

## Chapter 2

# Lateral Gene Transfer and the Evolution of Photosynthesis in Eukaryotes

Claudio H. Slamovits and Adrian Reyes-Prieto

**Abstract** Photosynthetic eukaryotes comprise the most visible and massive fraction of the biosphere. They have contributed to shaping land, oceans, and atmosphere during the last 2 billion years and their influence dominates every aspect of the existence of the rest of living beings, humans included. The introduction of photosynthesis into the eukaryotic domain and subsequent spread through various lineages by an endosymbiotic process are well-established facts, but the details implicated in allowing and driving the process remain under scrutiny. Relocation of genes from the intracellular symbiont into the host genome is critical to the origin of organelles by endosymbiosis, and an increasingly large body of evidence indicates that acquisition of genes from external sources can influence the organelle function to a large extent. In this chapter, we discuss the roles of gene transfer on the origins, evolution, and function of photosynthetic organelles in a wide range of eukaryotic organisms. A comprehensive review of recent studies devoted to elucidating the mechanisms involved in the migration of genes from endosymbiont to host nucleus is presented. In addition, we also mention the current controversies and recognize the difficulties faced by investigators working on this fascinating field. Finally, we identify several promising research questions that are likely to shed new light on our understanding of how gene flux has and does impact the evolution of photosynthetic eukaryotes.

## Photosynthesis in Eukaryotes

The origin of oxygenic photosynthesis is probably the single most important evolutionary event after the origin of life and the establishment of the first cells. The ability to assimilate inorganic carbon from the environment and turn it into organic matter ensured the

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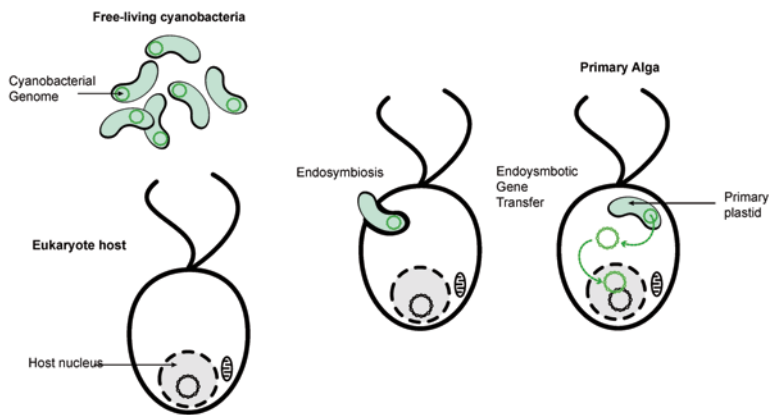
long-term survival of life, otherwise depending on the availability of organic molecules of abiotic origin. This complex molecular process originated about 2.4–2.3 billion years ago [1, 2] in the ancestors of the bacterial phylum known as cyanobacteria, a group that has achieved remarkable evolutionary success [3].

