

1

What are plastids and where did they come from?

Plastids are a group of organelles present in the cells of all higher and lower plants, including algae, which function in a variety of different ways to enable plants to grow and function. Although different types of plastids which are found in different types of cells have modified roles, according to the type of cell in which they reside, the foremost function of plastids is carrying out the process of photosynthesis. Photosynthesis is a fundamental feature of plants and is facilitated by the presence of green, pigmented chloroplasts within plant cells. Indeed, photosynthesis is a defining feature of plants and enables them to fix carbon from the gaseous carbon dioxide in the air and synthesise a variety of complex organic molecules which allows them to increase in stature and mass. Photosynthesis is carried out by chloroplasts, which by virtue of containing the green pigment chlorophyll, defines the phenotype of green plants. Photosynthetic Eukaryotes have increased in their complexity dramatically since the first land plants, termed Embryophytes, evolved from freshwater multicellular green algae, around 450 million years ago. The current-day group of algae that are most closely related to these ancient algae are the Chlorophytes (Fig. 1.1). From these have evolved the lower plants, which includes the liverworts, mosses, hornworts and ferns (Fig. 1.1). Subsequently, the Gymnosperms and then the flowering plants, the Angiosperms, evolved and the Angiosperms, in particular, have been highly successful in conquering the planet such that much of the Earth is covered in green swathes of vegetation containing countless numbers of photosynthetic chloroplasts within their cells. Although extensive research on plastid biology has been done on lower plants, the majority has been carried out using higher plants and particularly members of the Angiosperms, which include all of the world's crop plants. Thus, in this book the focus will be on an understanding of plastid biology in

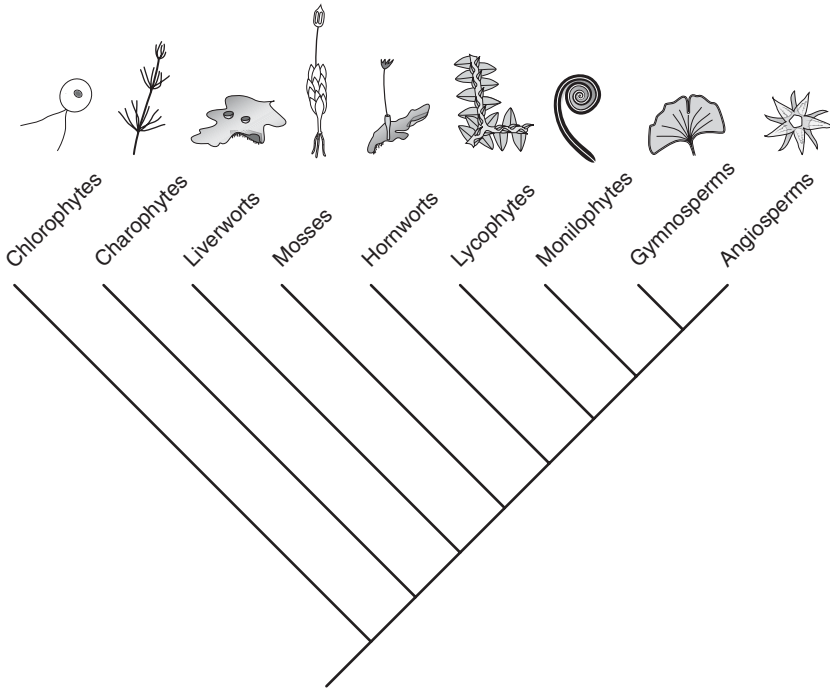


Fig. 1.1. Modern-day land plants, including the flowering plants or Angiosperms, evolved from an ancestral green alga which closely resembled today's Chlorophytes. This gave rise to the multicellular Charophyte algae, the most closely related relatives of land plants. Following invasion of the land, several distinct groups of land plants subsequently evolved: the liverworts, mosses, hornworts, Lycophytes (club mosses) and the Monilophytes (ferns). The most derived land plants bear seeds, and are represented by the Gymnosperms and the Angiosperms (the flowering plants).

