Review

Biogenesis and origin of thylakoid membranes

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Abstract

Thylakoids are photosynthetically active membranes found in Cyanobacteria and chloroplasts. It is likely that they originated in photosynthetic bacteria, probably in close connection to the occurrence of photosystem II and oxygenic photosynthesis. In higher plants, chloroplasts develop from undifferentiated proplastids. These contain very few internal membranes and the whole thylakoid membrane system is built when chloroplast differentiation takes place. During cell and organelle division a constant synthesis of new thylakoid membrane material is required. Also, rapid adaptation to changes in light conditions and long term adaptation to a number of environmental factors are accomplished by changes in the lipid and protein content of the thylakoids. Thus regulation of synthesis and assembly of all these elements is required to ensure optimal function of these membranes. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Evolution of oxygenic photosynthesis with its CO₂ fixation and oxygen release enabled life on earth as we experience it today. It is assumed that oxygenic photosynthesis developed several billion years ago in an ancestor of today’s cyanobacteria most likely from an already existing anoxygenic photosynthesis apparatus [1]. The capacity to perform oxygenic photosynthesis was passed on to algae and higher plants by an endosymbiotic event that turned a cyanobacterium into a cell organelle, the chloroplast. The photosynthetic machinery of both, cyanobacteria and chloroplasts, is located on a special internal membrane system, the thylakoids. Thanks to the unique architecture of this membrane cyanobacteria and chloroplasts convert solar energy into chemical energy with an efficiency significantly better than any man-made photovoltaic system. Therefore, the ability of the cell to build and alter this membrane system is essential for efficient oxygenic photosynthesis. Resulting from the combination of structural, biochemical, and genetic analysis, we have a well founded knowledge of the ultrastructure and composition of thylakoid membranes, but despite the importance that the thylakoid membrane system has for photosynthesis and the energy metabolism of plants and cyanobacteria, the molecular processes connected to the origin, synthesis, maintenance and adaptation of the thylakoids remain elusive. In this review we will discuss recent findings on thylakoid
biogenesis and evolution and their impact on our understanding. Since most studies concerning the biogenesis of thylakoids have been performed on chloroplasts of higher plants and green algae, this review will focus on these organisms. The last section will deal with internal membrane systems in bacteria, especially the thylakoids of cyanobacteria, and the evolution of the thylakoid membrane in these organisms.

2. Form and function in plastids

In 1848 chloroplasts were first described by Unger simply as pigment bound structures. Later in the 19th century Schimper [2,3] characterized these structures, which he called 'Chlorophyllkörner', in greater detail. With only the resolution of the light microscope available, he described plastids as cell components containing chlorophyll or other pigments which develop from colorless precursors. He already perceived the existence of various types of plastids and made the observation that they can pass through different stages during their development. Shortly after the invention of the electron microscope, the first electron micrographs of chloroplasts were published [4], and soon thereafter, this new technique was used for the first detailed studies of different plastid forms and their development. In the late 50s the basic structure of thylakoids had been described [5–7] and the means of thylakoid biogenesis were discussed.

Thylakoids are the dominating structure inside fully mature chloroplasts. The formation and alteration of the thylakoid membrane structure and composition are closely connected to the development of the chloroplasts from simple, undifferentiated proplastids. These are small round shaped organelles hardly distinguishable from mitochondria (Fig. 1), that contain very few internal membranes that are often found as vesicles or small saccular structures [7–9]. Occasionally these membranes are observed continuous with the inner envelope.

In the presence of light proplastids develop into mature chloroplasts. This transition has been intensively studied in grasses. The leaves of these monocotyledonous plants grow with a basal meristem and hence form a developmental gradient. Cells found at the base of the leaf are youngest and contain mainly proplastids while the oldest cells with fully developed chloroplasts are found close to the tip. Ultra-thin sections revealed that during the progress of chloroplast maturation the internal membrane system builds up in consecutive phases. First, long lamella are formed which are later complemented by smaller, disc-shaped structures that associate into so-called grana stacks (Fig. 1). At the same time the typical lens-shape form of the chloroplasts develops. Finally, mature plant chloroplasts contain a complex and intertwined internal membrane system which was named ‘thylakoids’ according to the Greek word ‘Θυλακοσκόμησις’ (sack-like) [10]. In fully mature chloroplast no continuation between the inner envelope and the thylakoids has been observed.