### *Plastids of Primary Origin*

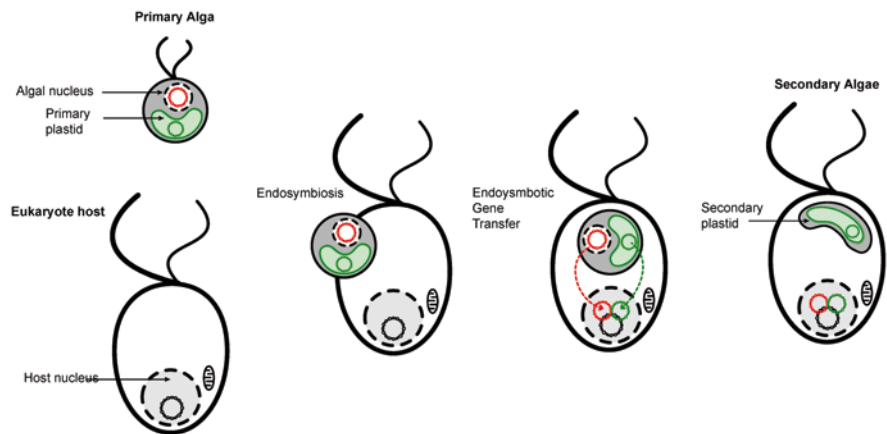
It is now well established that oxygenic photosynthesis arose only once in the ancestors of modern cyanobacteria, and it has been acquired by eukaryotes through a process of intracellular symbioses (i.e., endosymbiosis) involving a eukaryotic cell and photosynthetic cyanobacteria [4, 5] (Fig. 2.1a). There are some critical steps that presumably occurred during the ancient eukaryote–cyanobacteria endosymbiosis, such as survival of the cyanobacterial endosymbiont to the digestive process, the emergence of systems for cell-to-cell metabolite exchange, and the regulation of the endosymbiont cell division [4]. Exactly how this happened is still obscure as the remaining evidence of the process is buried in the intricacies of the genomes, proteins, and cellular structures of the enormous diversity of photosynthetic eukaryotes, when not completely lost. The existing evidence suggests that this process took a very long time and involved drastic changes in both partners that ended in the merger between two independent organisms into a single entity that incorporated the ability of harnessing solar power to synthesize its own building blocks and the flexibility of the eukaryotic cell. There is now a profuse body of genomic [6, 7] and cellular [8, 9] evidence indicating a single primary origin of all the plastids found in single-celled and multicellular photosynthetic eukaryotes, however, some contradictory results still maintain this scenario contentious and some authors consider the multiple origin as a likely explanation [10, 11]. However, a look at the diversity of plastids and plastid-bearing protists reveals that the subsequent evolution of plastids took a very complicated path. During the past decade, researchers have advanced significantly into the understanding of the origin and evolutionary history of photosynthetic eukaryotes and their plastids [6, 12–17]. In part, research in the field has been fueled by notable advances from several fronts, including biochemistry, ultrastructure, molecular biology, and more recently, bioinformatics and high-throughput sequencing technologies.

The primordial endosymbiotic event that gave rise to the first eukaryotic plastid spawned the diversification of a major eukaryotic lineage known as Archaeplastida [18], also referred to less formally as Plantae [19]. This monophyletic group contains three well-defined lineages: the viridiplants (comprising land plants and green algae), red algae, and glaucophytes, a lesser-known type of unicellular algae (Fig. 2.2). Plastids in these groups show distinctive signals of their primary origin: a double membrane, a prokaryotic-type genome with sequence features attesting to their cyanobacterial origin, and a complex arrangement of internal membranes (i.e., thylakoids) sustaining oxygenic photosynthesis. Several molecular phylogenies using plastidic [6, 20, 21] and nuclear [22–25] markers suggest the monophyly of the organelle and the hosts. Members of Archaeplastida contribute to a very important fraction of the global biodiversity, most

Primary endosymbiosis

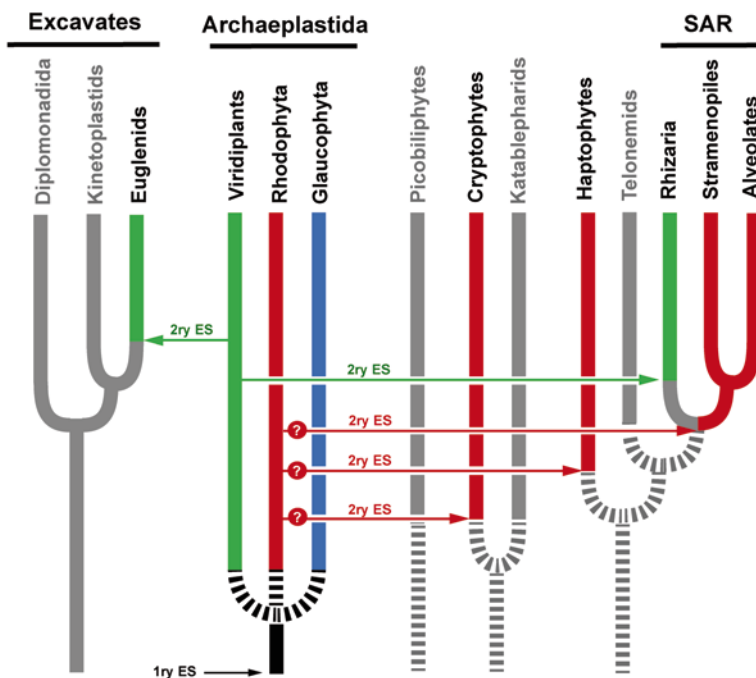


Secondary endosymbiosis



**Fig. 2.1** Origin of primary and secondary plastids in eukaryotes. The figure depicts the main steps of the process that led to the introduction and spread of photosynthesis among eukaryotes and the process of endosymbiotic gene transfer: **a** Primary endosymbiosis refers to the acquisition of a plastid by a heterotrophic eukaryote cell by engulfing, retaining, and assimilating cyanobacteria as a new cellular organelle. **b** In secondary endosymbiosis, which is known to have occurred several times, an eukaryotic alga is engulfed and assimilated by a heterotrophic cell and turned into a plastid

