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ENDOGENOSYMBIOSIS: FROM HYPOTHESIS TO EMPIRICAL EVIDENCE TOWARDS A UNIFIED SYMBIOGENETIC THEORY (UST)

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Contents: 1. Introduction. 2. The Theoretical Basis of the Endogenosymbiosis. 3. The Empirical Evidence of the Endogenosymbiosis. 4. The Evolutionary Mechanism of the Endogenosymbiosis. 5. Conclusion.

Keywords: Endosymbiosis, Endogenosymbiosis, Organelles, Gene carriers, Unified Symbiogenetic Theory.

Abstract: *In 1967 Lynn (Sagan) Margulis proposed that mitochondria, photosynthetic plastids and cilia were acquired prokaryotes and evolved symbiotically to form anaerobic bacteria, photosynthetic bacteria and eventually algae. Although most of this theory is well-accepted now, the hypothesis that endosymbiotic spirochaetes developed into eukaryotic flagella and cilia, and the following proposals of an endosymbiotic origin of other eukaryotic organelles such as peroxisomes, glyoxysomes, etc. have not received much acceptance, since evidence suggests they lack a genome and do not show ultrastructural similarities to bacteria or archaea. Nevertheless, the idea that over millennia mitochondria, plastids, prokaryotic and eukaryotic cells and even flagella and peroxisomes, as either primary or secondary endosymbionts, transferred some or all of their own DNA to the host cell's nucleus through a process called «endogenosymbiosis» (i.e. a symbiotic gene transfer, such as the internalisation of the endosymbiont's DNA with lateral transfer) has been recently suggested. This endogenosymbiosis could take place during the evolutionary transition from the symbiotic interacting community, invoked by Margulis, to a fully-integrated (either prokaryotic or eukaryotic) cell. This process could explain the missing evidence of the presence of DNA in flagella and peroxisomes whose ancestor endosymbionts, during the long endogenosymbiotic evolution, could have transferred their whole genome to the host cell that subsequently integrated it in its own genome, directly controlling its expression. Furthermore, the endogenosymbiosis hypothesis could be the explanation of the transition between an RNA to a DNA world and of some cases of true sympatric evolution of species, apparently inexplicable by the canonical speciation processes. Here, after an introduction to the theoretical basis of endogenosymbiosis and a discussion of the empirical confirming evidence, I show a graphical summary of the integration between this and the former endosymbiosis theories. The Serial Endosymbiosis Theory and the Secondary Endosymbiosis are merged with the Endogenosymbiosis Theory in a Unified Symbiogenetic Theory (UST).*

1. Introduction

In her masterpiece «On the Origin of Mitosing Cells», Lynn (Sagan) Margulis (1967) proposed that the organelles in eukaryotic cells are the result of an

ancient symbiosis between prokaryotes. She suggested that the mitochondria and the photosynthetic plastids were prokaryotes acquired and evolved symbiotically to form anaerobic bacteria, photosynthetic bacteria and eventually algae. This idea, which is well-accepted now, followed a difficult road to be published and found concerns and severe attacks at that time. A secondary endosymbiotic process has also been suggested (Gibbs 1978; McFadden and Gilson 1995; Okamoto and Inouye 2005), when a eukaryote cell engulfs another eukaryote that has undergone primary endosymbiosis. It seems, these processes have happened regularly throughout time and have led to the great genetic diversity we find on Earth (Margulis 1967).

Moreover, Margulis (1981) argued that eukaryotic cells originated as communities of interacting entities, including endosymbiotic spirochaetes that developed into eukaryotic flagella and cilia. So far, this last idea has not received much acceptance, since the evidence suggest that flagella lack DNA and do not show any kind of ultrastructural similarities to bacteria or archaea. Most recently, Christian de Duve (2007) proposed another debated example of endosymbiosis. He suggested that peroxisomes may have a symbiogenetic origin and that they may have been the first endosymbionts. This would have allowed cells to face the increasing amount of oxygen in the early Earth's atmosphere. However, the absence of DNA in peroxisome and the evidence that they evolutionarily originate from the endoplasmic reticulum (Gabaldón *et al.* 2006) challenges the hypothesis of the endosymbiotic origin of peroxisomes.

