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15. LIPIDS AND MEMBRANES

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Although much of cell biology focuses on proteins and the machines constructed from them, thousands of molecular forms of lipids are utilized across the Tree of Life, with dozens to hundreds frequently being deployed within individual species (Fahy et al. 2005; Oger and Cario 2013; Brügger 2014; Sohlenkamp and Geiger 2016; Buehler 2016). Although lipids are used for multiple cell functions, including energy storage and occasionally as cofactors for protein function, we focus here specifically on their deployment in membranes. Lipid membranes play a central role in all cells, providing both a barrier and a gateway to the external environment. By colocalizing genomes with the products they produce, cell membranes ensure individuality, a critical requirement for heritable evolutionary processes. In eukaryotes, they also circumscribe a wide variety of intracellular organelles, including the endoplasmic reticulum, the golgi, the nuclear envelope, mitochondria and plastids, and transport vesicles.

Consisting of millions to billions of noncovalently linked molecules, lipid membranes are typically highly fluid, effectively constituting a two-dimensional liquid with intrinsic biophysical features endowing both flexibility and resistance to breakage and leakage. As discussed below, specific structural features of lipid molecules play a central role in molding different cellular functions, leaving the impression that the universal use of lipids in biology is unlikely to be simply a frozen accident. Indeed, it is difficult to see how the establishment and diversification of cellular life would be possible without them.

Membranes also provide platforms for the residence of key proteins with diverse functions. Most notable are the trans-membrane proteins associated with channels, importers, and exporters used for ion and nutrient acquisition and balance (Chapter 18), electron transfer chains and ATP synthases used for energy production (Chapter 23), and components of signal transduction used for environmental sensing and communication (Chapter 22). Taken together, the proteins involved in these diverse functions typically comprise 10 to 30% of the total set of proteins encoded in the genomes of species.

The energetic costs of lipids are particularly germane to understanding the evolution of eukaryotic cells, which are typically laden with membrane-delineated organelles, and this will be taken up in detail in Chapter 17. As will be reviewed in the latter part of this chapter, establishment of the intricate system of vesicle transport in eukaryotes was also associated with a significant investment in a diverse repertoire of proteins required in vesicle formation, transport, and localization.

Molecular Structure

Rather than presenting an encyclopedic coverage of the various classes of membrane lipids, which can be found in Marsh (2013), the intention here is simply to provide an overview of the key relevant issues from an evolutionary perspective. The majority of membrane lipids in bacteria and eukaryotes reside in two families, the glycerophospholipids and the sphingolipids. In both cases, a polar (hydrophilic) head group is attached to a negatively charged phosphate, which in turn connects to a linker, glycerol in the case of glycerophospholipids and sphingosine in the case of sphingolipids (Figure 15.1). Glycerophospholipids have two fatty-acid chains attached to the glycerol linker, whereas in sphingolipids, sphingosine provides one built-in chain which joins with another fatty acid.

Such modular structure allows for enormous diversity of lipid types through the exchange of variable parts, including the nature of the head group. The most common head groups in glycerophospholipids are choline, ethanolamine, serine, glycerol, inositol, and phosphatidyl glycerol, and the resultant lipids are known as phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, phosphatidylglycerols, phosphatidylinositols, and cardiolipins. Additional structural diversity is associated with the number of carbon atoms and the number and locations of double C=C bonds in the fatty-acid chains. (Double bonds are referred to as unsaturated, as the carbon atoms are bound to only single hydrogens). The lengths of fatty-acid chains are typically in the range of 14 to 22 carbons, whereas the number of C=C bonds is usually between 0 and 5, and these features have a strong influence on membrane width and flexibility. In various phylogenetic groups, there are still other added layers of combinatorial complexity, with the head groups of some lipids being modified by additions of various small molecules, and some fatty acids containing methyl side branches and/or ring structures at the ends (Geiger et al. 2010; Buehler 2016). Although the precise functions of most such variants are unknown, they may play roles in thermal stability, permeability, and/or protection from various damaging agents.

In contrast to the water compatible head-groups of membrane lipids, the fatty-acid tails are highly hydrophobic, earning lipids the distinction of being amphipathic (or synonomously, amphiphilic) molecules. As a consequence of this structure, the roughly cylinder-shaped lipid molecules naturally self-associate into organized aggregates, with their hydrophobic tails lying parallel to each other in single sheets (Figure 15.2). Moreover, the most thermodynamically stable state is one in which two sheets (leaflets) align with their tails contraposed, minimizing the contact of hydrophobic tails with water, and leaving flexible walls of noncovalently bound head groups on the water-exposed sides. The internal hydrophobic environment of lipid bilayers makes them extremely impermeable to charged ions, which must then be imported / exported through gated channels comprised of proteins (Chapter 18).

Most classes of phospholipids are shared among bacteria and eukaryotes, although their relative usages can vary dramatically (Table 15.1), even between leaflets. The maintenance of such lipid diversity over billions of years of evolution may relate to the variation in structural flexibility endowed by alternative head groups and fatty-acid chains. Indeed, microbial species are generally phenotypically plastic with respect to the lipid profiles of their membranes, shifting them in response to

environmental change, e.g., using glyceroglycolipids instead of phospholipids when phosphorus is limiting. With increasing temperature, many cells physiologically remodel their membranes to contain lipids with longer and more saturated fatty acids or to incorporate different head groups. By this means, membrane fluidity and permeability is kept relatively constant, a process known as homeoviscous adaptation (Sinensky 1974; van de Vossenberg et al. 1995). Without a shift in lipid composition, increased temperature would magnify membrane permeability and fluidity, eventually leading to the loss of cell homeostasis. Homeoviscous adaptation has been observed in all domains of life (Haest et al. 1969; Arthur and Watson 1976; Hazel 1995; Toyoda et al. 2009; Nozawa 2011; Oger and Cario 2013; Ernst et al. 2016), and can be especially refined in organisms such as mammalian pathogens that regularly experience large shifts in temperature (external environment vs. host) (Li et al. 2012). The mechanisms for membrane monitoring, essential for an adaptive physiological response, involve proteins that regularly probe membranes for their fluidity (Harayama and Riezman 2018).

Finally, as noted in Chapter 3, the structures of lipid molecules in archaea differ significantly from those of eukaryotes and bacteria (Koga and Morii 2007; Chong 2010; Oger and Cario 2013; Buehler 2016). Most notably, archaea generally utilize isoprenoid hydrocarbon chains (which contain methyl side groups off the tails, rather than simple hydrogen atoms). Despite these differences, however, most of the head groups utilized in phospholipids in eukaryotes and bacteria are also deployed in their archaeal counterparts. A particularly unique aspect of archaeal membranes is the partial use of bipolar lipids , which span the entire width of the membrane. Some archaeal tails also contain cyclopentane carbon rings with head groups at both ends.

Table 15.1. Fractional contributions of lipid molecules to plasma membranes in select species. The surveys exclude contributions from sterols and proteins, and are generally given for optimal growth conditions. The central point is that distantly related species often utilize the same types of lipids, although at different frequencies.

Organism	PC	PE	PG	PI	PS	C	LPG	O
Bacteria:								
Bacillus subtilis	0.00	0.24	0.35	0.00	0.00	0.18	0.23	0.00
Caulobacter crescentus	0.00	0.00	0.88	0.00	0.00	0.12	0.00	0.00
Escherichia coli	0.00	0.75	0.19	0.00	0.00	0.06	0.00	0.00
Staphylococcus aureus	0.00	0.00	0.53	0.00	0.00	0.07	0.40	0.00
Zymomonas mobilis	0.13	0.62	0.20	0.00	0.00	0.01	0.00	0.03
Eukaryotes:								
Mus musculus, thymocytes	0.57	0.21	0.00	0.07	0.10	0.00	0.00	0.06
Vigna radiata, seedlings	0.47	0.35	0.05	0.05	0.08	0.00	0.00	0.00
Dictyostelium discoideum	0.29	0.55	0.01	0.08	0.02	0.02	0.00	0.03
Dunaliella salina	0.15	0.41	0.15	0.06	0.00	0.00	0.00	0.22
Saccharomyces cerevisiae	0.17	0.18	0.00	0.23	0.21	0.03	0.00	0.19
Schizosaccharomyces pombe	0.42	0.23	0.00	0.25	0.03	0.06	0.00	0.02

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinsoitol; PS, phosphatidylserine; C, cardiolipin; LPG, lysophosphatidylglycerol; O, other.

References: *Mm:* Van Blitterswijk et al. (1982); *Vr:* Yoshida and Uemura (1986); *Dd:*

Weeks and Herring (1980); *Ds:* Peeler et al. (1989); *Sc:* Zinser et al. (1991); Tuller et al. (1999); Blagović et al. (2005); *Sp:* Koukou et al. (1990); *Bs:* den Kamp (1969); Lopez et al. (1998); *Cc:* Contreras et al. (1978); *Ec:* Raetz et al. (1979); Rietveld et al. (1993); *Sa:* Haest et al. (1972); Mishra and Bayer (2013); *Zm:* Carey and Ingram (1983).

Membrane Structure

Although membranes have an enormous amount of flexibility and fluidity, they also have a high capacity for maintaining stable sheet-like structures. Owing to the difficulties of moving a polar headgroup through the hydrophobic interior of a bilayer, between-layer flip-flops of molecules are negligible unless promoted by specialized transport proteins. However, as the individual molecules are held together by non-covalent forces, lateral diffusive movement of molecules within a leaflet is essentially unavoidable.

This being said, lipid types are not homogeneously distributed within membranes. Rather, molecules tend to aggregate with their own types as they encounter each other by diffusion, leading to a sort of self-organizing phase separation, and generating patchy variation in membrane properties. Often referred to as lipid rafts, these island-like domains are themselves capable of diffusive lateral movement across the membrane. Such variation is relevant to the distribution of membrane proteins, as a stable platform for a membrane-spanning protein requires a good match between the membrane thickness and a protein's hydrophobic trans-membrane domains. Hence, particular types of proteins are associated with particular lipid rafts, further adding to membrane heterogeneity (Mitra et al. 2004).