In the absence of light proplastids turn into etioplasts which contain very few internal membranes but a characteristic prolamellar body [11,12]. The prolamellar body is a paracrystalline structure consisting of lipids and essentially a single protein, the NADPH-dependent protochlorophyllide oxidoreductase [13,14]. Shortly after the onset of illumination the prolamellar body is dispersed and thylakoids begin to form [7,12,15]. Since the start of illumination can easily be controlled in experimental setups, this system has often been used to study chloroplast development. Prolamellar bodies are mainly considered in connection with etioplasts but they are not restricted to them. Already after a short period of darkness ‘secondary’ prolamellar bodies form inside fully matured chloroplasts [16,17]. This results in the coexistence of prolamellar bodies and thylakoids and raises questions about the function of the prolamellar body for the mature chloroplasts.

Proplastids can further develop into chromoplasts or leucoplasts. These are specialized forms of plastids used for coloration or storage [18]. Chromoplasts are carotenoid-containing plastids found in many flower petals, fruits and roots. Coloration of these organs is often ascribed to chromoplasts and this might even be their main function. Leucoplasts are characterized by a lack of coloration and they can be distinguished by the substance that is stored, i.e. amyloplasts, proteoplas or elaioplasts. The final stage of a plastid’s life is the senescent or gerontoplast. These are plastids that have reached a stage of senescence that is not reversible. All plastids, independent of their sta-
tus, retain the ability to develop into each other (Fig. 1). Interconversion of different plastid forms requires dramatic changes of the ultrastructure, including the biogenesis, reorganization and regression of internal membranes.

3. Structure and composition of the thylakoid membrane

The additional compartment that the thylakoid network creates in cyanobacterial cells and in chloroplasts is an important feature that distinguishes these from bacteria performing anoxygenic photosynthesis. In these latter organisms, the internal membranes are invaginations still continuous with the plasma membrane [19,20]. In mature chloroplasts and in cyanobacteria it is assumed that the thylakoids are no longer connected to the inner envelope or the plasma membrane, respectively, because no such continuum can be observed in electron microscopic pictures.

How is this unique structural composition achieved? In cyanobacteria and many algae, thylakoids consist mainly of single layers formed by long lamellae. The structure of the thylakoid membrane in a fully mature chloroplast is more complex (Fig. 1). Initiated by earlier electron microscopic studies a model for the thylakoid structure as a huge intertwined network of stroma lamellae con-

Fig. 1. Overview of the development of chloroplasts. Chloroplast develop from undifferentiated proplastids. During maturation the complex internal thylakoid membrane network is formed. Proplastids can also develop into other plastid forms, such as etioplasts, chromoplasts and leucoplasts. Moreover, fully differentiated plastids retain the ability to develop into each other. Gerontoplasts are a final stage in plastid development in which a level of senescence is reached that is irreversible. The electron microscopic pictures of thin sections show several stages in the development of a chloroplast.
necting grana stacks was proposed which with little alterations is still valid today [21,22]. One can distinguish two major parts, the grana and the stroma lamellae. Grana are short, disc-shaped lamellae closely packed to form stacks. These stacks are interconnected by stroma lamellae which also form prolonged extensions into the stroma. Thus, the arrangement of the thylakoid membrane system creates a single huge compartment inside the chloroplast, the thylakoid lumen. Additionally to creating a single internal space this structure builds a membrane surface that is much larger than a simple invagination of the inner envelope would generate.

To understand the complexity of the task that the formation of thylakoids presents to the cell, it is important to figure the components that are required to build up this special photosynthetic membrane. Thylakoids are lipid bilayers with a unique glycerolipid composition different from other cell membranes. Thylakoid lipids have a high content, about 70–80%, of galactosyl diglycerides and both monogalactosyl diacylglycerol and digalactosyl diacylglycerol are lipids nearly exclusively found in plastidal membranes [23]. Notably, these galactolipids contain two highly unsaturated fatty acyl chains instead of one as is common in membrane lipids and are both non-bilayer forming lipids. Additionally the thylakoids contain phosphatidylglycerol and sulfoquinovosyl diacylglycerol together with other minor components [23]. All these lipids are not evenly distributed along the thylakoid membrane. Instead the lipid distribution differs between the leaflet that is exposed to the stroma and the inner leaflet that faces the thylakoid lumen [23]. It is not clear how this asymmetrical arrangement of the lipid distribution is achieved. Yet it has to be assumed that it is important for the function of the thylakoid membrane.

