Swimming, gliding, and rolling toward the mainstream: cell biology of marine protists

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ABSTRACT **Marine protists are a polyphyletic group of organisms playing major roles in the ecology and biogeochemistry of the oceans, including performing much of Earth's photosynthesis and driving the carbon, nitrogen, and silicon cycles. In addition, marine protists occupy key positions in the tree of life, including as the closest relatives of metazoans. Despite all the reasons to better understand them, knowledge of the cell biology of most marine protist lineages is sparse. This is beginning to change thanks to vibrant growth in the development of new model organisms. Here, we survey some recent advances in studying the cell biology of marine protists toward understanding the functional basis of their unique features, gaining new perspectives on universal eukaryotic biology, and for understanding homologous biology within metazoans and the evolution of metazoan traits.**

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INTRODUCTION

Because most of eukaryotic diversity is represented by unicellular organisms, their inclusion among model organisms is essential to understanding the evolutionary history, structure, and (dys)function of all eukaryotes. Unicellular eukaryotes are often lumped as "protists," a term that is useful despite its taxonomic irrelevance and origin as a definition by exclusion—a protist being any eukaryote that's not a plant, animal, or fungus. A few freshwater and terrestrial protists are considered to be model organisms, for example, by the National Science Foundation in their Proposal Classification Form: the chlorophytes *Acetabularia* and *Chlamydomonas*, the ciliates *Paramecium* and *Tetrahymena*, and the amoebozoan *Dictyostelium*. Another fairly well-developed model is the apicomplexan *Plasmodium*. There are no marine protists among the current model organisms, limiting our ability to understand how they achieve their essential functions in powering ocean ecosystems and global biogeochemical cycles (Caron *et al.*, 2011), and to exploit them for new cell biological insights. To address this gap, marine protists have been the focus of recent large and coordinated efforts, funded by the Gordon and Betty Moore Foundation, to build the genomic resources and to develop the methods for genetic manipulation needed to advance key species to the status of tractable model organism (for more information on these programs, see Keeling *et al.*, 2014; Waller *et al.*, 2018).

Marine protists include representatives of the diverse morphologies, physiologies, and life histories found among the major eukaryotic lineages. The rhizarian vampyrellids offer an example of the weird and wonderful biology to be found: vampyrellid trophozoites explore their surroundings using a form of rolling locomotion, piercing the cell walls of their chlorophyte prey and extracting the protoplast by phagocytosis, leaving behind an empty shell with one or more small holes; well-fed trophozoites then form digestive cysts, from which the next generation of young trophozoites emerge to resume the rolling hunt (Figure 1A; More *et al.*, 2019). Clarifying the phylogenetic relationships among the deeply divergent protist lineages is an active area of research (e.g., Adl *et al.*, 2019), and we display a view of eukaryotic diversity consistent with current understanding in Figure 1, highlighting marine protists that are developing models along with existing terrestrial and freshwater model protists. Some of the developing model marine protists are in the same class as established freshwater or terrestrial model protists, for example, *Micromonas* and other chlorophytes (Figure 1, B and C). But most represent major lineages of eukaryotes that otherwise lack tractable model organisms, including the Euglenozoa (Figure 1D), several groups of the stramenopiles-alveolates-rhizarians (often called SAR) lineage (Figure 1, E–K), and haptophytes (Figure 1L).

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Abbreviation used: SAR, stramenopiles-alveolates-rhizarians.