The individual lipid molecules within a membrane rotate around their long axes, with the lateral diffusion coefficients of individual glycerophospholipid molecules in a bilayer being $D \simeq 2$ to 4 μm^2/sec at 25° C (Devaux and McConnell 1972; Wu et al. 1977; Jin et al. 1999). Thus, letting $D = 3$, and assuming unbiased directional movement such that the mean squared distance traveled over a two-dimensional surface is $4Dt$, where t is measured in seconds (Chapter 7), the mean absolute distance traveled is $\sim 2\sqrt{Dt}$, or ~ 3.5 μm in 1 sec. This implies that individual molecules can diffuse the equivalent of full cell lengths in a matter of seconds. Rates of diffusion of lipid rafts are one to two orders of magnitude lower, declining with the size of the raft (Schütte et al. 2017; Zeno et al. 2018).

To put this into perspective, recall from Chapter 7 that diffusion rates of proteins are on the order of 10 to 40 μm^2/sec within a cytoplasmic environment. Thus, diffusion inhibition from molecular crowding within membranes is substantially greater than in the cytoplasm. The lateral diffusion coefficients of membrane proteins is even lower than that for lipids, e.g., ~ 0.04 to 0.3 μm^2/sec for mitochondrial proteins (Gupte et al. 1984), and such coefficients decline from ~ 0.2 to 0.02 μm^2/sec in *E. coli* as the number of membrane-spanning helices in proteins increases from 3 to 14 (Kumar et al. 2010). Thus, although membrane proteins are mobile in an absolute sense, they are effectively stationary from the perspective of cytoplasmic proteins.

Lipid molecules are not strictly cylindrical in shape. Rather, depending on the size of the head group relative to the tail width, the overall shape can be closer to a cone, with unsaturated fatty-acid tails tending to fan out. As a consequence,

curvature is induced when molecules of particular geometric shapes associate with each other (Figure 15.2). This serves as a simple structural mechanism to reduce the bending energy necessary to mold membranes into particular shapes, and in part explains the differential distribution of lipid types on the inner vs. outer leaflets of membranes.

Generation of stronger curvature typically requires additional sources of bending energy derived from ATP- or GTP-hydrolyzing processes (Helfrich 1973). For example, motor proteins moving along microtubules or actin filaments (Chapter 15) can pull membranes into tubular forms. A wide variety of membrane proteins have functions specifically associated with the bending and sculpting of membranes into specific forms (Shibata et al. 2009; Jarsch et al. 2016). For example, insertions of hydrophobic wedges naturally cause a membrane to bend towards the narrower end of the inserted protein, as occurs when ATP synthase molecules inhabit the tips of cristae on the internal membranes of mitochondria. Assemblies of scaffolding proteins with natural curvature and an affinity for lipid head groups can force lipid bilayers to conform to the same curvature, and are widely used in the formation of vesicles (as described in more detail below). Transmembrane proteins, which transverse the space between two membranes, help maintain specific distances between layered sheets.

Eukaryotes and the Endogenous Organellar Explosion

The proliferation of internal membrane-bound organelles is one of the hallmark features distinguishing eukaryotes from prokaryotes. Prominent in almost all eukaryotic cells are the endoplasmic reticulum (ER, the site of production of many proteins and lipids), the golgi (the site of secondary processing and transport), and lysozomes and peroxisomes (devoted to degradation), all of which must date to LECA. All such organelles are believed to be endogenous in origin, having developed by descent with modification, and hence containing no internal genomes. Special attention will be given to mitochondria and chloroplasts in Chapter 24, as these arose exogenously with endosymbiotic ancestries dating back to known bacterial lineages. Many organelles have membrane-contact sites (e.g., covering between 2 and 5% of the surface area of the ER and contacted mitochondria; Phillips and Voeltz 2016), with apparent functions in inter-organellar communication, further contributing to the complex interior of eukaryotic cells.

At both the cell-biological and evolutionary levels, there are numerous unsolved problems as to how individual organelle types achieve their distinctive shapes, identities, and interactions. Organelle assembly may in part be a consequence of the physical features of the component molecules described above, combined with the tendency for self-aggregation, but other evolved mechanisms may be involved. For example, major portions of the core endoplasmic reticulum have a layered, spiraling architecture, resembling a parking structure (Terasaki et al. 2013). The ER is also continuous with the nuclear envelope (Foundations 15.1), and the peripheral ER often exhibits a matrix-like structure involving narrow tubules (Nixon-Abell et al. 2016). It appears that these and many alternative morphologies can be generated by the relative concentrations of just two types of membrane-shaping proteins, one

encouraging flat sheets and the other curvature (Shemesh et al. 2014). Phylogenetic diversification extends to the golgi, the central clearing house for vesicle trafficking and post-translational modification. Many eukaryotes lack classical stacked golgi, and yet contain the genes associated with golgi trafficking, suggesting independent loss of this morphology at least eight times (Dacks et al. 2003; Mironov et al. 2007; Mowbrey and Dacks 2009).

Often, it is assumed that the invention of organelles led to fundamentally superior organisms, with increased cellular complexity being viewed as a positive endowment (Lane and Martin 2010; Gould 2018). However, although organelles allow eukaryotes to accomplish cellular tasks in novel ways relative to prokaryotes, there is no evidence that such mechanisms are more efficient in any absolute sense (Chapter 8), and as will be discussed in Chapter 17, the investment in internal membranes comprises a substantial energetic burden on cells. Thus, while one can marvel at the many intricacies of eukaryotic cell biology accomplished with organelles, there is no evidence that eukaryotic cell structure is intrinsically superior to that of prokaryotes.

Moreover, prokaryotes are not constrained from evolving internal cellular structures. For example, the planctomycetes, a group of aquatic bacteria, are endowed with substantial tubular networks of internal membranes (Fuerst and Sagulenko 2011; Acehan et al. 2014; Boedecker et al. 2017), reminiscent of but not clearly of the same origin as the endomembrane system of eukaryotes. Although the functions of such membranes are not fully resolved, one structure (the anammoxosome) sequesters a reaction that converts nitrite and ammonium ions to nitrogen gas (van Niftrik and Jetten 2012). The planctomycetes are also capable of endocytosis and reproduce by budding, both of which may involve the use of eukaryote-like mechanisms. Other related groups of bacteria (e.g., verrucomicrobia and chlamydiae) have endomembranes, and given the recent discoveries noted in Chapter 3, it would not be surprising if lineages of archaea are eventually found to have them.

There are many other examples of compartmentalized organelles in bacteria. The photosynthetic machinery in cyanobacteria is sequestered within a carboxysome (Savage et al. 2010). A microcompartment for ethanolamine metabolism consisting of hexameric protein subunits is present in *E. coli* (Tanaka et al. 2010), and *Salmonella* harbors another such structure for propanediol utilization (Chowdhury et al. 2015). In these particular cases, the intracellular compartment consists of an assembly of protein multimers, much like the capsids of viruses. However, magnetotactic bacteria contain crystals of magnetite or other iron-phosphate granules enclosed by phospholipid membranes (Byrne et al. 2010; Jogler et al. 2011), and the giant cells of *Epulopiscium,* a symbiotic bacterium (up to 1 mm in length) inhabiting triggerfish guts, contain stacked "vesicles" of unknown function near the cell membrane (Robinow and Angert 1998).

In short, given the energetic costs of internal membranes (Chapter 17), it is by no means clear that the proliferation of internal membranes endows eukaryotic cells with any baseline superiority. There is no evidence that prokaryotes are prohibited from evolving such structures were the selective pressures to do so present, as further discussed in the following section. In addition, once in place, an endocytic pathway provides a direct route for exploitative cellular entry and exit by pathogens (e.g., Heuer et al. 2009; Szumowski et al. 2014; Shen et al. 2015; Renard et al. 2015; Shi et al. 2016). Not ruling out an early role for adaptation, little more can be

said than that unknown historical contingencies led to the adoption and apparent permanent retention of the eukaryotic cell plan at some point on the path from FECA to LECA. The evolutionary conditions leading to the widespread proliferation of internal membranes in the ancestral eukaryote remain one of the greatest mysteries of evolutionary cell biology.

Vesicle Trafficking

Although most cellular functions in prokaryotes are governed by diffusion-like processes, eukaryotic cells rely extensively on active transport of macromolecules. Such processes include the internalization of extracellularly derived cargoes, the movement of molecules from one organelle to another, and the transport of proteins and RNA molecules across the nuclear envelope. Essentially every eukaryotic transport pathway involves one or more modes of intermolecular communication – correct substrates must be identified to the exclusion of erroneous and sometimes harmful cargoes, and must be delivered to their appropriate destinations. Thus, intracellular transport raises many of the same issues encountered in the consideration of metabolic (Chapter 19), transcription regulatory (Chapter 21), and signal-transduction networks (Chapter 22), most notably the specificity of the evolved languages underlying intermolecular interactions.

Eukaryotes deploy lipid-bound vesicles in a wide range of trafficking activities, including endocytosis and exocytosis, digestion, and transport between the endoplasmic reticulum and the golgi. The life cycle of a vesicle involves assembly at sites of initiation usually by pinching off a parental membrane, delivery through the intracellular domain, and docking and fusion to another lipid-bound compartment at the site of delivery (Figure 15.3). As these processes all occur simultaneously and bidirectionally, the quantitative partitioning of lipid membranes throughout the cell can remain in a roughly steady-state condition, such that membrane areas lost by donors are balanced by those gained by recipients, despite substantial traffic between compartments. The rate of membrane flux can be quite high. For example, amoeboid cells can internalize the equivalent of the entire surface membrane in the form of endocytic vesicles in just an hour (Ryter and de Chastellier 1977; Bowers et al. 1981; Steinman et al. 1983).

