Review

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The *Chlamydomonas* genome project: a decade on

Ian K. Blaby[1], Crysten E. Blaby-Haas[1], Nicolas Tourasse[2], Erik F.Y. Hom[3*], David Lopez[4], Munevver Aksoy[5], Arthur Grossman[5], James Umen[6], Susan Dutcher[7], Mary Porter[8], Stephen King[9], George B. Witman[10], Mario Stanke[11], Elizabeth H. Harris[12], David Goodstein[13], Jane Grimwood[14], Jeremy Schmutz[14], Olivier Vallon[2,15], Sabeeha S. Merchant[1,16], and Simon Prochnik[13]

[1] Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095, USA
[2] Centre National de la Recherche Scientifique (CNRS), Unité Mixte de Recherche 7141, Institut de Biologie Physico-Chimique, Paris, France
[3] Department of Molecular and Cellular Biology and FAS Center for Systems Biology, Harvard University, Cambridge, MA, USA
[4] Department of Molecular, Cell, and Developmental Biology, University of California, Los Angeles, CA, USA
[5] Department of Plant Biology, Carnegie Institute for Science, 260 Panama Street, Stanford, CA, USA
[6] Donald Danforth Plant Science Center, St Louis, MO, USA
[7] Department of Genetics, Washington University School of Medicine, St Louis, MO, USA
[8] Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN, USA
[9] Department of Molecular Biology and Biophysics, University of Connecticut Health Center, Farmington, CT, USA
[10] Department of Cell and Developmental Biology, University of Massachusetts Medical School, Worcester, MA 01655, USA
[11] Institut für Mikrobiologie und Genetik, Universität Göttingen, Göttingen, Germany
[12] Department of Biology, Duke University, Durham, NC 27708, USA
[13] US Department of Energy, Joint Genome Institute, Walnut Creek, CA 94598, USA
[14] HudsonAlpha Genome Sequencing Center, Huntsville, AL 35806, USA
[15] Université Pierre et Marie Curie, Paris, France
[16] Institute of Genomics and Proteomics, University of California, Los Angeles, CA 90095, USA

The green alga *Chlamydomonas reinhardtii* is a popular unicellular organism for studying photosynthesis, cilia biogenesis, and micronutrient homeostasis. Ten years since its genome project was initiated an iterative process of improvements to the genome and gene predictions has propelled this organism to the forefront of the omics era. Housed at Phytozome, the plant genomics portal of the Joint Genome Institute (JGI), the most up-to-date genomic data include a genome arranged on chromosomes and high-quality gene models with alternative splice forms supported by an abundance of whole transcriptome sequencing (RNA-Seq) data. We present here the past, present, and future of *Chlamydomonas* genomics. Specifically, we detail progress on genome assembly and gene model refinement, discuss resources for gene annotations, functional predictions, and locus ID mapping between versions and, importantly, outline a standardized framework for naming genes.

Chlamydomonas – a reference green alga

Chlamydomonas reinhardtii (herein referred to as *Chlamydomonas*) provides an excellent microbial platform for the investigation of fundamental biological functions. Both photosynthesis (a process associated with the plant lineage) and ciliary/flagellar function (associated with the animal lineage) are effectively studied using this organism

Glossary

Defline: a short (2–6 word) explanation of the encoded protein's function. For example, for *LAO1*, the defline is ''periplasmic L-amino acid oxidase, catalytic subunit''.
Description: a lengthier, but concise, explanation of the encoded protein's function with supporting evidence. For example, for *LAO1*, the description is ''L-amino acid oxidase, catalytic subunit Mα; induced by nitrogen starvation [PMID: 8344302]''.
Gene name: also known as the gene symbol. A series of letters and/or numbers assigned to a gene of known function or with known involvement in a biological process. The gene name is unique within *Chlamydomonas*, and for non-historically named genes it should be identical to orthologous gene names from other model organisms. For example, *FTR1* in *Chlamydomonas* and *FTR1* in *Saccharomyces cerevisiae*
Locus ID: defines the genomic region (nuclear, mitochondrial, or plastid) of a feature (typically a gene). In the absence of a gene name, the locus ID should be used to refer to a specific gene. Nuclear loci have the form Cre01.g123450.
Transcript ID: typically one or more transcripts are transcribed from a locus. These have .t1, t2 etc. appended to the locus name; for example, a locus that expresses two alternative spliceforms might be described by the following transcript IDs: Cre01.g123450.t1 and Cre01.g123450.t2. Strictly, a complete transcript ID ends with a version number that increases whenever the sequence of the transcript model changes, for example Cre01.g123450.t1.1. In everyday usage, the version number is often omitted for clarity.
User annotation: the 'gold standard' in gene function annotation. Applied to a gene by an expert in the relevant biological process and supported by experimental or non-automated informatic evidence.

