



REVIEW

A Series of Fortunate Events: Introducing *Chlamydomonas* as a Reference Organism^[OPEN]

Patrice A. Salomé^{a,1} and Sabeeha S. Merchant^{a,b}^a University of California, Los Angeles, Department of Chemistry and Biochemistry, Los Angeles, CA 90095^b University of California, Berkeley, Departments of Plant and Microbial Biology and Molecular and Cell Biology, Berkeley, CA 94720

ORCID IDs: 0000-0003-4452-9064 (P.A.S.); 0000-0002-2594-509X (S.S.M.)

The unicellular alga *Chlamydomonas reinhardtii* is a classical reference organism for studying photosynthesis, chloroplast biology, cell cycle control, and cilia structure and function. It is also an emerging model for studying sensory cilia, the production of high-value bioproducts, and in situ structural determination. Much of the early appeal of *Chlamydomonas* was rooted in its promise as a genetic system, but like other classic model organisms, this rise to prominence predated the discovery of the structure of DNA, whole-genome sequences, and molecular techniques for gene manipulation. The haploid genome of *C. reinhardtii* facilitates genetic analyses and offers many of the advantages of microbial systems applied to a photosynthetic organism. *C. reinhardtii* has contributed to our understanding of chloroplast-based photosynthesis and cilia biology. Despite pervasive transgene silencing, technological advances have allowed researchers to address outstanding lines of inquiry in algal research. The most thoroughly studied unicellular alga, *C. reinhardtii*, is the current standard for algal research, and although genome editing is still far from efficient and routine, it nevertheless serves as a template for other algae. We present a historical retrospective of the rise of *C. reinhardtii* to illuminate its past and present. We also present resources for current and future scientists who may wish to expand their studies to the realm of microalgae.

WHY ALGAE (IN GENERAL) AND CHLAMYDOMONAS (SPECIFICALLY)?

Algae represent a very large and diverse polyphyletic group of photosynthetic eukaryotes (Blaby-Haas and Merchant, 2019). They occupy all possible ecological niches on the planet, and therefore constitute a potential reservoir of untapped functional capabilities for adaptation to the environment. Algae are primary producers that contribute ~50% of total carbon fixation worldwide (Field et al., 1998), which makes their study fundamental to our understanding of global primary production and carbon flux. They also offer a low-cost option for the large-scale production of high-value molecules, since algae only require water, salts, air, and light. Unicellular algae such as the ciliated green alga *Chlamydomonas reinhardtii* offer high signal-to-noise during experiments due to the ease of growth in controlled medium and environments (temperature and light regimes) and the homogeneous nature of the cultures, and they grow much more rapidly than classic plant models.

With its haploid genome, *C. reinhardtii* is well suited for classical genetics, as loss-of-function mutations are immediately expressed and more likely lead to observable phenotypes compared with diploid organisms. *C. reinhardtii* not only responds to light, but it also anticipates environmental transitions like dawn and dusk under the supervision of a circadian system, which coordinates cell division, photosynthesis, and cilia biogenesis, representing

three fundamental research topics (Noordally and Millar, 2015). Other topics of research using *C. reinhardtii* include the carbon-concentrating mechanism (CCM) and in situ structure determination of protein complexes by electron cryotomography. Responses to excess light and the dissipation of light energy to prevent cellular damage are another research avenue that has benefitted enormously from the analysis of *C. reinhardtii*. Additional research questions being addressed by the study of *C. reinhardtii* focus on metabolism (starch, nitrogen, amino acids, sulfur, phosphorus, and metal micronutrients), biosynthetic pathways (glycerolipids, chlorophyll, hemes, and carotenoids), anoxia, thioredoxins, chaperones and proteases, and the control of chloroplast gene expression (Harris, 2008). Inside its 2000 pages and three volumes, *The Chlamydomonas Sourcebook* encompasses major research topics, history, and methodology (Harris, 2008). We will feature selected examples below where *C. reinhardtii* has played a key role before examining the events that promoted this alga to the forefront.

SELECTED RESEARCH HIGHLIGHTS

Cell Cycle and Cell Division

The eukaryotic cell cycle machinery was largely deciphered in budding yeast (*Saccharomyces cerevisiae*) using synchronized cultures. Despite conservation of the underlying proteins (for example, cyclins, cyclin-dependent kinases, and the anaphase-promoting complex), budding yeast is evolutionarily distant from algae and plants. *C. reinhardtii* makes an excellent photosynthetic

¹ Address correspondence to: salome@chem.ucla.edu.
^[OPEN]Articles can be viewed without a subscription.
 www.plantcell.org/cgi/doi/10.1105/tpc.18.00952

system whose progression through the cell cycle can be synchronized by daily light-dark cycles, as is common for many algae: cells enter G_1 during the day, followed by the S and M phases around dusk. This separation of phase along the diurnal cycle provides a predictable temporal cascade that starts with cell growth (fueled by photosynthesis), followed by the commitment to divide, resorption of cilia, doubling of DNA and histone contents, mitosis, and the growth of new cilia. With the help of robotics and semiautomated imaging, it is possible to identify temperature-sensitive mutants affected in some aspect of cell cycle progression, and whole-genome sequencing can be used to pinpoint the causal mutation. So far, ~150 mutants have been identified with a defect in division or exit from G_1 phase (Tulin and Cross, 2014; Breker et al., 2018). These mutants define genes with important roles in the cell cycle that may be applicable to plants as well, as ~75% of these genes have clear homologs in *Arabidopsis thaliana*. A clear challenge is to understand the role of each cell cycle gene, but looking at their expression windows over the diurnal cycle should help narrow down the options (Zones et al., 2015; Strenkert et al., 2019).

Chloroplast Biogenesis and Photosynthesis

C. reinhardtii is a facultative autotroph that can handle mutations in the photosynthetic apparatus if grown in the presence of a reduced carbon source such as acetate, offering a number of advantages when studying chloroplast biogenesis and function. Mutants with defects in the light reactions can be specifically enriched by the addition of the bactericidal agent metronidazole. Also known as Flagyl, this compound is reduced to its toxic form by ferredoxin; only cells that cannot reduce ferredoxin survive in its presence (Schmidt et al., 1977). Through classic genetic screens, *acetate-requiring* (*ac*) photosynthetic mutants were isolated based on the requirement for acetate as a reduced carbon source (hence the name *acetate requiring*) or increased chlorophyll fluorescence, a consequence of disrupted electron transport (Levine, 1960a; Bennoun and Levine, 1967; Bennoun et al., 1980). Chlorophyll fluorescence reflects the progression of electron flow through the electron transport chain: its blockage increases fluorescence, as light energy can no longer be used for photochemistry. The same fluorescence assay provides a powerful means to determine allelism and genomic rescue when identifying the underlying causal loci. In land plants, mutants in the homologous genes also display high chlorophyll fluorescence but are generally seedling-lethal and must be maintained as a segregating stock (Maiwald et al., 2003). Homozygous mutant plants can, however, survive on medium supplemented with sucrose, which is taken up by dedicated transporters (missing in *Chlamydomonas*).

Early evidence for nucleus- and plastid-encoded components of the photosynthetic apparatus came from radiolabeling *C. reinhardtii* cells blocked selectively in cytosolic or plastid translation (Chua and Gillham, 1977; Delepelaire, 1983; Merchant and Selman, 1984). Pulse-chase labeling of cells with ^{35}S in the presence or absence of a plastid translation inhibitor also revealed that the small subunit of Rubisco (translated in the cytoplasm) is rapidly degraded when the large subunit is missing (Schmidt and Mishkind, 1983), mirroring observations collected on photosystem II (PSII) proteins in the absence of plastid-encoded D1 and D2

proteins (Erickson et al., 1986; Jensen et al., 1986). The control of epistasis of synthesis (CES) model describes how the translation potential of individual components of a photosynthetic protein complex depends on the presence of the other constituents (Choquet and Wollman, 2002).

The genetic dissection of photosynthesis owes much to the collection of *ac* mutants from Levine, followed by new mutants from career-long screens from the Rochaix and Wollman laboratories. *ac* mutants were used to define nuclear loci that control chloroplast transcription, RNA stability, and translation. Analysis of some mutants provided experimental validation for *trans*-splicing of the plastid-encoded *psaA* gene (Kück et al., 1987; Choquet et al., 1988), which is transcribed as three separate RNAs that are later spliced together via the concerted action of at least 14 nucleus-encoded factors. Coordination between nucleus- and plastid-encoded subunits of photosynthetic complexes and enzymes is critical for proper chloroplast function. For example, the nucleus-encoded NAC2 protein is an RNA binding protein with tetratricopeptide-like repeats that stabilizes *psbD* RNA (Boudreau et al., 2000). Such RNA binding proteins exhibit sequence specificity for a given chloroplast RNA and are encoded by large gene families in land plants that have not yet expanded in algae (Jalal et al., 2015).

Photosynthesis and State Transitions

Analyses of other mutants have led to the genetic dissection of state transitions. At their core, light-dependent reactions drive electron flow from water to NADP^+ via PSII and PSI while generating a proton gradient via proton transfers at multiple steps. Under conditions referred to as state 1, each photosystem receives photons from its cognate chlorophyll binding antenna proteins: Light Harvesting Complex I (LHCI) or LHCII for PSI and PSII, respectively. When PSII becomes too excited, reduced plastoquinone accumulates, resulting in the phosphorylation of LHCII proteins and their subsequent dissociation from PSII and association with PSI: this is known as state 2. Healthy cells continuously shuttle LHCII proteins between PSII and PSI complexes as a function of light quality and quantity. State transitions balance the distribution of excitation energy, allowing the modulation of NADPH and ATP production.

Although state transitions were first discovered in other organisms, the biochemical underpinnings of this process were elucidated in *C. reinhardtii*, wherein as much as 80% of LHCII dissociates from PSII, in contrast to ~20% in land plants (Delosme et al., 1996). State transitions require cytochrome (Cyt) b_6/f to sense the redox status of the plastoquinone pool, as a Cyt b_6/f mutant is locked in state 1 and primarily accumulates unphosphorylated LHCII proteins (Wollman and Lemaire, 1988). The transition to state 2 involves the (reversible) phosphorylation of LHCII proteins (Wollman and Delepelaire, 1984). Mutants lacking state transitions display identical chlorophyll fluorescence whether the plastoquinone pool is highly oxidized (state 1) or reduced (state 2). In the *stt7* mutant (for *state-transition deficient*), LHCII proteins are only found in their nonphosphorylated form. The *stt7* mutant can still grow in the absence of acetate, albeit at a slower rate, indicating that state transitions contribute to fitness (Fleischmann et al., 1999). *STT7* encodes the kinase responsible for LHCII

phosphorylation (Depège et al., 2003). The interaction of Cyt b_6f with STT7 is promoted by reduced plastoquinone and results in the autophosphorylation and activation of the kinase. STT7 is then released from Cyt b_6f and diffuses to PSII within the thylakoid membrane to phosphorylate associated LHCII proteins, thereby initiating state 2 (Dumas et al., 2017). The STT7 homolog in Arabidopsis, STN7, also catalyzes LHCII phosphorylation in response to the redox status of plastoquinone, and the *stn7* mutant shows growth defects under a fluctuating light regime, which more accurately mimics real-life growth conditions (Bellafiore et al., 2005). Therefore, the dissection of a process in *C. reinhardtii* is applicable to plants. This process may allow an increase in photosynthetic yields by better recycling excess light photon energy away from dissipation.

Photosynthesis and the CCM

Oxygen and carbon dioxide compete for the active site of Rubisco, causing a decrease in photosynthetic efficiency. Rubisco evolved under low-oxygen conditions, but oxygenic photosynthesis altered the surrounding environment. Biological innovations increased local relative CO₂ concentrations to enhance photosynthetic performance. These innovations include carboxysomes in cyanobacteria, C₄ metabolism in land plants, and the CCM in *C. reinhardtii* and other algae. A functional CCM complex starts with the uptake of bicarbonate from the environment, its delivery to the pyrenoid (a site of concentrated Rubisco), and its conversion to CO₂ by carbonic anhydrases. The zinc-finger transcription factor CIA5/CCM1 was identified from genetic screens for mutants lacking the pyrenoid and unable to adapt to low CO₂ levels. CIA5/CCM1 orchestrates most transcriptional responses to variations in CO₂ levels (Fang et al., 2012). The putative membrane-bound transporters responsible for inorganic carbon uptake, the carbonic anhydrases, and the transcriptional network controlled by inorganic carbon availability were identified through comparative transcriptome analysis of cells grown under low and high CO₂ concentrations and through genetic screens (Jungnick et al., 2014; Memmola et al., 2014; Wang et al., 2015b). In addition, the biochemical identification of proteins in purified pyrenoid components was validated genetically (Mackinder et al., 2016; Zhan et al., 2018). Most CCM components were tentatively arranged along the bicarbonate chemical gradient created within the cell (from the cell wall to the pyrenoid matrix) based on their localization (Mackinder et al., 2017). Surprisingly, proteins can localize to distinct layers and puncta within the pyrenoid, in addition to the typical matrix localization of Rubisco and the Rubisco binding Essential Pyrenoid Component 1 (EPYC1). Time-course observations of the pyrenoid during cell division solved its mode of inheritance: most daughter cells acquire their pyrenoid by elongation and fission of the mother cell's pyrenoid (Freeman Rosenzweig et al., 2017). The direct observation of the Rubisco small subunit, RBCS1, fused to YFP also resolved the organization of the pyrenoid matrix as a liquid whose contents can mix internally, unlike the more rigid cyanobacterial carboxysomes (Freeman Rosenzweig et al., 2017). The introduction of the Rubisco linker protein EPYC1 into plants (or a version that can interact with plant Rubisco) may be an important

step toward the engineering of plants to form pyrenoids to enhance photosynthetic output.

Channelrhodopsins and Optogenetics

For a unicellular organism, *C. reinhardtii* possesses a surprisingly large number of putative photoreceptors, including 12 rhodopsins that bind retinal (Greiner et al., 2017). As suggested by their names, channelrhodopsins (ChRs) behave as light-activated ion channels to conduct protons and sodium cations across the membrane, normally to elicit cilia movement. Chlamydomonas ChRs decrease the local membrane potential in Chlamydomonas and after transient or stable transformation of the genetically encoded photoreceptor in cell cultures, nematodes, fruit flies (*Drosophila melanogaster*), and mammals via a method known as optogenetics (Deisseroth and Hegemann, 2017). The chromophore for ChRs, all-*trans*-retinal, is widespread from algae to mammals, although the feeding of retinal analogs can shift wavelength sensitivity (Foster et al., 1984).

When expressed in neurons and activated by light, ChRs elicit neuronal firing only in the cell types and within the membrane domain where they are expressed. Genetic engineering applied to ChRs created variants in the duration of the channel's open state, which drive longer neuronal activation upon stimulation by blue light and can be closed on command using green or yellow light pulses. Genome mining added to the optogenetics molecular toolkit, yielding rhodopsins that respond maximally to other wavelengths or cause hyperpolarization of the plasma membrane, resulting in the inhibition of neuronal impulses. Together with the technical innovations that supported the precise delivery of light pulses to single cells, optogenetics has opened up new avenues in the neurosciences by disturbing neural excitation and observing behavioral consequences, therefore going much beyond the simple recording of neuronal activity (Zhang et al., 2007; Rost et al., 2017).

Cilia Biogenesis and Function

C. reinhardtii uses cilia for phototaxis to optimize light exposure during photosynthesis and for cell-cell recognition during mating. It became clear during early mutant screens that cilia are dispensable in this organism in a laboratory setting. The same cannot be said for most eukaryotic systems that have retained cilia; mutations in human ciliary proteins lead to diseases (referred to as ciliopathies) that include male infertility, kidney and eye malfunctions, polydactyly, and brain disorders. Fungi (including budding yeast) and seed plants have lost genes for cilia, but the overall structure of cilia is well conserved between *C. reinhardtii* and other ciliated eukaryotes.

The biogenesis of *C. reinhardtii* cilia has been dissected genetically, biochemically, and microscopically over the past several decades. Transmission electron microscopy analysis of wild-type and mutant cilia helped identify affected structures associated with various motility defects in mutants (Goodenough and Heuser, 1982, 1985a). A comparison of the protein complement within the cilia core (the axoneme) between wild type and various *paralyzed flagella* (*pf*) mutants can provide clues about function.

Perhaps unique to *C. reinhardtii* is the analysis of dikaryons: newly fused gametes exist for several hours as tetraflagellate diploids whose cytoplasm is derived from two genotypes, allowing the study of rescue or complementation of mutant ciliary phenotypes (Dutcher, 2014). Intact cilia detach from cells following a pH shock or mechanical shearing, lending themselves to biochemical analysis: the cilium proteome is composed of over 1000 proteins, as identified by mass spectrometry (Pazour et al., 2005). Based on genome sequences, proteins found in ciliates (animals, *C. reinhardtii*, *Caenorhabditis elegans*) but missing in fungi and plants were compiled in the “CiliaCut” list, consisting of ~200 proteins (Li et al., 2004; Merchant et al., 2007), over half of which were detected in the cilium proteome. One major task will be to associate each protein with a function in cilia biogenesis and control, which will be aided by the availability of large insertion mutant libraries (Li et al., 2016; Cheng et al., 2017b).

Cilia grow from their tips, but their structural constituents are synthesized in the cytosol and must therefore be transported to the growing tip. Using differential interference contrast microscopy, researchers in Rosenbaum’s laboratory noticed particles moving up and down the length of cilia between the membrane and doublet microtubules, which were most readily visualized in mutants with paralyzed cilia (Kozminski et al., 1993). This intraflagellar transport (IFT) system was first described and characterized in *C. reinhardtii* (Piperno and Mead, 1997). Concurrently, technical advances in microscopy allowed the direct visualization of nonmotile (or primary) cilia in mammals, and a mouse (*Mus musculus*) model was developed for polycystic kidney disease using an insertional mutant (Moyer et al., 1994; Satir, 2017). The gene disrupted in the mouse model has one *C. reinhardtii* homolog, encoding a protein belonging to the IFT proteome: IFT88 (Pazour et al., 2000). *ift88* mutants in *C. reinhardtii* and mouse have short or no flagella. The major polycystin proteins PC1 and PC2 were later found to localize to the primary cilia membranes of kidney cells after being transported there along the IFT system (Pazour et al., 2002).

In Situ Electron Cryotomography

By necessity, researchers tend to focus on a handful of genes to uncover the mechanisms controlling their expression and the functions of their products, with the ultimate goal of placing the encoded proteins into the (much larger) context of a living cell. *C. reinhardtii* cells are composed of several compartments, each with a specialized function that is reflected in its structure. While microscopic observations of fixed cells have revealed much, sample preparation may cause artifacts and distort the underlying structures. Protocols based on living cells face their own challenges and are limited to locating single molecules within the cell at the expense of cellular structures. Quick-freeze, deep-etch techniques afford a glimpse of the substructure of the axoneme but remain limited to the exposed surface (Goodenough and Heuser, 1982, 1985a). Recent advances in in situ electron cryotomography and computer-assisted 3D reconstruction bring the promise of imaging organellar structures and protein aggregates. In the early days, this technique was hampered by the dense cytoplasm of yeast cells, but it worked very well in *C. reinhardtii*, especially for the *mat3* mutant, which is defective in the

algal homolog of the *RETINOBLASTOMA* gene (Umen and Goodenough, 2001a). Smaller cells are indeed advantageous during vitrification (Albert et al., 2017). Flash-frozen cells still need to be reduced to sections of 300 nm or less for optimal resolution, a process currently attained via focused ion beam-mediated thinning of vitrified cells (Schaffer et al., 2017). In recent years, Engel and coworkers have successfully visualized the chloroplast and pyrenoid, Golgi apparatus, COPI-coated vesicles, proteasome, and nuclear pore complex of *Chlamydomonas* (Engel et al., 2015; Albert et al., 2017; Bykov et al., 2017; Freeman Rosenzweig et al., 2017). These recent advances highlight the potential for visualizing organellar networks in unmatched detail, as well as protein complexes of moderate size in their natural state and place. For instance, thylakoid membranes are connected to the pyrenoid via tubules embedded into the starch sheath surrounding the pyrenoid and may therefore provide an access route to deliver CO₂ to the Rubisco enzymes concentrated inside the pyrenoid (Engel et al., 2015). These tubules also contain minitubules that are only wide enough for unidirectional trafficking, for example to deliver ATP to Rubisco activase and to export sugars from the pyrenoid after carbon fixation. Within the pyrenoid matrix, densely-packed Rubisco complexes were initially thought to assemble into hexameric ring-like structures (Mackinder et al., 2016). However, more recent higher-resolution imaging resolved Rubisco distribution within the matrix as liquid-like rose (Freeman Rosenzweig et al., 2017).

While a protein complex is tentatively identified based on its 3D shape, its true identity requires the confirmation that the corresponding *C. reinhardtii* genes exist. To bridge the gap between the observation and identification of proteins, it should be possible to add a tag to a protein of interest that is small enough to maintain the overall shape and structures within the cell but bulky enough to be observed in tomograms.

HISTORY OF *C. REINHARDTII*

Early Observations

Where did this unicellular alga come from and how did it emerge as a reference organism? The *Chlamydomonas* genus was first mentioned in the 1830s, when the German naturalist Christian Ehrenberg drew *Chlamydomonas* and *Volvox* (Ehrenberg, 1838). Naturalists described the world around them, and Ehrenberg was truly prolific (Jahn, 1998). His *Chlamydomonas* [sic] drawings are rather underwhelming and do not foretell its scientific future (Figure 1). Ehrenberg’s slides and samples reside at the Museum für Naturkunde in Berlin (Lazarus and Jahn, 1998).