Nevertheless, the idea that over millennia mitochondria, plastids, prokaryotic and eukaryotic cells and even flagella and peroxisomes, as either primary or secondary endosymbionts, transferred some or all of their own DNA to the host cell's nucleus through a process called «endogenosymbiosis» (*i.e.* a symbiotic gene transfer, such as the internalisation of the endosymbiont's DNA with lateral transfer) has been lately proposed (Cazzolla Gatti 2015; 2016). The endogenosymbiosis could take place during the evolutionary transition from the symbiotic interacting community, invoked by Margulis, to a full-integrated (either prokaryotic or eukaryotic) cell. This process could explain the missing evidence of the presence of DNA in flagella and peroxisomes whose ancestor endosymbionts, during the long endogenosymbiotic evolution, could have transferred their whole genome to the host cell that subsequently integrated it in its own genome, directly controlling its expression. Furthermore, the endogenosymbiosis hypothesis could be the explanation of the transition between an RNA to a DNA world and of some cases of true sympatric evolution of species, apparently inexplicable by the canonical speciation processes (Enard *et al.* 2007; Berlanga *et al.* 2007; Cazzolla Gatti 2016).

2. The Theoretical Basis of the Endogenosymbiosis

The endogenosymbiotic hypothesis, as an evolutionary process, suggests that «gene carriers» (viruses, retroviruses and bacteriophages), symbiotic prokaryotes (bacteria or archaea) and eukaryotic cells could not only be replicated (lytic cycle) and endosymbiotically integrated into other cells, respectively, but can share parts or even

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cede all of their genetic material in an endogenous symbiotic relationship with their hosts (*i.e.* through symbiotic lysogenic cycle and symbiotic lateral gene transfer).

The difference between endogenosymbiosis horizontal gene transfer (HGT) as processes resides in the possibility, taken into account by the former, that parts of «parasite DNA» (such as genes) are integrated into «host cells» in a relationship that, after some evolutionary time, becomes symbiotic (evolutionary advantageous for both host and parasite's genes), which has not been explicitly considered by the HGT. Endosymbiotic (horizontal) gene transfer is only the process that explains the movement of DNA from organelles to the host cell's nucleus, while endogenosymbiosis hypothesis points out that the transferred genes becomes themselves symbiotic component of the host cell, irrespectively if they come from endosymbiotic bacteria or viruses/phages. Here I review the past hypotheses and proposes a unified theory and definition of the whole symbiogenetic processes. Endogenosymbiosis embraces a comprehensive definition since it does not need to distinguish between endosymbiont gene transfer (EGT) and transfer of viral DNA to host genomes (horizontal transfer), because in either cases they both are part of the same process: an endogenous symbiotic integration of external DNA (*i.e.* endo-*geno*-symbiosis).

Lynn Margulis in 1967 argued that the process of symbiotic collaboration had run alongside the classical Darwinian cycle of mutation, natural selection and adaptation. Then, the endogenosymbiosis hypothesis (Cazzolla Gatti 2016) proposed that the main likely cause of the evolution of sexual reproduction, the parasitism, could also represent the origin of biodiversity (Hamilton and Zuk 1982). In other terms, this hypothesis suggests that sexual reproduction acts as a conservative system against the inclusion of new genetic variations into cell's DNA (supported by the DNA repair systems; Generoso *et al.* 1980) and, instead, the evolution of species can take place only when this preservative system fails to contrast the inclusion, within the host genome, of external parts of genetic material coming from obligate «parasitic» elements (viruses and phages) and prokaryotic and eukaryotic cells that establish an endogenous symbiosis with their hosts.

Hamilton *et al.* (1990) strongly advocate that: «Darwinian theory has yet to explain adequately the fact of sex. If males provide little or no aid to offspring, a high (up to 2-fold) extra average fitness has to emerge as a property of a sexual parentage if sex is to be stable. The advantage must presumably come from recombination but has been hard to identify. It may well lie in the necessity to recombine defenses to defeat numerous parasites».

As two parallel evolutionary processes, sexual reproduction seems to preserve what the endogenosymbiosis moves to diversify (Cazzolla Gatti 2016). With sexual reproduction, the species can adapt slowly and, potentially, indefinitely to the external factors, adjusting themselves (by natural selection), but not «creating» much novelty (speciation). Endogenosymbiosis, instead, could lead to speciation due to changes in the host-endogenosymbiont's integrated DNA. Not only bacteria and archaea can be endosymbiotic with other cells and have given rise to the evolution of eukaryotic cells, as suggested by Lynn Margulis, but entire pieces of genetic material coming from endosymbiotic cell parasites and endosymbiotic prokaryotes

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and eukaryotes, can be included in the host genome, changing the gene expression and addressing the speciation processes.

