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Abstract

Plastids (chloroplasts) possess their own genetic information and consequently, express heritable traits. The plastid genome (plastome) occurs at high copy numbers, with up to thousands of genome copies being present in a single cell. Although mapping as a single circular molecule, the plastid DNA shows great structural dynamics. Multiple copies of the plastome are packed together in large nucleoprotein bodies, referred to as plastid nucleoids. The plastomes of land plants harbor a rather conserved set of approximately 100-120 genes in a genome of 120-160 kilobase pairs (kb). In contrast, size and coding capacity of plastomes in algae are much more variable. In most plant species, plastids and their genetic information are inherited maternally and thus excluded from sexual recombination. The cytological mechanisms leading to uniparentally maternal inheritance are surprisingly diverse and can involve organelle exclusion by unequal cell division, plastid destruction or selective degradation of the plastid DNA from the paternal parent. Exceptions from maternal inheritance, i.e. biparental or paternal plastid transmission, have arisen multiple times during evolution.

1 Introduction

Already at the beginning of the last century, the German geneticist and plant breeder Erwin Baur proposed that the non-Mendelian inheritance of leaf variegations can be explained with the assumption that chloroplasts (plastids) contain their own genetic material (Baur 1909, Baur 1910; reviewed in Hagemann 2000, Hagemann 2002). More than half a century later, the discovery of plastid DNA (Chun et al. 1963, Sager and Ishida 1963, Tewari and Wildman 1966) ultimately confirmed Baur's ingenious hypothesis. During the following decades, the plastid genome (plastome), its coding capacity and gene expression have been the subject of extensive molecular studies and today, the chloroplast represents the by far best-studied genetic compartment of the plant cell.

Due to its cyanobacterial ancestry, the plastome has retained numerous prokaryotic features, including a bacterial-type circular genome structure, genome packaging in nucleoids, organization of genes in operons and a prokaryotic gene expression machinery. This chapter provides an overview of our current understanding of (i) the structural properties of the plastid DNA, (ii) structure and function of the plastome and (iii) the inheritance of plastids and their genomes.

1 Physical properties of plastid genomes

The plastid genome maps as a circular molecule of double-stranded DNA (ptDNA). In land plants, the genome size is typically in the range of 120-160 kb (Fig. 1), although some exceptions have been noted (see 3.1 and 3.2.4). Identical copies of this genome are present in all plastid types: the undifferentiated proplastids of meristematic tissues, the green chloroplasts in photosynthesis-performing cells, the colored chromoplasts of flowers and fruits and other plastid types specialized in storage of starch, proteins or lipids.

Chloroplast DNA can be extracted from isolated organelles (which are purified by gradient centrifugation; Jansen et al. 2005) and was found to have physical properties distinct from nuclear DNA. The distinguishing features include different buoyant density in CsCl gradients, different melting and renaturation behavior, different GC content and the absence of 5-methylcytosine from plastid DNA (Tewari and Wildman 1966). In spite of its small genome size, plastid DNA can make up a significant fraction of the total cellular DNA which is due to its presence in high copy numbers. For tobacco leaves, it was estimated that about 9% of the total DNA is chloroplast DNA representing about 4.7 x 10^{-15} g DNA per chloroplast (Tewari and Wildman 1966).

1.1 Copy number of plastid genomes

A single plant cell contains many plastids and each plastid contains numerous (identical) plastome copies. Thus, in contrast to the two copies of each gene in the nucleus of a diploid plant, the cell is highly polyploid for its plastid genome. Depending on species, tissue, developmental stage and environmental conditions, the ploidy level can easily reach more than 10,000 identical copies of the plastid genome per cell (Bendich 1987). In land plants, plastome copy numbers are usually highest in photosynthetically active cells, where plastids are present as green chloroplasts. In contrast, non-

green plastid types often possess fewer plastomes. The copy number in root plastids, for example, is only about one fifth of that in chloroplasts (Aguettaz et al. 1987, Isono et al. 1997). Likewise, chloroplast development from proplastids and etioplasts is associated with an increase in plastome copy number (from about 2000 to more than 8000 copies per cell in barley; Baumgartner et al. 1988). Changes in plastid genome copy numbers per cell during plastid differentiation and plant development most likely come from the combined action of two processes: changes in organelle number per cell and changes in the plastome copy number per plastid. For example, the copy number per plastids almost doubles during etioplast to chloroplast differentiation in barley leaf development (Baumgartner et al. 1988). Once plastid differentiation is completed, plastome copy numbers remain remarkably constant and do not vary significantly with leaf age or the plant's developmental stage (Li et al. 2006, Zoschke et al. 2007).

As plastids are asexual genetic systems and, in most species, excluded from sexual recombination (see 4), an intriguing question has been how plastid genomes can avoid evolutionary deterioration. Asexual reproduction is believed to be detrimental because of the accumulation of deleterious mutations over time, a hypothesis known as Muller's ratchet (Muller 1964). Since the vast majority of mutations are deleterious, an asexual genetic system is expected to suffer a continuous decline in fitness. Surprisingly, in spite of their asexual mode of reproduction, plastid genomes even have considerably lower mutation rates than nuclear genomes (Wolfe et al. 1987). A recent study has provided experimental evidence that it is the plastid's high degree of polyploidy which, together with a very active mutation-correcting activity by gene conversion, counteracts the detrimental effects of Muller's ratchet and keeps mutation rates in plastid genomes very low (Khakhlova and Bock 2006). These findings suggest a molecular link between asexual reproduction, high genome copy numbers and low mutation rates.

1.2 Organization of plastid genomes in nucleoids

The plastid genomes do not swim around as naked DNA in the plasmatic compartment (stroma) of the organelle. Instead, several copies of the plastome are densely packed together in large nucleoprotein bodies called plastid nucleoids (Kuroiwa 1989, Kuroiwa 1991). Nucleoids can be visualized by fluorescence microscopy after staining of cells or tissues with the DNA-intercalating fluorochrome DAPI (4',6-diamidino-2-phenylindole; Kuroiwa 1991). Number, shape and size of the nucleoids as well as their distribution in the chloroplast vary depending on the species. In algae and higher plants, five different subtypes of nucleoid morphology have been described, ranging from spherical to ring-like structures (Kuroiwa 1989). Likewise, plastome copy numbers per nucleoid are variable between species and in dependence on plastid differentiation. Proplastids, for example, often contain only a single nucleoid, whereas mature chloroplasts can easily contain several or even dozens of nucleoids.

The nucleoid, and probably each individual plastid genome, are membrane bound. In higher plants, evidence has been provided for both an association with thylakoid membranes and an anchoring to the inner envelope of the chloroplast (Liu and Rose 1992, Sato et al. 1993). Isolated nucleoids retain transcriptional activity *in vitro* (Sakai et al. 1991) suggesting that the transcriptional apparatus (RNA

polymerases and sigma factors) is tightly associated with the plastid genome (Krause and Krupinska 2000). Little is known about the molecular processes and mechanisms that organize plastid nucleoids. Notably, it is entirely unclear, how a defined number of genome copies are packed into one nucleoid and how higher-order structures of the plastid genome are built and regulated (Salvador et al. 1998). It is known, however, that nucleoid size and number per cell are controlled by nuclear genes. In the unicellular green alga *Chlamydomonas reinhardtii*, mutants were obtained that had increased or drastically decreased numbers of nucleoids (Ikehara et al. 1996, Misumi et al. 1999): While wild-type cells contain on average 7 nucleoids, mutants with increased nucleoid number had 14-23 nucleoids. At the other extreme, a mutant called *moc* (for 'monokaryotic chloroplast') had only a single huge nucleoid per chloroplast (Misumi et al. 1999). However, the genes and mutations responsible for these interesting nucleoid phenotypes have not been identified to date.