Angiosperms rather than in lower plants. Although chloroplasts are the major form of plastids in Angiosperm plant cells, other plastid types have evolved to take up modified roles in different types of cells and tissues, especially in relation to storage of different types of molecules. Different types of plastids and their roles are considered in detail in Chapter 2.

A major question therefore regarding the evolution of higher plants is from where photosynthetic plastids arose, since they are fundamental to the successful evolution of higher plants. The source of plastids in modern-day plants has been a much-debated subject for a long time. Andreas Schimper in 1883 first suggested that chloroplasts were

1. What are plastids and where did they come from? 3

a result of a symbiosis between a photosynthetic organism and a non-photosynthetic host, and this outrageous suggestion was further supported by the Russian botanist Constantin Mereschkowsky in 1905. Much debate continued through the twentieth century but was refocused as a hypothesis based on microbiological observations by Lynne Margulis in 1967 in her paper 'on the origin of mitosing cells' (*Journal of Theoretical Biology*, **14**, 255–274). At the time, this hypothesis was heavily criticised and Margulis showed great persistence in sticking with what was an unorthodox hypothesis, and which in the modern era of plant biology, with the availability of genome sequences from both the plant nucleus and from the genome present within the plastids (see Chapter 3), has become the generally accepted hypothesis of how plastids evolved. As such, it has become clear that plastids have a prokaryotic history, in that they most likely evolved originally from a free-living photosynthetic prokaryotic organism. Although the precise organism from which plastids evolved may no longer be found on the planet, through the course of its own evolution, the closest living modern-day group are the Cyanobacteria, the photosynthetic bacteria (Fig. 1.2). Cyanobacteria are free-living single-celled prokaryotic organisms, sometimes aggregated into clusters or filaments and capable of carrying out photosynthetic carbon fixation. Although they use the photosynthetic light absorption pigment chlorophyll, they also use other pigments, such as phycoerythrin and phycocyanin, and thus Cyanobacteria are so named due to their colour. Cyanobacteria are abundant across the planet and play a major role in global photosynthesis as well as in the fixation of atmospheric nitrogen. They are found largely in aqueous habitats, or at least in habitats which are damp and have rather confusingly been referred to extensively in the past as blue-green algae, even though they are not algae but bacteria. So how did Cyanobacteria give rise to chloroplasts found inside cells of modern-day higher plants?

The modern theory of endosymbiosis, as first put forward by Margulis, suggested that a free-living photosynthetic prokaryote was engulfed by phagotrophy and taken up into a proto-eukaryote cell, within which it evolved a symbiotic relationship with the host cell (Fig. 1.3). Margulis used various strands of evidence gathered by microscopical examination of chloroplasts, but a central element was the discovery that plastids contained their own DNA, which form their own plastid genomes. In addition, a significant number of features characteristic of modern-day plastids clearly arose from their prokaryotic history and many of these features are discussed in detail in other chapters in this book.

4 1. What are plastids and where did they come from?

