The Origin of the Eukaryotic Cell Based on Conservation of Existing Interfaces

Abstract  Current theories about the origin of the eukaryotic cell all assume that during evolution a prokaryotic cell acquired a nucleus. Here, it is shown that a scenario in which the nucleus acquired a plasma membrane is inherently less complex because existing interfaces remain intact during evolution. Using this scenario, the evolution to the first eukaryotic cell can be modeled in three steps, based on the self-assembly of cellular membranes by lipid-protein interactions. First, the inclusion of chromosomes in a nuclear membrane is mediated by interactions between laminar proteins and lipid vesicles. Second, the formation of a primitive endoplasmic reticulum, or exomembrane, is induced by the expression of intrinsic membrane proteins. Third, a plasma membrane is formed by fusion of exomembrane vesicles on the cytoskeletal protein scaffold. All three self-assembly processes occur both in vivo and in vitro. This new model provides a gradual Darwinistic evolutionary model of the origins of the eukaryotic cell and suggests an inherent ability of an ancestral, primitive genome to induce its own inclusion in a membrane.

Keywords  Evolution, nucleus, eukaryotes, self-assembly, cellular membranes

1 Introduction

The origin of eukaryotes is one of the major challenges in evolutionary cell biology. No intermediates between prokaryotes and eukaryotes have been found, and the steps leading to eukaryotic endomembranes and endoskeleton are poorly understood. There are basically two competing classes of hypotheses: the endosymbiotic and the autogenic. Endosymbiotic hypotheses posit that some membrane-bounded compartments within eukaryotic cells descend from other, once free-living cells that entered into the cytosol of their host as endosymbionts [16, 25–27, 31]. The exact nature of the host and endosymbiont(s) is currently unknown, and several hypotheses have been put forward [10, 26, 31, 38, 46, 47]. Autogenous, or karyogenic, hypotheses posit that the nucleus arose de novo in the cytosol, through the development of an endomembrane system that evolved into the nucleus. The endomembrane system could have evolved from a primitive endoplasmic reticulum (ER) [10, 43], or through invaginations of the plasma membrane [15, 65]. No conclusive evidence has been found to prove or disprove either theory.

In general, it is assumed that prokaryotes, being simpler organisms, evolved before eukaryotes. However, recent molecular genetic analyses point to an earlier origin of eukaryotes than prokaryotes [6, 21, 22, 27, 64] and challenge the idea that life evolved from simple prokaryotes to complex eukaryotes. Rather, they suggest that prokaryotes are not the earliest forms of modern life but rather the specialized derivatives of an earlier form [22, 57, 59]. This belief is based on the observations (see [58, 59]) that (a) eukaryotic metabolism has more relics of the RNA world, making a prokaryotic
model for the last common ancestor difficult to reconcile with the RNA world theory, (b) mRNA and rRNA processing is faster and more efficient in prokaryotes, indicating that the prokaryotic processing is the derived form, (c) there is no selective advantage for the recent origin of mRNA splicing and the spliceosome in eukaryotes, which suggests that the splicing machinery was part of the metabolic complexity of the RNA world, and (d) the presence of telomerases and their RNA components in eukaryotes favors an early origin for both telomeres and telomerase. Thus, the origin of the eukaryotic cell remains a matter of debate.

In endosymbiotic models, the evolution of the eukaryotic cell involved a continuous series of ancestors with a large number of intermediate states, all of which needed to be functional. Also, both the endosymbiotic and the karyogenic models require an extensive change in working processes, including those involved in transcription and translation that are physically separated in eukaryotic cells but not in prokaryotes [20]. A translocation of transcription mechanisms from the cytoplasm to the nucleus would require the implementation of a functional mRNA export mechanism and would involve a large number of functional intermediate states [1]. This transport of ribosomal subunits out of the nucleus through the nuclear pore is complex and involves many different proteins and transport mechanisms. It is unknown how these different steps in these translocation pathways were implemented starting from cytosolic transcription during the evolution of eukaryotes from their prokaryotic ancestors without affecting transcription or translation [1]. The transition to eukaryotic cells based on a model that would not affect or interrupt the basic transcription and translation processes would be intrinsically less complex and would provide a simpler mode of evolution.

