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Primer

Chytrid fungi

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Fungi have distinguishing traits, such as hyphae and cell walls, that evolved in a fungal ancestor over one billion years ago. Chytrid fungi are some of the earliest diverging fungal lineages that retained features of the opisthokont ancestor of animals and fungi (Figure 1). For example, chytrids make reproductive cells known as zoospores that swim with a motile cilium or crawl like an amoeba. The aim of this primer is to introduce the reader to the life cycle, biology, and ecology of chytrids and other zoosporic fungi. We highlight how chytrids are well positioned to elucidate both the cell biology of the animal–fungal ancestor and the evolution of derived fungal features.

Chytrids exhibit fungal and ancestral features

Life first evolved in the ocean and the last eukaryotic common ancestor (LECA) likely swam and engulfed organic matter via phagocytosis. Based on the shared features found across eukaryotes, LECA had a nucleus, mitochondria, an endo-membrane system, actin and tubulin cytoskeleton, and a centriole for building a mitotic spindle and cilium. LECA gave rise to diverse eukaryotes, some of which remained in aquatic environments and others which colonized land over 500 million years ago. Fungi (e.g. chytrids, rusts, molds, mushrooms, and yeast) are a large eukaryotic kingdom found in many environments and ecological niches. These eukaryotes are decomposers that live on organic matter or as parasites of plants and animals. Fungi are also important symbionts: they are partners of algae and cyanobacteria in lichens or they form mycorrhizae that colonize plant roots and extract water and nutrients from soil in exchange for sugars. The successful expansion and colonization of terrestrial environments by the plant and fungal kingdoms is likely the consequence of a symbiotic relationship between early fungi and photosynthetic algae.

Fungi are closely related to animals through a common opisthokont ancestor that lived in an aquatic environment over one billion years ago (Figure 1). Chytrids and other early-diverging fungi have persisted in this ancestral habitat and have retained traits that make them well adapted to foraging for resources in water. For example, chytrids produce spores (known as zoospores) that lack a cell wall and swim via a motile cilium and/or crawl on surfaces via amoeboid motion (Figure 1). The presence of a centriole and a motile cilium is unique to chytrids and other zoosporic fungi within the fungal kingdom. The cilium is attached to a basal body that contains a classic centriole with nine circularly arranged triplet microtubules that nucleate the axoneme. Similar to many animal cells, chytrids resorb the cilium and the centriole is repurposed as a centrosome to organize the mitotic spindle for nuclear division cycles.

The fungal ancestor evolved new traits (‘derived traits’) that are shared by all fungi including chytrids. For example, the chytrid life cycle includes a vegetative body (‘thallus’) with a cell wall and hyphal-like feeding structure known as a rhizoid (Figure 1). Fungal hyphae are branching, filamentous tubes that penetrate organic matter and secrete digestive enzymes to extract nutrients for cell growth. Hyphae grow into substrates by depositing cell wall materials and remodeling enzymes at the hyphal tip via directed vesicle trafficking on a cytoskeletal network. The cell wall is critical because it holds large, hydrostatic pressures caused by internal osmolytes, which generate the biomechanical forces that drive cell wall expansion at the hyphal tip. As in other fungi, the hyphal-like rhizoid is important for colonizing substrates and extracting nutrients to fuel chytrid cell growth.

Chytrid ecology and the evolution of zoosporic fungi

We use the term zoosporic fungi to describe chytrids and other early diverging fungi that have a zoospore stage during their life cycle (Figure 2A). Meta-genomic sequencing has shown that zoosporic fungi comprise much