This idea, together with the former symbiogenetic hypothesis, challenges the canonical natural selection models based on the gradualism of the mutation-adaptation pattern, providing more support to the punctuated equilibrium theory proposed by Stephen Jay Gould and Niles Eldredge (Gould and Eldredge 1977).

In the original formulation of the endogenosymbiosis hypothesis (Cazzolla Gatti 2016), it was proposed that during the early evolutionary events, by chance, some integrations of genetic parts, coming from successively evolved non-living endosymbiotic parasites of prokaryotes (such as viruses and bacteriophages), took place. The non-living prokaryotes' parasites could be considered as just fragments of genes encapsulated in protein coats moving among cells and using them to replicate. Then, after repeated interactions, the new genetic sequences could have been endogenosymbiotically integrated and later on transcribed, leading to the evolution of some cells that were more or less different from their ancestors. They became part of the so-called «polymorphic meta-populations» (Smith 1998). When these differences were big enough to separate the meta-populations reproductively (not only with genetic, physiological or anatomical barriers but, also, with simple behavioural aspects), the limits imposed by the environmental carrying capacity and density-dependent effects (Allee effect) could have forced some cells of the polymorphic meta-populations to exhibit phenotypic plasticity (Via *et al.* 1995). This would have led them to utilise different resources from those used by the individuals of the original population (*e.g.* by-products of them) or to metabolise some resources more efficiently. After the evolution of eukaryotic cells through the integration of organelles by endosymbiosis, viruses, prokaryotes and the eukaryotes themselves could have started an endogenosymbiotic integration of each other. This process, which started at the origin of life on Earth, seems to continue addressing the ongoing speciation events.

Therefore, the effect of acquiring symbiotic organelles through endosymbiosis (Margulis and Sagan 2008) and symbiotic genetic elements through endogenosymbiosis (Cazzolla Gatti 2016) could allow the adaptation to new niches by the consequent phenotypic plasticity (a variability made possible by the acquisition of external genetic material) and character displacement of some units of the different meta-population.

The successive evolution of sexual reproduction in eukaryotes (Kondrashov 1988), by recombining two different genetic pools, preventing the accumulation of deleterious of genetic mutations and increasing the probability of a favourable recombination (Michod *et al.* 2008, Bernstein *et al.* 2010; 2012), allowed the species to adapt to new environmental conditions but does not seem to have favoured the speciation of new ones. Sexual reproduction mainly results from a compromise between the need to transmit in the long-term as much genetic material as possible to future generations and the need to address the environmental variability, while halving its fitness potential (Hamilton 1993), but it does not generate diversity. In this perspective, the evolution of sexual reproduction appears as an extreme and ultimate action to preserve the species or, in other words, to adapt

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them to external changes and not as a mechanism capable of producing new species (Cazzolla Gatti 2006).

3. The Empirical Evidence of the Endogenosymbiosis

During the evolutionary process to become an organelle, an endosymbiont transfers most of its genes to the host cell genome (Keeling and Archibald 2008). In this way, the endosymbiont reduces its genome size. As a result of this transfer, a new transport mechanism to carry back the proteins needed by the new organelle but now transcribed and synthesised by the host cell is developed. It is well known that plastids and mitochondria show a huge reduction in their genome size when compared to their bacterial relatives (such as cyanobacteria and α-proteobacteria, respectively).

The genomic transfer between the endosymbiotic organelle and the host cell is well predicted by the endogenosymbiosis hypothesis, which implies that the longer the evolution of the symbiogenesis, the greater the amount of genome that is transferred from the organelle to the cell (Cazzolla Gatti 2016). The same applies to the «gene carriers» (viruses and phages) that, establishing long evolutionary endogenosymbiosis with their target cells, end up being gradually included in the host genome and being expressed under the host's control (Cazzolla Gatti 2016).

It can happen that, when the endogenosymbiosis is only partial (*i.e.* the transfer of DNA from the endosymbiont to the host cell is incomplete), another mechanism is implemented: the host cell simply regulates the former endosymbiont's division by synchronising it with its own division (Keeling and Archibald 2008).

For instance, another study revealed that chromatophores evolving from free-living cyanobacteria of the genus *Synechococcus* were subjected to a drastic genome shrinkage (Nowack *et al.* 2008). The lack of many relevant genes for biosynthetic functions in these endosymbiotic cells is a clear demonstration that after a long endogenosymbiosis they have become highly dependent on their hosts for their survival and growth mechanisms.