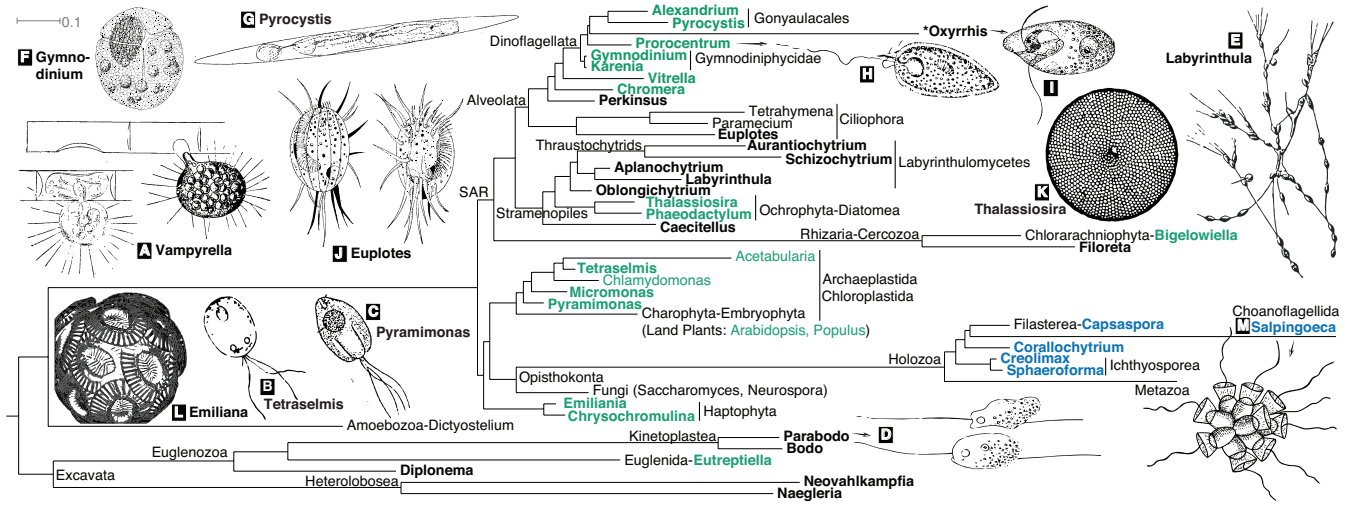

FIGURE 1: Overview of approximate phylogenetic relationships among developing marine protist models and established freshwater model systems, displayed at the genus level, as well as illustrations of select marine protists. On the phylogeny, marine protists are in bold, with marine models used to explore multicellularity and photosynthesis in blue and green, respectively. We include a subset of genera from Waller *et al.* (2018) as well as additional cases to highlight lineages discussed herein; see Supplemental Table 1 for a complete list. Small subunit rRNA alignment r132 from SILVA (Quast *et al.*, 2012) was used in IQ-TREE (Schmidt *et al.*, 2014) to produce the phylogeny, including an archaeal outgroup. Scale bar indicates average number of substitutions per site. Some lineages were rearranged in TreeGraph (Stöver and Müller, 2010) to reflect literature consensus. Drawings A–M (not to scale) are adapted from the following sources: *Tetraselmis* (Stokes, 1888), *Pyramimonas* and *Pyrocystis* (West, 1916), and *Prorocentrum* (Calkins, 1926). *Emiliana huxleyi* is adapted from a scanning electron micrograph image (CC BY 2.5; Taylor, 2011). *Thalassiosira* is adapted from microphotos of a fossil prepared by Anne Gleich (CC BY 2.0; Picturepest). The *Salpingoeca rosetta* colony is redrawn from a microscope image (CC BY-SA 3.0; Mark Dayel; redrawn by Tiago Pratas). *Labyrinthula* cells are illustrated (E) moving along the shared ectoplasmic network (Moore, 1911). Not shown on the phylogeny, *Vampyrella* is illustrated (A) boring into and sucking out (left) and completely emptying (right) a *Spirogyra* cell (Verworn, 1899, p. 148). All other drawings were adapted from Calkins (1901).

Cell biologists push their field forward via two general strategies: increasing depth and increasing breadth. Going deeper into long-studied biological systems such as *Saccharomyces cerevisiae* is often achieved by technical innovations that allow testing of previously inaccessible hypotheses. Gaining a broader perspective on long-studied processes is achieved by exploring biological diversity to identify different twists on known mechanisms as well as entirely new biology. Here, we highlight three aspects of marine protist cell biology that illustrate how the "breadth" strategy is yielding fresh insights. First, the cell biology of early-diverging holozoan lineages is being used to explore the evolution of multicellularity in animals. Second, some marine protists have evolved atypical mechanisms for performing essential and otherwise highly conserved tasks. Third, many marine protist lineages have unique cell biology that offers windows into the evolutionary heritage of all eukaryotes.

