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Chapter 4 Functional genomics of *Chlamydomonas reinhardtii*

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CHAPTER CONTENTS

ABSTRACT

Access to the *Chlamydomonas reinhardtii* genome provides the means to pursue fundamental genomics-based research of relevance to algae, land plants and animal lineages. Of the 17741 gene models, the function of most is only of limited knowledge, thus undermining our ability to truly understand the biology of this alga, and plants at large. Transcriptomes, proteomes and phenomes can each provide clues by presenting a global snapshot image of the behavior of the cell under specific conditions. Herein we describe the history of functional genomics, how it is being applied to *Chlamydomonas*, and discuss the resources available for the experimental validation of function predictions.

I. INTRODUCTION

The *Chlamydomonas* genome project was initiated during a number of genomic breakthroughs in the late 90s and early 2000s (Grossman et al., 2003), with the first bacterial, eukaryote, plant, mammalian and human draft genomes all published within less than a decade (Arabidopsis Genome, 2000; Fleischmann et al., 1995; Goffeau et al., 1996; Lander et al., 2001; Mouse Genome Sequencing et al., 2002). As an understanding of the genetic components underpinning biological processes became easier to attain, these watershed undertakings and achievements marked a new period of biological research. Access to the *Chlamydomonas* genome enabled the community to identify gene families shared with plant and animal lineages while leveraging sequence-based analyses to contextualize gene/protein-specific knowledge gained from other reference organisms and applying that information to *Chlamydomonas* genes/proteins. A new appreciation for biological complexity dawned since many coding sequences lacked similarity to proteins of known function. Although two decades have passed since the *Chlamydomonas* genome project was initiated, close to half of predicted *Chlamydomonas* proteins still fall into this “gene of unknown function” category. At the same time, the accuracy of many *predicted* functions remains unknown, while available functional annotations can be vague. Fortunately, as new genome-wide technologies have accelerated gene-specific data acquisition and decreasing costs have democratized acquisition, an increasing body of information is available to link genes and proteins with function and generate hypotheses for further testing. This chapter aims to describe the present state of functional knowledge associated with the *Chlamydomonas* genome, the role of post-genomics data types (transcriptomics, proteomics, phylogenomics, and genome-wide mutant screens) in

providing functional information, and the resources that have been developed to increase our understanding of the functional potential encoded in the *Chlamydomonas* genome.

II. The *CHLAMYDOMONAS* GENOME: STRUCTURAL GENOMICS

Following two early releases, the publication of the third version of the *Chlamydomonas* genome constituted a landmark achievement in algal research: a genomic touchstone became available to link multiple fields of biology from ciliary biogenesis to photosynthesis (Merchant et al., 2007). Constituting 120Mb spread across 1557 scaffolds with an average coverage of 30x Sanger-based sequences, the draft assembly provided the basis for post-genomics experiments and the broad application of bioinformatics and reverse genetics. Over the following decade a series of updates to both the draft genome sequence assembly and gene models was enabled with significant sequencing input of both genomic DNA and transcripts, culminating in the fifth genome (v5) release in 2012. This release consisted of 17 chromosomes plus an additional 37 unanchored scaffolds totaling 111Mb of DNA.

The raw genome sequence gives little insight into biology, and post-assembly analyses are needed to identify genomic elements, such as genes and protein-coding regions. Two releases of gene models were generated using v5 of the genome, namely, 5.3.1 and 5.5, which amplified previous releases by incorporating additional transcriptomic (i.e., RNA-Seq) data (I. K. Blaby et al., 2014). A revised draft genome sequence, v6, and accompanying gene models are actively being worked on with a view to release in 2022; a detailed presentation of v6 is found in Chapter 5 of this volume. For a comprehensive discussion of *Chlamydomonas* structural genomics (i.e., broadly, the raw genome sequence, assembly, and gene model prediction), the reader is referred to Chapter 5.

III. AN INTRODUCTION TO FUNCTIONAL ANNOTATION

Once structural annotations are available, the next step is functional annotation. Functional annotation is the curation of genes/proteins with biological information. However, there are no strict guidelines on how to functionally annotate a genome, and different research groups and different genome databases have their own approaches. The Gene Ontology Consortium (http://geneontology.org/docs/go-consortium/) curates gene/protein function with controlled vocabulary and categories based on 1) “molecular function,” a description of gene product’s activities that occur at the molecular level; 2) “biological process,” a description of the process to which the gene product (often in concert with other

gene products) contributes; and 3) “cellular component,” a description of the gene product’s location that can be a cellular compartment or macromolecular complex (Ashburner et al., 2000). This particular vocabulary-based structure aids in identifying enriched terms present in a genome-wide study, such as transcriptomics or proteomics.

In most other genome databases, functional annotations are presented as short deflines that may describe a characterized function, present a predicted function, or provide some type of functional information, such as family membership or the phenotype observed with the gene is disrupted. At one end of the spectrum, a functional annotation may be limited to the observation that the gene is upregulated under a certain condition, implicating the role of the encoded protein in response to that condition. At the other end, a fully characterized enzyme would be associated with knowledge of the reaction performed, in what pathway and in which cellular compartment the enzyme participates, the regulation of the gene and protein, and (ultimately) a mechanistic understanding as to how the reaction is catalyzed. Often this extent of functional annotation is not provided by a typical genome database and requires sourcing the primary literature.

IV. STATE OF FUNCTIONAL ANNOTATIONS IN *CHLAMYDOMONAS*

Based on community-driven curation efforts, 9% of *Chlamydomonas* genes are associated with a publication. In many cases, these publications present expert-derived bioinformatic analyses that place *Chlamydomonas* proteins within conserved families. Since the manual curation and experimental validation of each of the remaining 91% of genes/proteins is presently an impractical task, functional annotations, as with most other genomes, are largely based on genome-wide searches of sequence similarity to proteins in available databases, usually in an automated fashion via BLAST or sequence models that enable placing a protein within a conserved family (Altschul, Gish, Miller, Myers, & Lipman, 1990; Altschul et al., 1997).

Estimating a confidence level for such predicted functional annotations is difficult, and this type of functional annotation can lead to mis- and over-annotation (Danchin, Ouzounis, Tokuyasu, & Zucker, 2018; Promponas, Iliopoulos, & Ouzounis, 2015; Schnoes, Brown, Dodevski, & Babbitt, 2009). Specific metrics are not available, and there is no standard definition of what constitutes protein function (Rhee & Mutwil, 2014). Equally, the veracity of using sequence similarity alone to functionally annotate proteins is hotly debated (Radivojac et al., 2013) for a number of reasons: i) the source of the original, experimentally derived, annotation is only rarely preserved, thus obfuscating the evolutionary distance

between the experimentally characterized protein(s) and the protein being annotated; ii) functional annotations are often not transferred by similarity from the experimentally characterized progenitor of the annotation but from a sequence whose annotation is also computationally derived; iii) the E-value statistic used by BLAST is dependent on a number of parameters, including database size and protein length; hence using an arbitrary E-value cutoff as a proxy for functional conservation is a misappropriation of this statistic; iv) relatively minor amino acid changes can alter aspects of function, such as substrate-binding or localization (Rost, 2002; Zallot, Harrison, Kolaczkowski, & Crécy-Lagard, 2016).