Early observations of unicellular algae illustrated two key properties: sexual reproduction and phototaxis. The basis of sexual reproduction was a major topic of inquiry and curiosity, and unicellular algae were a prime research subject, as all steps of the sexual cycle can be readily observed by examining isolates from soil or freshwater ponds under a microscope (Johnson, 1914). The observation of aggregation and fusion of gametes under a microscope offered early evidence that *Chlamydomonas* cells are present as two mating types. Likewise, Faminzin (1866) described phototaxis in *Chlamydomonas* and *Euglena*. The alga was

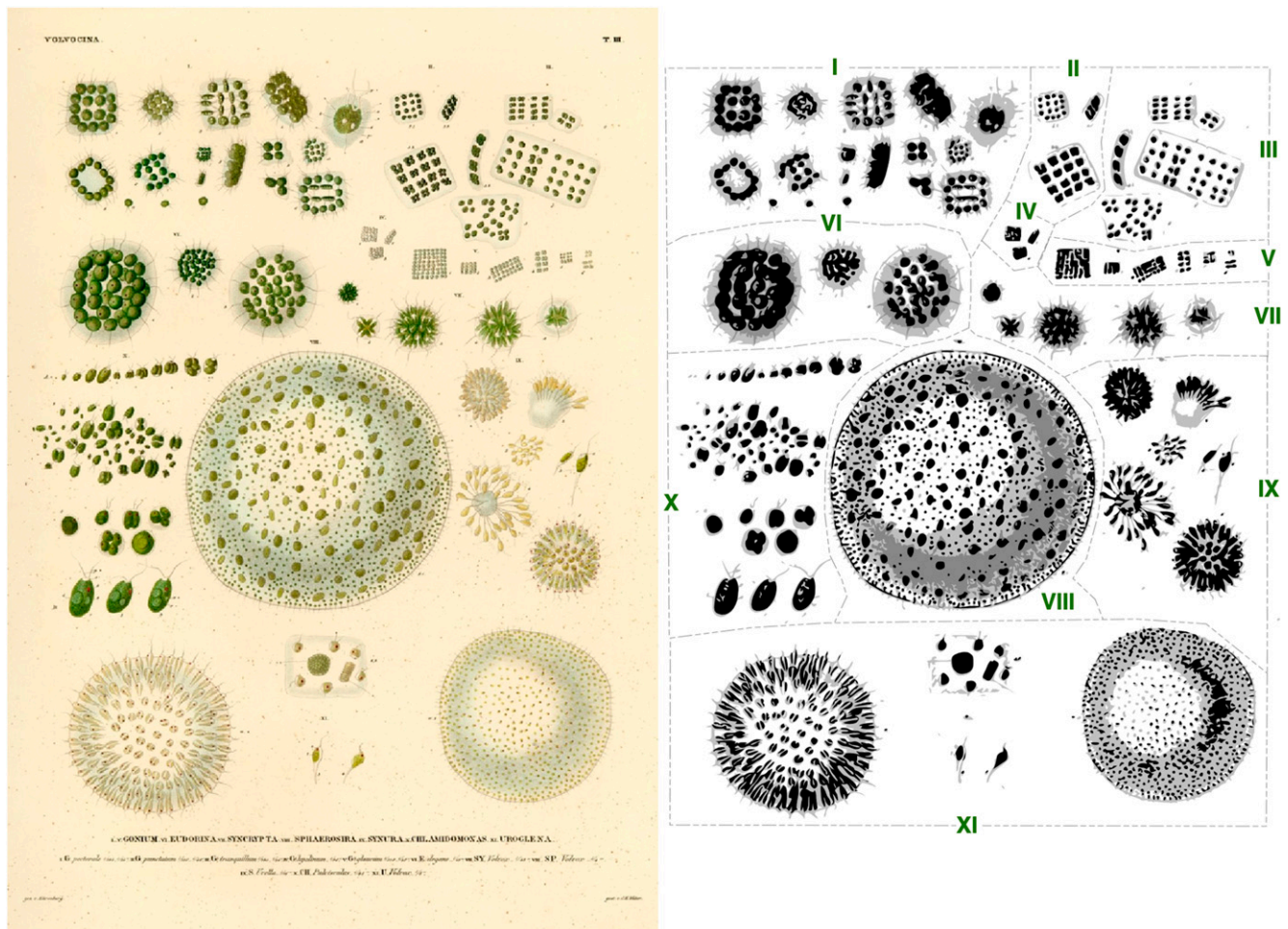


Figure 1. Taxonomic Basis of Chlamydomonas and Volvox.

Ehrenberg's drawings of Chlamydomonas and Volvox cells, published in 1838. Cells that belong to the same species are indicated by Roman numerals in the right panel. Reproduced with permission, Museum für Naturkunde, Berlin. I, *Gonium pectorale*; II, *Gonium punctatum*; III, *Gonium tranquillum*; IV, *Gonium hyalinum*; V, *Gonium glaucum*; VI, *Eudorina elegans*; VII, *Syncrypta volvox*; VIII, *Sphaerosira volvox*; IX, *Synura uvella*; X, *Chlamydomonas pulvisculus*; XI, *Uroglena volvox*. The species was identified as *Chlamydomonas pulvisculus* but renamed *Chlamydomonas reinhardtii* in 1888.

renamed from *Chlamydomonas pulvisculus* (the species drawn by Ehrenberg) to *C. reinhardtii* by the French botanist Pierre Dangeard in honor of the Ukrainian botanist Ludwig Reinhardt in 1888, and the new species name caught on.

This propelled Chlamydomonas to become a promising system to study sexual reproduction and phototaxis, possessing much of the convenience normally associated with microbes but with the added benefit of sexual dimorphism (Johnson, 1914). A report of phenotypic segregation in crosses between two Chlamydomonas species (Pascher, 1918) empirically confirmed Mendel's segregation rules, and it introduced tetrad analysis as a powerful tool for the genetic dissection of segregation.

Early Genetics in Chlamydomonas

Chlamydomonas research first entered the spotlight in the 1930s with Franz Moewus' biochemical genetics studies. Moewus proclaimed to have identified several sexual hormones related

to carotenoids in the Chlamydomonas strain *Chlamydomonas eugametos* that activated male or female gametes selectively. He also reported the isolation and genetic mapping of mutants along their biosynthetic pathway. He described the analysis of 200,000 zygotes for 10 phenotypes scored over a 10-year period, which represents an enormous amount of work for anyone (Gowans, 1976). Unfortunately, most of his results could not be reproduced, even by his own hands, casting doubt on his scientific claims.

The influence of Franz Moewus on modern Chlamydomonas research is, nevertheless, undeniable (Sapp, 1987). First, Moewus demonstrated that Chlamydomonas mutants could be isolated. His results, however implausible, remarkably supported the correct view that genes are located on chromosomes and that genetic mutations could result in the loss of enzymatic activity (the one gene-one enzyme concept). Second, as discussed below, these results motivated Gilbert Morgan Smith to isolate (and later share) *C. reinhardtii* strains in order to repeat Moewus' experiments.

The Modern *C. reinhardtii* Era and Gilbert Smith

Smith almost dropped out of high school and only discovered a keen interest in biology and botany when he was sent to a remedial academy. There, he gained enough confidence to enroll in college (Wiggins, 1962). During his Ph.D. work, he developed techniques to isolate pure cultures from field samples. He eventually moved to Stanford to become a Botany Professor until he retired in 1950. Smith was keen to repeat some of Moewus' experiments about *Chlamydomonas*, which he even made the focus of his address as the retiring President of the Botanical Society of America (Smith, 1946). Smith spent a year cultivating and separating mixed cultures into pure isolates. Of the over 700 soil samples collected all over the United States, he isolated several *Chlamydomonas* colonies. He specifically isolated spores rather than haploid vegetative cells, thereby producing the mating pairs required for genetic analysis. One of the 15 species Smith collected is the modern *C. reinhardtii* strain now in use worldwide.

Smith was not the only biologist isolating sexually competent algae, but he was lucky. On the East Coast, Luigi Provasoli identified a pair of *Chlamydomonas moewusii* strains (Provasoli et al., 1951) and shared them with Ralph Lewin, who was himself interested in recombination and sexual inheritance. Lewin isolated the first mutants with paralyzed flagella (Lewin, 1954) but quickly discovered that *C. moewusii* was not suited for mutants with photosynthetic defects, being an obligate photoautotroph (Lewin, 1950). Luckily for the field, *C. reinhardtii* is a facultative photoautotroph, can survive without photosynthesis if provided a reduced carbon source such as acetate, and remains green in the dark. Photosynthetic mutants emerged from work by Paul Levine using the Smith strains (Levine, 1960a).

Sharing Is Caring

Smith was indeed generous and shared his pure algal cultures. (Moewus was not, perhaps for the better, although his strains did end up in various hands over the years and are now available in algal repositories as *C. eugametos Moewus*.) Smith sent mating pairs to a number of eager colleagues (Ruth Sager at Rockefeller University; Bill Ebersold, David Regnery, Bill Eversole, and Edward Tatum, all at Stanford; John N. Hartshorne at the University of Manchester). Smith and Regnery determined that the inheritance of sexuality in *C. reinhardtii* was exclusively heterothallic (Smith and Regnery, 1950). At first, these experiments were limited to the segregation of the mating locus, but soon Smith, Tatum, Ebersold, and Eversole accumulated mutants to serve as phenotypically tractable markers.

Mutants in nuclear genes segregated in the 2:2 ratio typical for a haploid genome, but some mutants showed a 4:0 segregation ratio, providing evidence for non-Mendelian segregation. This piqued the interest of Sager, who isolated mutants conferring resistance to streptomycin whose causal locus mapped to the chloroplast genome (Sager, 1954; Ohta and Sager, 1975). She also discovered nutritional control of sexuality in *Chlamydomonas*: vegetative cells starved for nitrogen (and of opposite mating types) will readily fuse to form zygotes that, after a few days in the dark, will undergo meiosis and germinate when plated onto nitrogen-replete medium in the light (Sager and Granick, 1954).

Around the same time, Paul Levine, a *Drosophila* geneticist wishing to study recombination, was convinced by Sager and Lewin to switch to *C. reinhardtii* (Goodenough, 2015). Levine set up over 250 crosses between 45 mutants gathered by Smith, Tatum, Eversole, and Ebersold (Levine and Ebersold, 1960) and published the first algal genetic maps (Ebersold and Levine, 1959; Ebersold et al., 1962).

The isolation of many mutants between 1950 and 1960 laid the foundation for cilia biology and photosynthesis research. Analysis of *pf* mutants with paralyzed flagella demonstrated that cilia are dispensable for viability in laboratory settings and offered the promise of genetic analysis. Similarly, the *ac* mutants consolidated *C. reinhardtii*'s position as a system of choice for photosynthetic research decades before *Arabidopsis* research took off. Levine posited that *ac* mutants were defective in some step of the photosynthetic process because they could not fix radioactive CO₂, the prototypical mutant being *ac-21* (Levine, 1960b). This strain carries a mutation in the chloroplast Rieske iron-sulfur protein PETC. Other genes identified from the *ac* screen include those encoding plastocyanin (*ac-208*), factors involved in the biosynthesis of cytochromes *b* (*ac-141*) and *f* (*ac-206*) from the cytochrome *b₆f* complex, and factors involved in the biosynthesis of the PSII D2 protein (*ac-115*; Harris, 2008). All mutants were isolated in the strain CC-124 and CC-125 backgrounds (originally named 137c until they were deposited at the *Chlamydomonas* Resource Center; Table 1), one of the mating pairs from Smith.

Overcoming Limitations to Transformation and Transgene Expression

While the 1960s and 1970s were a fertile period for *C. reinhardtii* research, the 1980s witnessed the introduction of recombinant DNA, allowing the cloning and propagation of genes in bacteria, as well as DNA sequencing, paving the way for the molecular characterization of classical mutants. The functional rescue of a mutant via the introduction of an intact copy of the presumptive causal gene demands transformation protocols, which were developed fairly early on for yeast (Hinnen et al., 1978) and *Drosophila* (Rubin and Spradling, 1982) but initially seemed out of reach for the *Chlamydomonas* community, possibly due to poor penetrance of selection markers. The unusually high GC content of the *Chlamydomonas* genome (Chiang and Sueoka, 1967a) was thought to contribute to the poor expression of some selectable markers (Rochaix and van Dillewijn, 1982; Cerutti et al., 1997), but the expression of foreign genes with matching GC content fared just as poorly (Stevens et al., 1996), and the number of confirmed transformants was always low (Debuchy et al., 1989; Kindle et al., 1989; Diener et al., 1990; Mayfield and Kindle, 1990).

A major breakthrough in *Chlamydomonas* nuclear transformation came about in 1990, with the development of a modified yeast transformation protocol based on agitation of cells in the presence of glass beads coated with DNA (Kindle, 1990). Previous transformation methods had necessitated the use of biolistic gene guns to cross the cell wall and deliver DNA to the nucleus. A critical innovation was the weakening of cell wall strength to facilitate the entry of DNA into the cell, either genetically or following treatment with autolysin, a hydrolase secreted by gametes during mating that digests the cell wall. Electroporation was later applied to any

Table 1. Community Resources and Biological Repositories for Chlamydomonas Research

Genome information	
Phytosome	https://phytosome.jgi.doe.gov/pz/portal.html#info?alias=Org_Creinhardtii
European Nucleotide Archive	https://www.ebi.ac.uk/ena/data/view/GCA_000002595
UCLA MCDB/UCSC genome browser	http://genomes.mcdb.ucla.edu/index.html
Chloroplast genome	https://www.chlamycollection.org/chloro/genome.html
Metabolic pathways	https://www.genome.jp/kegg/genome/plant.html
PANTHER Gene Ontology	http://www.pantherdb.org
Algal repositories and stock centers	
Chlamydomonas Resource Center	https://www.chlamycollection.org
University of Texas at Austin	https://utex.org
University of Göttingen (SAG)	https://www.uni-goettingen.de/en/culture+collection+of+algae+%28sag%29/184982.html
CLiP mutants	https://www.chlamylibrary.org https://www.chlamycollection.org/products/clip-strains/
Niyogi CAL mutants	https://www.chlamycollection.org/products/niyogi-cal-strains/
ts mutants Cross	https://www.chlamycollection.org/products/tulin-and-cross-2014-strains/
Spatial interactome collection	https://www.chlamycollection.org/products/chlamydomonas-spatial-interactome-csi-collection/
Photosynthesis mutants	http://chlamystation.free.fr
Online cultivation and transformation protocols	
Chlamydomonas Resource Center	https://www.chlamycollection.org/methods/
EMBO Practical Course protocols	http://www.chem.ucla.edu/dept/Faculty/merchant/pdf/embo_protoc_2006.pdf
Other resources	
ZFN designer	http://bindr.gdcb.iastate.edu/ZFNGenome
microRNA designer	http://wmd3.weigelworld.org/cgi-bin/webapp.cgi
Christian Ehrenberg at Naturkunde Museum, Berlin	http://download.naturkundemuseum-berlin.de/Ehrenberg/
Early photosynthesis work by Wollman and colleagues	http://www.ibpc.fr/UMR7141/en/home/publications/

strain with efficiencies equal to or higher than the glass bead method (Shimogawara et al., 1998). Organellar transformation was achieved with biolistics (Boynton et al., 1988; Remacle et al., 2006) and was instrumental in the dissection of plastid and mitochondrial gene function; in this case, transgenes are not silenced and are expressed at high levels.

Sequencing the algal genome in the 2000s (Merchant et al., 2007) introduced the community to whole-genome transcriptome profiling, an approach made even more powerful by the microbe-like nature of *C. reinhardtii*: unicellular, fast-growing, and very amenable to quick changes in growth medium composition or growth conditions.

Such is the history of the rise of *C. reinhardtii* as a model for green unicellular algae.

CHARACTERISTICS

Morphology

Since the 1800s, algal taxonomy has been based on distinguishing cellular features. *C. reinhardtii* is unicellular, oval-shaped, and measures $\sim 5 \times 10 \mu\text{m}$. The cell is surrounded by a cell wall composed of glycoproteins and carbohydrates, arranged in seven layers, as seen in quick-freeze, deep-etched samples (Goodenough and Heuser, 1985b). During the course of sexual reproduction, gametes shed their cell wall before fusion. Many laboratory strains carry *cell wall* (*cw*) mutations and lack cell walls but are fully viable, although they are perhaps more sensitive to some stressors and may be more difficult to cross due to their

shorter cilia. Transmission electron microscopy best reveals the underlying cellular anatomy, as shown in Figures 2A and 2C and in the cartoon in Figure 2B. The cell is characterized by a pair of cilia of equal length located at one pole (Figures 2D and 2E). Chlamydomonas cilia (previously called flagella) are prototypes of motile animal cilia, and their axonemes show the same 9+2 symmetry consisting of nine microtubule pairs surrounding the central pair microtubules (Figure 2F). Close to the same pole, the cell accumulates vacuoles of various sizes, named contractile vacuoles, that control intracellular tonicity by expelling excess water that comes in through a passive gradient (as most Chlamydomonas algae are freshwater algae). These vacuoles are only dynamic under hypo-osmosis: small vacuoles fuse into a larger vacuole, which eventually fuses with the plasma membrane and releases its contents outside the cell (Luykx et al., 1997; Xu et al., 2016). The main osmolyte is thought to be potassium, while water molecules are brought into the contractile vacuole via aquaporin channels (Xu et al., 2016).

A single cup-shaped chloroplast occupies over half the cell volume (Sager and Palade, 1957). Starch granules are found between thylakoid membrane stacks and surrounding a spherical structure located at the pole opposite the cilia known as the pyrenoid. This structure is the site of CO₂ fixation, a process facilitated by high local concentrations of the enzymes Rubisco, Rubisco activase, and EPYC1 (Mackinder et al., 2016; Freeman Rosenzweig et al., 2017). Finally, the eyespot is a specialized domain of the chloroplast membranes containing carotenoid granules. The eyespot reflects light back onto ChR-type photoreceptors located in the overlaying plasma membrane to control light-mediated flagellar movement (Eitzinger et al., 2015;

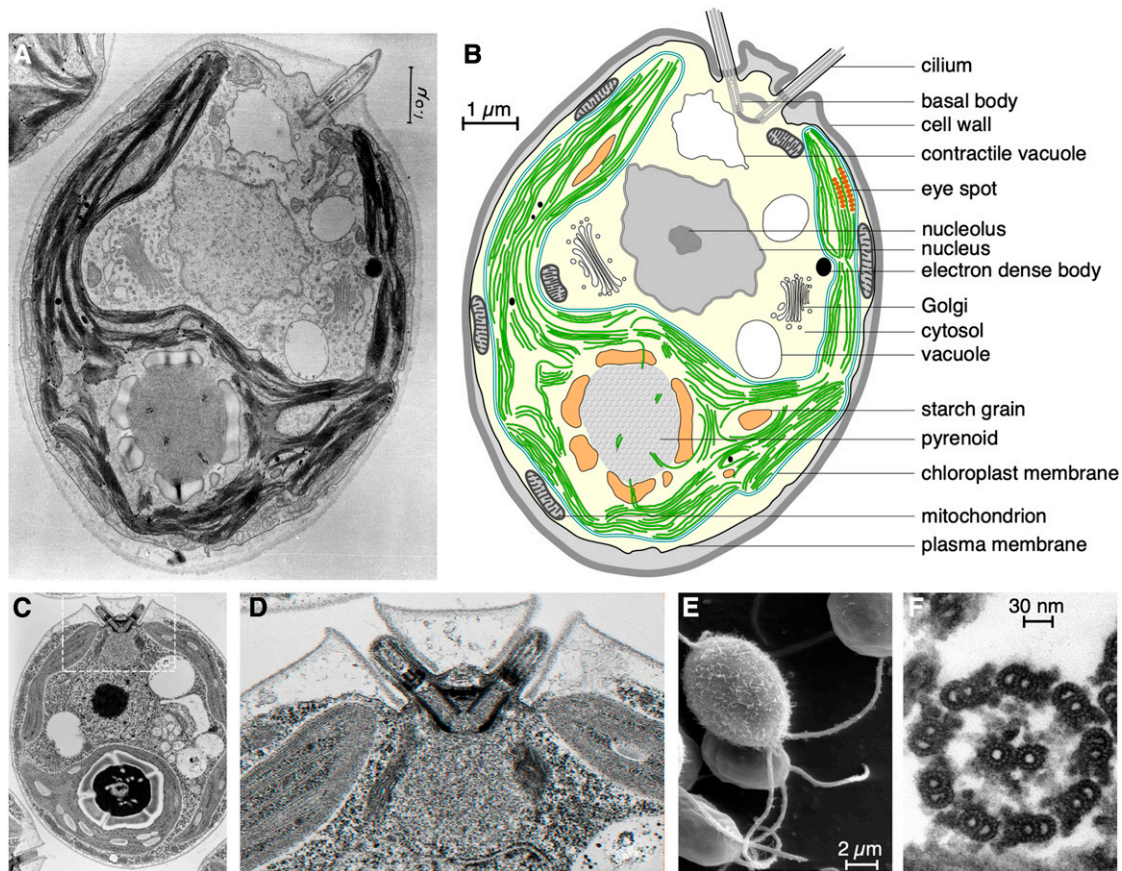


Figure 2. Chlamydomonas Anatomy.

(A) Transmission electron micrograph (TEM) of a cell. Originally published by Ohad et al. (Ohad et al., 1967) and made available on the Cell Image Library website (CIL:37252, *C. reinhardtii*. CIL. Data set: <https://doi.org/doi:10.7295/W9CIL37252>).

