# Genome hybridization: A universal way for the origin and diversification of organelles as well as the origin and speciation of eukaryotes

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# **Abstract**

The origin of organelles (mitochondrion, chloroplast and nucleus) remains enigmatic. The endosymbiotic hypothesis that chloroplasts, mitochondria and nuclei descend from the endosymbiotic cyanobacterium, bacterium and archaebacterium respectively is dominant yet uncompelling, while our discovery of de novo organelle biogenesis in the cyanobacterium TDX16 that had acquired its green algal host Haematococcus pluvialis's genome disproves this hypothesis. In light of organelle biogenesis in the cyanobacterium TDX16 in combination with the relevant cellular and molecular evidence, we propose genome hybridization hypothesis (GHH) that the origin of organelles and origin of eukaryotes as well as the diversification of organelles and speciation of eukaryotes are unified and achieved by genome hybridization: the endosymbiotic cyanobacteria/bacteria obtain their archaebacterial/eukaryotic hosts' genomes and hybridize with their own ones resulting in expanded genomes containing a mixture of hybrid prokaryotic genes and eukaryotic genes, and thus the cyanobacteria/bacteria have to compartmentalize to accommodate different genes for specialized function of photosynthesis (chloroplast), respiration (mitochondrion) and DNA preservation (nucleus), and consequently turn into photosynthetic/heterotrophic eukaryotes. Accordingly, eukaryotes and their organelles are of multiple origin, while the formation of cancer cells is the speciation of eukaryotes as cancer cells are new species of unicellular eukaryotes arising from bacteria. Therefore, GHH provides a theoretical framework unifying evolutionary biology, cancer biology and cell biology and directing the integrated multidisciplinary research.

**Keywords:** Origin, diversification, speciation, organelles, eukaryote, cancer cells, bacteria, cyanobacteria

# 1. Introduction

The origin of DNA-containing organelles (organelles, thereafter), including nucleus, mitochondrion and chloroplast, has been an enigma ever since their discovery with light microscopes. To explain this puzzle numerous hypotheses have been proposed, which are basically categorized into two types: the endosymbiotic hypothesis and the autogenous hypothesis.

# 1.1 The endosymbiotic hypothesis

The endosymbiotic hypothesis proposes that chloroplasts and mitochondria were derived from an endosymbiotic cyanobacterium and an endosymbiotic bacterium respectively. This hypothesis was initially proposed by Mereschkowsky for chloroplast in 1905 (Mereschkowsky, 1905) and Wallin for mitochondrion in 1923(Wallin, 1923) based on the similarities between chloroplasts/mitochondria and cyanobacteria/bacteria, restated in the early 1960s accompanying the detection of DNA in chloroplasts (Ris and Plaut, 1962) and mitochondria (Nass et al., 1965) with electron microscopes, and popularized in the late 1960s by Margulis (Sagan) (Sagan, 1967).

Aside from the endosymbiotic origin of chloroplast, Mereschkowsky also speculated that nucleus evolved from a prokaryote (mycoplasma) engulfed by an amoeboid cell (Mereschkowsky, 1905), which received less attention as nucleus, unlike chloroplast and mitochondrion, is unique in nature and not analogous to any prokaryotes. Yet with the identification of archaebacteria (Woese and Fox, 1977), it is found that the eukaryote genome is a chimera containing both archaebacterial and bacterial genes (Woese, 1981). As such, it is postulated that nucleus arose from an archaebacterium engulfed by or symbiotic with gram-negative bacteria (Lake and Rivera, 1994; Gupta and Golding, 1996; Moreira and López-Garc á, 1998; López-Garc á and Moreira, 2020).

#### 1.2 The autogenous hypothesis

The autogenous hypothesis postulates that mitochondrion and chloroplast originated by compartmentalizing a proto-eukaryote's cytoplasmic DNA with the invaginated cell membrane (Raff and Mahler, 1972; Uzzell and Spolsky, 1974) or thylakoid membrane within a cyanobacterial cell (Cavalier-Smith, 1975; Taylor, 1976). Similarly, nucleus arose by enclosing the cellular DNA with the internalized plasma membrane (Cavalier-Smith, 1988) or thylakoid membrane (Cavalier-Smith, 1975; Taylor 1976) and or bacterial vesicle membrane (Gould et al., 2016).

# 1.3 The endosymbiotic hypothesis is dominant but not compelling

The existence of DNA in chloroplasts and mitochondria and the resemblance of chloroplast/mitochondrial genes and protein synthesis systems (e.g., ribosome) to those of cyanobacteria/bacteria are in favor of the endosymbiotic hypothesis but not the autogenous hypothesis that imply that all genes in eukaryotic cells arose solely by gene duplication. Hence, the endosymbiotic hypothesis for the origin of chloroplast

and mitochondrion has become dominant since the late 1970s (Schwartz and Dayhoff, 1978), while the endosymbiotic hypothesis for the origin of nucleus is now prevailing. Nevertheless, the endosymbiotic hypothesis is countered by increasing cellular and molecular evidence and thus not compelling.

