

MICROBIOLOGY

A genetic toolbox for marine protists

A resource of detailed DNA delivery and expression protocols for marine protists will enable new studies to understand the fundamental and ancestral features of eukaryotic cells.

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Microbial life is most often associated with prokaryotes (Bacteria and Archaea). However, enormous biological diversity lies in protists, or eukaryotic microbes. For much of the history of biological investigation, protists have been the focus of far less intense research than complex multicellular eukaryotes. However, over the last twenty years the molecular biological revolution has brought the importance of protists to light. Faktorová et al.¹ now provide a set of tools for the genetic manipulation of diverse protists, which will enable many new types of studies.

Ecologically, protists are now known to be key players in the environment. They occupy nearly all ecological niches, they may be autotrophic or heterotrophic, and they are major drivers in global biogeochemical processes². They are the predominating biomass in the world's oceans as well as major contributors to biomass in terrestrial environments³. Recent efforts to holistically catalog the planktonic biodiversity of the world's oceans through environmental sequencing have revealed the sheer number of microbial eukaryotic taxa present in marine environments, highlighting their critical biological contribution. Even microbial protist lineages historically perceived to be species poor and rare in the environment are now known to be highly diverse and are major components of marine ecosystems⁴.

Perhaps the greatest shift in biological thinking regarding protists has come about through the use of molecular sequence data to reconstruct the eukaryotic tree of life^{5–7}. Historically, protists were placed into a grab bag of life, perceived as a separate entity: Protista. The classical eukaryotic kingdom-level lineages (Animalia, Fungi and Plantae) are now known to be sublineages within suprakindoms wherein most if not all other lineages are protists. This model of eukaryotic relationships is referred to as the supergroup model⁶. Thus, it is from protistan relatives that complex multicellular groups like animals, plants and fungi evolved.

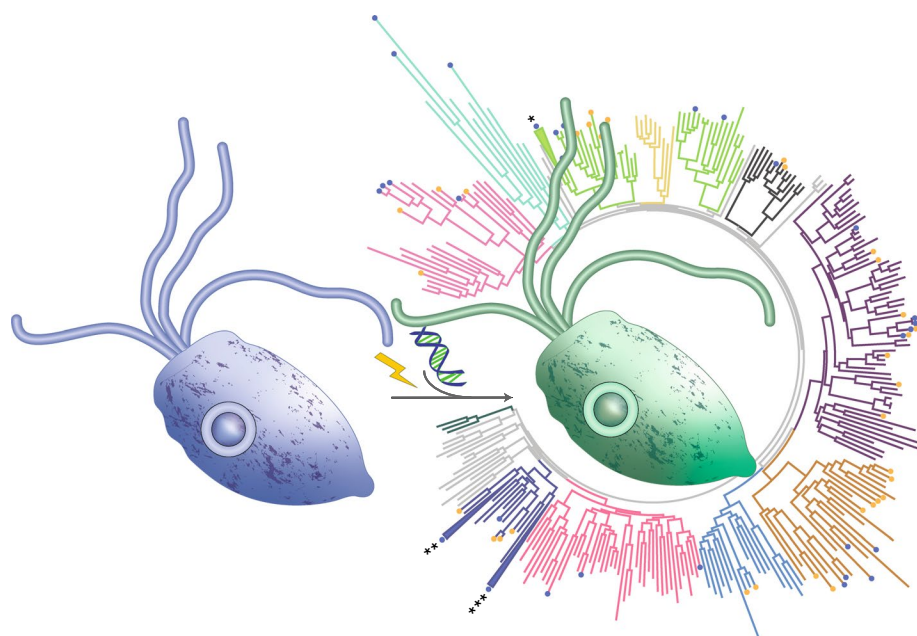


Fig. 1 | Genetic transformation across the tree of eukaryotes. The overlay is a drawing of a wild-type flagellated protist (blue) that is transformed (green) through foreign DNA delivery. The underlay is a phylogenomic-based tree of eukaryotes. Clades are colored as in ref. ¹. Blue dots at tips of branches represent taxa that have been genetically manipulated previously; yellow dots represent taxa for which Faktorová et al.¹ focused on developing or enhancing methods of genetic manipulation. Collapsed nodes represent plants (*), animals (**), and fungi (***). Light gray branches represent deep eukaryotic lineages not represented in the cladogram in ref. ¹; these remain 'dark' areas of the tree for which tools for genetic manipulation are not available. These taxa should be targeted in the future.

