Eukaryogenesis as a Process of DNA Packing: Review and Integration

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Review

Eukaryogenesis as a Process of DNA Packing: Review and Integration

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Abstract

The origin of eukaryotes represents one of the most profound unresolved questions in evolutionary biology. Although more than a dozen competing hypotheses have been put forward, no unified consensus has yet been reached. This paper critically reviews the major endosymbiotic and non-endosymbiotic theories, revealing a critical limitation shared by most existing models: the nearly 2.5-order-of-magnitude difference in genome size between prokaryotes and eukaryotes cannot be sufficiently explained by stochastic endosymbiotic events alone. I propose a new integrative framework in which eukaryogenesis is viewed as a process of subcellular structuralization driven primarily by DNA packing. This framework reconciles contradictions in current theories by clarifying the dynamic coupling between genome expansion induced by rising atmospheric oxygen and the emergence of sophisticated DNA packing mechanisms, which together supported the increase in cellular complexity, genome enlargement, and ordered biochemical processes. This study seeks to resolve long-standing controversies among competing paradigms, establish a robust theoretical framework for reconstructing eukaryogenesis, and provide critical direction for future interdisciplinary investigations.

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1. Introduction

The evolution of life on Earth has spanned approximately 4 billion years, progressing from the chemical evolution that preceded the origin of primitive life, through the era of prokaryotic dominance, to the eventual emergence and radiation of eukaryotes [1–5]. Each of these pivotal transitions has profoundly reshaped the structure and function of Earth's ecosystems [6,7]. The fundamental distinction between eukaryotes and prokaryotes lies in the presence of a nucleus, an endomembrane system, and semi-autonomous organelles such as mitochondria (or hydrogenosomes) [8]. These characteristics endow eukaryotes with enhanced environmental adaptability, more complex gene regulatory networks, and the evolutionary potential to develop multicellularity [9–11].

The evolutionary transition from prokaryotes to eukaryotes is termed eukaryogenesis. During eukaryogenesis, various mechanisms shaped the evolving eukaryotic genome, including HGT, EGT, gene duplication, gene loss and new gene creation, and accompanied by the invasion of introns and mobile selfish elements [12]. Molecular clock and fossil evidence indicate that the Last Eukaryotic Common Ancestor (LECA) emerged between approximately 1.0 and 2.7 billion years ago [13–18], though such estimates remain controversial [19,20]. The evolution of eukaryotes' core complex traits is closely linked to major shifts in Earth's environment (e.g., atmospheric oxidation events) [11,21–24]. The origin of eukaryotes represents a critical leap from the simple structural organization of prokaryotes to the complex compartmentalized architecture of eukaryotic cells, laying the foundation for the subsequent rise of multicellular organisms and the explosion of species diversity. It thus stands as a key milestone in the history of life on Earth [25–28].

The origin of eukaryotes has long been a focal point and major challenge across multiple disciplines, including evolutionary biology, cell biology, and paleontology [29–31]. Divergent perspectives on this issue have given rise to two primary theoretical paradigms: endosymbiotic theories and non-endosymbiotic theories. Endosymbiotic theories emphasize the role of symbiosis between distinct prokaryotic lineages, while non-endosymbiotic theories focus on intrinsic evolutionary processes within ancestral prokaryotes [32–34]. With the widespread application of technologies such as metagenomics, synthetic biology, and high-precision molecular clocks, research on eukaryogenesis has entered an era of interdisciplinary integration [35–37]. This has yielded a wealth of new evidence for various hypotheses, significantly enriching the theoretical framework underlying the study of eukaryotic origins.

