Chlamydomonas and Arabidopsis. A Dynamic Duo[1]

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A translational biology perspective on Chlamydomonas and Arabidopsis might be expected to focus on those genes, cellular components, or biological processes found first in Chlamydomonas and subsequently studied in Arabidopsis. There are indeed many such examples. There are also examples that flow from Arabidopsis to Chlamydomonas in terms of initial discovery and subsequent study. However, the differences can also be illuminating. In this brief essay, we make a case for the pairing of Chlamydomonas and Arabidopsis as model organisms that bracket a major subset of photosynthetic eukaryotes, the green plants (Mishler, 2000). By analogy with the yeast-mouse dyad, this green pair has tremendous potential as we enter an era of renewed interest in comparative biology.

A FOCUS ON MODEL ORGANISMS

In the time of Darwin, biology was done by naturalists. They studied life in its variety, cataloging differences in order to find larger patterns. Perhaps it was Mendel who conducted the first famous study of a model organism. Using only peas he discovered universal concepts of heredity that could later be extrapolated to other organisms. Since that time an increasing reliance has fallen on models, initially to study specific concepts, such as maize and Drosophila for genetics, Neurospora for biochemical genetics, *Caenorhabditis elegans* for development, yeast for cell cycle and metabolism, and Chlamydomonas for photosynthesis and flagella. As organism-centered research communities developed, many aspects of the biology of these organisms have been investigated, and these models have taken on even broader significance (for review, see Barr, 2003; Davis, 2004).

In many ways, *Escherichia coli* can be considered the universal model organism. Anything that can be studied with *E. coli* is studied with *E. coli*. It is simple, sequenced, and very well established. Beyond prokaryotes, the budding yeast, *Saccharomyces cerevisiae*, seems the predominant eukaryotic model. For understanding the cell cycle, basic physiology and metabolism, and principles of eukaryotic genetics, yeast has been incredibly useful. Moving down the list of models, the mouse, *Mus musculus*, is the most commonly used mammalian model and a frequent stand-in for humans. Other groups of organisms have their own premier models, such as Arabidopsis for plants.

As we advance into a new century of biology, it would seem pertinent to look at the models we use and how they can be used together or separately to provide the most powerful approaches for answering the questions that remain to be answered. Particularly in the age of genomics and comparative biology, a single model seems orders of magnitude less powerful than a pair or group of organisms. Recognition of this can be seen in the advent of the pufferfish (*Fugu rubripes*) as a model (Brenner et al., 1993) and the push to sequence the mouse genome (Waterston et al., 2002) as companions to the human genome.

THE POWER OF PAIRS

Together, *E. coli* and yeast illustrate both the consistencies and distinctions across a wide swath of all living things. As examples, the fact that each has ribosomes and tRNAs indicates that the genetic code and the basic mechanisms of translation are ancient and near universal features of life. On the other hand, the fact that operons and organelles are not conserved indicates some of the critical ways in which eukaryotes and prokaryotes differ and points to some significant events in evolution. This pairwise comparison is obviously limited in many respects, not least in the fact that the one constant in biology is that there are exceptions to everything. However, it does allow for a convenient and powerful way to recognize both the important traits and the degree of diversity within a selected grouping.

Yeast and mouse can be seen to bracket a branch of the (nonphotosynthetic) eukaryotes in the same incomplete but useful way in which yeast and *E. coli* bracket much of life. Yeast (fungi) and mouse (animals) are both classified as Opisthokonts (Simpson and Roger, 2002), which shared a common ancestor approximately 1.5 billion years ago (Fig. 1; reviewed in Hedges, 2002). In considering the yeast-mouse dyad, features such as membrane-bound organelles and a cytoskeleton show commonality, whereas multicellularity and cell specialization show diversity. Again, there is room for significant exceptions, but as a pair yeast and mouse have shown an admirable utility in the discovery of broad concepts. Such comparisons, by

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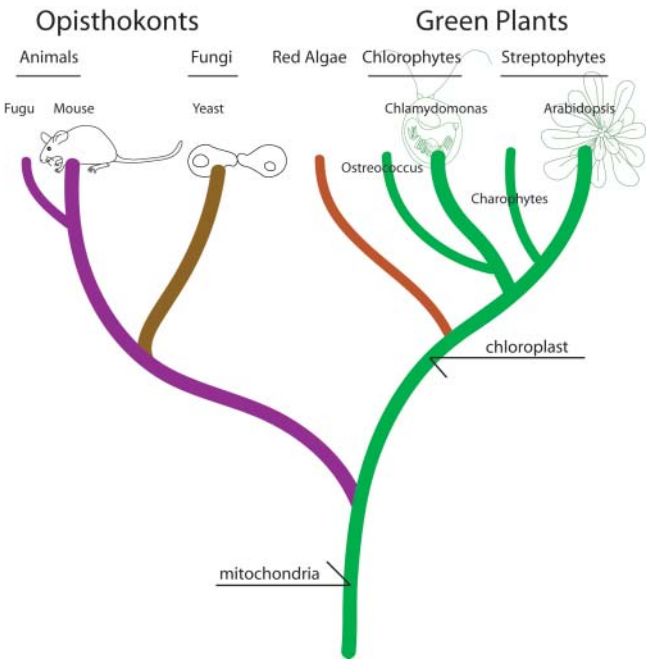

Figure 1. A simplified cladogram of the eukaryotes showing the phylogenetic relationships of the major organisms discussed in the text.

highlighting similarities and differences, can indicate topics and directions of interest, suggest which system might be best for studying them, and point to useful tools for understanding phylogeny and evolution.