# 2. Evidence contradicting the endosymbiotic hypothesis

# 2.1 The organelle envelopes are not derived from the prokaryotic cell envelops

The double-membraned envelopes of mitochondria and chloroplasts are interpreted to derive from the cell envelopes of the endosymbiotic bacterium and cyanobacterium respectively (Cavalier-Smith 1987; 1988). This interpretation is entirely false because the prokaryotic cell envelope consists of cell wall and cytoplasmic membrane that are structurally and functionally different compartments separated by the periplasmic space (Silhavy et al., 2010). The cell wall of cyanobacteria and gram-negative bacteria comprises an outer membrane, an inner peptidoglycan layer and an intervening space (Golecki, 1977; Beveridge, 1999). If the outer membrane becomes the corresponding membrane of chloroplast and mitochondrial envelopes, the peptidoglycan layer must be degraded, which is, however, impossible as synthesis inhibition (e.g., by penicillin and other  $\beta$ -lactam antibiotics) or degradation (e.g. by lysozyme) of the peptidoglycan layer results in cell lysis (Vollmer et al., 2008). So, the double-membraned mitochondrial and chloroplast envelopes are not derived form the cell envelopes of the endosymbiotic bacterium and cyanobacterium, which is why many enzymes for synthesizing chloroplast lipids are not of cyanobacterial origin (Sato, 2019). The double-membraned nuclear envelope derives neither from the cell envelope of the endosymbiotic archaebacterium since its membrane lipids are ether-linked instead of ester-linked as those of bacteria/cyanobacteria and eukaryotes (Woese, 1981), nor from the cell envelope of bacterial host for the same reason as discussed above for chloroplast and mitochondrial envelopes.

#### 2.2 The eukaryotic cytoplasmic matrix can not be formed

If the nucleus is derived from an archaebacterium within a bacterium, then, how the eukaryotic cytoplasmic matrix that is compositionally and functionally distinct from the prokaryotic cytoplasm forms and replaces the original bacterial cytoplasm? This is an unanswered yet crucial question as nucleus can not work in the prokaryotic cytoplasm and the eukaryotic cell is not simply the prokaryotic cytoplasm containing a nucleus and other organelles. If the endosymbiotic archaebacterium keeps intact, the eukaryotic cytoplasmic matrix can not be formed, while if the endosymbiotic archaebacterium ruptures for the presumptive merger with the bacterial host, the nucleus can not derive from the archaebacterium.

# 2.3 Only simultaneous origin of organelles is reasonable yet unattainable

The nucleus, mitochondrion and chloroplast are supposed to originate individually in separate symbiotic events. As such the origin order of these organelles has become an issue of debate, particularly the origin sequence of nucleus and mitochondrion. For

heterotrophic eukaryotes, it is traditionally assumed that the nucleus came first and later mitochondrion was formed in the nucleus-bearing cell (Cavalier-Smith, 1987), this scenario is argued to be unfeasible due to the insufficient energy supply in the absence of mitochondrion to underpin nucleus development; it is then proposed that mitochondrion predated nucleus in origin and was formed in a prokaryote (Lane and Martin, 2010). Such a scenario is, however, also unattainable, because mitochondria arose putatively from the endosymbiotic bacterium by losing and transferring genes into the nucleus and works only in the eukaryotic cytoplasmic matrix that is quite distinct from the prokaryotic cytoplasm, otherwise it is a bacterium rather than a mitochondrion. Hence, only simultaneous origin of nucleus and mitochondrion seems to be reasonable. Likewise, it is also the case for photosynthetic eukaryotes, in which the ancestral chloroplast, nucleus and mitochondrion were formed simultaneously. Nonetheless, simultaneous origin of these organelles means intact integration of an archaebacterium and a bacterium into a bacterial host cell for the origin of heterotrophic eukaryotes and an additional cyanobacterium into the same bacterial host cell at the same time for the origin photosynthetic eukaryotes, which is mechanically and cell biologically unachievable.

# 2.4 The genomes of mitochondrion, chloroplast and nucleus are not bacterial, cyanobacterial and archaebacterial ones

Different organelles have common DNA sequences termed promiscuous DNA (Ellis, 1982), particularly mitochondria and chloroplasts in photosynthetic eukaryotes (Stern and Lonsdale, 1982; Lonsdale et al., 1983; Stern and Palmer, 1984; Unseld et al., 1997; Notsu et al., 2002; Goremykin et al., 2009; Alverson et al., 2010; Iorizzo et al., 2012; Straub et al., 2013; Spooner et al., 2017; Zhang et al., 2019; Wee et al., 2022; Li et al., 2023), e.g. mitochondrial and chloroplast genomes of *Haematococcus lacustris* are made up of nearly identical repetitive DNA sequences (Zhang et al., 2019), and mitochondria and nuclei in heterotrophic eukaryotes (van den Boogaart et al., 1982; Farrelly and Butow, 1983; Gellissen and Bradfield, 1983; Kristensen and Prydz, 1986; Lopez et al., 1994; Bensasson et al., 2001; Richly and Leister, 2004; Hazkani-Covo et al., 2010; Wei et al., 2022; Kuprina et al., 2024), where even entire mitochondrial DNAs reside in the nuclei of human (Wei et al., 2022), rodent (Kuprina et al., 2024) and HeLa cell (cancer cell) (Kristensen and Prydz, 1986). Hence the genomes of mitochondrion, chloroplast and nucleus are not bacterial, cyanobacterial and archaebacterial ones. This is why the genome organization structures of chloroplasts and cyanobacteria are different (Sadovsky et al., 2020).