Corresponding author: Prochnik, S. (seprochnik@lbl.gov).
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Present address: Department of Biology, University of Mississippi, Oxford, MS 38677, USA

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as a reference system. A decade of work encompassing the publication of the genome draft sequence [1] has made this organism highly 'genome enabled'. Given the substantial recent and ongoing genomic improvements, their discussion in this article is timely.

As a unicellular haploid in the vegetative stage of its life cycle, *Chlamydomonas* shares the experimental advantages associated with microbes. These include rapid doubling time (~8–12 h), well-defined media and growth requirements, the ability to synchronize cultures with periodic light exposure, the capacity for classical genetic crosses to characterize mutant strains, and efficient long-term cryopreservation [2]. The *Chlamydomonas* molecular and genetic toolbox has grown over the years: irradiated or chemically mutagenized lines have been identified with classical genetic screens [3–5], and RNA interference (RNAi)-based knockdowns [6,7], zinc-finger nuclease-based mutagenesis [8], and efficient protocols for gene-specific mutant screens [9] are now available. A growing collection of laboratory generated and environmentally isolated strains are available at the *Chlamydomonas* resource center (http://chlamy.org/). Complementary to the use of mutants for ascribing gene function, cDNAs [10,11] and bacterial artificial chromosome (BAC) libraries [12] are available for rescuing mutant phenotypes.

Much of the interest in employing *Chlamydomonas* in the laboratory stems from its unique evolutionary history. Approximately 700 million years separate the Chlorophyte (green algae, including *Chlamydomonas*) and Streptophyte (non-chlorophyte green algae and land plants) lineages [13], but the photosynthetic apparatus and auxiliary components have remained remarkably similar. In addition, providing acetate as a fixed-carbon source fully overcomes the need to photosynthesize, such that strains with mutations in photosynthesis-related genes can complete the life cycle. This provides an advantage over land plant systems for determining photosynthetic gene function.

Although plants and animals diverged before the Chlorophyte–Streptophyte split, *Chlamydomonas* and animals have retained many features that were later lost in land plants. In particular, the flagella of *Chlamydomonas* are highly similar to cilia and flagella in mammals, making this alga an excellent system for studying ciliary disease [14,15]. Because the flagella of *Chlamydomonas* are not essential, mutants unable to assemble flagella can be isolated and studied, making this system uniquely useful. Furthermore, *Chlamydomonas* is one of very few model organisms from which it is possible to isolate the basal bodies and flagella, allowing biochemical and proteomic analyses of these organelles [16,17].

The ability of *Chlamydomonas* to bridge the plant and animal lineages, combined with access to the high-quality genome sequence, provides a powerful genetic and genomic platform for probing the function of uncharacterized genes such as the members of the 'green cut' [18,19] and the 'cilia cut' [1]. Consequently, hundreds of laboratories around the world exploit *Chlamydomonas* to address fundamental questions related to photosynthesis, flagella, and the photoproduction of commercial commodities including biofuels.

Version 3.1: a high-quality draft genome and gene predictions

Following two preliminary versions (reviewed in [20]), a draft *Chlamydomonas* genome (JGI v3.1) was published in 2007 [1]. CC-503, a cell wall-less strain of mating type +, was selected because the absence of a complete cell wall facilitated cell lysis and high DNA yields. An average of 13-fold coverage was achieved by sequencing 2.1 million paired-end reads of small insert plasmids, fosmids, and BACs on the Sanger platform. The major challenges presented by the high GC content (64%) were overcome by modifications to the sequencing protocols. Reads were assembled (Box 1) with the (JGI) JAZZ assembler (Table 1). A typical annotation strategy that combined evidence from ~250 000 expressed sequence tags (ESTs) and *de novo* prediction tools (Box 2) generated 15 143 gene models on the assembly. The *Chlamydomonas* community performed unprecedented

Box 1. Genome sequencing

Current technology cannot sequence entire chromosomes; instead, many copies of the chromosomes are randomly fragmented into millions of pieces and these fragments are sequenced. The challenging process of assembly involves recreating the starting chromosomes from millions or even billions of fragment sequences (or reads). Storing all the reads in memory and comparing their sequences to each other can require tens or hundreds of gigabytes of RAM and assembly software can run for days.

