the embryo is about 80 µm. Photo courtesy : J.H. Bothwell and J.M. Brownlee.

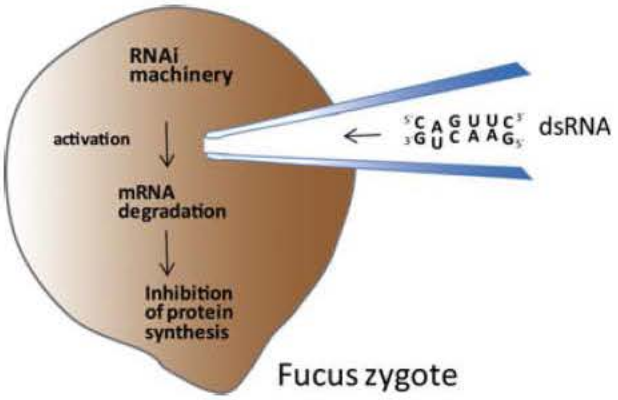

FIG. 2. Gene silencing approach developed by Farnham et al. (2013). Microinjection of double stranded RNA results in activa tion of the RNAi machinery, degradation of corresponding mRNAs, and inhibition of translation of these mRNAs.

interrupt that gene. This approach allows for the establishment of a correlation between a genotypic and phenotypic change, which could implicate a gene product in a specific cellular process and/or biochemical activity. Although random mutagenesis of brown algae like *E. siliculosus* by UV light or specific chemicals has been shown to be feasible (Coelho et al. 2011), such a forward genetics approach does not allow the disruption of a particular gene. The micro-injection of dsRNA in *Fucus* described here by Farnham et al. (2013) has the potential to silence individual genes at will. Notwithstanding the limitations of a transient approach – the change of expression is not transmitted to the next generation, but disappears after some cell divisions, making the protocol only applicable to certain cells and questions – it is the first demonstration of reverse genetics by targeted manipulation of single genes in brown algae. Therefore, the results reported by Brownlee et al. represent an important milestone in attaining the molecular resources required for elucidating the biology of macrophytic algae.

Genetic manipulation of eukaryotic algae has a long history. The *Acetabularia* transplant experiments by Hämmerling already resulted de facto (although the author did not know at the time, but still drew the right conclusions) in the transfer of mRNA from one cell type to another (Hämmerling 1934). Tools developed later on, such as random insertional mutagenesis, were powerfully applied to the haploid, genetically manipulable alga *Chlamydomonas reinhardtii*, which helped establish it as the first widely used algal model system ('green yeast'; Rochaix 1995). Currently, this organism has the largest repertoire of tools for studying photosynthesis, chloroplast biogenesis, and flagellar function (Harris 2001). A few years later, several groups demonstrated the genetic transformation of diatoms (Dunahay et al. 1995, Apt et al. 1996, Falciatore et al. 1999). Meanwhile, successful genetic transformations have been achieved for a number of algae (reviewed in Beer et al. 2009). Basic features of most transformation approaches involve the use of endogenous promoters driving resistance and reporter genes, the use of a biolistic device, stable integration of the transgene in the genome and the ability of the algal cells to grow on solid agar medium during the screening process. Depending on the genes of interest, both the nuclear and the plastid genomes have been targeted for insertion of exogenous DNA, the latter working to differing extents in the various algae (Takahashi et al. 1991, Doetsch et al. 2001, Materna et al. 2009, Purton et al. 2013).

Future developments, such as targeted mutagenesis and the development of gene knockdowns by new techniques that use meganucleases (Epinat et al. 2003), TALEN (Cermak et al. 2011) or CRISPR elements (Cong et al. 2013), will definitely broaden our toolset for genetic manipulation of the algae. Thus, while we are still waiting for the first stable genetic transformation system to be established in brown algae, Brownlee et al. have demonstrated that targeted genetic manipulation in these algae is feasible, paving the way for future sophisticated analyses of this exciting, novel system.

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