The origins of the vesicle-transport system remain obscure, with few obvious orthologs of any components known in prokaryotes. However, the planctomycete situation lends credence to the idea that some aspects of an endomembrane system may have been present in the primordial eukaryote, i.e., the first eukaryotic common ancestor (FECA) (Lonhienne et al. 2010). Such a hypothesis is consistent with phylogenetic analyses suggesting an origin of various aspects of endocytosis as well as the secretory system prior to LECA (Jékely 2003; Podar et al. 2008; Dacks et al. 2008; Makarova et al. 2010; Wideman et al. 2014; Klinger et al. 2016; Zaremba-Niedzwiedzka et al. 2017).

Pointing out that many bacteria release outer-membrane vesicles into the extracellular environment, Gould et al. (2016) suggested that the eukaryotic endomembrane system, along with the replacement of archaeal membrane components (and many other features) by those from bacteria (Chapter 2), originated via the mi-

tochondrial endosymbiont. One concern with this argument is the absence of any mechanistic evolutionary argument for how the simple production of vesicles by the primordial mitochondrion could have become transformed into a highly organized and nuclear-encoded vesicle transport system by the host cell. Given that any such modifications must have involved incremental evolutionary processes, and future understanding will require more than saltational arguments.

The following provides a brief overview of what little is known about the various steps from cargo uptake to delivery, the focus being on general principles. The enormously detailed molecular mechanisms can be explored further in many specialized publications.

Vesicle production. Rather than forming *de novo,* vesicles are typically derived via the invagination (endocytosis) or budding (inter-organelle transport) of a preexisting membrane, with the lipid molecules being constantly recycled via fission and fusion processes. Vesicle birth generally involves the recruitment of specific proteins dedicated to inducing membrane curvature. Such proteins generally assemble into cage-like lattices that support developing vesicles before they are eventually pinched off from parental membranes.

Three types of vesicle coating are known (Field et al. 2011). Clathrin-coated vesicles import cargoes across the cell membrane in the form of endosomes, and are also deployed in the trans-golgi network. COPI (coat protein I)-coated vesicles carry cargoes between different golgi compartments and from the golgi to the endoplasmic reticulum, and COPII-coated vesicles export cargoes from the endoplasmic reticulum. In all cases, large protein lattices are produced via the assembly of lower-order trimers (clathrin and COPI) or dimers (COPII), which then coassemble into higher-order structures with distinct geometric shapes and sizes (Figure 15.5).

Given the widespread presence of clathrin throughout the eukaryotic domain, the logical conclusion is that LECA deployed clathrin-coated vesicles (Field et al. 2007). Nevertheless, it is clear that substantial diversification of clathrin-coated vesicles has occurred, as their diameters range from ~ 30 to 200 nm among observed species (McMahon and Boucrot 2011; Kaksonen and Roux 2018). It remains to be seen whether a simple physical basis to such size differences resides in the architecture of the clathrin molecule itself, e.g., the numbers of α helices constituting the long connecting arms, whether turgor-pressure differences among cell types is involved (which would influence the membrane bendability), or both. Size variation is also known for COPI- and COPII-coated vesicles (Faini et al. 2012).

Although many of the details remain to be worked out, clathrin vesicle formation initiates when specialized adaptor proteins bind to the source membrane and then recruit the coat proteins (McMahon and Boucrot 2011; Boettner et al. 2011; Kirchausen et al. 2014). Adaptor proteins (often called adaptins) are thought to recognize specific cargo-recruitment molecules, which in turn have affinities to specific cargo types. In this sense, adaptins serve as an informational link between cargoes and coat recruitment, although this is a simplified view in that other ancillary proteins can be involved in clathrin recruitment, some of which appear to be lineage specific (Adung'a et al. 2013). The details on how clathrin-coated pits come to contain their cargoes or even whether cargoes are essential to trigger vesicle formation remain unclear (Kaksonen and Roux 2018), and pits may stochastically

develop and abort, with cargoes and their receptors diffusing laterally until they blunder upon a developing pit. Once within an incipient pit, stochastic dissociation becomes progressively more difficult as vesicle curvature becomes more pronounced (Weigel et al. 2013). Cargoes with higher affinities for such settings will naturally accumulate to a greater extent.

All adaptor proteins are heterotetramers comprised of two large, one medium, and one small subunit. Each subunit has orthologs across all adaptors and is also related to a particular protein involved in the COPI coat (which itself is a heptameric complex) (Schledzewski et al. 1999). Moreover, the two large subunits appear to have arisen by a gene duplication that preceded the origin of the different adaptor complexes, and the same is true of the medium and small subunits. These observations suggest that the ancestral adaptor may have been heterodimeric (or perhaps a dimer of heterodimers) consisting of just single small and large subunits (Schledzewski et al. 1999). Under this hypothesis, subsequent duplication of both subunits followed by divergence led to the heterotetrameric state, with further duplications and divergence of all subunits leading to the various classes of adaptors.

Because all five known adaptor proteins as well as COPI-coated vesicles are found throughout the eukaryotic domain, like clathrin, their diversification must have preceded LECA. Using the form of the genealogical relationships among the various complexes then provides a potential means for ordering events in the diversification of vesicle-trafficking pathways on the lineage connecting FECA to LECA (Figure 15.6). Such a perspective suggests a scenario in which an early ancestral adaptor diverged from the COPI coat, with the former then undergoing a series of duplications leading to five different adaptors that underwent further rounds of diversification, possibly prior to the emergence of clathrin (Hirst et al. 2011). The form of relationship between the gene-family members further suggests that the deployment of adaptors in endosomes emerged prior to the expansion of their use in the trans-golgi network.

Likely, other adaptor-like complexes remain to be discovered, given that a distantly related ortholog has recently been found based on structural information (rather than sequence divergence) (Hirst et al. 2014). Phylogenetically, this complex appears to be nestled between the adaptor proteins and the COPI subunits, and although the full complex is present in green plants and slime molds, it has been lost from several eukaryotic lineages (including metazoans). Unlike the complexes described previously, this new complex is a heterohexamer.

Central to the completion of vesicle formation, at least in metazoans, is the protein dynamin (Praefcke and McMahon 2004). After the development of clathrin-coated invaginations begin, dynamin assembles into collar-like helical structures and uses mechanical energy derived from GTP hydrolysis to pinch off the neck. Oligomerization of dynamin stimulates GTPase activity, resulting in a chain-like reaction that generates the overall mechanical force once a critical length of the collar has been achieved. Dynamin appears to be absent from most eukaryotic lineages, which nonetheless often harbor a separate clade of dynamin-like proteins that likely serve a similar function (Liu et al. 2012a; Briguglio and Turkewitz 2014).

Finally, an extreme form of membrane-mediated ingestion is the process of phagocytosis. Aided by their extensive cytoskeletons, most eukaryotes without cell walls are able to ingest large particles, including other cells, by invagination of the

surrounding cell membrane, followed by internalization and fusion with digestive vacuoles. Phylogenetic analysis suggests that LECA harbored many of the genes underlying the core machinery employed in the phagosome of today's species (Yutin et al. 2009; Boulais et al. 2010), with considerable independent additions and diversifications occurring in different descendant lineages, and complete losses in a few cases (e.g., chlorophytes and fungi). The logical conclusion is that LECA had no cell wall, and if not capable of phagocytosis, was primed for its subsequent emergence. Whether this capacity enabled the primordial eukaryote to ingest the ancestral mitochondrion remains unclear (Chapters 3 and 23).

Feeding by phagocytosis demands considerable membrane recycling. For example, when the ciliate *Euplotes* feeds on the smaller ciliate *Tetrahymena,* food-vacuole membrane equivalent to the entire surface area of the predator's cell is ingested every 5 minutes (Kloetzel 1974). This is also approximately the case for amoebae feeding on ciliates or other prey (Marshall and Nachmias 1965; Wetzel and Korn 1969). With no decline in cell volume during feeding, this implies a rate of membrane replenishment equal to that of membrane ingestion.

Vesicle delivery. Once formed, vesicles must find their way to an appropriate donor, and in doing so, avoid fusing with inappropriate membranes. The entire process entails multiple layers of information exchange, but central to such navigation are members of the RAB GTPase family of proteins, which help specify the locations to which vesicles are delivered. RABs act as switches by undergoing conformational changes when bound by GDP (inactive state) or GTP (active state). Specific GEFs (guanine exchange factors, which promote GDP release) catalyze conversion from the GDP- to GTP-bound forms, leading to activation, whereas GAPs (GTPase-activating proteins) do the reverse, leading to GTP hydrolysis and inactivation. Still other proteins are involved in RAB activation/deactivation cycles; e.g., RAB escort proteins deliver their cognate RABs to specific cellular locations, whereas after inactivation, RABs are recycled back to their membranes of origin via specific GDP-dissociation inhibitors (GDIs). The N-terminal residues of RABs contain vesicle specificity information, whereas the C-terminals are involved in targeting and adhesion to destination lipid membranes; still other enzymes endow these regions with post-translational modifications that confer specificity (Pylypenko and Goud 2012).

The main point here is that the transport of specific kinds of vesicles to specific locations involves an elaborate choreography of several layers of specialized protein-protein interactions – RABs and their various interactors. Eukaryotic species typically harbor 10 to 100 distinct RABs, and phylogenetic analysis suggests that LECA may have contained up to 23 RAB genes (Elias et al. 2012; Klöpper et al. 2012), with some lineages then experiencing losses of distinct family members. Fungi commonly encode no more than a dozen (Brighouse et al. 2010).