# 2.5 The proteomes of mitochondrion, chloroplast and nucleus are not bacterial, cyanobacterial and archaebacterial ones

Only a small fraction of the mitochondrial proteins have bacterial homologs, while the rest display no homology to bacterial proteins and thus is not of bacterial origin (Karlberg, et al., 2000; Kurland and Andersson, 2000; Gabald on and Huynen, 2003; Szklarczyk and Huynen, 2010; Huynen et al., 2013; Gray, 2015). Also, promiscuous proteins are widespread among organelles. A number of mitochondrial proteins reside

in chloroplasts of photosynthetic eukaryotes (Peeters and Small, 2001; Carrie and Small, 2013; Guan et al., 2020) but nuclei of heterotrophic eukaryotes (Monaghan and Whitmarsh, 2015; Tang, 2015). Thus, the proteomes of mitochondrion, chloroplast and nucleus are also not bacterial, cyanobacterial and archaebacterial ones.

# 2.6 Mitochondria present in chloroplasts of photosynthetic eukaryotes but nuclei of heterotrophic eukaryotes

The presence of mitochondria in chloroplasts of photosynthetic eukaryotes (Vesk et al., 1965; Pulich and Ward, 1973; Bonzi and Fabbri, 1975; Montes and Bradbeer, 1976; Brown et al., 1983; Carvalho et al., 2011) but nuclei of heterotrophic eukaryotes (Hoffman and Grigg, 1958; Brandes, 1965; Matsuyama and Suzuki, 1972; Oliva et al., 1973; Jensen et al., 1976; Takemura et al., 1997; Bakeeva et al., 2001; Eldarov et al., 2020) have been frequently observed, yet the reason and mechanism remain unclear. It is most likely that mitochondria are formed in the chloroplasts and nuclei, since detachment of mitochondria or mitochondrion-like bodies from chloroplasts in photosynthetic eukaryotes (Wildman et al., 1962; Bonzi and Fabbri, 1975) but nuclei in heterotrophic eukaryotes (Hoffman and Grigg, 1958) have also been detected.

Obviously, the endosymbiotic hypothesis fails to account for the above evidence. The origin of organelles is, in essence, the biogenesis of organelles in prokaryotes and thus is cellular events (a topic of cell biology). Yet, such cellular events or the like and even the intermediate state of cells had not been observed previously. In this circumstance, it is trying to infer the cellular events of organelle origin by comparing the similarity between extant eukaryotes' organelles and prokaryotes in morphology, structure, physiology, genes/genome, proteins/proteome and other molecular data, which is inevitably highly or even completely speculative and indeed unachievable, because how the extant eukaryotes/organelles descend from the ancestral eukaryotes/organelles, the basic mechanism necessary for inference, is unknown.

In our previous studies, we have found de novo organelle biogenesis in the endosymbiotic cyanobacterium TDX16 that had acquired its green algal host *Haematococcus pluvialis*'s total DNA (genome), resulting in the transition of prokaryote TDX16 into a new species of eukaryote TDX16-DE (Dong et al., 2020). This is the first case of organelle biogenesis in prokaryotes observed so far, which provides an unprecedented reference for inferring the origin of organelles.

# 3. De novo organelle biogenesis in the cyanobacterium TDX16

In brief, organelle biogenesis in the cyanobacterium TDX16 initiated with cytoplasm compartmentalization, followed by de-compartmentalization, DNA hybridization and allocation, and re-compartmentalization, as such only two composite organelles-the primitive chloroplast and primitive nucleus sequestering minor and major fractions of cellular DNA respectively were formed. Thereafter, the eukaryotic cytoplasmic matrix was built up from the matrix extruded from the primitive nucleus; mitochondria were assembled in and segregated from the primitive chloroplast, whereby the primitive nucleus and primitive chloroplast matured into nucleus and chloroplast respectively,

leading to the transition of prokaryotic cyanobacterium TDX16 into a new eukaryotic green alga TDX16-DE (Dong et al., 2020) that is taxonomically assigned as *Chroococcidiorella tianjinensis* (Dong and Xing, 2020). Hence, de novo organelle biogenesis in the cyanobacterium TDX16 is a cellular event for the formation of new organelles (organelle diversification) and new eukaryotic species (speciation), which disproves the endosymbiotic hypothesis and sheds light on the questions that is crucial for inferring the origin of organelles:

