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Quick guide

Chlamydomonas

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***What is* Chlamydomonas?** *Chlamydomonas reinhardtii* (hereafter *Chlamydomonas*) is a unicellular green microalga that has served as one of the major microbial reference systems for both plants and animals ever since the mid-20th century. The cell body is ovoid-shaped and 10–20 µm across, surrounded by a cell wall made primarily of glycoproteins, with two ~12 µm cilia at its anterior end. A single large chloroplast occupying >50% of the cell volume harbors the pyrenoid, a phase-separated organelle packed with the carbon-fixing enzyme Rubisco. A biochemical carbon-concentrating mechanism ensures that the levels of CO_2, the Rubisco substrate, are always high inside the pyrenoid. This organelle is found in many algae, giving algal cells a 10–50 times higher CO_2 fixation capability than the cells of land plants. As a motile, phototactic organism, *Chlamydomonas* senses incoming light with an eyespot containing light-gated channelrhodopsins (ChR1 and ChR2), which have been crucial in the development of optogenetic methods. Once activated, the channelrhodopsins initiate the transmission of membrane depolarization from the eyespot to the cilia, altering their beating pattern and allowing the cell to adjust its swimming direction according to the changing light conditions.

Where can* Chlamydomonas *be found? Most commonly used laboratory strains of *Chlamydomonas reinhardtii* derive from an isolate from a potato field near Amherst, Massachusetts, and all other confirmed isolates of this species are also from temperate soil environments. However, the genus *Chlamydomonas* is large and contains species that cover a wide range of habitats, such as streams, lakes, ocean, snow, ice, and even hyperacidic waters. This variety of species has facilitated comparative studies of adaptation to diverse environments and identification of traits that are suitable for biotechnological applications.

***What do we know about the life cycle of* Chlamydomonas?** *Chlamydomonas* alternates between a vegetative haploid phase and a non-dividing diploid phase (i.e. a haplontic life cycle). In the laboratory, gametogenesis, where mitotically dividing cells differentiate into mating-competent gametes, requires nitrogen starvation combined with light. Activated gametes of opposite mating types stick to each other via interactions between glycoproteins on their cilia, followed by cell and nuclear fusion. The resulting zygote develops into a thick-walled zygospore that is resistant to many types of harsh conditions, such as freezing and desiccation. Maturation of the zygospore takes place in the dark and, upon exposure to light, the zygospore undergoes meiosis and releases four haploid spores that resume vegetative growth. The observation that long photoperiods stimulate germination suggests that zygospores may represent a natural dormant state during the short days of winter.

Why do we use* Chlamydomonas *for research? Research on *Chlamydomonas* took off in the 1950s, after Gilbert Smith at Stanford University collected and shared the isolates that are now used in most laboratories. Phylogenetically, *Chlamydomonas* is favorably positioned as a model eukaryote to study a wide range of biological processes. On the one hand, *Chlamydomonas* is a member of the Viridiplantae supergroup (i.e., the green lineage), making it useful for the study of cellular processes in land plants and other photosynthetic organisms that are of ecological importance. On the other hand, *Chlamydomonas* also shares early evolutionary ancestry with animals and other non-green species, and many defining eukaryotic features are conserved in this organism. Thus, *Chlamydomonas* can serve both as a unicellular model to study plant biology (like yeast does for animal biology) and as a 'pan-eukaryotic' model to understand fundamental eukaryotic processes.

The early work focused on sexual reproduction and photosynthesis, for which *Chlamydomonas* turned out to be ideally suited. Most steps in the sexual cycle can be easily observed using a light microscope, from the initial attachment of cilia between opposite-sex gametes to cell fusion and zygospore formation.