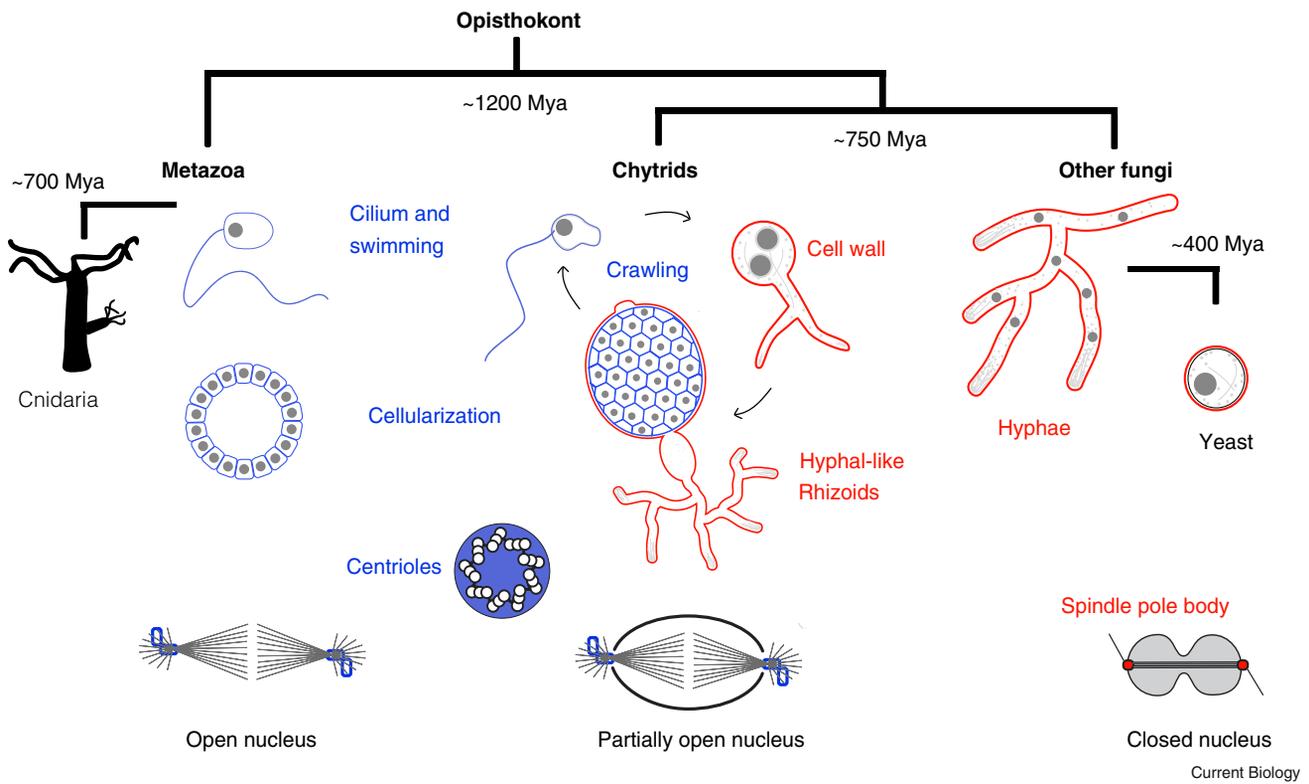


Figure 1. Chytrids are early-diverging fungi that exhibit both fungal features and ancestral features.

The common ancestor of the animal and fungal kingdoms is known as an opisthokont ('posterior pole') because of its posterior motile cilium present in animal cells and chytrid zoospores. Other ancestral features (shown in blue) are present during a chytrid life cycle, such as centrioles, actin-mediated crawling, chemotactic and phototactic swimming, and cellularization via membrane invagination to create zoospores from a field of nuclei. Fungal features (shown in red), such as a cell wall and hyphal-like structures, also occur during the chytrid life cycle. In non-chytrid fungi, centrioles have been lost and replaced by a microtubule organizing center known as the spindle pole body, which combines some centriolar and fungal-specific elements. Organelles and cell bodies not drawn to scale. Hydra Silhouette used with permission from Gareth Monger (CC BY 3.0).