(B) Drawing of a *C. reinhardtii* cell based on the TEM image in **(A)**.

(C) and **(D)** Another TEM of a *C. reinhardtii* cell, showing a complete basal body (white box, enlarged in **[D]**), nucleus, and pyrenoid. Image courtesy of Dr. William Dentler (University of Kansas).

(E) Scanning electron micrograph of *C. reinhardtii* cells. Note the two cilia per cell.

(F) TEM of a cross section through isolated axonemes.

The images in **(E)** and **(F)** were generated at the Dartmouth College Rippel Electron Microscope Facility by Louisa Howard, Elizabeth Smith, and Erin Dymek (Smith and Lefebvre, 1996).

Engel et al., 2015). The chloroplast envelope protein EYE2 connects pigment granules inside plastids to the cytoskeleton outside the organelle. In addition, EYE2 contributes to the positioning of the single eyespot to one side of the chloroplast so that cells can detect the direction and intensity of incoming light for phototactic movement (Boyd et al., 2011).

Vegetative Life Cycle

When resources are not limiting, Chlamydomonas multiplies asexually by simple mitotic division. Each daughter cell will carry the identical genetic information encoded by the nuclear, mitochondrial, and chloroplast genomes of its parent (Figure 3). The time to divide is dictated by two check-points during the G₁ phase of the cell cycle and is based on cell size. The first check-point, termed “commitment,” is equivalent to “start” in budding yeast

and marks the irreversible decision to divide at least once (Cross and Umen, 2015). The second check-point measures the volume of the mother cell and fixes the number of successive rounds of S phase and mitosis to produce daughter cells of equal size (Umen and Goodenough, 2001a). Therefore, like many chlorophyte algae, *C. reinhardtii* undergoes multiple fission divisions during mitosis, unlike budding yeast, where one daughter buds off the mother. Cells grown in constant light can divide every 8 to 10 h, but the cell cycle can be lengthened to 24 h by imposing daily light-dark periods. These conditions provide very good synchronization of cell populations and allow the biochemical, physiological, and molecular characterization of events leading to cell division (Chiang and Sueoka, 1967a, 1967b; Voigt and Münzner, 1987; Tulin and Cross, 2014; Zones et al., 2015). Cilia resorb before cell division and provide a visual marker for cell-cycle progression. After division(s), the daughter cells remain enclosed within the

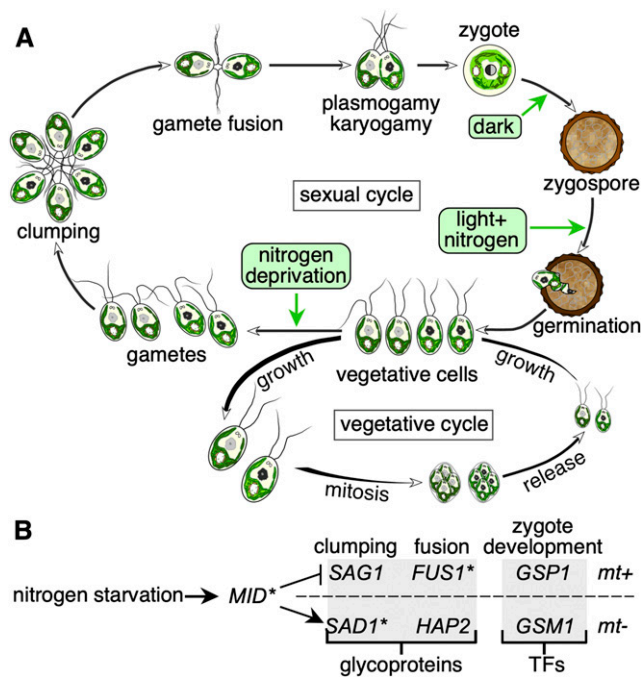


Figure 3. *Chlamydomonas* Life Cycles.

(A) The sexual and vegetative cycles of *C. reinhardtii*. See text for details. Adapted from a figure originally drawn by Karen VanWinkle-Swift and published in *The Chlamydomonas Sourcebook*, Volume 1 (Harris, 2008).

(B) Genes involved in gamete differentiation, agglutination, fusion, and zygote development as a function of mating type. *SAD1*, *SAG1*, *FUS1*, and *HAP2* encode glycoproteins critical for gamete mating type recognition, while *GSP1* and *GSM1* encode transcription factors (TFs) important for zygote development. *MT*-linked genes are indicated by asterisks. Adapted from Joo et al. (2017).

mother cell wall until they grow new cilia, which then release the hatching enzyme sporangin responsible for the degradation of the mother cell wall (Kubo et al., 2009). Therefore, under diurnal conditions, cells lack cilia at night but synthesize them anew before the next dawn (Figure 3A; Cross and Umen, 2015; Zones et al., 2015; Strenkert et al., 2019). Algae such as *C. reinhardtii* possess a circadian clock that shares some similarity with known *Arabidopsis* clock components, which generate oscillations with strong amplitudes and help synchronize cellular metabolism and cell division to imposed diurnal cycles (Matsuo et al., 2008; Noordally and Millar, 2015; Müller et al., 2017). Biosynthetic pathways for classical phytohormones are present in most algae, but the genes encoding their associated receptors are mostly missing from algal genomes (Lu and Xu, 2015; Wang et al., 2015a). The addition of individual phytohormones can affect growth rates, but the underlying molecular mechanisms are currently unclear. Perhaps hormones found in land plants have been coopted from existing biosynthetic pathways during evolution.

Reproductive Cycle

Under more adverse conditions of nutrient scarcity, vegetative cells differentiate into gametes of one of two mating types, *mt+* and

mt-, as determined genetically by the *MT* locus. Nitrogen deprivation induces gamete differentiation in the laboratory (Sager and Granick, 1954). Cells on solid medium may already be nitrogen-limited if grown for more than 5 d and are therefore sexually competent cells. Exposure to light, perceived by the blue light photoreceptor phototropin (Huang and Beck, 2003), commits nitrogen-starved cells to the gametic fate, but this step is reversible by incubation in darkness. *C. reinhardtii* is isogamous, as both gametes are identical in terms of size and morphology.

Gametic cilia are decorated by hydroxyproline-rich glycoproteins called agglutinins used for cell-cell recognition. Each mating type expresses a distinct agglutinin: *SEXUAL ADHESION1* (*SAD1*) in *mt-* gametes and *SEXUAL AGGLUTINATION1* (*SAG1*) in *mt+* gametes (Ferris et al., 2005). *SAD1* and *SAG1* share the same secondary structure but only weak sequence similarity (Ferris et al., 2005). Only *SAD1* maps to the mating locus. The proper expression of agglutinins is dictated by the transcription factor *MINUS DOMINANCE* (*MID*; Ferris and Goodenough, 1997; Lin and Goodenough, 2007). *MID* is found within the *mt-* mating locus, is expressed upon nitrogen starvation, and is necessary and sufficient to specify the *mt-* fate by contributing to *SAD1* induction in *mt-* gametes while preventing *SAG1* expression (Lin and Goodenough, 2007). The *mt+* mating locus encodes a third agglutinin critical for cell fusion: *FUS1*. As illustrated in Figure 3, *mt+* and *mt-* gametes first clump in oriented groups. Shortly thereafter, pairs of gametes of opposite mating types break off from the clump and fuse to form a diploid zygote. *sad1* and *sag1* mutants cannot form clumps, but *fus1* mutants can (Ferris et al., 1996, 2005).

Following agglutination and pairing, cells release an enzyme (autolysin) that digests the cell wall (Matsuda et al., 1978). Specialized mating structures between the cilia are activated to connect the two gametes: a fertilization tubule for the *mt+* gamete and a domed structure for the *mt-* gamete. In a parallel to ciliary recognition during agglutination, proteins decorate these mating structures and take on the appearance of a fringe in transmission electron micrographs (Goodenough et al., 1982). The glycoprotein *FUS1* is a fringe component from *mt+* gametes (Ferris et al., 1996). The *mt-* counterpart to *FUS1* is *HAPLESS2* (*HAP2*; Liu et al., 2008). *fus1* and *hap2* gametes cannot fuse to form a diploid zygote (Goodenough et al., 1978; Liu et al., 2008). Compatible mating structures fuse, followed by the shortening of the fertilization tube prior to cell fusion. The early zygote has four cilia that are resorbed after 1 to 2 h.

Agglutination is accompanied by a transient increase in intracellular cAMP levels. Treating cells with dibutyryl-cAMP (a more soluble form of cAMP) and with papaverine or isobutylmethylxanthine (inhibitors of phosphodiesterases) initiates the formation of the fertilization tubule and dome, triggers the release of the gametic cell wall, and leads to gametic fusion into a zygote. In fact, dibutyryl-cAMP and isobutylmethylxanthine can rescue agglutination-defective and paralyzed flagella gametes and allow progression through the sexual cycle (Pasquale and Goodenough, 1987; Dutcher, 1995).

Zygote development is initiated by the dimerization of the homeodomain transcription factors *GAMETE-SPECIFIC PLUS1* and *MINUS1*, which are contributed by the *mt+* and *mt-* gametes, respectively (Lee et al., 2008). *GSP1* and *GSM1* are

cytosolic proteins in gametes, but they translocate to the nucleus upon fusion and trigger the zygote developmental program; the regulatory cascade of the algal sexual cycle is summarized in Figure 3B. Diploid zygotes plated on solid growth medium lacking nitrogen quickly secrete a cell wall that acts as a chemical and physical protectant barrier (Figure 3). Cell wall formation starts soon after gamete fusion from hydroxyproline-rich glycoproteins (Suzuki et al., 2000) and later becomes more desiccation-resistant via the activity of the polyketide synthase PKS1 (Heimerl et al., 2018).

Based on electron microscopy observations, the two nuclei are the first to fuse following gamete fusion, followed by the two chloroplasts 1 h later (Blank et al., 1978). Inheritance of the chloroplast genome in *C. reinhardtii* is uniparental as a consequence of the complete digestion of the plastid genome originating from the *mt*⁻ parent, although parts of it can escape degradation (and recombine with the *mt*⁺ plastid DNA after fusion) ~10% of the time (Gillham, 1969). This genome elimination is performed within 24 h after mating (before chloroplast fusion) by the nonspecific DNA nuclease MDN (for *Mt*⁺-specific DNase; Nishimura et al., 2002). MDN is thought to reside in the cytosol of *mt*⁺ gametes before relocating to the *mt*⁻ chloroplast upon zygote formation, where high Ca²⁺ concentrations activate its enzymatic activity. It remains unclear how the *mt*⁺ plastid genome escapes degradation and whether plastid DNA methylation plays a role in this process (Lopez et al., 2015). Another nonexclusive scenario calls upon the differential replication rates of the chloroplast genomes derived from the *mt*⁺ and *mt*⁻ gametes during zygote germination (Umen and Goodenough, 2001b). The mitochondrial genome is itself inherited from the *mt*⁻ parent during sexual reproduction (Beckers et al., 1991; Nakamura, 2010).

The isolation and manipulation of zygotes are facilitated by plating mating mixtures on 4% agar plates, as zygotes adhere strongly to the surface of this medium. Zygotes are later induced to germinate (undergo meiosis) via transfer onto rich medium in the light, which is perceived by the blue light photoreceptors cryptochrome and phototropin (Huang and Beck, 2003; Müller et al., 2017; Zou et al., 2017). *Chlamydomonas* can be used for tetrad analysis via the dissection of the four germinated meiotic products, as they carry one or the other mating type, which segregate in a 2:2 ratio. The germinated products globally carry a mosaic of both parental nuclear genomes that have been shuffled during meiosis (Liu et al., 2018).

Systematics of the *Chlamydomonas* Family

Traditional taxonomic keys used to attribute algae to a given genus rely on the presence/absence and position of key cellular features described above (Ettl, 1976; Harris, 2008). Green algae containing a cell wall, two cilia of equal length near each other at one cellular pole, and a single cup-shaped chloroplast with a single pyrenoid at the pole opposite the cilia belong to the genus *Chlamydomonas* Ehrenberg, including *C. reinhardtii* and over 1000 other green algae. Based solely on morphological characteristics observed by microscopy, the Chlorophyceae class of green algae can be subdivided into 10 subgenera or sections, but several of the deciding criteria can also be found in algae that belong to other taxa. In addition, many early descriptions of algae were performed “in the wild” under natural conditions, which often meant small sample size and heterogeneity. Based on analysis

of rRNA and internal transcribed spacer sequences, the Chlorophyceae are now considered to be made up of 18 monophyletic lineages or clades. Representative species within these clades include well-studied algae: *Dunaliella*, *Polytoma*, *Chloromonas*, and *Scenedesmus* (Pröschold et al., 2001; Harris, 2008). These molecular criteria represent a departure from the taxonomic keys, as *Chloromonas* and *Polytoma* lack pyrenoids.

As in animals, members of the same algal species must be interfertile and form diploid zygotes that can later germinate, but interfertility has not been systematically tested. Approximately 300 *Chlamydomonas* strains are available in algae repositories, providing an opportunity to confirm relationships based on phylogenetic analyses and reproductive compatibility.

Modern *C. reinhardtii* is now the archetype for the *Reinhardtinia* clade, to which the multicellular green alga *Volvox carteri* also belongs (Pröschold et al., 2001). The genomes of *C. reinhardtii* and *V. carteri* share extensive synteny; with a sequence similarity of ~74% for mutual best BLAST hits, the two algae are as closely related to each other as humans are to chickens or *Arabidopsis* is to poplar (*Populus* spp; Prochnik et al., 2010). *Volvox* forms colonies of only two cell types: small vegetative cells (~2000) and large germ cells (~16), encapsulated within a spheroid body. Comparative analysis of the *Volvox* and *Chlamydomonas* genomes may provide clues about the evolution of multicellularity (Umen and Olson, 2012).

Most laboratory strains are derived from the isolates provided by Smith to Sager, Ebersold, and Hartshorne. The Ebersold lineage cannot utilize nitrate as its nitrogen source due to two unlinked mutations in the nitrate reductase gene *NIT1* and the transcription factor gene *NIT2*, whose expression is repressed by ammonium (Gallaher et al., 2015). It is not clear when this lineage acquired these mutations, although it probably happened shortly after the strains made their way to Ebersold, since all mutants isolated in this background are unable to grow on nitrate.

Ecology

The *Chlamydomonas* genus is found worldwide, although the presence or absence of an isolate may be more reflective of the distribution of phycologists than of the genus itself (Harris, 2008). The first *C. reinhardtii* isolates were collected in Europe, with more recent isolates coming from eastern North America (Massachusetts, Minnesota, North Carolina, Pennsylvania, Florida, and Quebec) (Harris, 2008; Flowers et al., 2015) and Japan (Nakada et al., 2014). All isolates are interfertile and are therefore considered to belong to the *C. reinhardtii* clade (Harris, 2008; Nakada et al., 2014).

Chlamydomonas species have been collected from a variety of habitats with a vast range in humidity, temperature, and salinity conditions. Species have been found on damp soil surfaces and in fresh and marine water. *Chlamydomonas* species are fairly tolerant to pollution and can also be found in sewage wastewater (Prescott, 1968). Algae can be picked up by strong winds and spread over land; viable algae (including *Chlamydomonas* spp) can be found in the air at high altitude (Brown et al., 1964).

Cobalamin (vitamin B12) is an important cofactor for the Met synthase METH. An alternative Met synthase, encoded by *METE*

(Helliwell et al., 2011), is present in half the eukaryotic unicellular algae, including *C. reinhardtii*. Only *Gonium pectoral* and *Volvox* species lack a functional *METE* gene (Helliwell et al., 2011) and thus depend on exogenous vitamin B12 scavenged from the environment or obtained via the establishment of beneficial interactions with bacteria. The secretion of potential carbon sources for nearby respiring prokaryotes in the form of organic compounds such as acetate, ethanol, and formate by *C. reinhardtii* at night (Yang et al., 2014; van Lis et al., 2017) also speaks to such an interaction.

Whether in a mutualistic interaction or alone, *Chlamydomonas* isolates originating from soil or water samples contain a mixture of cells at any stage along the algal vegetative or sexual cycle (illustrated in Figure 3). Pure *C. reinhardtii* cultures of vegetative haploid cells can be obtained by streaking, serial dilutions, or single-cell isolation with antibiotics to kill off bacteria. Phototaxis can be used to separate algae from bacteria when soil samples are resuspended in water (Gross et al., 1988), as can chlorophyll fluorescence, coupled with fluorescence-activated cell sorting. As a final purification step, single cells may be sprayed onto agar plates and green colonies selected (Wiedeman et al., 1964). Cultures can be maintained on solid agar plates or slants or in liquid cultures with or without bubbling (Figure 4A).

GENOMICS

The *Chlamydomonas* Genome: Sequencing-Aided Genealogy

Until recently, Smith's paired strains were presumed to be isogenic outside of the mating locus. The genealogy of all *Chlamydomonas*

strains was tentatively retraced based on whole-genome sequencing of 39 independent laboratory strains and 12 field isolates (Flowers et al., 2015; Gallaher et al., 2015). Field strains exhibited sequence diversity of ~2.8% between themselves and 2.4% when examined against laboratory strains. A comparison of laboratory strains yielded a number of surprises: (1) laboratory strains are not identical and can diverge by as many as 500,000 single-nucleotide polymorphisms (SNPs), or 0.4% of the genome; this number is lower than the ~2.8% diversity seen between field strains because (2) sequence divergence is concentrated inside polymorphic blocks that display ~2% sequence diversity; (3) these blocks are not random and can be shared by many strains; (4) these blocks come only in two flavors: haplotype 1 from the sequenced reference strain CC-503 and a polymorphic haplotype 2. Haplotype 2 only covers ~25% of the total genome. Laboratory strains can thus be thought of as limited recombinant inbred lines. Since CC-503 is *mt+*, strains of the opposite mating type are polymorphic at the beginning of chromosome 6, where the *MT* locus resides.

How did this happen? It is clear that the strains shared by Smith are not, in fact, equivalent. He sent strains as compatible mating pairs, but no pairs are isogenic, and different pairs were sent to different laboratories. The history of all lineages is likely further muddled by backcrosses since the original gifts. One Ebersold strain gave rise to CC-503, whose genome was sequenced as the reference. The apparent lack of polymorphisms in CC-503 is only an illusion, since polymorphic means "different from CC-503," just like polymorphic in *Arabidopsis* accessions really means "different from the reference accession Col-0." Nonetheless, CC-503 surely carries blocks derived from both its parents and may represent one of the four original zoospores.

One take-home message is "know thy strain." Laboratory strains can behave rather differently under various conditions (Gallaher et al., 2015). Haplotype 2 carries ~65,000 nonsynonymous codon changes as well as ~600 insertions/deletions predicted to have major effects. There is therefore ample room for loss-of-function alleles within haplotype 2, as revealed under the appropriate growth conditions (Flowers et al., 2015; Gallaher et al., 2015).

The *Chlamydomonas* Nuclear Genomic Space

Regardless of strain history, the nuclear genome of *C. reinhardtii* is ~111 Mb and is organized into 17 chromosomes (Figure 5A). These numbers are comparable to those of other algae such as *V. carteri* (Prochnik et al., 2010) and *Chromochloris zofingiensis* (Roth et al., 2017), although the *C. reinhardtii* genome is two to three times larger than those of other green algae such as *Chlorella* spp. The size of the *C. reinhardtii* genome sits between the small genomes of bacteria (*Escherichia coli*) and unicellular non-photosynthetic eukaryotes (yeast) and the larger genomes of plant and animal reference organisms (*Arabidopsis*, *Brachypodium*, rice [*Oryza sativa*], maize [*Zea mays*], and *Homo sapiens*; Figure 5B).

The quality and completion of a genome assembly can be measured based on the number of scaffolds and the sizes of the remaining gaps. The latest *C. reinhardtii* genome assembly release covers ~99.5% of the genome in only 30 scaffolds (17 chromosome-length scaffolds [1.9–9.7 Mb] and 13 smaller scaffolds ranging from 52 to 272 kb). Some gaps remain along the

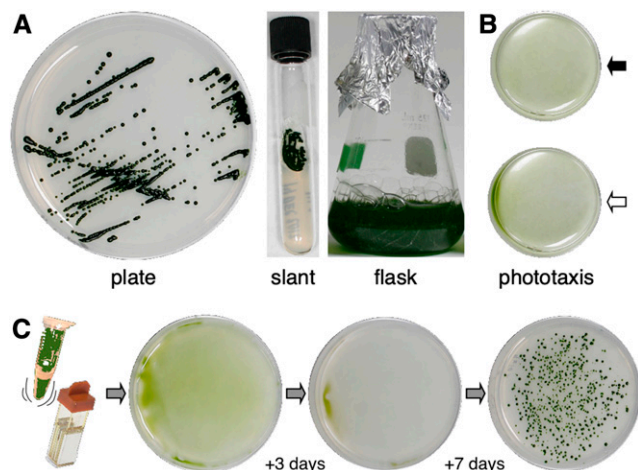


Figure 4. *Chlamydomonas* Culture and Transformation.

(A) *Chlamydomonas* growing on agar plates, slants, and liquid medium. **(B)** Example of negative phototaxis. The white arrow represents directed strong white light. After 30 min, most cells have swum away. **(C)** Transformation of *C. reinhardtii* by electroporation or glass beads. Cultures are grown to a cell density of 1 to 2×10^6 cells/mL and concentrated by light centrifugation before agitation with glass beads or electroporation. After recovery in fresh medium for 8 to 16 h, the cells are plated onto selective plates, and colonies derived from individual transformants develop within 7 to 10 d.