Also involved in vesicle delivery to specific sites are a large set of SNARE (soluble N-ethylmaleimide-sensitive factor-attachment protein receptor) proteins, which act in a zipper-like fashion, with coordination between specific sets on vesicles and on recipient membranes. The appropriate recognition groups attach to each other in four-helix bundles known as SNARE pins. The main types of SNAREs are classified into four subsets based on features of their four-helix bundles, which like the

coatamer proteins, diversified into subfamilies prior to LECA (Kloepper et al. 2007). Many of the subfamilies have expanded in lineage-specific ways, but with no obvious relationship to organismal complexity (Sanderfoot 2007; Kienle et al. 2009).

Evolutionary issues. Although many details remain to be elucidated, the information summarized above indicates that the vesicle trafficking system of eukaryotes diversified through gene duplication prior to LECA. This is true for essentially all of the components that have been subject to comparative sequence analysis, including the adaptor proteins, the RABs, and the SNARES. A possible contributor to such diversification (discussed further in Chapter 24) is the occurrence of one or more whole-genome duplication events on the path from FECA to LECA providing opportunities for coevolutionary coordination among duplicated parts. To this end, the organelle-paralogy hypothesis (Figure 15.4), invokes repeated rounds of gene duplication and joint coevolution of clusters of components toward more specialized functions (Dacks and Field 2007; Mast et al. 2014). However, although such descent with modification provides a logical argument for diversification (Ramadas and Thattai 2013), gene duplication does not ensure diversification in function, especially in a multilayered system that requires coordinated behavior of hundreds of component parts. At the very least, such evolution requires a series of mechanisms involving sub- or neofunctionalization to ensure the joint and coordinated preservation of mutually interacting pairs of components (Foundations 15.1). However, the population-genetic conditions permissive for such specialization have not been worked out and seem rather formidable, as the subcomponents of each descendent pathway must not only evolve pathway-specific features but also relinquish preduplication features that would promote pathway crosstalk.

Equally challenging is understanding how the multiple layers of communication necessary for specialized trafficking pathways evolve. Adaptor proteins provide the interface between various cargoes and the specific coat proteins of vesicles; different RAB proteins must specify unique types of vesicles as well as specialized effector molecules associated with subcellular localization; and specific pairs of vesicle and target SNARE proteins must recognize each other to ensure vesicle delivery to proper destinations. Although such a system might be viewed as exquisitely intricate, it comes at a substantial cost in terms of bioenergetic demand and mutational vulnerability.

The Nuclear Envelope

If there is an iconic feature of the eukaryotic cell, it is the housing of the genome inside the nucleus. Rather than floating free in the cytoplasm, the nucleus is surrounded by a double membrane (involving two bilayers), with the outer layer being continuous with the ER and periodically bending around at nuclear pores to form the inner nuclear membrane (Figure 15.7). There is also a proteinaceous support layer interior to the nuclear envelope, consisting of lamins in metazoa and amoebozoa (Simon and Wilson 2011; Burke and Stewart 2013) and apparently unrelated proteins in plants and other organisms (Cavalier-Smith 2005).

Among other things, genomic sequestration by the nuclear envelope separates

transcription (intranuclear) from translation (extranuclear), paving the way for the emergence of introns that must be spliced out of pre-messenger RNAs (Lynch 2007). It is through the nuclear pores that mRNAs are actively exported to the cytoplasm and nuclear proteins (e.g., transcription factors, histones, and DNA-repair enzymes) are imported. There is evidence in flies that clusters of proteins are sometimes exported as particles to the cytoplasm by budding of the inner nuclear membrane and vesicle transport to the outer membrane (Speese et al. 2012). However, it remains unclear whether this is a common phenomenon, and it is virtually certain that the bulk of transport proceeds through pores.

Nuclear-pore architecture. Nuclear pores are lined with a nuclear-pore complex (NPC), consisting of $\sim$ 500 to 1000 individual Nup (nucleoporin) proteins encoded by $\sim$ 30 separate genes. The NPC is the largest protein complex in most eukaryotic cells, exceeding the mass of a ribosome by more than ten-fold (Field et al. 2014; Devos et al. 2014; Beck and Hurt 2017). A brief excursion to describe the NPC is merited, as this will reinforce the contention that large complexes within eukaryotic cells are typically grown out of a series of gene duplication events (Chapter 13), while also illustrating that despite its conserved functions, the NPC has experienced considerable diversification at the architectural level. There are interesting lessons in coevolution to be learned as well, as pathogens that require entry into the nucleus (e.g., for replication and/or transcription) must successfully navigate the NPC.

The core of the NPC is both vertically and radially symmetrical, consisting of four stacked rings (two on the nuclear side and two on the cytoplasmic side), each comprised of eight spokes, which in turn consist of two parallel columns of several proteins (Figure 15.7). The proteins in adjacent columns are related as pairs, each of which is derived by gene duplication (Alber et al. 2007). This one-to-one correspondence of multiple pairs of duplicates is again consistent with a massive amount of duplication activity, if not a whole-genome duplication, in the ancestor leading to LECA.

Comparison of the parts lists from diverse species suggests that LECA had an NPC structure very much like that in today's species, with evolutionary roots associated with the proteins involved in vesicle production. Most notably, the core proteins of the inner rings appear to be related to the membrane-bending proteins involved in vesicle formation (COPI, COPII, and clathrin), motivating the hypothesis that all of these molecules are derived from a common ancestral protein, deemed the protocoatamer (Devos 2004; Mans et al. 2004; Alber et al. 2007a,b; Brohawn et al. 2008). Further evidence that the details of nuclear-pore construction are derived from predecessors in the vesicle-transport system is the use of a membrane-sculpting protein, known as ESCRT, for fusing the nuclear membranes at the pore junctions and in the development of internal vesicles of cells (Vietri et al. 2015).

Despite these common roots, there are component differences among lineages (Mans et al. 2004; Bapteste et al. 2005; DeGrasse et al. 2009; Neumann et al. 2010; Devos et al. 2014). As one example, the overall mass of the *S. cerevisiae* NPC is only $\sim$ 50% of that of the human NPC, owing to a reduction in the number of subunits banding together in the ring in *S. cerevisiae*. Even the two yeasts *S. cerevisiae* and *S. pombe* have different numbers of subunits in the multimeric complex (Liu et al. 2012b; Stuwe et al. 2015). Experiments have shown that changes

in the expression of subunit genes can lead to an alteration in the overall structure, suggesting a simple path to variation in pore composition/size within and among species (Rajoo et al. 2018). Larger compositional changes are known as well. For example, the deployment of proteins on the nuclear and cytoplasmic sides of the pore is asymmetrical in the case of yeast, animals, and land plants, but relatively symmetrical in the case of trypanosomes (Obado et al. 2016a,b).

Finally, it is worth noting that the NPC has evolved a number of secondary functions including involvement in chromosome organization and positioning and the mediating of transcription of tRNAs and mRNAs (Fahrenkrog et al. 2004; Xu and Meier 2007; Strambio-De-Castillia et al. 2010; Ikegami and Lieb 2013; Vaquerizas et al. 2010). In yeast, and likely many other species, a number of genes have short motifs that target them spatially to the nuclear periphery via interactions with the NPC (Ahmed et al. 2010). Thus, the NPC evolved to become the hub of many activities beyond cargo transport.

Nuclear transport. The nuclear pore is lined with a large number of FG Nups, each containing up to 50 phenylalanine (F)-glycine (G) repeats. These highly unstructured molecules can be viewed as a spaghetti-like sieve through which cargoes bound by appropriate nuclear transporter proteins are actively delivered and inappropriate molecules are excluded (Sorokin et al. 2007; Grünwald et al. 2011; Hülsmann et al. 2012; Vovk et al. 2016). Such selective filtering demands a means of molecular communication between the FG Nups and the transport mechanism, as well as between the cargoes and their transporters (Figure 15.7). The latter typically involves a nuclear localization signal on cargo protein molecules, which attracts a nuclear transporter protein, often called a karyopherin or importin/exportin. Such signals are generally quite simple, typically involving three or four consecutive basic amino acids (arginine or lysine), although the consensus sequence appears to vary among species (Kosugi et al. 2009). A separate set of transporter proteins is assigned to mRNA export.

The exact mechanisms of facilitated cargo transport are not fully resolved, but the process is fast, allowing the delivery of up to 1000 molecules per second per pore (Yang et al. 2004). Transport is governed by gradients of Ran-GTP and Ran-GDP, associated with the transporter-cargo complexes – Ran-GTP binds to the import complex on the nuclear side of the membrane, releasing the cargo, and then recycles to the cytoplasm during cargo export. Upon release, Ran-GDP is returned with its own carrier to the nucleus and converted to Ran-GTP, maintaining the Ran gradient necessary for efficient transport. Specific enzymes devoted to the Ran-GDP/GTP interconversion cycle reflect the need for still another means of molecular communication in the nuclear-transport pathway.

Although the basic mechanism of communication between karyopherins and FG Nups is conserved across taxa, there is drift in the language of communication across lineages. For example, human transport substrates are not imported into the nucleus of *Amoeba proteus* unless they are coinjected with human importins (Feldherr et al. 2002). Among yeast species, the FG Nups have diverged at the sequence level at much higher rates than other genes, with the greatest elevation arising in sequences interspersed between the Nup repeats (Denning and Rexach 2007). The ciliate *Tetrahymena thermophila* harbors two nuclei (the transcriptionally silent mi-

cronucleus and the transcriptionally active macronucleus), one of which has pores lined with FG Nups, while the other has Nups with novel NIFN repeats, implying distinct permeability of the two nuclear membranes (Iwamoto et al. 2017).

Evolutionary considerations. The universal presence of a nuclear envelope in eukaryotes presents two major evolutionary questions. First, what were the driving forces underlying the emergence of the nucleus? Second, once established, what secondary evolutionary challenges / opportunities did the nuclear envelope impose on other aspects of cellular evolution?