Overlapping identical sequences found on different fragments allow the smallest scale of assembly (known as contigs; contiguous runs with no gaps). Tricks such as sequencing both ends of a piece of DNA of known length help assembly at the next level (scaffolds, which link contigs across gaps). By combining sequences from a range of known-sized fragments it is usually possible to recapitulate Mb-sized runs of the genome sequence. Organizing scaffolds onto complete chromosomes currently requires integrating an optical or genetic map with the scaffold sequences. At this point the genome sequence is probably a draft. Finishing requires laborious manual experiments to target gaps that need filling and to correct sequence errors and misassemblies.

Serious problems exist: almost all genomes contain repeats (identical or nearly identical sequences that occur in many locations in the genome). If the sequencing reads are shorter than the repeat sequence, it is not possible to tell which copy of the repeat sequence generated the reads, because repeat sequences are identical (to within the limits of sequencing errors). Sequencing errors as well as variation caused by polyploidy can sometimes be corrected, but may interrupt contigs. Further, some regions of the genome (such as high %GC regions, whose DNA forms tight hairpins that cannot be accessed by the sequencing enzyme) are hard to obtain sequence data from. This and the random nature of sampling can lead to some regions of the genome that are only covered by a few reads (or, in extreme cases, none at all). Next-generation sequencing strategies try to mitigate these problems by sequencing at very high average depth, but, even so, poor coverage can generate a stretch of unknown sequence (a gap) in the assembly. There are a few very useful summary statistics for assessing genome quality. The simplest are the percentage of gaps and the percentage of the genome represented in the assembly. More complex are the N/L50: if all the pieces that make up the assembly are ordered from longest to shortest, these are the number (N50) of pieces needed to make up 50% of the assembly (fewer is better) and the length (L50) of the shortest piece in this set (longer is better) (Table 1).

Table 1. History of *C. reinhardtii* genome assemblies[a]

Genome version	Release date	New data compared to previous releases	Chromosomes	Total Scaffolds	Total sequence (including % gaps)	Scaffold N50/L50	Contig N50/L50
3	2006	Sanger sequencing optimized for high %GC genomes	N/a	1557	120.2 Mb (12.5%)	24/1.7 Mb	603/44.6 kb
4	2008	Complete reassembly with targeted Sanger sequencing of poor-quality regions, followed by manual finishing and further rounds of targeted genome completion. Repeats resolved with 3 kb to BAC-sized clone sequencing. A genetic map with 349 markers [22] was used to anchor scaffolds on chromosomes.	17	88[b]	112.3 Mb (7.5%)	7/6.6 Mb	322/90.6 kb
5	2012	New libraries generated at a wide range of insert sizes, sequenced with Sanger and 454, with every gap targeted for sequencing. Scaffolds integrated into 957 marker genetic map (Martin Spalding, personal communication), supported by Rymarquis *et al.* (2005) [22].	17	54[b]	111.1 Mb (3.6%)	7/7.8 Mb	140/219.4 kb

[a]Initial assemblies consisted of scaffolds (v3). From v4 onwards the scaffolds were mapped to chromosomes using data from genetic maps.

[b]Of which 17 are chromosomes (71 and 37 unanchored scaffolds in v4 and v5, respectively).

manual annotation of gene function, gene symbol (gene name), defline (see Glossary), and description for 2973 genes. This version was deposited in Genbank (accession ABCN01000000). However, gene models in this release were sometimes truncated or missing because supporting expression data were very limited at the time. As discussed below, dramatic improvements in assembly and annotation have taken place, and the most up-to-date version is maintained at Phytozome. Many sequence analysis studies were performed using this resource (reviewed in [21]), as well as comparative phylogenomic studies, culminating in the creation of the 'green cut' and 'cilia cut' [1].

Version 4: genome and annotation improvements

Subsequent improvements to the genome assembly and annotation were tackled systematically. Many gaps were filled by targeted sequencing of fragments appropriate to the size of the gap and manual analysis. The genome was completely reassembled and mapped onto a genetic map [22] that recapitulated the 17 chromosomes of *Chlamydomonas* with only 7.5% of the assembly represented by gaps (Table 1).

Gene models were predicted using a range of tools followed by manual review to reduce errors and increase annotation quality. Initially, gene models were predicted with the JGI pipeline (JGI v4; Table 2). Three annotations were generated with the Augustus algorithm [23], taking advantage of gradual improvements in its methods for integrating EST data. These updates (Aug u5, Aug u9, and Aug u10.2) gradually increased the number of gene models encoding complete proteins from a starting methionine to a terminal stop. This was particularly evident in Aug u10.2 in which expression data from over 7 million 454 ESTs were incorporated into the gene models, allowing extensive annotation of untranslated regions for the first time (Figure 1; Table 2). The Aug u10.2 update was incorporated into Phytozome v.8 as the official JGI v4.3 annotation for genome assembly v4 (Table 2).