There is evidence that the more organelle genomes have reduced over evolutionary time, the more nuclear genes have expanded and become more complex (Timmis *et al.* 2004). Because the integration of the endosymbiont's genome within its host's genome is mainly attributed to nuclear gene transfer (Archibald 2009) many organelle processes are directed by nuclear-encoded gene products (Timmis *et al.* 2004). To explain the gene transfer two main opposing hypotheses have been suggested. However, they are not mutually exclusive and could be integrated if considered under the framework of the endogenosymbiosis hypothesis.

The cDNA hypothesis proposes that genes can be transferred from organelles to the nucleus via messenger RNA (mRNA). Here, mRNA is converted to cDNA and included in the host cell's genome (Timmis *et al.* 2004; Leister 2005).

The alternative bulk flow hypothesis, stating that escaped DNA rather than mRNA is the mechanism of gene transfer, predicts that in the initial stages of endosymbiosis there is a lack of major gene transfer and the endosymbiont underwent cell division independently of the host cell (Timmis *et al.* 2004; Leister 2005).

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As a result, many 'copies' of the endosymbiont resides within the host cell and a high quantity of DNA can be incorporated into the nucleus (Barbrook *et al.* 2006).

These two proposed processes match well in the light of the endogenosymbiosis hypothesis. In fact, both the gene transfer operated by «gene carriers» (the obligate endosymbiotic parasites: viruses and phages) that carry DNA and RNA between host cells, and the endogenosymbiotic transport of genetic materials, as either DNA or RNA, from the endosymbiont reside within the host cells (Cazzolla Gatti 2006) are not in conflict if considered as different ways to share genes in an endogenosymbiotic relationship.

Furthermore, the endogenosymbiosis hypothesis could fill the gap left by the endosymbiosis hypothesis and so explaining the symbiotic origin of peroxisomes and flagella. How the cellular membrane complexity arises within cells during evolution and how the collection of organelles is maintained in multiplying cells to ensure that new cells retain a complete set of them (Tabak *et al.* 2006) could be clarified by endosymbiotic gene transfer (*i.e.* endogenosymbiosis).

Many studies occasionally showed that in different species peroxisomes are in close association with endoplasmic reticulum with possible membrane continuities between them, suggesting that they originated from the ER (Novikoff and Novikoff 1972; Tabak *et al.* 2006). New evidence better fits a concept of peroxisomes being autonomous organelles: peroxisomes take up most of their proteins post-translationally utilising peroxisomal targeting signals and a peroxisome-specific protein import machinery (Subramani 1998).

Mutations in certain genes result in the loss of the complete peroxisome population of a cell but they could reappear upon introduction of a wild-type version of the gene (Tabak *et al.* 2006). To explain this reappearance some authors postulated the existence of a proto-peroxisome as a source of regeneration of the peroxisome population (Lazarow 2003). During the last years, many groups have found significant indications that ER is the contributor to peroxisome formation. De Duve (1969) proposed that peroxisomes evolved as a line of defence when 2.5-2.0 billion years ago O_2 started to appear in the earth's atmosphere. However, the endosymbiotic origin of peroxisomes has been challenged by the evidence of a lack of DNA and the consequent impossibility of autonomous inheritability.

Now, in the light of the theoretical basis of the endogenosymbiosis, it could be possible to disentangle how some groups of organelles can be derived from other autonomous organelles instead to take care of its individual inheritance. The idea that the ER can be viewed as the autonomous donor compartment for peroxisomes could be explained by the hypothesis that peroxisomes were originally autonomous prokaryotes that were endosymbiotically included in the host cell. Then, after a long endosymbiotic evolution, the endogenosymbiosis took place and the whole peroxisome DNA was transferred and included in the host cell's genome. This could be the reason why peroxisomes heritability is still possible even without DNA included in these organelles. It is likely that the genes responsible for peroxisome synthesis are now integrated among those involved in ER formation (De Duve 1969).

Indeed, about 20% of the peroxisome's enzymes can be traced to an alpha-proteobacterial origin (Gabaldón *et al.* 2006). This percentage is close to that of the mi-

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tochondria's proteome, which is considered as support for the endosymbiotic origin of mitochondria and for DNA mixing between mitochondrial and nuclear genomes (Kurland and Andersson 2000; Gabaldón and Huynen 2003). Considering that the percentage of peroxisomal proteome of eukaryotic origin is higher in early organisms (56% in yeast) than in those lately evolved (38% in rat) and that two recent studies (Devos *et al.* 2004; Jékely 2003) suggest that the formation of secretory endomembrane systems originated before the endosymbiosis of mitochondria or chloroplasts, it seems likely that, very early in evolution, peroxisome endogenosymbiosis allowed these organelles to gradually transfer their whole genome to the host cell's DNA, leaving just a residual fingerprint of a proteobacterial proteome origin.