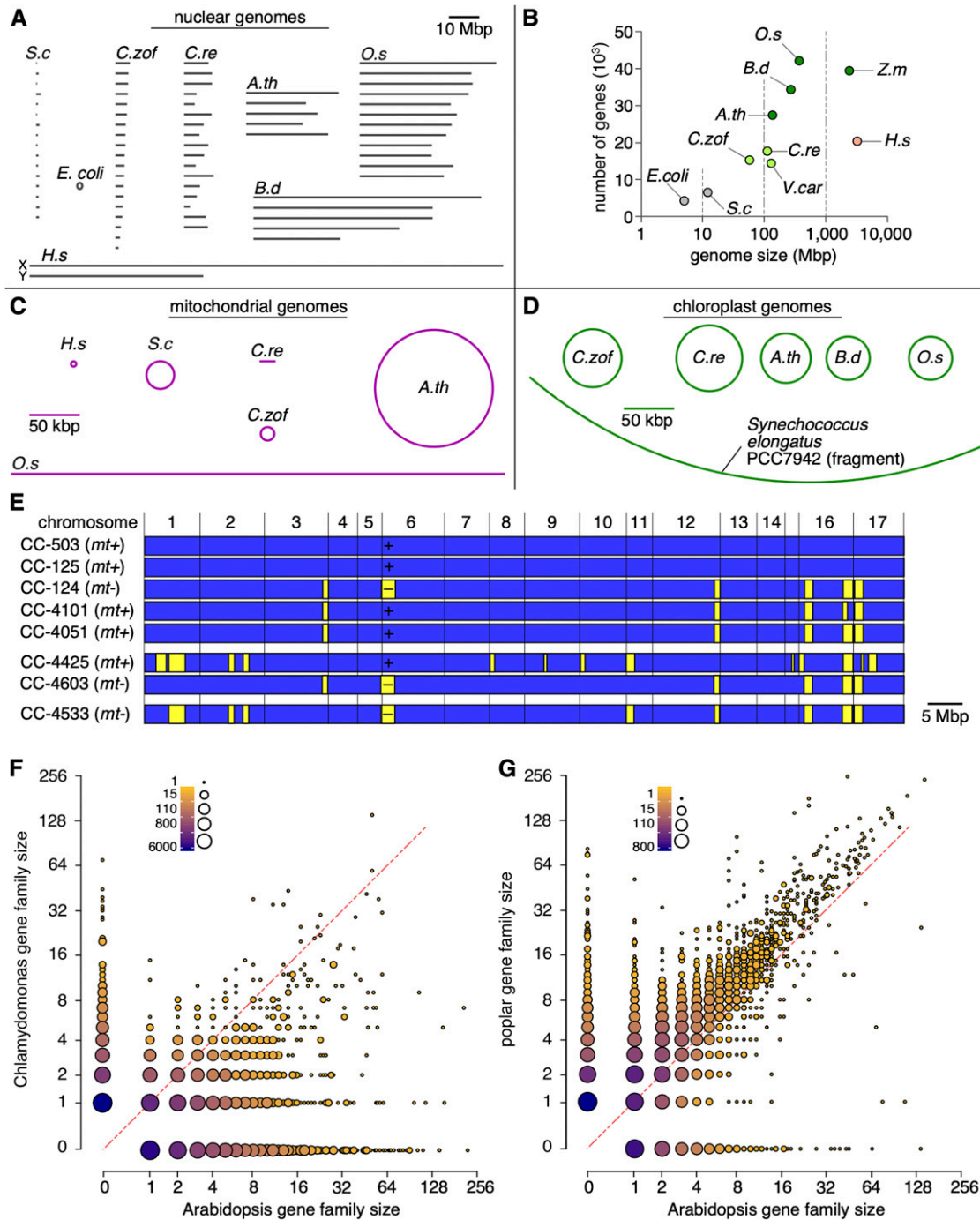


Figure 5. Genome Sizes and Chromosome Numbers in Bacteria, Green Algae, Plants, Yeast, and Humans.

(A) Nuclear genomes. All chromosomes were drawn to scale and ordered by their assigned number. Chromosomes in *C. zofingiensis* were sorted by length (Roth et al., 2017). The human X and Y chromosomes were drawn for reference.

(B) Genome size versus gene space, showing the number of predicted protein-coding loci as a function of nuclear genome size.

(C) The mitochondrial genome is a linear molecule (with inverted repeats at each end for replication) in *C. reinhardtii*, *C. zofingiensis*, and rice.

(D) The plastid genome is a circular molecule in green algae and plants. A portion of the single chromosome from *Synechococcus elongatus* PCC7942 is shown as a reference for the genome size of the cyanobacterium ancestor that gave rise to modern-day chloroplasts. *A.th*, *Arabidopsis thaliana*; *B.d*, *Brachypodium distachyon*; *C.re*, *Chlamydomonas reinhardtii*; *C.zof*, *Chromochloris zofingiensis*; *H.s*, *Homo sapiens*; *O.s*, *Oryza sativa*; *S.c*, *Saccharomyces cerevisiae*; *V.car*, *Volvox carteri*; *Z.m*, *Zea mays*.

(E) Similarities among the genomes of selected strains, represented within the context of the full genome. Haplotype 1 blocks are shown in blue, and haplotype 2 blocks are shown in yellow.

(F) and **(G)** Comparison of gene family sizes between Arabidopsis and Chlamydomonas **(F)** and poplar **(G)**. The number of genes associated with each gene family was extracted from Guo (2013).

genome, but they only account for ~3.6% of the genomic sequence, or half that of the previous 2009 release.

Wild isolates have proven to be very useful for generating genetic maps and mapping mutants (Gross et al., 1988; Kathir et al., 2003; Rymarquis et al., 2005; Liu et al., 2018). During reanalysis of published recombination data, we found that some markers are misplaced. For example, the phytoene synthase gene *PSY* is assigned to chromosome 2 in the latest genome release but maps to chromosome 11 based on our segregation and historical data (Kathir et al., 2003; McCarthy et al., 2004). The misallocation of the *PSY* locus to chromosome 2 happened between 2009 and 2012. The chromosomal locations of several additional markers are not supported by segregation data, suggesting that the physical map can be improved by incorporating recombination data.

The physical locations of centromeres are still unclear. In *Arabidopsis* and rice, centromeric regions contain few genes and are rich in transposons and repetitive sequences that are heavily methylated on cytosine residues (Zhang et al., 2006; Feng et al., 2010). Gene and transposable element densities are relatively uniform in *C. reinhardtii*: the reference genome contains ~850 transposable elements, while *Arabidopsis* boasts over 31,000 transposable elements heavily concentrated at and around centromeres (Zhang et al., 2006; Feng et al., 2010). Cytosine (CG) methylation of genomic DNA is much less prevalent in *C. reinhardtii* (~0.5 versus ~22% for *Arabidopsis*). However, CG methylation is clearly concentrated in discrete genomic regions (Lopez et al., 2015): for 15 of the 17 chromosomes, high CG methylation was detected over one to three genomic regions, often flanking known sequence gaps, themselves indicative of repetitive sequences that are notoriously difficult to assemble. The centromeres of the green alga *C. zofingiensis* were identified by mapping repetitive sequences (Roth et al., 2017). It is therefore reasonable to associate these regions of high CG methylation with *C. reinhardtii* centromeres.

Genome Annotation and Gene Prediction

To facilitate genome annotation, over 200,000 ESTs were generated from cells grown under various conditions and sequenced at both ends (Grossman et al., 2003; Shrager et al., 2003; Asamizu et al., 2004). ESTs were generated from two polymorphic *Chlamydomonas* strains to facilitate the design of molecular markers to anchor physical and genetic maps and for map-based cloning of mutated genes (Kathir et al., 2003; Rymarquis et al., 2005). The *Chlamydomonas* community also undertook a major manual annotation effort and provided functions and symbols for almost 3000 genes (Merchant et al., 2007). Some genes received names based on motifs present in their encoded proteins: for example, Ser-Thr kinases were given the generic name STK. Therefore, gene names do not always equate with functional information. Gene names anchored in functional data can supersede historical names (from the original mutant): *ac-208* is now known as plastocyanin (PCY1; Quinn et al., 1993). More recently, gene model predictions have been enriched and corrected with the help of RNA sequencing (RNA-seq) data sets, improved gene prediction algorithms, and sequence similarity and synteny with *Volvox*. Details on the

applied methods were described recently (Blaby et al., 2014). The latest genome annotation lists 17,741 loci, with the potential to encode 19,526 proteins (due to alternative splicing). This list excludes microRNAs (miRNA), long noncoding RNAs, and other small RNAs but does include all tRNAs.

Inspired by the *Arabidopsis* genetic nomenclature, the *Chlamydomonas* community uses a unique and permanent locus identifier that indicates the chromosome a gene maps to as well as a unique genomic “zipcode” along that chromosome. For instance, plastocyanin has the locus identifier Cre03.g182551: it is located on chromosome 3, between genes Cre03.g182550 and Cre03.g182560. Most often, the zipcode of two neighboring genes increases by a count of 50, which leaves more than enough room for new genes in the intervening space. Note that, contrary to *Arabidopsis*, where the zipcode resets for each chromosome, *Chlamydomonas* zipcodes keep increasing, such that the first gene on the last chromosome (chromosome 17) is Cre17.g692564 (and not Cre17.g000050 if following *Arabidopsis* rules).

Most *C. reinhardtii* genes contain at least one intron and are composed of an average of seven exons of 200 to 240 bp, which are comparable to exon lengths in *V. carteri* but ~50% shorter than those in *Arabidopsis*. Introns are over twice as long in *C. reinhardtii* as in *Arabidopsis* and almost three times as long in *Volvox* (Merchant et al., 2007; Prochnik et al., 2010; Kianianmomeni et al., 2014; Cheng et al., 2017a). In addition, both *Chlamydomonas* and *Volvox* transcripts are distinguished by long 3' untranslated regions (UTRs) relative to land plants, with a mean length of ~800 bp, compared with ~220 bp in *Arabidopsis* (Shen et al., 2008). The same trend is also observed for the 5' UTRs, although not as drastically: ~280 bp in *Chlamydomonas* and 131 bp in *Arabidopsis* (Kim et al., 2007). UTR sizes scale with gene length.

The wealth of sequence information (RNA-seq and ESTs) can be mined for consensus motifs for polyadenylation signals. For 65% of human and 60% of fruit fly genes, mRNAs present the consensus sequences AAUAAA or AUUAAA within the last 50 nucleotides before the start of the poly(A) signal (Graber et al., 1999). However, the same motifs are only detected in ~10% of yeast and *Arabidopsis* coding sequences (Graber et al., 1999; Loke et al., 2005), and no other consensus sequence has been identified. *Chlamydomonas* appears to favor the polyadenylation signal UGUAA, which is found in 50% of all genes (Wodniok et al., 2007). The motif UGUAA was also found in the handful of characterized *Volvox* genes (Dietmaier et al., 1995).

Chlamydomonas gene families are generally accepted to be much smaller than those of land plants, whose genomes have undergone multiple rounds of whole-genome duplications and rearrangements. Over 85% of gene families in *C. reinhardtii* are composed of single genes and represent almost 60% of the gene complement. Gene families of three genes or more account for only 6.5% of all gene families but contribute 28% of all genes; the situation is completely reversed in *Arabidopsis* (*Arabidopsis* Genome Initiative, 2000; Merchant et al., 2007; Guo, 2013). This picture is, however, incomplete: only one-third of *Arabidopsis* gene families have a match in *Chlamydomonas* (Figure 5E), whereas 7 out of 10 *Arabidopsis* gene families have a counterpart in poplar (Figure 5F). Of the gene families with a match in both organisms, the size does tend to be smaller in *C. reinhardtii*.

The Other Chlamydomonas Gene Space: The Mitochondrial and Chloroplast Genomes

The *C. reinhardtii* mitochondrial genome is a 15.8-kb linear molecule that encodes 8 proteins, 7 of which are either subunits of the respiratory chain or ATP synthase, as well as 3 tRNAs and 15 rRNA fragments (Boer et al., 1985; Gallaher et al., 2018). This small size came as a surprise, since mitochondrial genomes in land plants are 10 to 150 times larger (Figure 5C). Each cell contains ~130 copies of the mitochondrial genome. This organelle relies on importing missing tRNAs from the cytosol to sustain its translation needs (Salinas et al., 2012). Mitochondrial genes are expressed as two divergent and multicistronic RNAs that are subsequently processed into individual transcripts by endonucleolytic cleavage. RNA degradation in organelles is initiated by the addition of a poly(A) tail, but analysis of RNA-seq data sets revealed the preponderance of C-rich, polynucleotide tails for all protein-coding RNAs (Salinas-Giegé et al., 2017; Gallaher et al., 2018). Poly(C) tail length appears to be quite variable and may represent a previously undescribed early step in RNA maturation restricted to members of the Chlorophyceae lineage and absent in land plants (Salinas-Giegé et al., 2017).

The first cloned and sequenced *C. reinhardtii* chloroplast gene was *rbcL*, encoding the large subunit of Rubisco (Gelvin et al., 1977; Dron et al., 1982). The full chloroplast genome was assembled from National Center for Biotechnology Information (NCBI) sequences and targeted subcloning to fill in gaps into a circular molecule of 205 kb with two inverted repeats (each 21.2 kb) (Maul et al., 2002). This structure is similar to the structures of chloroplast genomes in *C. zofingiensis*, *Arabidopsis*, *Brachypodium*, and rice (Figure 5D). The *C. reinhardtii* plastid genome distinguishes itself by a high repeat content, which accounts for over 20% of the organellar genome and is limited to intergenic regions. The chloroplast genome is almost identical across all laboratory strains, which is in agreement with (1) uniparental chloroplast inheritance and (2) their common origin (Gallaher et al., 2018). The chloroplast genome sequence was recently improved by incorporating data initially filtered out while assembling the nuclear genomes of laboratory strains (Flowers et al., 2015; Gallaher et al., 2015), which corrected the orientation of some genomic fragments, in addition to a few polymorphisms present in earlier releases. This new genome release confirms the absence of RNA editing in the chloroplast transcriptome.

The chloroplast genome has a low GC content of 34.6%, with each cell containing ~80 copies (Gelvin et al., 1977; Gallaher et al., 2018), and is organized in large protein-DNA complexes called nucleoids. Plastid nucleoids are associated with DNA replication and repair, transcription, RNA processing, and translation (Krupinska et al., 2013). Unlike land plants, plastid gene transcription is performed solely by a plastid-encoded RNA polymerase (PEP); a single nucleus-encoded sigma factor participates in PEP-mediated transcription (Surzycki and Shellenbarger, 1976). The plastid genome contains 99 genes, including a full set of 30 tRNA genes, 5 rRNA genes, 17 ribosomal protein genes, 32 genes involved in photosynthesis (including the large subunit of Rubisco), and 5 genes for PEP subunits. One plastid gene contains cis-spliced introns (*psbA*), while another is present as three distinct loci that are independently transcribed (*psaA*) and posttranscriptionally

joined in a process known as *trans*-splicing. The number of intron-containing plastid genes increased during the evolution of land plants, from 2 in *C. reinhardtii* to 20 in *Arabidopsis* (Bonen and Vogel, 2001).

RESOURCES AND DATABASES

The cumulative information collected on the nuclear genome described above (sequences, annotations, and gene identifiers) is accessible via the Joint Genome Institute's platform Phytozome and the European Nucleotide Archive (Table 1). The six most recent genome releases, including genome assemblies and annotation files, can be downloaded from the Genome Portal at the Joint Genome Institute upon the creation of a (free) account. Also available for download are polymorphisms inferred from the resequencing of laboratory strains (Gallaher et al., 2015). The short reads for 12 field isolates (including S1 D2, used to generate roughly half of the sequenced ESTs deposited at NCBI) and 8 laboratory strains were uploaded at NCBI (Flowers et al., 2015).

The nuclear genome can be browsed on Phytozome, with optional tracks for Vista plots (for sequence conservation between *Chlamydomonas*, *Volvox*, *Dunaliella*, *Arabidopsis*, and rice), repeat elements, and remaining gaps in the current genome assembly. The positions of mapped reads from a subset of 13 RNA-seq experiments (out of the 46 published as of 2019) can be visualized at Phytozome and the UCSC's browser at UCLA (Table 1) and used as empirical support for gene annotation and gene models. We hope to launch a new visualization database with all RNA-seq data sets in the future, with an updated browser. Only 5% to 10% of genes have some functional annotation information. Metabolic pathways can be perused on the Kyoto Encyclopedia of Genes and Genomes (KEGG), while Gene Ontology enrichment can be tested at the Protein ANalysis THrough Evolutionary Relationships (PANTHER) Classification System website (Table 1). In both cases, annotations are based on the initial genome release from 2007 and use either the original gene identifier numbers (KEGG, with links to DNA and protein sequences, which need to be checked against the latest genome release for the Cre number) or corresponding Uniprot IDs (PANTHER).

The distribution of biological material is greatly aided by the availability of a dedicated stock center; for the *Chlamydomonas* community, this role is filled by the *Chlamydomonas* Resource Center (CRC). Strains from the core collection have the designation "CC" preceding a unique number. CRC maintains stocks for almost 3200 strains: laboratory strains and wild *C. reinhardtii* isolates, *C. reinhardtii* relatives, transgenic strains, and mutants, including original extant mutants isolated in the 1950s. CRC also holds ~400 plasmids, the indexed BAC genomic library produced from the reference strain CC-503 for genome sequencing, and several lambda phage cDNA libraries.

Several collections have been added to the CRC portfolio. The Jonikas lab recently deposited 185 strains expressing Venus YFP and/or mCherry fusions with a number of target proteins, many of them involved in the CCM, as well as cellular localization markers for microscopy and cloning vectors to fuse proteins of interest to the fluorescent proteins (Table 1; Mackinder et al., 2017). In addition, large-scale insertion mutant collections have been deposited. Insertion sites for all CLiP (*Chlamydomonas* Library

Project) mutants (Li et al., 2016) are indicated on the Chlamydomonas Phytozome web portal. This National Science Foundation-funded project generated ~54,000 mutants by random mutagenic insertion in the CC-4533 strain selected for good recovery from cryogenic storage, transformation rate by electroporation, and absence of clumping when grown in liquid cultures. As with other large-scale projects, ~80% of mutants carry an insertion; the presence of the insertion should be confirmed by PCR. This mutant collection joins a previous set of ~49,000 insertion mutants generated by Niyogi in the CC-4051 strain (Dent et al., 2015), ~450 of which are available at CRC (Table 1). Finally, the CRC also houses a collection of 383 temperature-sensitive mutants generated by Fred Cross by UV mutagenesis of strain CC-124. The putative causal mutations are known in over half the collection, leaving another ~160 mutations in unassigned loci (Tulin and Cross, 2014). One last mutant collection, not distributed by CRC but available, was generated in strain CC-4101 (T222+) and assembled in France by Wollman and colleagues and consists of ~500 photosynthetic mutants (Table 1).

Luckily for the community, the strains used for these large-scale mutant collections are closely related. CC-4533 (*mt*⁻) was derived from a cross between CC-4425 (*mt*⁺) and CC-4603 (*mt*⁻), itself essentially identical to CC-124 (*mt*⁻). CC-4051 (*mt*⁺) is isogenic to CC-4603 outside of the mating locus on chromosome 6 and is also very similar to CC-4101, except for a block at the end of chromosome 16 (Figure 5E; Gallaher et al., 2015). As a reminder, the historical *ac* and *pf* mutants were generated in CC-124. All but CC-4533 are thus closely related and only differ by ~20,000 SNPs, not counting those linked to the mating locus; CC-4533 is separated by an additional 100,000 SNPs due to haplotype 2 blocks on chromosomes 1, 2, and 11. Each haplotype block can be genotyped using allele-specific primers and selected when building double mutants (Gallaher et al., 2015).

CRC handles the distribution and long-term maintenance of the CLiP collection. In the past 2 years, CRC has distributed ~3,100 CLiP mutants (Matt Laudon, personal communication). Periodic passage of the collection has been accompanied by very minimal colony loss. In addition, cryopreservation is possible, although it is somewhat challenging and requires storage under liquid nitrogen, since frozen cells die within 24 h when stored at -80°C (Crutchfield et al., 1999). In both cases (cryopreservation and maintenance), fragile mutants may not recover past the initial isolation, first passage, or thawing.

In addition to CRC, the University of Texas at Austin in the United States and the Culture Collection of Algae at the University of Göttingen in Germany maintain stocks for almost 2600 and 2300 algal strains, respectively, including Chlamydomonas strains (177 in Austin and 320 in Göttingen; Table 1). Additional algal repositories are listed on the CRC website.

AVAILABLE TOOLS AND TECHNOLOGIES

Armed with the biology and genomics of the organism, we can now design some experiments. Many tested protocols are available online: for example, the 2006 EMBO practical course collection of protocols covers culture handling, crosses, transformation, and biochemical characterization of proteins. CRC has also compiled protocols and resources as well as unpublished

“wisdom” from researchers (Table 1). We recommend our readers familiarize themselves with these detailed protocols before starting experiments. Below, we highlight important technical aspects of *C. reinhardtii* research and some intrinsic caveats and work-arounds.

Classical Genetics and Mutagens

C. reinhardtii performs admirably well for classical forward genetics: mutations can be induced in each of the three genomes by chemical, radiation, or insertional mutagenesis using a selection cassette (such as a linearized plasmid or PCR product that includes a selection marker) (Jinkerson and Jonikas, 2015). Insertional mutants within coding sequences tend to be null alleles. This is problematic for studying essential genes, in which case temperature-sensitive or weak alleles produced with chemical or radiation mutagens may be more appropriate. However, weak alleles can be present in insertional collections if the cassette integrated in noncoding regions. One drawback of a haploid genome is the inability to distinguish between loss- and gain-of-function mutations. However, dominance can be tested in diploid vegetative cells (discussed below).