2 The Exomembrane Hypothesis

Although transcription and translation are physically separated in eukaryotes, the ribosomal subunits are generated and assembled in the nucleus and later exported to the cytoplasm. Translation has, however, also been shown to occur in the eukaryotic nucleus [32], and the nucleus also contains all the necessary components for translation. The two ribosomal subunits are assembled in the nucleolus; translation, initiation, and elongation factors reside in the nucleus; and also the addition of amino acids to transfer RNAs can take place in the nucleus [30]. The nucleus can therefore be considered as an independent cellular unit, since all basic life processes are comprised in it. From this point of view, the transition from the nucleus to a primitive eukaryotic cell can be made by the acquisition of an extra outer membrane, or exomembrane (Figure 1). This sequence of events only implies the translocation of the site of actual translation and would not affect basic existing transcription and translation.

![Figure 1. Origin of the eukaryotic cell explained by defining the current nucleus as the ancestor cell. Transition to a eukaryotic cell could be made by the simple acquisition of an extra membrane, the future plasma membrane. This scenario only requires the physical translocation of the molecules involved in translation—ribosomes and RNA—outside the protocyte. This transition can be made gradually, as the basic mechanisms stay intact.](http://direct.mit.edu/artl/article-pdf/12/4/513/1662352/artl.2006.12.4.513.pdf)
mechanisms. In fact, it would leave the entire ancestor cell (or protocyte) intact and would therefore be relatively simple to implement.

In this exomembrane hypothesis, the steps in ribosome assembly and transport still follow their evolutionary sequence. Ribosomes are assembled in the nucleolus, where the primary RNA polymerase I transcript assembles with many different large and small subunit proteins to form a pre-90S particle. This transcript is subsequently cleaved, apparently an evolutionary adaptation to the incompatibility of the size of the pre-90S ribosome with the diameter of the nuclear pore, and is separately exported to the cytoplasm [1, 20]. The subunits of early ribosomes may have been small enough to fit through the first nuclear pores and so have been able to exit the cell by facilitated diffusion. The steps leading to protein synthesis outside the nucleus could then be gradually implemented by slowly phasing out nuclear translation while transport mechanisms were implemented.

2.1 The Nucleus as Independent Unit

The exomembrane hypothesis proposes that the predecessor of the eukaryotic nucleus once existed in a free-living form. Mitosis is one of the most striking features of the nucleus that shows its potential to function as an independent organism. There are basically two types of mitosis, closed mitosis and open mitosis. In open mitosis, the nuclear membrane dissolves in the pro-metaphase and reassembles in the telophase. In closed mitosis, observed in primitive protists, the nuclear envelope is present throughout the mitotic cycle and the final genome partition is accomplished with a mid-constriction of this envelope, giving rise to two daughter nuclei [9, 23, 29, 63]. It is proposed that the closed mitosis of protists reflects the division process of the protocyte, giving it the capability to function as a replicating organism.

There are numerous situations in which nuclei can function as independent units. Multiple nuclei come to be present within the confines of a single plasma membrane when coenocytes are formed as a result of the uncoupling of mitosis from cytokinesis. Examples of coenocytic or syncytial development are yeast spores, the multinucleate algae, the gametophyte tissues of higher plants, and the syncytial blastoderm in early Drosophila development (reviewed in [5]). The coenocytic nuclei in these organisms organize nucleocytoplasmic domains that can behave like independent structural entities, despite lacking any obvious physical borders. It has been proposed that in plant supracellular structures, the fundamental cellular unit is the nucleus with associated microtubules; it is called the cell body [4, 5, 48] and is similar to the protocyte. In multinucleate algae, the naked nuclei that arise after wounding can survive in seawater for 10–20 minutes, before they are surrounded by a gelatinous envelope, after which a new cell membrane is synthesized around each of these aggregates [37]. This is an example of transient survival of life without a cell membrane, and shows the potential of the nucleus itself to behave as an independent unit.