Recently, several DNA-binding nucleoid proteins have been identified and/or biochemically characterized to some extent (Nakano et al. 1997, Sekine et al. 2002, Jeong et al. 2003, Cannon et al. 1999). The arguably best-studied nucleoid constituent is Hlp (also called HU), a histone-like DNA-binding protein that, similar to Hlp homologs in eubacteria, is believed to serve as a general architectural nuceoid protein (Kobayashi et al. 2002). Recently, a first step towards determining the proteome of chloroplast nucleoids has been taken in *Arabidopsis* (Pfalz et al. 2006): Nucleoid preparations obtained by a two-step chromatographic purification were subjected to mass spectrometric protein identification. Although the preparations were not absolutely pure, several good candidates for genuine nucleoid proteins could be identified, including RNA polymerase subunits, topoisomerases and DNA polymerase subunits (along with a number of novel proteins of unknown function; Pfalz et al. 2006). Interestingly, Hlp, the most abundant and major architectural nucleoid protein in bacteria and algae (Kobayashi et al. 2002) was not identified, possibly suggesting that nucleoid organization in higher plants is fundamentally different from that in eubacteria and algae. The systematic identification of nucleoid proteins and their functional characterization using the power of *Arabidopsis* nuclear genetics should pave the way to a better understanding of the higher-order structure of plastid DNA, its dynamics and impact on the regulation of plastid gene expression.

1.3 Structural conformations of plastid genomes

The finding that plastid genomes map as circular molecules and the identification of circular ptDNA molecules by electron microscopy (the contour length of which corresponded to the determined size of the plastome) in many independent studies led to the long-held belief that the structural conformation of the plastome *in vivo* is a simple circle of double-stranded DNA. However, more recent investigations have revealed that the ptDNA displays a surprisingly great structural plasticity with only a minority of the genome molecules being circular (25 to 45% in developing leaf tissue; Lilly et al. 2001). In addition to circles, both electron microscopic investigations and pulsed-field gel electrophoretic analyses have identified various linear genome conformations, including plastome multimers (resembling concatemers as arising during rolling-circle replication of bacteriophage genomes) and branched multimers (Bendich and Smith 1990, Lilly et al. 2001, Oldenburg and Bendich 2004). Linear ptDNA

molecules are unlikely to originate just from randomly broken circles: They were demonstrated to possess defined ends, some of which correspond to known origins of DNA replication (Oldenburg and Bendich 2004, Scharff and Koop 2006).

Interestingly, the fraction of genome molecules that is circular also shows a variety of different conformations. A substantial amount of the circles (>30% in tobacco; Lilly et al. 2001) is multimeric in that two or more copies of the genome form a single large circle (Deng et al. 1989). Multimers can come, for example, from rolling-circle replication and/or fusion of monomeric circles by homologous recombination. In addition to circles, lasso-like structures and suspected partially single-stranded molecules (showing D-loop-like bubbles) have been seen (Lilly et al. 2001). Similar to other circular genomes and episomes found in nature, the ptDNA can also adopt various supercoiled conformations and form catenanes (interlocking circles; Mukherjee et al. 1994, Kumar et al. 1995, Ahlert et al. 2003, Cho et al. 2004, Bendich 2004). Another structural peculiarity of the plastid genome is its presence in two isoforms due to flip-flop recombination of the two inverted repeat regions (3.1).

The functional relevance of most of the many different conformations of the ptDNA is still unclear. Some of them may simply represent replication intermediates, others may lack any functional significance. An interesting exception may be the degree of ptDNA supercoiling: Studies in the unicellular green alga *Chlamydomonas reinhardtii* have revealed that DNA topology fluctuates in dependence on the diurnal rhythm and that these fluctuations correlate with changes in the transcriptional activity (Salvador 1998). This finding may suggest that conformational changes of the ptDNA are involved in the regulation of plastid gene expression.

2 Fine structure of plastid genomes

The complete sequencing of two plastid genomes more than twenty years ago (Ohyama et al. 1986, Shinozaki et al. 1986) marks a milestone in structural genomics and has had a profound influence on our understanding of the genetics and molecular biology of plastids. In the following years, dozens of additional plastid genomes have been sequenced. The 88 plastomes fully sequenced by the end of 2006 (http://www.ncbi.nlm.nih.gov/genomes/static/euk_o.html) represent all major lineages of plant evolution. The picture that has emerged from these studies is that the plastome of land plants is a conservative genome while considerable variation in genome organization and coding capacity exists in algae (see 3.2.5).

In general, plastid genomes have a low GC content which is typically in the range of 30-40% (Ohyama et al. 1988, Shimada and Sugiura 1991). The low GC content is particularly pronounced in non-coding intergenic spacer regions where AT richness is often extreme and can reach values above 80% AT (Ohyama et al. 1988). Within coding regions, AT richness manifests as strong bias in codon usage, in that synonymous codons with an A or T in third codon position are strongly preferred over those with G or C in third position (Shimada and Sugiura 1991).

2.1 Inverted repeats and single-copy regions

In land plants, most plastomes display a tetrapartite genome organization with a large single copy region (LSC) and a small single copy region (SSC) separating tow inverted repeat regions (IR_A and IR_B; Fig. 1). The two IRs are identical in their nucleotide sequence, so that every gene contained within them is present in two copies per genome which only differ in their relative orientation (Fig. 1). The boarders between the IRs and the single copy regions are somewhat variable even between closely related species (Goulding et al. 1996). Expansion of the inverted repeat region is extreme in *Pelargonium*, the flowering plant species with the largest plastome (217 kb; Palmer et al. 1987, Chumley et al. 2006). Here, the IRs are 75 kb in size each and thus about three times as big as in most other higher plants. The functional significance of the presence of the IR region in two copies is not quite clear. Increasing the gene dosage of highly expressed genes (such as the ribosomal RNA genes; Fig. 1) and genome stabilization (Palmer and Thompson 1982) have been proposed as possible reasons why having this large inverted duplication could be beneficial. Its absence from some algal (Reith 1995) and even some higher plant plastomes (Palmer and Thompson 1982), however, indicates that the IR is not essential for plastome maintenance and/or function.

The presence of two large identical regions in the plastome facilitates two types of genetic interactions between homologous sequences: intramolecular recombination and gene conversion (Birky and Walsh 1992, Khakhlova and Bock 2006). Homologous recombination between the two IRs produces two isoforms of the plastid genome (dubbed flip-flop recombination; Palmer 1983, Stein et al. 1986) which differ in the relative orientations of LSC and SSC. Circumstantial evidence for the action of gene conversion in the IRs has come from the observation that the mutation frequency of genes in the IR regions is significantly lower than for genes located in the two single copy regions of the plastome (Wolfe et al. 1987, Maier et al. 1995). Gene conversion biased on average towards the wild-type sequence has been proposed to account for the lower mutation rate in the inverted repeats (Birky and Walsh 1992). The recent experimental demonstration of high gene conversion activity in plastids (Khakhlova and Bock 2006) lends support to this hypothesis.