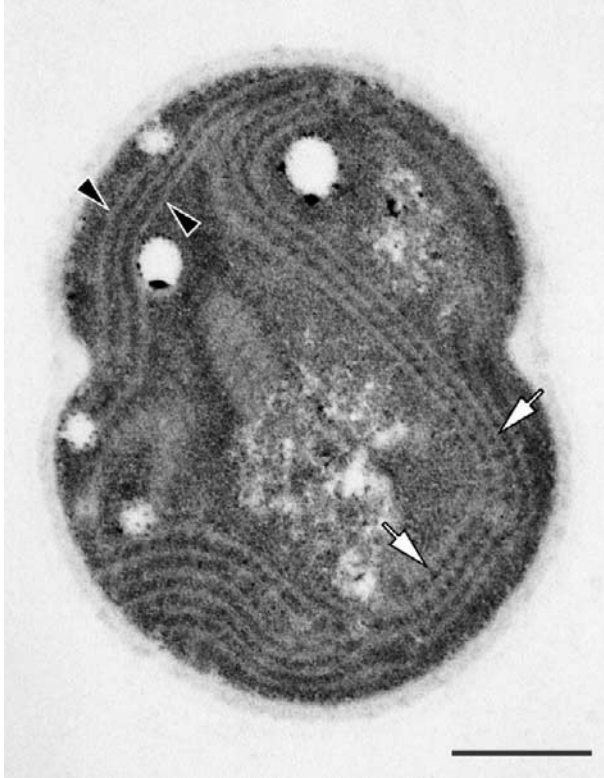


Fig. 1.2. A transmission electron micrograph of a section through the cell of a Cyanobacterium, *Synechocystis* sp. PCC6803. The thylakoid membranes within the cell (black arrows) and the light harvesting complexes, termed phycobilosomes (white arrows), are visible. Scale bar=1 μm . (Image courtesy of Conrad Mullineaux, Queen Mary, University of London.)

Foremost amongst these features are:

1. Plastids contain their own DNA, but much of that DNA has moved into the cell's nuclear genome through the course of evolution and is now missing from the plastid genome. Characteristics of plastid DNA structure and its molecular biology show many similarities with prokaryotic genomes from bacteria.
2. Proteins encoded by genes, which were originally plastid-encoded, and which are now present in the nucleus, are imported into the plastid after translation on ribosomes in the cytoplasm of the cell.
3. Plastids in higher and lower plants are surrounded by a double membrane, the inner membrane of which has features similar to