3 Evolution of the Eukaryotic Cell

3.1 From Chromosomes to the Nuclear Protocyte

The ability of membranes to self-assemble based on the interaction with proteins can provide a basic mechanism for the evolution to eukaryotic cells. The nuclear matrix is a scaffoldlike network of protein filaments surrounding the nuclear periphery and has a role in maintaining its shape and forming the nuclear envelope. It is intimately connected to both the chromatin and the nuclear membrane [71]. It is proposed that one of the fundamental steps in the evolution of life was the generation of a membrane structure around chromatin (Figure 2a), mediated by a primitive nuclear matrix and biomembranes that would already exist in an early protocell [11]. Since the formation of a tight lipid bilayer around the genome would prevent communication, a permeable membrane would have been a prerequisite to keep existing processes intact. This could be accomplished by the inclusion of (nuclear) pores in the developing membrane, as seen in eukaryotic cells. Thus, in the exomembrane scenario, chromatin induces its own inclusion in a porous nuclear membrane by the expression of proteins involved in nuclear membrane generation.
3.2 From the Nuclear Protocyte to the Eukaryotic Cell

The ER, the nuclear envelope, and the bacterial cytoplasmic membrane grow by the direct addition of lipids and proteins. The plasma membrane, however, grows exclusively by the fusion of ER-derived vesicles with the membrane [10, 60]. The phylogeny of small GTPases suggests that the first endomembranes had secretory function [34], indicating that vesicle budding was an early characteristic of a primitive ER. This suggests that the evolutionary origins of these membranes are different. This dependence on ER-derived vesicles for plasma membrane growth may indicate the evolutionary origin of the plasma membrane. Mechanistically, this would therefore place formation of the ER as an intermediate step between the protocyte and the eukaryotic cell. It is therefore proposed that the next step in the evolution towards eukaryotic cells was the proliferation of membrane from the outer nuclear membrane, giving rise to a primitive ER (Figure 2b), followed by the formation of the plasma membrane by fusion of vesicles.

The cytoskeleton is involved in the guiding or movement of vesicles shaping the cell [40, 66] and induces plasma membrane growth. The step from a protocyte with primitive ER to a eukaryote could be initiated or accompanied by the appearance of an extracellular cytoskeleton. Self-assembly of ER-derived vesicles along a network of cytoskeletal proteins would in effect generate an extra membrane around the protocyte, making a primitive eukaryotic cell with a nucleus (Figure 2b). In this way, the cytoskeleton would directly determine the cell boundaries or shape of the cell, illustrated by the intimate relation between the cytoskeleton and the plasma membrane in eukaryotic cells. This sequence of events follows the developmental mechanisms of the generation of the plasma membrane, which is exclusively fed by fusion of vesicles derived from the ER and golgi.

Figure 2. Mechanisms of evolution of the eukaryotic cell based on a self-assembly of membranes facilitated by membrane proteins. (a) Evolution of a nuclear protocyte by self-assembly of biomembranes on chromatin by an interaction with laminar proteins that couple the membrane to the chromatin. The process starts from an undefined protocell minimally consisting of chromatin and biomembranes in a protective environment such as a porous rock. This process is virtually identical to the regeneration of the nucleus in the mitotic telophase. The resulting double membrane is permeable or has primitive pores in order to preserve existing transport pathways. (b) Generation of a primitive ER from the outer membrane can be induced by the presence of membrane proteins similar to the formation of karmellae from the nuclear envelope and the self-assembly of the ER in general. Self-assembly of a plasma membrane on a cytoskeletal protein network can then form the eukaryotic cell. The initial formation and maintenance of the plasma membrane is similar to the mechanism of plasma membrane growth by the incorporation of vesicles as seen in eukaryotic cells.
4 Evolution on the Basis of Self-assembly of Membranes

4.1 The Self-assembly of the Nucleus
The exomembrane hypothesis is based on the enclosure of chromatin in a nuclear membrane. Basically, the fundamental required property of chromatin for self-assembly in a nuclear membrane can still be seen in vivo with every mitosis in eukaryotic cells when the nuclear envelope is reformed during telophase (see [24]). The in vitro assembly of functional nuclear pores is also well documented. A mixture of cytosol and membranes is able to form nuclei when chromatin is present [18, 41, 53]. In the presence of cytosolic extracts, a normal nuclear envelope can also form around DNA-coated magnetic beads, and formation of functional nuclear membranes can even occur in the absence of chromatin, showing the extreme robustness of nuclear envelope formation [28, 68, 79]. The ability of chromatin to govern the formation of its own nuclear membrane by the expression of proteins involved in membrane generation would be a solid basis for further development of cellular life.

