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3. THE MAJOR LINES OF DESCENT

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Once the foundational events for cellular life were established, various paths towards diversification were set in motion. Populations that are physically isolated from each other for sufficiently long periods will naturally accumulate independent mutations, in some cases promoted by natural selection for optimal phenotypes in local environments, and in others simply by random genetic drift. When the genomes of isolated lineages diverge to a sufficient degree and are then mixed back together by sexual processes, the internal coadaptive gene complexes will be mutually incompatible, preventing the production of downstream hybrids. Such genetic isolation ensures the survival and evolution of independent lineages on their own merits, as reflected in the millions of species now inhabiting the planet.

Understanding the genealogical relationships (phylogeny) of existing lineages is critical to cell biology as it provides a historical overview of what evolution has been able to accomplish. When large numbers of related species share the same trait, we can be fairly certain that their common ancestor (at the basal node of the clade) also carried the trait. Because sister taxa evolve from common ancestors, their differences also allow a minimal statement about the kinds of change that are possible from a shared beginning. For example, gains and losses of traits can be inferred when single lineages deviate from their surrounding relatives. Information on many pairs of taxa can then begin to reveal commonalities such mutual constraints among traits and parallel paths of evolution.

Analyses like these require well-resolved phylogenies, built from observations on traits other than those under investigation to avoid being circular. The modern age of whole-genome sequencing has brought us to the limits of such information. Nevertheless, despite the many millions of informative nucleotide sites in hundreds to thousands of species, many of the earliest branching patterns in the Tree of Life remain unresolved.

By outlining what we know about the relationships among the major lineages of life, this chapter sets the table for more in-depth comparative analyses in subsequent chapters. First, we will examine the degree of phylogenetic affinity between the two major functional groups of cellular life, the prokaryotes and eukaryotes. Although the two groups are readily distinguished by the absence/presence of a nuclear envelope and other membrane-bound organelles, this morphological distinction turns out to be misleading with respect to genealogical relationships. Not only are there two distantly related groups of prokaryotes, the bacteria and the archaea, but the eukaryotic lineage emerged from an ancestral archaeal lineage.

Second, the lineage with the most pronounced morphological diversification, the

eukaryotes, will be briefly considered. The main point here is that a very large suite of intracellular embellishments became established prior to the divergence of the major eukaryotic lineages, leaving no intermediate-state lineages, but followed fairly quickly by the emergence of major subclades with their own unique features. The antecedents of most shared eukaryotic traits can be found in one or more prokaryotic lineages, suggesting that their emergence was not strictly dependent on eukaryogenesis, but the stem eukaryote was unique in assembling such a unique mixture of features into a single lineage. The environmental conditions that might have driven the subsequent big bang of eukaryotic diversity remain unclear, and these may have been secondary, with the radical shift in the genetic system of the eukaryotic cell being the primary enabler of morphological and species diversification.

THE PRIMARY DOMAINS OF LIFE

Just as pairs of individuals within a population are related to various degrees in a pedigree sense, the relatedness of all species can be described in the form of a phylogenetic tree. Sibling species reside on adjacent branches separated by a single node (branch point), with pairs of species with lower affinities residing on more distant branches. Historically, the field of taxonomy sought to classify organisms by their physical appearances, but owing to the possibility of convergent phenotypic evolution, such an approach is fraught with interpretative problems. Information at the nucleotide level has an explicit genetic interpretation and is less ambiguous than phenotypic measures on complex, multilocus traits. Thus, almost all attempts to infer phylogenetic relationships are now based on observations on DNA-sequence divergence among extant species (Felsenstein 2004).

Although this is a highly technical field involving computationally demanding algorithms to obtain genealogical relationships that are most compatible with the data, the conceptual basis for these analyses is straight-forward. Because DNA naturally acquires nucleotide substitutions and rearrangements by mutation, some of which are nearly neutral (Chapter 4), related species experience DNA-sequence divergence over evolutionary time. This simple fact that species with higher levels of sequence similarity are expected to be more closely related forms the logical basis for virtually all statistical methods for estimating phylogenetic trees and dating evolutionary events.

With advent of molecular-genetic methods, insights into the broad form of the Tree of Life began to emerge in the 1970s. Up to this point, based on the obvious morphological void between prokaryotes and eukaryotes, the former had been viewed as one large, monophyletic group, ill-defined internally, but assumed to be deeply separated from the eukaryotes. However, noting that the genomes of all organisms encode for ribosomal RNAs, Woese and Fox (1977) reasoned that a higher degree of resolution could be obtained by comparative analysis of such sequences. They quickly discovered a deep phylogenetic furrow within the prokaryotes, implying the existence of two major lineages, seemingly as distinct from each other as they are from eukaryotes. Following Woese et al. (1990), these two prokaryotic groups came to be known as the archaea (often also called archaebacteria) and the bacteria (sometimes called the eubacteria).

This division of life into three major groups raised several questions about the base of the Tree of Life. Are the bacteria, archaea, and eukaryotes fully monophyletic (a three-domains of life model), or are one or more clades embedded within another (one or two major domains)? Assuming they are monophyletic, are eukaryotes more closely related to archaea or bacteria, or do they have affinities with both? Can the possibility that eukaryotes are ancestral to prokaryotes be ruled out?