To mitigate some of these challenges, Phytozome 13 furnishes automatically generated predictions derived from a handful of databases and preserves the name of those databases, the database identifiers, and provides a schematic of the detected similarity between each protein and the database-derived sequence models (Goodstein et al., 2012). Annotations tools such as MapMan (Thimm et al., 2004) and Mercator (Lohse et al., 2014) rely on manually curated classifications originating from green algae and land plants, which limits annotation error resulting from annotating *Chlamydomonas* proteins from very distantly related organism.

Since only a small proportion of *Chlamydomonas* genes/proteins are experimentally characterized, where is functional information available for annotating *Chlamydomonas* genes/proteins using bioinformatics? This is a difficult question to answer. Currently, only limited capabilities exist to automate data extraction from the primary literature via natural language processing techniques. As a result, reliable database entries have to be manually curated and updated with new discoveries and datasets as they are published (Ching et al., 2018; Pestian et al., 2007; Zhao, Su, Lu, & Wang, 2020). The best curated functional annotation database in AmiGO houses automated and curated GO terms and associated provenances (Carbon et al., 2009). Mining this database provides a snapshot of the state of functional annotations across the major phylogenetic lineages (Fig. 1). As part of the GO Consortium's Reference Genome Project, a comprehensive set of manually curated GO terms are available for the human genome and eleven reference organisms: *Arabidopsis thaliana, Caenorhabditis elegans, Danio rerio, Dictyostelium discoideum, Drosophila melanogaster, Escherichia coli, Gallus gallus, Mus musculus, Rattus norvegicus, Saccharomyces cerevisiae,* and *Schizosaccharomyces pombe (Consortium, 2009)*. This curation effort is evident in the distribution of captured experimentally supported functional annotations (Fig. 1A). For *Chlamydomonas*, a small number (~60) of gene products in v5.5 can be mapped to GO terms curated with experimental evidence from *Chlamydomonas* (Fig. 1B), while for ~30% of *Chlamydomonas* proteins, the most similar *Arabidopsis* protein is

associated with a GO term curated with experimental data (Fig. 1B). The fact remains that functional annotations for ~70% of *Chlamydomonas* genes are either absent or are computational predictions based on information outside the green lineage, which may or may not be appropriately, or correctly, ascribed.

Due to manual curation by the *Chlamydomonas* community at the time of the v3 genome release (Merchant et al., 2007), and the ongoing efforts of many groups in the field, 6554 loci (37%) are associated with biological information. In Phytozome v13 https://phytozome-next.jgi.doe.gov, 6092 loci are now associated with either a manually curated defline (a brief manual annotation usually comprising a few words) and/or a description (a more extensive description consisting of one or several sentences). A total of 5524 loci (31%) are associated with a manually curated gene symbol (i.e., gene name) and 3556 have a curated defline. In addition to these manual curations, Phytozome v13 provides automated curation for 9242, 4175, and 5685 loci based on PFAM, KOG and MapMan ontology, respectively[1]. These numbers are not additive, as many loci classified with one of these annotations are also labelled with another (Fig. 2). Also contained on Phytozome v13 are the predicted cellular localizations of proteins as determined by Predalgo https://mybiosoftware.com/predalgo-1-0-protein-subcellular-localization-prediction-green-algae.html, a tool trained specifically on curated green algal protein localizations (Tardif, 2012). To highlight the biology yet to be discovered in *Chlamydomonas*, ~ 6000 loci are devoid of a curated annotation, ontology or conserved PFAM/KOG domain (Fig. 2).

These statistics underscore the need for genome-scale investigations to provide locus-specific functional inferences at scale. While no single experiment can achieve this, a central concept to functional genomics is to integrate multiple sources of data. As experiments capturing cellular behavior under different conditions accumulate, hypotheses can be formed based on condition-specific expression or phenotypes (Ge, Walhout, & Vidal, 2003; Joyce & Palsson, 2006; Vidal, 2001), although computational and logistical complications have been noted (Palsson & Zengler, 2010). The following sections discuss the nature and extent of functional genomics experiments performed in *Chlamydomonas*, with a view to directing the reader to the accessibility and availability of each resource.

V. FUNCTIONAL GENOMICS OF *CHLAMYDOMONAS*: OVERVIEW

[1] These annotations, and bulk text files for download, are accessible via Phytozome v12, and were assessed in Nov 2020 (Goodstein et al., 2012). N.B. There was a superscript 1 on p. 3 that didn't refer to any footnote so I deleted it.

While defining and characterizing protein function constitutes much of biological research, functional genomics experiments set out to inform some aspect of function on a genome-wide scale. Functional genomics data sets, such as transcriptomics, proteomics, and genome-wide mutant screens, can provide layers of gene-specific data. On the one hand, each experiment provides a global snapshot of cellular behavior under different conditions. On the other hand, by comparing the results of multiple experiments, biological information can be derived by identifying the specific conditions under which a protein is expressed (e.g., when and in what situation the cell requires that transcript or protein) or needed for growth (e.g., which conditions under which the loss of a gene is detrimental), by determining co-expressed proteins (e.g., proteins possibly involved in the same process), and by localizing proteins to specific subcompartments within the cell (e.g., where a protein functions). Within this body of functional genomics resources, we also include phylogenomics techniques that provide a means to contextualize functional knowledge acquired with homologous, non-*Chlamydomonas*, genes and proteins. As with functional genomics experiments, techniques are available for informing on function, such as comparative genomic analyses that reveal whether a gene family is restricted to photosynthetic organisms and, therefore, may function in a photosynthesis-related process. Cumulatively, *Chlamydomonas* has been subjected to hundreds of functional genomics experiments, with thousands of samples. Since a functional genomics investigation provides a (usually quantified) datapoint for each gene in a relatively short time, each type of functional genomics experiment provides a snapshot of some functional data for many, if not all, genes with relatively little time or cost. Clearly such experiments do not individually offer the extensive level of characterization needed for a detailed functional annotation of a given locus, but they can provide the necessary information to generate a conjecture, allowing for further hypothesis-driven experiments to be performed.