noticeable for the land plants and multicellular green and red algae. Very small unicellular green algae such as *Ostreococcus* and *Micromonas* conform a fundamental layer of the marine ecosystem known as picoplankton (cells <3  $\mu\text{m}$  in diameter), which is now



**Fig. 2.2** Distribution of plastids in the different eukaryote “supergroups”. The schematic trees illustrate the origin of primary plastid by endosymbiosis (1ry ES) with cyanobacteria in the common Archaeplastida ancestor (*black arrow*). The spread of plastids via secondary endosymbiosis (2ry ES) from primary algae to other eukaryote lineages (Euglenids, Cryptophytes, Haptophytes, Rhizaria, and Alveolates) is illustrated with *color horizontal lines*. The *question marks* indicate uncertainties in the number of secondary endosymbiosis with red algae that have occurred during eukaryote evolution. *Gray lines* highlight nonphotosynthetic groups related with secondary algae and *dashed lines* indicate phylogenetic relationships that are not entirely discerned yet

known to be a critical protagonist of global cycling of nutrients, minerals, and gases, thus critical for the balance of atmospheric phenomena and climate [14, 26, 27].

### *Plastids of Secondary and Tertiary Origin*

In addition to the members of the Archaeplastida, the global diversity of plastid-bearing eukaryotes includes many other lineages, which collectively exert an even larger influence of the geo- and biological global cycles [28, 29]. Such photosynthetic lineages also acquired their plastids through endosymbiosis but unlike the archaeplastids, they did so by incorporating a plastid-bearing eukaryotic cell (i.e., unicellular alga). As this resulted from the second of two successive endosymbiotic events, we call these secondary plastids [30] (Fig. 2.1b). The telltale feature that best betrays the origin of secondary plastids is the presence of additional membranes surrounding the organelle [31]. As

predicted by the hypothesis, the secondary plastids in most algae have four membranes: two inner membranes that are homologous to the original (i.e., cyanobacterial) primary plastidic membranes and two outer membranes that originated from the plasma membranes of the engulfed alga and the host vacuole (eukaryotic). There are two exceptions to the four-membrane organization; the plastids of photosynthetic euglenids and some dinoflagellate plastids have three membranes. This apparent inconsistency complicated the interpretation of the structural evidence in the light of the endosymbiotic hypothesis, and prompted alternative explanations for the origin of secondary plastids. For instance, it has been proposed that dinoflagellates independently acquired a plastid by virtue of the ability of some species to feed by mizocytosis, a feeding strategy by which a predator pierces the membrane of its prey and sucks up its content without mediating typical phagocytosis [32]. Although this may explain the lack of a fourth membrane in some groups, recent accumulation of evidence from several fronts strengthened the support for the standard endosymbiotic origin via phagocytosis of all secondary plastids and subsequent loss of some membranes that underlies the triple-membrane bound plastids of particular lineages [17, 25, 31, 33]. The number of times secondary endosymbiosis occurred during the evolution of photosynthetic eukaryotes and the identity of the type of eukaryote lineages involved are hotly debated questions in the field of plastid evolution, although it is now accepted that secondary plastids originated at least three times independently (Fig. 2.2). On one side, the chlorarachniophytes and photosynthetic euglenids have secondary plastids with strong signs of having arisen from two distinct endosymbiotic green algae [34]. On the other side, a number of lineages conforming a major fraction of eukaryotic diversity have secondary plastids derived from red algae [31, 35, 36]. Beyond this, certainty vanishes: the lineages harboring “red plastids” are many and their plastids exhibit a variety of idiosyncratic features, which makes it difficult to establish correspondence among them (or lack of). One prominent hypothesis claims that all secondary plastids derived from red algae have a single origin [31]. This view, called “the Chromalveolate hypothesis,” received increasing support, primarily from phylogenetic evidence suggesting that all the lineages with red plastids conform a large monophyletic group [37–39], and also because of shared characters (e.g., plastid-targeted genes sharing a distinct evolutionary history) that result in difficulty to justify under a scenario of independent plastid acquisitions [35]. The biggest challenge faced by the Chromalveolate hypothesis comes from the fact that several lineages interspersed among photosynthetic groups are nonphotosynthetic, some of them with vestigial plastids [40–42], and in several cases they are also thought to lack plastids altogether [43], a scenario that has been interpreted as evidence of multiple independent gains of secondary plastids [44]. In some cases, nonphotosynthetic members of the Chromalveolate group were found to either contain a plastid (e.g., the apicoplast in apicomplexan parasites) or have convincing evidence to have evolved from a photosynthetic ancestor, indicating that absence of plastids in some lineages does not necessarily support multiple origins [17, 33, 40]. The question of the origin and spread of red plastids is an ongoing debate. Resolving it requires a better resolution of the backbone of the phylogenetic tree of eukaryotes on one side, and a better understanding of the conflicting signal that often arises in analyses of putative genes of plastid ancestry [45]. The recent explosion of sequence data from key Chromalveolate and related lineages is expected to greatly