- (1) Endosymbiosis is prokaryotes' way for bulk acquisition of foreign DNA (i.e., the host cell's total DNA), which is different from conjugation, transformation and transduction (Chen et al., 2005) that are prokaryotes' ways for piecemeal acquisition of DNA fragments.
- (2)Prokaryotes can hybridize the obtained eukaryotic genome with its own one, resulting in loss and retention of some genes as well as synthesis of new hybrid prokaryotic genes and eukaryotic genes, and allocate the retained and hybrid genes selectively into different compartments during compartmentalization.
- (3) Chloroplast, nucleus and mitochondrion are formed all at once in a short period of time by enclosing the allocated DNA with the membranes synthesized by fusion, flattening and extension of the small vesicles. Such that, the envelope of these organelles inevitably comprise two unit membranes if the vesicles are bounded by one unit membrane (e.g., vesicles derived from the cytoplasmic membrane and thylakoids), but four unit membranes (e.g., the nuclear envelop of TDX16-DE), if the vesicles are bounded by two unit membranes (e.g., vesicles derived from the primitive chloroplast envelope).
- (4)Chloroplast and mitochondrion are both developed from the primitive chloroplast.
- (5)The eukaryotic cytoplasmic matrix and nuclear matrix both originate from the matrix of primitive nucleus.
- (6)Thylakoids in cyanobacteria are developed initially from the osmiophilic granules assembled on the cytoplasmic membrane (primary thylakoids) and subsequently from the primary-thylakoid-derived vesicles or segments (secondary thylakoids).
- (7) Thylakoids in chloroplasts are developed originally from the modified osmiophilic granules-the plastoglobuli generated on the transitional secondary thylakoids.
- (8) The cristae in mitochondria are formed by fusing, flattening and elongating the single-membrane-bounded vesicles, and thus, like chloroplast thylakoids, are single membrane-bounded sacs, rather than the folds of inner mitochondrial membrane.

# 4. Presentation of the hypothesis

# 4.1 Concept definition: origin/diversification of organelles and origin/speciation of eukaryotes

Conceptually, the origin of organelles (biogenesis of organelles in prokaryotes without the involvement of eukaryotes) and diversification of organelles (biogenesis of organelles in prokaryotes with the involvement of eukaryotes) are two similar yet different cellular events resulting in the origin of eukaryotes (formation of eukaryotes from prokaryotes without the involvement of other eukaryotes) and speciation of

eukaryotes (formation of new eukaryotic species with the involvement of other eukaryotes) respectively.

# 4.2 The origin/diversification of organelles is the origin/speciation of eukaryotes

Currently, the relationship between the origin/diversification of organelles and the origin/speciation of eukaryotes remains controversial owing to the assumption of separate origin of organelles. While de novo organelle biogenesis in cyanobacterium TDX16 (Dong et al., 2020) demonstrates that nucleus, mitochondrion and chloroplast are formed all at once rather than separately, as such the origin/diversification of organelles surely leads to the origin/speciation of eukaryotes. Hence the origin of organelles and the origin of eukaryotes as well as the diversification of organelles and the speciation of eukaryotes are two sides of the same coin and thus unified.

# 4.3 The origin/speciation of eukaryotes is the origin/speciation of unicellular eukaryotes

Eukaryotes comprise unicellular eukaryotes (protists) and multicellular eukaryotes, while the latter derives from the former. So, the origin of eukaryotes is undoubtedly the origin of unicellular eukaryotes. While the speciation of eukaryotes is usually considered to be the speciation of multicellular eukaryotes (e.g., animals and plants) that are easy to observe owing to their visible morphological and structural traits. This view is, however, incorrect. In truth, the speciation of eukaryotes is the speciation of unicellular eukaryotes that determines the speciation of multicellular eukaryotes (or in other words, the speciation of multicellular eukaryotes depends on the speciation of unicellular eukaryotes), because multicellular eukaryotes descend from unicellular eukaryotes. This is really true as in the rare case that a new species of multicellular eukaryote is formed by sexual hybridization of two different species of multicellular eukaryotes (inter-species hybridization), e.g., a mule is the infertile hybrid species of a horse and a donkey, the hybrid multicellular species is also derived form a single celled zygote formed by fusion of two unicellular germ cells. As such, the origin/speciation of eukaryotes can only be observed microscopically and are extremely difficult to capture. So, it is not surprise that organelle biogenesis in cyanobacterium TDX16 (Dong et al., 2020) is the only case of eukaryote speciation observed so far.

# 4.4 The origin/diversification of organelles share the same or similar mechanism

Organelle biogenesis in cyanobacterium TDX16 (Dong et al., 2020) is, as described above, a cellular event of organelle diversification/eukaryote speciation, showing that a cyanobacterium (prokaryote) turns into a new species of alga (eukaryote) by de novo biogenesis of new organelles after endosymbiotic acquisition of a preexisting alga's genome and hybridization of the acquired genome with its own one. Such that, the diversification of organelles for the speciation of eukaryotes, like the origin of organelles for the origin of eukaryotes, is also achieved by de novo biogenesis of organelles in prokaryotes, suggesting that the origin and diversification of organelles share the same or similar cellular and molecular mechanism.

#### 4.5 Genome hybridization hypothesis

In light of organelle biogenesis in the cyanobacerium TDX16 in combination with cellular and molecular evidence, we propose genome hybridization hypothesis (GHH) that the origin and diversification of organelles as well as the origin and speciation of eukaryotes are unified and achieved by genome hybridization: the endosymbiotic cyanobacteria/bacteria obtain the senescent/necrotic archaebacterial/eukaryotic hosts' genomes and hybridize with their own ones resulting in expanded genomes containing a mixture of hybrid prokaryotic genes and eukaryote specific genes, and thus the cyanobacteria/bacteria have to compartmentalize to accommodate different genes for specialized function of oxygenic photosynthesis (chloroplast), aerobic respiration (mitochondrion) and DNA preservation (nucleus), and consequently turn into photosynthetic/heterotrophic unicellular eukaryotes.