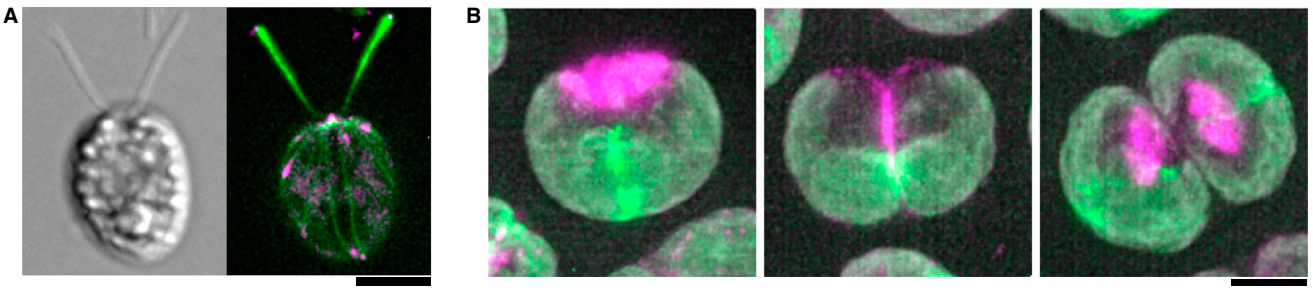

Figure 1. *Chlamydomonas* in different cell-cycle stages.
(A) An interphase *Chlamydomonas* cell, (left) in differential interference contrast and (right) labeled with alpha-tubulin-mNeonGreen (green) and EB1–mScarlet (magenta, marks the growing tips of microtubules). (B) Time-lapse series of a *Chlamydomonas* cell going through the first two divisions of multiple fission, in (left to right) metaphase, first division; telophase, first division; and metaphase, second division. Magenta, EB1–mScarlet; green, FtsZ2–mNeonGreen (marks the chloroplast division ring); gray, chlorophyll autofluorescence. Bars, 5 µm.

Nutritional control of gametogenesis, the existence of mating types, and the uniparental inheritance of the chloroplast genome were important early discoveries. This genetic tractability, combined with an ability to grow heterotrophically, made it possible to isolate and study 'acetate-requiring' mutants, many of which led to the identification of genes required for photosynthetic electron flow.

The structure and function of the cilia were another major focus of early research. The cilia of *Chlamydomonas* are compositionally similar to those of animal cells, and loss of critical ciliary proteins in *Chlamydomonas* often leads to easily detectable motility defects. Moreover, pure cilia can be easily isolated in large quantities for biochemical and ultrastructural analysis. Leveraging these approaches, research in *Chlamydomonas* has led to key insights into how eukaryotic cilia are assembled and maintain a defined length using a process called intraflagellar transport (IFT). The remarkable conservation of ciliary proteins between algae and humans has also made *Chlamydomonas* an important model system for ciliopathies, diseases that affect the assembly or function of human cilia. The first example of this was the isolation of a human gene required for ciliary beating and frequently mutated in the ciliopathy primary ciliary dyskinesia (PCD), based on the identification of *Chlamydomonas* mutants that had lost the ability to swim.

What research tools are available?

A high-quality genome (hosted at phytozome.org), efficient CRISPR-based editing, a library of deletion mutants, and high-throughput genetic screening platforms have meant that *Chlamydomonas* serves as a versatile system for all aspects of eukaryotic biology. The haploid-dominant lifestyle (i.e., each gene being present as a single copy) makes genetic screening straightforward because the phenotypic effects of a mutation are immediately visible. A comprehensive reference (*The* Chlamydomonas *Sourcebook*) was updated in 2024, and a well-maintained strain repository is available at chlamycollection.org.

With the research tools currently available and those being developed, *Chlamydomonas* is used as a new model system in emerging areas of biology. These areas include the carbon-concentrating mechanism and acclimation to high and fluctuating light levels; assembly and division of organelles like the chloroplast, pyrenoid, and basal body apparatus; development of new strains for production of high-value chemicals; evolution of microbial foodwebs; and regulation of the multiple fission cell cycle.