1. What are plastids and where did they come from?

5

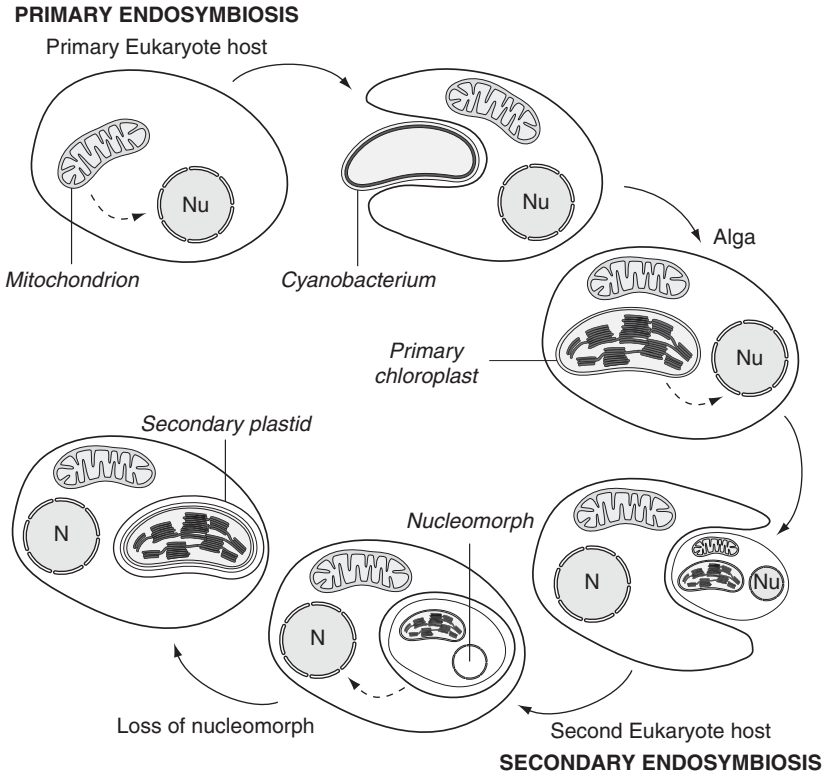


Fig. 1.3. The proposed origin of plastids in higher and lower plants occurred by a process of phagotrophy and the establishment of an endosymbiotic relationship. A photosynthetic Cyanobacterium was taken up by the primary eukaryotic host and incorporated into a stable endosymbiotic relationship, giving rise to a eukaryotic algal-like organism, containing mitochondria, a primary chloroplast and a nucleus (Nu). Subsequent secondary endosymbiotic events, in which an entire alga was taken up by another eukaryotic host cell also occurred. In this case, the algal nucleus forms the nucleomorph (Nu), which becomes reduced in size. N indicates the nucleus of the second eukaryote host. Broken arrows indicate gene transfer from organelles to the host nucleus. (Redrawn from Larkum WD, Lockhart PJ, Howe CJ., Shopping for plastids, *Trends in Plant Science* 12, 189–195. © Elsevier 2007.)

that of bacteria membranes and which is different to other cellular membranes.

4. The internal structure of the plastid, especially the thylakoid membrane and its protein complexes found in chloroplasts are similar to that found in Cyanobacteria.

6 *1. What are plastids and where did they come from?*

5. Plastids contain their own ribosomes, which have many features linking them with prokaryotic ribosomes and are different to those eukaryotic-type ribosomes found in the cytoplasm of the cell.
6. Plastids are capable of division within the plant cell and divide by a process of binary fission, similar in many ways to the division process of bacteria.

The theory of endosymbiosis has been supported extensively by the avalanche of new information about genes and proteins functional in plastids, which have been discovered in the last 20 years, especially as a result of the complete sequencing of nuclear and plastid genomes in plants, and it is now generally accepted that this was how plastids found in modern-day higher and lower plants originated. In addition, a previous endosymbiotic event involving non-photosynthetic bacteria is believed to have given rise to mitochondria, since they also show many prokaryotic features, similar to plastids.

In order for a successful endosymbiotic relationship to succeed, the symbiont has to be able to divide itself within the cytoplasm of the host cell and synchronise such divisions with the cell division of the host cell, such that the symbiont is maintained within the cells. Subsequent integration appears to have required extensive movement of genetic information to the nucleus, evolution of mechanisms to re-import those gene products along with the hijacking of nuclear genes for control of plastid function, and loss of the peptidoglycan cell wall from the symbiont.

A key question in the evolution of plastids is how many times did this original endosymbiotic relationship occur to give rise to the wide variety of plastid-containing organisms found on the planet today. Although this question has been much debated, it seems that the most likely scenario is that such a primary endosymbiosis occurred once and gave rise to three distinct groups of Eukaryotes, the Green algae, the Red algae and the Glaucophytes (Fig. 1.4). The precise order in which these groups have arisen through evolution is still open to significant debate, as is the discussion as to whether this original endosymbiotic event was a unique event or whether it occurred more than once. From this original event, the three groups of Red algae, Green algae and Glaucophytes have evolved into a large collection of organisms, consisting of around 15000 different species. Most of these are single-celled aquatic organisms but some have evolved significant multicellular structures and differentiated tissues, most notably in the seaweeds. From the Green algae eventually arose the evolutionary lineage, which gave rise to the first land plants (Fig. 1.1).