2.2 Information content of plastid genomes

Among the three genomes of the plant cell, the plastome is the most gene-dense one with more than 100 genes in a genome of typically only 120 to 160 kb (Sugiura 1989, Sugiura 1992, Wakasugi et al. 2001; Fig.1; Table 1). The plastid genome is the evolutionary remnant of a cyanobacterial genome. After endosymbiosis, the genome has undergone a dramatic size reduction and, thus, contemporary plastomes contain only a small proportion of the genes of their free-living cyanobacterial ancestors: Whereas the genome of the cyanobacterium *Synechocystis* contains more than 3000 genes (Kaneko et al. 1996, Kaneko and Tabata 1997), the plastid genomes of land plants harbor only approximately 115 genes.

Very obviously, the limited coding capacity of the plastome is by far insufficient to provide the thousands of components required to support its own gene expression system, photosynthesis and all

the many other plastid-localized metabolic functions. Therefore, all cellular functions fulfilled by present-day plastids are strictly dependent upon the products of nuclear genes that are synthesized on cytoplasmic ribosomes and post-translationally imported into the organelle. Nuclear-encoded proteins make up the by far largest fraction of the plastid proteome (Abdallah et al. 2000, Rujan and Martin 2001, Martin et al. 2002, Hippler and Bock 2004) and it is estimated that chloroplasts import more than 95 % of their proteins from the cytosol. Consequently, the spatial and temporal expression of nuclear and organellar genes must be tightly coordinated.

Plastid-encoded genes can be roughly classified into three major groups (Shimada and Sugiura 1991, Kahlau et al. 2006): genetic system genes, photosynthesis-related genes and other genes. The approximately 60 genetic system genes contained in land plant plastomes encode RNA and protein components of the plastid's gene expression machinery (Fig. 1, Table 1). Approximately 50 plastid genes encode protein products involved in photosynthesis (Fig. 1; Table 1). The heterogeneous third gene group comprises all other genes and conserved open reading frames of unknown function.

2.2.1 Photosynthesis genes

Chloroplasts are the site of photosynthesis, the conversion of solar energy to chemical energy. Photosynthesis consists of two stages, the light reactions and the dark reactions, both of which involve complex molecular machineries. A substantial number of plastome-encoded genes (47 genes in angiosperms; Table 1) is dedicated to the photosynthetic apparatus. These include 15 genes for subunits of photosystem II (PSII), the membrane protein complex catalyzing the light-driven oxidation of water. The products of another 7 genes are required for photosystem I (PSI) function, the membrane protein complex that catalyzes the light-driven transmembrane electron transfer from plastocyanin (or cytochrome c_6) to the ferredoxin-NADP complex. In addition to 5 genes for subunits of the PSI complex, the 7 PSI-related genes also include *ycf3* and *ycf4*, two genes for proteins involved in PSI assembly (Ruf et al. 1997, Boudreau et al. 1997). 6 plastid genes encode subunits of the cytochrome b_6f complex, the redox-coupling protein complex interconnecting the two photosystems. Another 6 genes encode subunits of the chloroplast ATP synthase, the enzyme that catalyzes the conversion of phosphate and adenosine diphosphate into adenosine triphosphate utilizing a proton gradient across the thylakoid membrane as energy source. 11 genes on the plastome encode subunits of a chloroplast NAD(P)H dehydrogenase, a thylakoid protein complex suggested to be involved in chlororespiration and cyclic electron flow around PSI (Burrows et al. 1998, Shikanai et al. 1998, Joet et al. 2001, Munekage et al. 2004). This complex is non-essential for photosynthesis and all genes for its subunits were found to be absent from the fully sequenced plastid genomes of the gymnosperm *Pinus thunbergii* and the green alga *Chlamydomonas reinhardtii* (Wakasugi et al. 1994, Maul et al. 2002). Finally, two plastid-encoded gene products are directly or indirectly involved in the dark reactions: *rbcL* encoding the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and *ycf10*, a conserved open reading frame encoding a chloroplast inner envelope membrane protein reportedly involved in inorganic carbon uptake (Sasaki et al. 1993a, Rolland et al. 1997).

None of the protein complexes involved in photosynthesis is composed entirely of plastome-encoded subunits. Instead, all of them require the products of nuclear genes which are of cyanobacterial origin and, during evolution, have been transferred from the plastid to the nuclear genome. The two-subunit enzyme Rubisco provides the classical example for this intimate plastid-nuclear cooperation: In all flowering plants, the large subunit of Rubisco is encoded in the plastome whereas the small subunit is encoded in the nuclear genome, typically by a small gene family.

2.2.2 Genetic system genes

The genetic system genes comprise the largest group of genes located on higher plant plastomes (62 genes; Table 1). To this group belong all genes whose products are involved in plastid gene expression (i.e., transcription, RNA processing, translation, protein degradation): 30 tRNA genes, 4 rRNA genes, 21 genes for ribosomal proteins (9 proteins of the large subunit and 12 proteins of the small subunit of the plastid 70S ribosome), 4 genes for subunits of the *E. coli*-like plastid RNA polymerase (PEP), *matK* suggested to encode an RNA maturase (i.e., a splicing factor involved in the removal of a subset of chloroplast group II introns; Hess et al. 1994, Liere and Link 1995, Mohr et al. 1993, Jenkins et al. 1997), *clpP* encoding a subunit of a chloroplast protease (Shanklin et al. 1995, Majeran et al. 2000) and *infA* encoding translation initiation factor IF-1 (Sijben-Müller et al. 1986).

A complete set of tRNAs for decoding all triplets in protein-coding genes is thought to comprise 32 tRNA species. Although only 30 tRNA genes are encoded in the plastome, they are nonetheless believed to be sufficient to read all codons (Jukes and Osawa 1990, Osawa et al. 1992). This is presumably achieved by an extended wobbling (referred to as 'four-way wobble') between the third codon position and the 5' nucleotide of the anticodon in the tRNA. In the case of the four alanine codons (GCU, GCC, GCA and GCG), for example, this means that the U in the first anticodon position of the single tRNA-Ala species (*trnA-UGC*; Table 1) can probably basepair with all four possible nucleotides in third codon position of the alanine triplets (Jukes and Osawa 1990, Osawa et al. 1992).

Remarkable differences between species exist concerning the essentiality of the plastid gene expression apparatus. Plastid translation has been demonstrated to be essential for cell survival in tobacco (Ahlert et al. 2003, Rogalski et al. 2006), but appears to be non-essential under heterotrophic culture conditions in at least some Brassicaceae species (Zubko and Day 1998, Zubko and Day 2002) and probably also in some cereals (Hess et al. 1993, Hess et al. 1994).

While the RNA components of the gene expression machinery (rRNAs and tRNAs) are exclusively encoded in the plastid genome (Lung et al. 2006), many of the protein components are encoded by nuclear genes. For example, only about one third of the plastid ribosomal proteins is plastome encoded in higher plants, the other two thirds are nuclear-encoded, made in the cytosol and imported into the plastid. A similar division of labor between the nucleus and the plastid occurs in the coding of the transcriptional apparatus: The four core subunits of the *E. coli*-like plastid RNA polymerase (plastid-encoded RNA polymerase, PEP) are encoded in the plastome, but the sigma factors, which are required for promoter recognition, are encoded in the nuclear genome. In addition, a second RNA-synthesizing activity in the plastid (nuclear-encoded RNA polymerase, NEP) provided by

bacteriophage-type enzymes is encoded by nuclear genes (Hedtke et al. 1997, Hess and Börner 1999).

