THE EVOLUTION OF FUNGI

Fungi are more closely related to animals than plants. The evolutionary origin of fungi is important in determining the phylogenetic relationships between fungi, animals, and plants, and in questioning a previous view of the origin of life, which stated that photosynthetic organisms were the first to evolve since they were utilized by heterotrophs as a food source. The key evidence in support of a fungi-animalia clade includes analysis of protein sequences biosynthetic pathways, cytochrome systems, mitochondrial genetic material, biochemical and structural cellular features, glycoproteins, mode of nutrition, and storage of nutritive materials. The hypothesis that fungi evolved from algae, the ancestor of photosynthetic plants is not well supported, The hypothesis that fungi evolved independently of both plants and animals is also not supported.

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Fungi are more closely-related to animals than plants. Although early studies and papers by mycologists suggested that fungi are members of the plant kingdom, new knowledge which has become available suggests that fungi share an evolutionary lineage with animals. This new knowledge, which will be addressed in the text to follow includes structural cellular features, particularly the structure of cell walls and cytoplasmic organelles; the chemical structure of hyphal walls, mitochondrial and nuclear DNA and ribosomal RNA; and nutritional and metabolic features, particularly metabolic pathways (Burnett, 1987).

The main questions to be addressed in our discussion are as follows: How are fungi similar to animals at the organismal, cellular, and biochemical levels? What do these similarities suggest about the phylogenetic relationships between fungi, animals, and plants? Finally, which evolutionary relationship is best supported by the evidence presented?

This topic is significant for several reasons. The origin of fungi has implications for the origin of life. One early theory stated that fungi, along with all other organisms, evolved from plants. An assumption of this theory is that photosynthetic organisms must have preceded heterotrophic organisms since heterotrophs are dependent on photosynthetic organisms as a source of food. Since algae were considered the simplest plants in terms of morphology (and thus the most primitive), it was concluded that fungi were derived from algae by loss of chlorophyll (Martin, **1955).** There are several problems with this hypothesis. First, we cannot assume that the atmospheric conditions on earth when life began were the same as the conditions on earth today. Second, there are other auxotrophs besides photosynthetic organisms, for example nitrogen-fixing bacteria, which could have existed before plants evolved and were utilized as a food source (Martin, 1955).

It was later concluded that connections with algae were "based on superficial resemblances. In the infancy of biochemistry, these resemblances were plausible to the casual observer, [but while fungi] showed considerable coherence in their physiology, they differed sharply from algae" (Sussman, 1966). In addition to physiological differences between the algae and fungi, "the differences in nuclear condition and in cellular organization and development are not satisfactorily explained" (Martin, 1955). It has since been largely agreed that derivation of the fungi from plants or algae would require more successive evolutionary changes than derivation of the fungi from protozoa, the unicellular ancestors of the animals (Sussman, 1966).

The biochemical evidence supporting the hypothesis of a fungi-animalia clade is particularly striking. First, the protein sequences of fungi show homology to those of animals. Recent results from multiple protein data sets from members of the Dictyosteliomycetes, the cellular slime molds, "show with statistical comidence that Dictyostelium is closely related to the animalia-fungi clade and is distantly related to plantae" (Kuma, 1995).

Elongation factors found in the fungal ribosomes also provide evidence for this thesis. Elongation factor 3 (EF-3) identified in a wide range of fungal species, was found to "display amino acid similarity to myosin proteins whose cellular function is to provide the motile force of muscle," and which work there mainly with actin. It was also stated that EF-3 "has at least ten regions of detectable amino acid sequence similar to the mammalian myosin heavy-chain proteins (MyHC). Although none of these regions is responsible for a defined activity of myosin, such as ATPase activity and actin binding, they "occur in equivalent positions in mammalian and yeast myosin proteins." EF-3 has also shown similarities to the product of the mammalian myosin gene MYO-1. The product of the MYO- 1 gene is a non-muscle cell form of myosin for which precise cellular function remains unreported (Belfield, 1995).