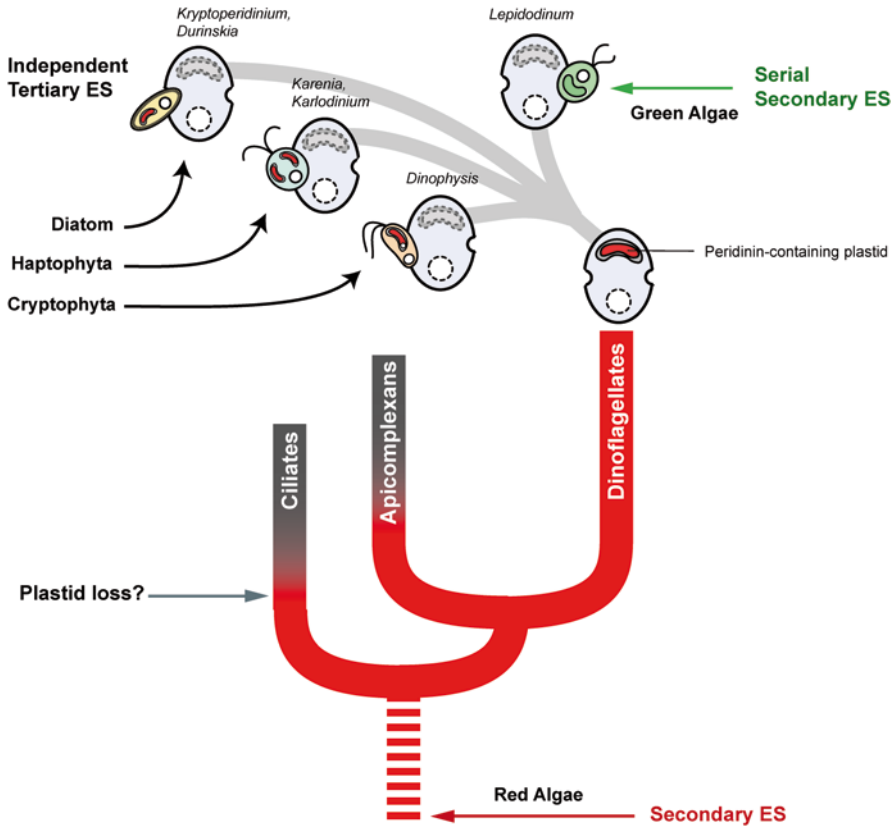
expand the available volume of evidence and hopefully spur a qualitative leap towards clarifying the evolution of secondary plastids.