4.2 The Self-assembly of the ER
The generation of an exomembrane or plasma membrane from ER-derived membrane vesicles requires that the ER evolved earlier than the plasma membrane. The ER is continuous with the nuclear envelope, and proliferation of membranes from the outer nuclear membrane has been shown to be a common mechanism in vivo. In yeast, characteristic multilayered structures that surround the nucleus, called karmellae, can be formed. Karmellae are continuous with the ER and nuclear envelope and can be induced by overexpression of a variety of integral membrane proteins [13, 56, 69, 78]. Networks of tubules can also be generated in vitro by incubation of microsomes with cytosolic factors [18, 39, 44]. The same fractions that give rise to ER networks can form nuclear envelopes when chromatin is added [19, 70], illustrating the similarity in these self-assembly processes. This shows that it is almost an intrinsic property of the nuclear membranes to form a primitive ER upon exposure to integral membrane proteins.

4.3 The Self-assembly of the Plasma Membrane
The last step in the proposed evolution of the eukaryotic cell is the generation of the plasma membrane based on fusion of ER-derived vesicles. There are many examples of the de novo generation of a plasma membrane. The de novo assembly of a unit membrane in the mother cell cytoplasm is for instance a unique feature of sporulation [62]. Similar phenomena exist in other biological systems, for example, the formation of the autophagosome in yeasts (reviewed in [54]), the formation of phragmoplasts in higher plants (reviewed in [52]), and the cellularization of syncytial blastoderm in Drosophila (reviewed in [49]). Interestingly, naked nucleocytoplasmic aggregates released from cut siphonous algae can even regenerate de novo a lost plasma membrane [37, 55, 61].

The involvement of the cytoskeleton in the formation of a plasma membrane is illustrated by the formation of a network of tubules in vitro by spontaneous hierarchical self-assembly of cytoskeletal filamentous actin (F-actin) and cationic lipid membranes [77]. The in vivo interaction with the cytoskeleton in the generation of the plasma membrane is illustrated in the cellularization of the syncytial blastoderm in Drosophila [24]. Each nucleus within the syncytial blastoderm is contained within its own little territory of cytoskeletal proteins [35]. The syncytium undergoes a process of cell formation, in which the individual nuclei become enclosed in individual cells. This process of cellularization is caused by a coordinated fusion of vesicles derived from the Golgi apparatus that generate a membrane that encircles the cell [8, 42, 49]. This process is similar to the evolutionary process described here in which a cytoskeleton precedes and guides the plasma membrane. Also, the individual nuclei in plant supracellular structures have radiating microtubules on the whole of their nuclear surface [4, 5, 48] that are similar to the proposed intermediate stage of a nucleus with associated cytoskeleton before the generation of the plasma membrane.
5 Discussion

5.1 Rooting of the Tree
The basic architecture of the nucleus, a functional genome enclosed by a double membrane, is identical to other ancient cellular life forms. Primitive true bacteria, such as proteobacteria, cyanobacteria, and green and purple bacteria, all belong to the gram-negative class of bacteria, characterized by their double membrane [7, 75], and also mitochondria and chloroplasts have a double membrane. It is therefore possible that the ancestor of the current eukaryotic nucleus has been the root of all cellular life. This is in agreement with recent molecular-genetic data suggesting that a eukaryotic ancestor preceded the prokaryotic bacteria in evolution. It is also compatible with data that has been put forward to prove an endosymbiotic origin of the nucleus, since in both cases the end result is a former prokaryote (cell without nucleus) that is enclosed by a plasma membrane.