It is not clear that genome sequestration would have any intrinsic advantage in a prokaryote-like ancestor, and the failure of any prokaryote to make such a transition over billions of years suggests that there is none. Nonetheless, two hypotheses have been proposed for the origin of a nuclear barrier based on a selection scenario surmised to be unique to eukaryotes. Martin and Koonin (2006) proposed that the origin of introns (intervening sequences of messenger RNAs that must be spliced out to yield a productive mRNA) forced the evolution of the nuclear envelope to prevent the early translation of inappropriate (not yet spliced) messages. Jékely (2008) suggested an alternative scenario, with the origin of the mitochondrion forcing the sequestration of nuclear-encoded genes. Here the idea is that once the ribosomal protein-coding genes of the primordial mitochondrion were transferred to the nucleus of the host cell (Chapter 21), there would have been a risk of constructing chimeric (and potentially malfunctional) ribosomes consisting of mixtures of proteins with host and endosymbiont functions. In principle, this problem could be avoided by assembling the cytosolic ribosomes prior to nuclear export, and addressing the mRNAs for the mitochondrial ribosomal protein-coding genes to the mitochondrion. A key difficulty with both of these arguments is the assumption of an initial evolved harmful condition that the host cell is unable to escape from. If a problem was deleterious enough to encourage a massive repatterning of cellular architecture, why weren't the original mutational variants that created such a dire situation simply removed from the population by purifying selection?

A more plausible alternative is that the nuclear envelope evolved prior to introns and mitochondrion-to-host genome transfer by an alternative form of positive selection, thereby paving the way for these changes secondarily. Alternatives involving direct promotion include the protection of the genome from shearing forces in cells with cytoplasmic streaming and/or invasive self-proliferating genomic parasites. Most bacterial genomes are largely devoid of mobile-genetic elements, in principle because of the typically large effective population sizes of such species enables them to resist the fixation of harmful insertions (Lynch 2007). In contrast, few eukaryotes are able to cleanse themselves entirely of such elements, with a large fraction of many eukaryotic genomes being a result of the activities of parasitic DNAs (Chapter 24).

Many intracellular parasites depend on access to the host genome for survival. Among the most prominent of these are the mobile genetic-elements that literally reside within the nuclear genome – transposons and retrotransposons. To produce their encoded mobilization factors necessary for proliferation, such elements must be transcribed and translated in the cytoplasm, and the resultant products must be able to return to susceptible genomic territories. The FG-Nup-gated nuclear pores

serve as a primary guardian against uncontrolled element spread.

Dozens of examples exist for the coevolution of Nups and genomic parasites. For example, two inner-channel Nups in *Drosophila* play a central role in a pathway for transposon silencing (Munafó et al. 2021). Yeast retrotransposons have a requirement for the host-cell FxFG repeats in Nup124 (Dang and Levin 2000; Kim et al. 2005), although nuclear-pore associated factors have also been found to have inhibitory effects on retrotransposition (Irwin et al. 2005). Notably, the same Nup protein is exploited by HIV-1 (Varadarajan et al. 2005; Woodward et al. 2009; Lee et al. 2010). On the other hand, Nup124 prevents entry of hepatitis B virus, specifically via the FxFG repeats (Schmitz et al. 2010). Many other exogenous viruses have been found to engage in genetic conflicts with Nups of their host species (e.g., Gallay et al. 1995, 1997; Strunze et al. 2005; Satterly et al. 2007; Bardina et al. 2009; Porter and Palmenberg 2009).

Given the potentially high evolutionary rates of the nuclear-pore components driven by infectious agents, and the NPC's involvement in chromosome organization and interactions with the spindle during meiosis, it would not be surprising if the divergence of the NPC at the sequence level played a central role in the emergence of species isolating barriers. This could happen if coevolutionary changes of interacting NPC components within species lead to cross-species assembly issues in hybrids with negative functional consequences. Although few isolating barriers have been elucidated at the molecular level in any species, in one of the major engines of speciation research, the genus *Drosophila,* negative interactions between heterospecific Nups have a direct role in hybrid incompatibility, with the causal genes having evolved at highly elevated rates, apparently driven by positive selection (Presgraves 2007; Presgraves and Stephan 2007; Tang and Presgraves 2009).

Finally, under the assumption that the rate of export of transcripts from the nucleus is limited by the surface area of the nuclear envelope, Cavalier-Smith (1978, 2005) suggested the need for a strong coordination between nuclear and cell volumes. Drawing from observations of an association between genome size and nuclear volume (mostly in land plants; Price et al. 1973), his nucleoskeletal hypothesis postulates that organisms with large cells evolve large genome sizes as a means to support a large nuclear membrane. Under this view, DNA has a pure structural role, independent of its coding content, with a larger nuclear envelope leading to an associated increase in the number of pores, which in turn supports an enhanced flow of mRNAs to maintain the needs imposed by large cell size. The limited amount of comparative data suggests a roughly constant scaling of total nuclear pore number with nuclear size, with a pore density generally between 5 and $15/\mu m^2$ (Figure 15.8).

A number of observations shed doubt about the validity of the nucleoskeletal hypothesis. First, it is unclear that transport rates through pores (as opposed to association rates between cargoes and transporters) is the limiting factor in material transport. Empirical studies suggest the latter, with transporter efficiency being greatly compromised by off-binding to nonspecific substrates (Riddick and Macara 2005; Timney et al. 2006). Second, as pointed in out in Chapter 9, nuclear volume does not appear to be regulated by the amount of DNA in a cell. Third, increases in genome size in organisms with larger cell sizes may simply be an indirect consequence of the latter experiencing higher levels of random genetic drift and being more vulnerable to the passive expansion of excess DNA (Chapter 6). The most no-

table source of genome expansion is mobile-element activity (Lynch 2007), a highly mutationally hazardous enterprise and hence less than ideal substrate for building a nuclear support structure.

One central issue with the nucleoskeletal hypothesis is that the data do not strongly support a general relationship between genome and cell sizes (Figure 15.8). Although some groups of eukaryotes do exhibit an increase in genome size with cell volume (Figure 15.8), the slopes of the scaling relationship are far below the value of 1.0 expected if nuclear volume is determined by bulk DNA. Moreover, there is a weak but significantly positive scaling between genome size and cell volume for both heterotrophic and photosynthetic bacteria, neither of which have nuclear envelopes. The latter pattern is largely due to the fact that bacteria with larger cells generally have genomes with larger numbers of genes.

Summary

- Lipids are an essential ingredient of life, as all cells, and all organelles within eukaryotes, are surrounded by bilayers of tightly packed lipid molecules, with hydrophobic tails facing the interior of the membrane and hydrophilic head groups on the outside.

- There is enormous combinatorial diversity of lipid molecules within and among species, with variation in head-group types, lengths of tails and numbers and locations of double carbon bonds within them, and various other embellishments.

- Most species appear to be capable of altering the composition of lipid profiles in response to environmental change in a presumably adaptive manner.

- Individual lipid molecules are free to diffuse laterally in a two-dimensional manner within their individual membranes, although they do so at rates that are an order of magnitude lower than diffusion rates for proteins within the cytoplasm.

- The hallmark of the eukaryotic cell plan is the presence of a network of internal membrane-bound organelles. However, although they are rarely deployed, there appear to be no barriers to the emergence of internal membranes in prokaryotes, and there is no evidence that internal cell structure endows eukaryotes with a baseline superiority in fitness.

- Rather than forming *de novo,* vesicles are typically pinched off from source membranes, with the assistance of cage-like assemblies of coat proteins, and then delivered to source membranes, where they fuse, with the overall gain/loss dynamics leading to an approximately steady-state distribution of cell constituents.

- Specificity in the vesicle trafficking system is a function of several layers of intermolecular crosstalk, including adaptor proteins for selecting cargo, RAB proteins for guiding delivery, and SNARE proteins for promoting appropriate membrane fusion. Although gene duplication is known to underlie such diversification in specificity, the precise population-genetic requirements for the stable emergence of such partitioning remain to be worked out.

- The nuclear envelope of eukaryotic cells is a continuous elaboration of the ER, which also serves as the substrate for the pores through which nuclear-cytoplasmic transport occurs. Within these openings are embedded nuclear-pore complexes, consisting of several hundred proteins, which guide the bidirectional passage of appropriate RNAs and proteins. Nuclear transport is a selective process involving proteins contained within the pore and transporter proteins that selectively bind to particular cargoes.

- Why the nuclear envelope evolved is not entirely clear, but once established it altered the intracellular environment in ways that created a permissive environment for the colonization of genomic elements previously forbidden by natural selection, e.g., introns and mobile genetic elements On-going molecular arms races between pore proteins dictate the success of intracellular pathogens that require access to host molecules residing within the nucleus.

Foundations 15.1. Probability of preservation and subdivision of labor by duplicated interactions. In Chapter 10, the concept of subfunctionalization of the two members of a duplicated gene pair was introduced. The central point is that genes often have multiple, independently mutable subfunctions; after gene duplication, these can become reciprocally silenced, leading to more specialized daughter genes. The question of interest here is how frequently pairs of interacting genes (e.g., members of a transport pathway) can partition up their functions after both members of the pair are simultaneously duplicated (as would occur following a whole-genome duplication event). In the extreme, this can lead to two independently operating pathways. The approach taken here is not fully general, in that it assumes as situation in which mutations with influential effects fix sequentially in the population, although it does highlight the basic principles that will need to be accounted for in a fuller development.