Despite the proposal of over a dozen mainstream hypotheses [38,39], the evolutionary scenarios put forward to date remain far from consensus, and no single framework has gained broad acceptance. Intense debates persist regarding the driving mechanisms of nuclear origin, the timing of key evolutionary nodes, and the formation mechanisms of core eukaryotic structures [40,41]. In particular, most eukaryogenetic hypotheses fail to advance convincing selective forces to explain why the nucleus evolved [42]. Although a handful of studies have touched upon the origin of nucleus [12], the issue of DNA compaction remains entirely unaddressed. Resolving these controversies will require not only technological innovations and the integration of interdisciplinary experimental evidence but also the construction of a unifying theoretical framework. Against this backdrop, the present paper first systematically reviews the two core theoretical frameworks of eukaryogenesis, then conducts an in-depth analysis of key contentious issues, highlights the limitations of existing theories, and finally focuses on the major challenges and breakthrough directions for future research. The goal is to provide a theoretical basis for a comprehensive understanding of this pivotal event in the evolution of life.

2. Two Paradigms of Eukaryogenetic Hypotheses

Over a dozen hypotheses regarding the origin of eukaryotes have been proposed, which can be categorized into two paradigms: symbiotic theories and non-symbiotic theories. These two paradigms focus on two core dimensions—"foreign cell integration and organelle origin" and "intrinsically driven differentiation of primitive cells"—jointly constructing a comprehensive theoretical framework for research on eukaryogenesis.

2.1. Symbiotic Paradigm

Symbioses, including endosymbioses, are not established within a single generation; instead, they evolve gradually over extended periods as symbiotic partners undergo adaptive modification and engage in genetic exchange via canonical horizontal gene transfer mechanisms: conjugation, transformation, and viral transduction [43].

2.1.1. Serial Endosymbiotic Theory

First proposed by Mereschkowski [44] for chloroplasts and Wallin [45,46] for mitochondria, the serial endosymbiotic theory was further refined by Margulis [47]. It posits that aerobic bacteria and cyanobacteria were phagocytosed by primitive eukaryotic ancestors and evolved into mitochondria and chloroplasts, respectively, through long-term symbiotic interactions [48]. Key supporting evidence includes: bacterial-like DNA in these organelles; ribosomes analogous to those of bacteria (which are susceptible to bacterial antibiotics); reproduction via binary fission; and phylogenetic evidence linking mitochondria to α-proteobacteria and chloroplasts to cyanobacteria [49,50].

2.1.2. Early vs Late Mitochondria Models

Most proposed explanations for endosymbiosis center on the timing and mechanisms of protomitochondrion acquisition, as well as its relevance to the origin of defining eukaryotic characteristics. These hypotheses are broadly categorized into two competing models. The mito-early model posits that mitochondrial endosymbiosis preceded the evolution of complex eukaryotic traits, such as the nucleus and cytoskeleton [51–54]. In contrast, the mito-late model argues that core eukaryotic features had already emerged before the integration of the mitochondrial endosymbiont [55,56]. Key lines of supporting evidence include the genetic and metabolic chimerism of eukaryotes—reflecting their mixed archaeal and bacterial ancestry—and the mosaic distribution of genes horizontally transferred from the bacterial endosymbiont to the host genome [54].

2.1.3. Metabolic Symbiosis Hypothesis

Several symbiogenetic models have been proposed, differing in metabolic interactions and the tempo and mode of key eukaryotic trait evolution [57–60].

① Searcy's model

This model proposes that eukaryotes originated from a sulfur-mediated symbiosis between a wall-less, sulfur-respiring Termoplasma-like archaeon and a photo- or chemoautotrophic H_2S-utilizing bacterium [61,62].

② Hydrogen hypothesis

This hypothesis suggests a hydrogen-mediated symbiosis between a hydrogenoclastic methanogenic archaeon and a hydrogen-producing α-proteobacterium, where hydrogen reduced the bacterium-released CO_2 for methanogenesis [51]. A recent revision replaces the methanogenic host with an autotrophic, non-methanogenic archaeon that uses the Wood–Ljungdahl pathway to fix carbon via hydrogen from the mitochondrial ancestor [63].

③ Reverse Flow model

This model posits that eukaryogenetic syntrophy relied on hydrogen (or electrons via formate/acetate) transfer from an anaerobic heterotrophic archaeon to an α-proteobacterium [64].