Version 5: further improvements

Version 5 of the genome assembly, released in 2012, improved on v4 by targeting remaining gaps and using new Sanger- and 454-based sequencing from a wide range of library sizes. This approach successfully filled approximately half of the gaps (Table 1) and, combined with a 957 genetic marker map (Martin Spalding, personal communication), allowed 34 of the 71 unanchored scaffolds in v4 to be incorporated into chromosomes (Table 1), leaving only 37 unanchored scaffolds in the v5 assembly.

The v5 gene models were generated by integrating new expression data from 59 RNA-Seq experiments totaling 1.03 billion reads. These included 239 million read pairs from JGI, roughly a quarter of which were strand-specific, allowing the direction of transcription and hence the strand of the gene model to be inferred. Gene models were based on Augustus update 11.6 (Aug u11.6) predictions. However, these predictions were made without repeat masking (because the 67% GC content of *Chlamydomonas* coding regions [1] leads to excessive repeat masking; Box 2). They were filtered to remove gene models with $\geq$30% overlap to known transposable elements, open reading frames of $<$50 amino acids, or internal stop codons.

Annotation version JGI v4.3 consisted of 17 114 gene loci (Table 1). A preliminary mapping of 12 263 (72%) of the stable locus identifiers from v4 (see below) was released (JGI v5.3.1, Table 2). The latest version (JGI v5.5) used a more robust mapping algorithm that used local synteny to map loci (12 647 loci, 74%). In addition, genes on the 34 scaffolds that were integrated into chromosomes were given a new locus updated to reflect their new location (2487 loci, 15%). Owing to large changes in the gene models between versions, the remaining loci (1980, 12%) could not be mapped from v4 to v5 in a straightforward manner,

Box 2. Gene modeling, or finding needles in a haystack

The raw genome sequence (Box 1) tells us little about biological function. A series of algorithms with varying degrees of accuracy must be employed to tease this information out of the genome. More than half of a typical plant consists of repetitive sequences, in other words it comprises up to thousands of stretches of sequence that are identical or nearly identical to each other. Repetitive sequences that are similar to each other comprise a repeat family; it is common to have thousands of different repeat families. The presence of many Mb of repetitive sequences greatly increases the computational time it takes to annotate the gene models in the genome (see below) because these regions do not often encode proteins but still have to be scanned. Furthermore, some gene finding algorithms will annotate large and spurious families of genes in repetitive sequences. In a process known as repeat masking, the genome is scanned for repetitive sequences and all occurrences are 'masked' from further analysis.

The next step is gene prediction, which builds 'models' of the genes on the genome from statistical algorithms that recognize likely splice sites, translation starts and stops, open reading frames, typical intron and exon numbers, and lengths per transcript. Modern algorithms also weave in homology data: regions of the assembly that can be translated into a sequence that is similar to a protein from a different organism are likely to encode a gene, and expression data (to confirm predicted splice junctions and add untranslated regions (UTRs) and putative alternative splice forms to transcript predictions). Toolkits such as PASA [25], EVM [41], and MAKER2 [42] are commonly used to integrate expression and homology data into gene models. EST sequences do not usually identify full-length mRNAs, and predictive algorithms therefore range from conservative (giving a minimum combination of exons) and inclusive (giving all possible combinations of exons). A reasonably simple strategy is to generate the 'best' model at a locus, at least as a starting point for downstream analysis. Sometimes the longest model at the locus is used, assuming it is the most complete, however this approach is also subject to errors of locus merging. Finding the beginning and end of transcripts is also tricky, particularly in compact genomes including that of *Chlamydomonas*. Gene models that split or merge gene loci are the result of errors in predicting transcription starts and ends. Errors in gene models are caused equally by too little EST information (where no transcript evidence is available to help delineate exon–intron structure of the gene model) as by too much EST/RNA-Seq data, where noise and inaccuracies in transcription or RNA processing (e.g., intron retention) start to confound what data correspond to functional transcripts. It is important to note that, even with high-quality EST data and robust gene prediction, the gene models are merely that – models.

As genome projects mature, updated (and hopefully improved) assemblies and gene models are generated. It is of great interest to be able to map gene models from previous versions to the new data to leverage published work that references the old data and provide new insights from more complete/detailed updated datasets. However, mapping annotations is challenging: previous models can be fragmented or incomplete, and resolution of collapsed repeats in the new genome sequence can cause particular problems when trying to map paralogs correctly. Gap filling and assembly rearrangements cause additional problems. That being said, in a typical genome two-thirds or more of the gene models can be mapped straightforwardly, and most of the rest can be mapped to some degree, leaving several percent unmapped.

