COMMENTARY

Waking sleeping algal cells

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Our growing energy demands and decreasing reserves of fossil fuels call for the development of renewable energy solutions. Biodiesel derived from plant triacylglycerol (TAG) storage lipids can be used in diesel engines directly and is thus a drop-in solution to offset the increasing demand for transportation oils (1). Microalgae are promising feedstocks for biodiesel production because they can accumulate large amounts of TAGs (up to 70% of their dry weight), and their production does not have to compete with food crops for land or freshwater (2). However, a major challenge facing commercial production of algal biodiesel is that TAG production is maximal under nutrient deprivation conditions, where cell growth slows down or stops completely. This coupling of TAG accumulation to growth arrest greatly limits the total yield of biodiesel. In PNAS, Tsai et al. (3) present the discovery of a factor required for exit from growth arrest in the green algal model *Chlamydomonas reinhardtii*. This finding could be a critical first step toward improving algal biofuel yields by uncoupling growth arrest from TAG accumulation.

The cells of all organisms are thought to be capable of a state known as quiescence, where cells are not proliferating but retain the ability to grow and divide at a later time (4) (Fig. 1). In unicellular organisms, quiescence is often induced by nutrient starvation and typically represents a state where cells are more resistant to stress (5). The decision to enter or exit quiescence is carefully regulated. Extensive studies have revealed many regulators involved in the entry, maintenance, and exit from quiescence in the budding yeast *Saccharomyces cerevisiae* (5) and several other microbes. However, the mechanisms regulating quiescence in algae have hitherto remained unknown.

Tsai et al. identify CHT7, a protein regulating quiescence in algae, through a screen for mutants with defects in TAG degradation on quiesence exit. Quiescence can be induced in *C. reinhardtii* by depriving cells of nitrogen (N) (6). On entering quiescence, *C. reinhardtii* cells accumulate TAG in lipid droplets (7, 8). When N is resupplied after 2 or 3 d of N deprivation, cells exit quiescence and degrade most of the TAG within 1 d (9). The *cht7* mutant was initially isolated by Tsai et al. because it showed a delay in TAG degradation on N resupply. Tsai et al. observe that *cht7* cells take much longer to resume growth after N resupply. Vital dye staining of quiescent *cht7* cells argues against the possibility that a large fraction of cells died during N deprivation. Thus, the *cht7* mutant exhibits a defect in exiting N deprivation-induced quiescence. A more general defect of *cht7* in exiting quiescence is revealed by its inability to restart cell growth after phosphate deprivation and rapamycin treatment, two alternative ways of inducing quiescence (3). By mutation mapping and mutant complementation, Tsai et al. find that disruption of the *CHT7* gene is responsible for the delay in the exit from quiescence caused by N deprivation. This leads them to a model where the CHT7 protein promotes exit from quiescence (Fig. 1).

By what mechanism does CHT7 promote exit from quiescence? CHT7 contains a CXC domain, which has been found to mediate DNA binding in animal and plant CXC-containing proteins (10, 11). Additionally, Tsai et al. find that CHT7 is located in the nucleus (3). It therefore seems possible that CHT7 functions as a transcription factor regulating global transcriptional changes between quiescence and proliferation. Although a direct role in transcription for CHT7 remains to be tested, the observation of massive transcriptome changes in the *cht7* mutant is consistent with a possible transcription factor activity. Specifically, Tsai et al. find that proliferating *cht7* mutants exhibited a transcriptome profile with striking overlap with that of its quiescent N-starved parental line, suggesting a role for CHT7 in repressing the quiescence program (3) (Fig. 1). The reciprocal comparison of transcriptomes during refeeding of *cht7* and its parental strain was not reported, but might be difficult to interpret because growing and nongrowing cells will necessarily exhibit many transcriptome differences regardless of the underlying cause.

CHT7 could be a master regulator of the full quiescence exit program, or it could be specifically required for a critical component of the exit program. Hints about the specific role of CHT7 might be obtained from identifying its genomic binding sites and putative transcriptional targets. If CHT7 mediates a subset of the regulatory changes required for exit from quiescence, can the *cht7* mutant be rescued by direct execution of these changes, e.g., by overexpression of a lipase required for exit from quiescence? If CHT7 is a master regulator that promotes exit from quiescence, could its overexpression overrule other signals that promote entry into quiescence? This possibility could be explored by asking whether CHT7 overexpressors are more reluctant to enter quiescence on nutrient starvation or are resistant to rapamycin-induced quiescence. In fact, some hint of such effects may be apparent in figure 3 *B* and *D* of Tsai et al. In these figures, some of the complemented lines (which may overexpress CHT7 under the experimental conditions) appear to grow faster than WT under N-deprived and N-replete conditions and possibly also in the presence of rapamycin.

The mechanism by which CHT7 activity might respond to nutrient availability and cell growth cues also remains unclear. Tsai et al. find that CHT7 is constitutively nuclear and is part of a large stable complex whose relative abundance does not change when cells are

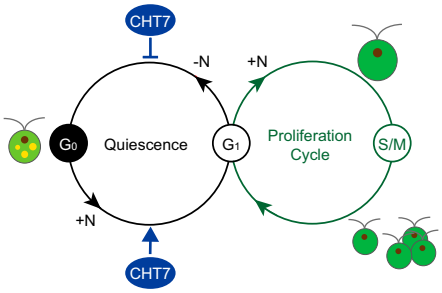

Fig. 1. CHT7 is required for exiting quiescence caused by nutrient deprivation. Under N-replete (+N) conditions, cells grow mitotically, alternating between growth in the G_1 phase and division through a multiple fission mechanism (12) involving rapid alternating rounds of DNA synthesis and mitosis (S/M). On N deprivation (–N), cells enter quiescence (G_0) and accumulate triacylglycerol in lipid droplets. CHT7 is required for cells to exit quiescence and reenter the proliferative cycle when nutrients are resupplied.

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growing vs. quiescent. The authors speculate that if CHT7 is regulated by nutrients, it might be through posttranslational modifications such as phosphorylation, which are a common means of controlling transcription factor activity (3). Such posttranslational modifications could be identified by proteomic analysis of CHT7 under quiescence and normal growth.

The discovery of CHT7 is a key first step toward understanding the exit from quiescence in algae. It will be interesting to see how the wiring of the green algal quiescence machinery differs from that of other microorganisms, like yeast. Furthermore, it will be exciting to see whether it is possible to use these insights to uncouple TAG accumulation from growth arrest to ultimately create more productive biofuel strains.

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