Crosses: Double Mutants, Dominance, and Allelism Tests

Mutants may be combined, or sorted into complementation groups, by crossing. This assumes that mutants are available in both mating types, either by performing the screen in two isogenic strains differing at the mating locus or by crossing mutants to an isogenic strain of opposite mating type and selecting appropriate genetic combinations in the progeny. As detailed earlier, gamete formation is induced by nitrogen depletion, preferably on plates, before mixing gametes in distilled water. The mating reaction is plated and kept in the dark for 4 to 7 d to prevent premature germination. Zygotes adhere strongly and partially embed themselves into the agar surface, and are resistant to chloroform fumes, whereas unmated haploid cells can be washed off, scraped off with a razor blade, or killed by chloroform fumes.

Mapping a mutation by genotyping large numbers of progeny is possible but somewhat limited by the number of tetrads one is willing and able to dissect. At a smaller scale, for example allelism tests, two mutants are crossed and multiple tetrads are phenotyped. If all progeny display only the mutant phenotype, the two mutants are allelic (or tightly linked, depending on how many tetrads are analyzed). A second technique is based on non-complementation in vegetative diploids, which form during mating reactions at low rates. To facilitate the selection of vegetative diploids, *arg2* and *arg7* are crossed into the mutants of interest; both mutations map to *ARG7* but result in intragenic complementation and therefore confer Arg autotrophy in diploids (Debuchy et al., 1989). The same strategy can be undertaken for dominance tests, using a wild-type strain as one of the mating partners.

In yeast, mating type is controlled by the *MAT* locus and switches back and forth when the allele present at *MAT* is replaced by the opposite mating type from a duplicated but silenced copy found on the same chromosome by homologous recombination

(HR; Haber, 2012). Each *Chlamydomonas* cell harbors only one copy of the *MT* locus and cannot simply switch. Nevertheless, gametes induced from an *mt+* strain transformed with the *MID* gene differentiate as *mt-* and are competent to mate with *mt+* gametes, although with reduced efficiency (Ferris and Goodenough, 1997). While this remains to be empirically tested, it may therefore be possible to transform *mt+* mutants by introducing *MID* by transformation and using the transformants for crosses with other *mt+* mutants for allelism tests or for generating double mutants.

Complementation by Transformation of Genomic Libraries

Causal genes may be identified by genomic rescue, whereby random genomic fragments subcloned into cosmids or BACs are introduced into the mutant. The Arg auxotrophy mutant *arg7* is crossed with the mutant of interest; the resulting double mutant is then cotransformed with the *ARG7* locus and pools of cosmid or BAC DNA clones. Alternatively, dominant selectable markers that confer resistance to antibiotics or herbicides may be used in place of *ARG7*-based auxotrophic complementation (Purton and Rochaix, 1994; Zhang et al., 1994). This method is best suited for mutants amenable to plate screening techniques, such as chlorophyll fluorescence or autotrophy rescue. Only 1% to ~20% of transformants typically exhibit full rescue due to a combination of low expression of transgenes and diluted individual clone representation within DNA pools. These frequencies are obviously higher than those that might be expected of spontaneous second-site suppressors, but they may startle researchers new to *C. reinhardtii*. As in microbes, mutational reversion does occur at variable frequencies. Intragenic reversion can provide support for the identification of the causal gene, while extragenic suppressors will complicate matters and potentially lead to heterogeneous stocks.

Transformation Methods and Selectable Markers

Stable transformation methods for *C. reinhardtii* were developed in the 1980s and 1990s. Recombinant DNA is introduced into cells by vortexing in the presence of glass beads coated with DNA, by electroporation, or by bombardment of tungsten or gold particles coated with DNA (Figure 4C; Kindle et al., 1989; Diener et al., 1990; Kindle, 1990; Mayfield and Kindle, 1990; Shimogawara et al., 1998; Yamano et al., 2013). The glass bead method works best for strains without a cell wall, which otherwise limits the amount of DNA reaching the nucleus. The cell wall may be removed by autolysin digestion (Neupert et al., 2012). Strains with a cell wall can be transformed at higher frequencies by electroporation. A recent study using the NEPA21 electroporator from NepaGene reported transformation efficiencies at least 10 times higher than those obtained using the Gene-Pulser from Bio-Rad (Yamano et al., 2013). Biolistics-mediated transformation requires specialized equipment and is generally low-efficiency but is the prime method for manipulating the chloroplast (Boynton et al., 1988) and mitochondrial (Remacle et al., 2006) genomes. *Chlamydomonas* is the only system where all three genomes can be genetically modified. In fact, the *Chlamydomonas* chloroplast genome was

the first chloroplast genome to be manipulated by transgenesis (Boynton et al., 1988).

Selectable markers that confer antibiotic or herbicide resistance or complement auxotrophic growth facilitate the selection of stable integration of recombinant DNA into the genome(s) (Tables 2–4). Popular markers for nuclear transformation confer growth on medium lacking Arg (*ARG7*; see above) or ammonium (*NIT1*) or resistance via antibiotic detoxification: paromomycin (*aphVIII*), spectinomycin (*aadA*), and zeocin (*ble*). A gene encoding a mutant form of ribosomal protein S14, known as *cry1-1*, provides a dominant resistance marker against the drug emetine (Tables 2–4). The effective dosages of zeocin and emetine that kill all nontransformants should be tested empirically due to strain-by-strain variation. The selection of chloroplast transformants is possible using *aadA* (Goldschmidt-Clermont, 1991) or a mutant allele of the 16S-23S plastid rRNA that prevents antibiotic binding (Newman et al., 1990). The selection of mitochondrial transformants relies on the rescue of respiration capacity in respiration-deficient mutants by reintroducing a fragment of the mitochondrial genome (Remacle et al., 2006). Organellar transformation is a very rare event but is mitigated by the absence of transgene silencing.

Multiple selection markers can be used sequentially, but this is not routinely performed, possibly due to the likelihood of transgene silencing upon serial transformation. Gene synthesis is strongly recommended for all selectable markers that are not derived from *C. reinhardtii* to match codon usage and promote high-level expression and translation of the transgene (Barahimipour et al., 2015).

Adequate transgene expression has indeed long been an issue when generating nuclear transformants in *C. reinhardtii*, but a number of strong promoters and regulatory sequences derived from *C. reinhardtii* genes have been adopted. Strong constitutive promoters include *PSAD*, *RBCS2*, and a chimeric *RBCS2* promoter combined with regulatory elements from the *HSP70A* promoter (Table 4). Inducible promoters may prevent silencing. The 5' UTR of the *THIAMINE4* (*THI4*) gene contains a riboswitch. In the presence of thiamine, the 5' UTR undergoes alternative splicing and includes a premature stop codon in the resulting transcript, effectively providing vitamin B1-dependent repression of gene expression (Croft et al., 2007). The nitrate-repressible, ammonium-inducible *NIT1* promoter offers tight control for transgene expression, but it only works in strains that have not lost the ability to grow on nitrate, as have most laboratory strains (Schmollinger et al., 2010). The *CYC6* promoter confers copper-dependent repression and nickel-dependent induction of gene expression. While copper deficiency is not associated with visible phenotypes, nickel may cause an upregulation of autophagy (Pérez-Martín et al., 2015). The applicability of a steroid-inducible system (Aoyama and Chua, 1997) has yet to be demonstrated. Adding introns to transgenes increases their expression, for instance *RBCS2* intron 1. This intron is included in many selection marker cassettes, as one (*aphVIII*) or two (*ble*) copies, and is often included in the chimeric *RBCS2/HSP70A* promoter mentioned earlier. The construction of shuttle vectors is based on decades of observations associated with efficient transgene expression (Tables 2 and 4). Most recently, a series of constructs based on Golden Gate technology was developed that allows combinatorial cloning of regulatory and selection elements (promoter, 5' UTR,

Table 2. Selectable Markers in Use for Chlamydomonas Transformation

Marker Name	Selection Type	Marker Origin	Organelle	Reference
<i>NIT1</i> ^a	Auxotrophy rescue (nitrate use)	Chlamydomonas	Nucleus	(Kindle et al., 1989)
<i>ARG4</i> ^a	Auxotrophy rescue (Arg)	Budding yeast	Nucleus	(Rochaix et al., 1984)
<i>ARG7</i> ^a	Auxotrophy rescue (Arg)	Chlamydomonas	Nucleus	(Debuchy et al., 1989)
<i>ARG9</i> ^a	Auxotrophy rescue (Arg)	Chlamydomonas	Nucleus	(Remacle et al., 2009)
<i>ARG9</i> ^a	Auxotrophy rescue (Arg)	Arabidopsis	Chloroplast	(Remacle et al., 2009)
<i>NIC7</i> ^a	Auxotrophy rescue (nicotinamide)	Chlamydomonas	Nucleus	(Ferris, 1995)
<i>THI10</i> ^a	Auxotrophy rescue (thiamine)	Chlamydomonas	Nucleus	(Ferris, 1995)
<i>OEE1</i> ^{a,b}	Auxotrophy rescue (acetate requiring)	Chlamydomonas	Nucleus	(Mayfield and Kindle, 1990)
<i>psbA</i> ^{a,b}	Herbicide resistance (DCMU)	Chlamydomonas	Nucleus	(Erickson et al., 1984)
<i>PDS1</i> ^b	Herbicide resistance (norflurazon)	Chlamydomonas	Nucleus	(Brueggeman et al., 2014)
<i>PPO</i> ^b	Herbicide resistance (oxyfluorfen)	Chlamydomonas	Nucleus	(Brueggeman et al., 2014)
<i>GAT</i>	Herbicide resistance (glyphosate)	Agrobacterium spp	Nucleus	(Brueggeman et al., 2014)
<i>SUL</i>	Herbicide resistance (sulfadiazine)	<i>E. coli</i>	Nucleus	(Tabatabaei et al., 2019)
<i>CRY1-1</i> ^c	Drug resistance (emetine)	Chlamydomonas	Nucleus	(Nelson et al., 1994)
<i>aphVIII</i>	Antibiotic resistance (paromomycin)	<i>Streptomyces rimosus</i>	Nucleus	(Sizova et al., 2001)
<i>aphA-6</i>	Antibiotic resistance (kanamycin)	Acetobacter	Chloroplast	(Bateman and Purton, 2000)
<i>aadA</i>	Antibiotic resistance (spectinomycin)	<i>E. coli</i>	Nucleus, chloroplast	(Goldschmidt-Clermont, 1991)
<i>ble</i> ^c	Antibiotic resistance (bleomycin, zeocin)	<i>Streptoalloteichus hindustanus</i>	Nucleus	(Stevens et al., 1996)
<i>aph7''</i>	Antibiotic resistance (hygromycin)	<i>Streptoalloteichus hindustanus</i>	Nucleus	(Berthold et al., 2002)
<i>NPTII</i>	Antibiotic resistance (kanamycin)	<i>E. coli</i> Tn5 transposon	Nucleus	(Barahimipour et al., 2016)
<i>er, spr, sr</i> (16S, 23S cp rRNA) ^d	Antibiotic resistance (spectinomycin, erythromycin)	Chlamydomonas	Chloroplast	(Newman et al., 1990)
<i>cob-nd4-nd5</i>	Auxotrophy rescue (respiration)	Chlamydomonas	Mitochondrion	(Remacle et al., 2006)
<i>cob^{mud2}</i>	Drug resistance (myxothiazol)	Chlamydomonas	Mitochondrion	(Remacle et al., 2006)

^aRequire the mutation to be present in the relevant background for auxotrophy rescue.

^bHerbicide resistance is conferred by a point mutation in the presumed target of the herbicide, but the mutants have no phenotype on their own.

^cCan potentially damage DNA.

^dAntibiotics target the plastid ribosomes, and the resistance-conferring mutations are in the plastid 16S or 23S rRNAs.

intron, 3' UTR, and selection marker; Crozet et al., 2018) and decreases the cloning bottleneck. This study also illustrates the importance of the choice of promoter and untranslated regulatory sequences for expression potential. It should be noted that the lengths of common 5' UTRs have not been optimized and are rather short, which may hinder ribosome landing onto mRNAs.

Reverse Genetics Approaches for the Nuclear Genome

The wealth of RNA-seq-generated expression data sets for *C. reinhardtii* covers many growth conditions and can be mined

to generate hypotheses that can be tested by reverse genetic approaches. Insertional mutant libraries (see above) present one route; targeted knockdowns or gene inactivation offer another route.

C. reinhardtii possesses most proteins necessary for the biogenesis of short RNAs, small interfering RNAs, and miRNAs. *C. reinhardtii* miRNAs can induce mRNA cleavage and/or translational repression depending on the miRNA and target. Cleavage by the Argonaute complex is not very accurate, and mutants that lack all small RNAs and miRNAs are completely viable with no clear phenotypes (Voshall et al., 2017; Yamasaki and Cerutti, 2017).

Table 3. Selectable Reporters in Use in Chlamydomonas Transformation

Reporter	Reporter Origin	Reference
<i>ARS</i> ^a	Chlamydomonas	(Davies et al., 1992)
<i>luciferase</i>	<i>Gaussia princeps</i> , <i>Renilla reniformis</i> , firefly	(Matsuo et al., 2006; Shao and Bock, 2008)
<i>GFP, BFP, CFP, YFP</i>	<i>Aequorea victoria</i> ^b	(Rasala et al., 2013)
<i>mCherry</i>	Sea anemone	(Rasala et al., 2013)
<i>mRuby</i>	Sea anemone	(Rasala et al., 2013)
<i>tdTomato</i>	Sea anemone	(Rasala et al., 2013)
<i>aequorin</i>	<i>Aequorea victoria</i>	(Aiyar et al., 2017)

^a*ARS* can be used to dissect promoter elements, when cloning them upstream of the β -tubulin minimal promoter.

^b*GFP* was cloned from the jellyfish, but the other colored variants were engineered in the laboratory by mutagenesis.

Table 4. Selectable Promoters in Use in Chlamydomonas Transformation

Promoter	Expression Type	Origin	Reference
<i>RBCS2</i>	Constitutive	Chlamydomonas	(Stevens et al., 1996)
<i>PsaD</i>	Constitutive	Chlamydomonas	(Fischer and Rochaix, 2001)
<i>HSP70A</i>	Constitutive, heat-inducible	Chlamydomonas	(Schroda et al., 2000)
<i>RBCS2:HSP70A</i>	Constitutive, heat-inducible	Chlamydomonas	(Schroda et al., 2000)
<i>NIT1</i>	Ammonium-inducible, nitrogen-repressible	Chlamydomonas	(Ohresser et al., 1997)
<i>CYC6</i>	Nickel-inducible, copper-repressible	Chlamydomonas	(Pérez-Martín et al., 2015)
<i>THI4^a</i>	Thiamine-repressible	Chlamydomonas	(Croft et al., 2007)
<i>METE</i>	Vitamin B12-repressible	Chlamydomonas	(Helliwell et al., 2014)
35S	Constitutive	CaMV	(Díaz-Santos et al., 2013)
Nopaline synthase (NOS)	Constitutive	Agrobacterium	(Díaz-Santos et al., 2013)

^a*THI4* is a riboswitch found in the *THI4* transcript that provides thiamine-repressible control of gene expression when fused to a promoter.

Most of the miRNAs initially described do not survive stringent reevaluation for annotation as true miRNAs (Tarver et al., 2012). Of the six remaining robust miRNAs, the highly expressed Cre-miR1157 miRNA has provided the backbone for knocking down candidate genes by modifying the miRNA target site to match a gene of interest following plant miRNA target selection rules (Schwab et al., 2005). Success rates are variable, partially due to transgene silencing. This can be minimized with the use of an inducible promoter such as *NIT1* but is restricted to *NIT+* strains (Schmollinger et al., 2010) or by selecting for luciferase activity derived from a bicistronic message combining *Gaussia princeps* luciferase and the artificial microRNA (amiRNA; Hu et al., 2014). In addition, amiRNA design may be hindered by the high GC content of the genome, which places a strong burden on the nucleotide composition of putative target sites: natural plant amiRNAs indeed tend to have an adenine at the presumptive cleavage site (Schwab et al., 2005). RNA interference via expression of a double-stranded RNA hairpin construct can be effective and offers the advantage of enabling signal amplification over the entire coding sequence (Rohr et al., 2004). Small RNA-seq also revealed putative *trans*-acting small interfering RNAs that may be produced by phased cleavage downstream of a miRNA target site (Zhao et al., 2007). In Arabidopsis, the 22-nucleotide miR173 sequence is sufficient to trigger the formation of *trans*-acting small interfering RNAs from a gene fragment cloned downstream (Felippes et al., 2012) but is dependent on the activity of the RNA-dependent RNA polymerase RDR6, which *C. reinhardtii* apparently lacks.

Genome editing by zinc-finger nucleases (ZFNs; Sizova et al., 2013; Greiner et al., 2017) and transcription activator-like effectors (TALEs) has been reported in *C. reinhardtii* but is not routine. ZFNs are modified versions of an endonuclease that consists of a nonspecific DNA cleavage domain and zinc-finger DNA binding domains. Each zinc finger targets three nucleotides, so they are stacked to attain desired specificity. Target sequences have been matched with potential ZFNs for eight organisms, including *C. reinhardtii* (Reyon et al., 2011). Sequence specificity should be tested in vitro against the intended target prior to transformation. TALEs from *Xanthomonas* offer another avenue for genome editing. Each module recognizes 1 bp and can be combined to achieve sequence specificity. The C terminus of a TALE acts as a transcriptional activator. Constitutive expression of a gene of interest can therefore be achieved by targeting its promoter with a suitably designed TALE,

but the C terminus can also be substituted for an endonuclease domain to generate breaks in the DNA (Gao et al., 2015).

Gene inactivation and genomic manipulation by HR were reported in *C. reinhardtii* as early as 1993. Using this technique, a selection cassette is flanked by 200 bp or more of genomic sequence from a gene of interest. HR borrows the endogenous DNA repair machinery to replace the gene with the selection cassette. The efficiency is poor, with 1000 random insertion events for each true HR event (Sodeinde and Kindle, 1993; Gumpel et al., 1994; Nelson and Lefebvre, 1995). The establishment of HR would partially alleviate technical difficulties associated with the expression of engineered nucleases such as ZFNs (see above) and Cas9 (see below) but will require higher efficiency. Complementation of auxotrophic mutants by HR-mediated replacement of the inactive allele with a functional copy may provide a positive selection method that does not rely on the random insertion of a selection cassette (Gumpel et al., 1994; Mages et al., 2007). In *Neurospora crassa* and other fungi, the detection of HR was greatly improved by performing all manipulations in a background that lacked the nonhomologous end-joining protein KU70 or KU80 (Colot et al., 2006). The *C. reinhardtii* genome contains a single copy of each *KU* gene, and insertion mutants are available.

Cas9/CRISPR technology has quickly been adapted for use in many reference organisms since the discovery of this bacterial immune system, including *C. reinhardtii* (Baek et al., 2016; Ferenczi et al., 2017; Greiner et al., 2017). Target specificity is achieved with an RNA molecule, the single guide RNA (sgRNA), which binds to and activates the Cas9 nuclease. While several groups have published successful genome editing with Cas9 (and related nucleases such as Cpf1) and sgRNAs where Cas9/Cpf1 is either transiently expressed in cells or delivered preloaded with the sgRNA by electroporation, the efficiency remains low to very low. A robust selection method for edited cells is clearly needed; thus far, the Cas9 and sgRNA constructs have been cotransformed with an antibiotic selection cassette, or two sgRNAs have been cotransformed (one for the gene of interest and one targeting FKBP12, whose loss of function leads to rapamycin resistance).

Genetic Manipulation of Organellar Genomes

HR is highly efficient for organellar genomes, using homologous sequences as short as 25 bp (Esland et al., 2018). Transgenes may

be targeted to “neutral sites” that do not affect the expression of nearby genes or to endogenous organellar genes to replace them by versions carrying point mutations or deletions to study gene function. The design of transformation constructs should be given considerable thought to ensure successful transgene expression. Several rounds of selection might be needed to reach homoplasmy (when all organellar genome copies carry the transgene). Lingering heteroplasmy may indicate that the gene under study is essential; a conditional system will then be more appropriate.

Rochaix’s laboratory devised such a system involving the nucleus-encoded, chloroplast-localized protein NAC2, which binds to and stabilizes the *psbD* RNA via its 5′ UTR. The conditional repression system exploits and diverts NAC2-dependent stabilization of *psbD* RNA with two modifications. First, the expression of NAC2 is driven by the *METE* promoter, which is turned off by vitamin B12 (Helliwell et al., 2014), and the construct is introduced into a *nac2* mutant. Second, the 5′ UTR of the selected plastid gene is replaced by that of *psbD*, thereby becoming NAC2-dependent. The 5′ UTR of the *psbD* gene itself is replaced by the *petA* 5′ UTR to maintain an otherwise functional chloroplast (Ramundo and Rochaix, 2015). The role of a plastid gene can be uncovered by the addition of vitamin B12, resulting in the repression of NAC2 and the destabilization of the chimeric plastid transcript carrying the *psbD* 5′ UTR.

(Heterologous) Protein Expression

The production of metabolites or peptides of high pharmaceutical or nutritional value would be ideal in large-scale bioreactors if not for common nuclear transgene silencing in *C. reinhardtii*. Matching codon usage and GC content to the *Chlamydomonas* nuclear genome increases protein accumulation dramatically (Barahimipour et al., 2015, 2016), as does the use of strains selected for high-level transgene expression. The UVM11 strain was identified by UV mutagenesis-induced derepression of a transgenic *cry1-1* gene, which confers resistance to emetine. This strain does not exhibit silencing even after long-term cultivation (Neupert et al., 2009). Identifying the causal mutation will pave the way for conducting parallel loss- and gain-of-function studies in the same background, assuming that a CLiP insertion allele exists.