Another finding amongst the fungal translation elongation factors was that EF-1 alpha showed remarkable similarity to human EF- 1 alpha protein, showing 81% identity. In the same way, proteins such as Beta-tubulin and calmodulin are all "fairly evenly conserved across the fungal kingdom and even with their human counterparts" (Belfield, 1995).

Fungi also display similarities to animals in the biosynthesis of polyunsaturated fatty acids. Two distinct pathways are recognized, one leading to the production of alpha-linolenic acid, and the other to gamma-linolenic acid and arachidonic acid. Interestingly enough, "the alpha-linolenic acid appears to be predominantly synthesized by higher plants.. .not by protozoa and metazoa" (Lejohn, 1974). The gamma linolenic acid is synthesized by animals. In 1965, Shaw in his studies showed that "out of thirty-one fungal species.. [seven orders of the Ascomycetes and live orders of the Basidiomycetes] all produce gamma linolenic acid, not the alpha linolenic acid (Lejohn, 1974).

Fungi also show similarities to animals in their cytochrome systems. In 1957, Boulter and Derbyshire, using intensive spectroscopic methods, studied the visible absorption of the cytochromes of fungi and compared their spectra with those obtained for yeast.Forty-five fungal species represented all major classes and possessed a cytochrome structure similar to the cytochromes of mammalian and avian [bird] cells. On the other hand, they were not nearly as similar to the cytochrome systems of green plants. Fungi have b-type and c-type cytochromes which higher plants lack and which are found in metazoa. Studies also showed that "cytochrome c is the only hemoprotein of all cytochromes that displays a constancy of physical and chemical properties (Lejohn, 1974). Therefore, the conclusion came that these features may be a biochemical parameter for phylogenetic affinities between fungi and animals.

Unlike plants, but like animals, in thefungal mitochondrial code, 'UGA codes for tryptophan, not chain termination" (Cavalier-Smith, 1987). It was said that "because none of the positions where UGA is found happen to [code]... for a known product, it is difficult to decide whether this codon codes for tryptophan or serves as a stopcodon, as it does in the universal code." However, studies developed by Lang in 1984 suggest that these codons are read as tryptophan, though inefficiently. This situation was compared to a number of organisms from different phylogenetic groups. Results showed that "in all animal mitochondria UGA is also read as tryptophan" (Scazzacchio, 1987).

Fungi share common morphological and structural cellular features with animals. Both have non-discoidal plate-like mitochondrial cristae, while plants have mostly tubular mitochondrial cristae. This finding "explains the origin of othercristal types as the consequence ofcoevolution of the plastid and mitochondrial trans-envelope protein systems during conversion of [one kind of plastid]. ..into the different kinds of plastids." In both fungi and animals, plastids are absent, while plants possess plastids with two envelopes, usually containing starch (Cavalier-Smith, 1987).

Microfibrils and matrix polysaccharides are another piece of evidence supporting the hypothesis of a closer resemblance betweenfungi and animals. The fungal cell wall is mostly made up of polysaccharides, andchitin is the most characteristic of the polysaccharides. The fungal chitin is made up of an unbranched polymer of B-14, 4 linked N-acetyglychosamine units, which is distinct from higher plants. Chitin is found in protozoa and higher animals. Fungi also contain chitin only in the Alpha form and none in the Beta form. Alpha-chitin is also the most abundant form present in animals (Ruiz-Herrera, 1992).

Polysaccharide microfibrils are randomly oriented in fungi. This differs from plants and algae in which the microfibrils are laid down in parallel arrays (Ruiz-Herrera, 1992). Treatment of the

polysaccharides with an alkali containing nitrogen resulted in its degradation, along with the production of acetic acid. This differs from similar reactions carried out with the polysaccharides present in plants. Several other chemical reactions of the polysaccharides showed distinctness from the properties of plant polysaccharides (Ruiz-Herrera, 1992).

There are two types of bonding in the glycoproteins of fungi that are shared with glycoproteins of animal origin: oligosaccharides are joined toserine/threonine residues through an O-glycosidic linkage, and sugar present at the reducing end of the oligosaccharide moiety is joined to an asparagine residue through a N linkage (Ruiz-Herrera, 1992).