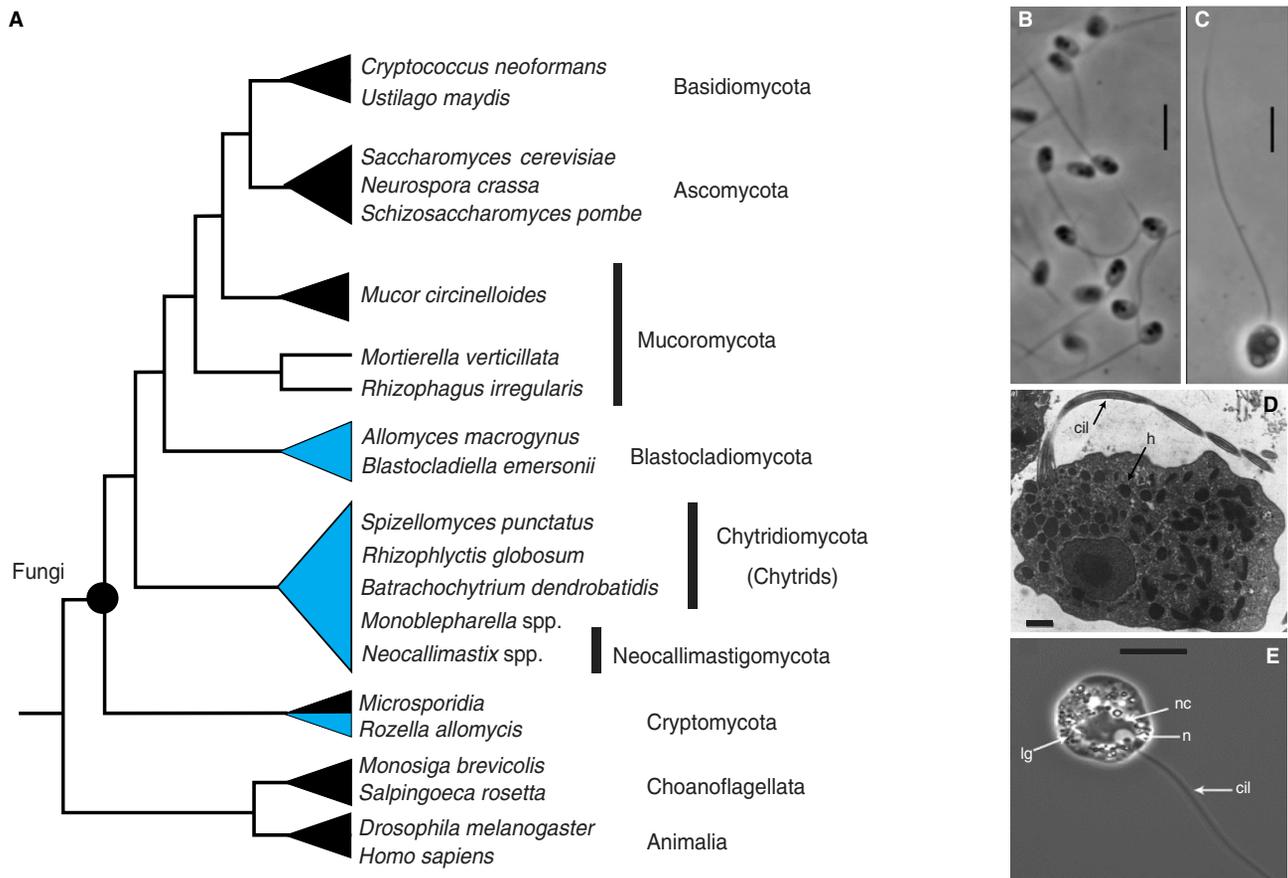
of the unknown fungal diversity in aquatic environments. Zoosporic fungi span at least three phyla (*Cryptomycota*, *Chytridiomycota*, and *Blastocladiomycota*). The *Cryptomycota* are the deepest lineage and include the genus *Rozella*, which parasitizes chytrids (Figure 2B), and other uncultured parasites of fungi, amoeba, oomycetes and algae. The *Cryptomycota* also include the *Microsporidia*, which are common animal parasites that have small, fast-evolving eukaryotic genomes and that have lost their cilium. Despite their lack of a zoospore stage, phylogenetic analyses place *Microsporidia* within the *Cryptomycota*.