Transgenes inserted into the chloroplast genome by HR are not silenced and offer a platform for the production of recombinant proteins at high titer (Dyo and Purton, 2018). Increasing the number of cloning vectors and selectable markers specifically designed for chloroplast genome transformation would broaden the range of heterologous protein expression within the organelle (Esland et al., 2018).

Metabolic Studies in a Green Microbe

One great advantage of *C. reinhardtii* is the ease with which growth medium composition can be modulated to study responses to nutrient deficiency or to label proteins with stable or radioactive tracer isotopes. Cells can be grown in replete medium and then transferred to a medium lacking an element such as sulfur, nitrogen, or phosphorus. As these elements are fundamental building blocks for proteins, their absence will severely limit cell

growth and metabolism within 24 h (Saroussi et al., 2017). Cellular and physiological responses to various essential metal concentrations can also be investigated; iron deficiency is the easiest, as the chloroplast constitutes the major iron sink in the cell and requires a high iron quota to sustain photosynthesis. Deficiencies in other metals (zinc, copper, and manganese) are more difficult to observe and require the use of ultra-pure water and acid-washed glassware to remove any trace contamination from glass surfaces (Blaby-Haas and Merchant, 2017). Trace element solutions can be purchased from CRC (therefore alleviating the significant effort of preparing them); alternatively, cells grow very well when using a revised mineral nutrient supplement that more than covers cellular requirements and is easy to prepare (Kropat et al., 2011).

Large numbers of cells can easily be collected and assayed for transcriptomics, proteomics, and metabolomics studies. While yeast two-hybrid screens are technically possible and have been performed with *C. reinhardtii* genes (Lee et al., 2008), this technique suffers from the high GC content of the cDNA library and the ensuing low expression levels of baits and preys. The exploration of protein complexes and organellar proteomes has greatly benefited from proteomics approaches and was bolstered following the completion of the genome. The flux of carbon, nitrogen, and sulfur within the cell may be followed by pulse-labeling with stable isotopes.

CONCLUSION AND OUTLOOK

Chlamydomonas has had a long, rich history as a model organism for studying photosynthesis and cilia biology. More recently, studies of *Chlamydomonas* have branched out into light responses, metabolism, and the cell cycle, in large part due to its microbe-like nature. This alga is a choice model for cell biology and microscopy, as well as for defining growth conditions to study the effect of nutrient deficiencies or to bend cellular metabolism to suit our needs. Foundational work on chloroplast and cilia biogenesis should be quantitatively revisited at a global scale using isotope labeling. Certainly, genome sequencing, deep transcriptomics, and proteomics studies have reenergized the community. The state of completion of the *Chlamydomonas* genome (sequence, assembly, and annotation) currently stands as a standard to which other algae should aspire. *Chlamydomonas* now finds itself at another turning point in its long history: large-scale mutant collections bring hypothesis-driven science and reverse genetics within grasp, while we wait for the optimization of genome-editing methods.

Many classical mutants remain uncharacterized (at the molecular level), while large insertional mutant collections are being screened for phenotypes of interest, with thousands of algal genes not annotated or associated with a clear function. In the near future, *Chlamydomonas* studies will involve the integration of both forward and reverse genetics approaches within the technical challenges still facing the organism. The upcoming bounty of algal genomes (Cheng et al., 2018) may advocate for a focus on conserved algal genes; longer term, they may offer a new algal model with easier genome-editing possibilities and minimal transgene silencing. As the most thoroughly characterized unicellular alga, *C. reinhardtii* can transcend its impact in algal biology by becoming a model for future microalgae and biotechnology for decades to come.

Accession Numbers

Sequence data from this article can be found in the Phytozome and TAIR databases under the accession numbers listed in the Supplemental Data Set.

Supplemental Data

Supplemental Data Set. Accession numbers of the major genes mentioned in this article.

ACKNOWLEDGMENTS

We thank four anonymous reviewers as well as Jean-David Rochaix, Alison Smith, Ursula Goodenough, Ralph Bock, and Sean Gallaher for comments and insightful discussions on earlier versions of this article. We apologize to those whose work could not be mentioned due to word count limits. We thank the reviewers for their comments on an earlier version of this article. Some work in the Merchant lab on the *Chlamydomonas* genome was supported by a cooperative agreement with the U.S. Department of Energy Office of Science, Office of Biological and Environmental Research program, under award DE-FC02-02ER63421.

AUTHOR CONTRIBUTIONS

P.A.S. prepared all figures and wrote the article, with supervision from S.S.M.

Received December 14, 2018; revised May 20, 2019; accepted June 8, 2019; published June 19, 2019.

REFERENCES

- Aiyar, P., Schaeme, D., García-Altare, M., Carrasco Flores, D., Dathe, H., Hertweck, C., Sasso, S., and Mittag, M. (2017). Antagonistic bacteria disrupt calcium homeostasis and immobilize algal cells. *Nat. Commun.* **8**: 1756.
- Albert, S., Schaffer, M., Beck, F., Mosalaganti, S., Asano, S., Thomas, H.F., Plitzko, J.M., Beck, M., Baumeister, W., and Engel, B.D. (2017). Proteasomes tether to two distinct sites at the nuclear pore complex. *Proc. Natl. Acad. Sci. USA* **114**: 13726–13731.
- Aoyama, T., and Chua, N.H. (1997). A glucocorticoid-mediated transcriptional induction system in transgenic plants. *Plant J.* **11**: 605–612.
- Arabidopsis Genome Initiative (2000). Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* **408**: 796–815.
- Asamizu, E., Nakamura, Y., Miura, K., Fukuzawa, H., Fujiwara, S., Hirano, M., Iwamoto, K., Matsuda, Y., Minagawa, J., Shimogawara, K., Takahashi, Y., and Tabata, S. (2004). Establishment of publicly available cDNA material and information resource of *Chlamydomonas reinhardtii* (Chlorophyta) to facilitate gene function analysis. *Phycologia* **43**: 722–726.
- Baek, K., Kim, D.H., Jeong, J., Sim, S.J., Melis, A., Kim, J.S., Jin, E., and Bae, S. (2016). DNA-free two-gene knockout in *Chlamydomonas reinhardtii* via CRISPR-Cas9 ribonucleoproteins. *Sci. Rep.* **6**: 30620.
- Barahimipour, R., Strenkert, D., Neupert, J., Schroda, M., Merchant, S.S., and Bock, R. (2015). Dissecting the contributions of GC content and codon usage to gene expression in the model alga *Chlamydomonas reinhardtii*. *Plant J.* **84**: 704–717.
- Barahimipour, R., Neupert, J., and Bock, R. (2016). Efficient expression of nuclear transgenes in the green alga *Chlamydomonas*: Synthesis of an HIV antigen and development of a new selectable marker. *Plant Mol. Biol.* **90**: 403–418.
- Bateman, J.M., and Purton, S. (2000). Tools for chloroplast transformation in *Chlamydomonas*: Expression vectors and a new dominant selectable marker. *Mol. Gen. Genet.* **263**: 404–410.
- Beckers, M.C., Munaut, C., Minet, A., and Matagne, R.F. (1991). The fate of mitochondrial DNAs of mt+ and mt– origin in gametes and zygotes of *Chlamydomonas*. *Curr. Genet.* **20**: 239–243.
- Bellaïf, S., Barneche, F., Peltier, G., and Rochaix, J.D. (2005). State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. *Nature* **433**: 892–895.
- Bennoun, P., and Levine, R.P. (1967). Detecting mutants that have impaired photosynthesis by their increased level of fluorescence. *Plant Physiol.* **42**: 1284–1287.
- Bennoun, P., Masson, A., and Delosme, M. (1980). A method for complementation analysis of nuclear and chloroplast mutants of photosynthesis in *Chlamydomonas*. *Genetics* **95**: 39–47.
- Berthold, P., Schmitt, R., and Mages, W. (2002). An engineered *Streptomyces hygrosopicus* aph 7" gene mediates dominant resistance against hygromycin B in *Chlamydomonas reinhardtii*. *Protist* **153**: 401–412.
- Blaby, I.K., et al. (2014). The *Chlamydomonas* genome project: A decade on. *Trends Plant Sci.* **19**: 672–680.
- Blaby-Haas, C.E., and Merchant, S.S. (2017). Regulating cellular trace metal economy in algae. *Curr. Opin. Plant Biol.* **39**: 88–96.
- Blaby-Haas, C.E., and Merchant, S.S. (2019). Comparative and functional algal genomics. *Annu. Rev. Plant Biol.* **70**: 605–638.
- Blank, R., Grobe, B., and Arnold, C.G. (1978). Time-sequence of nuclear and chloroplast fusions in the zygote of *Chlamydomonas reinhardtii*. *Planta* **138**: 63–64.
- Boer, P.H., Bonen, L., Lee, R.W., and Gray, M.W. (1985). Genes for respiratory chain proteins and ribosomal RNAs are present on a 16-kilobase-pair DNA species from *Chlamydomonas reinhardtii* mitochondria. *Proc. Natl. Acad. Sci. USA* **82**: 3340–3344.
- Bonen, L., and Vogel, J. (2001). The ins and outs of group II introns. *Trends Genet.* **17**: 322–331.
- Boudreau, E., Nickelsen, J., Lemaire, S.D., Ossenbühl, F., and Rochaix, J.D. (2000). The Nac2 gene of *Chlamydomonas* encodes a chloroplast TPR-like protein involved in psbD mRNA stability. *EMBO J.* **19**: 3366–3376.
- Boyd, J.S., Mittelmeier, T.M., and Dieckmann, C.L. (2011). New insights into eyespot placement and assembly in *Chlamydomonas*. *Bioarchitecture* **1**: 196–199.
- Boynton, J.E., Gillham, N.W., Harris, E.H., Hosler, J.P., Johnson, A.M., Jones, A.R., Randolph-Anderson, B.L., Robertson, D., Klein, T.M., Shark, K.B., and Sanford, J.C. (1988). Chloroplast transformation in *Chlamydomonas* with high velocity microprojectiles. *Science* **240**: 1534–1538.
- Breker, M., Lieberman, K., and Cross, F.R. (2018). Comprehensive discovery of cell-cycle-essential pathways in *Chlamydomonas reinhardtii*. *Plant Cell* **30**: 1178–1198.
- Brown, R.M., Jr., Larson, D.A., and Bold, H.C. (1964). Airborne algae: Their abundance and heterogeneity. *Science* **143**: 583–585.
- Brueggeman, A.J., Kuehler, D., and Weeks, D.P. (2014). Evaluation of three herbicide resistance genes for use in genetic transformations and for potential crop protection in algae production. *Plant Biotechnol. J.* **12**: 894–902.
- Bykov, Y.S., Schaffer, M., Dodonova, S.O., Albert, S., Plitzko, J.M., Baumeister, W., Engel, B.D., and Briggs, J.A. (2017). The structure of the COPI coat determined within the cell. *eLife* **6**: e32493.

- Cerutti, H., Johnson, A.M., Gillham, N.W., and Boynton, J.E.** (1997). A eubacterial gene conferring spectinomycin resistance on *Chlamydomonas reinhardtii*: Integration into the nuclear genome and gene expression. *Genetics* **145**: 97–110.
- Cheng, S., et al.** (2018). 10KP: A phylodiverse genome sequencing plan. *Gigascience* **7**: 1–9.
- Cheng, C.Y., Krishnakumar, V., Chan, A.P., Thibaud-Nissen, F., Schobel, S., and Town, C.D.** (2017a). Araport11: A complete re-annotation of the *Arabidopsis thaliana* reference genome. *Plant J.* **89**: 789–804.
- Cheng, X., Liu, G., Ke, W., Zhao, L., Lv, B., Ma, X., Xu, N., Xia, X., Deng, X., Zheng, C., and Huang, K.** (2017b). Building a multipurpose insertional mutant library for forward and reverse genetics in *Chlamydomonas*. *Plant Methods* **13**: 36.
- Chiang, K.S., and Sueoka, N.** (1967a). Replication of chloroplast DNA in *Chlamydomonas reinhardtii* during vegetative cell cycle: Its mode and regulation. *Proc. Natl. Acad. Sci. USA* **57**: 1506–1513.
- Chiang, K.S., and Sueoka, N.** (1967b). Replication of chromosomal and cytoplasmic DNA during mitosis and meiosis in the eucaryote *Chlamydomonas reinhardtii*. *J. Cell. Physiol.* **70** (suppl.): 89–112.
- Choquet, Y., and Wollman, F.A.** (2002). Translational regulations as specific traits of chloroplast gene expression. *FEBS Lett.* **529**: 39–42.
- Choquet, Y., Goldschmidt-Clermont, M., Girard-Bascou, J., Kück, U., Bennoun, P., and Rochaix, J.D.** (1988). Mutant phenotypes support a trans-splicing mechanism for the expression of the tripartite *psaA* gene in the *C. reinhardtii* chloroplast. *Cell* **52**: 903–913. 3280139
- Chua, N.H., and Gillham, N.W.** (1977). The sites of synthesis of the principal thylakoid membrane polypeptides in *Chlamydomonas reinhardtii*. *J. Cell Biol.* **74**: 441–452.
- Colot, H.V., Park, G., Turner, G.E., Ringelberg, C., Crew, C.M., Litvinkova, L., Weiss, R.L., Borkovich, K.A., and Dunlap, J.C.** (2006). A high-throughput gene knockout procedure for *Neurospora* reveals functions for multiple transcription factors. *Proc. Natl. Acad. Sci. USA* **103**: 10352–10357.
- Croft, M.T., Moulin, M., Webb, M.E., and Smith, A.G.** (2007). Thiamine biosynthesis in algae is regulated by riboswitches. *Proc. Natl. Acad. Sci. USA* **104**: 20770–20775.
- Cross, F.R., and Umen, J.G.** (2015). The *Chlamydomonas* cell cycle. *Plant J.* **82**: 370–392.
- Crozet, P., et al.** (2018). Birth of a photosynthetic chassis: A MoClo toolkit enabling synthetic biology in the microalga *Chlamydomonas reinhardtii*. *ACS Synth. Biol.* **7**: 2074–2086.
- Crutchfield, A.L.M., Diller, K.R., and Brand, J.J.** (1999). Cryopreservation of *Chlamydomonas reinhardtii* (Chlorophyta). *Eur. J. Phycol.* **34**: 43–52.
- Davies, J.P., Weeks, D.P., and Grossman, A.R.** (1992). Expression of the arylsulfatase gene from the beta 2-tubulin promoter in *Chlamydomonas reinhardtii*. *Nucleic Acids Res.* **20**: 2959–2965.
- Debuchy, R., Purton, S., and Rochaix, J.D.** (1989). The argininosuccinate lyase gene of *Chlamydomonas reinhardtii*: An important tool for nuclear transformation and for correlating the genetic and molecular maps of the ARG7 locus. *EMBO J.* **8**: 2803–2809.
- Deisseroth, K., and Hegemann, P.** (2017). The form and function of channelrhodopsin. *Science* **357**: 357.
- Delepelaire, P.** (1983). Characterization of additional thylakoid membrane polypeptides synthesized inside the chloroplast in *Chlamydomonas reinhardtii*. *Photobiochemistry and Photobiophysics* **6**: 279–291.
- Delosme, R., Olive, J., and Wollman, F.A.** (1996). Changes in light energy distribution upon state transitions: An in vivo photoacoustic study of the wild type and photosynthesis mutants from *Chlamydomonas reinhardtii*. *Biochim. Biophys. Acta* **1273**: 150–158.
- Dent, R.M., Sharifi, M.N., Malnoë, A., Haglund, C., Calderon, R.H., Wakao, S., and Niyogi, K.K.** (2015). Large-scale insertional mutagenesis of *Chlamydomonas* supports phylogenomic functional prediction of photosynthetic genes and analysis of classical acetate-requiring mutants. *Plant J.* **82**: 337–351.
- Depège, N., Bellafiore, S., and Rochaix, J.D.** (2003). Role of chloroplast protein kinase Stt7 in LHCII phosphorylation and state transition in *Chlamydomonas*. *Science* **299**: 1572–1575.
- Díaz-Santos, E., de la Vega, M., Vila, M., Vigara, J., and León, R.** (2013). Efficiency of different heterologous promoters in the unicellular microalga *Chlamydomonas reinhardtii*. *Biotechnol. Prog.* **29**: 319–328.
- Diener, D.R., Curry, A.M., Johnson, K.A., Williams, B.D., Lefebvre, P.A., Kindle, K.L., and Rosenbaum, J.L.** (1990). Rescue of a paralyzed-flagella mutant of *Chlamydomonas* by transformation. *Proc. Natl. Acad. Sci. USA* **87**: 5739–5743.
- Dietmaier, W., Fabry, S., Huber, H., and Schmitt, R.** (1995). Analysis of a family of *ypt* genes and their products from *Chlamydomonas reinhardtii*. *Gene* **158**: 41–50.
- Dron, M., Rahire, M., and Rochaix, J.D.** (1982). Sequence of the chloroplast DNA region of *Chlamydomonas reinhardtii* containing the gene of the large subunit of ribulose biphosphate carboxylase and parts of its flanking genes. *J. Mol. Biol.* **162**: 775–793.
- Dumas, L., Zito, F., Blangy, S., Auroy, P., Johnson, X., Peltier, G., and Alric, J.** (2017). A stromal region of cytochrome *b₆f* subunit IV is involved in the activation of the Stt7 kinase in *Chlamydomonas*. *Proc. Natl. Acad. Sci. USA* **114**: 12063–12068.
- Dutcher, S.K.** (1995). Mating and tetrad analysis in *Chlamydomonas reinhardtii*. *Methods Cell Biol.* **47**: 531–540.
- Dutcher, S.K.** (2014). The awesome power of dikaryons for studying flagella and basal bodies in *Chlamydomonas reinhardtii*. *Cytoskeleton (Hoboken)* **71**: 79–94.
- Dyo, Y.M., and Purton, S.** (2018). The algal chloroplast as a synthetic biology platform for production of therapeutic proteins. *Microbiol. Today* **164**: 113–121.
- Ebersold, W.T., and Levine, R.P.** (1959). A genetic analysis of linkage group I of *Chlamydomonas reinhardtii*. *Z. Vererbungsl.* **90**: 74–82.
- Ebersold, W.T., Levine, R.P., Levine, E.E., and Olmsted, M.A.** (1962). Linkage maps in *Chlamydomonas reinhardtii*. *Genetics* **47**: 531–543.
- Ehrenberg, C.G.** (1838). *Die Infusionstierchen als Vollkommene Organismen: Ein Blick in das Tiefere Organische Leben der Natur.* (Leipzig, Germany: L. Voss).
- Eitzinger, N., Wagner, V., Weisheit, W., Geimer, S., Boness, D., Kreimer, G., and Mittag, M.** (2015). Proteomic analysis of a fraction with intact eyespots of *Chlamydomonas reinhardtii* and assignment of protein methylation. *Front. Plant Sci.* **6**: 1085.
- Engel, B.D., Schaffer, M., Kuhn Cuellar, L., Villa, E., Plitzko, J.M., and Baumeister, W.** (2015). Native architecture of the *Chlamydomonas* chloroplast revealed by in situ cryo-electron tomography. *eLife* **4**: e04889.
- Erickson, J.M., Rahire, M., Bennoun, P., Delepelaire, P., Diner, B., and Rochaix, J.D.** (1984). Herbicide resistance in *Chlamydomonas reinhardtii* results from a mutation in the chloroplast gene for the 32-kilodalton protein of photosystem II. *Proc. Natl. Acad. Sci. USA* **81**: 3617–3621.
- Erickson, J.M., Rahire, M., Malnoë, P., Girard-Bascou, J., Pierre, Y., Bennoun, P., and Rochaix, J.D.** (1986). Lack of the D2 protein in a *Chlamydomonas reinhardtii* *psbD* mutant affects photosystem II stability and D1 expression. *EMBO J.* **5**: 1745–1754.
- Esland, L., Larrea-Alvarez, M., and Purton, S.** (2018). Selectable markers and reporter genes for engineering the chloroplast of *Chlamydomonas reinhardtii*. *Biology (Basel)* **7**: 7.
- Ettl, C.G.** (1976). *Die Gattung Chlamydomonas Ehrenberg.* (Vaduz, Liechtenstein: J. Cramer).
- Faminzin, A.** (1866). *Die Wirkung des Lichtes auf die Bewegung der Chlamydomonas pulvisculus Ehr., Euglena viridis Ehr. und Oscillatoria insignis Tw.* *Mélanges Biologiques tirés du Bulletin de l'Académie Imperial des Sciences de St-Petersbourg* **6**: 73–93.