Before addressing this point, it will be useful to understand the quantitative expectations for the single-gene situation. Consider the case illustrated in Figure 15.9, where initially a single protein-coding gene has a coding region and two regulatory elements for different subfunctions. It will be assumed here that all mutations with significant effects on gene activity are degenerative in nature, with loss of single subfunctions occurring at rate μ_s for each regulatory element, and mutations that eliminate whole-gene function arising at rate μ_n. Following gene duplication under this model, there are two possible fates: one of the genes will become completely silenced (nonfunctionalization), returning the system to the initial state of a single active gene, or the two genes will become mutually preserved by subfunctionalization, as in this case joint retention is necessary to retain the full complement of gene activity. It will be assumed that the loss of single gene features is a neutral process owing to the redundancy of the two-gene system, so that each step of permissible mutations proceeds at a rate equivalent to the mutation rate.

If subfunctionalization is to occur, the first mutation to fix must be of the subfunctionalizing type, the probability of which is $2\mu_s/(\mu_n+2\mu_s)$. This expression follows from the fact that there are three ways to mutate each fully endowed gene, two of which eliminate single subfunctions. Conditional upon arriving at this initial state, the remaining fully intact gene cannot be nonfunctionalized, as this would fully eliminate one subfunction entirely, although it can lose the remaining redundant subfunction. In addition, the partially partially incapacitated copy can be completely silenced by either a nonfunctionalizing mutation or by a mutation to the remaining subfunction. The total permissible mutation rate during the second step is then $\mu_n + 2\mu_s$, with the probability that the second mutation leads to joint subfunctionalization being $\mu_s/(\mu_n + 2\mu_s)$. The total probability of subfunctionalization is equal to the product of the two stepwise probabilities,

$$P_{\text{sub},1} = \frac{2\mu_s^2}{(\mu_n + 2\mu_s)^2}, \tag{15.1.1a}$$

with the probability of nonfunctionalization being

$$P_{\text{non},1} = 1 - P_{\text{sub},1}. \tag{15.1.1b}$$

Now consider the situation in which a pair of interacting genes (e.g., a donor and its recipient) is duplicated simultaneously, with each pair having two independently mutable interactions (as indicated by the different colors and complementary shapes in Figure 15.9). Following the same mutation scheme noted above, there are four possible final fates of this system: 1) complete subfunctionalization and the preservation of two specialized single-subfunction interactions; 2) one fully endowed donor gene, and two specialized recipients; 3) two specialized donors, and one fully endowed recipient (not

shown); and 4) nonfunctionalization of one donor and one recipient and return to the single-pair (ancestral) situation.

Multiple paths involving multiple steps lead to each of these final outcomes, rendering the book-keeping difficult, so only a few of the results will be sketched out. It is relatively straight-forward to obtain the probability of complete subfunctionalization, as this requires that a series of four subfunctionalizing mutations occur before any gene is completely nonfunctionalized. Moreover, specific subfunctions must be retained in each gene – the two donor genes must preserve alternative subfunctions, as must the two recipient genes. The probability of each specific subfunctionalizing mutation is $\mu_s/(\mu_n + 2\mu_s)$, and because there are two ways by which the donor copies can be resolved (blue in one, and green in the other, in either order), and likewise for the recipient genes, the probability of preservation of the four-gene set by subfunctionalization is

$$P_{\text{sub},2} = \frac{4\mu_s^4}{(\mu_n + 2\mu_s)^4}, \tag{15.1.2}$$

which is equivalent to $P_{\text{sub},1}^2$.

We next consider the probability of return to a single-pair system, which requires the complete loss of function of one donor and one recipient gene. There are three ways by which this endpoint can come about. First, if the initial mutation is nonfunctionalizing, which occurs with probability $\mu_n/(\mu_n + 2\mu_s)$, the system effectively returns to a one-gene system, as only the remaining pair of duplicates is now capable of further evolution. The net probability of return to the ancestral state by this path is then simply

$$P_{\text{non,a}} = \frac{\mu_n P_{\text{non},1}}{\mu_n + 2\mu_s}. \tag{15.1.3a}$$

Second, there are two additional paths to a one-pair system if the first mutation is of the subfunctionalizing type (probability $2\mu_s/(\mu_n + 2\mu_s)$) and the second is nonfunctionalizing. When there are three fully functional and one subfunctionalized genes, the total rate of permissible mutations in the next step is $d = 3(\mu_n + 2\mu_s)$. The probability that the subfunctionalized copy is silenced in the next step is then $(\mu_n + \mu_s)/d$, and this returns the system to the identical situation noted in the previous paragraph – one fully endowed gene of one type and two of the other, with a probability of nonfunctionalization of $P_{\text{non},1}$ in the final step. Alternatively, a member of the pair of fully endowed genes will be nonfunctionalized with probability $(2\mu_n)/d$, in which case the remaining single-subfunction gene will be lost with probability $(\mu_n + \mu_s)/(\mu_n + 2\mu_s)$, leaving one fully endowed donor and recipient gene. Collecting terms, the probability of complete nonfunctionalization by these two path types is

$$P_{\text{non,b}} = \frac{2\mu_s(\mu_n + \mu_s)}{3(\mu_n + 2\mu_s)^2}\left(P_{\text{non},1} + \frac{2\mu_n}{\mu_n + 2\mu_s}\right). \tag{15.1.3b}$$

The third potential path to complete nonfunctionalization follows when the first two mutations are of the subfunctionalizing type. This can only occur if one of each such mutations is allocated to a donor and the other to a recipient gene (as otherwise, both members of donor and/or recipient would be permanently preserved by subfunctionalization). The probability of this starting point is $[2\mu_s/(\mu_n + 2\mu_s)] \cdot (4\mu_s/d)$. Completion of the path to complete nonfunctionalization then requires that one of the single-subfunction genes is silenced by the next mutation, the probability of which is $(\mu_n + \mu_s)/(\mu_n + 2\mu_s)$, and that the final single-subfunction gene is also silenced in the remaining step, which also occurs with probability $(\mu_n + \mu_s)/(\mu_n + 2\mu_s)$. Collecting terms,

$$P_{\text{non,c}} = \frac{8\mu_s^2(\mu_n + \mu_s)^2}{3(\mu_n + 2\mu_s)^4}. \tag{15.1.3c}$$

Summing up terms, the total probability of return to a single-pair system by random silencing of one donor and one recipient gene is

$$P_{\text{non},2} = P_{\text{non,a}} + P_{\text{non,b}} + P_{\text{non,c}}. \tag{15.1.4}$$

The probability of partial preservation is

$$P_{\text{par}} = 1 - P_{\text{sub},2} - P_{\text{non},2}, \tag{15.1.5}$$

with half of these cases involving two specialized donors and one two-subfunction recipient, and the other half the reciprocal situation.

The solutions of the above formulae, given in Figure 15.9, are simple functions of the ratio μ_s/μ_n. As noted already, the probability of complete subfunctionalization of a two-component pathway is substantially smaller than that of subfunctionalization of a single two-function gene, being equivalent to the square of the latter. On the other hand, the probability of partial preservation of a pathway (involving just one member of the pair) is $\simeq 2P_{\text{sub},1}$, provided $\mu_s < \mu_n$ (which is likely to be the usual case). Thus, given that there are two genes involved, only one of which will be preserved by subfunctionalization, the probability of preservation per gene is very nearly the same with the duplication of a two-gene system as in the case of single-gene duplication.

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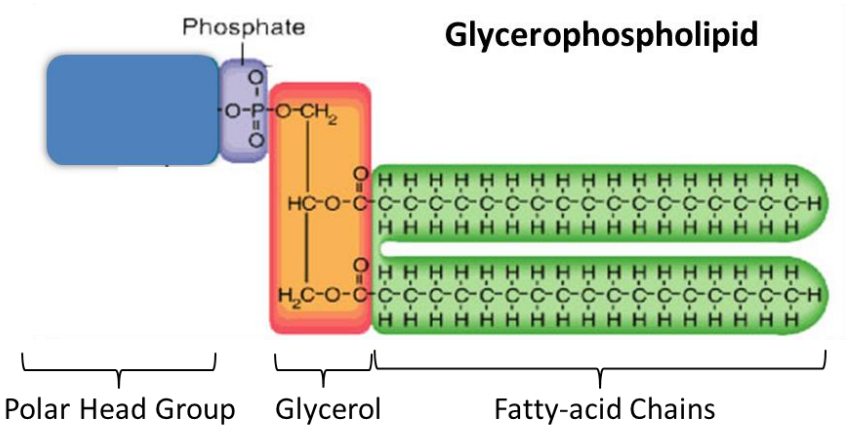
Figure 15.1. Schematics of the structures of the two major classes of lipid molecules. The glycerophospholipid depicted here is saturated, as the fatty-acid tails contain only single carbon-carbon (C-C) bonds. The sphinoglipid has a single double bond, denoted by the double line in the tail, where each kink denotes a C. A third common group of membrane lipids (not shown) consists of a diverse array of sterols, which lack head groups and intercalate between the fatty-acid tails of membranes.



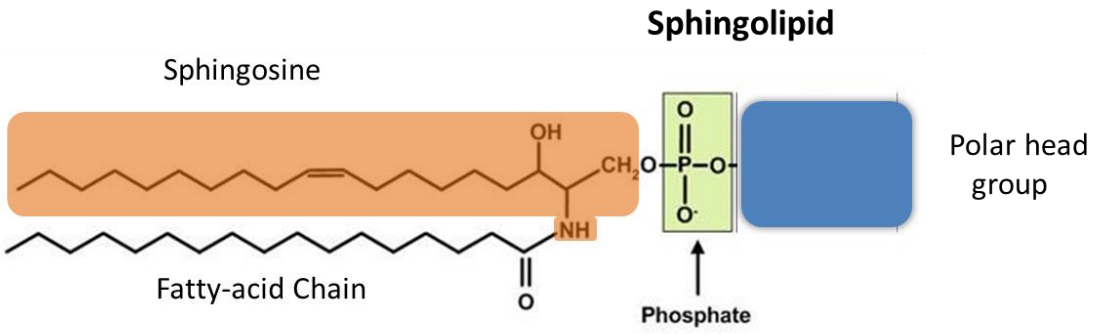


Figure 15.2. Left) Lipid bilayers, with the hydrophilic head groups in red and the hydrophobic fatty-acid tails in black. In the lower left, some individual molecules (blue) have C=C (unsaturated) bonds, yielding slightly kinked tails and a more open membrane. **Right)** The width of the tail region relative to the head group determines the tendency of a membrane to curve inwardly vs. outwardly.