The *Chytridiomycota* and *Blastocladiomycota* are later-diverging phyla that are better studied than the *Cryptomycota*. The *Chytridiomycota* (commonly called 'chytrids') are

found in aquatic and terrestrial habitats, and are saprotrophs as well as parasites of algae, plants and animals (e.g. the amphibian pathogen *Batrachochytrium*). These chytrids play an important role in aquatic food webs by infecting large, inedible algae and producing small zoospores (Figure 2C) that are edible to zooplankton. Anaerobic, multi-ciliated *Neocallimastigomycota* in ruminants (e.g. sheep, cattle) are a well-characterized subgroup of this phylum that have evolved hydrogen-producing organelles known as hydrogenosomes (Figure 2D). The rumen microbiome contains eubacteria, archaea, ciliates, and chytrids that collectively ferment plant material to produce volatile fatty acids and microbial protein for their animal host. The rumen chytrids penetrate plant tissue with their hyphal-like structures, secrete cellulases, and

help breakdown highly recalcitrant carbohydrates for the microbiome.

The *Blastocladiomycota* include saprotrophs as well as parasites of fungi, algae, plants and invertebrates (Figure 2E). Although zoosporic, and once classified as *Chytridiomycota*, the *Blastocladiomycota* differ from the other chytrids in the complexity of their thallus and life cycle: they can have haplontic alternation of generations (much like land plants) and exhibit multicellular haploid (gametophyte) and multicellular diploid thalli (sporophyte). While asexual reproduction is through zoospores, sexual reproduction involves motile gametes of opposite sexes with different sizes and coloration that attract and swim towards each other through pheromone signaling. The *Blastocladiomycota* have diverse body plans with some species



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Figure 2. Chytrids and other zoosporic fungi are early-diverging fungal lineages.

(A) Many early-diverging lineages (shown in blue) produce zoospores. Later-diverging lineages, such as *Mucoromycota* and dikaryotic fungi (*Ascomycota*, *Basidiomycota*), lost ancestral features and further elaborated upon fungal characters, such as hyphae that form a mycelium and build complex sexual structures. (B) Brightfield microscopy of a *Rozella rhizoclosmatii* (Cryptomycota) zoospore. (C) *Rhizoclosmatium globosum* (Chytridiomycota) zoospore. Scale bar = 5 μm. Modified from (Letcher *et al.* 2017). (D) Electron microscopy of *Neocallimastix patriciarum* zoospore. Scale bar = 1 μm; cil = cilia, h = hydrogenosomes. Panel used with permission of the Biochemical Society from (Yarlett *et al.* 1986). (E) Brightfield microscopy of *Allomyces javanicus* (Blastocladiomycota) zoospore. Scale bar = 10 μm; lg = lipoid granules, nc = nuclear cap, n = nucleus, cil = cilium. Reprinted with permission from Springer-Verlag Berlin Heidelberg from (James *et al.* 2014).