- Fang, W., Si, Y., Douglass, S., Casero, D., Merchant, S.S., Pellegrini, M., Ladunga, I., Liu, P., and Spalding, M.H. (2012). Transcriptome-wide changes in *Chlamydomonas reinhardtii* gene expression regulated by carbon dioxide and the CO₂-concentrating mechanism regulator CIA5/CCM1. *Plant Cell* **24**: 1876–1893.
- Felippes, F.F., Wang, J.W., and Weigel, D. (2012). MIGS: MiRNA-induced gene silencing. *Plant J.* **70**: 541–547.
- Feng, S., et al. (2010). Conservation and divergence of methylation patterning in plants and animals. *Proc. Natl. Acad. Sci. USA* **107**: 8689–8694.
- Ferenczi, A., Pyott, D.E., Xipnitou, A., and Molnar, A. (2017). Efficient targeted DNA editing and replacement in *Chlamydomonas reinhardtii* using Cpf1 ribonucleoproteins and single-stranded DNA. *Proc. Natl. Acad. Sci. USA* **114**: 13567–13572.
- Ferris, P.J. (1995). Localization of the nic-7, ac-29 and thi-10 genes within the mating-type locus of *Chlamydomonas reinhardtii*. *Genetics* **141**: 543–549.
- Ferris, P.J., and Goodenough, U.W. (1997). Mating type in *Chlamydomonas* is specified by mid, the minus-dominance gene. *Genetics* **146**: 859–869.
- Ferris, P.J., Woessner, J.P., and Goodenough, U.W. (1996). A sex recognition glycoprotein is encoded by the plus mating-type gene fus1 of *Chlamydomonas reinhardtii*. *Mol. Biol. Cell* **7**: 1235–1248.
- Ferris, P.J., Waffenschmidt, S., Umen, J.G., Lin, H., Lee, J.H., Ishida, K., Kubo, T., Lau, J., and Goodenough, U.W. (2005). Plus and minus sexual agglutinins from *Chlamydomonas reinhardtii*. *Plant Cell* **17**: 597–615.
- Field, C.B., Behrenfeld, M.J., Randerson, J.T., and Falkowski, P. (1998). Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* **281**: 237–240.
- Fischer, N., and Rochaix, J.D. (2001). The flanking regions of PsdD drive efficient gene expression in the nucleus of the green alga *Chlamydomonas reinhardtii*. *Mol. Genet. Genomics* **265**: 888–894.
- Fleischmann, M.M., Ravanel, S., Delosme, R., Olive, J., Zito, F., Wollman, F.A., and Rochaix, J.D. (1999). Isolation and characterization of photoautotrophic mutants of *Chlamydomonas reinhardtii* deficient in state transition. *J. Biol. Chem.* **274**: 30987–30994.
- Flowers, J.M., et al. (2015). Whole-genome resequencing reveals extensive natural variation in the model green alga *Chlamydomonas reinhardtii*. *Plant Cell* **27**: 2353–2369.
- Foster, K.W., Saranak, J., Patel, N., Zarilli, G., Okabe, M., Kline, T., and Nakanishi, K. (1984). A rhodopsin is the functional photoreceptor for phototaxis in the unicellular eukaryote *Chlamydomonas*. *Nature* **311**: 756–759.
- Freeman Rosenzweig, E.S., et al. (2017) The eukaryotic CO₂-concentrating organelle is liquid-like and exhibits dynamic reorganization. *Cell* **171**: 148–162.e119.
- Gallaher, S.D., Fitz-Gibbon, S.T., Glaesener, A.G., Pellegrini, M., and Merchant, S.S. (2015). *Chlamydomonas* genome resource for laboratory strains reveals a mosaic of sequence variation, identifies true strain histories, and enables strain-specific studies. *Plant Cell* **27**: 2335–2352.
- Gallaher, S.D., Fitz-Gibbon, S.T., Strenkert, D., Purvine, S.O., Pellegrini, M., and Merchant, S.S. (2018). High-throughput sequencing of the chloroplast and mitochondrion of *Chlamydomonas reinhardtii* to generate improved de novo assemblies, analyze expression patterns and transcript speciation, and evaluate diversity among laboratory strains and wild isolates. *Plant J.* **93**: 545–565.
- Gao, H., Wang, Y., Fei, X., Wright, D.A., and Spalding, M.H. (2015). Expression activation and functional analysis of HLA3, a putative inorganic carbon transporter in *Chlamydomonas reinhardtii*. *Plant J.* **82**: 1–11.
- Gelvin, S., Heizmann, P., and Howell, S.H. (1977). Identification and cloning of the chloroplast gene coding for the large subunit of ribulose-1,5-bisphosphate carboxylase from *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. USA* **74**: 3193–3197.
- Gillham, N.W. (1969). Uniparental inheritance in *Chlamydomonas reinhardtii*. *Am. Nat.* **103**: 355.
- Goldschmidt-Clermont, M. (1991). Transgenic expression of aminoglycoside adenine transferase in the chloroplast: A selectable marker of site-directed transformation of *Chlamydomonas*. *Nucleic Acids Res.* **19**: 4083–4089.
- Goodenough, U. (2015). Historical perspective on *Chlamydomonas* as a model for basic research: 1950–1970. *Plant J.* **82**: 365–369.
- Goodenough, U.W., and Heuser, J.E. (1982). Substructure of the outer dynein arm. *J. Cell Biol.* **95**: 798–815.
- Goodenough, U.W., and Heuser, J.E. (1985a). Substructure of inner dynein arms, radial spokes, and the central pair/projection complex of cilia and flagella. *J. Cell Biol.* **100**: 2008–2018.
- Goodenough, U.W., and Heuser, J.E. (1985b). The *Chlamydomonas* cell wall and its constituent glycoproteins analyzed by the quick-freeze, deep-etch technique. *J. Cell Biol.* **101**: 1550–1568.
- Goodenough, U.W., Hwang, C., and Warren, A.J. (1978). Sex-limited expression of gene loci controlling flagellar membrane agglutination in the *Chlamydomonas* mating reaction. *Genetics* **89**: 235–243.
- Goodenough, U.W., Detmers, P.A., and Hwang, C. (1982). Activation for cell fusion in *Chlamydomonas*: Analysis of wild-type gametes and nonfusing mutants. *J. Cell Biol.* **92**: 378–386.
- Gowans, C.S. (1976). Publications by Franz Moewus on the Genetics of Algae. (Berkeley: University of California Press).
- Graber, J.H., Cantor, C.R., Mohr, S.C., and Smith, T.F. (1999). In silico detection of control signals: mRNA 3'-end-processing sequences in diverse species. *Proc. Natl. Acad. Sci. USA* **96**: 14055–14060.
- Greiner, A., Kelterborn, S., Evers, H., Kreimer, G., Sizova, I., and Hegemann, P. (2017). Targeting of photoreceptor genes in *Chlamydomonas reinhardtii* via zinc-finger nucleases and CRISPR/Cas9. *Plant Cell* **29**: 2498–2518.
- Gross, C.H., Ranum, L.P., and Lefebvre, P.A. (1988). Extensive restriction fragment length polymorphisms in a new isolate of *Chlamydomonas reinhardtii*. *Curr. Genet.* **13**: 503–508.
- Grossman, A.R., Harris, E.E., Hauser, C., Lefebvre, P.A., Martinez, D., Rokhsar, D., Shrager, J., Silflow, C.D., Stern, D., Vallon, O., and Zhang, Z. (2003). *Chlamydomonas reinhardtii* at the crossroads of genomics. *Eukaryot. Cell* **2**: 1137–1150.
- Gumpel, N.J., Rochaix, J.D., and Purton, S. (1994). Studies on homologous recombination in the green alga *Chlamydomonas reinhardtii*. *Curr. Genet.* **26**: 438–442.
- Guo, Y.L. (2013). Gene family evolution in green plants with emphasis on the origination and evolution of *Arabidopsis thaliana* genes. *Plant J.* **73**: 941–951.
- Haber, J.E. (2012). Mating-type genes and MAT switching in *Saccharomyces cerevisiae*. *Genetics* **191**: 33–64.
- Harris, E.C. (2008). The *Chlamydomonas* Sourcebook: Introduction into *Chlamydomonas* and Its Laboratory Use. (Oxford, UK: Elsevier Academic Press).
- Heimerl, N., Hommel, E., Westermann, M., Meichsner, D., Lohr, M., Hertweck, C., Grossman, A.R., Mittag, M., and Sasso, S. (2018). A giant type I polyketide synthase participates in zygospore maturation in *Chlamydomonas reinhardtii*. *Plant J.* **95**: 268–281.
- Helliwell, K.E., Wheeler, G.L., Leptos, K.C., Goldstein, R.E., and Smith, A.G. (2011). Insights into the evolution of vitamin B12 auxotrophy from sequenced algal genomes. *Mol. Biol. Evol.* **28**: 2921–2933.
- Helliwell, K.E., Scaife, M.A., Sasso, S., Araujo, A.P., Purton, S., and Smith, A.G. (2014). Unraveling vitamin B12-responsive gene regulation in algae. *Plant Physiol.* **165**: 388–397.
- Hinnen, A., Hicks, J.B., and Fink, G.R. (1978). Transformation of yeast. *Proc. Natl. Acad. Sci. USA* **75**: 1929–1933.
- Hu, J., Deng, X., Shao, N., Wang, G., and Huang, K. (2014). Rapid construction and screening of artificial microRNA systems in *Chlamydomonas reinhardtii*. *Plant J.* **79**: 1052–1064.

- Huang, K., and Beck, C.F.** (2003). Phototropin is the blue-light receptor that controls multiple steps in the sexual life cycle of the green alga *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. USA* **100**: 6269–6274.
- Jahn, R.** (1998). C. G. Ehrenberg: The man and his contribution to botanical science. In Christian Gottfried Ehrenberg (1795–1876): The Man and His Legacy, D.M. Williams and R. Huxley, eds (London, UK: Special Publication of The Linnean Society, Academic Press), pp. 15–29.
- Jalal, A., Schwarz, C., Schmitz-Linneweber, C., Vallon, O., Nickelsen, J., and Bohne, A.V.** (2015). A small multifunctional pentatricopeptide repeat protein in the chloroplast of *Chlamydomonas reinhardtii*. *Mol. Plant* **8**: 412–426.
- Jensen, K.H., Herrin, D.L., Plumley, F.G., and Schmidt, G.W.** (1986). Biogenesis of photosystem II complexes: Transcriptional, translational, and posttranslational regulation. *J. Cell Biol.* **103**: 1315–1325.
- Jinkerson, R.E., and Jonikas, M.C.** (2015). Molecular techniques to interrogate and edit the *Chlamydomonas* nuclear genome. *Plant J.* **82**: 393–412.
- Johnson, D.S.** (1914). The history of the discovery of sexuality in plants. *Annual Report Smithsonian Institution* **69**: 383–406.
- Joo, S., Nishimura, Y., Cronmiller, E., Hong, R.H., Kariyawasam, T., Wang, M.H., Shao, N.C., El Akkad, S.E., Suzuki, T., Higashiyama, T., Jin, E., and Lee, J.H.** (2017). Gene regulatory networks for the haploid-to-diploid transition of *Chlamydomonas reinhardtii*. *Plant Physiol.* **175**: 314–332.
- Jungnick, N., Ma, Y., Mukherjee, B., Cronan, J.C., Speed, D.J., Laborde, S.M., Longstreth, D.J., and Moroney, J.V.** (2014). The carbon concentrating mechanism in *Chlamydomonas reinhardtii*: Finding the missing pieces. *Photosynth. Res.* **121**: 159–173.
- Kathir, P., LaVoie, M., Brazelton, W.J., Haas, N.A., Lefebvre, P.A., and Silflow, C.D.** (2003). Molecular map of the *Chlamydomonas reinhardtii* nuclear genome. *Eukaryot. Cell* **2**: 362–379.
- Kianianmomeni, A., Ong, C.S., Rättsch, G., and Hallmann, A.** (2014). Genome-wide analysis of alternative splicing in *Volvox carteri*. *BMC Genomics* **15**: 1117.
- Kim, B.H., Cai, X., Vaughn, J.N., and von Arnim, A.G.** (2007). On the functions of the h subunit of eukaryotic initiation factor 3 in late stages of translation initiation. *Genome Biol.* **8**: R60.
- Kindle, K.L.** (1990). High-frequency nuclear transformation of *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. USA* **87**: 1228–1232.
- Kindle, K.L., Schnell, R.A., Fernández, E., and Lefebvre, P.A.** (1989). Stable nuclear transformation of *Chlamydomonas* using the *Chlamydomonas* gene for nitrate reductase. *J. Cell Biol.* **109**: 2589–2601.
- Kozminski, K.G., Johnson, K.A., Forscher, P., and Rosenbaum, J.L.** (1993). A motility in the eukaryotic flagellum unrelated to flagellar beating. *Proc. Natl. Acad. Sci. USA* **90**: 5519–5523.
- Kropat, J., Hong-Hermesdorf, A., Casero, D., Ent, P., Castruita, M., Pellegrini, M., Merchant, S.S., and Malasarn, D.** (2011). A revised mineral nutrient supplement increases biomass and growth rate in *Chlamydomonas reinhardtii*. *Plant J.* **66**: 770–780.
- Krupinska, K., Melonek, J., and Krause, K.** (2013). New insights into plastid nucleoid structure and functionality. *Planta* **237**: 653–664.
- Kubo, T., Kaida, S., Abe, J., Saito, T., Fukuzawa, H., and Matsuda, Y.** (2009). The *Chlamydomonas* hatching enzyme, sporangin, is expressed in specific phases of the cell cycle and is localized to the flagella of daughter cells within the sporangial cell wall. *Plant Cell Physiol.* **50**: 572–583.
- Kück, U., Choquet, Y., Schneider, M., Dron, M., and Bennoun, P.** (1987). Structural and transcription analysis of two homologous genes for the P700 chlorophyll a-apoproteins in *Chlamydomonas reinhardtii*: Evidence for in vivo trans-splicing. *EMBO J.* **6**: 2185–2195.
- Lazarus, D., and Jahn, R.** (1998). Using the Ehrenberg Collection. *Diatom Res.* **13**: 273–291.
- Lee, J.H., Lin, H., Joo, S., and Goodenough, U.** (2008). Early sexual origins of homeoprotein heterodimerization and evolution of the plant KNOX/BELL family. *Cell* **133**: 829–840.
- Levine, R.P.** (1960a). Genetic control of photosynthesis in *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. USA* **46**: 972–978.
- Levine, R.P.** (1960b). A screening technique for photosynthetic mutants in unicellular algae. *Nature* **188**: 339–340.
- Levine, R.P., and Ebersold, W.T.** (1960). The genetics and cytology of *Chlamydomonas*. *Annu. Rev. Microbiol.* **14**: 197–216.
- Lewin, J.C.** (1950). Obligate autotrophy in *Chlamydomonas moewusii* Gerloff. *Science* **112**: 652–653.
- Lewin, R.A.** (1954). Mutants of *Chlamydomonas moewusii* with impaired motility. *J. Gen. Microbiol.* **11**: 358–363.
- Li, J.B., et al.** (2004). Comparative genomics identifies a flagellar and basal body proteome that includes the BBS5 human disease gene. *Cell* **117**: 541–552.
- Li, X., Zhang, R., Patena, W., Gang, S.S., Blum, S.R., Ivanova, N., Yue, R., Robertson, J.M., Lefebvre, P.A., Fitz-Gibbon, S.T., Grossman, A.R., and Jonikas, M.C.** (2016). An indexed, mapped mutant library enables reverse genetics studies of biological processes in *Chlamydomonas reinhardtii*. *Plant Cell* **28**: 367–387.
- Lin, H., and Goodenough, U.W.** (2007). Gametogenesis in the *Chlamydomonas reinhardtii* minus mating type is controlled by two genes, MID and MTD1. *Genetics* **176**: 913–925.
- Liu, H., Huang, J., Sun, X., Li, J., Hu, Y., Yu, L., Liti, G., Tian, D., Hurst, L.D., and Yang, S.** (2018). Tetrad analysis in plants and fungi finds large differences in gene conversion rates but no GC bias. *Nat. Ecol. Evol.* **2**: 164–173.
- Liu, Y., Tewari, R., Ning, J., Blagborough, A.M., Garbom, S., Pei, J., Grishin, N.V., Steele, R.E., Sinden, R.E., Snell, W.J., and Billker, O.** (2008). The conserved plant sterility gene HAP2 functions after attachment of fusogenic membranes in *Chlamydomonas* and *Plasmodium* gametes. *Genes Dev.* **22**: 1051–1068.
- Loke, J.C., Stahlberg, E.A., Strenski, D.G., Haas, B.J., Wood, P.C., and Li, Q.Q.** (2005). Compilation of mRNA polyadenylation signals in Arabidopsis revealed a new signal element and potential secondary structures. *Plant Physiol.* **138**: 1457–1468.
- Lopez, D., Hamaji, T., Kropat, J., De Hoff, P., Morselli, M., Rubbi, L., Fitz-Gibbon, S., Gallaher, S.D., Merchant, S.S., Umen, J., and Pellegrini, M.** (2015). Dynamic changes in the transcriptome and methylome of *Chlamydomonas reinhardtii* throughout its life cycle. *Plant Physiol.* **169**: 2730–2743.
- Lu, Y., and Xu, J.** (2015). Phytohormones in microalgae: A new opportunity for microalgal biotechnology? *Trends Plant Sci.* **20**: 273–282.
- Luykx, P., Hoppenrath, M., and Robinson, D.G.** (1997). Structure and behavior of contractile vacuoles in *Chlamydomonas reinhardtii*. *Protoplasma* **198**: 73–84.
- Mackinder, L.C., et al.** (2016). A repeat protein links Rubisco to form the eukaryotic carbon-concentrating organelle. *Proc. Natl. Acad. Sci. USA* **113**: 5958–5963.
- Mackinder, L.C.M., Chen, C., Leib, R.D., Patena, W., Blum, S.R., Rodman, M., Ramundo, S., Adams, C.M., and Jonikas, M.C.** (2017). A spatial interactome reveals the protein organization of the algal CO₂-concentrating mechanism. *Cell* **171**: 133–147.e114.
- Mages, W., Heinrich, O., Treuner, G., Vlcek, D., Daubnerova, I., and Slaninova, M.** (2007). Complementation of the *Chlamydomonas reinhardtii* arg7-8 (arg2) point mutation by recombination with a truncated nonfunctional ARG7 gene. *Protist* **158**: 435–446.
- Maiwald, D., Dietzmann, A., Jahns, P., Pesaresi, P., Joliot, P., Joliot, A., Levin, J.Z., Salamini, F., and Leister, D.** (2003). Knock-out of the genes coding for the Rieske protein and the ATP-synthase delta-subunit of Arabidopsis: Effects on photosynthesis,