④ Entangle–Engulf–Endogenize (E^3) model

In microoxic environments, an Asgard archaeon (degrading amino acids to short-chain fatty acids and hydrogen) forms a dual symbiosis with a sulfate-reducing bacterium (SRB) and an aerobic organotrophic α-proteobacterium (scavenging toxic O_2). As oxic levels increase, the α-proteobacterium is engulfed, while the transient SRB symbiosis is eventually lost [65].

⑤ HM Syntrophy hypothesis

This hypothesis assumes a tripartite symbiosis: first, H_2 transfer syntrophy between a fermentative, ancestral sulfate-reducing myxobacterium (δ-proteobacteria) and a methanogenic archaeon; second, stable incorporation of a versatile α-proteobacterium (facultative aerobic respiration, methanotrophy) into the consortium [66,67].

⑥ HS Syntrophy hypothesis

This model proposes symbiosis between a hydrogen-releasing Asgard archaeon (degrading small organics) and a myxobacterial-like δ-proteobacterium (scavenging hydrogen for sulfate reduction). The mitochondrial α-proteobacterial ancestor was a sulfide-oxidizing facultative aerobe (recycling sulfur) and possibly a mixotrophic anoxygenic photosynthesizer using H_2S as an electron donor [58].

Modern eukaryotic metabolism preserves information about the nature of the original asgardarchaeal–alphaproteobacterial interactions and supports syntrophy scenarios [68]. Synthetic biology studies have demonstrated compatibility between eukaryotic nucleosomes and bacterial chromosome machinery [37], and membrane remodeling has been observed during syntrophic interactions in archaeal-bacterial co-cultures [69]. Yet, symbiosis theory fails to explain how the eukaryote condition itself (that is, the nucleus) evolved [70]. Archaeal ancestors of the eukaryotes show no evidence of a nucleus [71]. The archaeal-origin eukaryogenesis scenarios annot explain several eukaryote-specific features of the last eukaryotic common ancestor, such as glycerol-3-phosphate-type membrane lipids, large cells and genomes, and endomembrane formation [72].

2.1.4. Inside-Out Model

This model posits that a progenitor prokaryotic cell—homologous to the nucleus of modern eukaryotes—extruded membrane-bound blebs outward across its cell wall. These blebs mediated material exchange with ectosymbiotic proto-mitochondria. Subsequently, the eukaryotic cytoplasm arose from the expansion of these blebs to enwrap proto-mitochondria; the interstices persisting between adjacent blebs then differentiated into the endoplasmic reticulum, a precursor of the eukaryotic secretory system. Further fusion of these blebs generated a continuous plasma membrane, which sequestered the endoplasmic reticulum from the extracellular environment [73,74].

2.1.5. Viral Eukaryogenesis Model

This model suggests that the eukaryotic nucleus originated from the symbiotic integration of large double-stranded DNA viruses (e.g., nucleocytoplasmic large DNA viruses, NCLDV) with primitive archaea [75–78]. Following infection, the virus co-opted the host’s metabolic machinery, with its capsid evolving into a primitive nuclear membrane; integration of host and viral genomes ultimately led to the formation of the nucleus. While, mitochondrial acquisition occurred subsequent to nucleus formation.

2.2. Non-Symbiotic Paradigm

2.2.1. Autogenous Model

This model proposes that eukaryotic cells evolved from prokaryotic cells (bacteria) by an autogenous process of complexification, involving the development of already existing elements or the evolution of new ones in an originally prokaryotic cell, for example, not only the endomembrane system and the nucleus, but also membranous organelles such as mitochondria and plastids, would derive from thylakoids in a cyanobacterial-like ancestor of eukaryotes [70].

Cavalier-Smith [70] suggests that the evolution of phagocytic capability in a wall-less cyanobacterium generated selective pressures that directly drove the emergence of all hallmark eukaryotic organelles and cellular traits . Its core significance lies not in enabling endosymbiont sequestration (endosymbiosis is merely a secondary, nearly inevitable byproduct of phagocytosis, not the trigger of eukaryogenesis) but in providing both the selective pressure and physical machinery (membrane budding and fusion) required for intracellular membrane-based compartmentalization. This compartmentalization framework offers a more parsimonious explanation than the endosymbiosis theory for both the origins and defining properties of mitochondria, plastids, and the nucleus.

2.2.2. Exomembrane Hypothesis

This hypothesis postulates that the evolutionary transition toward the first eukaryotic cell can be delineated as a three-step model, driven by the self-assembly of cellular membranes via lipid-protein interactions. First, the sequestration of chromosomes within a nuclear envelope is mediated by interactions between lamin proteins and lipid vesicles. Second, the biogenesis of a primitive endoplasmic reticulum (or exomembrane) is triggered by the expression of integral membrane proteins. Third, a plasma membrane is generated through the fusion of exomembrane vesicles on a cytoskeletal protein scaffold. Notably, all three self-assembly processes are recapitulated both in vivo and in vitro. This novel framework establishes a gradual Darwinian evolutionary paradigm for eukaryotic origins, while also implying that an ancestral primitive genome inherently possessed the capacity to direct its own encapsulation within a membrane-bound compartment [79,80].

2.2.3. Eukaryomorpha Hypothesis

According to this hypothesis, the Asgard archaea are more appropriately categorized as early-diverging lineages within the broader clade Eukaryomorpha—a taxon that also encompasses eukaryotes. Subsequent to the evolutionary split between Asgard archaea and the eukaryotic lineage, stem eukaryotes underwent extensive acquisition of derived phenotypic traits, among which mitochondria represent a key innovation; these characters collectively defined the archetype of the modern eukaryotic cell [81].

2.2.4. Thermotogales Hypothesis

According to this hypothesis, the spheroidal cells of Thermotogales that produce immature progeny are considered proto-eukaryotes. Multicellularity, which arises within the intermembrane space between the inner and outer membranes, facilitates the formation of large genomes with multicopy gene integration. Furthermore, ancestral Thermotogales spheroids acquired archaeal genes via horizontal gene transfer (HGT), thereby evolving into the first eukaryotic common ancestor (FECA). Within the FECA, an archaeal-derived cellular system emerged through the random assembly of DNA fragments, which was subsequently followed by genomic reorganization. Importantly, this process of random DNA assembly is postulated to have provided the core molecular mechanisms that fueled the early evolution of eukaryotes [72].

2.2.5. Nuclear Pore Timing Hypothesis

This hypothesis argues that organisms on the path from the first eukaryotic common ancestor to the last common ancestor of all eukaryotes did not possess a nucleus at all—at least not in a form we would recognize today—and that the nucleus in fact arrived relatively late in the evolution of eukaryotes. The clues to this alternative evolutionary path lie, most of all, in recent discoveries concerning the structure of the nuclear pore complex [82].

2.2.6. DNA Packing Hypothesis

Wang et al. [11] focused on genome packaging and atmospheric oxygenation, proposing four key points: (1) atmospheric oxygenation provided the energy required for the evolution of genome complexity; (2) innovations in genome packaging overcame the size limitations of prokaryotic genomes; (3) the formation of the nuclear membrane and an increase in chromosome number enabled orderly management of complex genomes (coordinated with oxygenation-driven metabolic expansion); (4) protein neofunctionalization supported chromatin compaction. Supporting evidences include: the emergence of eukaryotes coinciding with a critical C-value threshold (a 2.5-order-of-magnitude leap in genome size from prokaryotes); and high chromosome compaction ratios in eukaryotes (10,000:1 in humans and up to 65,600:1 in fungi).