### ***Spread of Plastids Beyond Secondary Endosymbiosis***

Even though there are numerous examples of protists harboring temporary photosynthetic endosymbionts, the presence of bona fide endosymbiotic organelles is not abundant, and the vast majority of the known cases trace back their origin to at least 1 billion of years in the past [20, 46]. The available evidence suggests that the genesis of a cellular organelle by endosymbiosis is an extremely rare event, attested by the fact that for some deep eukaryotic lineages (e.g., fungi, animal, and amoebozoa), nothing like this has occurred since the origin of the mitochondrion. There is, however, one group that seems to have a penchant for acquiring plastids. The ancestor of dinoflagellates inherited a plastid of red-algal origin, which has been lost (or highly reduced) and replaced by another plastid in at least four independent occasions (Fig. 2.3). In three of them, the newly assimilated plastids originated from a diatom, a haptophyte, and a cryptophyte, respectively [47–49]. Since all these organelle ancestors were already secondary plastids, these events are referred to as “tertiary” endosymbiosis [50, 51]. In the fourth case, the new plastid resulted from the assimilation of a green alga. Since the series of events involved in the last case is different from that in the previous three, this one is called “serial secondary” endosymbiosis [52]. Exactly why dinoflagellates take up and replace plastids so frequently is unknown, but it could be related to a combination of factors that are presumed to increase the probability for DNA movement between endosymbiont and host. For example, a voracious phagotrophic feeding behavior and elevated genomic plasticity that may be favoring rapid molecular integration of the ingested cell to the new intracellular habitat [53–56].

There are instances of interactions between colorless and photosynthetic protists that are not permanent and inextricable, like the plastid and its host, but could nonetheless leave long-term evolutionary consequences. Kleptoplastidy is the ability of some phagotrophic protists feeding on unicellular algae to retain undigested plastids from their prey [57]. The sequestered plastids remain on a physiologically active state providing the host with nutrients that sustain or supplement the host’s metabolic necessities for days to even months [57–59]. This phenomenon has largely been viewed as a curiosity and remained relatively obscure, but growing interest in lateral gene transfer (LGT; see further) has brought it into attention. It has been suggested that kleptoplastidy could represent an intermediate stage in the process of secondary endosymbiosis [60, 61], and an underlying process of gene transfers from the alga to the host would facilitate the apparent longevity of the plastids inside the host cell. This attractive hypothesis can provide an adaptive explanation for the intermediate stages of endosymbiotic organellogenesis, but few cases of alleged kleptoplastidy have been analyzed using massive sequencing, hence data supporting this view are still scarce [59, 62].

Plastids have been spread horizontally between eukaryotes several times. In contrast, the origin of plastids from photosynthetic bacteria (i.e., primary origin) is a much rarer



**Fig. 2.3** Multiple independent plastid replacements in dinoflagellates. The tree depicts the multiple cases of plastid replacements occurred during dinoflagellate evolution. Dinoflagellates are one of the three major Alveolate branches and considerable amount of molecular data suggest that all dinoflagellates share a common ancestor that harbored a secondary plastid (peridinin-containing) of red-algal origin. At least in three independent events, the ancestral peridinin-containing plastid has been replaced with organelles derived from tertiary endosymbiosis with secondary algae (diatoms, haptophytes, and cryptophytes). The case of a serial secondary endosymbiosis with green algae that replaced the ancestral peridinin-containing plastid is illustrated as well. Names of representative dinoflagellate genera that harbor tertiary and secondary plastid replacements are indicated

phenomenon, so much that the widely accepted view is that it occurred only once, early in the history of eukaryotes in the Archaeplastida [22, 50]. However, recent studies looking at microbial biodiversity using modern approaches have uncovered one ongoing case of a cyanobacterium–protist symbiotic system that is totally independent from the ancient and widely established chloroplast-based eukaryotic photosynthesis. The widely distributed freshwater filose amoeba *Paulinella chromatophora* has discarded the ancestral phagotrophic feeding style still maintained by its marine relatives, which feed on cyanobacteria, and harbors two conspicuous blue-green organelles called chromatophores. These structures were initially described more than a century ago [63], and their



role in phototrophy has been elucidated back in 1979 [64]. The chromatophores exhibit several features common with cyanobacteria of the genus *Synechococcus* and seem to have reached a high level of integration with the amoeba host cell, as evidenced by the synchronization of the division cycles of chromatophores and host cell [64]. This implies that systems for signaling and metabolites exchange must have developed in both partners. More recently, molecular phylogenetic analyses have shown that the cyanobacterial entity that gave rise to the chromatophores in *Paulinella* is not closely related to the group that gave rise to the ubiquitous plastid from plants and all known unicellular algae [65]. Moreover, the chromatophore genome has suffered considerable size reduction (>60%) as result of multiple gene losses and gene transferences into the host genome, resembling the process occurred in typical plastids of plants and algae. This finding means that the ancient event of endosymbiotic origin of a photosynthetic organelle from a prokaryotic free-living cell, although highly unlikely, occurred again more recently (approximately 60 million years ago; [66]). This “take two” of an ancient evolutionary performance is of tremendous importance, as it constitutes a window into the intermediate stages of the process of organellogenesis by endosymbiosis [12, 66–71].