Table 1 Symbioses involved in the origin of organelles and eukaryotes

	Endosymbionts	Hosts (aerobic)	Origin of organelles	Origin of eukaryotes
1	Cyanobacteria	Archaebacteria	Nucleus, mitochondrion,	Photosynthetic
			chloroplast	eukaryotes
2	Bacteria	Archaebacteria	Nucleus, mitochondrion	Heterotrophic
				eukaryotes

Table 2 Symbioses involved in the diversification of organelles and speciation of eukaryotes

	Endosymbionts	Hosts (aerobic)	Diversification of organelles		Speciation of eukaryotes
1	Cyanobacteria	Photosynthetic	Nucleus, mitocho	ndrion,	Photosynthetic
		eukaryotes	chloroplast		eukaryotes
2	Cyanobacteria	Heterotrophic	Nucleus, mitocho	ndrion,	Photosynthetic
		eukaryotes	chloroplast		eukaryotes
3	Bacteria	Heterotrophic	Nucleus, mitochondrion		Heterotrophic
		eukaryotes			eukaryotes
4	Bacteria	Photosynthetic	Nucleus, mitocho	ndrion,	Heterotrophic
		eukaryotes	abnormal compartments*		eukaryotes

<sup>\*</sup> occasionally formed structures with irregular shape and function.

Cyanobacteria and bacteria symbiotic with archaebacteria are involved in the origin of organelles and origin of eukaryotes (Table 1); while cyanobacteria and bacteria symbiotic with eukaryotes are responsible for the diversification of organelles and speciation of eukaryotes (Table 2). The origin and diversification of nucleus and mitochondrion take place in both bacteria and cyanobacteria, while the origin and diversification of chloroplast occur only in cyanobacteria. This is because the capacity for organelle biogenesis depends on the metabolic capability of genome-recipient cyanobacteria and bacteria, but not genome-donor archaebacteria and eukaryotes. Bacteria are capable of respiration in which only mitochondrion can be formed; while cyanobacteria are capable of photosynthesis and respiration in which chloroplast and mitochondrion can be developed.

Archaebacteria and eukaryotes (hosts) involved are all aerobic, because the endosymbiotic cyanobacteria/bacteria are oxygen-generating/requiring prokaryotes (aerobic) and thus can not inhabit oxygen-sensing/free anaerobic hosts. The archaebacteria and eukaryotes shelter the endosymbiotic cyanobacteria/bacteria from harsh conditions, e.g., high or low temperature; and in turn the endosymbiotic cyanobacteria/bacteria facilitate hosts' metabolism (e.g., synthesis of metabolites or nitrogen-recycling) rather than confer hosts the ability of photosynthesis or respiration. The hosts impose efficient control over the endosymbiotic cyanobacteria/bacteria under normal conditions, but turn senescence and lose control of these endosymbionts under adverse conditions. Such that the endosymbiotic cyanobacteria/bacteria take up their senescent/necrotic host's total DNA (genome) and retain the obtained DNA in a membrane-bounded heterogenous globular body (HGB) like those of TDX16 (Dong et al., 2020) so as to keep from degradation.

After liberation from the ruptured necrotic host cell, the endosymbiotic cyanobacteria/bacteria hybridize the obtained DNA that was released from HGB with their own one, resulting in an expanded genome comprising the hybrid prokaryotic genes (e.g., hybrid 16S rRNA) and the completely novel or modified eukaryote specific genes (e.g., 18S rRNA). Such that, the cyanobacteria/bacteria are confronted with two problems:

- (1) the expanded genome, a mixture of prokaryotic and eukaryotic genes, can no longer be deposited simply in the nucleoid, and must be categorized and preserved separately.
- (2) the prokaryotic systems of respiration and photosynthesis are energetically less efficient to operate the expanded genome (genome expression) for upgraded cell functions. The endosymbiotic bacteria, like their archaebacterial hosts, perform respiration in cytoplasmic membranes, which have a low energy-generating capacity due to the limited respiratory membrane surface, and so a specialized compartment with large and adjustable surface area for respiration is needed. The endosymbiotic cyanobacteria perform both photosynthesis and respiration in thylakoid membranes (Lea-Smith, et al., 2013), which are also incapable of producing enough energy, because the concurrence of photosynthesis and respiration in thylakoid membranes reduce their energetic capacity and efficiency, and so photosynthesis and respiration must be performed separately in different compartments.

To tackle these two problems, DNA fragments with hybrid prokaryotic genes encoding constituents specific for photosynthesis and respiration (e.g., photosynthetic and respiratory electron transport chains), and essential components of prokaryotic protein synthesis systems (e.g., 16SrRNA, tRNA and ribosomal proteins) are sorted out and enclosed separately with membrane for specialized function of photosynthesis (chloroplast) and respiration (mitochondrion); while all the rest of DNA fragments containing eukaryote specific genes and nonfunctional DNA sequences are totally encased with membrane for management (nucleus). As a result, the organelle-less cyanobacteria and bacteria suddenly turn into organelle-containing photosynthetic eukaryotes and heterotrophic eukaryotes respectively.