Multiple fission sounds interesting. Can you tell us some more about this? During the light phase of a typical 12 hour light/12 hour dark cycle in the lab, a small daughter newborn *Chlamydomonas* cell can increase more than 10-fold in size by photosynthesis-driven growth. When a size threshold is reached, the cell enters a phase of successive rounds of S phase, mitosis, and cytokinesis — the so-called S/M/C phase — resulting in the generation of up to 16 daughter cells. Uncoupling of the long growth phase from the S/M/C phase (referred to as 'multiple fission') makes *Chlamydomonas* a powerful system for studying cell-size control.

Green plants contain two core cyclin-dependent kinases, CDKA and CDKB, which play important roles in regulating the cell cycle. In *Chlamydomonas*, CDKA controls the upper size threshold by linking G1 growth to S/M/C entry; *cdka* null mutants grow abnormally large before initiating S phase. The Rb protein (homolog of human Retinoblastoma protein) regulates the number of divisions that occur during the S/M/C cycles. The CDKB kinase binds to a mitotic cyclin and controls events within each M phase.

Cytokinesis in *Chlamydomonas* is achieved by animal-like cleavage furrow formation. Unlike the situation in animals, however, this process does not involve myosin II (which is absent from the genome) or require F-actin. Instead, microtubule-based 'phycoplast' structures appear to play an important role. This actomyosin-independent cytokinesis probably shares some commonalities with many other eukaryotes that also lack myosin II. In summary, multiple fission is one of many areas in which *Chlamydomonas* can serve as a model to study the fundamental principles of cell biology, such as cell-size control, cell-cycle regulation, and evolution of cytokinesis mechanisms.

Where can I find out more?

Cross, F.R., and Umen, J.G. (2015). The *Chlamydomonas* cell cycle. Plant J. *82*, 370–392.

Dutcher, S.K. (2023). The *Chlamydomonas* Sourcebook: Volume 3: Cell Motility and Behavior (New York: Elsevier).

Ehler, L.L., and Dutcher, S.K. (1998). Pharmacological and genetic evidence for a role of rootlet and phycoplast microtubules in the positioning and assembly of cleavage furrows in *Chlamydomonas reinhardtii*. Cell Motil. Cytoskeleton *40*, 193–207.

Fauser, F., Vilarrasa-Blasi, J., Onishi, M., Ramundo, S., Patena, W., Millican, M., Osaki, J., Philp, C., Nemeth, M., Salomé, P.A., *et al.* (2022). Systematic characterization of gene function in the photosynthetic alga *Chlamydomonas reinhardtii*. Nat. Genet. *54*, 705–714.

Goodenough, U. (2023). The *Chlamydomonas* Sourcebook: Volume 1: Introduction to Chlamydomonas and Its Laboratory Use (New York: Elsevier).

Grossman, A.R., and Wollman, F.-A. (2023). The *Chlamydomonas* Sourcebook: Volume 2: Organellar and Metabolic Processes (New York: Elsevier).

Hegemann, P., and Nagel, G. (2013). From channelrhodopsins to optogenetics. EMBO Mol. Med. *5*, 173–176.

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.

Mackinder, L.C.M. (2018). The *Chlamydomonas* CO2-concentrating mechanism and its potential for engineering photosynthesis in plants. New Phytol. *217*, 54–61.

Onishi, M., Umen, J.G., Cross, F.R., and Pringle, J.R. (2020). Cleavage-furrow formation without F-actin in *Chlamydomonas*. Proc. Natl. Acad. Sci. USA *117*, 18511–18520.

Salomé, P.A., and Merchant, S.S. (2019). A series of fortunate events: introducing *Chlamydomonas* as a reference organism. Plant Cell *31*, 1682–1707.

Sasso, S., Stibor, H., Mittag, M., and Grossman, A.R. (2018). From molecular manipulation of domesticated *Chlamydomonas reinhardtii* to survival in nature. eLife *7*, e39233.

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. (2001). Control of cell division by a retinoblastoma protein homolog in *Chlamydomonas*. Genes Dev. *15*, 1652–1661.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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