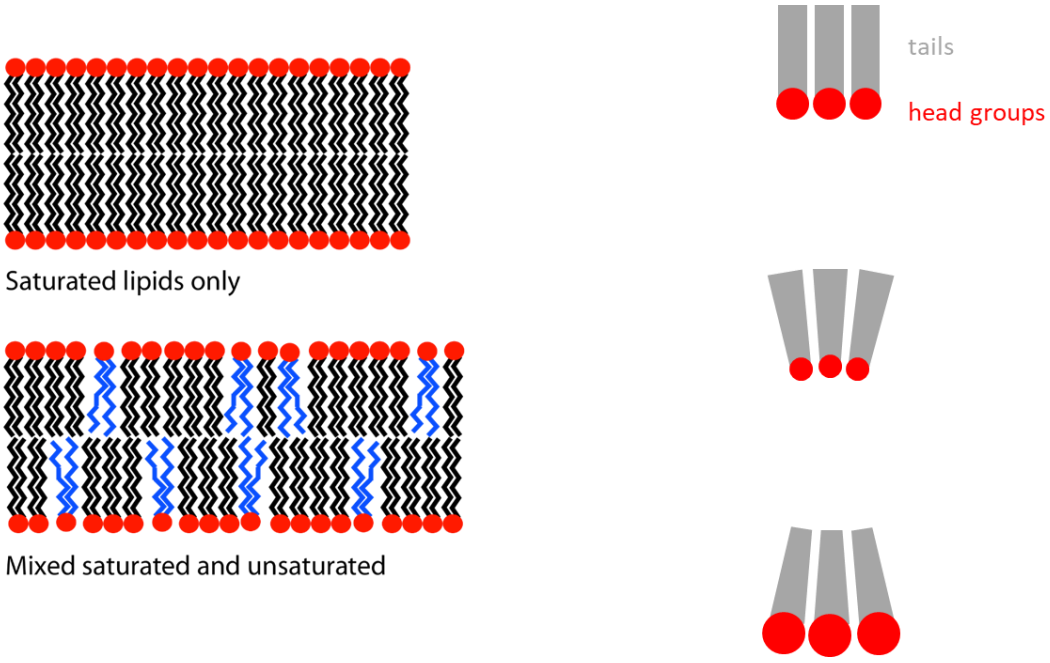
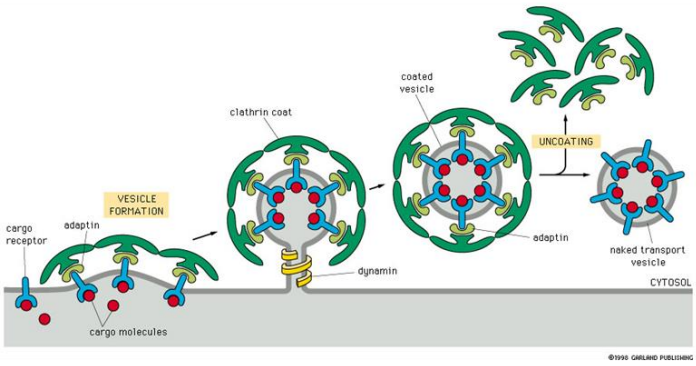


Figure 15.3. Generic schematic of a vesicle-transport pathway with several stages. **Left)** Cargo capture: external cargo molecules (red) are initially bound by specific cargo-transport proteins (blue). Vesicle budding and coating: specific adaptor proteins (light green) bind to the cargo receptors, and in turn recruit vesicle coating proteins (dark green), which induce membrane curvature (dark gray). Vesicle scission: coat proteins continue to be recruited, and the stem is eventually squeezed off with a concatamer of dynamin molecules (yellow coil). Vesicle uncoating: the coat proteins are removed, leaving the lipid-bound vesicle free to bind to a recipient membrane. **Right)** Tethering: a specific RAB protein (blue) provides recognition between the vesicle and a tethering effector molecule (green), and vesicle and target SNARE proteins (orange and lavendar) join to seal the final connection. Fusion: after docking with the recipient membrane, the cargo is unloaded.



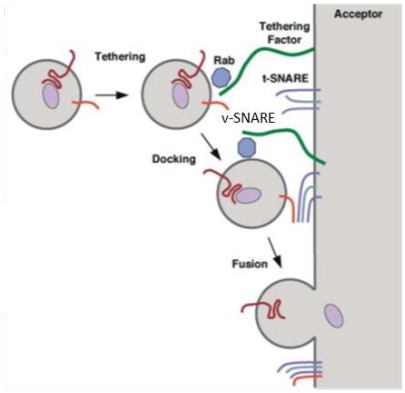


Figure 15.4. The organelle paralogy hypothesis (Dacks and Field 2007; Mast et al. 2014). **Left)** An ancestral communication mechanism between two molecular structures, e.g., an adaptor protein and its cargo receptor; a RAB protein and a tethering molecule; or a v-SNARE and a t-SNARE. (See Figure 15.3). **Center)** Nested sets of duplications of the genes for both participants, followed by molecular evolution, eventually lead to interacting pairs with specific functions isolated from other such pairs. **Right)** These specializations lead in turn to partitioning with respect to subcellular functions.

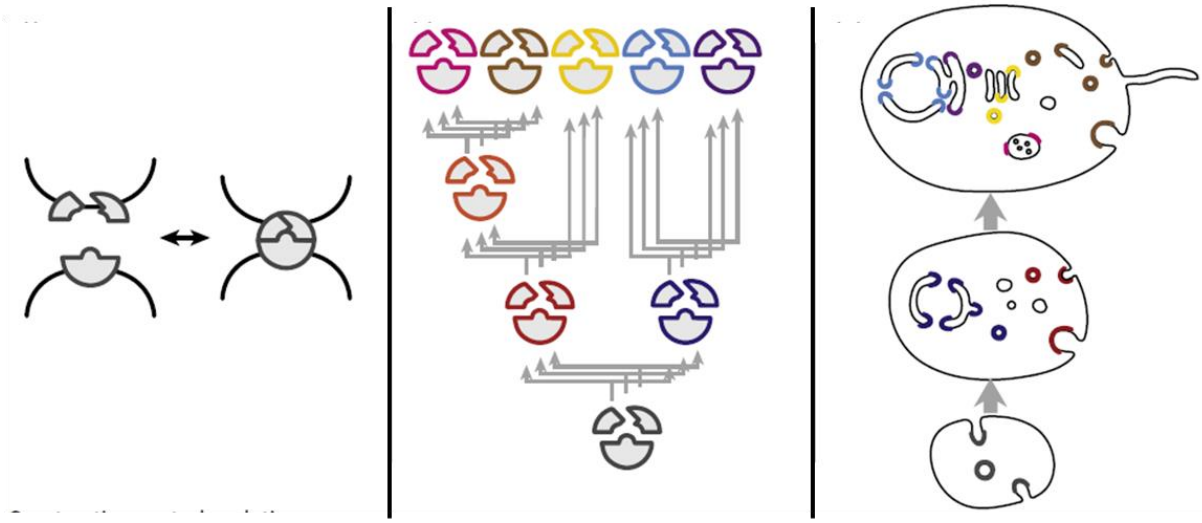
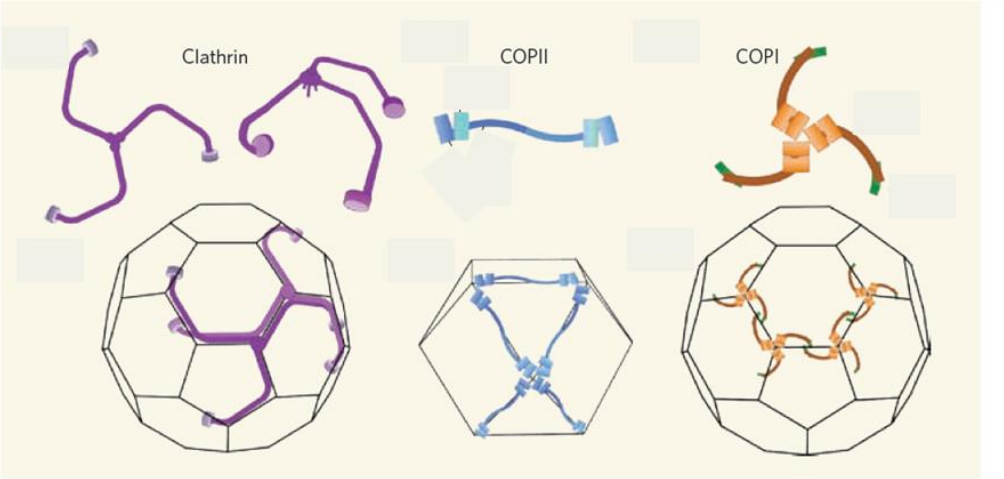


Figure 15.5. Schematics of the higher-order structure of the proteinaceous coats of eukaryotic lipid vesicles. **Top)** The basic structures of clathrin and COPI coats are homotrimeric subunits, whereas that for COPII vesicles is a heterodimer. These subunits organize into lattices with distinct geometric shapes, the specific dimensions of which are defined by the lengths of the domains of the monomeric subunits. **Bottom)** A monomeric subunit of clathrin. Each linear domain of the arm consists of a long series of α helices, the numbers of which define the overall dimensions of the lattice. It remains to be seen whether such structures vary in any meaningful way phylogenetically. From Edeling et al. (2006) and Harrison and Kirchhausen (2010).