YEAST:MOUSE::GREEN YEAST:MOUSE-EARED CRESS

Among the photosynthetic eukaryotes, *Chlamydomonas reinhardtii* and Arabidopsis are well poised in terms of both phylogenetics and genetics to serve the role of bracketing the green branch of the plant kingdom. Each has proven incredibly useful on its own as a model organism. Together, however, they nicely encompass the commonality and variety of green plants. The green plants are a monophyletic taxon comprising two major subclades, the Chlorophyta and Streptophyta (Bremer, 1985). These two green lineages are represented by Chlamydomonas and Arabidopsis, respectively, analogous to the situation for yeast and mouse as representatives of two other sister groups, the fungi and animals (Fig. 1). Chlamydomonas and Arabidopsis shared a common ancestor approximately 1.1 billion years ago, which is similar in order of magnitude to the time of divergence of yeast and mouse (for review, see Hedges, 2002).

Both Chlamydomonas and Arabidopsis are exemplary model organisms (reviewed by Meinke et al., 1998; Dent et al., 2001; Harris, 2001; Weigel and Glazebrook, 2002), but in unique and complementary ways. Here again the yeast-mouse analogy is informative. Like yeast, Chlamydomonas is unicellular, haploid, and amenable to tetrad analysis. It was through tetrad analysis, for instance, that the concepts of chloroplast DNA and its maternal inheritance were first illuminated in Chlamydomonas (Sager, 1954). The ability to screen mutagenized haploids in the first generation on a timescale of hours to days allows for very rapid forward genetic screens. As the mouse is the complex, multicellular eukaryote to yeast's simpler unicellular version, the multicellularity of Arabidopsis allows for studies of development and cell-cell communication, whereas Chlamydomonas is the pared down unicellular model with the unique advantage of having three transformable genomes. Like the mouse, Arabidopsis has a large and established community of researchers and powerful reverse genetics tools (Colbert et al., 2001; Sessions et al., 2002; Alonso et al., 2003). Mouse is the close relative of the all-important human; yeast is the far divergent, but easily manipulated companion. Likewise, Arabidopsis is closely related to the all-important agricultural crops, whereas Chlamydomonas is its more distantly related but easily manipulated cousin.

OSTREOCOCCUS AS THE GREEN PUFFERFISH

While unicellular Chlamydomonas might represent a simpler organism compared to Arabidopsis, the genome size of Chlamydomonas is approximately the same as that of Arabidopsis. Similar to the way in which Fugu has been developed as a genomic companion to mouse and human because of its small and densely packed genome (Brenner et al., 1993), *Ostreococcus tauri* is being developed and has just been sequenced (Derelle et al., 2004) as a genomic model for green plants (Derelle et al., 2002). This smallest of known eukaryotes resides on an early branch of Chlamydomonas' chlorophyte lineage (Courties et al., 1998). It has a genome of only 11.5 Mb, which is miniscule compared to Chlamydomonas' approximately 100 Mb (Harris, 2001) or Arabidopsis' 125 Mb (The Arabidopsis Genome Initiative, 2000). This should make Ostreococcus a very useful organism for comparative gene identification and annotation, and it might provide insight into the minimal set of genes for a photosynthetic eukaryote. However, as Fugu is more companion to, than replacement for, the mouse, it seems unlikely that Ostreococcus—lacking the useful tools and established community—will surpass Chlamydomonas as a workhorse model organism any time soon.

EXAMPLES FOUND IN TRANSLATION BETWEEN CHLAMYDOMONAS AND ARABIDOPSIS

Historically, Chlamydomonas has informed Arabidopsis, and much of plant science, as a particularly useful model of photosynthesis and chloroplast-nucleus interactions (for review, see Rochaix et al.,

1998; Harris, 2001). The key advantages of Chlamydomonas for studies of photosynthesis are its abilities to grow either photoautotrophically or heterotrophically on acetate and to assemble its photosynthetic apparatus in the dark, allowing for isolation of mutants impaired in all aspects of photosynthesis and chloroplast biogenesis (Davies and Grossman, 1998; Dent et al., 2001). Chlamydomonas has been critical in elucidating the structure and organization of the photosystems, the biosynthesis of chlorophyll, and in understanding the complex regulation of these components.