The key to answering these questions is a correctly rooted phylogenetic tree denoting the location of the most recent common ancestor from which all species in the tree ultimately descend. This hypothetical taxon is often referred to as LUCA (for Last Universal Common Ancestor) (Figure 3.1). Last common ancestors for bacteria, archaea, and eukaryotes are designated LBCA, LACA, and LECA. The first common ancestor for a lineage (e.g., FUCA, FBCA, FACA, and FECA) denotes the most remote point on the branch leading to the last common ancestor not containing any other major clade. Traits that are shared by all members of a clade were almost certainly present in the last common ancestor of the clade, but one cannot rule out an earlier origin on the branch leading to the last common ancestor. For example, a feature shared by all bacteria may have arisen anywhere along the FBCA-LBCA branch.

Several significant problems conspire to make the ascertainment of the relationships between the three major domains a difficult enterprise. First, although placing a root on a phylogeny is usually a simple matter of including in the analysis a confident outgroup (i.e., a bird for a mammalian phylogeny), this is not an option when the entire Tree of Life is being considered. Second, the amount of molecular divergence among all three groups is so vast that the signal of genealogical relationships has been greatly diluted by the accumulation of multiple nucleotide substitutions per site.

This being said, a consensus seems to have emerged on the deepest branches of the Tree of Life. Virtually all analyses indicate that the bacterial lineage is monophyletic and separate from the lineage containing the archaea and eukaryotes (e.g., Raymann et al. 2015). As the archaeal lineage also appears to be monophyletic (Williams et al. 2017), this leaves the positioning of eukaryotes as the main issue. In principle, eukaryotes could simply join as a separate monophyletic clade at a single node outside of bacteria and archaea. Alternatively, one of the groups might emerge as a sublineage within the other. The first pattern would arguably imply a Tree of Life consisting of three primary domains, as postulated by Woese and colleagues, whereas the second condition would imply a two-domain scenario in which eukaryotes are simply a derived lineage of one of the prokaryotic groups or vice versa.

Resolving this issue has been challenging, owing to biological complications beyond the statistical problems outlined above. Most notable are the occurrence of substantial horizontal gene transfer among lineages early in the history of life (Doolittle et al. 2003), and the additional massive transfer of bacterial genes to their eukaryotic host cells following the endosymbiotic establishment of the mitochondrion (derived from an α-proteobacterium on the FECA-LECA branch; Chapter 23). Nonetheless, most analyses now seem to support the eocyte hypothesis of Lake et al. (1984), which postulates eukaryotes as being most closely related to one particular archaeal group (Cox et al. 2008; Guy and Ettema 2011; Kelly et al. 2011;

Thiergart et al. 2012; Williams et al. 2012, 2013, 2020; Raymann et al. 2015). This hypothesis essentially eliminates the possibility that eukaryotes are the primordial cellular lineage, rejecting the three-domains of life view, and implicating a member of the archaea as the ultimate source of the eukaryotic nuclear genome.

The closest relatives to eukaryotes reside within the archaeal subgroup lokiarchaeota, known mainly from the sequencing of environmental samples from deep-sea sediments (Spang et al. 2015; Hug et al. 2016; Zaremba-Niedzwiedzka et al. 2017), with one isolate now growing in the lab being more closely related to eukaryotes than any other archaeal species (Imachi et al. 2020). The genomes for members of the lokiarchaeota imply the presence of actin (cytoskeletal) proteins as well as components associated with vesicle trafficking and membrane remodeling, all of which are classical attributes of eukaryotic cells, and none of which are known in any bacterium (Ettema et al. 2011; Yutin and Koonin 2012; Akil and Robinson 2018). Notably, eukaryotic proteins involved in information processing (e.g., transcription and translation) tend to be more similar to those in archaea than bacteria, as expected if the nuclear genome is derived from a member of the archaea. In contrast, proteins involved in house-keeping functions (e.g., metabolism) tend to most closely resemble those in bacteria (Brown and Doolittle 1997; Rivera et al. 1998; Leipe et al. 1999; Brown et al. 2001; Horiike et al. 2001), many of which may be derived from the colonizing bacterium that founded the mitochondrion (Chapter 23).

One concern with the two-domains hypothesis draws from observations on the types of phospholipids deployed in the cell membranes of the different major lineages (Figure 3.2). All cells are enveloped by phospholipid bilayers, with the individual molecules comprised of a glycerol-phosphate sandwiched between a head group and two hydrocarbon chains (Chapter 15). However, whereas glycerol-1-phosphate (G1P) is bound to methyl-branched isoprenoid chains by ether linkages in archaea, in both bacteria and eukaryotes, glycerol-3-phosphate (G3P) is bound to straight fatty-acid chains by ester linkages (Boucher et al. 2004; Peretó et al. 2004). This affiliation of membrane composition in bacteria and eukaryotes is clearly inconsistent with the topology of the Tree of Life suggested above, unless LACA and its early descendants had membranes containing a mixture of both types of lipids (Lombard et al. 2012).

The latter idea has some support. The dehydrogenase enzymes that make G1P and G3P (respectively, G1PDH and G3PDH) are found in all major lineages, raising the possibility of a nonspecific GPDH in LUCA. In addition, the phospholipids in some bacteria and eukaryotes have ether linkers; some archaea have fatty acids; and isoprenoids are universally distributed, although they are synthesized by different pathways in the three major groups (Lange et al. 2000; Lombard and Moreira 2011; Villanueva et al. 2018). Although it has been argued that a mixed population of lipid molecules will reduce membrane stability, there have been doubts about this idea (Shimada and Yamagishi 2011), and indeed, *E. coli* has been engineered to contain up to 30% archaeal lipids with little negative effects on growth rate (Caforio et al. 2018). Thus, it is plausible that LUCA or its early descendants had a membrane consisting of a mixture of the molecules found in modern-day prokaryotic lineages, with alternative mechanisms for catalyzing pure populations of G1P or G3P molecules evolving independently in isolated lineages (Koga et al. 1998; Martin and Russell 2003; Wächtershäuser 2003).

