

can be done outside the body – *ex vivo*, as we say. Any condition involving blood cells would be a candidate: thalassemia (hemoglobin deficiencies), severe combined immune deficiencies (SCID), and others. When it comes to delivering the nucleases to intact organs, the challenge becomes greater, but people are working on it. Researchers are also using these nucleases to modify human stem cells in culture. As we learn enough about such cells to have some confidence in putting them into the body, we will also have the tools to correct genetic defects in them. You can imagine manipulating neural stem cells in culture to help reverse neurodegenerative diseases that have a genetic cause.

**What's next?** There is a lot of work ahead to bring the applications I've hinted at to fruition. But beyond the slogging, there is a new nuclease technology, with the catchy name of CRISPR. This system uses a single bacterial protein to do the DNA cutting, and it is guided to its target by an RNA molecule using Watson-Crick base pairing. In that way, it is simpler than ZFNs and TALENs, and it threatens to take over entirely. The CRISPR approach has already been used in a number of systems very effectively, but there are some concerns about whether it will be specific enough for use in people. Keep your eyes open. The future will also be full of surprises.

#### Where can I find out more?

- Carroll, D. (2011). Genome engineering with zinc-finger nucleases. *Genetics* 188, 773–782.
- Gaj, T., Gersbach, C.A. and Barbas, C.F. III (2013). ZFN, TALEN and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol.* 31, 397–405.
- Joung, J.K. and Sander, J.D. (2013). TALENs: a widely applicable technology for targeted genome editing. *Nat. Rev. Mol. Cell Biol.* 14, 49–55.
- Ramalingam, S., Annaluru, N. and Chandrasegaran, S. (2013). A CRISPR way to engineer the human genome. *Genome Biol.* 14, 107.
- Tan, W., Carlson, D.F., Walton, M.W., Fahrrenkrug, S.C. and Hackett, P.B. (2012). Precision editing of large animal genomes. *Adv. Genet.* 80, 37–97.
- Urnov, F.D., Rebar, E.J., Holmes, M.C., Zhang, H.S. and Gregory, P.D. (2010). Genome editing with engineered zinc finger nucleases. *Nat. Rev. Genet.* 9, 636–646.
- Voytas, D.F. (2013). Plant genome engineering with sequence-specific nucleases. *Annu. Rev. Plant Biol.* 64, 327–350.

Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT 84112-5650, USA.  
E-mail: [dana@biochem.utah.edu](mailto:dana@biochem.utah.edu)

## Primer

### Rhizaria

Fabien Burki and Patrick J. Keeling

Have you ever stumbled across Ernst Haeckel's stunning 19th century art prints representing complex symmetrical forms that look like snowflakes, armored knights, or even futuristic space stations? Or maybe walking down an indo-pacific beach, you have taken a closer look at the warm sand only to realize that the 'sand' is really countless, minute earthly stars? Chances are you did not realize it, but in both cases you were looking at the skeletons of single-celled organisms belonging to Rhizaria, a large group, or 'supergroup', of eukaryotes. Various kinds of rhizarians have long been known to biologists, as evidenced by the fame and frequency with which Haeckel's illustrations have been reproduced, but the idea that these organisms are all related to one another emerged only recently. And this means that Rhizaria, as a whole, is one of the most poorly understood supergroups of eukaryotes.

That Rhizaria lags far behind more studied groups is of course a challenging statement to prove, but a fair approximation to illustrate the depth of our knowledge, or rather the lack of it, is to look at the available genetic and genomic information. **Figure 1** depicts our current understanding of the evolutionary tree of eukaryotes, as assembled by more than 20 years of molecular phylogenetics and advanced microscopy. In addition to positioning Rhizaria among its 'peers', this figure also shows how researchers have allocated the genomic resources across the whole phylogenetic spectrum of eukaryotes. In this tree, the thickness of each branch corresponds to the number of complete nuclear genomes available from members of those lineages collectively. Looking at the last common ancestor of eukaryotes (here at the center, or the unresolved base of the tree), one can see that we have sequenced a lot of nuclear genomes, but as we move out to the tips it becomes apparent that most sequencing effort has focused on a few lineages only, primarily animals,