Tools such as Interproscan [43] are commonly used to do a first pass in predicting function based on sequence similarity or motifs. Although having some notion of putative function is desirable, caution must be exercised because inaccuracies are commonplace [39] and computational prediction is no substitute for experimental verification.

and new loci were generated. Expert annotation of gene symbols, deflines, and descriptions was carried forwards during the mapping process.

Owing to the high-quality genome sequence and the substantial amount of expression data available, as well as the functional annotation efforts of the community, gene models in the JGI flagship genome of *Chlamydomonas* represent the most highly curated genomic data for any alga.

Future work

Developments in the *Chlamydomonas* genome project will continue. A systematic review of gene symbols is nearing completion and will form the basis of an updated *Chlamydomonas* GenBank submission. A more involved update of deflines and gene descriptions will come later in 2014, together with methods for users to contribute new information to the database.

As sequencing technologies develop, new types of data, such as chromatin state, will be incorporated into the *Chlamydomonas* genome project will enable novel and exciting analyses on gene regulation.

Resources for gene identifier conversion and bulk annotations

Gene identifier conversion

As *Chlamydomonas* assembly versions and gene models are refined, updated annotations with new locus and transcript identifiers have been generated. This necessitates the ability to convert between versions. For instance, if an RNA-Seq experiment was published with JGI v4 transcript IDs, a researcher would need to convert the old IDs for comparison to present work being performed using the new Aug u11.6 IDs. For small tasks this can be done manually with BLAT [BLAST (Basic Local Alignment Search Tool)-like alignment tool] [24] searches of transcripts against the genome. However, for longer lists of genes, The Algal Functional Annotation Tool offers a Batch Identifier Conversion tool (Table 3). Currently, the tool can convert between JGI v3, JGI v4, Augustus u5, u9 u10.2 (JGI v4.3), and u11.6 (JGI v5.3.1 and v5.5). The Program to Assemble Spliced Alignments (PASA) tool [25] was used to map previous gene models to the v5 assembly; this was aided by a BLAT [24] and BLASTP (BLAST–protein)-based approach [26] that used neighboring genes to help map loci. Future releases of *Chlamydomonas* gene models will be integrated into the tool.

However, automated mapping is impossible or misleading if the underlying genomic sequence (and hence the gene model and, potentially, the protein sequence) for a particular locus has changed drastically between versions, such as in split/merged genes (Box 2) or the filling of large exon coding gaps.

Bulk retrieval of gene function annotation

Whole-genome scale datasets of gene function annotations must be downloaded to perform global 'omics studies. Several online resources provide this functionality (Table 3). The Phytozome database [27] has integrated the Intermine tool [28] for bulk download of sequence and annotation information. Phytozome maintains the gold standard, experimentally validated user annotations, descriptions,

Table 2. History of gene models and locus identifiers

Gene model version[a]	Release date	Transcripts (alternative forms)	New data compared to previous releases	Locus ID format and example	Transcript ID example	Data available at:
JGI v3	2005	15 143 (82[b])	204 k Sanger ESTs	Protein ID, unique number	196029	http://genome.jgi-psf.org/Chlre3/Chlre3.home.html
JGI v4	2008	16 709 (0)	New v4 assembly	Protein ID, unique number	334127	http://genome.jgi-psf.org/Chlre4/Chlre4.home.html
Aug u5	2008	15 818 (1070)	Includes alternate transcript predictions. Transcriptional starts and stops inferred from EST data [44] and trained on a set of manually inspected 5′ and 3′ UTR regions.	Au5.gYYYYY_t1; YYYYY is a serial number along the assembly starting at 1 at the beginning of chromosome 1.	au5.g5896_t1	http://augustus.gobics.de/predictions/chlamydomonas/
Aug u9	2009	15 935 (0)	Augustus algorithm improvements	Au9.CreXX.gZZZZZZZ.t1; XX is the chromosome or scaffold number and ZZZZZZZ is a serial number along the assembly, increasing by 50.	Au9.Cre01.g003650.t1	http://augustus.gobics.de/predictions/chlamydomonas/ http://www.phytozome.net/chlamy
JGI v4.3 (Phytozome 8)	2012	17 114 (0)	Based on Augustus u10.2. Incorporates 6.32 M JGI and 0.69 M Genoscope 454 ESTs, homology to *Volvox carteri*, proteomics data.	CreXX.gZZZZZZZ.t1.B; XX and ZZZZZZ as for Aug u9, B is the version number of this transcript sequence.	Cre01.g042500.t1.2	http://genomes.mcdb.ucla.edu/cgi-bin/hgGateway
JGI v5.3.1 (Phytozome 9.1)	2012	17 737 (1789)	New v5 assembly. Based on Augustus u11.6. Incorporates 1.03 M 454 ESTs and 239 M 2×100 bp Illumina read pairs[c] and other Illumina data totaling 1.03 B reads. Alternative splice forms are included in prediction. Initial partial mapping forwards of v4.3 locus IDs.	CreXX.gZZZZZZ.tA.B; XX and ZZZZZZ as for Aug u9, A is the number of the splice form, B is the version number of this splice form sequence. 13 448 models have stable IDs of this form. The remaining 6078 models are of the form gYYYYY.tA where YYYYY is a serial number along the assembly and A is the number of the splice form.	Cre01.g006450.t2.1 or g200.t1	http://www.phytozome.net/chlamy
JGI 5.5 (Phytozome 10)	2014	17 741 (1785)	Based on Augustus u11.6. Improved mapping forwards from v4.3. All loci have stable locus ID.	CreXX.gZZZZZZ.tA.B	Cre08.g386100.t3.1	http://www.phytozome.net/chlamy