VI. TRANSCRIPTOMICS

A transcriptome, whether captured by cDNA sequencing (i.e., RNA-Seq) or by microarray, provides a measure of transcript abundance under defined conditions. The resulting expression data can be used in a number of ways to inform function. Since gene expression is often regulated in response to the environment, a comparative analysis of two or more growth conditions can be used to infer the involvement of genes in that condition. For example, greater transcript abundance of a given gene collected from cells limited for nitrogen compared to cells replete for nitrogen suggests the expression of that locus, and by extension, the encoded protein, is necessary for cell maintenance when restricted for nitrogen. In addition to assessing when a transcript is needed, co-expression analyses can

reveal sets of co-expressed genes, which are more likely to function in the same pathway or process than genes that behave differently (Niehrs & Pollet, 1999). Thus, a functional inference may be made for a gene of unknown function whose transcript abundance alters to similar degrees as another gene of known function. Yet the investigator should be aware of expression-based assumptions: a change in transcript abundance may be post-transcriptional, and changes in transcript abundance do not always correlate with protein abundance. As an example, transcript abundance of some metal-dependent proteins increases during metal deficiency, but protein abundance actually decreases (Blaby-Haas & Merchant, 2011); the increase in transcript abundance is proposed to serve as a feedback loop responding to lower protein activity in the absence of the necessary metal cofactor. In other cases, the altered transcript level may not result directly from the environmental cue but may be a consequence of a downstream or indirect process. These types of changes are seen most often for expression that is responding to a stress caused by the growth condition, such as photooxidative damage during iron limitation (Glaesener, Merchant, & Blaby-Haas, 2013).

The first large-scale interrogations of gene expression in *Chlamydomonas* were performed by microarray. Using an array containing probes representing around 2700 gene sequences derived from Expressed Sequence Tags (ESTs), a comparative analysis was performed to identify genes whose expression profiles differed in response to light intensity and CO_2 levels (Grossman et al., 2003; Im, Zhang, Shrager, Chang, & Grossman, 2003). Other early experiments sought to investigate the influence of other environmental factors, such as sulfur deprivation and reactive oxygen species (ROS) exposure, as well as nutrient deprivation and toxicity (Jamers et al., 2006; Ledford et al., 2004; A. V. Nguyen et al., 2008; Z. Zhang et al., 2004). As access to genomic resources expanded (Asamizu et al., 2004; Eberhard et al., 2006), so did the size of microarrays, as was used to capture a transcriptome-level evaluation of ciliary regeneration (Stolc, Samanta, Tongprasit, & Marshall, 2005).

While the arrays continued to advance coordinately with improved genome releases, and their data was integrated more broadly with other ‘omic scale datasets (Mettler et al., 2014), the technological breakthrough of parallelized second-generation sequencing, and, in particular, the ability to quantitatively sequence cDNA, resulted in RNA-Seq largely replacing microarrays. In addition to being able to detect a higher dynamic range of transcript abundance, reduced cost and cDNA requirements, and availability of standardized analysis tools, RNA-Seq is not limited to a defined set of probes and can capture transcript isoforms as well as novel transcripts. As a consequence, RNA-Seq is routinely exploited to advance

gene model accuracy (Van Verk, Hickman, Pieterse, & Van Wees, 2013; Z. Wang, Gerstein, & Snyder, 2009). Indeed, v5 of the *Chlamydomonas* gene models have benefited from this input (I. K. Blaby et al., 2014). A further advantage is that, since the RNA-Seq read data can be saved digitally as raw sequence, reads can be realigned to the genome and transcripts re-quantified as updated genome drafts and gene models are released (this also allows for data from different investigators to be re-processed using identical computational pipelines, assuming they are made available, allowing for direct comparison and integration of datasets). These advantages have resulted in RNA-Seq becoming a routine technique in many *Chlamydomonas* laboratories. A decade since the first RNA-Seq-based transcriptomes in this alga, there are now hundreds of accessible datasets investigating all areas of *Chlamydomonas* research (examples in Table 1).

The large number of available datasets for a wide breadth of physiological processes is due in part to development of *Chlamydomonas* as a facile experimental system. Carefully controlled and defined manipulations can be made to the growth environment (e.g., precise alterations to temperature, light quality and intensity) and culture media (through chemical supplement or micro/macronutrient dropout). Comparisons of mutants and parental strains (e.g., for regulon analysis with a strain disrupted for a transcription factor) are not complicated by ploidy. While not exhaustive, Table 1 attempts to capture the breadth of availability data by cataloguing *Chlamydomonas* RNA-Seq publications, with particular attention to those datasets that are publicly archived in either the Gene Expression Omnibus (GEO) or the Short Read Archive (SRA) databases, thus enabling access to sequenced reads for future analyses. Some RNA-Seq data can be visualized and accessed in bulk via the *Chlamydomonas* pages of Phytozome v13.

Mining this data can be performed in multiple ways. While each individual study focuses on those genes differentially expressed based on a specific experimental design, deeper investigation can be afforded by collating multiple datasets from different publications or reanalyzing data with different computational techniques. One approach is to identify emergent properties from the hierarchical complexity of the data. For example, a manifold-learning method showed that *FDX7* may have different roles in the day vs. night by treating light- and dark-period transcriptomes of a diurnal transcriptome as two distinct datasets and simultaneously clustering the differentially expressed genes (N. D. Nguyen, Blaby, & Wang, 2019). This approach does not assume a phenotype arises from a single gene. By assessing the behavior of genes under different conditions, cohorts with similar expression profiles can be identified.

As noted above, gene co-expression analysis can be a powerful means to inferring gene function, since genes of similar expression profiles may be functionally linked, such as involved in the same pathway or process. Two databases have been developed allowing mining of gene co-expression networks (Aoki, Okamura, Ohta, Kinoshita, & Obayashi, 2016; Romero-Campero, Perez-Hurtado, Lucas-Reina, Romero, & Valverde, 2016), and more recently, a comprehensive study of 58 transcriptomes has illuminated the extent of gene co-expression in *Chlamydomonas*, and provides significant opportunity for further gene-function mining (Salomé & Merchant, 2020).

VII. Proteomics

As valuable as transcriptomes are, their value is limited to inferring transcriptionally and post-transcriptionally regulated mRNA abundance. As the predominant molecular machines in the cell, proteins are prone to additional levels of post-translational regulation. Thus, transcriptome-based expression estimates do not constitute the perfect proxy for protein concentration or enzyme activity, illustrating the benefit of proteomic datasets. The logic for linking proteins with function is identical for proteomes as it is for transcriptomes: proteins enriched in response to an experimental perturbance vs. control cells may perform a role in response to that perturbation. Unlike transcriptomes, proteomic analysis can be performed on biochemically fractionated cells and purified compartments, providing protein localization data. Multiple cellular compartments have been isolated from *Chlamydomonas* and subjected to proteomics to determine the protein composition of cilia, the chloroplast, lipid droplets, nucleus and mitochondria (Table 2). As with transcriptomics, the quality and depth of proteomics experiments in *Chlamydomonas* have increased with revisions to the genome and gene models and new technological developments (Rolland et al., 2009).

Nevertheless, while proteomic data captures a truer estimate of protein levels in the cell, this benefit is offset by a lower dynamic range and an inability to detect all expressed proteins. Consequently, many studies investigating different phenomena in *Chlamydomonas* perform both transcriptomic and proteomic analyses on the same samples (Table 2). Indeed, multiple *Chlamydomonas* studies have additionally captured metabolomics data, providing a systems-level view of the cell that attempts to quantify transcript, protein and metabolite abundances[2]. Integrating multi-disciplinary datasets is a powerful approach for understanding cellular behavior. Not only does the likelihood of a gene exhibiting differential expression increase, but the biological significance of a gene's involvement in a specific

[2] While a valuable tool for gene-function analyses, metabolomics data cannot necessarily be correlated to a specific single genetic locus, and consequently are not discussed in this chapter, but are considered elsewhere in *The Sourcebook* (Volume 2, Chapters ***).

process or response increases if consistent behaviors are observed at the transcript and protein levels. Several *Chlamydomonas* studies have combined proteomes and transcriptomes, often with samples being taken from the same cultures (Tables 1 & 2). Even so, and especially when comparative analyses are performed across datasets resulting from different studies or laboratories, carefully controlled conditions, and the recording of experimental metadata, are crucial.

VII. Phylogenomics

Phylogenomics is a term coined by Eisen and colleagues (Eisen, 1998; Eisen, Kaiser, & Myers, 1997) and refers to a strategy for improving function predictions based on protein family relationships rather than just sequence similarity. This approach encompasses phylogenetics (i.e., reconstruction of family member relatedness and common ancestry) as a means to weigh the significance of functional information derived from different family members. The underlying assumption is that orthologs (i.e., genes/proteins in different species that evolved during speciation) are more likely to share the same function, while paralogs (i.e., genes/proteins that have diverged from one other due to a duplication event) may have diverged in function. In addition to phylogenetics, phylogenomics encompasses a suite of comparative-genomic techniques that use associations between genes of unknown/uncertain function and genes with known function to provide insight into the function of the former. Sometimes referred to as “guilt-by-association” (Aravind, 2000), a common analysis is the generation of phylogenetic profiles, which are used to identify functional linkages between sets of genes and a phenotype or metabolic capability (Pellegrini, Marcotte, Thompson, Eisenberg, & Yeates, 1999).

Two major phylogenomics projects undertaken in *Chlamydomonas* have been seminal to our understanding the organism’s photosynthetic and ciliated characteristics. The GreenCut is a phylogenetic inventory, originally comprising 349 genes and since expanded to nearly 600, that are only found in the genomes of organisms capable of photosynthesis (Karpowicz, Prochnik, Grossman, & Merchant, 2011; Merchant et al., 2007). The conservation of these genes in all photosynthetic organisms across nearly a billion years of evolution signifies the paramount importance of their encoded proteins’ functions in the maintenance and efficient operation of photosynthesis. Surprisingly, however, given their apparent importance, 46% of these proteins have only vague functional annotations and 32% are annotated simply as conserved or predicted proteins; hence they are the focus of intense study (Heinnickel & Grossman, 2013; Karpowicz et al., 2011; Merchant et al., 2007; Wittkopp, Saroussi, Yang, & Grossman, 2016). Similarly, the CiliaCut is an assemblage of

195 genes conserved only in the genomes of ciliated organisms; this phylogenetic profile is suggestive of involvement of the proteins encoded by these genes in the biosynthesis, structure or regulation of cilia (Merchant et al., 2007). Both the GreenCut and CiliaCut are discussed at length in chapters X and Y of this publication.

IX. Genome-wide phenotype screens

As with the above approaches, the identification of a gene-specific phenotype can provide an additional layer of biological information for a gene. Since *Chlamydomonas* can be grown in microplates, easily performed screens can involve one mutant and hundreds of growth conditions or thousands of mutants and one or more growth conditions. As underscored by the catalogued collections of *E. coli*, *S. cerevisiae* and *A. thaliana* mutants (Alonso et al., 2003; T. Baba et al., 2006; Winzeler et al., 1999), mapped mutant libraries constitute invaluable resources, especially once they reach genome saturation. When such a library is generated, the entire assemblage (or targeted subgroups) can be subjected to a condition-based selection, identifying genes whose gene products play a role in acclimating to the given selection either because they are essential under the prescribed selection or their loss leads to a fitness advantage. Screens can be conducted under different conditions, such as, for *Chlamydomonas*, heterotrophic vs. photoautotrophic growth regimes, enabling the recorded growth/no growth phenotypes to be attributed to carbon metabolism generally, and photosynthesis specifically. Performing such large-scale experiments with sufficiently large collections of mutants allows for the systematic identification of genotype-to-phenotype relations. Sequenced mutant libraries also allow researchers to cherry-pick specific mutant(s) of interest to their investigations.

To be representative of the genome, such a resource is considered to be approaching genome-saturation once ~85% of loci are disrupted; by definition, genes encoding proteins essential to the cell's viability under the conditions in which the mutants were generated cannot be isolated (Carpenter & Sabatini, 2004). Given the relative ease with which insertional mutants can be produced in *Chlamydomonas* (Chapter **), further facilitated by the haploid status of its genome, this has been a lucrative approach to illuminating key enzymes in a number of pathways and processes in *Chlamydomonas*.

More recently, deep-sequencing technologies have provided opportunities beyond a digital grow/no grow. Second-generation sequencing can be used to quantify the abundance of individual mutants in a population of mutations, obtaining more granular and quantitative strain fitness data of each strain exposed to a given condition (Price et al., 2018). Although

only just becoming available for *Chlamydomonas*, CRISPR-based mutagenesis has enabled genome-scale gene editing in other microbes, overcoming limitations associated with relying on near-random integration of a selection cassette (and the difficulty of identifying disrupted loci) -- the most popular technique in *Chlamydomonas* for generating mutant libraries (as discussed below). Recent progress in applying CRISPR to *Chlamydomonas* (Chapter 18) will presumably lead to the development of such a resource in the near future.