fungi, plants, and their respective parasites. Microbial eukaryotic lineages (aka the protists) are generally undersampled, but with only one complete genome available, that of the chlorarachniophyte *Bigeloviella natans* and the foraminifer *Reticulomyxa filosa*, the entire supergroup Rhizaria still stands out among protists in the extreme paucity of genomic data. It is not surprising that large, multicellular organisms are better studied, as these have always been the main subjects of biological research. By extension, parasites of animals and plants have also attracted much attention, and among protists the vast majority of sequenced genomes are from pathogens of humans or economically important animal and plant species. For example, the apicomplexan parasites, such as the malaria agent *Plasmodium falciparum*, top the list of protist groups with complete genomes (currently about 30). Another, slightly weaker bias comes from our preference for sequencing genomes from photosynthetic species, for instance green and red algae with 11 and 5 genomes, respectively, or diatoms with 4 genomes (**Figure 1**).

So why is Rhizaria so understudied? One reason is that they almost entirely run contrary to the above noted biases. For a start, only two relatively small subgroups are photosynthetic, one being the chlorarachniophytes, which not surprisingly include one of the two rhizarian genomes currently available (*B. natans*). Moreover, no rhizarian parasite of human is currently known, and only a handful parasitize commercially relevant species, mostly crops or invertebrates (see below). Aside from these biases, Rhizaria also faces a problem of a more technical nature: they are hard to cultivate, and until recently a relatively large scale cell culture was a prerequisite for launching major sequencing projects in order to meet the material (DNA or RNA) requirements.

So does that mean that rhizarian genomics, and by extension rhizarian research as a whole, faces a dark future, being labeled 'difficult and not sufficiently sexy'? On the contrary, our awareness of the global significance of Rhizaria is on the rise, just as the technical difficulties are rapidly receding. Rhizaria itself represents a fascinating reservoir of unexplored diversity. At the environmental and

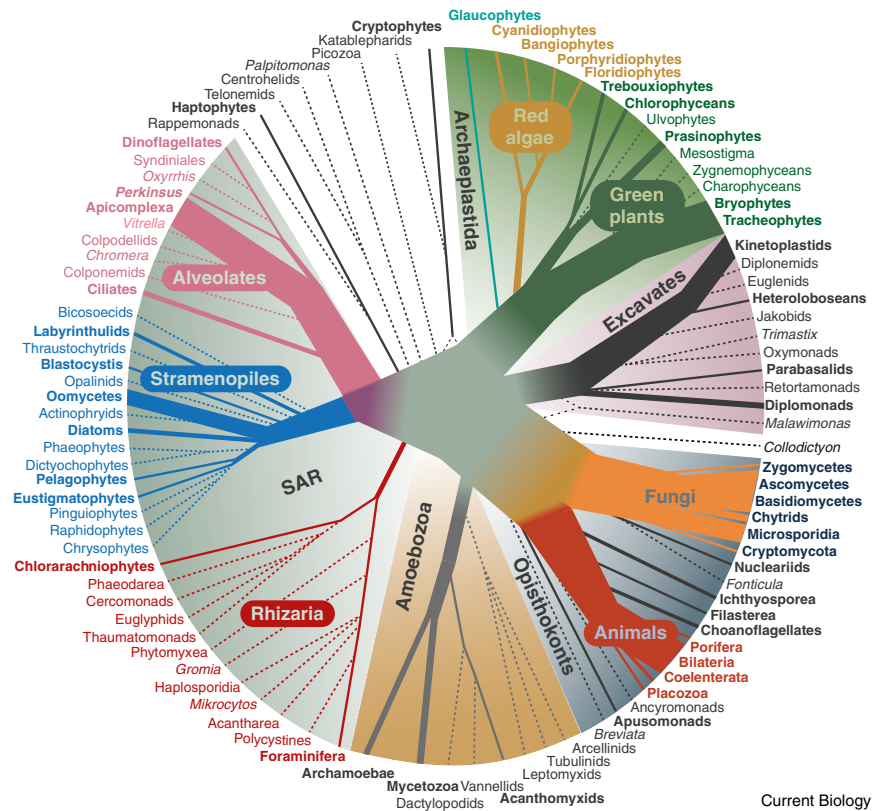


Figure 1. Evolutionary relationships among eukaryotes, showing the focus of genomic sequencing efforts.