[a]All previous versions are mapped forward and can be browsed at http://www.phytozome.net/chlamy.

[b]Alternative transcripts annotated by hand.

[c]Of these, four sequencing runs (116 million reads) used strand-specific sequencing.

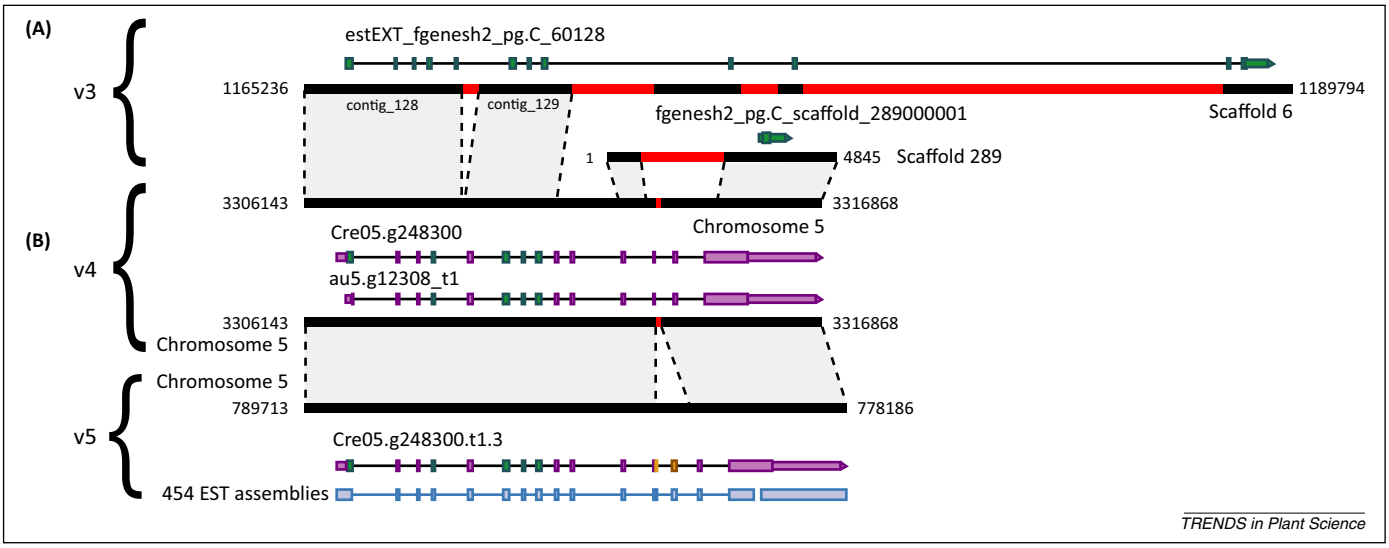

Figure 1. Refinement of the NRAMP4 gene model. NRAMP4 is a divalent cation transporter and member of the 'natural resistance associated macrophage protein' family. Black and red boxes represent genome sequence and gaps respectively on portions of scaffolds or chromosomes (coordinates in bp indicated at the edges) for genome assembly versions as labeled on the left. Gene models are depicted as filled boxes (exons) along horizontal lines (introns). Box fill color indicates the first assembly version an exon was predicted in (green is v3, mauve is v4, orange is v5); wide and narrow sections represent coding sequence and untranslated regions, respectively, and an arrowhead indicates the direction of transcription. Shading between dotted lines represents identical nucleic acid sequence between genome assemblies. **(A)** Comparing assembly v3 to v4, note the amount of gap sequence (red) that was filled, thereby allowing more accurate gene loci to be predicted. The sequences from contig_128 and contig_129 from scaffold 6 were placed on chromosome 5, as was all of scaffold 289. The gap between contig_128 and contig_129 was filled (by addition of 17 bp) in v4, whereas the gap in scaffold 289 was partially filled (by addition of a further 1178 bp). **(B)** The gap in v4 was filled in the v5 assembly (899 bp), which is near-finished quality, allowing the extension of exon 12 and prediction of a new exon (both represented by orange boxes), and generating a gene model that is completely consistent with assembled 454 expressed sequence tag (EST) evidence (lilac track at the bottom).