The dominant protein complexes of the thylakoids are photosystems I [24] and II [25] and their associated light harvesting antenna, the cytochrome b₆f complex [26] and the proton-translocating ATP synthase [27]. These complexes comprise not only many peripheral and integral proteins but also a variety of pigments and co-factors [28]. Their assembly is, therefore, a complex process and requires a larger number of auxiliary and regulatory factors [28,29]. These factors are involved in the membrane integration, modification and later degradation of the proteinaceous components and are also required for the addition of the pigments and co-factors. To complicate matters, certain components, like the two photosystems, are unevenly distributed in the thylakoid membrane network. While photosystem I is most abundant in the non-stacked stroma lamellae, photosystem II is the dominating component of the grana stacks [30]. Thus thylakoid biogenesis and maintenance have to assure not only the arrangement of a functional but at the same time asymmetric architecture of both the lipid and the protein components of this membrane.

4. Thylakoid membrane formation

One of the most elusive aspects of thylakoid formation is the exact mechanism by which the membrane itself is formed. In young, not yet differentiated plastids a continuum can sometimes be observed between the inner envelope and the developing internal membrane structures [7–9]. Thus the synthesis of early thylakoid membranes might be achieved by invagination of the inner envelope. Even in fully mature chloroplasts the thylakoid membrane is a very dynamic system. Short-term adaptation to changing light conditions is obtained by movement of proteins, especially the light harvesting complex, within the thylakoid membrane. Long-term adaptation on the other hand is achieved by a change in the protein and lipid content of the thylakoids. Although in mature chloroplasts a continuum between the inner envelope and the thylakoids can no longer be observed, the membrane material required for synthesis and maintenance of the thylakoids originates from the chloroplast’s inner envelope [7,31,32] and not from de novo synthesis on already existing thylakoids.

How these lipids are transferred from the inner envelope to the thylakoids is controversially discussed. One possibility would be the transfer by vesicles which is a common phenomenon in the cytosol, where vesicle traffic is involved in many different cellular processes including the secretory pathway, endocytosis, neural transmission and vacuole formation [33]. A similar vesicle transfer from the inner envelope to the thylakoids has been implicated in the synthesis of thylakoid membranes.
Vesicles inside plastids have been observed in early electron microscopic studies [7–9]. They are common in proplastids and have also been observed on the inner envelope of etioplasts in dark-grown cells of the Chlamydomonas y-1 mutant, shortly after illumination when chloroplast development sets in [36]. On the other hand vesicles are very rarely detected in mature chloroplasts. They do accumulate in the stromal space between the inner envelope and the thylakoids after a low temperature incubation of leaf tissue [34,35]. A similar phenomenon is described for vesicle transport in animal cells, i.e. endoplasmic reticulum to Golgi and Golgi to plasma membrane, where low temperature blocks the fusion of vesicles with their target membrane [37]. Further indication for vesicle transfer in chloroplasts comes from mutant analysis. In several plant mutants that are affected in thylakoid biogenesis, an accumulation of vesicles can be observed. Others, like the vipp1 mutant of Arabidopsis, no longer exhibited low temperature vesicle accumulation [35].

The possibility of vesicle transfer inside the chloroplast raised the additional question whether solely membrane lipids would be transported by these vesicles. As in vacuole formation the vesicle transport in chloroplasts could be limited to the supply of thylakoid lipids that are either synthesized at the inner envelope, i.e. galactolipids, or imported from the cytosol. It is also possible that non-lipid components of the thylakoid membrane might be transported by means of vesicle traffic [38,39]. Several of the non-lipid components required for the biogenesis and maintenance of thylakoids are synthesized on the envelope, i.e. carotenoids, or in the cytosol [40,41]. Especially hydrophobic components would require a system to travel through the aqueous stroma.

During chloroplast maturation an extensive formation of thylakoid membranes occurs in concert with the accumulation of the photosynthetic complexes. Several of the proteinaceous components are nuclear encoded and post-translationally imported into the chloroplasts. It was suggested that in Chlamydomonas the nuclear encoded light harvesting complex proteins are inserted into newly developing membranes at the inner envelope immediately upon their entrance in the organelle [42]. Later on, the development of the thylakoid system continues with the formation of grana stacking. Again, integration of the light harvesting complex into the thylakoid membrane might play an important role in this structural reconstruction [43]. This early speculation was supported recently by Simidjiev and coworkers, who showed that delipidated light harvesting complexes would restructure into ordered lamellae by the addition of monogalactosyl diacylglycerol [44]. They concluded that the light harvesting complex together with monogalactosyl diacylglycerol is responsible for lamellae organization of the thylakoid membrane. Therefore interaction between thylakoid proteins and thylakoid lipids seems important for the formation of the lipid bilayer in a membrane whose main components are non-bilayer forming lipids.

5. Regulation of thylakoid biogenesis

How is the formation of the thylakoid lipid bilayer coordinated with the expression of proteins and the biosynthesis of pigments and co-factors? It became obvious quite early after the identification of DNA and genome structure that plastid development and thylakoid formation is controlled by both the genome of the cell (nucleome) and the organelle (plastome). Plastids contain up to several hundred copies of a circular chromosome with a size between 120 and 220 kb. Encoded on the plastome is an average of about 100–200 proteins in addition to a full set of ribosomal and transfer RNAs [45,46]. Chloroplasts are, however, estimated to house about 2000–5000 different proteins; consequently only 5–10% of the plastidial proteins are encoded within the plastome [46,47] and the majority of proteins required for plastid development and function are encoded in the nucleus. These nuclear encoded proteins are translated on cytoplasmic ribosomes and have to be post-translationally transported to the chloroplast ([48]; Jarvis and Soll, this issue).

Many protein complexes and biosynthetic pathways of the chloroplast contain components encoded both in the nucleome and in the plastome and virtually all chloroplast functions require the concerted action of nuclear and plastidial encoded factors (Fig. 2). Complex regulatory processes are required to ensure that gene expression of proteins encoded in the nucleome is properly coordinated with the expression
of plastome encoded proteins. At the same time the coordinated development of all plastids in one cell has to be guaranteed.

Thylakoids become photochemically competent very early in their development [49,50]. The level of transcription, which is quite low in proplastids, increases drastically when the chloroplast begins to mature [51]. At the same time the translational apparatus inside the plastids is built up [52]. It is believed that the nucleus has the control over the onset of chloroplast differentiation and also takes the leading part in further developmental stages. To execute this control most regulatory components have been transferred to the nucleus [53]. At the same time the plastids signal back their development stage and condition to the nucleus. These signals, often called the ‘plastidal factor’, influence the expression of nuclear encoded plastid proteins [54–56]. The biogenesis and function of the chloroplast are therefore an integral part of the plant cell and the development of the cell and its organelle are interdependent [57,58]. This is supported by the fact that plastids cannot easily be exchanged into a different cell background [59–61].

This interdependence of the cell and its organelle is further strengthened by the fact that two different RNA polymerases are required to transcribe plastidal genes [62,63]. This includes a phage-type RNA polymerase of nuclear origin [64] and an eubacterial, multisubunit enzyme whose core subunits are encoded by the plastome [63] while its sigma factor subunits are encoded by nuclear genes [65]. The nuclear encoded RNA polymerase is primarily responsible for transcription of so-called ‘housekeeping’ genes of the chloroplasts, while the bacterial-type enzyme preferentially transcribes genes encoding components of the photosynthetic machinery [66].

Very little is known so far about the regulation of plastidal import in relation to plastid development. Most studies on the regulation of plastidal import have been done on fully mature, photosynthetically active chloroplasts (Jarvis and Soll, this issue). A recent publication indicates a direct influence of assembly of the light harvesting complex on the import of the chlorophyll binding protein into the chloroplast [42]. A similar regulation could be envisioned for other nuclear encoded chloroplast proteins since also in mature chloroplasts the thylakoid composition is very dynamic and undergoes constant changes in order to adapt to changing environmental conditions. The ability for adaptation is specially important since plants are not mobile and can therefore not escape unfavorable conditions. Only a constant

Fig. 2. Schematic display of nucleus–chloroplast interaction. Synthesis of plastid encoded proteins is regulated by nuclear encoded factors from the point of gene expression and translation until the final incorporation into the thylakoid membrane. At the same time the chloroplast signals the nucleus about its state of development. This signal influences the expression of nuclear encoded genes. This figure is based on a scheme presented by Rochaix [102].
communication between the organelle and the nucleus can ensure a coordinated supply of all the different factors required.

6. Analysis of thylakoid biogenesis through mutants

Mutants are a powerful tool to study the involvement of gene products on specific processes. Many different mutants that display deficiencies in plastid development and thylakoid formation exist in a wide range of species. Many of these mutants are randomly occurring natural variations, others are man-made. In early work, new mutants were produced by treatment of plants or algae with radiation or chemical mutagens [43,67]. Later, the necessary genetic tools became available for random insertional mutagenesis by T-DNA of Agrobacterium tumefaciens [68-70] or transposable elements. Only a limited set of these mutants can be discussed within this section. For a more detailed summary of mutants see [43,71,72].

There are many different types of mutations that affect both plastid development and thylakoid formation and the effect that a mutation has on either is often difficult to distinguish. Often these mutants are blocked in a step of a biosynthetic pathway located inside the chloroplasts. The resulting loss of a functional component of the plastid then extends its effect on the macromolecular structures. In other mutants structural components of the thylakoid membrane are missing or defective. Mutations can affect plastids in all stages of thylakoid formation. In several cases plastids are blocked very early in development. These mutants include dcl from tomato [73], dag from Antirrhinum [74], cla1-1 from Arabidopsis thaliana [75] and several albina mutants of barley [65,76]. Plastids in dcl, dag and cla1-1 seem to be arrested in the proplastid stage while plastids in some of the barley albina mutants can reach the size of mature chloroplasts but remain fully depleted of internal membrane structures except for vesicles that accumulate in some of them.

A similar phenotype can be observed in ΔroA, B and CI mutants that lack the bacterial-type RNA polymerase and consequently the ability to transcribe the photosynthetic genes which encode subunits of thylakoid protein complexes [62,77]. Other mutants can be found that are blocked in later stages of plastid development, anywhere from the proplastid to mature chloroplasts. Because of the close connection between plastid development and thylakoid formation it is often difficult to distinguish pleiotropic effects of these mutations. In some cases mutants seem to suffer from a secondary destruction of the internal membrane structure rather than a defect in thylakoid synthesis [78,79].

Defects in thylakoid formation are often caused by mutations that result in a depletion of major proteinaceous components of the thylakoid membrane, e.g. major components of the photosystems. For instance, the hcf136 mutant of A. thaliana cannot assemble a functional photosystem II, and this defect is associated with a drastically disturbed thylakoid membrane system [80]. Mutations of the protein import apparatus of chloroplasts cause similar defects in thylakoid formation [81]. Other mutations that have a great impact on thylakoid formation are mutations that affect the import pathways by which proteins are inserted into the thylakoid membrane [82-84]. Examples for such mutants can be found in maize in the form of tha1 and tha5 which inhibit the SecA-type import pathway and hcf106 and tha4 where the Δph or Tat pathway is disrupted [85,86]. Not surprisingly, mutants that affect the synthesis of important thylakoid lipids display alterations in the chloroplast ultrastructure. Arabidopsis dgd1 and mdg1 mutants lack the enzymes monogalactosyl diacylglycerol synthase or digalactosyl diacylglycerol synthase that are required for the formation of the two major thylakoid membrane lipids. These mutants show a wide range of alterations including changes in the chloroplast ultrastructure and protein composition [87-89].

Also very common is the connection between deficiencies in thylakoid formation and disruption of pigment biosynthesis [43,67,90]. While pleiotropic effects of these mutations cannot be excluded in some cases, many investigations have supported the potential influence of chlorophyll production on chloroplast development [43,90-93]. This connection is especially interesting in light of the ‘plastidal factor’ that is discussed as a signal from the chloroplasts to the nucleus (Fig. 2). As described above, the ‘plastidal factor’ is thought to signal the developmental stage of the plastid to the nucleus and affect the
expression of many different nuclear encoded genes [55,56]. So far the nature of this plastidial factor remained elusive and indication for the existence of more than one signaling pathways exists. A recent paper by Chory and coworkers on the Arabidopsis mutant uncoupled 5, together with earlier studies by other groups, provides evidence that one of these factors might have been found ([94] and references therein). Their findings indicate that a subunit of Mg-chelatase, the enzyme that converts protoporphyrin IX into Mg-protoporphyrin, has an additional, distinct function in the plastid–nucleus signaling pathway.

Especially interesting are mutants that affect thylakoid formation in otherwise fully developed chloroplasts. One recent example is the vipp1 mutants of Arabidopsis and Synechocystis. Mutant analysis showed that the gene product of vipp1 is involved in the biogenesis of thylakoids in Arabidopsis and cyanobacteria [35,95]. Interruption of the vipp1 gene locus results in a complete loss of thylakoid membranes. It seems that Vip1 is directly involved in the process of thylakoid biogenesis. Even more, phylogenetic analysis indicated that the presence of this protein might be a prerequisite to the ability of cyanobacteria and chloroplasts to form internal membranes. Interestingly, the Arabidopsis vipp1 mutant additionally lost the ability for vesicle formation. A vesicle transport system might thus be important for thylakoid formation in mature chloroplasts.