Tree of eukaryotes derived from a consensus of phylogenetic evidence (mostly phylogenomics) and morphological characteristics. The taxa shown are not all of the same taxonomic ranks (i.e., genus or higher taxonomic ranks); they were chosen to depict the eukaryotic diversity and how it all comes together in an evolutionary framework. Several large groups are highlighted, such as Rhizaria, and our current understanding of the relationships between these groups is tentatively presented. The thickness of each branch represents the number of full genome sequences available in that group and its descendants. So working inwards from the tips, the thickness of the branches grows as groups with complete genomes coalesce. Dashed lines denote no genome is available. The main patterns to emerge from this are that, while there are many nuclear genomes available, they are strongly biased to a few lineages, mostly animals, fungi, plants, and parasites. On the other end of the spectrum, Rhizaria (with two genomes) is the worst sampled of any supergroup. Genome information was gathered from several online resources: GOLD (genomesonline.org); DOE Joint Genome Institute (genome.jgi.doe.gov); Broad Institute (broadinstitute.org); Ensembl (ensembl.org); EuPathDB (eupathdb.org); Sanger Institute (sanger.ac.uk).

ecological levels, rhizarians are increasingly found to be abundant bacterial grazers, and therefore hold key positions in microbial food webs. Rhizaria also sits at a crossroads in the eukaryotic tree of life, as it is closely related to two abundant and extremely diverse lineages (stramenopiles and alveolates), and for that reason is an attractive place to seek answers to outstanding evolutionary questions surrounding these lineages. And since culturing is no more the *sine qua non* of genomics (and soon transcriptomics) due to recent technological advances, rhizarian genomes will doubtlessly begin to flow

more freely (and in fact several are already underway).

The last decade has seen the birth and rise of Rhizaria, and this group has quickly been established as one of the major lineages of eukaryotes, encompassing a huge diversity of microbes (Figure 2). Here, we will summarize some of the key biological features of various rhizarian groups in order to introduce the reader to a 'rising star' of the microbial world.

#### Making a long story short: birth and rise of Rhizaria

The formal recognition of Rhizaria as an evolutionary lineage of eukaryotes

represented a late stage in a series of phylogenetic aggregations, mainly based on the small subunit ribosomal DNA (SSU rDNA). It all started rather modestly; in the 90s, a short paper reported that two lineages of otherwise very different protists appeared to be related: the euglyphid testate amoeba and the photosynthetic chlorarachniophytes (see Figure 2A and 2F for representatives of these lineages). This new little branch proved to be the beginning of something bigger. Soon a whole host of lineages as diverse as heterotrophic flagellates and obligate intracellular plant parasites were added to the growth of what rapidly became the phylum Cercozoa. But this was not the end of it: Cercozoa was shown to be related to foraminiferans (or forams, for those in the know), which are cells with long, root-like cytoplasmic extensions known as reticulate pseudopodia (Figure 2D); Cercozoa itself was expanded by the addition of various species as different as haplosporidian parasites, more zooflagellates and testate amoebae, cells with branch-like filose pseudopodia, or radiolarians (the giant amoebae with beautiful and delicate skeletons made famous by Haeckel; Figure 2B). In almost no time, the diversity had outgrown existing taxonomy — the new lineage needed a name, and Rhizaria was born.

Currently, Rhizaria includes these groups and a number of others that have since been shown to fall within the lineage by molecular phylogeny — Rhizaria is a magnet for new lineages whose positions in the tree have historically been mysterious. Some progress has also been made in understanding how the various subgroups are related to one another, mostly through phylogenomics, which is the use of hundreds of genes to reconstruct phylogenetic relationships. The swelling diversity now recognized to belong to Rhizaria is impressive, but as we shall see in the next section, it appears to be only the tip of a large iceberg; environmental surveys have indeed started to reveal that the rhizarian diversity yet to be found is likely enormous.

#### Cercozoa in the environment

Very little is known of the global impact of Rhizaria as a whole in nature, but molecular ecology has begun to show that some lineages are more important than we once thought. Culture-based

approaches and environmental clone libraries have shown that Cercozoa, for example, is composed of thousands of distinct lineages thriving in various ecosystems. More than anywhere else, it is in soils that the greatest environmental impact of Cercozoa can be unearthed, where they often represent the largest protist biomass (together with Amoebozoa). But cercozoans are also abundant in marine ecosystems. A recent environmental survey combined with new culturing methods revealed the existence of minute unflagellated heterotrophs, named *Minorisa minuta*, which are among the smallest grazers known to date. If size sometimes matters, in this case tiny does not mean insignificant: *M. minuta* flourishes in the ocean, feasting on bacteria and accounting for up to 5% of all heterotrophic flagellates in coastal waters of the world. At a global scale, the impact of this species alone must be quite significant, playing an important role in controlling bacterial populations and carbon fluxes.

As was the case with the discovery of Rhizaria, however, current data on the environmental importance of Cercozoa suggest that we are just at the beginning of something bigger. Even though high-throughput sequencing studies specifically targeted at cercozoan environmental diversity are not yet available, next-generation sequence surveys of eukaryotes as a whole have already revealed that these taxa are extremely diverse and present in the environment in huge numbers. For example, vampire amoebae (vampyrellids; [Figure 2C](#)) are protist predators best known for their peculiar mode of feeding: they typically bore through the prey's cell membrane and suck up its content, or sometimes invade the prey cell to digest it from the inside out. Despite having been described over 150 years ago, vampyrellids have been considered a relatively small group of Cercozoa, apparently inhabiting only non-marine environments. But that was what we thought without the insights of massive environmental sequencing. Now, hundreds of thousands of environmental sequences have revealed that the genetic diversity of vampyrellids is similar to that of fungi! They also appear to represent a significant proportion of the eukaryotic community in marine habitats, where they had never been previously

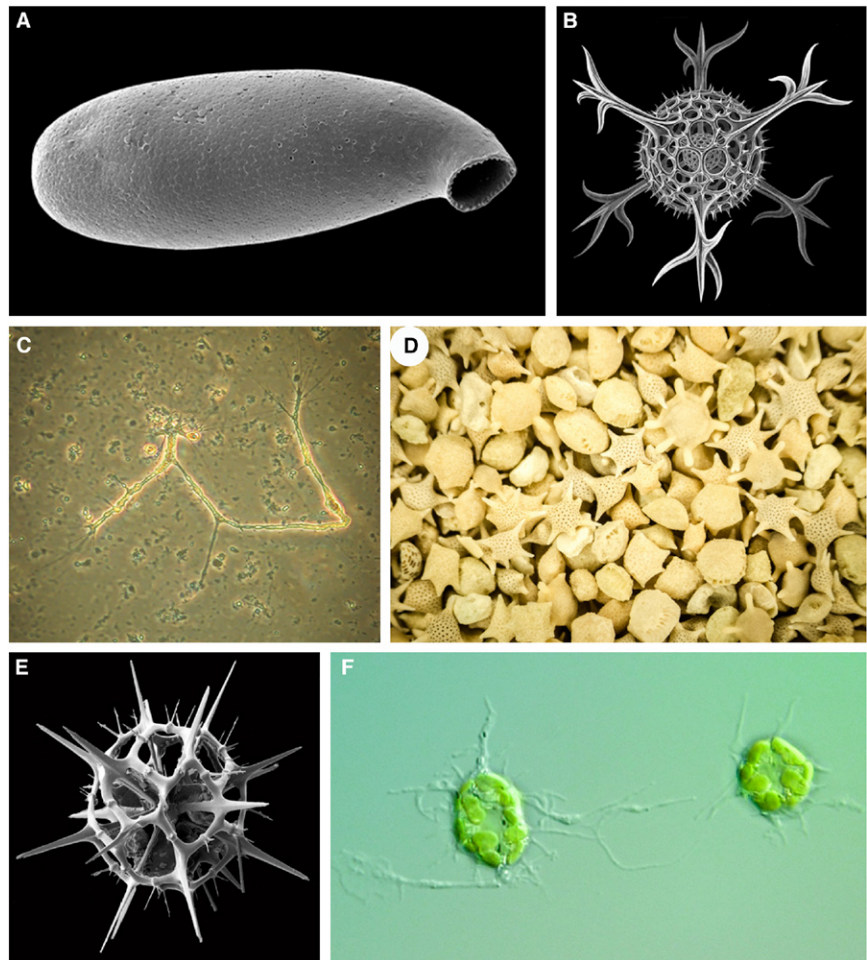


Figure 2. A montage of rhizarian morphology.

(A) Silica shell of the euglyphid testate amoeba *Cyphoderia ampulla* (SEM image courtesy of Thierry Heger). (B) Drawing of the skeleton of the spumellarian (Radiolaria) *Hexancistras quadricuspis*, from Haeckel's plate 91 in *Kunstformen der Natur* (1904). (C) Light microscopy image of a vampyrellid showing cytoplasmic extensions known as filose pseudopods (image courtesy of Cedric Berney). (D) Star sand from Okinawa, composed of the calcareous shell of foraminifera (image from the authors). (E) Intracellular celestite skeleton of the acantharian *Pleuraspis* sp. (SEM image courtesy of Johan Decelle). (F) Light microscopy image of the plastid-containing chlorarachniophyte *Chlorarachnion reptans*, showing its reticulose pseudopods (image from the authors).

observed. Transposing this result onto the rest of Cercozoa, or indeed to the whole of Rhizaria, suggests that a vast reservoir of biodiversity is currently hidden from our eyes, but will be unveiled by more deliberate exploration of the biodiversity of Rhizaria.

#### Wanted: dead or alive

Two groups that have been better-studied than other rhizarians, although from a different angle, are forams and Radiolaria (now grouped together in Retaria). The star sand mentioned above is in fact composed of dead forams ([Figure 2D](#)), and all that remains of a happy life in the sea is

their calcium carbonate shells, which accumulate by billions on star sand beaches, or, if they died long ago, in rocks like those used to build the great pyramids. Most forams are protected by such calcareous shells, which often take complex multi-chambered shapes, but others have organic or agglutinated shells, or lack shells altogether. Radiolarians, which are divided into acanthareans and polycystines, are also skeletonized sea creatures, but unlike forams their shell is intracellular ([Figure 2E](#)), and made of strontium sulfate (celestite) in the case of acanthareans and silica in polycystines. Some of these skeletons and shells preserve well in the sediment, and

their fossils extend continuously to the Cambrian, more than 500 million years ago. Few other protists and no larger organisms have left behind such a well-preserved and continuous microfossil record, and so they have been for decades the favorite tools of paleontologists in paleostratigraphic and paleoclimatic reconstructions. Indeed, many of the divisions between geological periods we use today are defined based on the appearance and disappearance of these fossils.

When they are not dead or fossilized, retarians are still important. Forams, acanthareans, and polycystines are all important grazers, 'munching' on other protists, bacteria, and even crustacean larvae and copepods, and all three punch well above their weight in global geochemical cycling. Forams, for example, can be very abundant in marine waters and on the sea floor, and their calcium carbonate walls are a critical carbon sink that plays a major role in carbon cycling. They absorb huge quantities of dissolved CO<sub>2</sub> from the water, which they lock into their shells, trapping the carbon when they sink to the deep ocean floor. Forams also play a more surprising role in the nitrogen cycle: they are unique among eukaryotes in that they can accumulate intracellular nitrate, and respire it through denitrification in the absence of oxygen. This singular characteristic places forams together with prokaryotes on the list of organisms capable of removing fixed nitrogen from the environment (nitrate) and returning it to the atmosphere as nitrogen gas (N<sub>2</sub>).