This empirical approach, matched with the theoretical basis provided by the endogenosymbiosis hypothesis, might be also applied to better understand the origin of glyoxysomes. It is believed that these organelles that contain glycolytic enzymes have evolved from the peroxisome (Reinhold *et al.* 2016). This brings the ultimate origin back to the endogenosymbiotic origin of peroxisomes. There is increasing support for the idea that glyoxysomes in plants originate from the ER (González 1986; Gabaldón 2010). A study on Trypanosomatids shows that some proteins in the glyoxysomes of this taxon can be traced back to cyanobacteria, the other group of bacteria giving rise to endosymbionts (Hannaert 2003).

Explaining the origin of flagella by the endogenosymbiosis hypothesis poses a more difficult task.

However, the symbiotic origin of eukaryotic cilia and flagella from a symbiotic spirochete (Margulis 1967) may be supported by the fact that some eukaryotes, such as Mixotricha and Trichonympha, use symbiotic spirochetes as their motility organelles (Berlanga *et al.* 2007). At the same time, the lack of DNA and the homology of tubulin to the bacterial replication and cytoskeletal protein FtsZ are two major arguments against the endosymbiotic origin of cilia and flagella. Nevertheless, the fact that FtsZ-like proteins are found natively in archaea, suggests an endogenous ancestor to tubulin, could be better explained by the endogenosymbiosis process. It could still be plausible that archaea acquired tubulin from a symbiotic spirochete (the original Margulis' hypothesis) if we consider this event as a long evolved endosymbiotic gene transfer (*i.e.* an endogenosymbiotic process). The presence of FtsZ-like protein in the native archaea could be due to the total inclusion of the symbiotic spirochete's genome into that of the archaea. This could have led to the complete loss of direct gene expression control by the spirochete and the consequent integration of genes for the FtsZ-like protein in the host cell's genome.

The loss of ultrastructure and functions by the endogenosymbiotic spirochete may not be an isolated event. One of the most common «spirochete-like» cells, the sperm cell, which formed early during the evolution of sexual reproduction, completely lack the endoplasmic reticulum (ER). Nevertheless, Nakamura *et al.* (1993) discovered a Ca(2+)-binding protein from rat spermatogenic cells, which was identified as calreticulin and is a resident protein of the endoplasmic reticulum (ER). Their immunohistochemical studies revealed that calreticulin was present in the acrosome of both round spermatids and mature sperm, although they do not include

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any organelle similar to the somatic cell's endoplasmic reticulum (ER). Furthermore, Northern blot analysis of RNAs from purified populations of spermatogenic cells indicated that the calreticulin mRNA was present in both pre- and post-meiotic cells (Nakamura *et al.* 1993).

Although this ER protein is not produced directly in the sperm cell, Nakamura *et al.* (1993) speculated that it may be incorporated into the acrosomal vesicle via the Golgi apparatus, without glycosylation, during spermiogenesis, and may play an important role in the regulation of cell functions such as sperm motility and the acrosome reaction. As predicted by the endogenosymbiosis theory, the lack of DNA coding for sperm's ER proteins does not prevent their synthesis and utilisation. Similarly to the loss of flagellum's DNA, the genetic information for calreticulin is not encoded in the sperm cell itself but is closely associated with spermatogenesis of rats.

The fact that some organelles of endosymbiotic origin (*e.g.* hydrogenosomes and mitochondria) still contain genomes (Akhmanova *et al.* 1998, Embley *et al.* 2003) suggests that only some specific genes of a symbiont may become endo-*geno*-symbiotic, while other genes may rest part of the endosymbiont (*i.e.* only endo-symbiotic) without integration into host's DNA. The evidence that some endosymbionts (including viruses/phages) can almost completely 'donate' their own DNA to host cells, while others can just transfer some selected genes, highlights the importance to unify under a general evolutionary theory the two similar, but yet substantially evolutionary different, processes of endo*geno*symbiosis and endosymbiosis.