Chlamydomonas has a rich history of exploiting screens to obtain mutant strains with particular phenotypes (i.e., forward genetic screens). Since *Chlamydomonas* can grow heterotrophically when provisioned with fixed carbon (i.e., acetate), mutants in genes essential for photosynthesis can be isolated (Levine, 1960b). Taking advantage of this characteristic, early experiments exposed cells to UV and selected acetate-requiring mutants, leading to the discovery of genes encoding components of the photosynthetic electron transport chain and photosystem II (Fork & Urbach, 1965; Levine, 1960a). Ethyl methanesulfonate (EMS) and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) have also been employed in concert with appropriate screens to identify the genetic basis of arginine biosynthesis (and also generating the Arg- auxotrophic strains) (Loppes, 1968), cell wall biosynthesis (Davies & Plaskitt, 1971) and ciliary assembly (Davies & Plaskitt, 1971; Huang, Rifkin, & Luck, 1977). A thorough consideration of mutagenesis in *Chlamydomonas* is provided in Chapter 15 of this volume.

The use of mutagens as a tool to generate mutants was later joined by the development of protocols enabling efficient DNA delivery into *Chlamydomonas* and integration of that DNA into the genome by illegitimate recombination in a near-random fashion. The transforming DNA can be as minimal as a drug resistant or auxotrophic marker, of which many have been described in *Chlamydomonas* (Scaife et al., 2015). Since the inserted DNA is of known sequence, selected mutants can be mapped (Cheng et al., 2017; Rachel M Dent, Haglund, Chin, Kobayashi, & Niyogi, 2005; R. M. Dent et al., 2015; Li et al., 2016). Furthermore, if one assumes random integration, and that the genome is 111Mb with an average gene size of ~5kb, a library of 100,000 mutants might be expected to contain several integrations per gene (although integrations in non-coding regions, such as introns and UTRs, may not impair function of the encoded protein). While dependent upon the ability to select for an observable or measurable phenotype, screens based on this approach have identified genes in many pathways. For example, a collection of approaching 50,000 mutants generated by the random insertion of either zeocin or paromycin resistant markers (conferred by *ble* and *aphVIII* respectively) was subjected a series of screens designed to identify genes related to photosynthesis and related metabolism (R. M. Dent et al., 2015).

Mutants were isolated displaying a phenotype as a consequence of one of twelve conditional screens, including acetate-dependency (thus null for photosynthesis), deviant growth rates vs. when subjected to high- or low-light, and sensitivity to reactive oxygen species. Using PCR-based methods (David Gonzalez-Ballester et al., 2011; Liu, Mitsukawa, Oosumi, & Whittier, 1995) to enrich for and identify the flanking regions of the lesion, these studies resulted in the identification of 439 mapped mutants displaying known phenotypes (R. M. Dent et al., 2015). A similar study mapped by PCR-based methods (D. Gonzalez-Ballester, de Montaigu, Galvan, & Fernandez, 2005) yielded 26 mutants in 20 genes defective for motility and a further 10 mutants incapable of intraflagellar transport complex assembly (Cheng et al., 2017). Other screens have identified key proteins involved in starch biosynthesis, flagellar biosynthesis, the carbon concentrating mechanism and the circadian system). Another collection of mutants with disrupted cell division, an essential process, overcame the limitation of disrupting essential genes by screening specifically for temperature sensitive mutants (Breker, Lieberman, & Cross, 2018). By screening at both restrictive and permissive temperatures, 350 mutants were isolated, and 260 were determined to have resulted from one or occasionally two mutagenized loci. This and other collections of mapped mutants from such genome-wide screens are available to researchers *en masse* (Table 3), allowing access to these mutants for reverse-genetics based investigations.

X. Use of mutant collections for reverse-genetics

Despite their utility, most libraries built for forward genetic screens are ultimately disposable resources: a handful of mutants with desired phenotypes are maintained, some are successfully mapped to a specific locus, but the remainder (likely tens of thousands of mutant strains) are discarded. Beginning with the publication of a method enabling moderate throughput identification of the disrupted locus (David Gonzalez-Ballester et al., 2011), high-throughput reverse genetics took off in *Chlamydomonas.*

Specifically, reverse genetics conceptually differs from forward-genetic screens, since in the latter, investigators hope to identify mutants with a specific phenotype, while in the former researchers seek a phenotype associated with a given gene disruption. The relative ease with which investigators could screen this collection by PCR-based methods enabled a series of genetic mutant-based gene function analyses to be performed (Beel et al., 2012; N. R. Boyle et al., 2012; Catalanotti et al., 2012; Duanmu et al., 2013; Magneschi et al., 2012; Meuser et al., 2012; Yang et al., 2015). Building upon these achievements, several large-scale *Chlamydomonas* mutant collections have been generated by insertional mutagenesis, the affected loci mapped, and the collection preserved, allowing for interrogation of the

affected region. . A thorough consideration of *Chlamydomonas* mutant libraries is provided in Chapter 16 of this volume.

A significantly larger collection of known site-of-insertion *Chlamydomonas* mutants has been generated by the Jonikas laboratory. The group has mapped the sites of >80,000 integrants; thus, assuming random integration, the collection is approaching the theoretical point of targeting all loci. The first method to identify the sites of integration exploited a type IIS restriction enzyme, *Mme*I, which cleaves DNA 20 nucleotides downstream of the recognition site (R. Zhang et al., 2014). Since the recognition site, TCCRAC, is incorporated at the extreme of the end of the introduced cassette, 20 nt of the flanking genomic DNA can be captured. Following enrichment, this signature 20bp can be determined by second-generation sequencing methods to identify the genomic insertion site, resulting in roughly 12,000 mapped mutants. As with other protocols to map insertions described above, this technique does not fully capture the genotype of the resultant strains, since only the region immediately flanking the insertion is determined. Notably, the site of insertion can be associated with unmapped deletions or insertions (Rachel M Dent et al., 2005; R. M. Dent et al., 2015; Tam & Lefebvre, 1993; R. Zhang et al., 2014). Multiple integration events, disrupting more than one locus, are also possible. Linking genotype to phenotype requires the implicit assumption that the genotype is known. Therefore, because of the potential for unmapped mutations in these mutants, additional due diligence is required.

In some organisms, rescue of the phenotype can be performed by expressing a wild-type copy of the target gene *in trans*. Since *Chlamydomonas* has a plasmid system for this purpose, complementation can be attempted by introducing a non-replicating expression cassette containing the wild-type gene, which integrates into the genome by illegitimate recombination. This method results in non-uniform expression levels across transformants, sometimes requiring the screening of 100 independent transformants to acquire one or two lines with suitable levels of expression (Ian K Blaby & Blaby-Haas, 2018). Furthermore, the high GC-skew of the genome presents challenges to cloning *Chlamydomonas* genes, although this has been offset to some degree by advances in obtaining synthetic DNA.