7. Evolution of the thylakoid membrane system

Cyanobacteria are the only phototrophic prokaryotes that carry out oxygenic photosynthesis with two photosystems. They very much resemble chloroplasts and it is assumed that at the time of the endosymbiotic event they had already invented oxygenic photosynthesis and developed most of the photosynthetic features found in chloroplasts today. Like chloroplasts, most cyanobacteria contain an internal membrane system in which the photosynthetic apparatus is located. Extensive stacking of grana lamellae is not found in these organisms. Their thylakoids are organized in layers often paralleling the contour of the cells. Algae are probably the organism most similar to the early endosymbiotic cells. Similar to cyanobacteria, most algae do not contain grana stacks. Chloroplasts of red algae contain a simple thylakoid structure similar to cyanobacteria. In green and brown algae regions of closely appressed thylakoid membranes occur similar to grana stacks in chloroplasts of higher plants [15]. Also many algae contain only a single chloroplast per cell. These structural similarities fit well with an evolutionary position between the cyanobacterial endosymbiont and higher plants.

It is still a point of debate where photosynthesis developed in the first place. Recent results favor an origin of photosynthesis in anoxygenic bacteria [1]. Phototropic green and purple bacteria carry out anoxygenic photosynthesis with a single photosystem strongly resembling photosystem I. In green-sulfur bacteria the photosynthetic machinery is located in the cytoplasmic membrane and the antenna complexes reside in a special non-membranous structure, the chlorosomes, closely attached to the cytoplasmic membrane [96]. Purple bacteria on the other hand often display strong invagination of the cytoplasmic membrane and their photosystems are concentrated in these intracytoplasmic membrane regions [97,98]. It is believed that these membranes are not fully separated from the cytoplasmic membrane and still form a continuum with the latter [20,21]. It is therefore tempting to speculate that the development of oxygenic photosynthesis is connected to two different events: the invention of the second photosystem and the biogenesis of an internal membrane system disconnected from the cell membrane. Support for this speculation arose from the identification of Vipp1, a protein essential for thylakoid formation in higher plant chloroplasts and cyanobacteria [35,95]. Phylogenetic analysis showed that Vipp1 can be found in organisms that carry out oxygenic photosynthesis, i.e. plants, algae and cyanobacteria. No Vipp1 homologue has been found so far in bacteria including those that are capable of anoxygenic photosynthesis, such as Rhodobacter or Chlorobium. Vipp1 shares sequence homology with a subunit of the bacterial phage shock, psPA, and might have originated from a gene duplication of the latter in an ancestor of cyanobacteria. It subsequently obtained an additional C-terminal domain that seems essential for its function in thylakoid formation. In Arabidopsis the vipp1 mutation also interrupts...
vesicle traffic between the inner envelope and the thylakoids. No such vesicle transport has yet been shown in any prokaryotic organism including cyanobacteria. Further studies are needed to show whether vesicle transport is a feature that developed only in chloroplasts.

At least one cyanobacterium performs oxygenic photosynthesis without having thylakoids. *Gloeobacter violaceus* was first isolated in 1972 from a limestone rock in Switzerland [99]. Electron microscopic studies revealed the complete lack of internal membranes. Not even invaginations of the plasma membrane were observed. Nevertheless, these cells perform oxygenic photosynthesis [100,101]. The photosystems are located on the plasma membrane and, similar to purple and green-sulfur bacteria, they form their proton gradient along the plasma membrane. This organism might be a cyanobacterium at a stage before biogenesis of thylakoids was invented or has resulted from a secondary loss of thylakoid membranes. Compared to cyanobacteria with thylakoids their photosynthetic capacity is very low. Thus, efficient oxygenic photosynthesis may require the presence of an internal membrane system.

While it is easy to envision the evolution of chloroplasts from a cyanobacterium, it is much more difficult to understand the evolutionary processes that created the multiple forms of plastids. There is no indication that the structures found in proplastids, chromoplasts or leucoplasts have been part of the genetic plan that the endosymbiont transferred to the host cell. It must be assumed that this development took place after the endosymbiotic event and was imposed on the plastid by the host cell. It will be for future research to elucidate the evolutionary true origin of the thylakoid membrane and its evolution from simple single membrane layers to the complex system present in plant chloroplasts.

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