The cellulose of fungi is also slightly different from that found in the cell walls of green plants. When examined by X-ray diffraction and compared to X-ray diffraction images of the cellulose of green plants, fungal cellulose is less crystalline than plant cellulose(Ruiz-Herrera, 1992)

Fungi lack chloroplasts, as do animals. Thus the process of photosynthesis, which is very common and important in plant life because it serves as their source for food, is absent.Fungi do not produce chlorophyll pigments like green plants do. Therefore, a brief summaryof the modes of nutrition is as follows: photosynthesis in plants, ingestion in animals, and absorption in fungi (Moore-Lander, 1987).

Specifically, the absorption of food materials byfungi occurs as follows:

> In order to utilize certain fractions of potentially nutritive materials in their environment, they must secrete enzymes into their surroundings. These exoenzymes degrade those substances to which they are adapted, and theresulting.,.compounds must be absorbed through the wall of the cell. They must pass through the cytoplasmic membranes before reaching the cytoplasm in which they are carried to locations in which the endoenzymes react with them, reorganizing them into forms in which they can be combined with other elements, ions, or compounds to produce new protoplasm and cell wall material (Cooke, 1979).

This digestion of food materials at the surface of the organism, followed by absorption of the products of digestion through membranes while leaving the residues outside, has been used in the past to characterize fungi as plants. However, this conclusion ignores the existence of protozoa and worms with a similar, absorptive nutrition (Martin, 1955). It also does not take into account the animal-like amoeboid phase in some fungi, particularly the cellular slime molds, which "lack cell walls during the phase in which they obtain nutrients and grow, and are capable of digesting nutrients in particulate form by phagocytosis" (Carlile, 1994).

For example, the amoebae of *Dictyostelium discoideum* (a cellular slime mold) "can be grown readily in a two-membered culture with a variety of bacteria.. .The bacteria multiply and the amoebae feed on them by phagocytosis, taking the bacteria into food vacuoles within which the bacteria are digested...The nutrition of cellular slime molds is.. mainly ingestive" (Carlile, 1994).

D. discoideum is able to efficiently locate bacteria by chemotactic responses. The amoebae release an unknown factor which allows them to repel each other, thus avoiding high concentrations of amoebae. The amoebae show positive chemotaxis to folic acid, however, which is released as a waste product by the bacteria. Upon sensing the presence of folic acid, the amoebae release an enzyme which destroys folic acid, presumably in order to prevent build-up of folic acid in the environment. Such a build-up would probably not give as clear an indication of the direction from which the folic acid is coming, and therefore would not accurately locate the bacteria (Carlile, 1994). Fungi secrete enzymes to break down polysaccharides, protein, nucleic acids, lignin, and lipids in order to absorb nutrients. Enzyme production is also a characteristic in which fungi resemble animals and differ from plants(Ruiz-Herrera, 1992).

The Myxomycetes, or plasmodial slime molds (given the name because of a transient stage in their life cycle in which they form a plasmodium, a multinucleated mass of protoplasm not divided

into cells), also have an amoeboid phase in their life cycle. Myxomycete amoebae are similar to those of the cellular slime molds, but they can also form flagella and swim. Myxomycete amoebae are also phagotrophic, resembling protists in their mode of nutrition rather than photosynthetic algae or plants (Carlile, 1994).

Fungi which are members of the order Blastocladiales (a division of the class Chytridiomycetes) also display protist-like nutrition. This order has a zoospore stage in the life cycle. The zoospores are able to swim via a posterior flagellum, and show chemotaxis to amino acids (Carlile, 1994). Only two other types of eukaryotes have a posterior flagellum and mitochondrial cristae: metazoan animals and choanoflagellate protozoa (Cavalier-Smith, 1987).

Fungi resemble animals not only in their mode of nutrition, but in their storage of digestive nutritive materials. Like many eukaryotic organisms, fungi can accumulate lipids as a carbon reserve (Carlile, 1994). However, another means of storage common to fungi, bacteria, protozoa, and higher animals is the polysaccharide glycogen. Plants, on the other hand, use starch as a carbon reserve (Ruiz-Herrera, 1992).