deflines, and *in silico* functional predictions. Alternatively, the IOMIQS framework [29] utilizes MapMan ontologies to provide a visual output that 'bins' genes into various metabolic groupings. More specific types of annotation can be found on the *Chlamydomonas* section of BioCyc, which maps genes onto metabolic pathways, the *cis*-regulatory element prediction database [30], and PredAlgo [31], providing green algae-specific protein localization predictions (Table 3).

Uniform and stable gene names for *Chlamydomonas*

Following in the footsteps of the reference plant, *Arabidopsis*, once the *Chlamydomonas* assembly was mapped to chromosomes in version 4, every genetic locus in the genome was given a permanent address or locus identifier (e.g., Cre01.g123450; Table 2). These identifiers ensure continuity in the nomenclature going forwards. Such frameworks are widespread for other commonly used organisms and have undoubtedly contributed to their adoption as model systems [32–38].

In addition to the following guidelines, we recommend that researchers use Phytozome as the primary repository for name and annotation data. A mechanism for manual annotation of genes is under active development.

To name or not to name?

Over-annotation in databases, whether of an automated origin, or user-initiated, is common and detrimental: errors can proliferate as computer algorithms map data to new genomes [39]. We therefore propose that genes should only be named (i.e., given what geneticists formally call a gene symbol, such as *ODA11* or *RBCS2*) if one of the following is true: (i) a function or involvement in a specific biological process is associated with a publication. In this case, a pubmed ID (PMID) or other citation should accompany the gene symbol, which should be included in the Phytozome Description. (ii) A gene is associated with a high-throughput screen or global study; for example, proteomes of flagella resulting in the naming of flagellar associated proteins (FAP) or the conserved green lineage (CGL)-associated genes. (iii) The gene function is confidently predicted by a rigorous bioinformatic study. Previously, annotation by investigators with extensive knowledge of particular pathways has been very valuable [40].

If the above criteria are not met, then a gene symbol should not be created. This includes genes encoding proteins with poor similarity to sequences in other organisms (forcing an annotation) or for which the naming is only based on a single conserved domain. In a similar vein, genes should not be named on the basis of homology to proteins involved in a process that does not (or has not been shown to) exist in *Chlamydomonas*. For example, the protein encoded by Cre02.g116900 displays high similarity to small hydrophilic plant seed proteins in *Arabidopsis*. In the absence of seed production, this protein clearly cannot perform this function in *Chlamydomonas*, and therefore should not be named after the Arabidopsis gene *ATEM1*. Genes without an assigned symbol should be referred to by their locus ID because every locus has a unique and stable ID. To distinguish between a gene and an encoded protein, we suggest italicizing locus IDs (*Crex.gyyyyyy*) and non-italicizing proteins (Crex.gyyyyyy).

How to devise a gene symbol

Gene nomenclature guidelines have been established by the *Chlamydomonas* community (http://www.chlamy.org/nomenclature.html) but are not always strictly followed.