Several genomic and transcriptomic sequencing efforts have focused on protists^{8,9}. These data have allowed deep comparisons of genome content and the development of reference databases for environmental sequencing studies. They simultaneously serve as the first steps in the modern-day development of emerging model organisms. However, these mostly in silico studies have also generated questions regarding gene and protein functions that require experimental evidence to validate and further explain their bioinformatic findings. Genetic transformation is a key tool that can be used for alteration of observable ecological, life-cycle, pathogenicity and cell-biological phenotypes.

Key findings have been made by critical advances in genetic manipulations in microbial eukaryotes. Take, for example, the first to be genetically manipulated, the yeast *Saccharomyces cerevisiae*¹⁰. Most well-established model organisms such as *S. cerevisiae* were originally selected for study on the basis of characteristics such as ease of growth and generation time. While studies using existing model systems have provided pivotal advances in our knowledge of life, they lack the taxonomic breadth to access deep comparisons of gene function in eukaryotes. This in turn has limited our understanding of processes such as gene retooling, neofunctionalization, and co-option of genes for complex biological functions in eukaryotes.

While transformation has become commonplace for animals, plants and fungi, it still represents a substantial hurdle for most protists. Using a phylogenetically informed framework, Faktorová et al.¹ set out to establish or improve existing transfection protocols for 38 strategically selected marine microbial eukaryotes (Fig. 1). These taxa represent important phytoplankton and heterotrophs in marine environments. The authors were successful in the development or improvement of protocols for 21 of these taxa and provide valuable negative data on what did not work for the remaining 17 organisms. This represents the first community effort to bring a taxonomically broad sampling of protists to genetically tractable emerging model status across the breadth of the tree of eukaryotic life. Nearly every major deep lineage on the tree now has a representative emerging model.

Each organism had its own set of challenges with regards to DNA delivery. Target taxa included organisms that are free-living or parasitic, grow individually or in the presence of another organism, grow in liquid or solid media, are naked cells (without shells or cell walls) or have cell walls of different compositions, from cellulose to silica. The authors also employ an innovative approach to select microbial eukaryotes from natural planktonic communities that show a propensity for DNA uptake for later cultivation attempts,

as opposed to selection of potential models based on the classical criteria mentioned above. They provide a starting point with detailed methods, including an extensive set of easily accessible protocols on <https://www.protocols.io/> listed for each targeted taxon. Their methods of gene delivery are multifaceted, ranging from electroporation to bacterial conjugation to biolistics. In addition, they provide a ‘do-it yourself’ roadmap targeting new protistan organisms for which models may be developed.

This community-based effort was made possible by a major investment into marine protists by the Gordon and Betty Moore Foundation. It is a major step forward in the rapidly evolving and exciting field of protistology. With this diverse set of emerging models under development in deliberately selected areas of the tree of eukaryotes, questions surrounding the molecular underpinnings of ecological niche invasion, cellular phenotype, and phenomena such as convergence versus ancient homology can be explored at evolutionarily deep levels, beyond gene-inventory and RNA-seq studies.

The diversity of eukaryotic microbes that continues to be discovered and characterized is immense. On the basis of environmental sequencing data it is clear that much more unseen diversity exists⁴. Faktorová et al.¹ provide an ample starting point for a new frontier in protistan science. However, there still exist ‘dark’ areas on the eukaryotic

tree of life (Fig. 1) that have yet to be explored. We hope that, through investments and initiatives like this, the cell biological, ecological and evolutionary developmental biology communities will consider the rich diversity that these organisms provide to ask deeper questions in their respective fields. □

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Competing interests

The authors declare no competing interests.