A case has been made for at least two alternative hypotheses to the one which has been presented regarding the phylogeny of fungi. One, suggested by D.P. Rogers, is that fungi are phylogenetically distinct from all other eukaryotic animals, and evolved in an entirely separate lineage (D.P. Rogers, in Cooke, 1979).

Fungi appear to be unique in their requirement for a third soluble translation elongation factor 3 (EF-3). EF-3 exhibits ribosome dependent ATPase and CTPase activities that are not intrinsic to the fungal ribosome but are essential for translation elongation in vivo. The EF-3 polypeptide has been identified in a wide range offungal species, and the gene encoding EF-3 (YEF-3) has been isolated from four fungal species (Belfield, 1995).

However, the similarities between EF-3 and the myosin proteins of animals cannot be ignored. EF-3 was found to "display amino acid similarity to myosin proteins [found in animals] whose cellular function is to provide the motile force of muscle" (Belfield, 1995). In addition, at least 10 regions of EF-3 show amino acid sequence similarities to the mammalian myosin heavy-chain protein (Belfield, 1995).

In 1960, Vogel discovered that fungi are unique in their distribution of two distinct pathways known to lead to the biosynthesis of L-lysine. One path, the diaminopimelic acid (DAP) route, is the most common among both mono- and dicotyledonous plants. The second, aminoadipic acid (AAA) route, is confined to the fungi. On this basis, he concluded that fungi are distinct from both animals and plants. While the DAP route is the most common among plants, it can generally be regarded as universal. The complete AAA route is confined to fungi, but elements of the AAA pathway of lysine degradation are found in animals as well (Lejohn, 1974). "The fungal theory explains the origin of the AAA lysine pathway as the result of the loss of DAP during the conversion of the peptidoglycan wall into a eukaryotic [animal-like] chitinous [exoskeleton]" (Cavalier-Smith, 198 1).

The difference between the major sterols in animals and fungi has also been cited as evidence against a phylogenetic relationship between fungi and animals. Studies show that cholesterol is the main sterol found in animals and ergosterol in fungi. However, closer examination of these two sterol biosynthetic pathways indicates that "the key intermediates in sterol biosynthesis diverge, with lanosterol being a key intermediate in fungi and vertebrates," while the precursor of phytosterols in green plants is cycloartenol. It was also found that a variety of chitin-walled fungi contain ergosterol, and cellulose-walled fungi contain cholesterol derivatives similar to those in

animals (Lejohn, 1974). A deeper understanding of this biosynthetic pathway proves that this evidence is actually in favor of a closer phylogenetic relationship between fungi and animals.

Another hypothesis that has been suggested, as previously mentioned, is that fungi share a common ancestor with plants, particularly algae, and that animals evolved separately from the fungi-plantae clade. Most of the early evidence in support of this hypothesis was superficial, based mainly on morphological similarities with algae (Lejohn, 1974). However, molecular evidence has been cited in support of this theory as well. For example, it has been noted that "most fungal mitochondrial genomes are circular...This is a characteristic of the chloroplast genome of higher plants" (Scazzocchio, 1987). Although this shape of the mitochondrial genome of fungi is similar to that of higher plants, it has already been noted that in translation of the mitochondrial RNA of fungi, UGA is read as tryptophan rather than as a stop codon. Mitochondrial RNA of animals is read in the same manner (Scazzacchio, 1987).

Although fungi are unique in many ways and have similarities to both plants and animals, the overwhelming evidence we have presented favors a closer phylogenetic relationship between fungi and animals. The evidence we have cited in support of our hypothesis regarding t-RNA and the mitochondrial genome (particularly the translation of UGA as tryptophan), as well as the similarities in biosynthetic pathways of animals and fungi are especially convincing. There certainly is a need for further research, particularly regarding the composition of the cell wall, which is an evolutionary novelty for fungi since it contains both chitin and cellulose. "The most promising techniques... are those which measure true homology, i.e., those employing comparisons of nucleic acids. Nuclear and mitochondrial DNA, either complete single-copy sequences or restriction endonuclease fragments, together with molecular weight or sequence studies of ribosomal RNA, are all potential materials for study" (Burnett, 1987).

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