Table 3. Online *Chlamydomonas* resources

Database	URL	Summary
Phytozome [27]	http://www.phytozome.net	Primary repository of *Chlamydomonas* genome/gene models. Bulk retrieval of annotation data. Structured to enable comparative genomics with other plants and algae. Contains user validated annotations, and PFAM, Panther, and GO predicted annotations.
UCLA algal genomics portal	http://genomes.mcdb.ucla.edu/	*Chlamydomonas* genome browser. Repository for multiple transcriptomic datasets.
Algal Annotation Tool [45]	http://pathways.mcdb.ucla.edu/algal/index.html	Batch conversion of gene identifiers. Bulk annotation prediction via KEGG, MapMan, GO, Panther, Metacyc.
GIAVAP	https://giavap-genomes.ibpc.fr/chlamydomonas	Comparison of v5.5 gene predictions with previous versions, browser with BAC and fosmid ends.
IOMIQS [29]	http://iomiqsweb1.bio.uni-kl.de	Bulk annotation prediction via MapMan with visual output.
Predalgo [31]	https://giavap-genomes.ibpc.fr/cgi-bin/predalgodb.perl?page=main	Green algal-specific protein localization predictions.
BioCyc [46]	http://biocyc.org/CHLAMY/organism-summary	Maps gene products onto metabolic pathways.
Chlamydomonas Connection	http://www.chlamy.org/	A Gateway to Resources for *Chlamydomonas* Research: news, methods, jobs, gene nomenclature, etc.
Chloroplast genome [47]	http://www.chlamy.org/chloro	Map and gene lists.
Flagellar proteome [17]	http://labs.umassmed.edu/chlamyfp/index.php	Based on version 3, but lists JGIv4 equivalence; UMASS Amherst.
Kazusa Institute [10,11]	http://est.kazusa.or.jp/en/plant/chlamy/EST	Distributes cDNA clones corresponding to their EST collection.
Chlamydomonas Resource Center	http://chlamycollection.org/	Distributes strains, plasmids, cDNA libraries, kits, etc.
ChlamyStation	http://chlamystation.free.fr/	Paris (IBPC) Collection of photosynthesis mutants.
Transcription factors	http://plntfdb.bio.uni-potsdam.de/v3.0/index.php?sp_id=CRE4	Part of the Plant Transcription Factor Database, University of Potsdam.
Silencing RNAs [48]	http://cresirna.cmp.uea.ac.uk/	From the Sainsbury Laboratory, D.C. Baulcombe group.
GreenGenie2 [49]	http://stormo.wustl.edu/GreenGenie2/	GreenGenie gene models.
Plant TFDB [50]	http://planttfdb.cbi.pku.edu.cn/index.php?sp=Cre	Database of *Chlamydomonas* transcription factors.

We hereafter recall the basic rules, and when it is acceptable to depart from them.

(i) The preferred format for gene symbols in *Chlamydomonas* is a 3–5 letter root, in uppercase for nuclear genes, or lower case for organelle genes; this is followed by a number denoting isoform, or occasionally subunits (although, for historically named genes, a combination of letters or numbers has been used and can denote numbered mutants recovered in a genetic screen. Alternatively, the gene symbol, including a number, has on occasion been maintained exactly from the orthologous gene of another organism). In general, three letters are preferred, but may not always be possible (for example when using an *Arabidopsis* gene name, which does not conform to a three-letter standard, the name should not be abbreviated). The root should indicate or abbreviate some aspect of function or phenotype. For example genes *GPD1–4* encode 4 isoforms of glycerol-3-phosphate dehydrogenase, *ASA1–9* encode the nine Chlorophyceae-specific subunits of the mitochondrial ATP synthase, and *ACLA1* and *ACLB1* encode ATP citrate lyase subunits A and B, respectively. For historical reasons, some names depart from this scheme; for example *HSP70A, HSP70B, HSP70C* encode three isoforms of HSP70. Nuclear genes for photosynthesis will retain their cyanobacterial name, followed by a number to denote isoform, unless several isoforms exist (e.g., *RBSCS1–2*, *PSBP1–9*).

To make nomenclature more intuitive, gene symbols can be adapted from those of orthologs in other organisms where characterized orthologs exist. This will ensure related gene symbols across organisms, simplifying comparisons between organisms and retrieval of associated literature.

(ii) Potential confusion should be avoided by confirming the proposed gene symbol is not already in use in *Chlamydomonas*. The authors of this manuscript are available to help researchers verify this. Ideally, it should also not be used in another organism for a different function. The global gene hunter tool (http://www.yeastgenome.org/help/community/global-gene-hunter) enables six databases to be searched simultaneously for this purpose. The Gene database (http://www.ncbi.nlm.nih.gov/gene),

at the National Center for Biotechnology Information (NCBI), is also useful for this purpose and can be used to trace gene name roots across different organisms.

(iii) Historically, many genes were discovered following genetic studies of mutants named on the basis of a phenotype, expression or localization studies (e.g., *LF5* mutants have long flagella, *LCI5* is low-CO_2 inducible). Whenever informative of function, these names are to be preferred as the primary gene symbol over names describing molecular functions. Alternative gene symbols are stored as aliases in Phytozome, allowing the gene to be found if any of its symbols is used as a search term. This effectively links genes to all related literature and vice versa.

Concluding remarks

The culmination of the substantial efforts over a decade is a near-finished *Chlamydomonas* assembly at the scale of complete chromosomes annotated with high-confidence gene models (JGI v5.5), and mappings from previous versions [25]. In addition, our gene naming guidelines provide an empirical framework in which gene names are both likely to reflect function and searchable. If future gene naming follows the policy outlined above, this will help maximize the benefits that the *Chlamydomonas* community derives from its genome project, particularly as refinements and developments continue into the future.

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