- thylakoid protein composition, and nuclear chloroplast gene expression. *Plant Physiol.* **133**: 191–202.
- Matsuda, Y., Tamaki, S.-I., and Tsubo, Y.** (1978). Mating type specific induction of cell wall lytic factor by agglutination of gametes in *Chlamydomonas reinhardtii*. *Plant Cell Physiol.* **19**: 1253–1261.
- Matsuo, T., Onai, K., Okamoto, K., Minagawa, J., and Ishiura, M.** (2006). Real-time monitoring of chloroplast gene expression by a luciferase reporter: evidence for nuclear regulation of chloroplast circadian period. *Mol. Cell. Biol.* **26**: 863–870. 16428442
- Matsuo, T., Okamoto, K., Onai, K., Niwa, Y., Shimogawara, K., and Ishiura, M.** (2008). A systematic forward genetic analysis identified components of the *Chlamydomonas* circadian system. *Genes Dev.* **22**: 918–930.
- Maul, J.E., Lilly, J.W., Cui, L., dePamphilis, C.W., Miller, W., Harris, E.H., and Stern, D.B.** (2002). The *Chlamydomonas reinhardtii* plastid chromosome: Islands of genes in a sea of repeats. *Plant Cell* **14**: 2659–2679.
- Mayfield, S.P., and Kindle, K.L.** (1990). Stable nuclear transformation of *Chlamydomonas reinhardtii* by using a *C. reinhardtii* gene as the selectable marker. *Proc. Natl. Acad. Sci. USA* **87**: 2087–2091.
- McCarthy, S.S., Kobayashi, M.C., and Niyogi, K.K.** (2004). White mutants of *Chlamydomonas reinhardtii* are defective in phytoene synthase. *Genetics* **168**: 1249–1257.
- Memmola, F., Mukherjee, B., Moroney, J.V., and Giordano, M.** (2014). Carbon allocation and element composition in four *Chlamydomonas* mutants defective in genes related to the CO₂ concentrating mechanism. *Photosynth. Res.* **121**: 201–211.
- Merchant, S.S., et al.** (2007). The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science* **318**: 245–250.
- Merchant, S., and Selman, B.R.** (1984). Synthesis and turnover of the chloroplast coupling factor 1 in *Chlamydomonas reinhardtii*. *Plant Physiol.* **75**: 781–787.
- Moyer, J.H., Lee-Tischler, M.J., Kwon, H.Y., Schrick, J.J., Avner, E.D., Sweeney, W.E., Godfrey, V.L., Cacheiro, N.L., Wilkinson, J.E., and Woychik, R.P.** (1994). Candidate gene associated with a mutation causing recessive polycystic kidney disease in mice. *Science* **264**: 1329–1333.
- Müller, N., Wenzel, S., Zou, Y., Künzel, S., Sasso, S., Weiß, D., Prager, K., Grossman, A., Kottke, T., and Mittag, M.** (2017). A plant cryptochrome controls key features of the *Chlamydomonas* circadian clock and its life cycle. *Plant Physiol.* **174**: 185–201.
- Nakada, T., Tsuchida, Y., Arakawa, K., Ito, T., and Tomita, M.** (2014). Hybridization between Japanese and North American *Chlamydomonas reinhardtii* (Volvocales, Chlorophyceae). *Phycol Res* **62**: 232–236.
- Nakamura, S.** (2010). Paternal inheritance of mitochondria in *Chlamydomonas*. *J. Plant Res.* **123**: 163–170.
- Nelson, J.A., and Lefebvre, P.A.** (1995). Targeted disruption of the NIT8 gene in *Chlamydomonas reinhardtii*. *Mol. Cell. Biol.* **15**: 5762–5769.
- Nelson, J.A., Savereide, P.B., and Lefebvre, P.A.** (1994). The CRY1 gene in *Chlamydomonas reinhardtii*: Structure and use as a dominant selectable marker for nuclear transformation. *Mol. Cell. Biol.* **14**: 4011–4019.
- Neupert, J., Karcher, D., and Bock, R.** (2009). Generation of *Chlamydomonas* strains that efficiently express nuclear transgenes. *Plant J.* **57**: 1140–1150.
- Neupert, J., Shao, N., Lu, Y., and Bock, R.** (2012). Genetic transformation of the model green alga *Chlamydomonas reinhardtii*. *Methods Mol. Biol.* **847**: 35–47.
- Newman, S.M., Boynton, J.E., Gillham, N.W., Randolph-Anderson, B.L., Johnson, A.M., and Harris, E.H.** (1990). Transformation of chloroplast ribosomal RNA genes in *Chlamydomonas*: Molecular and genetic characterization of integration events. *Genetics* **126**: 875–888.
- Nishimura, Y., Misumi, O., Kato, K., Inada, N., Higashiyama, T., Momoyama, Y., and Kuroiwa, T.** (2002). An mt(+) gamete-specific nuclease that targets mt(−) chloroplasts during sexual reproduction in *C. reinhardtii*. *Genes Dev.* **16**: 1116–1128.
- Noordally, Z.B., and Millar, A.J.** (2015). Clocks in algae. *Biochemistry* **54**: 171–183.
- Ohad, I., Siekevitz, P., and Palade, G.E.** (1967). Biogenesis of chloroplast membranes. I. Plastid dedifferentiation in a dark-grown algal mutant (*Chlamydomonas reinhardtii*). *J. Cell Biol.* **35**: 521–552. 6064364
- Ohresser, M., Matagne, R.F., and Loppes, R.** (1997). Expression of the arylsulphatase reporter gene under the control of the nit1 promoter in *Chlamydomonas reinhardtii*. *Curr. Genet.* **31**: 264–271.
- Ohta, N., and Sager, R.** (1975). Identification of a chloroplast ribosomal protein altered by a chloroplast mutation in *Chlamydomonas*. *J. Biol. Chem.* **250**: 3655–3659.
- Pascher, A.** (1918). Über die Beziehung der Reduktionsteilung zur Mendelschen Spaltung. *Ber. Dtsch. Bot. Ges.* **36**: 163–168.
- Pasquale, S.M., and Goodenough, U.W.** (1987). Cyclic AMP functions as a primary sexual signal in gametes of *Chlamydomonas reinhardtii*. *J. Cell Biol.* **105**: 2279–2292.
- Pazour, G.J., Dickert, B.L., Vucica, Y., Seeley, E.S., Rosenbaum, J.L., Witman, G.B., and Cole, D.G.** (2000). *Chlamydomonas* IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. *J. Cell Biol.* **151**: 709–718.
- Pazour, G.J., San Agustin, J.T., Follit, J.A., Rosenbaum, J.L., and Witman, G.B.** (2002). Polycystin-2 localizes to kidney cilia and the ciliary level is elevated in orpk mice with polycystic kidney disease. *Curr. Biol.* **12**: R378–R380.
- Pazour, G.J., Agrin, N., Leszyk, J., and Witman, G.B.** (2005). Proteomic analysis of a eukaryotic cilium. *J. Cell Biol.* **170**: 103–113.
- Pérez-Martín, M., Blaby-Haas, C.E., Pérez-Pérez, M.E., Andrés-Garrido, A., Blaby, I.K., Merchant, S.S., and Crespo, J.L.** (2015). Activation of autophagy by metals in *Chlamydomonas reinhardtii*. *Eukaryot. Cell* **14**: 964–973.
- Piperno, G., and Mead, K.** (1997). Transport of a novel complex in the cytoplasmic matrix of *Chlamydomonas* flagella. *Proc. Natl. Acad. Sci. USA* **94**: 4457–4462.
- Prescott, G.W.** (1968). *The Algae: A Review*. (Boston: Houghton Mifflin).
- Prochnik, S.E., et al.** (2010). Genomic analysis of organismal complexity in the multicellular green alga *Volvox carteri*. *Science* **329**: 223–226.
- Pröschold, T., Marin, B., Schlösser, U.G., and Melkonian, M.** (2001). Molecular phylogeny and taxonomic revision of *Chlamydomonas* (Chlorophyta). I. Emendation of *Chlamydomonas* Ehrenberg and *Chloromonas* Gobi, and description of *Oogamochlamys* gen. nov. and *Lobochlamys* gen. nov. *Protist* **152**: 265–300.
- Provasoli, L., Hutner, S.H., and Pintner, I.J.** (1951). Destruction of chloroplasts by streptomycin. *Cold Spring Harb. Symp. Quant. Biol.* **16**: 113–120.
- Purton, S., and Rochaix, J.D.** (1994). Complementation of a *Chlamydomonas reinhardtii* mutant using a genomic cosmid library. *Plant Mol. Biol.* **24**: 533–537.
- Quinn, J., Li, H.H., Singer, J., Morimoto, B., Mets, L., Kindle, K., and Merchant, S.** (1993). The plastocyanin-deficient phenotype of *Chlamydomonas reinhardtii* Ac-208 results from a frame-shift mutation in the nuclear gene encoding preapoplastocyanin. *J. Biol. Chem.* **268**: 7832–7841.
- Ramundo, S., and Rochaix, J.D.** (2015). Controlling expression of genes in the unicellular alga *Chlamydomonas reinhardtii* with a vitamin-repressible riboswitch. *Methods Enzymol.* **550**: 267–281.
- Rasala, B.A., Barrera, D.J., Ng, J., Plucinak, T.M., Rosenberg, J.N., Weeks, D.P., Oyler, G.A., Peterson, T.C., Haerizadeh, F., and**

- Mayfield, S.P.** (2013). Expanding the spectral palette of fluorescent proteins for the green microalga *Chlamydomonas reinhardtii*. *Plant J.* **74**: 545–556.
- Remacle, C., Cardol, P., Coosemans, N., Gaisne, M., and Bonnefoy, N.** (2006). High-efficiency biolistic transformation of *Chlamydomonas* mitochondria can be used to insert mutations in complex I genes. *Proc. Natl. Acad. Sci. USA* **103**: 4771–4776.
- Remacle, C., Cline, S., Boutaffala, L., Gabilly, S., Larosa, V., Barbieri, M.R., Coosemans, N., and Hamel, P.P.** (2009). The ARG9 gene encodes the plastid-resident N-acetyl ornithine aminotransferase in the green alga *Chlamydomonas reinhardtii*. *Eukaryot. Cell* **8**: 1460–1463.
- Reyon, D., Kirkpatrick, J.R., Sander, J.D., Zhang, F., Voytas, D.F., Joung, J.K., Dobbs, D., and Coffman, C.R.** (2011). ZFNGenome: A comprehensive resource for locating zinc finger nuclease target sites in model organisms. *BMC Genomics* **12**: 83.
- Rochaix, J.D., and van Dillewijn, J.** (1982). Transformation of the green alga *Chlamydomonas reinhardtii* with yeast DNA. *Nature* **296**: 70–72.
- Rochaix, J.D., van Dillewijn, J., and Rahire, M.** (1984). Construction and characterization of autonomously replicating plasmids in the green unicellular alga *Chlamydomonas reinhardtii*. *Cell* **36**: 925–931.
- Rohr, J., Sarkar, N., Balenger, S., Jeong, B.R., and Cerutti, H.** (2004). Tandem inverted repeat system for selection of effective transgenic RNAi strains in *Chlamydomonas*. *Plant J.* **40**: 611–621.
- Rost, B.R., Schneider-Warme, F., Schmitz, D., and Hegemann, P.** (2017). Optogenetic tools for subcellular applications in neuroscience. *Neuron* **96**: 572–603.
- Roth, M.S., Cokus, S.J., Gallaher, S.D., Walter, A., Lopez, D., Erickson, E., Endelman, B., Westcott, D., Larabell, C.A., Merchant, S.S., Pellegrini, M., and Niyogi, K.K.** (2017). Chromosome-level genome assembly and transcriptome of the green alga *Chromochloris zofingiensis* illuminates astaxanthin production. *Proc. Natl. Acad. Sci. USA* **114**: E4296–E4305.
- Rubin, G.M., and Spradling, A.C.** (1982). Genetic transformation of *Drosophila* with transposable element vectors. *Science* **218**: 348–353.
- Rymarquis, L.A., Handley, J.M., Thomas, M., and Stern, D.B.** (2005). Beyond complementation: Map-based cloning in *Chlamydomonas reinhardtii*. *Plant Physiol.* **137**: 557–566.
- Sager, R.** (1954). Mendelian and non-Mendelian inheritance of streptomycin resistance in *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. USA* **40**: 356–363.
- Sager, R., and Granick, S.** (1954). Nutritional control of sexuality in *Chlamydomonas reinhardtii*. *J. Gen. Physiol.* **37**: 729–742.
- Sager, R., and Palade, G.E.** (1957). Structure and development of the chloroplast in *Chlamydomonas*. I. The normal green cell. *J. Biophys. Biochem. Cytol.* **3**: 463–488.
- Salinas, T., Duby, F., Larosa, V., Coosemans, N., Bonnefoy, N., Motte, P., Maréchal-Drouard, L., and Remacle, C.** (2012). Co-evolution of mitochondrial tRNA import and codon usage determines translational efficiency in the green alga *Chlamydomonas*. *PLoS Genet.* **8**: e1002946.
- Salinas-Giegé, T., Cavaiuolo, M., Cognat, V., Ubrig, E., Remacle, C., Duchêne, A.M., Vallon, O., and Maréchal-Drouard, L.** (2017). Polycytidylation of mitochondrial mRNAs in *Chlamydomonas reinhardtii*. *Nucleic Acids Res.* **45**: 12963–12973.
- Sapp, J.** (1987). What counts as evidence or who was Franz Moewus and why was everybody saying such terrible things about him? *Hist. Philos. Life Sci.* **9**: 277–308.
- Saroussi, S., Sanz-Luque, E., Kim, R.G., and Grossman, A.R.** (2017). Nutrient scavenging and energy management: Acclimation responses in nitrogen and sulfur deprived *Chlamydomonas*. *Curr. Opin. Plant Biol.* **39**: 114–122.
- Satir, P.** (2017). Cilia: Before and after. *Cilia* **6**: 1.
- Schaffer, M., Mahamid, J., Engel, B.D., Laugks, T., Baumeister, W., and Pitzko, J.M.** (2017). Optimized cryo-focused ion beam sample preparation aimed at in situ structural studies of membrane proteins. *J. Struct. Biol.* **197**: 73–82.
- Schmidt, G.W., and Mishkind, M.L.** (1983). Rapid degradation of unassembled ribulose 1,5-bisphosphate carboxylase small subunits in chloroplasts. *Proc. Natl. Acad. Sci. USA* **80**: 2632–2636.
- Schmidt, G.W., Matlin, K.S., and Chua, N.H.** (1977). A rapid procedure for selective enrichment of photosynthetic electron transport mutants. *Proc. Natl. Acad. Sci. USA* **74**: 610–614.
- Schmollinger, S., Strenkert, D., and Schroda, M.** (2010). An inducible artificial microRNA system for *Chlamydomonas reinhardtii* confirms a key role for heat shock factor 1 in regulating thermotolerance. *Curr. Genet.* **56**: 383–389.
- Schroda, M., Blöcker, D., and Beck, C.F.** (2000). The HSP70A promoter as a tool for the improved expression of transgenes in *Chlamydomonas*. *Plant J.* **21**: 121–131.
- Schwab, R., Palatnik, J.F., Rieger, M., Schommer, C., Schmid, M., and Weigel, D.** (2005). Specific effects of microRNAs on the plant transcriptome. *Dev. Cell* **8**: 517–527.
- Shao, N., and Bock, R.** (2008). A codon-optimized luciferase from *Gaussia princeps* facilitates the in vivo monitoring of gene expression in the model alga *Chlamydomonas reinhardtii*. *Curr. Genet.* **53**: 381–388.
- Shen, Y., Ji, G., Haas, B.J., Wu, X., Zheng, J., Reese, G.J., and Li, Q.Q.** (2008). Genome level analysis of rice mRNA 3'-end processing signals and alternative polyadenylation. *Nucleic Acids Res.* **36**: 3150–3161.
- Shimogawara, K., Fujiwara, S., Grossman, A., and Usuda, H.** (1998). High-efficiency transformation of *Chlamydomonas reinhardtii* by electroporation. *Genetics* **148**: 1821–1828.
- Shrager, J., Hauser, C., Chang, C.W., Harris, E.H., Davies, J., McDermott, J., Tamse, R., Zhang, Z., and Grossman, A.R.** (2003). *Chlamydomonas reinhardtii* genome project: A guide to the generation and use of the cDNA information. *Plant Physiol.* **131**: 401–408.
- Sizova, I., Fuhrmann, M., and Hegemann, P.** (2001). A *Streptomyces rimosus* aphVIII gene coding for a new type phosphotransferase provides stable antibiotic resistance to *Chlamydomonas reinhardtii*. *Gene* **277**: 221–229.
- Sizova, I., Greiner, A., Awasthi, M., Kateriya, S., and Hegemann, P.** (2013). Nuclear gene targeting in *Chlamydomonas* using engineered zinc-finger nucleases. *Plant J.* **73**: 873–882.
- Smith, G.M.** (1946). The nature of sexuality in *Chlamydomonas*. *Am. J. Bot.* **33**: 625–630.
- Smith, E.F., and Lefebvre, P.A.** (1996). PF16 encodes a protein with armadillo repeats and localizes to a single microtubule of the central apparatus in *Chlamydomonas* flagella. *J. Cell Biol.* **132**: 359–370.
- Smith, G.M., and Regnery, D.C.** (1950). Inheritance of sexuality in *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. USA* **36**: 246–248.
- Sodeinde, O.A., and Kindle, K.L.** (1993). Homologous recombination in the nuclear genome of *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. USA* **90**: 9199–9203.
- Stevens, D.R., Rochaix, J.D., and Purton, S.** (1996). The bacterial phleomycin resistance gene *ble* as a dominant selectable marker in *Chlamydomonas*. *Mol. Gen. Genet.* **251**: 23–30.
- Strenkert, D., Schmollinger, S., Gallaher, S.D., Salomé, P.A., Purvine, S.O., Nicora, C.D., Mettler-Altmann, T., Soubeyrand, E., Weber, A.P.M., Lipton, M.S., Basset, G.J., and Merchant, S.S.** (2019). Multiomics resolution of molecular events during a day in the life of *Chlamydomonas*. *Proc. Natl. Acad. Sci. USA* **116**: 2374–2383.
- Surzycki, S.J., and Shellenbarger, D.L.** (1976). Purification and characterization of a putative sigma factor from *Chlamydomonas reinhardtii*. *Proc. Natl. Acad. Sci. USA* **73**: 3961–3965.

- Suzuki, L., Woessner, J.P., Uchida, H., Kuroiwa, H., Yuasa, Y., Waffenschmidt, S., Goodenough, U.W., and Kuroiwa, T.** (2000). A zygote-specific protein with hydroxyproline-rich glycoprotein domains and lectin-like domains involved in the assembly of the cell wall of *Chlamydomonas reinhardtii* (Chlorophyta). *J. Phycol.* **36**: 571–583.
- Tabatabaei, I., Dal Bosco, C., Bednarska, M., Ruf, S., Meurer, J., and Bock, R.** (2019). A highly efficient sulfadiazine selection system for the generation of transgenic plants and algae. *Plant Biotechnol. J.* **17**: 638–649.
- Tarver, J.E., Donoghue, P.C., and Peterson, K.J.** (2012). Do miRNAs have a deep evolutionary history? *BioEssays* **34**: 857–866.
- Tulin, F., and Cross, F.R.** (2014). A microbial avenue to cell cycle control in the plant superkingdom. *Plant Cell* **26**: 4019–4038.
- Umen, J.G., and Goodenough, U.W.** (2001a). Control of cell division by a retinoblastoma protein homolog in *Chlamydomonas*. *Genes Dev.* **15**: 1652–1661.
- Umen, J.G., and Goodenough, U.W.** (2001b). Chloroplast DNA methylation and inheritance in *Chlamydomonas*. *Genes Dev.* **15**: 2585–2597.
- Umen, J.G., and Olson, B.J.** (2012). Genomics of volvocine algae. *Adv. Bot. Res.* **64**: 185–243.
- van Lis, R., Popek, M., Couté, Y., Kosta, A., Drapier, D., Nitschke, W., and Atteia, A.** (2017). Concerted up-regulation of aldehyde/alcohol dehydrogenase (ADHE) and starch in *Chlamydomonas reinhardtii* increases survival under dark anoxia. *J. Biol. Chem.* **292**: 2395–2410.
- Voigt, J., and Münzner, P.** (1987). The *Chlamydomonas* cell cycle is regulated by a light/dark-responsive cell-cycle switch. *Planta* **172**: 463–472.
- Voshall, A., Kim, E.J., Ma, X., Yamasaki, T., Moriyama, E.N., and Cerutti, H.** (2017). miRNAs in the alga *Chlamydomonas reinhardtii* are not phylogenetically conserved and play a limited role in responses to nutrient deprivation. *Sci. Rep.* **7**: 5462.
- Wang, C., Liu, Y., Li, S.S., and Han, G.Z.** (2015a). Insights into the origin and evolution of the plant hormone signaling machinery. *Plant Physiol.* **167**: 872–886.
- Wang, Y., Stessman, D.J., and Spalding, M.H.** (2015b). The CO₂ concentrating mechanism and photosynthetic carbon assimilation in limiting CO₂: How *Chlamydomonas* works against the gradient. *Plant J.* **82**: 429–448.
- Wiedeman, V.E., Walne, P.L., and Trainor, F.R.** (1964). A new technique for obtaining axenic cultures of algae. *Can. J. Bot.* **42**: 958–959.
- Wiggins, I.L.** (1962). Gilbert Morgan Smith, a Biographical Memoir. (Washington, D.C.: National Academy of Sciences).
- Wodniok, S., Simon, A., Glöckner, G., and Becker, B.** (2007). Gain and loss of polyadenylation signals during evolution of green algae. *BMC Evol. Biol.* **7**: 65.
- Wollman, F.A., and Delepelaire, P.** (1984). Correlation between changes in light energy distribution and changes in thylakoid membrane polypeptide phosphorylation in *Chlamydomonas reinhardtii*. *J. Cell Biol.* **98**: 1–7.
- Wollman, F.A., and Lemaire, C.** (1988). Studies on kinase-controlled state transitions in photosystem II and b6/f mutants from *Chlamydomonas reinhardtii* which lack quinone-binding proteins. *Biochim. Biophys. Acta* **933**: 85–94.
- Xu, F., Wu, X., Jiang, L.H., Zhao, H., and Pan, J.** (2016). An organelle K⁺ channel is required for osmoregulation in *Chlamydomonas reinhardtii*. *J. Cell Sci.* **129**: 3008–3014.
- Yamano, T., Iguchi, H., and Fukuzawa, H.** (2013). Rapid transformation of *Chlamydomonas reinhardtii* without cell-wall removal. *J. Biosci. Bioeng.* **115**: 691–694.
- Yamasaki, T., and Cerutti, H.** (2017). Cooperative processing of primary miRNAs by DUS16 and DCL3 in the unicellular green alga *Chlamydomonas reinhardtii*. *Commun. Integr. Biol.* **10**: e1280208.
- Yang, W., et al.** (2014). Alternative acetate production pathways in *Chlamydomonas reinhardtii* during dark anoxia and the dominant role of chloroplasts in fermentative acetate production. *Plant Cell* **26**: 4499–4518.
- Zhan, Y., et al.** (2018). Pyrenoid functions revealed by proteomics in *Chlamydomonas reinhardtii*. *PLoS One* **13**: e0185039.
- Zhang, F., Wang, L.P., Brauner, M., Liewald, J.F., Kay, K., Watzke, N., Wood, P.G., Bamberg, E., Nagel, G., Gottschalk, A., and Deisseroth, K.** (2007). Multimodal fast optical interrogation of neural circuitry. *Nature* **446**: 633–639.
- Zhang, H., Herman, P.L., and Weeks, D.P.** (1994). Gene isolation through genomic complementation using an indexed library of *Chlamydomonas reinhardtii* DNA. *Plant Mol. Biol.* **24**: 663–672.
- Zhang, X., Yazaki, J., Sundaresan, A., Cokus, S., Chan, S.W., Chen, H., Henderson, I.R., Shinn, P., Pellegrini, M., Jacobsen, S.E., and Ecker, J.R.** (2006). Genome-wide high-resolution mapping and functional analysis of DNA methylation in *Arabidopsis*. *Cell* **126**: 1189–1201.
- Zhao, T., Li, G., Mi, S., Li, S., Hannon, G.J., Wang, X.J., and Qi, Y.** (2007). A complex system of small RNAs in the unicellular green alga *Chlamydomonas reinhardtii*. *Genes Dev.* **21**: 1190–1203.
- Zones, J.M., Blaby, I.K., Merchant, S.S., and Umen, J.G.** (2015). High-resolution profiling of a synchronized diurnal transcriptome from *Chlamydomonas reinhardtii* reveals continuous cell and metabolic differentiation. *Plant Cell* **27**: 2743–2769.
- Zou, Y., Wenzel, S., Müller, N., Prager, K., Jung, E.M., Kothe, E., Kottke, T., and Mittag, M.** (2017). An animal-like cryptochrome controls the *Chlamydomonas* sexual cycle. *Plant Physiol.* **174**: 1334–1347.