The katablepharid flagellate *Hatena arenicola* constitutes another example of possible “plastid-in-the-making” process [72]. *H. arenicola* harbors a symbiont that has been identified as a green alga of the genus *Nephroselmis*. Among the many interesting features of this system, the most tale-telling of an ongoing organellisation are a suite of defined and consistent morphology changes in both the host and the symbiont in response to the presence or absence of the symbiont [73]. Interestingly, *H. arenicola* exhibits a dual life cycle where the host cell can sustain heterotrophic (phagotrophic) lifestyle when the symbiont is not present, whereas the presence of the *Nephroselmis* cell induces a drastic modification resulting in the loss of the feeding apparatus, which is replaced by the eyespot [73]. This observation implies that the symbiont-bearing cell’s ability to feed is at least partially impaired and thus it is probably depending on the photosynthetic machinery of the symbiont. There exist several other known cases of associations showing different stages of integration and coadaptation (see [74] for a review), and many more will surely be discovered, but the extent to which they represent stages of organellogenesis ultimately depends on our capacity to differentiate between an organelle and a symbiont, but that is a blurry concept that needs deep study and discussion.

## Lateral Gene Transfer and Endosymbiotic Gene Transfer

A working definition of lateral (or horizontal) gene transfer (LGT) as it can be seen in the Wikipedia entry for the term reads “the transfer of genetic material between organisms other than the vertical gene transfer” ([http://en.wikipedia.org/wiki/Horizontal\\_gene\\_transfer](http://en.wikipedia.org/wiki/Horizontal_gene_transfer)). As a definition by the negative, it implicitly includes a wide array of biological processes that result in the transfer of genetic material between individuals, excluding only the passing of genetic material from parents to offspring (i.e., phyletic) during reproduction. Given the fundamental differences between prokaryotes and eukaryotes, certain mechanisms of LGT are likely to be lineage-specific. For example,



DNA transfers mediated by phages and integron cassettes will tend to occur between bacteria sharing a habitat [75, 76]. Among the known situations that are conducive to the transfer and establishment of genetic material between eukaryotes or from bacteria to eukaryotes, endosymbioses can potentially result in massive gene transfer, usually from the bacterial endosymbiont to the host, with drastic and long-lasting effects for the two lineages involved in the relationship (Fig. 2.1). This chapter appraises the current understanding about the impact of endosymbiotic gene transfer (EGT), which has to be appreciated as a particular case of LGT, in lineages of photosynthetic eukaryotes.