### The 'green' Rhizaria

In addition to grazing, many retarians make ends meet by also hosting symbiotic microalgae; in these photosymbiotic associations, the symbionts are often highly specialized organisms that rarely occur outside host cells. Some acanthareans, however, have evolved an unconventional mode of symbiosis in which the symbionts (a tiny alga named *Phaeocystis*) are also active and abundant in their free-living stage. By establishing partnerships with organisms that do not depend on them, acanthareans struck a good deal in the otherwise vast and barren open oceans, gaining an almost unrestricted access to energy and nutrients from their symbiotic photosynthesizing algae.

Other rhizarians have formed alliances based on photosynthesis, but have taken the association further,

and are now themselves considered to be 'algae'. The evolutionary history of photosynthesis and the plastid organelles in eukaryotes remains a matter of some debate, but it is generally accepted that all plastids trace back to a single endosymbiotic cyanobacterium. Or rather, all plastids except that of the rhizarian *Paulinella chromatophora*. This euglyphid amoeba harbors what it says on the tin: chromatophores. These are large blue-green bodies of cyanobacterial origin that are in charge of the photosynthetic reactions and absolutely dependent on their host for survival. Like the genomes of other plastids, the chromatophore genome is highly reduced, encoding less than 900 protein-coding genes (their free-living closest cyanobacterial relatives encode over 3,000). But the chromatophore genome is also very unlike that of plastids, because it originated in an independent and relatively recent endosymbiotic event. The independent origin of chromatophores and plastids opens a window on the transformations that occur when endosymbionts reduce and transform into organelles, and the events that precipitate this integration. This is no small matter because plastid integration has changed the face of the planet by first transferring photosynthetic capabilities from prokaryotes to eukaryotes, then spreading photosynthesis across the eukaryotic tree by eukaryote-to-eukaryote endosymbioses.

The other photosynthetic rhizarian lineage, the chlorarachniophytes (meaning green spiders), is famous for having stolen its plastids from another eukaryote, in a so-called secondary endosymbiosis. These green amoebae (Figure 2F) stand out evolutionarily because in addition to their stolen plastid, they also retained a miniaturized version of the nucleus (the nucleomorph) of their green algal endosymbiont. Nucleomorphs are known in only one other lineage, the cryptophytes, which retain a nucleomorph derived from a red algal endosymbiont. Nucleomorph genomes have become models for the drastic reduction process that followed endosymbiosis, and today include the smallest known nuclear genomes. The *B. natans* nucleomorph is the smallest of the small, encoding a mere 284 genes wrapped in only 380 kb of DNA. Paradoxically, it has retained over 800 introns, because reductive

evolution shrank the introns rather than eliminating them, making *B. natans* a record holder for intron size at only 18–21 bp in length.

### Parasitism in Rhizaria

As with photosynthesis, only a few rhizarian lineages are parasitic, and no infamous human pathogens can be found, which is a unique situation among the eukaryotic supergroups. The best known rhizarian parasites, plasmodiophorids and haplosporidians, infect plant crops and commercially important invertebrates, respectively. For example, the plasmodiophorid *Plasmodiophora brassicae* is the causative agent of the club root disease of cruciferous vegetables, such as cabbage or kale, and *Spongospora subterranea* causes powdery scab of potato. Haplosporidians parasitize several kinds of marine invertebrates, including oysters and mussels, and can devastate aquaculture when a severe infection occurs. Other more enigmatic parasitic groups are also known, such as the phagomyxids, which infect diatoms and filamentous brown algae, and the copepod parasite *Paradinium*.

Recently, another parasite was found to belong to Rhizaria, and in some ways its story represents the history of the group as a whole. *Mikrocytos mackini*, an intracellular microcell parasite that infects Pacific oysters, was an enigmatic lineage that had eluded robust phylogenetic placement for more than 20 years. Even with molecular data, its position in the tree remained unknown because its genes are some of the most divergent sequences known. It took the comparison of more than 100 protein-coding genes to show that it is a new member of Rhizaria, which not only added a new parasitic assemblage to this supergroup, but also the first 'amitochondriate' organism. Indeed, *Mikrocytos* lacks canonical mitochondria, and possesses instead highly reduced organelles similar to the 'mitosomes' found in *Giardia* and microsporidia.