1. What are plastids and where did they come from?

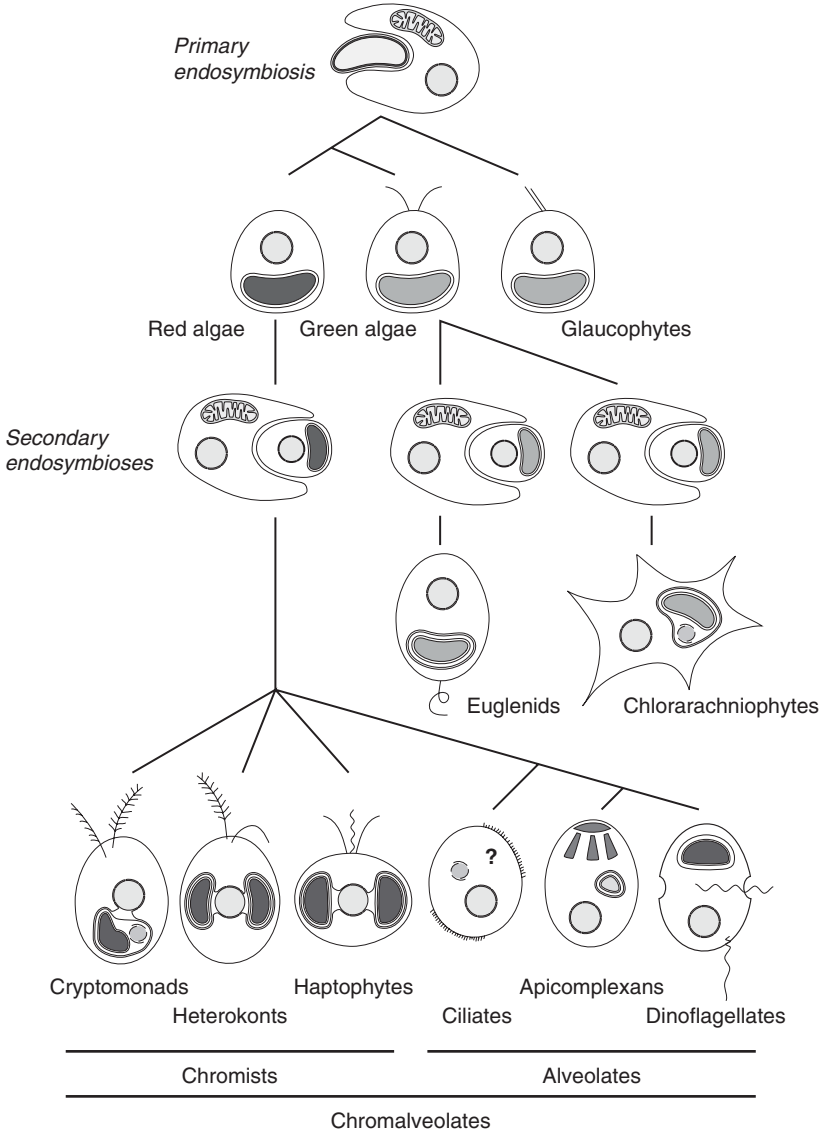


Fig. 1.4. A diagram of plastid evolution in Algae. Primary endosymbiosis took place once to yield Glaucophytes, Red algae and Green algae, which eventually gave rise to land plants and Angiosperms. Secondary endosymbiosis involving Green algae occurred twice, giving rise to Euglenids and Chlorarachniophytes. A single secondary endosymbiosis involving a Red alga gave rise to the many groups called Chromalveolates. In many of these groups, the ability to carry out photosynthesis has been lost. (Redrawn from *Protist 155*, Keeling P, A brief history of plastids and their hosts 3–7. © Elsevier 2004.)

8 *1. What are plastids and where did they come from?*

A key feature of all of the plastids in these three groups of organisms is that they are surrounded by a double membrane, which reflects the fact that they arose by a primary endosymbiotic event (Fig. 1.3). The extent of genetic transfer from the endosymbiont plastid to the cell's nucleus has been massive, such that modern-day plastid genomes are significantly smaller in size than those found in current-day Cyanobacteria (see Chapter 3). Indeed, analysis of the fully sequenced genome of the higher plant *Arabidopsis* reveals that around 18% of all genes in its nuclear genome were acquired from the original prokaryotic endosymbiont.

A significant aspect of plastid evolution, which has resulted in the diverse array of plastid-containing organisms found today, is the process of secondary endosymbiosis whereby an existing algal cell containing a plastid is engulfed by another eukaryotic cell (Fig. 1.3). This results in the plastid having more than two envelope membranes as well as the cell having, at least transiently, two nuclei. Subsequently, in most secondary endosymbiotic events, the secondary nucleus is lost, although in some groups it remains and is termed a nucleomorph (Fig. 1.3). Such events have given rise to a wide variety of organisms evolved from Red algae (Fig. 1.4), including Dinoflagellates, Heterokonts, Haptophytes and Cryptomonads. In such cases, the number of membranes surrounding the original plastid can be three or even four, reflecting the number of engulfing events that have taken place. In some of these groups, their photosynthetic capability as provided by the endosymbiont plastid has been lost.