# 4.6 The way of organelle biogenesis in cyanobacteria and bacteria

The biogenesis of chloroplast, nucleus and mitochondrion in cyanobacteria that had acquired their archaebacterial/eukaryotic hosts' genomes for the origin/speciation of photosynthetic eukaryotes proceeds in the same way as organelle biogenesis in the cyanobacterium TDX16 (Dong et al., 2020); while the biogenesis of nucleus and mitochondrion in bacteria that had obtained their archaebacterial/eukaryotic hosts' genomes for the origin/speciation of heterotrophic eukaryotes follows the hypothetical way of organelle biogenesis in bacteria for the formation of cancer cells (Dong and Xing, 2018).

# 5. Testable predictions

# 5.1 Multiple origins of eukaryotes and organelles

A photosynthetic/heterotrophic eukaryote origins from a cyanobacterium/bacterium by de novo biogenesis of organelles after acquisition of an archaebacterium's genome. There is no reason to think that only one or a limited number of cyanobacteria, bacteria and archaebacteria are involved in the origin of organelles and eukaryotes. In fact, the simple prokaryotes have a natural tendency to upgrade to complex eukaryotes and theoretically all aerobic bacteria, archaebacteria and cyanobacteria can participate in the origin of organelles and eukaryotes. So, eukaryotes and their organelles are of multiple origins, which sets the fundamental basis for biodiversity.

# 5.2 Organellar genomes are patchworks of diverse organisms' DNA sequences

During the origin and speciation of eukaryotes, genomic DNAs of diverse organisms including archaebacteria, bacteria, cyanobacteria and eukaryotes are intermingled, fragmented and hybridized resulting in a mixture of DNAs, which is subsequently partitioned into different compartments. So, each organelle's genome is a patchwork of diverse organisms-derived DNA sequences (so is the eukaryote's whole genome) and each organism-derived DNA sequences exist in all organelles' genomes. For example, a photosynthetic eukaryote's three organellar genomes are all assembled with diverse organisms' DNA sequences and if a cyanobacterium's gene presents in the mitochondrial genome, then similar or different DNA sequences derived from this cyanobacterium also exist in the chloroplast genome and nuclear genome.

# 5.3 Single source of archaebacterial DNAs in each eukaryote

Archaebacteria involve only in the origin but not the speciation of eukaryotes, and so all archaebacterial DNAs in each eukaryotic species come definitely form a single archaebacterium, which is important for identifying the archaebacterium and inferring the origin or speciation of the eukaryotic species.

# 5.4 Multiple sources of eubacterial and eukaryotic DNAs in each eukaryote

During eukaryote speciation, genomic DNAs of one eubacterium (cyanobacterium or bacterium) and one eukaryote that contains other eubacteria and euakaryotes' DNAs are introduced into the new species. Hence, eubacterial and eukaryotic DNAs in each eukaryotic species come from multiple eubacteria and eukaryotes except that the

eukaryotic species is formed in origin (one of the ancestral eukaryotes).

# 5.5 Different cellular traits of photosynthetic and heterotrophic eukaryotes

Photosynthetic eukaryotes and heterotrophic eukaryotes are different in origin and speciation and thus exhibit different cellular traits:

- (1) The cytoplasmic membrane comprises one unit membrane in heterotrophic eukaryotes, but two unit membranes in photosynthetic eukaryotes.
- (2) The nucleus is enclosed with two unit membranes (one set of envelope) in heterotrophic eukaryotes but four unit membranes (two set of envelopes) in photosynthetic eukaryotes. The outer nuclear envelope is flexible in structure. For example, the chloroplasts in some algae are surrounded by four unit membranes (two set of envelopes) (Gibbs, 1981) and inferred as the secondary chloroplasts formed in the secondary endosymbiosis, with the two membranes of the outer envelope being derived from the plasma membrane of the endosymbiotic algae and the phagosomal membrane of the secondary host (Archibald and Keeling, 2002). The inference is false as the endosymbiotic hypothesis is disproved by our discovery (Dong et al., 2020). Virtually, these chloroplasts have only one double-membraned envelope, while the additional envelope is the outer nuclear envelope that extends around the chloroplast. (3) Mitochondria can be formed de novo in the nuclei of heterotrophic eukaryotes but in the chloroplasts of photosynthetic eukaryotes.

# 5.6 Different content of heterotrophic and photosynthetic eukaryotes' genomes

Heterotrophic eukaryotes' genomes contain only bacterial and archaebacterial but no cyanobacterial DNAs except those bearing the so-called non-photosynthetic plastids and their descendants. By contrast, photosynthetic eukaryotes' genomes contain cyanobacterial and archaebacterial or additional bacterial DNAs if heterotrophic eukaryotes are involved in speciation. So, photosynthetic eukaryotes fall into two categories: category1 contain only cyanobacterial and archaebacterial DNAs, while category 2 contain cyanobacterial, archaebacterial and bacterial DNAs.