Although based on completely different mechanisms, the exomembrane model does not exclude endosymbiotic events or fusion of endosymbionts with the nucleus and allows extensive gene transfer [17]. Also, the nuclear protocyte is not likely to be the origin of cellular life, since protein-membrane reactions require advanced processes that could probably arise only in an advanced stage of cellular life [11].

5.2 The Origin of Life: From an Open to a Semi-closed System
Some form of compartmentalization is a necessary prerequisite for maintaining the integrity of interdependent molecular systems that are associated with metabolism. However, membrane lipid bilayers form tight barriers, which require membrane-spanning proteins serving as carriers or channels that permit the transport of polar or charged molecules and ions. The appearance of such a closed system would therefore have required the simultaneous evolution of membrane permeability to permit uptake of nutrients and secretion of waste products [73]. The scenario sketched in this article starts with a semi-open system that consists of a microenvironment with genetic material, biomembranes, and a primitive nuclear matrix, a prerequisite of the first cellular life [11]. The exomembrane hypothesis proposes that also the next stage in the evolution of cellular life, the nuclear protocyte, was not a completely closed system but contained pores and would therefore not affect existing transport of metabolites.

From the energetically disadvantageous situation of a semipermeable system of the nuclear protocyte, two basic directions can be taken. First, the further regulated closure by the development of specific membrane transporters as seen in prokaryotes, driven by the evolutionary advantages of a semi-closed system, which could evolve into prokaryotes and primitive mitochondria and chloroplasts. Second, the development of an extra plasma membrane as seen in eukaryotes that would ensure the required compartmentalization of complex life. In the latter case, an early symbiosis of ancient mitochondria could replace a primitive ATP-generating system in the protocyte through the existing semipermeable nuclear pores. This dependence on external energy sources would also prohibit the closure of the nuclear pores. During the development of the eukaryotic cell, chloroplasts and mitochondria could have been recruited to the exocytoskeleton, even before the plasma membrane evolved, thereby ensuring continuous supply of energy to the nuclear protocyte.

The established (passive) flow of nutrients can remain intact when the first membranes that would be formed were permeable to most of these molecules. This could have been accomplished by the introduction of large non-selective pores that would ensure passive transport of molecules in concert with ongoing compartmentalization. Primitive pores would then be a prerequisite for the development of the first protocyte by the enclosure of genetic material by lipid bilayers. The nuclear pore complex as seen in all eukaryotes with a molecular size limit of about 5 kDa would ensure passive diffusion of most nutrient and waste products, while establishing a base for the containment of larger macromolecules such as ribozymes or proteins. The inclusion of pores in the protocyte would allow the continuous supply of energy sources. The functional link that the integral membrane...
nucleoporin pom121 has in nuclear pore complex assembly and nuclear envelope formation [2] may illustrate the need for pores in an otherwise closed system.

5.3 Evolutionary Driving Forces
The acquisition of an extra membrane and cytoplasm would give the potential to develop a range of new processes, for instance the functional separation of transcription from translation, that would increase the complexity of cellular life. The recruitment of the extracellular space and the resulting increase in cellular complexity may therefore represent the driving force for the evolution of the eukaryotic cell. The genetic complexity of eukaryotes, which allowed the differentiation of cells into the complex tissues and organs seen in higher organisms, can be further increased by symbiotic interactions, polyploidy, and genetic recombination (see [12, 65, 76]). Thus, the increase in cellular complexity may have been an important driving force for the evolution of the eukaryotic cell and multicellularity.

Another of the essential characteristics and requirements for cellular life is a regulated microenvironment, or closed system, for example a tight membrane with transporters and channels. The proposed steps towards the evolution of the eukaryotic cell show a gradual process towards such a closed system. The proposed transition from a simple protocyte to a eukaryotic cell may have been initiated by the development of a nuclear matrix and lamina. The nuclear matrix is an ancestral nuclear protein scaffold in eukaryotes that serves in essential housekeeping functions for the cell and has been very well conserved throughout the eukaryotic kingdom [51, 63, 72, 74]. Such a protein scaffold could give protection to ancient DNA and provide a microenvironment that would give structural support. This evolutionary advantage to develop a nuclear matrix would in a later stage provide the substrate for the first membrane biogenesis.