While in angiosperm plants, the set of genes encoded in the plastome is usually highly conserved between species, a small number of genetic system genes, including *rpl23* and *infA* (Table 1), provide notable exceptions in that they have been transferred to the nucleus or replaced by nuclear genes of non-plastid origin in some lineages of evolution (Bubunenko et al. 1994, Millen et al. 2001). The presence in the plastome of pseudogenic remnants of the genes suggests that these events occurred only relatively recently in evolution. The *infA* gene encoding the plastid translation initiation factor 1 provides a particularly striking example: It had long been known that *infA*, while being a functional gene in the plastome of the liverwort *Marchantia polymorpha* and the higher plant rice (Ohyama et al. 1986, Hiratsuka et al. 1989), exists only as a pseudogene in the tobacco ptDNA (Shinozaki et al. 1986, Shimada and Sugiura 1991). A systematic phylogenetic analysis of *infA* structure in the plastomes of angiosperms revealed that the gene has repeatedly become non-functional in approximately 24 separate lineages of angiosperm evolution. Search for nuclear *infA* copies in four of these lineages identified expressed nuclear *infA* genes whose gene products are targeted to plastids (Millen et al. 2001).

2.2.3 Other genes and conserved open reading frames

A small number of genes on the plastome of land plants are not directly involved in photosynthesis or gene expression. These include the *accD* gene which encodes a subunit of acetyl-CoA carboxylase, the key enzyme in fatty acid biosynthesis (Sasaki et al. 1993b, Sasaki et al. 1995). A second example is *ccsA* (*ycf5*), the protein product of which is required for heme attachment to chloroplast c-type cytochromes (Orsat et al. 1992, Xie et al. 1998, Xie and Merchant 1996).

The plastome of pland plants also harbors a few conserved open reading frames (ORFs) of unknown function (Table 1). Interspecific conservation of an ORF is usually taken as good indication that it constitutes a genuine gene. In plastids, such conserved ORFs are referred to as *ycf* (hypothetical chloroplast reading frame). Although during recent years, the functions of most *ycf*s could be determined by reverse genetics in *Chlamydomonas reinhardtii* and tobacco (e.g., Monod et al. 1994, Ruf et al. 1997, Hager et al. 1999; reviewed in Rochaix 1997, Bock and Hippler 2002), there are a few left whose functions have not yet been elucidated. Among them are two giant open reading frame, *ycf1* and *ycf2*, which in tobacco, potentially encode protein products of 1901 and 2280 amino acids, respectively. Attempts to inactivate *ycf1* and *ycf2* in tobacco have revealed that both reading frames are essential genes (Drescher et al. 2000). This excludes a function of the gene products in photosynthesis (because photosynthesis is non-essential under *in vitro* culture conditions), but leaves the possibility of a function in plastid gene expression (which has been demonstrated to be essential in tobacco; Ahlert et al. 2003, Rogalski et al. 2006) or in some essential metabolic pathway.

All plastid genomes also harbor a number of non-conserved open reading frames, most of which are shorter than 150 codons. Lack of evolutionary conservation even among closely related species is usually interpreted as evidence for these ORFs fortuitously forming contiguous reading frames that have no functional significance (Kahlau et al. 2006).

2.2.4 Plastid genomes of parasitic plants

With the exception of only very few genes, the above-described set of plastome-encoded genes (Table 1) is highly conserved among angiosperm plant species. There is, however, one group of angiosperms whose plastid genomes differ radically in gene content: parasitic plants. A limited number of plant species grows heterotrophically by exploiting green plants as carbon source. Many of these holoparasites have lost the capacity to carry out photosynthesis and also lack photosynthetic pigments. The ability to obtain sugars from a host plant releases the selective pressure on the maintenance of photosynthesis-related genes. Consequently, the plastomes of such parasites suffer dramatic size reductions, mainly caused by the loss of photosynthesis genes or their degeneration to pseudogenes (dePamphilis and Palmer 1990, Wimpee et al. 1991, Bungard 2004). For example, the plastome of the root holoparasite *Epifagus virginiana* (an Orobanchaceae species) is less than half the size of that in photoautotrophic angiosperms (dePamphilis and Palmer 1990, Wolfe et al. 1992). It contains only 21 intact protein-coding genes, 18 of which belong to the genetic system genes and the remaining three falling into the category 'other genes' (*accD*, *ycf1* and *ycf2*; see 3.2.3 and Table 1). Remarkably, also some genetic system genes have been lost or degraded to pseudogenes (Morden et al. 1991, Wolfe et al. 1992). It is currently unclear, whether or not these missing genes have been substituted by functional nuclear gene copies the protein products of which are imported into plastids. Nonetheless, plastid genes in *Epifagus* are actively transcribed and their mRNAs are faithfully processed by intron splicing and RNA editing suggesting that the vestigial plastid genome is indeed expressed (Ems et al. 1995). However, whether or not also the translational apparatus in these plastids has remained functional, is not yet clear.

Parasitism in seed plants has evolved several times independently (presumably at least ten times; Bungard 2004). Interestingly, not all parasitic plants grow exclusively heterotrophically. A number of parasitic species have retained at least some photosynthetic activity and thus, strictly speaking, grow mixotrophically: They fix a limited amount of carbon by themselves through photosynthesis, while obtaining the bulk of carbon as sugars from their host plant. Such species are believed to represent evolutionary intermediates that are in the process of losing their ability to photosynthesize. The genus Cuscuta (dodders) provides a particularly striking example for this evolutionary transition: Its more than 150 species vary greatly in their residual photosynthetic activities and also show great interspecific variation with respect to the extent of plastid genome degeneration by gene deletion or gene decay to inactive pseudogenes (Berg et al. 2004, Revill et al. 2005). Thus, the analysis of ptDNA evolution in Cuscuta provides a unique opportunity to follow the molecular changes associated with the gradual transition to heterotrophy and to study the mechanisms of plastid genome streamlining as triggered by the loss of photosynthesis.

2.2.5 Plastid genomes of algae

While structure and coding capacity of the plastid genome are highly conserved among land plants, the plastome has experienced many architectural changes during the evolution of algae (Simpson and

Stern 2002). The most dramatic change appears to have occurred in some dinoflagellates, where single genes are contained on small (2-3 kb) minicircles and the sum of the minicircles may make up the plastid genome (Zhang et al. 1999, Barbrook and Howe 2000). This unusual multipartite plastid genome structure seems to be confined to dinoflagellates: The genomes of most other algae map as circular molecules of 100-300 kb (Reith 1995, Simpson and Stern 2002).

The inverted repeat region present in most higher plant plastomes and containing the ribosomal RNA (rRNA) operon (3.1; Fig.1) has undergone many structural changes in algae. While, for example, many green and red algae have rRNA operon-containing IRs, some green and red algae have lost one of the IRs and thus possess only a single copy of the rRNA operon. Again other green and red algal species have direct repeats rather than inverted repeats (Reith 1995). The perhaps most unusual structure of the rRNA operon is found in *Euglena gracilis*, a unicellular flagellate-like protist with a green algal plastid acquired via secondary endosymbiosis, In *Euglena*, multiple copies of the rRNA operon are arranged as a tandem array of three complete and one partial operons (Hallick et al. 1993).