INSIGHTS INTO ORIGIN OF MULTICELLULARITY

The availability of more, and more diverse, protist genomes and transcriptomes has revealed that machinery previously thought to be unique to metazoans is not. For example, genes encoding lamin-like proteins have now been detected in diverse protists including choanoflagellates and the marine rhizarian *Corallomyxa* (now *Filoreta*) *tenera* (Kollmar, 2015). Choanoflagellates are of particular interest to developmental biologists because they are one of the closest living relatives of animals in many molecular phylogenetic analyses (see Figure 1M) and share with other holozoans a variety of genes required for embryogenesis in animals (e.g., cadherins, tyrosine kinases, and Myc; Booth *et al.*, 2018). Taking a genome-wide approach, Richter *et al.* (2018) identified hundreds of additional gene families formerly considered to be animal-specific that instead are present in the common ancestor of metazoans and choanoflagellates. This includes examples that will be familiar to many cell biologists, including Notch signaling pathway receptors and ligands, components of the innate immune system including Toll-like receptors, and enzymes responsible for the hydrolytic cleavage of glycosaminoglycans in the extracellular matrix. The opportunity to gain insight into the ancestral functions of such genes is now becoming a reality with the development of transformation methods for the ichthyosporean *Creolimax fragrantissima* (Suga and Ruiz-Trillo, 2013) and the choanoflagellate *Salpingoeca rosetta* (Figure 1M). Booth *et al.* (2018) introduced recombinant septin and tubulin genes into *S. rosetta*, and localization of the fluorescently tagged proteins to the basal poles of cells in rosette-shaped colonies was consistent with the behavior of homologous genes in fungi and metazoan epithelial cells, suggesting conserved functions in establishing and maintaining cell polarity.

The critical role of the microbiome in determining the morphology and function of animals and plants is now widely recognized (McFall-Ngai *et al.*, 2013; Vandenkoornhuyse *et al.*, 2015). Free-living bacteria may also have important impacts on diverse eukaryotes, with examples from marine systems including animals, seaweeds, and protists. For example, rosette formation in *S. rosetta* occurs in response to several bacterial compounds released in outer membrane vesicles, and sexual reproduction is triggered by bacterial chondroitin lyase (Woznica and King, 2018). With continued development of additional tools, marine protists offer new models for investigating bacteria-mediated development.

NEW WAYS TO SOLVE OLD PROBLEMS

Some marine protists have unusual cell biological features that reveal alternative, derived solutions for conserved tasks. Perhaps the classic example is the dinokaryon fibrillar chromosomes of dinoflagellates (Figure 1, F–H) that are always condensed, replacing the nucleosomal histones found in other eukaryotes (Iwamoto *et al.*, 2016). Dinoflagellates utilize a nucleoprotein derived from a viral homologue as a bulk protein for packing DNA, even while retaining divergent (and not highly expressed) but still recognizable versions of the ancestral histone genes (Gornik *et al.*, 2012). The acquisition of this virus-derived gene corresponds with a tremendous increase of genome size in dinoflagellates relative to their alveolate relatives, and may enable them to manage so much DNA. Perhaps relatedly, dinoflagellates have so far proved refractory to genetic manipulation, and the development of such tools would enable deeper study of this intriguing phenomenon.

Another case of innovation in marine protists is in the regulation of nonreceptor tyrosine kinase signaling in the ichthyosporean *C. fragrantissima*. Universally, Src kinase is negatively regulated by Csk, but in *C. fragrantissima* the Csk gene has been has lost (Suga and Miller, 2018). Transient overexpression of Src in transfected *C. fragrantissima* prevented growth, whereas coexpression of Src with one of the *C. fragrantissima* protein–tyrosine phosphatases rescued the phenotype, showing that another protein–tyrosine phosphatase has been coopted in place of Csk. An intriguing additional detail is that the Src kinases of *C. fragrantissima* and other symbiotic (with marine invertebrates) ichthyosporeans have a bulky gatekeeper residue in the active site cleft, which mimics that of drug-resistant mammalian Src kinases and may have ecological relevance.