### Concluding remarks

Rhizaria is the last-born in the current family of eukaryotic supergroups, and we are only starting to come to grips with its diversity. Rhizaria remains the least studied supergroup, and, importantly, the lineages about which we know the most are the least representative of the group as a whole. There are only a couple small groups

of photosynthetic rhizarians, and yet a genome and most of our molecular data come from one of these. There are relatively few parasites, but again they are better studied than their free-living sisters. We know more about the fossil record of dead foraminiferans than we do of the biology of living ones. This is really just a symptom of a larger problem — free-living heterotrophic protists are one of the most difficult kinds of life to study, and this is precisely what most rhizarians are. What makes these bugs tick, and how they affect their environment is a tricky business to resolve, but resolving it will surely provide the highlights for the next stage of rhizarian research.

#### Further reading

- Bass, D., and Cavalier-Smith, T. (2004). Phylum-specific environmental DNA analysis reveals remarkably high global biodiversity of Cercozoa (Protozoa). *Int. J. Syst. Evol. Microbiol.* 54, 2393–2404.
- Berney, C., Romac, S., Mahé, F., Santini, S., Siano, R., and Bass, D. (2013). Vampires in the oceans: predatory cercozoan amoebae in marine habitats. *ISME J.* 7, 2387–2399.
- Bhattacharya, D., Helmchen, T., and Melkonian, M. (1995). Molecular evolutionary analyses of nuclear-encoded small subunit ribosomal RNA identify an independent Rhizopod lineage containing the Euglyphina and the Chlorarachniophyta. *J. Eukaryot. Microbiol.* 42, 65–69.
- Burki, F., Corradi, N., Sierra, R., Pawlowski, J., Meyer, G.R., Abbott, C.L., and Keeling, P. (2013). Phylogenomics of the intracellular parasite *Mikrocytos mackini* reveals evidence for a mitosome in Rhizaria. *Curr. Biol.* 23, 1541–1547.
- Curtis, B.A., Tanifuji, G., Burki, F., Gruber, A., Irimia, M., Maruyama, S., Arias, M.C., Ball, S.G., Gile, G.H., Hirakawa, Y., et al. (2012). Algal genomes reveal evolutionary mosaicism and the fate of nucleomorphs. *Nature* 492, 59–65.
- Decelle, J., Probert, I., Bittner, L., Desdevises, Y., Colin, S., de Vargas, C., Gali, M., Simó, R., and Not, F. (2012). An original mode of symbiosis in open ocean plankton. *Proc. Natl. Acad. Sci. USA* 109, 18000–18005.
- del Campo, J., Not, F., Forn, I., Sieracki, M.E., and Massana, R. (2012). Taming the smallest predators of the oceans. *ISME J.* 7, 351–358.
- Keeling, P.J. (2001). Foraminifera and Cercozoa are related in actin phylogeny: two orphans find a home? *Mol. Biol. Evol.* 18, 1551–1557.
- Nikolaev, S.I., Berney, C., Fahmi, J.F., Bolivar, I., Polet, S., Mylnikov, A.P., Aleshin, V.V., Petrov, N.B., and Pawlowski, J. (2004). The twilight of Heliozoa and rise of Rhizaria, an emerging supergroup of amoeboid eukaryotes. *Proc. Natl. Acad. Sci. USA* 101, 8066–8071.
- Nowack, E.C.M., Melkonian, M., and Glöckner G. (2008). Chromatophore genome sequence of *Paulinella* sheds light on acquisition of photosynthesis by eukaryotes. *Curr. Biol.* 18, 410–418.
- Pawlowski, J., and Burki, F. (2009). Untangling the phylogeny of amoeboid protists. *J. Eukaryot. Microbiol.* 56, 16–25.