To help mitigate these effects, the *Mme*I-based method was subsequently built upon. A paired-end sequencing approach, combined with sequencing the region immediately flanking the insertion site, provides sequence information for a region up to 1.5kb distal to the insertion (Li et al., 2016). If all paired-end reads match the expected genome sequence, a higher level of confidence can be placed on the presence of a single insertion site for the cassette. The added sequence coverage also aids with mapping integrants whereas the 20bp signature DNA did not invariably yield unique sequence (Li et al., 2016). While this approach does not rule out the possibility of rearrangements, deletions and insertions distal the identified integration site, ~83,000 mutants have been determined using these

techniques, and are available as the Chlamydomonas Library Project (CLiP). These mutants plus an additional 439 mutants identified by Dent et al, (R. M. Dent et al., 2015) are available where they are maintained to the community via the Chlamydomonas Resource Center, constituting a reverse-genetics resource (See Vol 1 Chapter 17 for a detailed presentation of these libraries). These mutant collections have been analyzed using large numbers of parameters, resulting in the creation of comprehensive datasets for each mutant strain's phenome (Vilarrasa-Blasi et al., 2020). At this depth of experimentation, the same logic can be practiced as with gene co-expression: when multiple mutants carrying deletions for different genes exhibit similar phenotypes when exposed to the same conditions, they may be functionally linked.

Many of these mapped strains are of potential interest to multiple researchers. Even when only those mutants mapping to exons are considered, 14,650 mutants are accessible, affecting 5078 of the 17741 loci in v5.5 (~28%), or conversely, 12,663 genes do not contain an insertion within an exon. If one assumes the sequencing approach devised to map the mutants captured all insertions, including those landing within non-coding regions, 4,876 loci are not detected at all, suggesting that these genes constitute the essentiome (i.e., genes whose proteins are essential to viability under the conditions in which the library was generated lies).

Of particular relevance are those mutants lying within specific inventories of genes noted earlier, such as the GreenCut, CiliaCut or genes highlighted by transcriptomes to be differentially expressed under certain conditions. For example, of the 596 GreenCut genes mapped to v5.5, 226 exon-specific insertions (or 451 total) are available in the Chlamydomonas Library Project (CLiP) and 108 are accessible in the Niyogi collection (R. M. Dent et al., 2015). Mutants are available for 70 of the 161 CiliaCut genes that can be dependably mapped to v5.5. A further 134 mapped single mutants and 45 double mutants that have been instrumental in studying photosynthesis are available through the ChlamyStation database (See tools section below). Nevertheless, only a minute subset of all available mutants have been sequenced at the whole-genome level, and the methods for mapping the CLiP collection have been found to be inaccurate for some mutants. Due to the possibility of offsite, non-detected, genetic aberrations occurring beyond the sequenced region, caution should be exercised by the researcher before assuming an observed phenotype is directly due to proposed lesion, and newly received strains should be confirmed prior to subjecting them to extensive analysis.

XI. Databases and resources for *Chlamydomonas* genomics and functional genomics

Central to the ability to interpret large, complex, datasets are the computational tools to both warehouse and analyze/visualize those data. Fortunately, multiple web-based resources are available to aid with the curation and dissemination of genomics data for *Chlamydomonas*. Table 3 describes tools that continue to be maintained, as well as detailing websites enabling distribution of strains and plasmids. It should be noted that several of these tools were implemented during previous releases of the *Chlamydomonas* genome, and consequently cater predominantly to earlier genome versions. Therefore, using some tools, and indeed datasets as described in Tables 1 and 2, may necessitate converting gene model generations. This can be automated via the algal annotation tool or on a local machine using the correspondence tables accessible from Phytozome (Table 3).

XII. Outlook and future directions

As presented in this chapter, functional genomics datasets provide a means to collect biological information for genes and proteins on a genome-wide scale. Because each dataset typically focuses on a specific biological question and a limited number of growth conditions, mining multiple datasets is needed to build a more complete picture of gene/protein expression and condition-specific phenotypes. When contextualized with biological information acquired through homology searches and phylogenetics, these resources can be used to generate, support, or refute a functional annotation. At the same time, leveraging these data can lead to a hypothesis with respect to gene/protein function, which can be tested at the bench.

Resources are available for acquiring or generating *Chlamydomonas* gene-disruption mutants and/or mutants with the gene of interest overexpressed. Presently, reverse genetics (i.e., the ability to delete or disrupt loci in a targeted manner without affecting any non-targeted region) in *Chlamydomonas* is not as routine as for other microbial reference systems like *S. cerevisiae* (which lacks chloroplasts and cilia), but several techniques are available.

Several studies have employed gene silencing (RNAi) as an approach to knock down the expression of targeted genes (Cerutti, Johnson, Gillham, & Boynton, 1997; E.-J. Kim & Cerutti, 2009). This has the unique benefit of allowing the phenotypic analysis of essential genes; however, the extent of down-regulation tends to diminish over time, making these mutants short-term resources. Down-regulation is likely often due to the fact that *Chlamydomonas* has an effective mechanism to recognize and silence transgene expression

– e.g. expression of the artificial microRNA used for directed knock-down (Neupert et al., 2020). Still, multiple mutants have been made in this way, provisioning researchers with the strains required to assess gene function (Kumar et al., 2017; Oey et al., 2013; S. Schmollinger, Strenkert, & Schroda, 2010).

Zinc-finger nucleases (ZNFs), transcription activator-like effector nucleases (TALENS) and clustered regularly interspaced short palindromic repeat (CRISPR) together with its associated nuclease, Cas, represent progressive generations of genetic tools that can be exploited to induce double-strand breaks in DNA in a targeted manner (Christian et al., 2010; Jinek et al., 2012; Y.-G. Kim, Cha, & Chandrasegaran, 1996). The means of gene targeting differs between each of these tools. Both ZFNs and TALENs are dependent on DNA-binding domains to recruit the nuclease component to the intended locus, and consequently their use necessitates assembly of the DNA constructs, as well as in vivo transcription and translation of the protein machinery, to ensure proper recruitment of the nuclease. Both this complex assembly as well as a propensity for off-target lesions has limited the utilization of either ZFNs or TALENs in *Chlamydomonas*, despite both being demonstrated to viable approaches, with efficiencies of 1% being reported (Gao et al., 2014; Greiner et al., 2017; I. Sizova, Greiner, Awasthi, Kateriya, & Hegemann, 2012, 2013).