The Chlamydomonas *stt7* mutant, which is defective in a photosynthetic regulatory mechanism called state transition, provides a specific example of how Chlamydomonas can drive discovery in Arabidopsis and beyond. State transition involves the reversible reallocation of the light-harvesting complex proteins ordinarily associated with photosystem II (PSII) toward PSI in response to a shift in incident wavelengths of light (Wollman, 2001). Chlamydomonas exhibits a particularly large, and thus easily detectable, shift in chlorophyll fluorescence during state transition, making Chlamydomonas the initial experimental organism of choice for studying state transition. The *stt7* mutant was identified in a video imaging screen for mutants that were incapable of making the fluorescence shift (Fleischmann et al., 1999). Using traditional complementation and molecular biology techniques, *STT7* was cloned and found to encode a chloroplast-localized Ser/Thr protein kinase (Depège et al., 2003). It has been implicated in phosphorylating, either directly or indirectly, light-harvesting complex II proteins, a process shown to be involved in state transition. *STT7* has two homologs that can be recognized in the Arabidopsis genome (Depège et al., 2003). These genes, which would have been much more difficult to recognize and isolate directly among the plethora of kinases in the Arabidopsis genome (Wang et al., 2003), can now be targeted and studied in both organisms.

Mutants defective in a protective response to excess light called nonphotochemical quenching have been isolated and studied in our laboratory, and they provide a good example of the parallel utility of Chlamydomonas and Arabidopsis. The *npq1* mutant, which is defective in the high-light-dependent synthesis of zeaxanthin via the xanthophyll cycle, was isolated first in Chlamydomonas (Niyogi et al., 1997) and subsequently in Arabidopsis, where it proved easier to identify the mutated gene (Niyogi et al., 1998). Although the phenotypes of the *npq1* mutants are broadly similar, there is a difference in the relative contributions of the xanthophyll cycle to nonphotochemical quenching (Niyogi et al., 1998) that underscores the value of comparative studies using Chlamydomonas and Arabidopsis. Working in the opposite direction, the critical role of the PsbS protein of PSII was first identified and characterized using an Arabidopsis mutant (Li et al., 2000). It was only recently through genome sequencing that a Chlamydomonas homolog of PsbS was found (X.P. Li, D.A. Martinez, and K.K. Niyogi, unpublished data). Now there are two fronts on which to attack this protein and get at the other components of this important physiological process. It will be exciting to see not only where PsbS fits in the overall framework of PSII, and what other factors work with it, but also how the process differs between photosynthetic organisms.

Studies of chloroplast-localized RecA have also shown how these two models, Chlamydomonas and Arabidopsis, work well as companions. A RecA homolog was first identified and cloned in Arabidopsis (Cerutti et al., 1992). However, because Chlamydomonas has an easily transformable chloroplast, the function of the RecA homolog could be tested much more easily in Chlamydomonas than in Arabidopsis. By transforming a known dominant-negative form of the *E. coli* protein into the Chlamydomonas chloroplast genome, Cerutti et al. (1995) were able to show that recombination in the chloroplast was impaired, as was viability in the presence of DNA-damaging agents. A RecA homolog (*REC1*) has now been cloned from Chlamydomonas too and shown to have a functional chloroplast-targeting peptide as well as expression patterns that are consistent with roles in chloroplast DNA replication and repair (Nakazato et al., 2003).

One area in which the unique attributes of Chlamydomonas as a photosynthetic model have proven especially fruitful is that of nucleus-encoded factors involved in translation of chloroplast-encoded photosynthetic proteins. A great number of such factors, often specific to a single chloroplast gene, have been identified genetically in Chlamydomonas. In Arabidopsis, investigation of these chloroplast-nucleus interactions lags behind because of the relative difficulty in isolating nonphotosynthetic mutants (Barkan and Goldschmidt-Clermont, 2000). Going forward, as one organism informs the other, it will be interesting to see how conserved these factors and regulatory processes are across the green plants. Variety might be expected, as there are significant differences in chloroplast genome organization and gene structure (Maul et al., 2002).

The joint utility of Chlamydomonas and Arabidopsis is also enhanced by some of their significant differences, and CO_2 fixation provides an interesting example of a fundamental process in which Chlamydomonas does something that Arabidopsis cannot. Unlike Arabidopsis (a C3 plant), but somewhat analogous to C4 plants, Chlamydomonas has a means of concentrating inorganic carbon, particularly under low CO_2 conditions, and thus favoring carboxylation by Rubisco over oxygenation and limiting the losses due to photorespiration. Carbon concentrating mechanisms (CCMs) are common in algae that grow in aquatic habitats where the diffusion of CO_2 would otherwise limit photosynthesis. The biochemistry and regulation of the CCM have attracted considerable research attention, and a key regulator of CCM-related gene expression has been identified recently (Fukuzawa et al., 2001; Xiang et al., 2001).

CONCLUDING REMARKS

There are many other such examples of the similarities and differences between Chlamydomonas and Arabidopsis, indicating both their complementarity as experimental organisms and the diversity of green plants (and the world of photosynthetic eukaryotes in general). Looking to the future, these complementary models should continue to inform each other and the field of plant biology. This interaction will likely increase as new tools are developed for each, as the transformability of organelles is improved in Arabidopsis and as reverse-genetics tools become widely available in Chlamydomonas. With the completion of the Chlamydomonas nuclear genome sequence, plant biologists eagerly await a full annotation and a comparative genomic analysis with Arabidopsis that should yield a wealth of new information and exciting new research directions for this dynamic duo of model organisms.

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