UNIQUE CELL BIOLOGY

Other marine protists possess cell biology that has no obvious counterpart in plants or animals, such as the vampyrellids described earlier. Perhaps the most widely known example is the siliceous cell walls, or frustules, of diatoms (Figure 1K), which come in a bewildering array of species-specific, finely detailed, hierarchically porous variants. There is much interest in understanding the ontogeny of diatom cell walls not only for biological insight, but for biotechnological applications. There has been a great deal of progress in identifying components of diatom frustule biosynthesis, and this knowledge has recently been applied to support in vitro synthesis of hierarchically porous silica (Pawolski *et al.*, 2018). The essentiality of the frustule in diatoms has presented a challenge to in vivo manipulation. The closest relatives of diatoms, the parmales, do not require their silica shells and the biosynthesis of the silica plates in *Triparma laevis* can be controlled by simply manipulating silicate availability. Yamada *et al.* (2019) demonstrated that the early steps of silica wall formation in *T. laevis* are homologous to the process of frustule formation in diatoms, confirming that parmales provide an alternative system in which to study frustule formation.

Diatoms and other photosynthetic stramenopiles and alveolates offer independent examples from the chlorophytes of the integration of a photosynthetic organelle into the heterotrophic physiology of the host (see Figure 1), and thus insight into other ways that complex evolutionary event might be accomplished. Despite the convergence of photoautotrophy, diatoms use pathways for circadian regulation of gene expression more like those of mammals than plants (Annunziata *et al.*, 2018), and have distinct mechanisms to coordinate mitochondrial and plastid metabolism (Murik *et al.*, 2019).

Another unique aspect of cell biology exists in a group of nonphotosynthetic stramenopiles, the Labyrinthulomycetes (Figure 1E). These organisms have a unique organelle, the bothrosome (sagenogenetosome), that is responsible for the production of an ectoplasmic network involved in cell motility and the search for and attachment to food sources (Fossier Marchan *et al.*, 2018; Iwata and Honda, 2018; Hamamoto and Honda, 2019). Iwata *et al.* (2017) suggest that the bothrosome may be related to the endoplasmic reticulum–plasma membrane junctions with diverse functions in eukaryotic cell biology (Stefan, 2018). Genetic tools are being developed for some Labyrinthulomycetes (e.g., Okino *et al.*, 2018), and promise to help uncover the molecular mechanisms of such novel biology.

THE FUTURE OF MARINE PROTIST CELL BIOLOGY

The few examples mentioned here of recent progress in the cell biology of marine protists only touch the surface of the questions that further work on these organisms will open to investigation. For example, it appears that many interspecific interactions among marine protists (e.g., host–parasite and predator–prey) are highly specific, but very little is known about either the cell biological mechanisms underlying such specificity or their ecological and evolutionary ramifications. Two promising model species among the euglenozoan phagotrophic marine flagellates, the kinetoplastid *Parabodo caudatus* (Figure 1D; Gomaa *et al.*, 2017) and the diplonemid *Diplonema papillatum* (Kaur *et al.*, 2018), have recently been transformed, opening the door to investigating how these micropredators hunt and choose their prey. Continued progress along the "breadth" pathway to advancing cell biology will require developing these and other protists as model systems.

There are many challenges along that road. Moving even fairly standard and universal methods into new species can be challenging for many reasons, for marine protists including typically small cell size, the need for seawater, and diverse cell wall composition. Progress can be slow with too few people focusing on each species to provide critical mass or shared resources. We encourage cell biologists experienced in working on well-established model organisms to team up with investigators developing new models for processes of interest to them; the synergy of such collaborations promises to expand an already golden age of cell biology.

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