The cytoskeleton is a eukaryote-specific attribute, and its most basic components, tubulin and actin, have an ancient origin, for homologues are found among prokaryotes [17, 45, 47, 67]. The formation of a cytoskeletal exoskeleton could also help to provide a microenvironment around the protocyte, giving protection, support, and a buffer region around it. The evolutionary acquisition of a plasma membrane enables a tightly regulated environment around the nucleus: the cytoplasm. In some algae, the primary envelope that surrounds naked nuclei and is formed after injury has some characteristics of a cell membrane, including semipermeability and selective transport of materials [37]. This indicates the potential for non-membrane structures to form a microenvironment and could provide the driving force for translocation of protein translation outside the nuclear protocyte.

The generation of membranes from the outer membrane requires membrane protein targeting to this outer nuclear membrane. The nuclear pore complex provides a way of connecting the inner and outer membrane, and membrane proteins can diffuse freely through the pore membrane domain between the inner and the outer membrane [23, 33, 68, 72]. Therefore, the development of a nuclear pore complex, starting with the existing interaction from laminar proteins and the nuclear membrane, could have triggered the outer membrane’s expansion into a primitive ER. Although the connection of the inner nuclear membrane with the outer membrane and the ER could have a function in shuttling membrane proteins from the ER to the inner nuclear membrane, it could also aid in the creation of a protective microenvironment around the nucleus.

5.4 Evolution of Membrane Proteins
It has been shown that many of the protein components of the nuclear pore complex and nuclear envelope are paralogues descending from a limited set of ancestral forms and that many of these forms have a relative among prokaryotes [43]. Components of coated vesicles and nuclear pore complexes share a common architecture of beta-propeller and alpha-solenoid folds. These motifs have a role in membrane curvature and are proposed to be involved both in the curvature of the membrane in nuclear pores and in the ER-to-Golgi signaling pathway (COPII pathway) [3, 15, 36, 73]. Curvature of membranes is involved in the formation of pores in the nuclear membrane, the generation of ER stacks, and the budding of vesicles from the ER that later fuse with the plasma membrane. Therefore,
the proposed introduction of nuclear pores could have later been followed by the ability to form a primitive ER and budding of vesicles from the ER. Thus, one protein family can be involved in many of the hallmarks of the eukaryotic cell: the nuclear membrane, the ER, and the plasma membrane.

5.5 Design by Contract as a Framework

The new mechanism proposed here is based on an evolutionary model in which self-contained modules interact with each other by constant interfaces [14], a general method for reducing complexity and could give evolution the necessary robustness, flexibility, and extensibility. This design pattern is abstracted in the software development methodology known as design by contract [50] in which the interface is viewed as specifications of the mutual obligations, or contracts. The effect of constant interfaces across self-contained modules is a reduction of the interdependences across modules or components and a reduction of the risk that changes within one module will create unanticipated changes in other modules. The constancy of interfaces in evolution is enforced by the dependence of all downstream processes on an established interface. Self-assembly processes are by definition self-contained and they decrease complexity by reducing dependences on external factors. The proposed sequence of events during the acquisition of an exomembrane, including the export mechanism of ribosomal subunits, is in line with a model based on the conservation of existing interfaces during evolution. The evolutionary model of self-contained modules that interact with conserved interfaces can provide a general framework for the creation of artificial life.

6 Conclusion

A model for the origin of the eukaryotic cell is proposed that does not require drastic changes in existing interfaces and provides a Darwinian gradualism in the evolution of the eukaryotic cell. The logical sequence of events is based on self-assembly mechanisms that are driven by the evolutionary advantages of the creation of microenvironment and a drive towards greater cellular complexity. It shows that the ability to form a robust form of cellular life could be initiated by the genetic information carriers after inducing the generation of its own nucleus and plasma membrane. The evolution of a plasma membrane would enable the development of a complete new set of processes and interfaces based on a cytoplasm and plasma membrane. This would give the eukaryote the required flexibility and extensibility for evolutionary development and development of multicellular organisms.

References