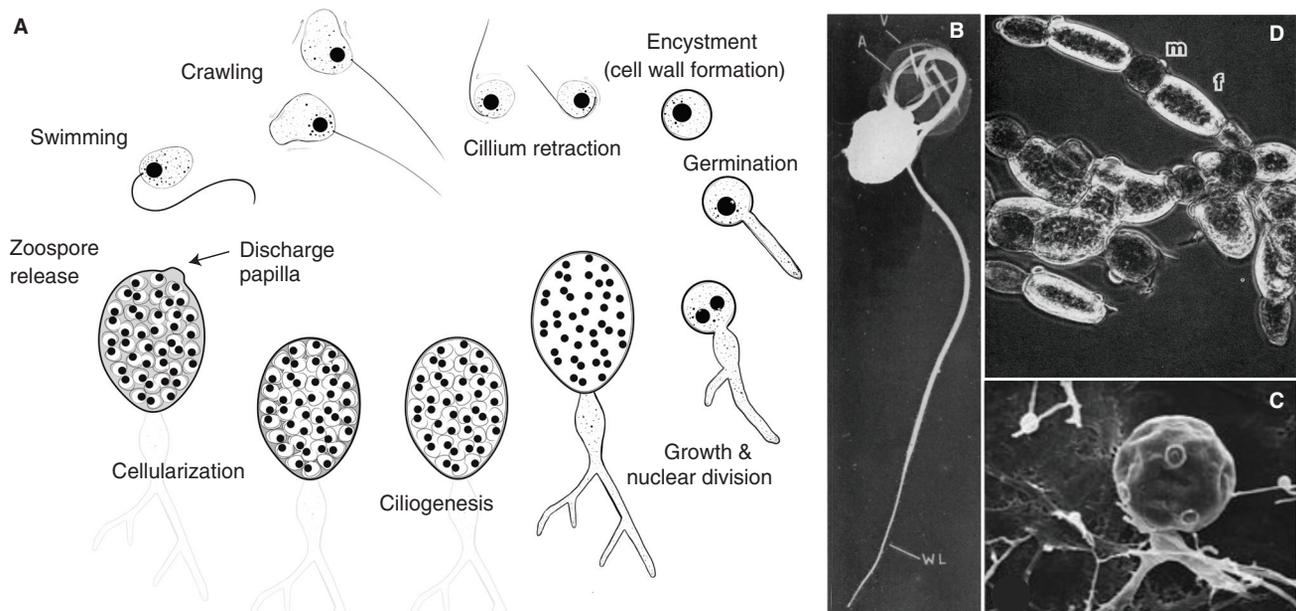
(e.g. *Allomyces*) developing true hyphae (nucleated, with pseudo-septa and polarized indeterminate growth with an apical organizing center, similar to the Spitzenkörper found in filamentous fungi).

Chytrid life cycle

Chytrid species can differ considerably in their life history, morphology, metabolism, and sub-cellular organelles. However, many chytrids exhibit a similar life cycle that progresses from zoospore to thallus to sporangium (Figure 3A). Chytrid zoospores range from 2–10 microns in diameter and have a single posterior motile cilium, although anaerobic chytrids of the rumen can have

multiple cilia (Figure 2D). Zoospore ultrastructure (e.g. basal body and associated sub-structures) is diverse and is often used to identify and classify chytrid species. Chytrids swim with a motile cilium and some species can switch to amoeboid crawling when attached to a surface. Zoospores have a single nucleus and are quiescent, i.e. inactive cell division cycle and no growth. They sustain the energetic demands for motility by catabolizing lipids and storage carbohydrates that were maternally provisioned by the chytrid sporangium during zoosporogenesis in the previous life cycle. Lipid droplets are often visible when observing chytrid zoospores by light microscopy (Figure 2E).

Although chytrid zoospores are metabolically active, they do not produce new DNA, RNA, or proteins until after germination. Zoospores are translationally inactive and contain inactive ribosomes pre-loaded with maternal mRNAs. In the *Blastocladiomycota*, inactive mRNA–ribosomes are packaged into an organelle associated with the nucleus called the nuclear cap (Figure 2E). Ribosome activity in the zoospore is blocked in the elongation stage by an inhibitor whose identity remains unknown. It is unclear how universal this mechanism might be across all chytrids; however, it has been established that some *Chytridiomycota* zoospores are also translationally inactive.



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Figure 3. General chytrid life cycle.