The plastomes of algae also vary greatly with respect to gene density and information content. While in some algal lineages, plastid genomes are extremely compact and gene-dense (such as the plastome of the cryptophyte alga *Guillardia theta* with 180 genes in a genome of only 122 kb; Douglas and Penny 1999), genome expansion by accumulation of non-coding DNA has occurred in other algae. The model green alga *Chlamydomonas reinhardtii* provides a particularly striking example for such a genome expansion: Its plastome is 203 kb large, but contains only 99 genes. The presence of repetitive DNA (i.e., short dispersed repeats) in intergenic regions accounts for more than 20% of the genome size (Maul et al. 2002). The selective forces that have led to extreme genome streamlining in some algae, but genome expansion in others are currently totally unknown.

Green algae share a common ancestry with land plants and it is therefore unsurprising that the gene content of their plastomes is similar to that of higher plants. Exceptions include, for example, *petN* which is nuclear encoded in green algae (Hager et al. 1999; Table 1), and two genes for proteins involved in plastid division which are present in the plastome of the green alga *Chlorella vulgaris*, but absent from the ptDNA of higher plants and another green alga, *Chalmydomonas reinhardtii* (Wakasugi et al. 1997). In contrast to green algae, the plastomes of non-green algae have retained many genes that are absent from the plastid genomes of land plants (Reith and Munholland 1993, Reith and Munholland 1995, Kowallik et al. 1995, Reith 1995, Ohta et al. 2003). It is generally assumed that these additional genes were transferred to the nucleus in the common ancestor of land plants and green algae which, in this respect, represent a more derived state of plastome evolution. Among the algal plastomes fully sequenced to date, the red alga *Porphyra purpurea* has the highest number of genes (250 genes in a genome of 191 kb, Reith and Munholland 1995). Expansion of the two main gene classes in plastid genomes (photosynthesis genes and genetic system genes; see 3.2.1 and 3.2.2) as well as presence on the plastome of additional groups of genes (e.g., for amino acid, fatty acid, pigment and vitamin biosyntheses) account for this enlarged gene repertoire.

3 Inheritance of plastid genomes

DNA-containing organelles (plastids and mitochondria) are inherited in a non-Mendelian fashion in all eukaryotes. In most organisms, organellar genomes are inherited from only one parent, with maternal inheritance being much more widespread than paternal inheritance. Plastids and their DNA can be inherited maternally, paternally or biparentally (reviewed, e. g. in Mogensen 1996, Birky Jr. 1995, Hagemann 2002). At least in higher plants, plastid genomes do not normally undergo sexual recombination, even when they are inherited biparentally. This means that, except in very rare cases (which may be considered accidents; Medgyesy et al. 1985, Thanh and Medgyesy 1989, Baldev et al. 1998), chloroplast fusion and genetic recombination do not occur (an exception is the green alga *Chlamydomonas* reinhardtii; see 4.1.4).

Uniparentally maternal plastid inheritance has long been considered the rule, although the first exception (*Pelargonium* as a species with biparental inheritance; Baur 1909) was discovered simultaneously with the rule (maternal inheritance of plastids in several angiosperm species; Correns 1909, Baur 1910). Although it is still true that the majority of flowering plants transmit their plastids uniparentally from the female parent to the progeny, exceptions are found in nearly all major lineages of plant evolution (Mogensen 1996, Birky Jr. 1995). This suggests that maternal inheritance as the presumably ancestral mode of plastid transmission has been broken many times independently in plant evolution (Birky Jr. 1995).

While the different modes of plastid inheritance (maternal, paternal, biparental) are cytologically reasonably well described (see below), the characterization of the molecular mechanisms underlying plastid inheritance is still in its infancy. While in *Chlamydomonas*, a model alga in which plastid inheritance is genetically tractable (, but mechanistically very different from higher plants; see 4.1.4), some of the molecular components involved in maternal inheritance have been identified, next to nothing is known about the factors involved in the various modes of plastid inheritance existing in flowering plants. Plastid transmission is very likely controlled by nuclear genes (Tilney-Bassett 1984, Tilney-Bassett 1994), but to date, not a single gene involved in plastid inheritance has been identified in any higher plant.

3.1 Maternal inheritance

The vast majority of angiosperms and at least some gymnosperms display a maternal mode of plastid inheritance and thus do not regularly transmit plastids and plastid genes through pollen. Cytological investigations have revealed that there is not a unique mechanism how maternal inheritance of plastids is brought about. Instead, different species can utilize very different mechanisms of eliminating paternal plastids and/or paternal plastid genomes. The correlation of the cytological mechanisms leading to maternal inheritance with plant phylogeny is rather poor, and therefore, the mechanism operating in a given species is hardly predictable. Similarly to the mode of plastid inheritance, the cytological mechanism of maternal inheritance must be determined on a species-by-species basis.

According to the mechanism of paternal plastid elimination, at least four different subtypes of maternal inheritance can be distinguished (Hagemann and Schröder 1989, Hagemann 2002). This classification

is largely based on electron microscopic investigations of plastid fate during male gametophyte development. The subtypes are named after the first species discovered to realize the respective cytological mechanism.

3.1.1 Maternal inheritance: Lycopersicon type

In angiosperms, development of the male gametophyte starts out with meiotic division of the microspore mother cell generating four haploid microspores. Subsequently, the haploid microspores undergo two mitotic divisions, referred to as pollen mitoses. The first pollen mitosis involves an asymmetric division of the haploid microspore resulting in a large vegetative cell and a small generative cell. The vegetative cell receives most of the cytoplasm from the microspore and completely envelopes the generative cell (Fig. 2). The generative cell then gives rise to two sperm cells by another mitotic division (second pollen mitosis). During fertilization, the two sperm cells move towards the ovule through the growing pollen tube. One of them fuses with the egg cell giving rise to the zygote, whereas the other fuses with the central cell to produce the precursor cell of the endosperm (double fertilization).

The Lycopersicon type of maternal plastid inheritance involves plastid exclusion during the first pollen mitosis. The extremely asymmetric division of the microspore results in a vegetative cell that contains all plastids and a generative cell that is free of plastids (Fig. 2). Consequently, also both sperm cells lack plastids. It is generally assumed that plastid inheritance in the majority of angiosperm species follows the exclusion mechanism of the Lycopersicon type (Hagemann and Schröder 1989, Hagemann 2002; Table 2).

3.1.2 Maternal inheritance: Solanum type

In a number of angiosperm species, maternal inheritance is brought about by degradation of paternal plastids. During male gametophyte development in species belonging to the Solanum type of maternal inheritance, plastids in the generative cells are selectively destructed whereas plastids in the vegetative cell remain intact (Fig. 2). Consequently, as in the Lycopersicon type, the two sperm cells carrying out the double fertilization are free of plastids.

The examples of species utilizing the plastid exclusion mechanism of the Lycopersicon type and those utilizing the plastid degradation mechanism of the Solanum type (Table 2) illustrate that even closely related species can differ in the cytological mechanisms conferring maternal plastid inheritance: Tomato (*Solanum lycopersicum*, formerly *Lycopersicon esculentum*) and potato (*Solanum tuberosum*) belong to the same genus of Solanaceae, but yet exhibit different modes of maternal plastid inheritance.

3.1.3 Maternal inheritance: Triticum type

In pea and at least some monocotyledonous species, both the generative cell and the sperm cells regularly contain plastids. Nonetheless, these species do not normally transmit paternal plastids into

the zygote. It is generally believed that, during fertilization of the egg cell by one of the two sperm cells, the plastids are stripped off together with most of the cytoplasm and do not enter the zygote along with the sperm cell's nucleus (Hagemann and Schröder 1989; Fig. 2; Table 2). Such a mechanism would be somewhat reminiscent of the exclusion of sperm cell mitochondria during fertilization of the egg in animals. However, 'smoking gun' evidence for a stripping-off mechanism underlying maternal inheritance in the Triticum type is largely lacking. This is mainly due to the difficulty to catch in the act sperm and egg by electron microscopy. Therefore, alternative mechanisms, such as degeneration of the cytoplasm surrounding the sperm cell nucleus (including the demise of plastids and mitochondria) shortly before the fertilization process, presently cannot be excluded.

3.1.4 Maternal inheritance: Chlamydomonas type

In no other plant, chloroplast inheritance has been as thoroughly studied as in the unicellular green alga *Chlamydomonas reinhardtii*. *Chlamydomonas* has a single large (cup-shaped) chloroplast per cell. There exist 'male' and 'female' algae which are morphologically indistinguishable and commonly referred to as mating type + (mt+, 'female') and mating type – (mt–, 'male'). Organelle inheritance in *Chlamydomonas* exhibits several interesting features (Umen and Goodenough 2001). First, chloroplast and mitochondrial genomes are oppositely inherited: The chloroplast DNA is transmitted maternally whereas the mitochondrial DNA is transmitted paternally. Second, during syngamy the maternal chloroplast fuses with the paternal chloroplast. Third, chloroplast DNA in mt+ *Chlamydomonas* gametes is methylated by a DNA methyltransferase converting cytosine to 5-methylcytosine (Nishiyama et al. 2002, Nishiyama et al. 2004). In contrast, plastid DNA in higher plants is nowadays believed to be unmethylated (at least in somatic tissues: Marano and Carrillo 1991, Fojtová et al. 2001), although some early reports had suggested that cytosine methylation can occur also in higher plant plastomes (Ngernprasirtsiri et al. 1988a, Ngernprasirtsiri et al. 1988b).

If the two parental chloroplasts fuse upon mating, how then is maternal inheritance of chloroplast DNA in *Chlamydomonas* secured? Following syngamy, a zygotic maturation program sets in which leads to selective destruction of chloroplast DNA from the mt– parent, while the mt+ chloroplast genomes survive (Nishimura et al. 1999). Degradation of the paternal chloroplast genomes (by a specific endonuclease; Nishimura et al. 2002) is largely completed before fusion of the two parental chloroplasts occurs, thus resulting in uniparental inheritance of the maternal plastid DNA. It was reasonable to speculate that the difference in DNA methylation could be causally responsible for the selective degradation of the chloroplast genomes in mt– chloroplasts: By analogy to the restriction-methylation systems operating in eubacteria, this model posited that cytosine methylation protects mt+ plastid genomes from endonucleolytic degradation. However, recent studies have cast considerable doubt on this idea. Apparently, DNA methylation is not necessary for protection of mt+ plastid genomes in early zygotes and instead, may affect the relative rates of plastid genome replication in mt– and mt+ cells (Umen and Goodenough 2001). Thus the mechanistic details of how mt+ plastid genomes are protected from decay remain to be elucidated.

Maternal plastid DNA inheritance in *Chlamydomonas* is not absolute in that occasionally, paternal plastid DNA molecules (or fragments thereof) survive until chloroplast fusion occurs and thus can recombine with the maternal plastid genomes. These so-called 'exceptional zygotes' occur spontaneously at a frequency of a few percent (1-9 %, depending on the genotype of the algal strains and on the experimental conditions). Interestingly, UV irradiation of mt+ gametes can significantly increase the intake rate of paternal ptDNA into the zygote. This discovery made in the mid-sixties of the last century has facilitated the recombination mapping of the chloroplast genome in *Chlamydomonas* (by R. Sager, J. Boynton, N. Gillham and E. Harris; e.g. Sager and Ramanis 1976) and, in this way, contributed greatly to the development of plastid genetics in the pre-genomics era.
Together with the availability of antibiotic resistance markers encoded in the plastid genome, the low-level transmission of paternal plastid genes provides a powerful tool to quantify plastid inheritance (Bolen et al. 1982) and moreover, makes the unique system of chloroplast DNA inheritance in *Chlamydomonas* amenable to rigorous genetic analysis by selecting mutants with altered chloroplast genome transmission.

3.2 Biparental inheritance

A small number of angiosperms transmit their plastids biparentally. Working with *Pelargonium, Mirabilis, Melandrium, Antirrhinum* and *Aquilegia*, already Erwin Baur and Carl Correns noted in their first experiments on the inheritance of leaf variegations almost hundred years ago (Baur 1909, Correns 1909, Baur 1910) that the mode of plastid inheritance may differ between species: While *Melandrium, Antirrhinum* and *Aquilegia* mutants transmitted their altered leaf color (which, as we now know, represented plastome mutations) purely maternally, similar traits could also be transmitted via pollen in *Pelargonium* (for review see Hagemann 2000). Baur concluded that the plastids (or the 'chromatophors', as they were called at that time) must be biparentally inherited in *Pelargonium zonale*. Later, other examples of species with biparental chloroplast inheritance were found (Table 2), including *Oenothera* (evening primrose), *Hypericum* (St. John's wort) and *Medicago* (alfalfa).
Extensive genetic work has determined the relative contributions of maternal and paternal plastids to the organelle population in the progeny in these species and revealed striking differences. In *Oenothera* and *Hypericum*, the rate of paternal transmission is relatively low, as evidenced by reciprocal crosses between white plastome mutants and green wild-type plants: When the plastome mutant served as maternal parent, many white and variegated seedlings were obtained, but almost no green progeny. In contrast, when the plastome mutant was the paternal parent (i.e., the pollen donor), most F1 seedlings were uniformly green or variegated and only very few were white. In *Pelargonium* and *Medicago*, the paternal contributions are much greater. Whereas in *Pelargonium*, sperm and egg seem to make about equal plastid contributions to the zygote, paternal plastids are even predominantly inherited in alfalfa (Shi et al. 1991, Hagemann 2002).
Cytological investigations confirmed that, as expected, biparental plastid inheritance correlates with (i) the distribution of microspore plastids between vegetative cell and generative cell during the first

pollen mitosis, (ii) the regular presence of viable plastids in sperm cells and (iii) their entry into the zygote.