That CRISPR can be programmed with a single guide RNA, circumventing much of the complexity associated with either ZFNs or TALENs, has made this system an indispensable tool for high throughput gene function interrogation in many organisms. Presently, the technology is in its relative infancy in *Chlamydomonas* (Chapter 18). An early investigation suggested toxicity of high levels of Cas expression (Jiang, Brueggeman, Horken, Plucinak, & Weeks, 2014), and, consistent with this, most success has been observed by pre-assembling the RNA protein complex in vitro prior to transformation (Baek et al., 2016; Ferenczi, Pyott, Xipnitou, & Molnar, 2017; Greiner et al., 2017; S. E. Shin et al., 2016). Using this approach, many groups have described success in Chlamydomonas genome editing (Baek et al., 2016; Findinier, Delevoye, & Cohen, 2019; Greiner et al., 2017; Guzmán-Zapata et al., 2019; Jiang et al., 2014; J. Kim, Lee, Baek, & Jin, 2020; S.-E. Shin et al., 2016; Shin et al., 2019), indicating this technique holds promise as a routine reverse genetics approach. CRISPR/Cas9 knockout mutants in *C. reinhardtii* are described in Joo et al., 2022).

Table 1 Examples of *Chlamydomonas* RNASeq studies

Experimental variable	Genome version aligned to	*Chlamydomonas* strain(s)	Data availability[1]	Reference
bilin signalling	4	D66, *hmox1, hmox2, hmox1hmox2*	GSE40031	(Duanmu et al., 2013)
cell cycle	5	not indicated	GSE43004	(Albee et al., 2013)
cell development, strain comparison	5	R3, CJU10	Supplemental Table 2 [2]	(Lopez et al., 2015)
ClpP protease depletion	4	A31, DCH16	GSE56295	(Ramundo et al., 2014)
CO_2 limitation, strain comparison	4	CC-125, *cia5*	GSE33927	(Fang et al., 2012)
copper deprivation, strain comparison	3	CC-1021, *crr1-2*, *crr1*:*CRR1*	GSE25124	(Castruita et al., 2011)
diurnal cycle	5	CC-5152	GSE71469	(Zones, Blaby, Merchant, & Umen, 2015)
diurnal cycle	5	CC-5390	GSE112394	(Strenkert et al., 2019)
diurnal cycle	4	dw15.1	PRJNA264777	(Panchy et al., 2014)
haploid to diploid transition	5	JL28	GEO91400	(Joo et al., 2017)
hypoxia	4	CC-124	GSE42035	(Hemschemeier et al., 2013)
iron starvation	4	CC-4532	GSE44611	(Urzica et al., 2012)
light dark transition	4	CC-1609	GSE62690	(Fu et al., 2015)
longterm culturing under constant light	4	4A +	Supplemental Table 1[3]	(Lv et al., 2013)
mercury toxicity	5	CPCC11	GSE70066	(Beauvais-Flück, Slaveykova, & Cosio, 2016)
nickel addition	5	CC-3960, CC-5073, PST35, CC-5071	Supplemental Data [2]	(Blaby-Haas, Castruita, Fitz-Gibbon, Kropat, & Merchant, 2016)
nitrogen deprivation	4	dw15	GSE24367	(Miller et al., 2010)

nitrogen deprivation, additional acetate added	4	CC-4348, CC-4349, CC-4565, CC-4566, CC-4567	GSE55253	(Blaby et al., 2013)
nitrogen deprivation, strain comparison	5	CC-4348, CC-4349	GSE51642	(Goodenough et al., 2014)
nitrogen deprivation, strain comparison	4	CC-4532, CC-4348, CC-4349	SRX038871, GSE51602	(Schmollinger et al., 2014)
oxidative stress, hydrogen peroxide treatment	5	CC-4532	GSE34826	(Blaby et al., 2015)
oxidative stress, rose bengal treatment, strain comparison	5	CC-4348, *gpx5*	Supplemental Table 1[3]	(Simon et al., 2013)
oxidative stress, rose bengal treatment, strain comparison	4	4A+, sak1	KF985242	(Wakao et al., 2014)
oxidative stress, singlet oxygen acclimation	3	4A+	GSE33548	(Fischer et al., 2012)
salt stress		GY-D55	PRJNA490089	(Wang et al., 2018)
solid and liquid media in light and dark	5	137C	SRP132684	(Bogaert et al., 2018)
sulfur nutrition, strain comparison	3	D66, *snrk2.1*	GSE17970	(González-Ballester et al., 2010)
zinc deficiency	4	CC-4532	GSE25622, GSE41096	(Malasarn et al., 2013)
zinc deficiency and resupply	5	CC-4532	GSE58786	(Hong-Hermesdorf et al., 2014)

[1] Unless otherwise indicated, accession numbers are provided for Gene Expression Omnibus (GEO) (Edgar, Domrachev, & Lash, 2002) or Sequence Read Archive (SRA) (Leinonen, Sugawara, Shumway, & Collaboration, 2010)
[2] Partially available in supplemental data
[3] Supplemental data includes complete transcriptome aligned to the indicated genome version

Table 2 Examples of *Chlamydomonas* proteomic studies

Study summary	Strain	Additional 'omics[1]	Proteins identified	Genome version	Data availability[2]	Reference
anaerobic response	CC-424		2315	3	SM	(Terashima, Specht, Naumann, & Hippler, 2010)
Comparative analysis of WT and salt tolerant strains	CC-503, salt tolerant strain		683 (detected in both strains)	5	SM	(Sithtisarn et al., 2017)
copper, iron, manganese, zinc deprivation	CC-4532			4	SM	(Hsieh et al., 2013)
diurnal	CC-5390	T, M	6403	5	PXD010794[3]	(Strenkert et al., 2019)
glutathionylation	D66		225	4	SM	(Zaffagnini et al., 2012)
heat stress	CF185		3433	3	SM	(Mühlhaus, Weiss, Hemme, Sommer, & Schroda, 2011)
high CO_2	CC-400			4	SM	(M. Baba, Suzuki, & Shiraiwa, 2011)
high light	CC-125		444	3	SM	(Förster, Mathesius, & Pogson, 2006)