Finally, it should be noted that the two-domains view makes the implicit assumption that the root of the entire Tree of Life falls between the bacterial and archaeal domains. A more formal way of evaluating the problem uses genes that duplicated prior to the divergence of the main lineages, as each member of a such gene pair can serve to root the phylogeny of the other. In the ideal scenario, both trees resulting from such reciprocal rooting would yield the same topology. To exploit this strategy, Gogarten et al. (1989) used anciently duplicated subunits of ATP synthase to show that archaea and eukaryotes consistently group together to the exclusion of bacteria, and the same result has been obtained with several other pairs of ancient duplicate genes (Iwabe et al. 1989; Brown and Doolittle 1995; Baldauf et al. 1996; Lawson et al. 1996; Gribaldo and Cammarano 1998; Zhaxybayeva et al. 2005). Although there has been some dissent on the matter (Philippe and Forterre 1999), and there remain a number of reasons to be cautious (Lynch 2007, Chapter 1), the consensus view is that the Tree of Life is rooted as illustrated in Figure 3.1.

TIMES OF ORIGIN

The preceding discussion provides a description of the basic topology of the main trunks of the Tree of Life, but leaves unresolved the times of origin of various lineages, e.g., the temporal positions of the first and last common ancestors of the key clades. The gold standard for such estimates is a fossil record. Unfortunately, however, only a small fraction of species leave fossil traces, and even in the best cases, the vagaries of geological activity generally result in substantial gaps and uncertain time horizons in the fossil record. Whereas there is a well-established fossil record for many groups of land plants and animals, few unicellular organisms are fossilizable, and a wide range of abiotic events can leave traces that can be nearly indistinguishable from those induced by real cells (Javaux 2019). Today's smallest bacteria have diameters $< 1\,\mu\text{m}^3$, and the earliest cells were likely even smaller, further reducing the likelihood of detection. Rock formations older than 3.5 BY are extremely rare, further restricting the opportunities of directly inferring the earliest stages of evolution, which almost certainly unfolded before this time.

The development of chemical methods for detecting organic molecules in ancient rocks expands the potential window for inferring life's presence (Brasier et al. 2015), but as noted in Chapter 2, numerous geological mechanisms can yield organic molecules in the absence of any biology. Given that the presence of photosynthesis earlier than 3.4 BYA has been inferred (Javaux 2019), and the sophisticated nature of photosynthesis, this would further imply the establishment by this time of the full repertoire of metabolic/molecular processes from which all subsequent cellular lineages were built. Thus, it is not far-fetched to suggest that cells were present as early as 4.0 BYA, and some indirect evidence for biological activity as early as 4.1 BYA has been suggested (Bell et al. 2015).

The first evidence of eukaryotic cells appears in shale deposits containing putative diagnostic biomarkers of membrane components from ~ 2.7 BYA (Brocks et al. 1999), with the first presumptive algal fossils dating to ~ 2.1 BYA (Han and Runnegar 1992). Many other fossils of unicellular eukaryotes with well-developed cytoskeletons date to 1.5 to 1.7 BYA (Knoll 1992; Shixing and Huineng 1995; Javaux

et al. 2001), but even after the emergence of eukaryotes, complex multicellularity remained absent for at least another billion years. A dramatic shift occurred ~ 550 MYA, when all of the major groups of multicellular animals appear essentially simultaneously in the fossil record, in what is popularly known as the Cambrian Explosion. The most visible biota on today's Earth, the jawed vertebrates and land plants, emerged only ~ 440 and ~ 400 MYA, respectively.

Of course, the time of first appearance of a group in the fossil record must postdate the the actual time of origin. Attempts have been made to estimate key early divergence points in the Tree of Life using molecular clocks for protein-coding sequences calibrated with more recent fossils from well-understood taxonomic groups. Although numerous assumptions underlie these analyses, the current prognosis is an initial point of divergence of the eukaryotic branch from its archaeal ancestor of ~ 1.9 (FECA, the First Eukaryotic Common Ancestor), with the Last Eukaryotic Common Ancestor (LECA, at the base of the tree of diverging eukaryotic lineages) occurring ~ 1.0 to 1.7 BYA (Parfrey et al. 2011; Shih and Matzke 2013; Eme et al. 2014). These dates are roughly compatible with the fossil-record data noted above. If this is correct, the first two billion years or so of biological history was written entirely by prokaryotes, with $> 80\%$ of biological history involving a world containing only single-celled organisms. Significant surprises may still be in store, as genome sequences from environmental samples continue to reveal new microbial lineages (Hug et al. 2016; Zaremba-Niedzwiedzka et al. 2017).

THE EMERGENCE OF EUKARYOTES

Evolutionary cell biology is equally concerned with prokaryotes and eukaryotes, but given the disproportionate devotion of cell biology to the latter group (mostly just yeast, plant, and animal cells) as well as the massive expansion of morphological complexity, a brief excursion on the unity and diversity of the main lineages of eukaryotes is warranted.