Canadian Institute for Advanced Research,  
Botany Department, University of British  
Columbia, 3529–6270 University Boulevard,  
Vancouver, BC, V6T 1Z4, Canada.  
E-mail: [fabien.burki@botany.ubc.ca](mailto:fabien.burki@botany.ubc.ca),  
[pkeeling@mail.ubc.ca](mailto:pkeeling@mail.ubc.ca)

## Correspondences

# Generation of infectious virus particles from inducible transgenic genomes

Mathias F. Wernet, Martha Klovstad, and Thomas R. Clandinin\*

Arboviruses like dengue virus, yellow fever virus, and West Nile virus are enveloped particles spread by mosquitoes, infecting millions of humans per year, with neither effective vaccines, nor specific antiviral therapies [1,2]. Previous studies of infection and virus replication utilize either purified virus particles or deficient genomes that do not complete the viral life cycle [1,2]. Here we describe transgenic *Drosophila* strains expressing *trans*-complementing genomes (referred to as ‘replicons’) from the arbovirus Sindbis [2]. We use this binary system to produce, for the first time in any metazoan, infectious virus particles through self-assembly from transgenes. Such cell-type specific particle ‘launching’ could serve as an attractive alternative for the development of virus-based tools and the study of virus biology in specific tissues.

Arbovirus genomes are positive-stranded RNA molecules that are capped and polyadenylated, thereby resembling cellular mRNAs (Figure 1A). However, unlike most eukaryotic messages, the genomic RNAs of some arboviruses like Sindbis are bicistronic, containing two open reading frames (ORF1 and ORF2) [2]. We have generated transgenic Sindbis genomes stably inserted into the fly genome, under the control of an inducible promoter. After inducing host-cell transcription of virus ORF1, which encodes the viral RNA-dependent polymerase (RdRP, or ‘replicase’), viral replication can be induced in *Drosophila* [3]. We sought to induce self-assembly of infectious virus particles *in vivo* by adding the viral structural proteins (which include the glycoproteins necessary for membrane fusion). Structural proteins are encoded

by virus ORF2, and translated from a ‘subgenomic RNA’ [2] generated by the viral replicase (Figure S1A). Analogous ‘launching’ of infectious particles has previously been reported in yeast [4] and in plants [5], but never in any metazoan.

We first generated a transgenic replicon reporter (SinR-GFP), by replacing ORF2 with GFP, under the control of the viral replicase (translated from ORF1 of the same RNA). As previously reported, expression of such a replicon was universally low [3]. We therefore inhibited the cellular RNA interference (RNAi) pathway, which has a strong antiviral role in *Drosophila* [6,7], using protein B2, a dominant inhibitor of RNAi from flock house virus [8]. We observed high levels of GFP expression when SinR-GFP and B2 were coexpressed in such diverse tissues as gut, muscles, salivary glands, trachea, and photoreceptors (Figure 1B). Similarly high levels were obtained when RNAi was inhibited using *Dcr2* mutants (Figure S1B).

We next provided structural proteins by expressing a template for the ‘subgenomic RNA’ *in trans* from a second ‘deficient’ transgene lacking ORF1, which encodes the viral replicase (DH-BB; Figure 1C). We homogenized flies expressing the *trans*-complementing transgenes SinR-GFP, DH-BB and B2 under *GMR*-GAL4 control. This fly debris was then used to infect a monolayer of cultured baby hamster kidney (BHK) cells, in which Sindbis virus replicates efficiently (see Supplemental Information published with this article online). Robust expression of GFP was detected in 1.5–5.4% of total BHK cells (Figure 1D), indicating that these cells had been infected with Sindbis, and translated the self-replicating GFP-expressing replicon. As the absolute number of GFP-positive cells depended on fly rearing conditions and BHK cell passage number, we calculated an ‘infection coefficient’ (Inf.; see Supplemental Information). An Inf. >1 indicates an experimental condition that produced more infectious particles than the positive control, while values <1 correspond to reduced infectivity. Virtually identical results were observed when RNAi was inhibited using *Dcr2* mutants (Inf. = 0.94 ± 0.53; Figure S1G) and lower numbers were observed when different tissues were targeted (neurons, photoreceptors, glia,