(A) In the right conditions, motile zoospores retract their cilium and transition to a fungal-like stage with a thallus and hyphal-like rhizoids to colonize a new resource. The zoospore is mitotically inactive, whereas cell growth and nuclear divisions occur during the ‘fungal’ stage. Multiple rounds of nuclear division without cytokinesis will create a multi-nuclear compartment (‘coenocyte’) in the growing thallus, which will become a sporangium. Each nucleus and new cilium (‘ciliogenesis’) in the mature sporangium will be encapsulated through a process of membrane invagination (‘cellularization’) to create zoospores that are eventually released, thus completing the chytrid life cycle. There are important life cycle variations between chytrid species, such as nutrient and host preference, thallus morphology, nuclear migration into the rhizoids, location and number of sporangia, formation of a resting spore, and sexual reproduction. Cell bodies not drawn to scale. (B) Electron micrograph of vesicular retraction of a motile cilium in *Chytridiomyces hyalinus* (Chytridiomycota). A = axoneme, V = vesicle (inflated membrane of cilium), WL = whip-lash or endpiece of cilium. Image used with permission from (Koch 1968). (C) Electron micrograph of empty *Spizellomyces punctatus* (Chytridiomycota) sporangium with multiple discharge papilla. Image used with permission from (Chen and Chien 1998). (D) Lower magnification brightfield microscopy of alternating male (m) and female (f) gametangia from *Allomyces macrogynus* (Blastocladiomycota). Image used with permission from (Fuller and Jaworski 1987). Scale bars were not included in the original figures.

Once chytrid zoospores find an appropriate niche, they encyst by retracting their motile cilium and building a fungal cell wall (Figure 3A). The mechanics of ciliary retraction are diverse with at least four scenarios (lash-around, body-twist, straight in, and vesicular) that can vary depending on the species or the environment. In the lash-around retraction, the cilium lashes around the immobile zoospore body resulting in a sheath-less axoneme coiled inside the membrane. In the body-twist retraction, the zoospore body twists or rotates while the cilium remains passive, with the same resulting axoneme coiled under the membrane. In the straight-in retraction, the immobile cilium slowly reduces length, entering the immobile zoospore body at the point of attachment. Finally, for vesicular retraction, the axoneme coils or loops within itself in a vesicle of the cilia

membrane, progressively shortening the cilium until the vesicle reaches and fuses into the main zoospore body (Figure 3B). The cell biology and mechanisms used by zoospores for retraction are still an open question, but its diversity of form and plasticity may reflect the structural diversity seen in the chytrid zoospore basal body and associated structures. Much like metazoan cells, retraction of the cilium liberates the centrioles for cell division that occurs during chytrid growth. It may also repurpose ciliary components for germination or other cellular processes until new protein is synthesized in the chytrid.

Upon encystment, changes in the regulation of the actin cytoskeleton shift the chytrid from a naked motility specialist (ciliary swimming and crawling) to a foraging specialist with a fungal cell wall and turgor-driven polarized growth. The germinating

cyst usually forms a single germ tube that later expands and branches into a hyphal-like rhizoidal system (Figure 3A). Zoospores in some *Chytridiomycota* crawl using pseudopod-based alpha-motility, which is driven by the expansion of branched-actin filament networks via the Arp2/3 complex. Chytrid species whose zoospores crawl contain activators of branched-actin assembly (WASP, SCAR/WAVE), which are correlated with crawling and alpha-motility in other eukaryotes. Once the zoospores encyst and germinate, there is a shift in actin cytoskeleton organization to actin patches and cables that extend into the germ tube and rhizoids. This architecture is typical of fungi, where actin patches are associated with endocytosis and cell wall deposition, whereas actin cables are pathways for targeted delivery of exocytic vesicles.