The stem eukaryote. Provided a group of species is monophyletic, as is the case for eukaryotes, we can generally be confident that any feature that is shared across all members of the clade must have been present in its most recent common ancestor (in this case LECA). Based on the logic that highly complex cellular features are unlikely to have arisen independently in multiple lineages, comparative biology tells us that LECA was a flagellated heterotroph, capable of phagocytosis, with quite complex internal structure.

Eukaryotes distinguish themselves from prokaryotes in numerous ways at the level of cellular structure, intracellular processes, gene structure, and genome organization – Cavalier-Smith (2009) suggests ~ 60 eukaryote-wide innovations. Although all such features must have been present in LECA, the order in which they emerged is less clear, and will likely remain so unless basal lineages lacking subsets of such traits are discovered. This raises significant challenges for determining the key innovations that might have precipitated the evolutionary cascade of events known as eukaryogenesis. The following provides just a brief overview of the primary changes, with fuller details appearing in subsequent chapters.

The most celebrated eukaryotic attributes are physical ones, most notably a nuclear envelope that allows a spatial separation between gene transcription within the nucleus and cytoplasmic translation of transcripts after export through the nuclear pore. Unique cytoskeletal structures based on actin and tubulin provide physical support for a variety of cellular functions, including a platform for membrane bending essential for food engulfment by phagocytosis and osmotic regulation via contractile vacuoles, a scaffold for the ordered transport of chromosomes during cell division, and a highway for the travel of molecular motors for transporting vesicles and powering flagella. Molecular motors are also a eukaryotic invention, and the eukaryotic flagellum is completely different from that in bacteria. Internal membrane-bound structures such as the endoplasmic reticulum and the golgi provide sites for molecular processing unique to eukaryotes.

A key eukaryotic organelle is the mitochondrion, which became established at some point between FECA and LECA, and is one of the only eukaryotic features whose origin is known. Unlike other organelles, mitochondria contain genomes, and from sequence information, we know that these trace to a colonizing α-proteobacterium, which eventually transformed to an obligate endosymbiont that became the powerhouse of eukaryotic cells. Prior to the establishment of the mitochondrion, ATP synthase (Chapter 2), resided on the cell membrane (as it does in all of today's prokaryotes), but in eukaryotes ATP synthase is sequestered to internal mitochondrial membranes. Some have argued that this relocation provided a solution to the reduced surface:volume ratio in larger cells, essentially generating a bioenergetics revolution necessary for the establishment of all other things eukaryotic, including an expansion in gene number (Lane 2002, 2015; Lane and Martin 2010). Under this view, colonization of the mitochondrion would have been the causal event in eukaryogenesis, and therefore the first key innovation to appear on the branch from FECA to LECA.

Although the origin of the mitochondrion may have been a watershed event, the idea that it spawned a quantum leap in bioenergetic capacity will be questioned in subsequent chapters. Once established, however, the mitochondrion generated numerous secondary effects to accommodate its use. For example, massive transfer of mitochondrial genes to the nuclear genome occurred prior to LECA. Many of these transferred genes produce products that must be sent back to the mitochondrion, and some provide components to mitohondrial protein complexes that also contain mitochondrially encoded subunits. This necessitates reliable mechanisms for coordinating the activities of organelle and nuclear genomes and targeting the transport of proteins to their appropriate destinations.

The transition to eukaryotes was also accompanied by major alterations in the mode of genome replication and transmission (Lynch 2007). The genomes of almost all bacteria consist of single circular chromosomes that replicate in two continuous streams from a single origin of replication, with the daughter genomes moving to opposite ends of the parental cell by fairly simple mechanisms. In contrast, the nuclear genomes of eukaryotes consist of multiple linear chromosomes, spooled around protein complexes called histones, with multiple origins of replication and ends capped by repetitive arrays of short motifs called telomeres.

Eukaryotic cell division requires an organized set of events, known as mitosis, by which multiple chromosomes duplicate simultaneously, with complete offspring

sets then being dragged to opposite poles along a microtubule-based spindle apparatus. Moreover, eukaryotes have another specialized form of genome replication called meiosis. During this process, homologous pairs of chromosomes (one set from each parent) in a diploid cell line up in parallel, and reciprocally exchange material with each other by recombination, ultimately producing four haploid daughter cells (with single copies of each chromosome). The fusion of two such haploid cells reconstitutes the diploid form, completing the sexual life cycle, and providing a means for generating variation that is not possible in prokaryotes.

The mode of transcript processing also underwent considerable modification in the stem eukaryote (Lynch 2007). Most, if not all, prokaryotic genomes contain operons (cassettes of cotranscribed and often functionally related genes), but polycistronic transcripts constitute a significant challenge for the membrane-bound genomes of eukaryotes, as the multigene transcript would have to be either exported from the nucleus in its entirety or pre-processed into single-gene messages prior to export. The few known cases of eukaryotic operons do, in fact, involve such processing, along with the splicing of a small leader sequence to the front end of each individual transcript, a process called *trans*-splicing, which is unknown in prokaryotes.

Finally, the nuclear envelope provided a genomic environment that promoted the emergence of more complex gene structure, most notably the pre-LECA (and subsequent) colonization of genes by intragenic spacers called introns. Because introns are transcribed along with their surrounding exons, this genes-in-pieces architecture imposes another significant challenge for information processing – introns must be neatly excised and exons spliced back together (*cis*-splicing) prior to the export of mature mRNAs through the nuclear pore to the cytoplasm. Splicing is carried out by a complex molecular machine unique to eukaryotes, the spliceosome, consisting of five small RNA subunits and more than 100 proteins. In striking contrast, nearly all prokaryotic genes consist of a single uninterrupted coding region, and in the very few instances where this is not the case, the introns are self-splicing.