# 5.7 The heterotrophic eukaryote's nucleus contains a mitochondrial genome

Mitochondria are assembled in the primitive nucleus during the origin and speciation of heterotrophic eukaryote, and after origin or speciation an entire mitochondrial genome (intact or fragmented mitochondrial DNA) remains in the nuclear genome enabling de novo biogenesis of mitochondria in the nucleus under stress conditions. So, the presence of entire mitochondrial DNA (mitochondrial genome) in nuclei is not confined to the known cases (Kristensen and Prydz, 1986; Wei et al., 2022; Kuprina et al., 2024), but a general phenomenon in heterotrophic eukaryotes.

# 5.8 The photosynthetic eukaryote's chloroplast contains a mitochondrial genome

Different from the case of heterotrophic eukaryotes, mitochondria are assembled in the primitive chloroplast during the origin and speciation of photosynthetic eukaryote. So, after origin or speciation, the chloroplast genome retains an entire mitochondrial DNA (a backup copy) so as to develop mitochondria from scratch in the chloroplast under abnormal conditions, e.g., no functional mitochondria is available.

# 5.9 Mitochondrial proteomes reside in nuclei of heterotrophic eukaryotes but chloroplasts of photosynthetic eukaryotes

The reason for the presence of some mitochondrial proteins in nuclei of heterotrophic eukaryotes (Monaghan and Whitmarsh, 2015; Tang, 2015) but chloroplasts of photosynthetic eukaryotes (Peeters and Small, 2001; Carrie and Small, 2013; Guan et al., 2020) as described in section 2.5 is unclear. In fact, like the case of DNA, it is also because mitochondria can be formed de novo in nuclei of heterotrophic eukaryotes but chloroplasts of photosynthetic eukaryotes. So, not just some but actually all of the mitochondrial proteins, the mitochondrial proteomes, reside in nuclei of heterotrophic eukaryotes but chloroplasts of photosynthetic eukaryotes.

#### 5.10 The formation of cancer cells is the speciation of eukaryotes

It has been proposed that cancer cells are new species of protists (e.g., HeLa cell) arising from bacteria (Dong and Xing 2018): the endosymbiotic bacteria turn into primary cancer cells for cancer initiation after acquisition and hybridization of the normal somatic cell's genome and de novo biogenesis of organelles; likewise the endosymbiotic bacteria turn into secondary cancer cells (new species of protists different from the primary cancer cells) for cancer progression (e.g., invasion and metastasis) after acquisition and hybridization of the primary cancer cell's genome and de novo biogenesis of organelles. Hence, the formation of cancer cells is the speciation of eukaryotes that is achieved by de novo biogenesis of organelles in bacteria after acquisition and hybridization of the eukaryotic host's genome. Such that, cancer biology, evolutionary biology and cell biology are unified, while cancer cells provide the experimental material not only for cancer research but also for studying the de novo biogenesis of organelles in prokaryotes and speciation of eukaryotes.

# 5.11The non-photosynthetic plastids are not plastids

The presence of chloroplast-related genes in unicellular heterotrophic parasites, e.g., apicomplexan parasites (McFadden et al., 1996; Köhler et al., 1997; Wilson 2002) and heterotrophic algae (Sekiguchi et al., 2002; Tartar and Boucias 2004; Borza et al., 2005) is putatively ascribed to the non-photosynthetic plastids. These putative non-photosynthetic plastids are, however, not plastids but abnormal compartments. As summarized in Table1and 2, chloroplasts can not be developed in bacteria due to their incapacity of photosynthesis. So, if bacteria endosymbiotically acquire photosynthetic eukaryotes' genomes, the chloroplast-related genes might be lost or deposited in abnormal compartments and or in nuclei or mitochondria if there is no abnormal compartments. For example, the putative genome-less non-photosynthetic plastid of the heterotrophic green alga *Polytomella* spp (Smith and Lee, 2014) does not exist, while the chloroplast-related genes were lost or deposited in mitochondria. Therefore, the so-called non-photosynthetic plastids are not plastids but abnormal compartments formed in bacteria that aberrantly obtain photosynthetic eukaryotes' genomes and in essence the heterotrophic algae are not algae but bacteria-derived protists.

# 5.12 The amitochondriate eukaryotes congenitally lack mitochondrion

The presence of mitochondrion-related genes in amitochondriate eukaryotes (anaerobic protists) is ascribed to the putatively lost mitochondria. In truth, these putative mitochondria do not exist for the similar reason as discussed above for non-photosynthetic plastids. Anaerobic bacteria are incapable of aerobic respiration in which mitochondria can not be formed. Such that the anaerobic bacteria that endosymbiotically obtain their aerobic eukaryotic hosts' genomes turn into amitochondriate anaerobic protists with the mitochondrion-related genes being lost (Karnkowska et al., 2016) or deposited after hybridization in nuclei (Hirt et al., 1997; Roger et al., 1998; Arisue et al., 2002) and or in abnormal compartments, e.g., hydrogenosome (Akhmanova et al., 1998). Therefore, the amitochondriate eukaryotes congenitally lack mitochondrion, and the putative mitochondrion-derived organelles (Mai et al., 1999) and mitochondrial remnants (Tovar et al., 1999; Williams et al., 2002; Tovar et al., 2003) are not true.