### *Evidence of EGT*

The idea that the process of endosymbiosis may entail relocation of symbiont genes to the nucleus of the host was originally formulated during the second half of the 20th century. In the midst of Lynn Margulis' intellectual battle for the recognition of the endosymbiotic origin of the eukaryotic organelles [77], Jostein Goksøyr put forward a model for the evolution of the eukaryotic cell from prokaryotic forms in which he explicitly mentions the possibility of symbiont DNA being incorporated into the nucleus of the eukaryotic cell, giving it control over its partner [78]. Considering that Margulis' own view emphasized the autonomous character of the enslaved organelle, Goksøyr's foresight was remarkable. In 1981, Norman F. Weeden postulated a more explicit, well-supported model for gene transfer between the endosymbiont and the nucleus in the particular case of the plastid (chloroplast) and its host that takes into account the genetic and biochemical characteristics of the plastid and their similarities and differences with those of cyanobacteria and the eukaryotic (cytoplasmic) processes [79]. The key evidence for the occurrence of EGT comes from the study of the plastid genomes. Present day free-living cyanobacteria carry several thousands of genes in their genomes, thus it is reasonable to assume that the ancestor of plastids would have had a gene repertoire comparable to that range (between 2,000 and 5,000 genes). However, the genomes of plastids typically contain a few dozens to just about over 200 genes, which is clearly insufficient to sustain the physiology and perpetuation of the organelle, moreover considering that plastids require approximately 3,000 different proteins for their function and maintenance. These missing plastid genes must then have been relocated to the nucleus of the host cell, a process that involved the evolution of mechanisms to direct the products of those genes from the host's cytoplasm to the plastid [5, 80, 81]. A natural implication of this scenario is that the host nuclear genome must contain a number of genes with a phylogenetic signature resembling their cyanobacterial origin rather than the host's own ancestry [82]. In fact, whole genome sequencing of plants and algae has revealed several hundreds to thousands of genes of clear cyanobacterial affiliation and whose predicted functions are consistent with proteins and pathways that are thought to have been moved to the host during the establishment of the plastid [6, 81, 83]. Likewise, the nuclei of organisms with secondary or tertiary plastids contain a large proportion of genes originated, not only from the cyanobacterial ancestor or the original plastid but also from the nucleus of the alga that became the secondary plastid of the new host [12, 13, 84–86] (Fig. 2.1).

## ***Mechanisms of EGT in Land Plants and Green Algae***

Nuclear genome sequences of photosynthetic eukaryotes, including plants and algae, show that the contribution of EGT has been extensive [6, 81], but how did the genes from organelles move to the nucleus, and how long did this take? When both the source and destination of genes coexist in the same cell, as is the case of endosymbiotic associations, there are no obstacles involving acquisition of foreign genetic material. This draws a drastic difference with interorganism gene transfer because the movement of genes or genomic fragments is not only facilitated by physical proximity in EGT but also because the temporal permanency of the organelle provides continuous supply of a particular, functionally adjusted set of genes, increasing the likelihood of successful integration in the host genome and further reprogramming (e.g., gene expression and translation) to service the organelle. In principle, transfer of genes between genomes can occur via the integration of retrotranscribed processed (spliced and edited) transcripts (complementary DNA) or segments of genomic DNA. Some EGT events, which presumably occurred via RNA intermediaries, have been reported [87, 88], however it seems the most frequent EGT mechanism is mediated by genomic DNA [89–91]. To distinguish between cDNA and genomic DNA transfers one can examine sequences of nuclear encoded genes of plastid ancestry and look for telltale signs such as the presence of organellar introns in the case of a transfer of DNA, or evidence for RNA processing such as editing, which would indicate that the gene in question has been transferred in the form of cDNA [92]. The problem is that once a gene has established in the host genome, sequence divergence in a new genetic context would quickly blur these or other features. Even though those genes can still be recognized as former plastid (i.e., cyanobacterial-derived) genes, the molecular mechanism that drove them into the host genome is unknown. To answer this question, recent cases of EGT have been examined by two different approaches, both of which have been very fruitful in understanding the mechanistic aspects of EGT. One approach consists of analyzing DNA sequences from plant and algal nuclear genomes to look for recent, naturally occurring incorporations of plastid (chloroplast) DNA, whereas the second approach involves experimental reconstruction of the EGT process in the laboratory.

The first type of surveys revealed the presence of numerous fragments of plastidic DNA embedded in the nuclear genomes of plants. These fragments, dubbed NUPTs for Nuclear PlasTid DNA, represent random insertions of plastidic DNA ranging from a few tens of base pairs up to complete plastidic genomes (see [89] for review). The nuclear genome of *Arabidopsis thaliana*, the most thoroughly studied among viridiplants, carries 35 kb in NUPT sequences, which represent 19% of its 154.5 kb plastid genome [93]. The larger nuclear genome of rice, however, contains much more NUPT DNA: NUPTs cover 99% of its 134 kb plastid genome with a total of just over 800 kb [94]. As more genomic data become available, the characteristics and patterns of NUPT distribution and variation are revealing interesting clues on the process of EGT. One general observation is that NUPTs and NUMTs (Nuclear MiTochondrial DNA) exhibit similar behaviors regarding the dynamics of their genomic distribution, sequence evolution etc. This indicates that once they become integrated in the nuclear genome, their behavior is governed by the nuclear genomic dynamics, regardless of their origin. Another general



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