These are just a few of the many features unique to the eukaryotic lineage, the main point being that an enormous remodeling of cell biology occurred on the lineage from FECA to LECA, and yet the ordering of events remains unknown. Parallels of many "eukaryotic-specific" attributes can be found in isolated prokaryotic lineages, so one need not invoke *de novo* invention. For example, as already noted, many proteins previously thought to be restricted to eukaryotes are now known to have orthologous relatives in the lokiarchaeota. In addition, internal membranes and lipid-bounded organelles are known for several members of the bacteria and archaea (Clark et al. 2018). These types of observations, along with other indirect inferences (Pittis and Gabaldón 2016), indicate that many of the embellishments of eukaryotic cells did not have to await the origin of the mitochondrion as an energetic support system.

What remains unclear, however, is how so many odd features of prokaryotic cells came to be located in the same FECA-LECA lineage. Although one might argue that FECA was a highly polymorphic species, with different individuals harboring subsets of traits (O'Malley et al. 2019), it is difficult to conceive of individuals with different constellations of complex traits still being reproductively compatible. Thus, the early steps of eukaryogenesis remain a mystery of mysteries. We do not

know the events that triggered eukaryogenesis, nor do we know the extent to which the peculiar features that arose did so via the encouragement of natural selection. Some, such as intron colonization, may have emerged in population settings that enabled mildly deleterious mutations to accumulate passively by mutation pressure alone. Once established, however, the vast set of changes bestowed upon LECA provided the substrate for an extraordinary evolutionary explosion in cell architectural diversity that is the hallmark of eukaryotes.

The eukaryotic radiation. As in investigations of the prokaryote-eukaryote divide, progress on revealing phylogenetic relationships among the major eukaryotic groups has largely relied on comparative gene-sequence analysis. However, even with whole-genome analyses, a variety of issues (including idiosyncratic changes in rates of evolution, divergent nucleotide compositions across lineages, possibilities of early horizontal gene transfer, gene duplications, and inadequate taxon sampling) still conspire to cloud our understanding of the phylogeny of eukaryotes. Two things can be agreed upon – the primary eukaryotic lineages are deeply branching in time, and the major groups upon which most biological research is performed (metazoans, fungi, and plants) constitute only a small fraction of the eukaryotic phylogenetic diversity. Moreover, although these three favored sets of study organisms are sometimes viewed as members of a "crown group" of eukaryotes or "higher forms" of life, there is now compelling evidence that they do not even comprise a monophyletic lineage.

An attempt to summarize what is known about eukaryotic phylogeny is presented in Figure 3.3, with two caveats. First, this description is by no means complete, as it contains only the groups that will be encountered in the following chapters. Even if all of the major known groups of eukaryotes were included, the story would be an abstract at best, as agnostic searches for ribosomal RNA sequences from environmental samples suggest that many novel lineages of microbial eukaryotes, never before visualized, reside in our midst (Dawson and Pace 2002). Second, the phylogenetic relationships of many of the main eukaryotic groups remain unresolved. Depending on the authors, between five and eight monophyletic supergroups are recognized (e.g., Baldauf et al. 2000; Richards and Cavalier-Smith 2005; He et al. 2014; Derelle et al. 2015; Katz and Grant 2015; Ren et al. 2016; Burki et al. 2019; Wideman et al. 2020), and these will likely change to some degree as further data emerge.

The vast majority of eukaryotes are often viewed as falling into two major morphological groups based on the ancestral number of flagella being one or two (Cavalier-Smith 1998). The first of these, the unikonts, are united by the general presence of cells with a single flagellum at some stage of the life cycle (Cavalier-Smith 1998; Steenkamp et al. 2006; Paps et al. 2013). The unikonts contain the opisthokont group, an assemblage of metazoans, choanoflagellates, and fungi (top of Figure 3.3), as well as the amoebozoan group, comprised of the lobose amoeba and the slime molds (Bapteste et al. 2002). Along with a few biflagellate lineages, the unikonts appear to be separated from the remaining supergroups (all of which are biflagellate, and referred to as bikonts) by the root of the eukaryotic tree (Derelle et al. 2015), leading to the suggestion that LECA was biflagellate.

The large bikont assemblage contains the remaining supergroups, the inter-

relationships of which remain unresolved. One of these groups, encompassing the chloroplast-bearing green plants (including the green algae) and red algae (rhodophytes), is often referred to as the archaeplastida. The excavate supergroup contains the euglenozoa, which unites the euglenoids (e.g., *Euglena*) with the parasitic kinetoplastids (e.g., the trypanosomes *Trypanosoma* and *Leishmania*), as well as several other groups of flagellates.

A large supergroup is the SAR clade, based on its component lineages, the stramenopiles, alveolates, and rhizarians. The diverse stramenopile subclade contains the diatoms, brown algae, and oomycetes, whereas the alveolate subclade (united by the presence of alveoli, a system of sacs underlying the cell surface), contains the ciliates (e.g., *Paramecium* and *Tetrahymena*), the dinoflagellates (a group of aquatic flagellates), and the obligately parasitic apicomplexans (e.g., the malarial parasite *Plasmodium*) (Fast et al. 2002). The rhizaria consists of cercozoans, foraminiferans, and radiolarians, most of which are amoeboid and produce external skeletons (Nikolaev et al. 2004).