Determined for all her life, Lynn Margulis (1998) continuously argued in favour of her idea of the endosymbiotic origin of cilia and flagella. The idea that the integration of the centriole-kinetosome bacteria constituted the fundamental step for the evolution of a nucleated eukaryotic cell was supported by a study of the basal body and centriolar DNA of Chlamydomonas (Hall *et al.* 1989). These authors were able, for the first time, to capture some photos of reproducing Chlamydomonas where the centriole-kinetosome DNA, which is distinguishable from the remnant nuclear DNA in some developmental stages, was connected to the other chromosomal DNA during the mitotic division (Margulis 1998). The endogenosymbiotic hypothesis is reinforced and *vice versa* reinforces this empirical evidence and Margulis' argument (supported by the «Henneguy-Lenhossek theory»): the spirochetes could have established a long endogenosymbiotic relationship with their hosts, transferring their whole genome for the regulation of the centriole-kinetosome complex and allowing the evolution of the eukaryotic cells with their cilia. However, as for most scientific discussions, the debate is still open.

Furthermore, the hypothesis that viruses have played a critical role in the origin of DNA is based on the conception that retroviruses were relics of the RNA/DNA world transition (Lazcano *et al.* 1992; Forterre *et al.* 2004). The evolution of DNA from an RNA virus seems to be more likely than the invention of DNA by an RNA cell for protection against infection by viral RNAses. This seems justified by the fact that viruses have managed to multiply with very different types of genetic material (single and double strands of RNA and DNA, modified DNA) whereas, apart from

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localized methylation, all types of cells have the same kind of dsDNA genome (Forterre *et al.* 2004).

The endogenosymbiosis hypothesis endorses the idea of a viral origin for DNA. Although, the fact that many DNA viruses encode their own ribonucleotide reductase and/or thymidylate synthase is usually interpreted as the recruitment of cellular enzymes by viruses (Forterre *et al.* 2004) if DNA appeared in viruses, the opposite could be true as well. When placed into phylogenetic trees, many viral ribonucleotide reductases and thymidylate synthases branch off far from ribonucleotide reductases and thymidylate synthases of their hosts, confirming that the viral versions of these enzymes are indeed as ancient as their cellular versions (Forterre *et al.* 2004).

More recently, other independent studies provided empirical support to the endogenosymbiosis hypothesis. Henzy and Johnson (2016) demonstrated that the complex evolutionary history of the IFIT (Interferon Induced proteins with Tetratricopeptide repeats) family of antiviral genes has been shaped by continuous interactions between mammalian hosts and their many viruses.

Moreover, Enard and colleagues (2016) estimated that viruses have driven close to 30% of all adaptive amino acid changes in the part of the human proteome conserved within mammals. Their results suggest that viruses are one of the most dominant drivers of evolutionary change across mammalian and human proteomes.

Previously, it was estimated that about 7-8% percent of the entire human genome carries about 100,000 pieces of DNA that came from endogenous retroviruses but this may be an underestimate (Coffin 2004).

Finally, in 2016 the biologists Sarah R. Bordenstein and Seth R. Bordenstein, studying the bacteriophage WO particles of *Wolbachia*, indirectly confirmed the theoretical claim of the endogenosymbiosis hypothesis that genes are frequently and endosymbiotically transferred between hosts and parasites. Because eukaryotic genes are often co-opted by viruses and bacterial genes are commonly found in bacteriophages, they stressed that «the presence of bacteriophages in symbiotic bacteria that obligately reside in eukaryotes may promote eukaryotic DNA transfers to bacteriophages».

4. The Evolutionary Mechanism of the Endogenosymbiosis

A graphical summary of the integration between endosymbiosis and the endogenosymbiosis theories is shown in Fig. 1. The Serial Endosymbiosis Theory (SET, after Margulis 1967) and the Secondary Endosymbiosis (SE, after Gibbs 1978) are merged with the Endogenosymbiosis Theory (EGST, after Cazzolla Gatti 2016) in a Unified Symbiogenetic Theory (UST).

Initially, a simple (anaerobic) bacterium with a proto-DNA genome could have established an endosymbiosis with a spirochete-like bacterium (step 1) and evolved into the first flagellate cell. The bacteriophages, transporting genetic sequences collected from prokaryotic cells and integrating them into the host's genome, after repeated lytic cycles (a) could have evolved endogenosymbiotically (during some ly-