In some chytrids, the nucleus remains in the cyst during germ tube expansion and the cyst will develop into a spherical reproductive structure called the sporangium (Figure 3A). In other species, the nucleus can migrate into the rhizoid and eventually trigger the growth and formation of a sporangium outside the original cyst. During the formation of the sporangium, a nucleus goes through multiple rounds of nuclear division without cytokinesis to create a shared compartment of nuclei known as a coenocyte. This is later followed by ciliogenesis (i.e. the conversion of centrioles into basal bodies and the building of motile cilia), membrane invagination and the coordinated encapsulation of individual nuclei, cilia and other organelles into single cells to form new zoospores. Membrane cellularization of a coenocytic compartment is an ancestral process that occurs in pre-metazoan lineages and animal embryogenesis. In appropriate environmental conditions, the mature zoospores are released through one or multiple pores (called discharge papillae) that open in the cell wall (Figure 3C).

Zoospore phototaxis and chemotaxis

Upon release from a sporangium, chytrid zoospores swim or crawl to find new niches. The zoospores can swim for hours to days with speeds of up to 100 microns per second. The motion consists of swimming in mostly straight lines with rapid changes in direction, interspersed with long breaks of crawling in some species. Zoospores sense both chemical cues and use light cues to locate hosts or substrates. Chemotaxis assays have shown that zoospores will swim towards specific nutrients (e.g. sugars, proteins, fatty acids, amino acids) usually associated with target hosts or substrates. Likewise, phototaxis assays have shown that zoospores of different species will swim towards green or blue light. A type-I opsin (also known as bacteriorhodopsin) fused to guanylate-cyclase drives the phototaxis of zoospores in *Blastocladiella emersonii* (Blastocladiomycota). The mechanism of phototransduction of this

type-I opsin is reminiscent of the phototransduction pathway of animal G-protein-coupled ciliary opsins. This type-I opsin is homologous to the one used by pre-metazoan choanoflagellates to drive ciliary movement and contractility of the multi-cell colony. The diversity of type-I opsins seen in chytrids and choanoflagellates have optogenetic potential. For example, *Blastocladiella* ‘CyclOp’ was recently developed for sensitive and fast control of cGMP levels in target cells and animals.

Chemotaxis also drives sexual reproduction in the *Allomyces* (Blastocladiomycota) in which motile male and female gametes produced by male and female gametangia (Figure 3D) swim towards each other using pheromone signaling. Although the chemical structure of the male pheromone (parisin) is unknown, the female pheromone (sirenin) is a sesquiterpene. Strikingly, sirenin can activate the human sperm CatSper calcium channel, much like progesterone. CatSper is essential for hyperactivation of the sperm’s cilium and plays a role in chemotaxis towards the egg. Interestingly, chytrids have orthologs of CatSper and calcium signaling is involved in sirenin signaling. The chytrid pheromone receptor and its mechanism of action remain unknown, but we anticipate that chytrids will be useful organisms for understanding the conserved mechanisms of sexual chemotaxis, calcium signaling, and the regulation of swimming motility via CatSper.

Evolutionary outlook

Basic research in animal and fungal model organisms elucidated conserved mechanisms and regulators of eukaryotic cell biology (e.g. cell cycle). However, these closely related eukaryotes also evolved new features and adaptations over the last one billion years. The common ancestor of most fungi committed to a cellular morphology and sessile life cycle that produces hyphae that grow into their substrates and durable spores that disperse via air currents or ejection. These adaptations are useful for a saprophytic or parasitic lifestyle in a terrestrial environment, but they emerged when the fungal ancestor was still living in aquatic environments.

Chytrids are an early-diverging fungal lineage that likely reflect a transitional phase in the evolution of terrestrial fungi, not unlike amphibious animals. Chytrid genomes are also unique because they contain ancestral, animal-like genes and regulatory networks that were lost in most other fungi. For example, the fungal cell cycle was rewired by a viral domain that eventually replaced the ancestral G1/S regulator in most fungi. The ancestral and viral regulators and G1/S pathways still coexist in chytrids. This same viral domain also created a large family of transcription factors that regulate fungal-specific processes, such as hyphal morphogenesis. As such, chytrids are promising organisms to help understand the molecular evolution of derived fungal features and the conservation of ancestral features.

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