Monophyly of the entire bikont group is supported by a unique fusion between two key genes (dihydrofolate reductase and thymidylate synthase), which are encoded separately in all unikonts and prokaryotes (Stechmann and Cavalier-Smith 2002). However, two groups of protists, the amitochondriate diplomonad (including *Giardia*) and trichomonad lineages, appear not to contain either gene and so cannot be assigned phylogenetic positions on this basis. Most molecular phylogenies place these two lineages at the very base of the eukaryotic tree, but as with the other bikont groups, significant uncertainty remains over their exact position.

A eukaryotic big bang? Given that the deep lines of descent between bacteria, archaea, and eukaryotes have been resolved with far less data, the inability to fully decipher the more recent relationships among the main lines of eukaryotes is unlikely to be a matter of statistical limitations alone. The relatively short internal branches of the eukaryotic tree, which imply a rapid early radiation of such groups, is the major issue – shorter branches between related groups means lower discriminating power. This form of the eukaryotic tree has inspired a "big-bang" hypothesis suggesting that most of the major lineages became established in a period of 10 to 100 million years (Philippe et al. 2000; Cavalier-Smith 2002). If this idea is correct, the arguments presented above along with other molecular estimates of the age of LECA would suggest a radiation set down in a window roughly between 1.7 and 2.0 BYA (Wang et al. 1999; Yoon et al. 2004; Parfrey et al. 2011; Eme et al. 2014).

What might have precipitated such an active phase of lineage isolation? Most attempts at explaining evolutionary radiations resort to ecological arguments, either invoking a dramatic change in the environment or the chance appearance of an evolutionary novelty allowing the exploitation of new ecological niches. The evolution of eukaryotes was a singular event, generating an entirely new "body plan" that might have served as a potential launching pad for subsequent diversification, as phagocytosis opened up novel ways of living. However, a species radiation requires more than ecological opportunity. There must also be genetic isolating mechanisms to keep lineages distinct. Opportunities for speciation arise when populations are isolated for long enough periods to allow the accumulation of sufficient mutational changes that hybrid viability and/or fertility will be compromised by parental-genome incompat-

ibilities.

Post-reproductive isolating barriers can arise by many different mechanisms (Coyne and Orr 2004), but microchromosomal rearrangements in which genes relocate from one chromosome to another are of particular relevance to the early eukaryotic radiation, which experienced two novel forms of genomic upheaval. Consider first the primordial mitochondrion. Because most prokaryotic genomes contain a few thousand genes, while mitochondrial genomes contain no more than a few dozen, it is clear that hundreds of organelle-to-nuclear gene transfers occurred early in the establishment of mitochondria, although many were probably simply lost. Because mitochondrial genomes are haploid and generally inherited uniparentally, a relocation of an essential mitochondrial gene to the nuclear genome would create an imbalance in hybrid progeny resulting from a cross with any lineage having the ancestral (non-rearranged) type (Figure 3.4). Regardless of the direction of the cross, both types of hybrid would be presence/absence heterozygotes for the nuclear gene, but one would also harbor a mitochondrial genome devoid of the gene, and half of the gametes produced by such an individual would be lacking the gene entirely.

Although a single genomic transfer of this sort does not produce complete reproductive isolation, just a few independent transfers would have a powerful effect. Imagine an incipient pair of species experiencing n independent organelle-to-nuclear gene transfers in each lineage. Assuming independent assortment of the nuclear genes during meiosis, then the fraction of F_1 gametes entirely lacking in a functional gene at one or more loci is $1 - 0.5^n$, which is 0.969 for $n = 5$. Thus, when one considers the hundreds of organelle-to-nuclear gene transfers that may have occurred soon after the colonization of the primordial mitochondrion, and probably over several million years, it is plausible that such gene traffic played a significant role in the passive development of isolating barriers among the earliest eukaryotes. Note that such microchromosomal rearrangements only yield reproductive isolating barriers in species with multiple chromosomes and sexual reproduction, as both are necessary for the independent segregation of unlinked loci. As noted above, both features were among the novelties that emerged on the branch from FECA to LECA.

A second mechanism of gene relocation relevant to the eukaryotic radiation involves nuclear gene-duplication events, which can passively lead to rearrangements when the original copy is silenced and a descendant copy is preserved on a separate chromosome (Chapter 6). Such events are of interest here because, as discussed in subsequent chapters, there was a massive amount of gene duplication at the base of eukaryotes, possibly a result of one or two complete genome duplications. Such activities left their imprint on a wide variety of cellular features, including mitosis and meiosis (Ramesh et al. 2005; Malik et al. 2007; Liu et al. 2015; Onesti and MacNeill 2013), the cytoskeleton (Goodson and Hawse 2002; Dutcher 2003; McKean et al. 2001) and the flagellum (van Dam et al. 2013), proteasomes (Bouzat et al. 2000) and chaperones (Fares and Wolfe 2003), the nuclear-pore complex (Alber et al. 2007), and other organelles (Hirst et al. 2011; Schledzewski et al. 1999; Mast et al. 2014).

Thus, the indirect consequences of two of the defining cytological attributes of the stem eukaryote, a genome-bearing mitochondrion and meiotic recombination, along with rampant duplication in the nuclear genome, may have played a central role in the passive and relatively rapid emergence of the basal eukaryotic lineages.