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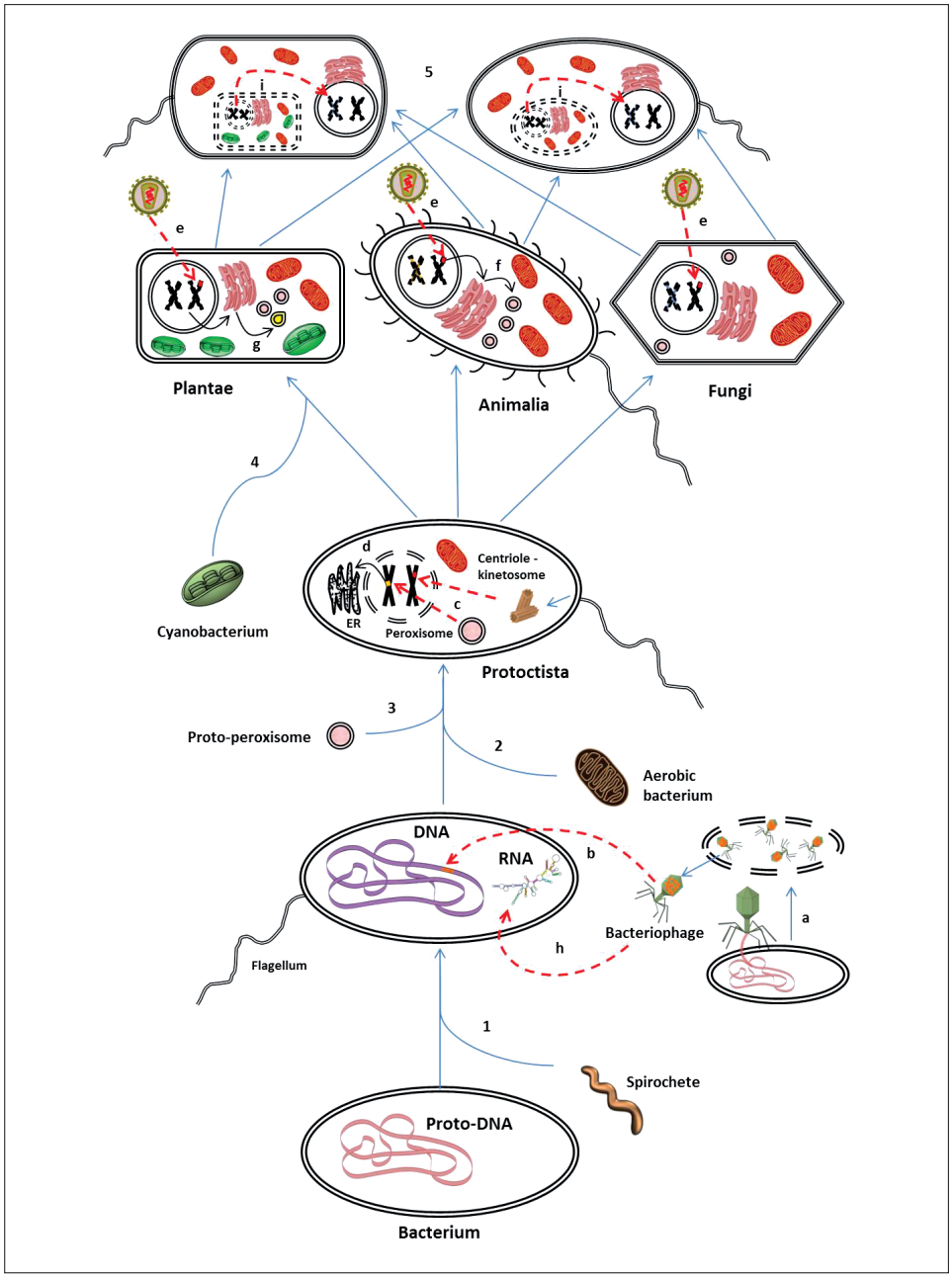

FIG. 1. A graphical summary of the integration between the endosymbiosis and the endogenosymbiosis theories. The Serial Endosymbiosis Theory (SET; after Margulis 1967) and the Secondary Endosymbiosis (SE, after Gibbs 1978) are merged with the Endogenosymbiosis Theory (EGST, after Cazzolla Gatti 2016) in a Unified Symbiogenetic Theory (UST). Blue arrows represent endosymbiotic events; red dotted arrows represent endogenosymbiotic events.
The description of the processes is in the text.

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sogenic cycles). This process could have led to a coevolution of cells and viruses in the transition from the RNA to the DNA world (b). Different replication mechanisms originated among various viral lineages after the invention of U-DNA and T-DNA by viruses and evolved through the endogenosymbiosis of RNA and the related enzymes (polymerases, helicases, nucleotide binding proteins; see Cornish-Bowden (2012) for a detailed description of enzyme kinetics) to produce the first proto-ribosome involved in DNA replication (h).

This prokaryote (with a cytoplasmic DNA and a ribosome-like RNA) by endosymbiosis with an aerobic bacterium (step 2) and a proto-peroxisome (step 3) evolved a 'package' including an aerobic metabolism and an oxidation protection system to cope with the increasing O_2 in the Earth's atmosphere.