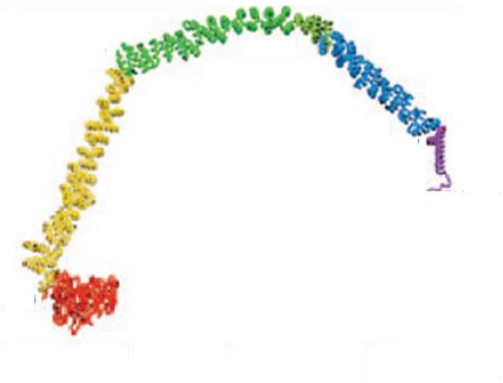


Figure 15.6. A view of the timing of the evolutionary diversification of the five known adaptor proteins and the COPI coat subunit. All of the nodes on the tree emerged prior to the last eukaryotic common ancestor (LECA), as all components are distributed throughout the entire eukaryotic phylogeny. Thus, the structure of the tree yields a hypothesis about the order of events in the functional diversification of adaptor proteins, e.g, implying a likely early role for COPI, but a relatively late recruitment of adaptor proteins to the trans-golgi network. From Hirst et al. (2011).

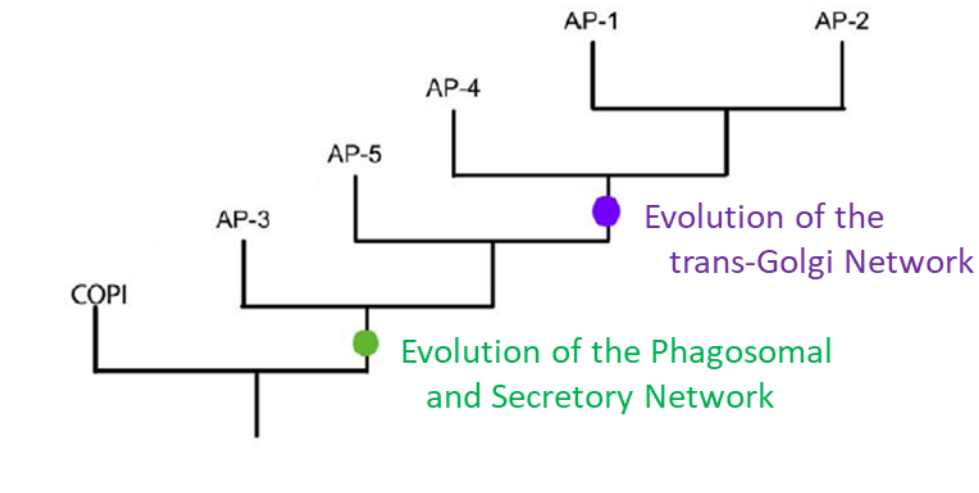
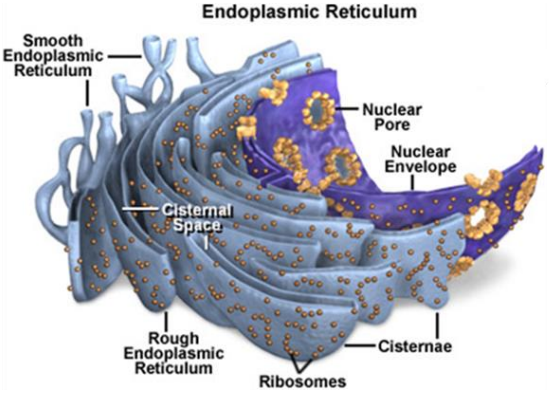
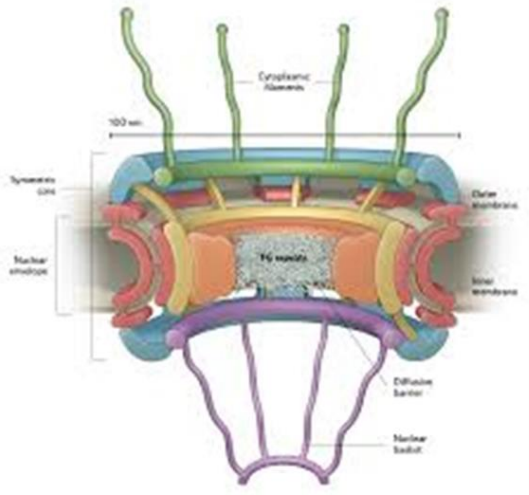


Figure 15.7. A hierarchical view of the nuclear envelope (NE) and the nuclear pore complex (NPC). **Upper left)** The outer layer of the NE is continuous with the endoplasmic reticulum. Perforating both sides of the NE are nuclear pores. **Lower left)** The NPC, through which cargos must be transported, is a complex structure consisting of hundreds of proteins encoded by multiple loci. **Right)** The central core of the NPC consists of eight spokes, each consisting of two columns, for a total of 16 columns per pore, all of which are organized into four cylindrical layers.





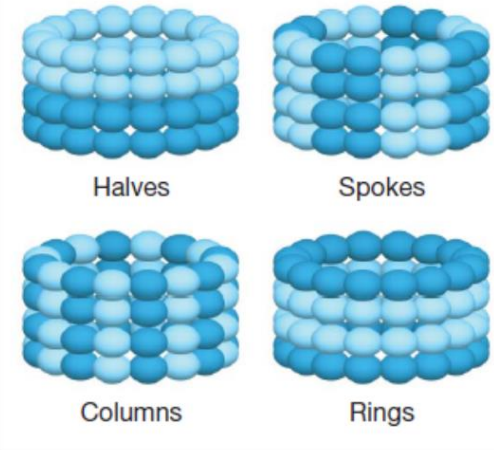


Figure 15.8. Left) Phylogenetic scaling of the total number of nuclear pores with nuclear volume, $33.1V^{0.78}$ ($r^2 = 0.94$). Because area scales with $V^{2/3}$, this implies that the mean number of pores per area scales very weakly with an increase in nuclear volume. Data are from Keddie and Baraja (1969), Atkinson (1974), Maul and Deaven (1977), Maul (1997), Winey et al. (1997), and Henderson et al. (2007). **Right)** The scaling of genome size with cell volume. For both heterotrophic bacteria and cyanobacteria, there is a significant positive scaling, with genome size (in megabases) being $4.3V^{0.17}$ ($r^2 = 0.19$) and $2.7V^{0.17}$ ($r^2 = 0.71$), respectively. In eukaryotes, only the regrssions for chlorophytic algae and diatoms are significant, with respective slopes of 0.29 and 0.59. Data are contained in Supplemental Table 15.1.

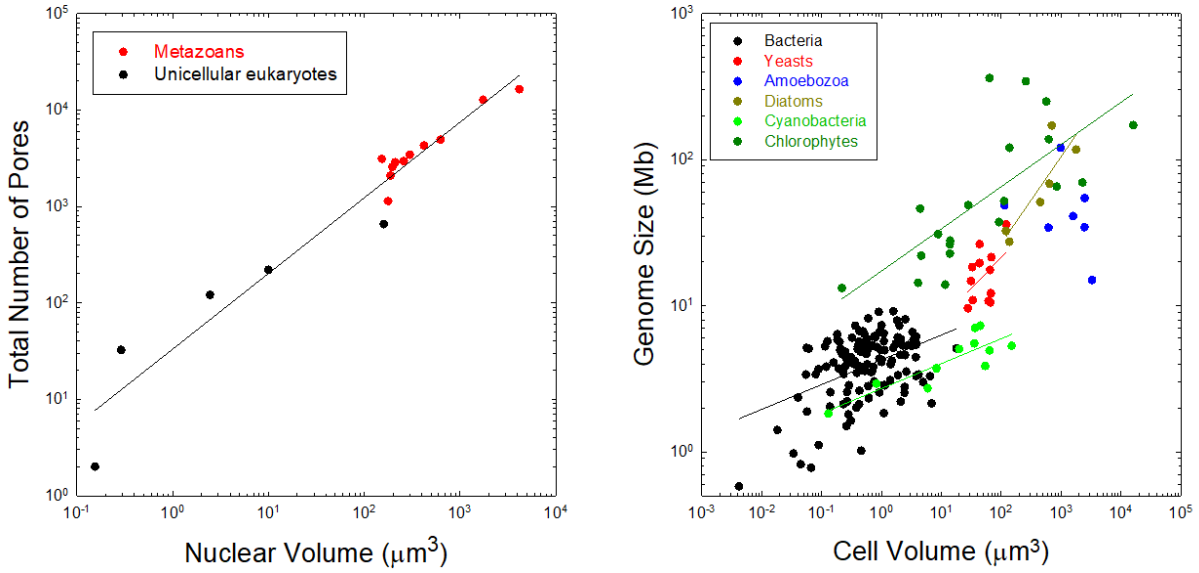
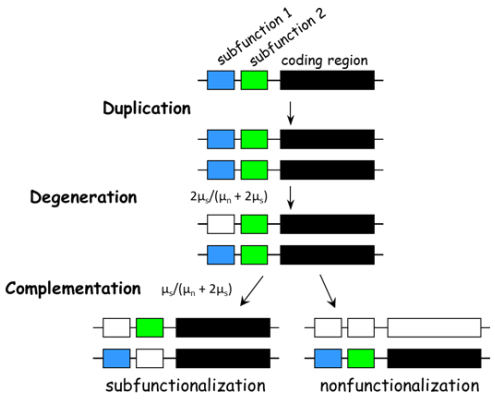


Figure 15.9. Above) The probabilities of the individual steps involved in the preservation of two duplicate genes, each with two independently mutable subfunctions. **Below)** The alternative fates of duplicated pairs of interacting genes, and their probabilities. In both cases, the fate probabilities are functions of the ratio of rates of subfunctionalizing to nonfunctionalizing mutations (μ_s/μ_n).

Single-gene Duplication:



Gene-pair Duplication: