# 1

# THE CELLULAR ORIGIN OF VERTEBRATES





## THE ORIGINS OF UNICELLULAR LIFE ON EARTH

Life has existed on Earth for billions of years, starting with primitive cells that evolved into unicellular organisms (Fig. 1.1) over the course of the first 4.5 billion years of Earth's existence. The evolution of complex biologic organisms began with the symbiotic relationship between prokaryotes and eukaryotes. This relationship gave rise to mitochondria, and the resulting diversity of unicellular

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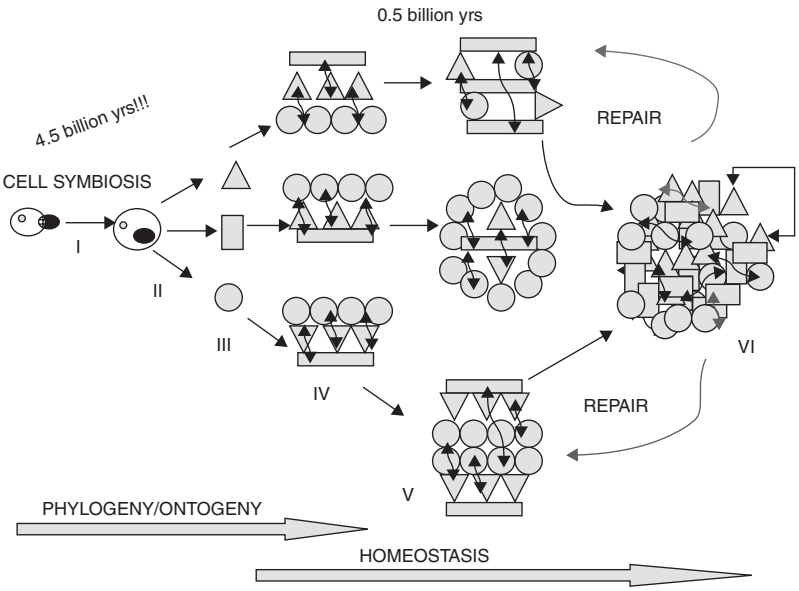


Figure 1.1. Cooperative cells as the origin of vertebrate evolution. The evolution of complex biologic oroganisms began with the symbiotic relationship between pro- and eukaryotes **(I)**. This relationship gave rise to mitochondria **(II)**. The resulting diversity of unicellular organisms **(III)** led to their metabolic cooperativity **(IV)**, mediated by ligand–receptor interactions and cell–cell signaling. Natural selection generated increasing complexity **(V)**. Failed homeostatic signaling **(VI)** recaptulates phylogeny and ontogeny, offering pathology and repair as the inverse of phylogeny and ontogeny. From Torday (2004). (See insert for color representation.)

organisms led to their metabolic cooperativity, mediated by ligand–receptor interactions and cell–cell signaling. Natural selection generated increasing complexity. Failed homeostatic signaling recapitulates phylogeny and ontogeny, offering pathology and repair as the inverse of phylogeny and ontogeny. How life on Earth actually began can only be speculated, unless we can witness it unfolding on "other Earths," and even then the process would probably differ from what has transpired on Earth since it is still contingent on the prevailing environmental conditions.

The question of the origins of life was first formally addressed by A. I. Oparin in 1924, and then by J. B. S. Haldane in 1929. They reasoned that if the early Earth environment lacked atmospheric oxygen, a variety of organic compounds could have been synthesized in reaction to energy from the sun, and by electrical discharges generated by lightning. Haldane suggested that in the absence of living organisms feeding on these putative organic compounds, the oceans would have attained a hot, soupy consistency.

The formation of boundaries through which things cross is the domain of cellular processes. Metabolic theories of the origins of life such as those of Oparin

and Fox (Fox 1965) assume the existence of a primitive cell-like compartment, or protocell, in which metabolism may have emerged. Metabolism, in turn, caused the growth of the cell and its division into daughter cells when its physical limits of gas and nutrient exchange had been reached or surpassed.

One way in which cellular life has been postulated to have originated was through the well-recognized process by which the repeated wetting and drying of lipids naturally generates micelles, which are spheres composed of semipermeable membranes. Perhaps this occurred on the shores of the primordial oceans, waves depositing lipids derived from plant life at the water's edge (algae have been around for 3.5 billion years and are rich in lipids) and drying out, only to be wetted and dried again and again, repeatedly over eons. Within these primitive cells, catalytic reactions that would have reduced entropy within them could have resulted from random interactions between molecules generated by the electrical discharges during thunderstorms passing through the primordial atmosphere. In 1953, Miller and Urey tested this hypothesis experimentally by passing an electrical charge through an airtight glass reaction chamber containing water, methane, ammonia, hydrogen, and carbon monoxide, modeling the composition of the prebiotic Earth atmosphere. After days of refluxing, the apparatus was opened and the contents of the reaction vessel were analyzed. They identified a wide variety of organic compounds, including amino acids (the building blocks of proteins), sugars, purines and pyrimidines (the building blocks for DNA), fatty acids, and a variety of other organic compounds, suggesting that the conditions in the primitive Earth's atmosphere gave rise to the origins of life.

Wachtershauser (1988) refined this concept by suggesting that chemical reactions may have taken place between ions bonded to a charged surface. Advocates for this school of thought maintain that the emergence of such a structure that walled itself off from its environment by a membrane gave rise to the partitioning between life and nonlife. Membrane proponents focus on the primordial role of lipids in this process, and the fundamental role of membranes in the conversion of light energy into chemical, electrical, or osmotic energy, fostering the growth of protocells through metabolic processes within them (Morowitz 1992). Morowitz suggested that the prebiotic environment contained hydrocarbons, some of which were composed of long chains of carbon and hydrogen. These compounds accumulated on the surface of the ocean, where they interacted with minerals to generate amphiphiles, such as phospholipids, which are molecular dipoles, one end of which was hydrophilic and the other end, hydrophobic. These molecules condensed into various structures, including mono- and bilayers, or lipid sheets. Amphiphilic bilayers spontaneously form spheres in an aqueous solution, with the polar heads of the two layers pointing outward into the adjoining aqueous phase. The nonpolar ends of the bilayer point inward toward the center. This is the basic structure of biological membranes that form the outer surfaces of all cells, allowing active transport of chemicals across the membrane in conjunction with proteins interspersed in the lipid bilayer.

It has been suggested that this spontaneous formation of closed vesicles is the origin of triphasic systems consisting of a polar interior, a nonpolar membrane core, and a polar exterior, creating an interior environment. Morowitz went on to empirically demonstrate that the advent of life processes depended on these properties of amphiphilic vesicles. Nonpolar molecules such as chromophores, which absorb light energy, tend to dissolve in the nonpolar lipid core of the membrane, where the light energy is converted into electrical energy that drives various chemical reactions, including the generation of even more amphiphiles. In contemporary cells, such reactions are mediated by phosphate bond energy, whereas in their primitive condition these reactions were facilitated by pyrophosphates. The generation of new amphiphiles through this mechanism increased the vesicle size. Once the vesicle reached a critical size, it broke up into smaller, more stable vesicles in the same way that soap bubbles do. This process is thought to be the origin of cell division.

## PROKARYOTES VERSUS EUKARYOTES

Eukaryotes are organisms with a membrane envelope around their nuclei like our own cells. They are assumed to have evolved from prokaryotes, such as bacteria, which lack a nucleus. Eukaryotes have numerous organelles that are absent from prokaryotes, including mitochondria, or plastids, which play an important role in energy metabolism. The unusual structure and self-replication of mitochondria and plastids had suggested to some scientists back in the nineteenth century that perhaps these structures descended from bacteria. It was subsequently determined that the mitochondrial and plastid mutations were independent of nuclear DNA. We now know that these organelles are related to bacteria, forming the basis for the *endosymbiotic theory*, which was first put forward by the Russian botanist Mereschkowski in 1905. Mereschkowski knew of the work by botanist Andreas Schimper, who had observed in 1883 that the division of chloroplasts in green plants closely resembled free-living cyanobacteria. Schimper had proposed that green plants arose from the symbiotic union of two organisms. Wallin extended this concept of endosymbiosis to mitochondria in the 1920s. At first these theories were either dismissed or ignored. More detailed electron microscopic comparisons between cyanobacteria and chloroplasts, combined with the discovery that plastids and mitochondria contain their own DNA, led to a reprise of endosymbiosis by Lynn Margulis in a 1967 paper entitled “The origin of mitosing eukaryotic cells.” In her 1981 book, entitled *Symbiosis in Cell Evolution*, she postulated that eukaryotic cells originated as communities of interacting entities, including endosymbiotic spirochetes that developed into eukaryotic flagella and cilia. This last idea has not received much acceptance, because flagella lack DNA and do not show ultrastructural similarities to prokaryotes.

## COEVOLUTION OF TRAITS

The most significant functional difference between pro- and eukaryotes is that prokaryotes have a rigid cell wall, while eukaryotes have a compliant plasma membrane, allowing them to easily change shape. The deformability of the plasma membrane allows for cytosis, a process by which membrane-bound vesicles inside the cell can fuse with and become part of the cell membrane. The prototype for this function is phagocytosis, or "cell eating," which allows eukaryotic cells to ingest solid particles that fuse with lysosomes containing digestive enzymes. Bacteria can feed on solid nutrients by secreting digestive enzymes into their immediate surroundings, and then absorb the molecular nutrients across the cell wall, molecule by molecule. This adaptation for feeding efficiently may have been the first step in the successful evolution of eukaryotes. This may also have been the basis for endosymbiosis by the ingestion and compartmentation of bacteria. Evidence for such a mechanism has come from studies of Archezoa, the most ancient eukaryotes. These protists have a nucleus containing chromosomes, but remain unicellular and lack mitochondria or plastids. Molecular phylogeny has documented the relationship between archezoans and eukaryotes. Another adaptation is the nuclear envelope, the structural hallmark of eukaryotes, which may have derived from invagination of the outer cell membrane. This concept is consistent with the fact that the nuclear envelope is a double membrane, and that the nuclear outer membrane is continuous with the endoplasmic membrane.

The loss of the external skeleton would have been offset by balancing selection for internal skeletal filaments and microtubules, along with mitosis, the dividing of chromosomes, and their segregation into daughter cells, since bacterial chromosomal segregation is dependent on attachment of the chromosome to the cell wall. The selection advantage is for more efficient means of feeding on solid food, and the acquisition of novel organelles. The concomitant reproductive mechanism of chromosome segregation, aided by the newly evolved microfilaments, provided a selection advantage over bacterial chromosomal replication, which must start at a single point, allowing for an exponential increase in genetic material.

(*Note*: This recurrent theme of overlapping selection for phenotypes of feeding with other complementary, coevolved adaptations is key to understanding the cellular origin of metazoan evolution. Such coevolved traits represent the emergence and contingence of the evolutionary process, all the way from molecular oxygen and cholesterol to complex physiologic traits. This concept will be repeated throughout this book.)

The loss of the prokaryotic stiff outer cell wall by eukaryotes is important in providing a possible new way of feeding, and also for other adaptations made possible by this newly evolved trait. The cytoskeleton comprises two main classes of molecules: actin filaments and microtubules. They perform complementary

functions; actin filaments resist pulling forces, and microtubules resist compression and shearing forces. These properties enable the cytoskeleton to maintain the cell's form in the absence of a rigid cell wall. The cytoskeleton can also change the shape of the cell, and move things around within it. Microtubules are used for the intracellular trafficking of particles and vesicles. They also pull chromosomes apart during cell division, and are constituents of such locomotor organs as cilia and flagella. Actin filaments are active in cell division and in phagocytosis. To move things around, molecules must exert *mechanochemical activity*, meaning that they must convert chemical activity into mechanical motion. This requires that the protein molecules be able to actively change shape—in effect, they must be able to extend themselves, attach to something, and retract.

Where did this ability come from? It already existed in a rudimentary form in eubacteria. When bacteria divide, a furrow is formed in the cell membrane. This requires a mechanically active molecule; the gene sequences of certain eukaryotic mechanochemical proteins show some resemblance to this bacterial molecule. So the "fission"-aiding protein turns out to be preadapted to a cytoskeletal function.

In modern eukaryotic cells, there are several different kinds of membranes. How could they have evolved? The initial evolution of the food vacuole, budding off by endocytosis to form the cell membrane, presented just such an opportunity. In bacteria, the digestive enzymes that are secreted are synthesized by ribosomes attached to the cell membrane. If, in the original eukaryotes, a food vacuole formed randomly in response to the phagocytosis of a food particle, aided by the primitive cytoskeleton (the furrow-forming bacterial molecules), then the digestion of food within the vacuole and the ingestion of nutrients would be carried out by the original bacterial machinery.

One school of thought is that the presence of cholesterol, or related sterols, where appropriate, modified the physical properties of membranes in a manner that was crucial to the evolution of eukaryotic cells into their present form. The lipid composition of the plasma membranes of eukaryotic cells invariably includes a substantial amount of cholesterol. Konrad Bloch (1979) asked whether one could discern in the contemporary sterol pathway, and in the temporal sequence of modifying events, a directed evolutionary process operating on a small molecule and, if so, whether each step of the sequence produces a molecule functionally superior to its precursor, or molecular evolution. He demonstrated that cholesterol evolved on the appearance of oxygen in the atmosphere, facilitated by the cytochrome P450 family of enzymes necessary for cholesterol synthesis. He speculated that the biological advantage associated with cholesterol may have been due to the "reduced fluidity" or "increased microviscosity" that the addition of cholesterol imparts to the liquid crystalline state of phospholipid bilayer membranes.

Bloom and Mouritsen (Miao et al. 2002) proposed that the biosynthesis of cholesterol in an aerobic atmosphere removed a bottleneck in the evolution of

eukaryotic cells. This proposed role of the physical properties of membranes in the evolution of eukaryotes is compatible with Cavalier-Smith's (2010) characterization of the evolution of eukaryotic cells, in which he identifies "twenty-two characters universally present in eukaryotes and universally absent from prokaryotes." He presents detailed arguments that, of these, the advent of exocytosis and endocytosis is the most likely to have provided the driving force for the evolution of eukaryotic cells into their present form. Bloom and Mouritsen have hypothesized that, in addition to influencing the cohesive strength of membranes, the main role of cholesterol in this evolutionary step was to relax an important constraint on membrane thickness imposed by the biological necessity of membrane fluidity—the introduction of cholesterol into phospholipid bilayer membranes increases the orientational order, but does not increase the microviscosity. Such fluidlike properties would allow large membrane curvatures without abnormal increases in permeability. As a result, with the appearance of large amounts of molecular oxygen in Earth's atmosphere between 2.3 and 1.5 billion years ago, a bottleneck in the evolution of eukaryotic cells was removed by the resultant incorporation of sterols into the plasma membrane. This interrelationship between cholesterol, membrane thickness, and cytosis is recapitulated later in this book through the overlapping of the evolution of pulmonary surfactant, the increased oxygenation of the lung, and feeding efficiency (Chapter 6). Such a reprise of a trait that served one purpose in evolution, only to serve a homologous purpose later in evolution, is referred to as an *exaptation*. Cholesterol represents a molecular phenotypic trait that has been positively selected for, beginning with unicellular organisms, all the way up through the complex physiologic properties of lung surfactant, cell–cell signaling via G-protein-coupled receptors, and endocrine regulation of physiology, all of which are catalyzed by cytochrome P450 enzymes.

## CHOLESTEROL FACILITATES LIPID RAFTS FOR CELL–CELL COMMUNICATION

Plasma membrane aggregates formed by cholesterol and sphingomyelin are referred to as *lipid rafts*. Lipid rafts are composed of twice the amount of cholesterol found in the surrounding membrane, and are enriched in sphingolipids such as sphingomyelin, which is typically elevated by 50% compared to the bilayer. Phosphatidylcholine levels are decreased to offset the elevated sphingolipid levels, resulting in similar choline-containing lipid levels between the rafts and their surrounding plasma membrane. Cholesterol interacts preferentially with sphingolipids, due to their structure and the saturation of the hydrocarbon chains. Although not all phospholipids within the raft are fully saturated, the hydrophobic chains of the lipids contained in the rafts are more saturated and tightly packed than the

surrounding bilayer. Cholesterol holds the raft together. Because of the rigid nature of the sterol group, cholesterol partitions preferentially into the lipid rafts where acyl chains of the lipids tend to be more rigid and in a less fluid state. One important property of membrane lipids is their amphipathic character. Amphipathic lipids have a polar, hydrophilic headgroup and a nonpolar, hydrophobic region. Cholesterol can pack between the lipids in the rafts, serving as a molecular spacer, filling gaps between associated sphingolipids.

*Lipidomics* is the systematic study of pathways and networks of cellular lipids. The term *lipidome* is used to describe the complete lipid profile within a cell, tissue, or organism, and is a subset of the *metabolome*, which also includes the three other major classes of biological molecules: proteins/amino acids, sugars, and nucleic acids. Lipidomics is a relatively recent research field that has been driven by rapid advances in technologies such as mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, fluorescence spectroscopy, dual-polarization interferometry, and computational methods, coupled with the recognition of the role of lipids in many metabolic diseases such as obesity, atherosclerosis, stroke, hypertension, and diabetes. This rapidly expanding field is seen as a complement to the huge progress made in genomics and proteomics, all of which constitute systems biology. Yet, biology has evolved from simple lipid micelles in conjunction with that of cholesterol in response to rising oxygen tension in the atmosphere; we suggest that the lipidome may be the organizing principle for the other aspects of biology. This perspective is 180° out of phase with the conventional, descriptive way of thinking about biology and medicine, providing a novel perspective for the vertical integration of biologic data that is predictive.

Lipidomics research involves the identification and quantitation of the thousands of cellular lipid molecular species and their interactions with other lipids, proteins, and other metabolites. Investigators in lipidomics examine the structures, functions, interactions, and dynamics of cellular lipids and the changes that occur during perturbations of the system.

Han and Gross (2003) first defined the field of lipidomics through integration of the specific chemical properties inherent in lipid molecular species with a comprehensive MS approach. Although lipidomics is under the umbrella of the more general field of *metabolomics*, lipidomics is a distinct discipline because of the uniqueness and functional specificity of lipids relative to other metabolites.

In lipidomic research, a vast amount of information quantitatively describing the spatial and temporal alterations in the content and composition of different lipid molecular species is accrued after perturbation of a cell through changes in its physiological or pathological state. Information obtained from these studies facilitates mechanistic insights into changes in cellular function. Therefore, lipidomic studies play an essential role in defining the biochemical mechanisms of lipid-related disease processes by identifying alterations in cellular lipid metabolism, trafficking, and homeostasis. The growing attention on lipid research is also seen in initiatives under way such as the *lipid m*etabolites *a*nd *p*athways *s*trategy (LIPID MAPS consortium) and the *E*uropean *l*ipidomics *i*nitiative (ELIfe).

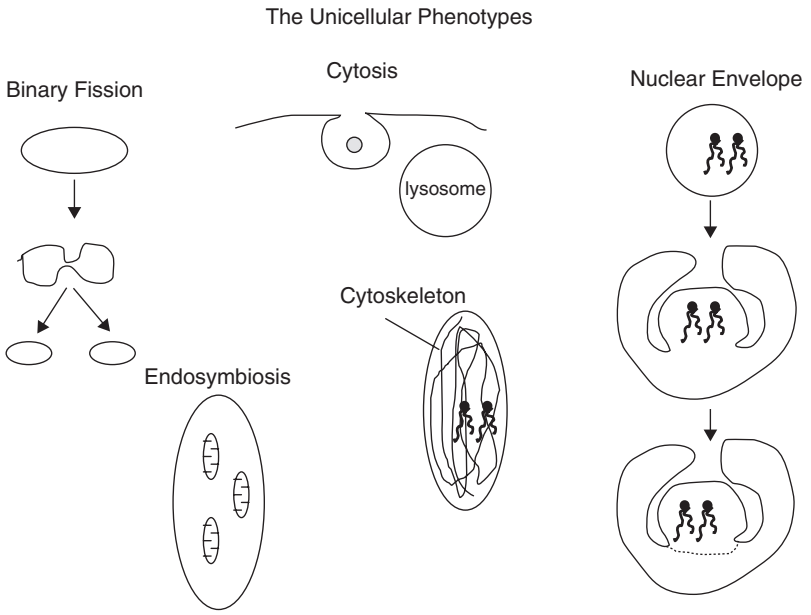


Figure 1.2. The endosymbiosis theory gives rise to specialized structures in unicellular organisms. The evolution of such structures as the mitochondria, cytoskeleton, and nuclear envelope may have resulted from cytosis.

## THE ENDOMEMBRANE SYSTEM

The evolution of the thinner, more fluid eukaryotic membrane may well have accommodated the engulfment of bacteria, otherwise known as the *endosymbiosis theory* (Fig. 1.2).

The eukaryotic cell contains a variety of membranes. In bacteria, there are ribosomes attached to the outer cell membrane, synthesizing proteins that form part of that membrane—for example, proteins involved in the passage of nutrients and waste products through the membrane, and digestive enzymes secreted into the surrounding medium. It has been speculated that when a food vacuole was formed in a primitive eukaryote, it resembled the cell membrane in its ability to synthesize digestive enzymes and absorb nutrients. This is no longer true of contemporary eukaryotes—there are no ribosomes attached to the cell membrane. As a result, a food vacuole formed from the cell membrane cannot digest the food it contains. Before food can be digested, the vacuole must combine with a lysosome, which contains digestive enzymes.

If the cell membrane lacks ribosomes, and therefore the ability to synthesize proteins, how does it grow? It essentially grows by a process that is the reverse of the formation of a food vacuole—a vesicle formed within the cell fuses with the outer membrane. These vesicles originate from the endoplasmic reticulum,

which is a system of double membranes within the cell. It consists of two membranes stuck to one another and formed by the flattening of spherical vesicles. Ribosomes are attached to the endoplasmic reticulum, so proteins can by synthesized. Vesicles of various kinds bud off of the endoplasmic reticulum, with the appropriate proteins embedded within them. Among these vesicles are those that fuse with the outer membrane and contribute to its growth, and also to the formation of lysosomes.

In eukaryotes there is a distinction between the cell membrane, lacking ribosomes, and the endoplasmic reticulum, which contains ribosomes and is therefore able to synthesize new cell membrane material. This may have been the first difference between these membranes as they evolved, but it has been followed by many others. The envelope surrounding the nucleus, like the endoplasmic reticulum, is formed from flattened vesicles, and so it consists of two adjacent membranes. The inner one has no ribosomes (there is no protein synthesis inside the nucleus); the outer membrane is continuous with the endoplasmic reticulum.

## THE CELLULAR MECHANISM OF EVOLUTION

According to the fossil record, unicellular organisms dominated Earth for the first 4.5 billion years after this planet formed as a result of the biologic "Big Bang," adapting to their physical environment in the process. Then some 500 million years ago multicellular organisms evolved from unicellular organisms through metabolic cooperativity (see Schematic in Fig. 1.1). The cause of this cooperativity may have been due merely to the chance failure of the daughter cells to separate after mitosis (Muller and Newman 2003), or to the ongoing competition between pro- and eukaryotes, referred to as the *endosymbiosis theory* (Margulis 1981), as described above, or more likely both. The subsequent symbiotic adaptation of bacteria, leading to the formation of mitochondria, provided the energy source for the emergence of multicellular organisms. Metabolic cooperativity, and the evolution of multicellular organisms was facilitated by the production of paracrine factors for cell–cell signaling. The target cell was near the signaling cell that mediated homeostasis in primitive multicellular organisms. Such paracrine mechanisms were subsequently exploited by eukaryotes in the emergence of multicellular organisms.

At this point, we should mention that specific unicellular traits were mimicked by multicellular organisms. The progressive increase in size was a survival strategy (Bonner 2000), in tandem with host defense mechanisms such as the nuclear envelope to safeguard and protect the increasing surface area. Such adaptations were undoubtedly fostered by the functionally linked increases in oxygenation and cellular metabolism, although this would have been limited by nutritional requirements, necessitating the evolution of mitochondria. At this stage in evolution, multicellularity may have evolved to accommodate the combined selection pres-

sures of the size–oxygenation–metabolism demands. After all, unicellular organisms had already immortalized themselves through the mechanism of binary fission. In response, multicellular organisms devised a way of immortalizing themselves through asexual and sexual reproduction.

## WHY EVOLVE?

With these concepts for the beginnings of life on Earth in mind, why would the process of evolution be necessary? To understand this mechanism, please refer to Figure 1.3.

As alluded to above, the formation of lipid micelles as semipermeable membranes would have generated an internal space in which catalysis would have reduced the entropy of the molecules within it. Over eons, these primitive cell-like entities would have adapted to their physical environment through communication, during which nucleic acids would have emerged to form the database for

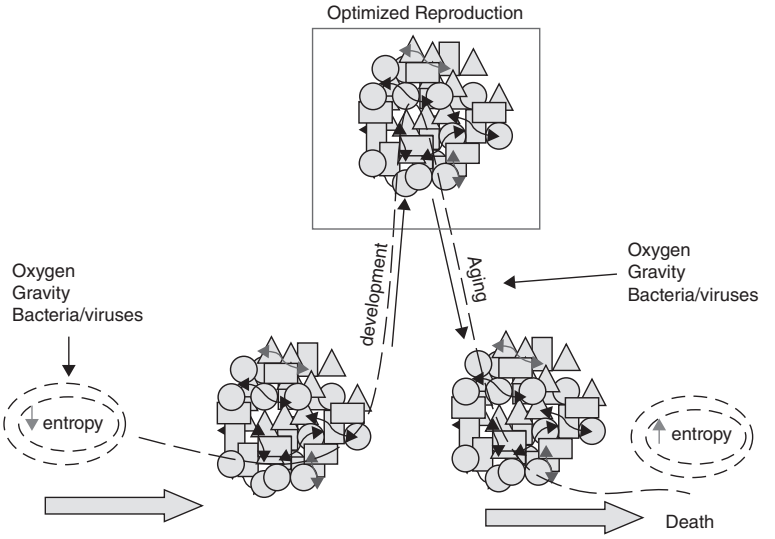


Figure 1.3. Evolutionary selection pressure, development, homeostasis, and aging. The schematic depicts the evolution of vertebrates, starting with the generation of micelles (far left), or semipermeable membrane spheres in which entropy decreased as a result of catalysis. Selection pressure due to external forces (oxygen, gravity, bacteria, viruses) gave rise to multicellular organisms through cell communication (depicted by arrow between cells). Cell–cell communication evolved into mechanisms of development, homeostasis, and repair, optimized (red box) for reproduction. Eventually this system fails as payback for defying the second law of thermodynamics, shunting energy allocation toward reproductive success, resulting in decreased cell communication (aging) and increased entropy (death). (See insert for color representation.)

storing biologic information, allowing the transfer of information from one generation to the next. This, and the advent of multicellular organisms made possible by cell–cell communication, have facilitated metabolic cooperativity. Therefore, the essence of evolution can be reduced to forms of communication: unicellular communication with the environment, cell–cell communication for multicellularity, and the communication of biologic information from generation to generation, or reproduction.

There is evidence for the continued existence of some of these properties in present-day organisms. The unicellular stage of slime molds, for example, is as free-swimming amoebas. But when there is a lack of food in the environment, the amoebas will form colonial organisms, signaling to one another through the generation of the chemical second messenger cyclic adenosine monophosphate. Similarly, sponges exist in an independent, unicellular form, generating the familiar bath sponge colonial organisms during their lifecycle. Importantly, Nicole King has shown that the unicellular form of the sponge has all of the genes necessary to form the colonial sponge, providing evidence that single-celled organisms probably evolved the metazoan toolkit (King et al. 2003).

Yet, this still does not explain the mechanism(s) of evolution. To answer that question, we can go back to the wetting and drying of lipids to form micelles, the wetting due to wave action, and the drying due to the heat from the sun. The drying would have been further affected by the 23.45° degree tilt of Earth's axis, causing seasonal variations, or the environmental "bias" (skewness) that resonates throughout animal life, as the determinant of evolutionary change according to Wallace Arthur (2004). *It is that resonance that this book seeks to identify*.

Moreover, the dynamic nature of Earth's atmosphere is due to the interaction between its organic and inorganic components. This is why evolution was necessary for life on Earth—consider a thought experiment in which an organism was perfectly adapted to its environment—that organism would have become extinct over generations because the environmental conditions that initiated and perpetuated life on Earth are constantly changing, requiring a mechanism for life to be able to adapt to such changes. Perhaps this is the mechanistic principle behind Darwin's metaphoric survival of the fittest.

## CELL–CELL COMMUNICATION AND AGING

Organisms have survived because they have devised adaptive genomes that allow them to change in response to the ever-changing nature of Earth's environments (please refer to Fig. 1.3). This has come in the form of their reproductive strategy, which is optimized to generate the largest number of offspring suited for the environment into which they are born. This comes at a cost, because the energy of reproduction is selected to optimize the organism's internal physiologic milieu, namely, the cell–cell signaling mechanisms of both somatic cells and gametes.

But that energy debt must somehow be repaid because the Second Law of Thermodynamics cannot be violated—the first and second laws of thermodynamics state that the total energy content of the universe is constant, and that total entropy is continually increasing.

This assumes that there is a finite amount of energy during the lifecycle. Hayflick (2007) has unequivocally stated that longevity is genetically determined, whereas aging is epigenetic. Therefore, by definition, there must be a finite amount of energy generated during the lifecycle of any organism, which is then distributed throughout the period between birth and death in response to selection pressure for reproductive success. As a result, the bioenergetics are optimized during the reproductive phase, followed by a progressive loss of energy during the postreproductive phase of life, leading to the breakdown in cell–cell communication, aging, and ultimately death, as a result of the progressive increase in entropy.

This mechanistic explanation for the process of aging is consistent with descriptive theories of aging such as the mutation theory, antagonistic pleiotropy, and the disposable soma, as follows.

## The Mutation Accumulation Theory of Aging

Peter Medawar (1952) thought that aging resulted from neglect. Since nature is highly competitive, and virtually all animals in nature die before they age, there is no selection advantage for traits that maintain life beyond the phase of the lifecycle when most animals would be dead, anyway, killed by predators, disease, or accidentally.

This theory, referred to as *mutation accumulation,* proposes that there are random, maladaptive mutations that occur late in life, unlike most deleterious mutations that are lost through the process of natural selection. As a result, they accumulate over time, causing the deterioration and damage that are conventionally attributed to aging. The mutation accumulation theory suggests that evolution is a function of the age at which the organism is able to reproduce. Traits adversely affecting an organism before that age would severely compromise the organism's ability to pass such characteristics on to its offspring. Therefore, such traits would be selected against. Characteristics that cause the same adverse effects beyond the reproductive phase would have relatively little effect on the organism's ability to reproduce, and therefore might be tolerated by natural selection. This concept fits well with the observed diversity in mammalian lifespans (and differing ages of sexual maturity), and is relevant to all of the other theories of aging discussed below.

## The Antagonistic Pleiotropy Theory of Aging

The mutation accumulation theory of aging was extended by George Williams (1957), who proposed that senescence might directly cause death. Senescence

would compromise the animal's ability to fight off predators, rendering them susceptible, whereas the younger animals would have been able to escape. Or senescence could undermine the animal's immunity, rendering it vulnerable to fatal infection. Williams thought that because nature was so highly competitive, due to the evolutionary process, even the smallest senescent changes in fitness would put one at risk in the wild.

Williams proposed the theory of antagonistic pleiotropy. *Pleiotropy* is defined as one gene having more than one phenotypic effect. *Antagonistic pleiotropy* means that some effects of the gene are beneficial while others are not. Such genes are selected for their beneficial effects early in life, but they then become deleterious in later life. Since evolution selects for reproductive success, the increased fertility could be selected for, but could result in premature death. Therefore aging is a natural consequence of adaptive physiology.

Antagonistic pleiotropy continues to be a popular and testable theory of aging. Williams' concept of aging has been validated by more recent studies of animal populations, in which senescence contributes substantially to the death rate. These studies, along with genomic studies, have undermined the mutation accumulation theory since the genes that cause aging are not random mutations. The aging genes are highly conserved across yeast, worms, fruit flies, and mice.

An inherent problem with antagonistic pleiotropy and other theories that assume that aging is an adverse side effect of some beneficial function is that the linkage between adverse and beneficial effects would need to be *rigid* in the sense that the evolutionary process would not be able to evolve a way to accomplish the benefit without incurring the adverse effect, even over a very long timespan. Such a rigid relationship has not been demonstrated experimentally and, in general, evolution is obviously able to independently and individually adjust myriad organismal characteristics.

## The Disposable Soma theory of Aging

First proposed by Kirkwood (1977), the *disposable soma theory* states that the body budgets the amount of energy available to it. The body uses nutrient energy for metabolism, reproduction, repair, and maintenance. Because of the limited supply of nutrients, the body compensates by doing none of these things optimally. By limiting the amount of energy used for repair, the body gradually deteriorates with age. The disposable soma theory is attractive because it seems intuitively correct, yet there is evidence that refutes this idea. Whereas lack of food should hasten aging, food restriction experiments have shown that experimental animals live longer. It is true that restricting food intake would slow metabolism, decreasing the damage done by free radicals, while the repair process remains unaffected. But dietary restriction has not been shown to increase lifetime reproductive success (fitness), because when food availability is low, reproductive output is also low. So food restriction does not completely disprove the disposable soma theory.

Experimentally, some animals lose fertility when their lifespan is protracted by food restriction, while others are unaffected. Males typically remain fertile when they are food-restricted, whereas females do not. Moreover, the females present a paradox because their loss of fertility does not correlate well with the increase in lifespan.

## The Cell–Cell Signaling Model of the Lifecycle and Aging

The success of the cell–cell interaction mechanism is in its proven ability to communicate knowledge acquired during the lifecycle from one generation to the next in adaptation to the ever-changing environment. As shown in Figure 1.3, agents that initially fostered evolution, such as oxygen, gravity, and bacteria, are omnipresent in the environment, but through the evolving reproductive strategy, organisms have adapted to their ever-changing environment. However, those same agents that gave rise to the evolutionary strategy continue to affect the organism throughout its lifecycle, so the system must fail, that is, age and die, as a result of the breakdown in cell–cell signaling as the mirror image of development and homeostasis. This phenomenon has been described as *molecular fidelity and antagonistic pleiotropy*. The difference between these metaphoric descriptions of aging and the model that we are proposing in Figure 1.3 is that the mechanistic hypothesis that the ligand–receptor mechanisms of development fail during biologic aging is testable and refutable. The implications of such a mechanism are manifold, not the least of which is the confusion between aging and cumulative pathology, as has been pointed out by Michael Rose (2009). Moreover, by determining how evolution has been optimized for reproductive success at the cell–molecule level, we can apply those same mechanisms to the postreproductive period to actively and effectively promote healthy aging based on tried and true evolutionarily adaptive biological mechanisms, rather than on *ex post facto* reasoning and results-oriented pharmacology.

As mentioned earlier, studies by King et al. (2003) have indicated that unicellular organisms had the entire genetic complement to form multicellular organisms, suggesting that the principles of evolution go all the way back to one-celled organisms. In the following chapters we will show how unicellular organisms generated multicellular organisms through cell–cell molecular coupling mechanisms acting as evolutionary transducers. As a result, the mechanisms of evolution are continuous from uni- to multicellular organisms; we will refer to those highly conserved mechanisms as the evolutionary *data operating system*.

In Chapter 2 we will describe the cellular–molecular basis for lung alveolar development and homeostasis, which forms the basis for our evolutionary approach to the physiology of the lung as the platform for other evolved physiologic traits. During the process of determining the cellular–molecular basis for alveolar physiology, we have come to the realization that cell–cell signaling mechanisms are far

more likely to have evolved under Darwinian selection pressure than as a result of random genetic mutations.

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