# 5.13 The inferred organelle-to-organelle gene transfers do not exist

The homologous DNA sequences/genes in different organelles as described above in section 2.4 is inferred as the results of organelle-to-organelle gene transfers during or after the transition of the endosymbiotic cyanobacterium and bacterium into the chloroplast and mitochondrion, termed endosymbiotic gene transfer (Timmis et al., 2004), interorganellar/intercompartmental gene transfer (Schuster and Brennicke, 1988; Kleine et al., 2009) and intracellular gene transfer (Park et al., 2015). The inferred organelle-to-organelles gene transfers, however, do not exist, because chloroplasts and mitochondria are not derived from the endosymbiotic cyanobacteria and bacteria as evidenced by de novo organelle biogenesis in the cyanobacterium TDX16 (Dong et al., 2020). Indeed, the homologous DNA sequences/genes in different organelles result from genome hybridization and allocation of the hybrid DNA for organelle biogenesis during the origin and speciation of eukaryotes. Hence, the homologous DNA sequences/genes exist in organelles from the start of their assembly, reflecting their relationships in biogenesis. For example, mitochondria are assembled in the primitive nuclei of heterotrophic eukaryotes but the primitive chloroplasts of photosynthetic eukaryotes, and so mitochondria share more common DNA sequences with nuclei in heterotrophic eukaryotes but chloroplasts in photosynthetic eukaryotes.

# 5.14The inferred prokaryote/eukaryote-to-eukaryote gene transfers do not exist

The presence of homologous DNA sequences/genes in a eukaryote and a prokaryote or in two different eukaryotes is inferred as the results of prokaryote-to-eukaryote or eukaryote-to-eukaryote horizontal/lateral gene transfers (HGT/LGT) (Keeling and Palmer, 2008). The inferred HGT/LGT are, however, unattainable, because eukaryotes are naturally resistant to foreign DNA. In fact, a eukaryote's genome is shaped during its origin or speciation by hybridizing the genomes of two parental organisms (two prokaryotes or a prokaryote and a pre-existing eukaryote) and thus inevitably contains DNA sequences/genes homologous to those of its parental prokaryote and eukaryote. Therefore, the homologous DNA sequences/genes exist in a eukaryote from the start

of its origin or speciation but are not transferred from other organisms after its origin or speciation, which provides clues for inferring the eukaryote's origin or speciation.

# 5.15 The inferred phylogenies of eukaryotes and organelles are false

Each eukaryotic species arises directly from a cyanobacterium or a bacterium by de novo biogenesis of organelles after endosymbiotic acquisition and hybridization of an archaebacterium or a pre-existing eukaryote's genome. So, each eukaryote has two parental organisms, one is a cyanobacterium or a bacterium (genome-recipient), and the other is an archaebacterium or a eukaryote (genome-donor), and is formed by genomically hybridizing two into one instead of splitting one into two, a process similar in mechanism to sexual hybridization and sexual reproduction of eukaryotes, and thus the origin of sex seems to start from the origin and speciation of eukaryotes. While, current phylogenetic analyses are based on the wrong fundamental assumption that all eukaryotes and organelles descend directly from other eukaryotes and other organelles by putative gene duplication and point mutation. Therefore, the currently inferred phylogenies of eukaryote and organelles are false and misleading regardless of what algorithms have been applied.

#### 5.16 The prokaryote-eukaryote evolutionary relationship is a web but not a tree

As described above, each heterotrophic/photosynthetic eukaryote arises from a bacterium/cyanobacterium that endosymbiotically acquires an archaebacterium or a eukaryote' genome. Thus, all eukaryotes (heterotrophic eukaryotes and photosynthetic eukaryotes) are evolutionarily linked with prokaryotes (archaebacteria, bacteria and cyanobacteria) in a non-hierarchical manner, and the evolutionary relationship of prokaryotes and eukaryote represents a truly interconnected web (web of life) rather than a bifurcating tree (tree of life), which is different, in essence, from the putative LGT/HGT-based net (reticulated tree) (Doolittle, 1999) or web (Soucy et al., 2015).

# 6. Conclusion

The discovery of de novo organelle biogenesis in the cyanobacterium TDX16 (Dong et al., 2020) invalidates the endosymbiotic hypothesis and prompts an evidence-based reconsideration of the true origin of organelles and its association with the origin of eukaryotes and relationship with the diversification of organelles and speciation of eukaryotes that are important but unclear. GHH is a paradigm shift in understanding these questions. According to GHH, the origin/diversification of organelles and the origin/speciation of eukaryotes are unified and achieved by de novo biogenesis of organelles in bacteria/cyanobacteria after endosymbiotic acquisition and hybridization of their archaebacterial/eukaryotic hosts' genomes, while the formation of cancer cells is the speciation of eukaryotes as cancers are new species of unicellular eukaryotes arising from bacteria (Dong and Xing, 2018). Thus, GHH unifies evolutionary biology, cancer biology and cell biology and can account for all the evidence contradicting the endosymbiotic hypothesis and be verified with the testable predictions.

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