iron deprivation	CC-424		203	2	SM	(Naumann et al., 2007)
light intensity	CC-1690	T (microarray), M	644	4	SM	(Mettler et al., 2014)
nitrogen deprivation	CC-4532	T, M		5	SM	(Stefan Schmollinger et al., 2014)
nitrogen deprivation	dw15		259	3	SM	(Moellering & Benning, 2010)
nitrogen deprivation	not indicated		248	4	16644[3]	(H. M. a. B. M. a. C. Nguyen, 2011)
nitrogen deprivation	CC-400		2853	5	PXD019491[3]	(Smythers, McConnell, Lewis, Mubarek, & Hicks, 2020)
routine culturing	CC-503	M	1069	3	SM	(May et al., 2008)
phosphoproteome	SAG 73.72		328	2	SM	(Wagner et al., 2006)
secretome of mating gametes	CC-124, CC-125		1216	5	SM	(Luxmi et al., 2018)
thiolation target proteins	CW15		25		MAT	(Michelet et al., 2008)
thioredoxin target proteins	CW15		55	1	MAT	(Lemaire et al., 2004)
TOR kinase inhibition effect on phosphoproteome	CC-1690		1432	5	PXD007221[3]	(Werth et al., 2019)
compartment/machinery: thylakoid	CW15		2622	2	SM	(Allmer, Naumann, Markert, Zhang, & Hippler, 2006)
compartment/machinery: stroma	XS1		274	4	SM	(Bienvenut et al., 2011)
compartment/machinery: chloroplast	n/a		2315		SM	(Terashima, Specht, & Hippler, 2011)

compartment/machinery: eyespot	CW15		202	2	SM	(Schmidt et al., 2006)
compartment/machinery: centriole	CW15		61	2	SM	(Keller, Romijn, Zamora, Yates III, & Marshall, 2005)
compartment/machinery: flagellar	137c, *oda1-1*		360	2	SM	(Pazour, Agrin, Leszyk, & Witman, 2005)
compartment/machinery: transition zone of flagellar	*pf18* mt−		115	4	SM	(Diener, Lupetti, & Rosenbaum, 2015)
compartment/machinery: mitochondria	83.82		496	2	SM	(Atteia et al., 2009)
compartment/machinery: nucleus	CC-503		672	4	13764[3]	(Winck, Riaño-Pachón, Sommer, Rupprecht, & Mueller-Roeber, 2012)
compartment/machinery: 70S ribosome	CC-3395		30	ESTs & plastid genome (Maul et al., 2002)	MAT	(Kenichi Yamaguchi et al., 2003) (K. Yamaguchi et al., 2002)
compartment/machinery: small subununit of chloroplast ribosome	CC-3395		21	ESTs & plastid genome (Maul et al., 2002)	MAT	
compartment/machinery: pyrenoid	CC-1690	T	368	5	SM	(Mackinder et al., 2016)

[1] T=transcriptome, M=metabolite
[2] SM=Supplemental Material; MAT=Main article table
[3] Accession number for Proteomics Identification Database or proteomeXchange (Martens et al., 2005; Vizcaíno et al., 2014)

Table 3 Summary of data repositories and online tools enabling functional genomics in Chlamydomonas

	Tool	website	genome version	summary	reference
Genome databases	Phytozome 13	https://phytozome-next.jgi.doe.gov	5	Repository of Chlamydomonas genome/gene models allowing bulk-download of data. Gene annotations include user validated annotations, and PFAM and GO predicted annotations, as well as Predalgo predicted protein localizations. Gene co-expression tool utilizing some transcriptomes. Also allows comparative analysis with >200 plants/photosynthetic organisms	(Goodstein et al., 2012)

			https://bioinformatics.psb.ugent.be/plaza/versions/pico-plaza		Comparative genomics of Chlamydomonas and other photosynthetic organisms	(Vandepoele et al., 2013)
Co-expression	picoPlaza			4		
	ChlamyNET		http://viridiplantae.ibvf.csic.es/ChlamyNet	5	Chlamydomonas gene coexpression networks and putative transcription factor binding site predictions	(Romero-Campero et al., 2016)
	ALCOdb		http://alcodb.jp	5	Gene coexpression networks for Chlamydomonas and *Cyanidioschyzon merloae*	(Aoki et al., 2016)
	Algal annotation tool		http://pathways.mcdb.ucla.edu/algal/index.html	3-5	Bulk annotation prediction via Kegg, MapMan, GO, Panther, Metacyc and interconversion of gene model IDs	(Lopez, Casero, Cokus, Merchant, & Pellegrini, 2011)
	Predalgo		https://giavapgenomes.ibpc.fr/cgibin/predalgodb.perl?page=main		Protein localization predictions.	(Tardif, 2012)

Plant transcription factor database	http://planttfdb.gao-lab.org/	5	Predictions for 230 Chlamydomonas transcription factors, and for >160 additional photosynthetic organisms	(Tian, Yang, Meng, Jin, & Gao, 2020)
PlnTFDB	http://plntfdb.bio.uni-potsdam.de/v3.0/index.php?sp_id=CRE4	4	Predictions for 348 Chlamydomonas transcription factors, and for 19 other photosynthetic organisms	(Jin, Zhang, Kong, Gao, & Luo, 2014)
ChlamyCyc 9.0	https://plantcyc.org/content/chlamycyc-9.0	5	Metabolic pathways mapped to genome	(Schläpfer et al., 2017)
Chlamydomonas Resource Center	https://www.chlamycollection.org/		Distributes thousands of catalogued Chlamydomonas strains, in addition to mutant collections (Breker et al., 2018; R. M. Dent et al., 2015; Li et al., 2016). Also maintains plasmids and cDNA libraries	
ChlamyStation	http://chlamystation.free.fr/		IBPC Collection of Chlamydmonas photosynthesis mutants	

Screen data	ChlamyChem	http://chlamychem.utoronto.ca/ChlamyChem/method.php	small molecule screen data repository	(Alfred et al., 2012)

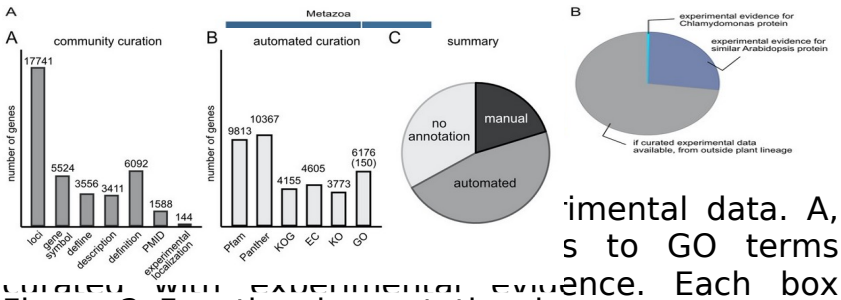

Figure 2 Functional annotation in Chlamydomonas. A, bar chart representing number of loci with associated information as given. Numbers on top of each bar refer to number of loci with given information. B, bar chart representing number of loci whose gene products contain similarity to the given databases. C, pie chart summarizing information in panel A and B. Of the 17,741 loci in v5.5, 20% have been manually curation with a functional annotation. 47% have an automated annotation from one or more of the databases from panel B, and 33% are not associated with an annotation. These 33% are typically either uncharacterized proteins specific to Chlamydomonas, specific to green algae, or belong to a protein family not recognized by one of the databases in panel B.

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