3.3 Paternal inheritance

Thus far, only a single angiosperm species has been found to inherit its plastids uniparentally paternally: the kiwi plant *Actinidia deliciosa* (Testolin and Cipriani 1997). By contrast, in gymnosperms, paternal inheritance (or biparental inheritance with a strong predominance of paternal transmission) seems to be widespread (Szmidt et al. 1987, Neale et al. 1989, Mogensen 1996). Distinction between purely paternal inheritance and biparental inheritance with a strongly prevailing paternal component has been difficult, because most studies on plastid inheritance in gymnosperms suffer from statistically limited datasets. This is due to the lack of suitable phenotypic markers (i.e., plastome mutations resulting in pigment deficiencies and thus providing visible markers) in most species analyzed to date which restricts the assay of progeny plants to RFLP analysis employing phenotypically neutral polymorphisms in the paternal and maternal ptDNAs. Naturally, this limits the number of progeny seedlings that can be analyzed and makes it difficult to exclude maternal plastid transmission below a certain level (Hagemann 2004).
Electron microscopic investigations confirmed the absence of plastids from egg cells (and the presence of them in sperm cells) in gymnosperm species displaying paternal plastid inheritance. In analogy to the diverse cytological mechanisms leading to maternal plastid inheritance (Fig. 2), at least two distinct mechanisms can contribute to paternal inheritance: Plastid exclusion by unequal organelle distribution during female gametophyte development and/or plastid degradation in the egg cell (Mogensen 1996, Hagemann 2004).

3.4 Paternal leakage

As evident from the above-mentioned exceptional transmission of paternal plastid genes in *Chlamydomonas* and the discussion of paternal vs. biparental inheritance in gymnosperms, there is a grey zone between uniparental inheritance and biparental inheritance. In most instances, the conclusion that a given species transmits its plastids uniparentally is based on the phenotypic analysis of at most a few thousand progeny plants from reciprocal crosses (see 4.2). Failure to detect variegated seedlings is usually interpreted as uniparental mode of inheritance. However, in this approach, occasional plastid transmission from the other parent goes undetected if it occurs only at a very low level. How strict maternal plastid inheritance can be has been a highly controversial issue, particularly in the context of the level of transgene containment provided by plastid transformation technology (see below). Paternal leakage, the low-level paternal transmission of plastids in species believed to inherit their plastids maternally, is known to occur at least in some plant species (Avni and Edelman 1991, Medgyesy et al. 1986, Horlow et al. 1990, Wang et al. 2004). A large-scale genetic study in foxtail millet, *Setaria italica*, employed crosses between male-sterile yellow- or green-leafed herbicide susceptible lines (as maternal parent) and a line with chloroplast-inherited atrazine

resistance as pollen donor (Wang et al. 2004). Assaying more than 780,000 hybrid offspring for atrazine resistance as it would be caused by paternally transmitted plastid genomes, paternal leakage was detected at a frequency of $3x10^{-4}$. Unfortunately, similarly reliable quantitative data in other plant species are largely lacking. It seems reasonable to suspect that the rate of paternal leakage can be very different in species representing the different subtypes of maternal inheritance (Fig. 2; Table 2), but this remains to be established experimentally.

The laborious and time-consuming genetic analyses required to establish low-level paternal leakage make it desirable to develop faster assays suitable to assess a species' potential to occasionally transmit paternal plastids via pollen. A rapid screening method that has been widely used employs staining of pollen with the DNA fluorochrome DAPI to identify plastid DNA in generative cells (Corriveau and Coleman 1988, Zhang et al. 2003). DAPI stains intensely plastid nucleoids which then can be readily detected by fluorescence microscopy. Absence of stainable plastid DNA from generative and sperm cells was taken as evidence for strictly maternal inheritance, whereas species with detectable ptDNA in generative and/or sperm cells were classified as potentially capable of occasional or regular biparental plastid ransmission (which, however, does not mean that these species indeed display biparental plastid transmission: species of the Triticum type regularly have plastids in their sperm cells, but yet transmit their plastids maternally; Fig. 2 and 4.1.3). The latter was the case for roughly one fifth of the species investigated (Corriveau and Coleman 1988). Generally, how reliable DAPI staining of pollen grains can predict paternal leakage will require confirmation by rigorous genetic analysis.

Finally, it seems possible that environmental factors influence the rate of occasional paternal plastid transmission. Experimental evidence for this has been obtained already in *Chlamydomonas* where exposure of mt+ gametes to UV light increases the rate of occasional paternal chloroplast DNA transmission (Hagemann 2004). Whether or not abiotic stress conditions also affect plastid inheritance in higher plants (which is mechanistically very different from *Chlamydomonas*; Table 2) remains to be investigated.

3.5 Biotechnological implications of plastid inheritance

With very few exceptions (e.g., alfalfa), all major food and fodder crop species fall into the large group of angiosperm plants exhibiting maternal plastid transmission. Maternal inheritance excludes plastid genes from pollen transmission. Consequently, putting transgenes into the plastid genome instead of the nuclear genome (as done in conventional transgenic plants) can greatly reduce the risk of unwanted transgene spreading via pollen. Uncontrolled transgene transmission through pollen dispersal represents a major concern in the public debate on transgenic technologies in agriculture and plant biotechnology. In this respect, two scenarios are frequently discussed: (i) pollen flow from fields with genetically modified (GM) cultivars to neighboring fields with non-GM cultivars and (ii) unwanted transgene spreading via pollen from GM plants to related plant species (through hybridization with sexually compatible wild or weed species). As maternal transgene inheritance can potentially prevent outcrossing via pollen flow, plastid genetic engineering has recently stirred

tremendous interest among plant biotechnologists (reviewed, e.g., in Bock 2001, Bock and Khan 2004, Maliga 2004, Bock 2007).

To critically assess the level of transgene confinement attainable by chloroplast transformation technology, knowledge about the reliability of maternal inheritance and the possible frequency of paternal leakage in a given crop species is of paramount importance. In view of the many different cytological and molecular mechanisms involved in maternal plastid inheritance (Fig. 2; Table 2) and the significant variation in them even between closely related species, general conclusions and statements are inappropriate here. How strict maternal inheritance is and whether or not paternal leakage occurs must be assessed on a species-by-species basis and requires genetic analyses (crosses and phenotypic analysis of the progeny) at a very large scale (Wang et al. 2004). The possibility of occasional paternal leakage notwithstanding, it is self-evident that chloroplast transformation offers greatly increased transgene containment compared with conventional (nuclear-transgenic) plants which would transmit the transgene with every single pollen grain. However, if paternal leakage occurs in a given species and pollen transmission of the transgene must be prevented altogether, stacking of plastid transformation with other containment methods will be necessary to eliminate the residual outcrossing risk (Daniell 2002, Lee and Natesan 2006).