Although ecological divergence need not have played any role in such processes, the resultant reproductive isolation allowed such lineages to descend down independent evolutionary pathways driven by adaptation to local environmental settings as well as by simple fixation of random of effectively neutral mutations.

Summary

- Although life has classically been divided into eukaryotes and prokaryotes, molecular analyses indicate that these are not meaningful phylogenetic labels. Instead, there appear to be two domains of life, with two prokaryotic groups, bacteria and archaea, appearing on opposite sides of the root of the Tree of Life, and eukaryotes being the most recent newcomer, derived from a member of the archaea.

- Prokaryotic life was established on Earth $\sim$ 4 billion years ago (BYA), with eukaryotes appearing $\sim$ 3 BYA. Although many eukaryotic lineages may have coexisted during this early period, only one eventually gave rise to todays eukaryotes, forming the base of the tree of extant lineages $\sim$ 2 BYA. The bases of the bacterial and archaeal trees must be older than this. Each of these time points has a level of uncertainty of a few hundred million years.

- From the standpoint of morphological diversification, the emergence of eukaryotes marked a dramatic event in Earth's history. With some 60 eukaryote-specific changes having become established prior to LECA, this keystone species was extraordinarily unique in terms of cellular and genomic architecture. The order in which these features arose remains unknown, and many of them are difficult to explain with adaptive arguments.

- Once established, LECA gave rise to an explosive radiation of the major eukaryotic groups on a relatively short time scale. This rapid episode of lineage isolation may have had little to do with any change in the environment, instead being an inevitable consequence of two pre-LECA genomic upheavals – the origin of the mitochondrion and a period of rampant nuclear gene duplication. Combined with the evolution sex and independently segregating chromosomes, these changes would have led to the passive accumulation of microchromosomal rearrangements and reproductive isolation in ways that would have been inoperable in prior asexual lineages of prokaryotes.

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Figure 3.1. An idealized view of the two-domains view of the Tree of Life. The Last Universal Common Ancestor (LUCA) diverged into the bacterial and archaeal lineages, with eukaryotes then emerging out of the archaeal clade. Horizontal lines with blunt ends denote extinct lineages, and the relative temporal positions of lineage origins are not meant to be taken literally. The vertical blue line denotes the origin of the mitochondrion via endosymbiosis of a colonizing bacterium. Abbreviations used in acronyms: F = first; L = last (or most recent); U = universal; B = bacterial; A = archaeal; E = eukaryote.

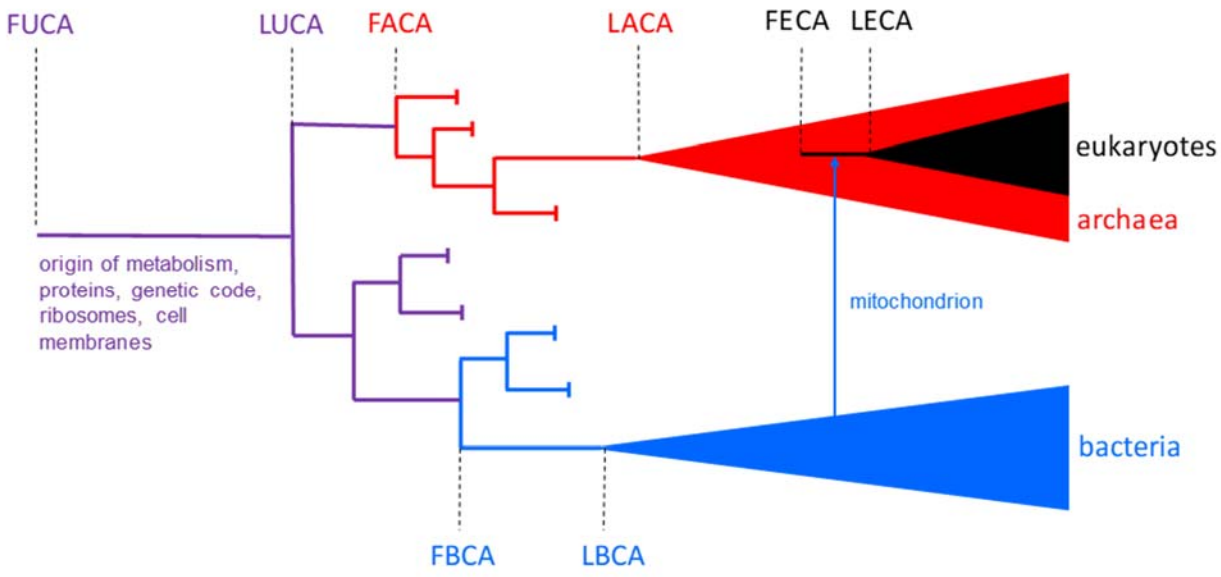

Figure 3.2. Alternative forms of phospholipids deployed by the three major lineages of life. The jagged lines represent chains of carbon atoms.

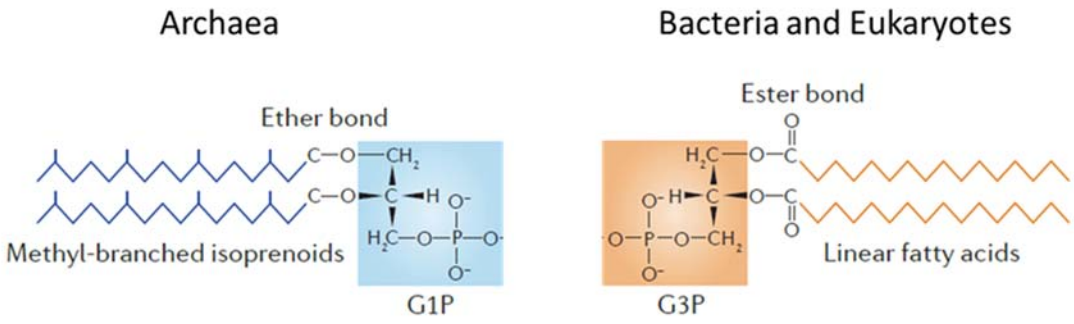

Figure 3.3. An approximate phylogenetic tree for some of the major eukaryotic "supergroups," generalized from the references in the text. The branch lengths are not proportional to time, although all external branches are expected to be in excess of 700 million years in length. Grey lines denote areas of uncertainty.

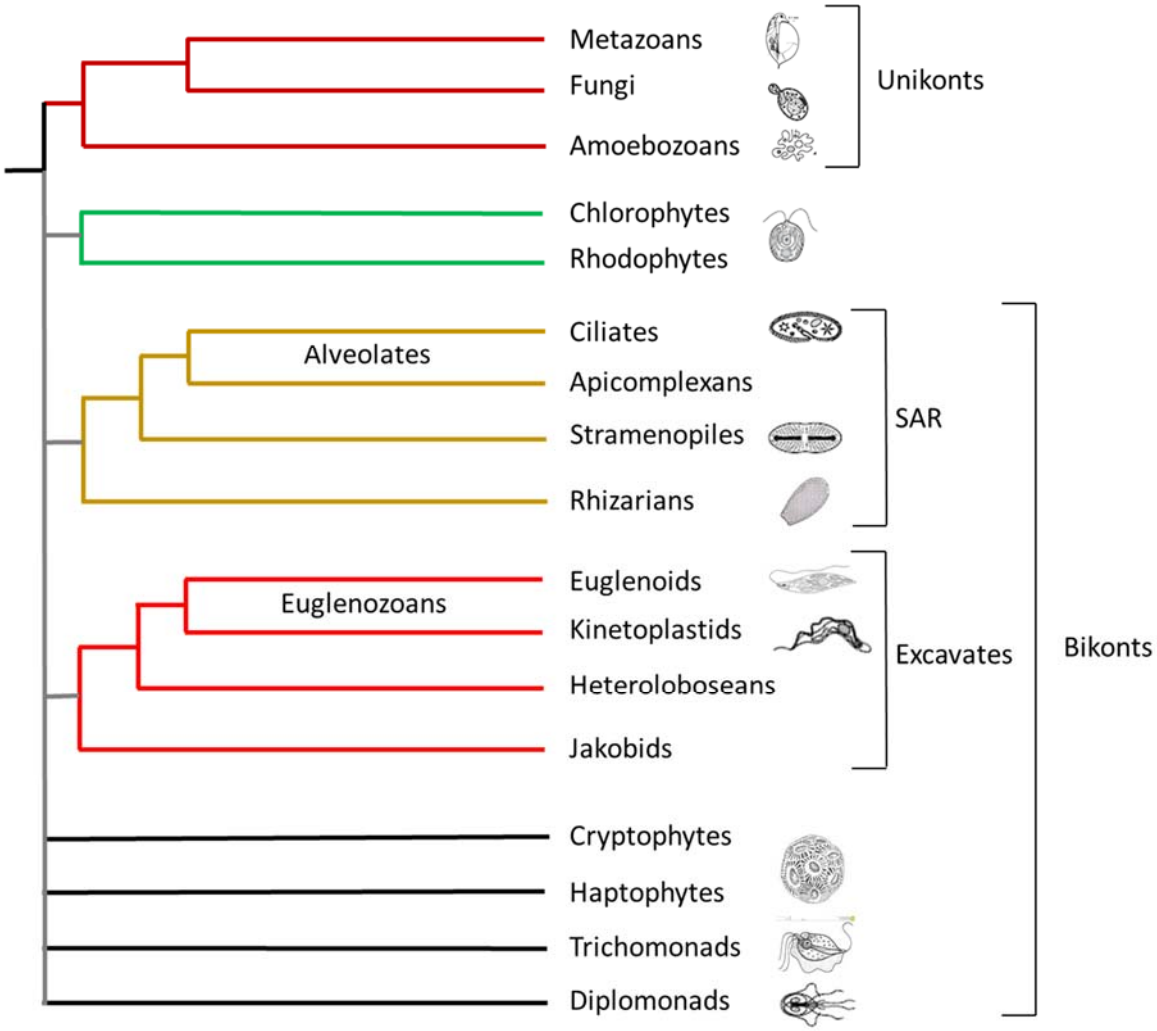

Figure 3.4. The development of a reproductive incompatibility following the relocation of a mitochondrial gene. A diploid nuclear genome is assumed. Rectangles and circles denote autosomal and organelle gene copies respectively, with open symbols indicating gene absence. Following a geographic isolating event, the incipient species on the left experiences an organelle-gene transfer to the nucleus. Subsequent hybridization yields presence/absence heterozygotes at the autosomal locus, with the status of the maternally inherited mitochondrial genome depending on maternal identity. As a consequence of Mendelian segregation of the diploid autosomal locus, half of the gametes of the individual in the lower left will lack the gene entirely, and half of those on the right will have a double dose of the gene.