3. Genomic and Structural Debates on Eukaryogenesis

3.1. The Phagocytosis Paradox: Limits of the Endosymbiotic Narrative

The endosymbiotic theory offers a robust account for the acquisition of energy-producing organelles [83]. However, extending this framework to explain the origin of the nucleus gives rise to a critical biological paradox [84]. If eukaryotes emerged through phagocytosis, the host cell must have been a phagocyte—yet phagocytosis is a complex eukaryotic trait that itself depends on a sophisticated cytoskeletal network and membrane trafficking system. Further compounding the issue is the challenge of genetic integration: phagocytosed DNA is typically degraded in the host cell. Even if such DNA persists, a fundamental question remains unresolved: What was the origin of the phagocyte’s own nucleus?

Alternative hypotheses have been proposed to resolve this paradox, including the fusion of archaeal and bacterial genomes to form a karyomastigont complex [83] and the endosymbiosis between methanogens and myxobacteria [85]. Nevertheless, these models fail to address key evolutionary transitions, such as the transformation of cell wall architectures—from the flexible membranes of animal-like phagocytes to the rigid cell walls of green algae. While symbiosis

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undoubtedly facilitates horizontal genetic exchange [28], it is insufficient to explain the de novo assembly of the nuclear compartment, a defining feature of eukaryotic cells.

3.2. Genomic Mosaics and the Limits of Phylogeny

Genomically, eukaryotes display a distinct "mosaic" architecture: information-processing genes share homology with those of Archaea, while metabolic genes cluster phylogenetically with Eubacteria [86]. This pattern has led to the widely accepted view of eukaryotes as genetic chimeras. However, the pervasive nature of LGT complicates this simplistic characterization [87–92]. Microorganisms operate as transient genetic collectives, with genes flowing freely across traditional phylogenetic boundaries [93]. As a result, conventional phylogenetic analyses face significant challenges in delineating a single, unambiguous Last Eukaryotic Common Ancestor (LECA).

Structurally, a profound discontinuity persists between these two domains of life. Prokaryotes organize their DNA into nucleoids via nucleoid-associated proteins (NAPs), whereas eukaryotes employ histones to assemble nucleosomes [94]. This fundamental distinction in DNA packaging indicates that the evolution of the eukaryotic genome entailed more than mere gene acquisition; it necessitated a comprehensive restructuring of the genetic storage and organization system.

3.3. Genome Expansion and the Need for Ordering

The transition to eukaryotes was inextricably linked to genome enlargement. Yet, to date, few hypotheses have addressed the striking C-value disparity (i.e., the difference in genome size) between prokaryotes and eukaryotes—a gap of nearly 2.5 orders of magnitude. This divergence reflects an evolutionary trajectory driven by processes such as replication errors, polyploidization, and lateral gene transfer (LGT) [88]. Prokaryotes prioritize replication speed, achieved through coupled transcription and translation, whereas eukaryotes evolved specialized mechanisms to manage their increasing genetic complexity. Key pathways driving genome size expansion are known to include polyploidization—either autopolyploidy resulting from failed cell division or allopolyploidy arising from interspecific hybridization—and intercellular recombination [95,96].

It is hypothesized that the evolutionary transition from prokaryotes to eukaryotes may have critically depended on the relocation of ATP synthesis from the cell surface to mitochondrial membranes, which in turn boosted the energetic capacity of eukaryotic cells [97]. While the causal relationship between this relocation and genome expansion remains debated [98], the rise in atmospheric oxygen levels—a byproduct of cyanobacterial photosynthesis—furnished the energetic surplus necessary to sustain these information-dense eukaryotic genomes [99–101].

The evolution of the nuclear membrane, which is derived from prokaryotic precursor genes [102], was not merely an adaptive trait but a biological imperative. This structure created a protected compartment for the genome, thereby facilitating the emergence of introns, alternative splicing, and elaborate regulatory networks—all of which underpin the complexity that distinguishes eukaryotes from prokaryotes [103].

3.4. The Solution: Hierarchical DNA Packing

Genome expansion posed a physical conundrum: how to encapsulate meters of DNA within a cell measuring just a few micrometers. Eukaryogenesis addressed this challenge through a sophisticated hierarchical DNA packing mechanism, which proceeds in distinct stages:

First, nucleosomes (forming the 10 nm fiber) are assembled when DNA wraps approximately 1.75 turns around histone octamers—composed of two copies each of histones H2A, H2B, H3, and H4—resulting in the characteristic "beads-on-a-string" structure and achieving a 7-fold compaction of the DNA. Next, the solenoid (30 nm fiber) forms as nucleosomes coil into a helical conformation, a process mediated by the linker histone H1, which further compacts the DNA by an additional 6-fold. Finally, ATP-dependent Condensin complexes and structural maintenance of chromosomes

(SMC) proteins drive the formation of topologically associating domains (TADs) and supercoiled loop domains, which ultimately organize into chromatids [104,105].

Genome regulation in eukaryotes centers on the nucleosome—the fundamental building block of eukaryotic chromatin [106]. A pivotal evolutionary innovation in this context was the emergence of histone variants, such as H2A.Z, which selectively modulates chromatin accessibility to fine-tune gene expression [93].

Collectively, this hierarchical packing process achieves a total DNA compaction of approximately 8,400-fold, converting disorganized DNA strands into discrete, structurally stable chromosomes. This elaborate structural organization is indispensable for maintaining genome integrity and enabling precise spatiotemporal gene regulation [107].

The evolution of the nucleus further complemented this packing system by compartmentalizing the expanded genome. This spatial separation segregates the DNA "library" from the ribosomal "factory," mitigating transcriptional conflicts and laying the molecular foundation for the development of complex multicellular organisms.

3.5. Segregation: From Membrane Anchors to Mitotic Spindles

Accurate genetic segregation represents the ultimate challenge of cell division. While prokaryotes utilize simple membrane anchors and segregation motors, the eukaryotic transition—with its requirement to segregate multiple linear chromosomes simultaneously—demanded the evolution of the mitotic spindle [108–110].

A central innovation in this process was the modulation of the nuclear envelope, which in many lineages involves dissolution and reformation to permit microtubule-kinetochore attachment [41,111]. Transitional forms, such as the membrane-bounded nucleoids found in Planctomycetes, imply that intracellular compartmentalization arose prior to the emergence of true mitosis [94]. The spindle apparatus thus constituted a critical evolutionary leap, guaranteeing the precise transmission of the complex eukaryotic genome. Building on this foundation, the subsequent appearance of meiotic sex introduced a mechanism for genetic diversity, marking a pivotal milestone in eukaryotic evolution [112].

3.6. Not a Single Event but Multiple Steps

Eukaryogenesis was likely not a singular event—such as a chance occurrence of phagocytosis or endosymbiosis—but rather a cascade of structural adaptations triggered by genomic complexification. This process can be viewed as a sequence of evolutionary pressures and responses:

- **Genome Expansion**: Replication errors and fusion events increased DNA length beyond the organizational limits of prokaryotic architectures.
- **Structural Accommodation**: To manage this length, the cell evolved histones and chromatin folding.
- **Compartmentalization**: A nuclear membrane formed to protect and organize this complex genome, thereby establishing the nucleus.
- **Mechanical Innovation**: The cytoskeleton and mitotic spindle evolved to manage the increased cellular volume and ensure precise chromosome segregation.

Consequently, the eukaryotic cell is a product of cellular modularization. The nucleus is not merely a passive container, but a regulatory hub that enables the precise control of gene expression necessary for development and environmental response. Thus, eukaryogenesis represents an evolutionary trajectory of order emerging from genomic complexity.

4. Integration of Eukaryogenetic Theories

Although many competing models of eukaryogenesis have been advanced, each explaining certain facets of the process, a unified consensus remains lacking. A defining characteristic of eukaryotic genomes is their substantial DNA length, which requires extensive condensation to form chromosomes. To date, however, only one hypothesis has explicitly incorporated DNA packaging [11], while all others neglect this essential issue, constituting a major conceptual gap.

During the transition from prokaryotes to eukaryotes, total genomic DNA content expanded by approximately 2.5 orders of magnitude [11]. This dramatic genomic enlargement renders it implausible to attribute eukaryogenesis solely to isolated events such as accidental phagocytosis, symbiosis, or parasitism. In fact, overreliance on endosymbiotic theory may have misled researchers, as this framework fails to explain the substantial genomic disparity between prokaryotes and eukaryotes. Endosymbiosis thus likely constitutes only one event or fragment within the broader evolutionary trajectory of eukaryogenesis.

Based on the above analysis, this paper proposes that eukaryogenesis emerged from the synergistic interaction of multiple evolutionary pathways, including endosymbiosis, cellular fusion, membrane encapsulation and internalization (or protrusion externalization), and autogenous evolution. Among these drivers, atmospheric oxygenation is identified as the most critical evolutionary impetus [11]. In this oxidative evolutionary context, mitochondria likely evolved as adaptations to aerobic respiration: without an oxidizing environment, mitochondria could not have arisen, establishing them as products of oxidative adaptation [53,113,114]. Efficient oxidative metabolism, in turn, facilitated the elaboration of cellular structure and function—complexity that required additional genetic material to sustain genomic expansion.

In response to oxidative stress and the demands of enhanced metabolism, a series of adaptive processes unfolded, including metabolic complementarity, genetic integration and coordinated regulation, and metabolic co-evolution. Ultimately, to enable orderly management of this increasingly complex cellular system, a novel subcellular structure—the nucleus—evolved, alongside a new reproductive mode: meiotic sex, which is linked to a polyploid ancestor generated by cell-cell fusion [115].

This integrative framework unifies existing theories by incorporating valid insights from each, thereby delineating a comprehensive and coherent evolutionary trajectory of eukaryogenesis.

5. Perspectives

The development of DNA packing mechanisms represents a pivotal step in the trajectory toward eukaryogenesis. This evolutionary process was inherently coupled with the gradual differentiation of intracellular structures and the emergence of sophisticated cell division machineries—traces of which are presumably preserved in the evolutionary record. Consequently, a primary priority for future research is to reconstruct the sequential evolutionary pathway underlying the sequence "genome expansion→increased demand for DNA packing→formation of nuclear membrane/chromosomal structures", thereby elucidating the core driving forces of early eukaryotic evolution.

A second key priority is to clarify the temporal and causal relationships between endosymbiotic events and DNA packing. A critical unresolved question persists: Did DNA packing precede endosymbiosis, laying the structural foundation for the integration of endosymbionts? Or did these two processes evolve synergistically, with each amplifying the selective pressures driving the other? Resolving this temporal sequence will yield crucial insights into the coordinated evolution of eukaryotic cellular complexity.

Third, it is essential to decipher the intrinsic evolutionary mechanisms of DNA packing. The striking congruence between the eukaryote-to-prokaryote C-value ratio and the DNA compaction ratio in modern eukaryotes is unlikely to be coincidental. Instead, this correspondence may serve as a "digital signature" of nuclear origin, encapsulating evolutionary truths otherwise obscured by the myriad complex phenomena of life. Unraveling this signature will require dissecting the molecular and selective forces that shaped the evolution of packing machinery.

Fundamentally, advancing these research priorities demands interdisciplinary integration. For instance, can paleogenomic approaches validate the evolutionary timing of the emergence of DNA packing mechanisms? Can synthetic biology strategies simulate the synergistic co-evolution of DNA packing and nuclear membrane formation? Moving forward, establishing a multidisciplinary platform for data sharing and integration—encompassing paleontology, molecular biology, ecology, evolutionary biology, and earth sciences—will be imperative. Through cross-validation across these disciplines, the complementarity and integration of multi-dimensional evidence can be achieved, facilitating the construction of a unified theoretical framework that fully delineates the evolutionary trajectory of eukaryotes.

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