This first Protoctista could have taken advantage of the new endosymbionts and, after some evolutionary time, could have integrated – with endogenosymbiosis – part of the proto-peroxisome and sprirochete's genome into its own gnome (c). Endogenosymbiosis with the proto-peroxisome could have been the basis of the evolution of the endoplasmic reticulum (ER). In fact, after the inclusion of some genes from the proto-peroxisome, the host cell nucleus could have directly transcribed the synthesis of the ER (d). Similarly, the endogenosymbiosis with the spirochete could have led to the formation of the centriole-kinetosome complex and, later on, of the nucleus typical of eukaryotic cells.

Subsequently, by natural selection, three main types of cell evolved. Those of animals and fungi directly followed from the protoctist with minor changes (the loss of cilia and flagella in some, the chitinization of the cell's wall in some others, etc.). Those of plants and photosynthetic algae, instead, evolved after another endosymbiosis with a cyanobacterium-like cell (step 4), which added the photosynthetic complex to the eukaryotic host.

Viruses, by sharing parts of genetic material among eukaryotes, after repeated interactions can become endogenosymbiotic (e), firmly integrated into host's DNA, and could have increased the genetic variability that has led to the evolving biological diversity.

After the endogenosymbiosis with proto-peroxisomes and the inclusion of part of their genes into the host's genome, eukaryotic cells could have evolved the capacity to transcribe the synthesis of ER from their nuclear DNA and the following production of eukaryotic peroxisomes (f) and plant glyoxysomes (g).

Eukaryotic cells continued to endogenosymbiotically integrate other genetic material from viruses and from secondary endosymbiosis (SE, step 5). SE takes place when a eukaryote cell engulfs another eukaryotic cell that has undergone primary endosymbiosis. During secondary endosymbiosis, the DNA of the primary endosymbiont can be transferred (partially or totally) to the host cell genome (i) in a secondary endogenosymbiotic (SEGS) process. Evident examples of secondary endosymbiosis are diatoms (Lucentini 2005). Here, an ancestor of a red alga engulfed a cyanobacterium that became the alga's chloroplasts and transferred, with a primary endogenosymbiosis, most of its genes to the host's nucleus. Then, an ancestral diatom engulfed both organisms and took over most of their genes (Armbrust *et al.* 2004), with a secondary endogenosymbiosis. The endosymbiotic red alga is now al-

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most undetectable within the diatom cell, where just a pair of extra membranes around the chloroplast are visible (Lucentini 2005). Here the possibility that secondary endosymbiosis and endogenosymbiosis could happen even within and among prokaryotic and eukaryotic cells is proposed (step 5, FIG. 1).

5. CONCLUSION

Primary and secondary (and serial) endosymbiosis and endogenosymbiosis have continued since the beginning of life and, with an autocatalytic process (Cazzolla Gatti 2017), allow the expansion of our planets' biodiversity (Cazzolla Gatti 2018).

In this new Unified Symbiogenetic Theory (UST) the non-trivial problem of the evolution of cooperation (in the even stronger form of endogenosymbiosis) is still present. Certainly, the host cell has enormous advantages in engulfing the endosymbionts (oxidation protection, motility, energy production, photosynthesis, etc.). A potential cost for the host, which also justifies the need for endogenosymbiosis over simple endosymbiosis, is a need for central coordination by the host's nucleus because of the presence of free radicals in energy-producing organelles that could damage sensitive nuclear genes during their fast asexual reproduction.

However, the evolutionary costs undertaken by the endogenosymbiont, ceding its own genome to the host cells and thus losing the replicating control and a «selfish reproductive advantage», could be balanced by the reduced energy needed for its own protein synthesis and increased fitness due to the higher physico-chemical protection by belonging to another cell and the more 'comfortable' cytoplasmic environment (including the reduced costs of searching for nutrients).

In the case of diatoms, for instance, a clear example of a gene's primary and secondary endogenosymbiosis have been shown (Nisbet *et al.* 2004). The gene called «cbbX» entered into cyanobacteria, probably through a plasmid, and moved to the red alga's nucleus. Then, it finally settled down in the diatom nucleus after secondary endosymbiosis and endogenosymbiosis.

Fitting serial endosymbiosis and endogenosymbiosis into a bigger picture, calming the debates about these cellular symbiotic processes, will surely require more empirical investigations, and more genomic and cytogenetic studies. However, this first attempt to define a Unified Symbiogenetic Theory (UST) could serve as a general framework and as a concrete basis for building up the next evolutionary research approaches.

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