What is most remarkable is that the original endosymbiont, which was essentially chloroplast-like in nature, has evolved into many different types of plastids found in different types of tissues in higher plants and these are considered in detail in the next chapter.

2

Different types of plastids and their structure

Since the first endosymbiotic event that enabled a free-living photosynthetic organism to take up residence in a eukaryotic cell, the organisms that we know of as plants have evolved in a dramatic and highly varied way. In particular, plants have evolved from their single-celled ancestors into complex multicellular structures. A key characteristic of these multicellular plants is that they contain cells of different types, which are distinguishable from each other in the functions that they perform within the whole complex organism. With increasing complexity of form through the evolution of Bryophytes, Monilophytes and into the higher plants, Gymnosperms and Angiosperms, large numbers of different types of cells have been developed such that, in the more complex members of the flowering plants, the Angiosperms, there are over 50 different types of cells. As a result of this diversification of cell types in plants, the original endosymbiotic plastid found itself being manipulated by the host cell to take on a variety of different roles in these different types of cells. Plastids evolved from their original photosynthetic function after the original endosymbiosis to take up a key role in the cell as a whole, particularly in relation to biochemical interactions in the cell's metabolism. As a result, in modern-day higher plants, there are a variety of different types of plastids, which fulfil different roles in different types of plant cells. The situation, however, is not clear-cut. There is significant interaction between different plastid types in different types of cells in that the plastids can interconvert between different types according to molecular and environmental signals. In addition, the plastid populations in many types of cell are not entirely homogeneous and may be composed of a mixture of different types of plastids. Here we will consider the basic types of plastids found in higher plants.

Proplastids

All of the cells in a plant are derived from cells produced in highly organised areas of tissue called meristems. Meristems are found at the tips of shoots and at the tips of roots and act as a source of undifferentiated cells, which are recruited from the meristem by cell division and cell expansion to form complex organs such as leaves, roots and flowers. During the formation of such organs, these undifferentiated cells differentiate into distinct cell types and contribute functionality to the organ in question. All of the plastids in all the cells in a plant are derived from those plastids found in the meristem cells within that plant and these progenitor plastids are called proplastids. Proplastids are essentially undifferentiated plastids, and are found extensively in root and shoot meristems, as well as in other tissues such as cells in early embryo development in seeds and other tissues which contain young dividing cells. Proplastids are also present in cultured plant cells and callus tissues on cultured plant explants, which are non-green. Most of the knowledge about proplastids has come from observing sections of meristem tissues with the electron microscope (Fig. 2.1), since proplastids are small, about 1–2 μm in length and contain little, if any, pigment. Thus they are difficult to study with conventional light microscopy. They can be viewed more effectively, however, by introducing into them a fluorescent molecule, and then observing them using fluorescence or confocal microscopy (Fig. 2.2). As with all plastids, proplastids are surrounded by a boundary double membrane, called the plastid envelope and, internally, they contain small pieces of a membrane system called the thylakoid membrane (Fig. 2.1), which becomes considerably more extensive in other differentiated types of plastid. Plastids resident in the shoot apical meristem contain a more organised array of thylakoid membrane than those in the root apical meristem. In addition to thylakoid membrane, proplastids contain their own ribosomes and, in seeds, proplastids often contain grains of starch, which are laid down during seed development and form a source of nutrition during seed germination. In specific tissues such as the plumule of wheat seedlings and in the stolon tissues of potato, many proplastids contain starch grains whilst others contain none. This difference relates to the presence or absence of a key starch biosynthetic enzyme, starch synthase, in different subgroups of proplastids within a cell.

The extent of variability of proplastids between meristem cells or within the same cell is difficult to examine because of the small size of the meristem cells and the dense meristem tissue. However, there is some