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4 References

Abdallah F, Salamini F, Leister D (2000) A prediction of the size and evolutionary origin of the proteome of chloroplasts of Arabidopsis. Trends Plant Sci 5:141-142

Aguettaz P, Seyer P, Pesey H, Lescure A-M (1987) Relation between the plastid gene dosage and the levels of 16S rRNA and rbcL gene transcripts during amyloplast to chloroplast change in mixotrophic spinach cell suspensions. Plant Mol Biol 8:169-177

Ahlert D, Ruf S, Bock R (2003) Plastid protein synthesis is required for plant development in tobacco. Proc Natl Acad Sci USA 100:15730-15735

Avni A, Edelman M (1991) Direct selection for paternal inheritance of chloroplasts in sexual progeny of Nicotiana. Mol Gen Genet 225:273-277

Baldev A, Gaikwad K, Kirti PB, Mohapatra T, Prakash S, Chopra VL (1998) Recombination between chloroplast genomes of Trachystoma ballii and Brassica juncea following protoplast fusion. Mol Gen Genet 260:357-361

Barbrook AC, Howe CJ (2000) Minicircular plastid DNA in the dinoflagellate Amphidinium operculatum. Mol Gen Genet 263:152-158

Baumgartner BJ, Rapp JC, Mullet JE (1988) Plastid transcription activity and DNA copy number increase early in barley chloroplast development. Plant Physiol 89:1011-1018

Baur E (1909) Das Wesen und die Erblichkeitsverhältnisse der "Varietates albomarginatae hort." von Pelargonium zonale. Z indukt Abstammungs- u Vererbungslehre 1:330-351

Baur E (1910) Untersuchungen über die Vererbung von Chromatophorenmerkmalen bei Melandrium, Antirrhinum und Aquilegia. Z indukt Abstammungs- u Vererbungslehre 4:81-102

Bendich AJ (1987) Why do chloroplasts and mitochondria contain so many copies of their genome? BioEssays 6:279-282

Bendich AJ (2004) Circular chloroplast chromosomes: the grand illusion. Plant Cell 16:1661-1666

Bendich AJ, Smith SB (1990) Moving pictures and pulsed-field gel electrophoresis show linear DNA molecules from chloroplasts and mitochondria. Curr Genet 17:421-425

Berg S, Krause K, Krupinska K (2004) The rbcL genes of two Cuscuta species, C. gronovii and C. subinclusa, are transcribed by the nuclear-encoded plastid RNA polymerase (NEP). Planta 219:541-546

Birky CWJr, Walsh JB (1992) Biased gene conversion, copy number, and apparent mutation rate differences within chloroplast and bacterial genomes. Genetics 130:677-683

Birky Jr. CW (1995) Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. Proc Natl Acad Sci USA 92:11331-11338

Bock R (2001) Transgenic chloroplasts in basic research and plant biotechnology. J Mol Biol 312:425-438

Bock R (2007) Plastid biotechnology: prospects for herbicide and insect resistance, metabolic engineering and molecular farming. Curr Opin Biotechnol:in press

Bock R, Hippler M (2002) Extranuclear inheritance: Functional genomics in chloroplasts. Prog Bot 63:106-131

Bock R, Khan MS (2004) Taming plastids for a green future. Trends Biotechnol 22:311-318

Bolen PL, Grant DM, Swinton D, Boynton JE, Gillham NW (1982) Extensive methylation of chloroplast DNA by a nuclear gene mutation does not affect chloroplast gene transmission in Chlamydomonas. Cell 28:335-343

Boudreau E, Takahashi Y, Lemieux C, Turmel M, Rochaix J-D (1997) The chloroplast ycf3 and ycf4 open reading frames of Chlamydomonas reinhardtii are required for the accumulation of the photosystem I complex. EMBO J 16:6095-6104

Bubunenko MG, Schmidt J, Subramanian AR (1994) Protein substitution in chloroplast ribosome evolution. A eukaryotic cytosolic protein has replaced its organelle homologue (L23) in spinach. J Mol Biol 240:28-41

Bungard RA (2004) Photosynthetic evolution in parasitic plants: insight from the chloroplast genome. BioEssays 26:235-247

Burrows PA, Sazanov LA, Svab Z, Maliga P, Nixon PJ (1998) Identification of a functional respiratory complex in chloroplasts through analysis of tobacco mutants containing disrupted plastid ndh genes. EMBO J 17:868-876

Cannon GC, Ward LN, Case CI, Heinhorst S (1999) The 68 kDa compacting nucleoid protein from soybean chloroplasts inhibits DNA synthesis in vitro. Plant Mol Biol 39:835-845

Cho HS, Lee SS, Kim KD, Hwang I, Lim J-S, Park Y-I (2004) DNA gyrase is involved in chloroplast nucleoid partitioning. Plant Cell 16:2665-2682

Chumley TW, Palmer JD, Mower JP, Fourcade HM, Calie PJ, Boore JL, Jansen RK (2006) The complete chloroplast genome sequence of Pelargonium x hortorum: Organization and evolution of the largest and most highly rearranged chloroplast genome of land plants. Mol Biol Evol 23:2175-2190

Chun EHL, Vaugham MH, Rich A (1963) The isolation and characterization of DNA associated with chloroplast preparations. J Mol Biol 7:130-141

Correns C (1909) Vererbungsversuche mit blass(gelb)grünen und buntblättrigen Sippen bei Mirabilis jalapa, Urtica pilulifera und Lunaria annua. Z indukt Abstammungs- u Vererbungslehre 1:291–329

Corriveau JL, Coleman AW (1988) Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. Am J Bot 75:1443-1458

Daniell H (2002) Molecular strategies for gene containment in transgenic crops. Nature Biotechnol 20:581-586

Deng X-W, Wing RA, Gruissem W (1989) The chloroplast genome exists in multimeric forms. Proc Natl Acad Sci USA 86:4156-4160

dePamphilis CW, Palmer JD (1990) Loss of photosynthetic and chlororespiratory genes from the plastid genome of a parasitic flowering plant. Nature 348:337-339

Douglas SE, Penny SL (1999) The plastid genome of the cryptophyte alga, Guillardia theta: complete sequence and conserved synteny groups confirm its common ancestry with red algae. J Mol Evol 48:236-244

Drescher A, Ruf S, Calsa Jr. T, Carrer H, Bock R (2000) The two largest chloroplast genome-encoded open reading frames of higher plants are essential genes. Plant J 22:97-104

Ems SC, Morden CW, Dixon CK, Wolfe KH, dePamphilis CW, Palmer JD (1995) Transcription, splicing and editing of plastid RNAs in the nonphotosynthetic plant Epifagus virginiana. Plant Mol Biol 29:721-733

Fojtová M, Kovarik A, Matyásek R (2001) Cytosine methylation of plastid genome in higher plants. Fact or artefact? Plant Sci 160:585-593

Goulding SE, Olmstead RG, Morden CW, Wolfe KH (1996) Ebb and flow of the chloroplast inverted repeat. Mol Gen Genet 252:195-206

Hagemann R (2000) Erwin Baur or Carl Correns: Who really created the theory of plastid inheritance? J Hered 91:435-440

Hagemann R (2002) Milestones in plastid genetics of higher plants. Prog Bot 63:1-51

Hagemann R (2004) The sexual inheritance of plant organelles. In: Daniell H, Chase C (eds) Molecular Biology and Biotechnology of Plant Organelles. Springer, Heidelberg, pp. 93-113

Hagemann R, Schröder M-B (1989) The cytological basis of the plastid inheritance in angiosperms. Protoplasma 152:57-64

Hager M, Biehler K, Illerhaus J, Ruf S, Bock R (1999) Targeted inactivation of the smallest plastid genome-encoded open reading frame reveals a novel and essential subunit of the cytochrome b6f complex. EMBO J 18:5834-5842

Hallick RB, Hong L, Drager RG, Favreau MR, Monfort A, Orsat B, Spielmann A, Stutz E (1993) Complete sequence of Euglena gracilis chloroplast DNA. Nucleic Acids Res 21:3537-3544

Hedtke B, Börner T, Weihe A (1997) Mitochondrial and chloroplast phage-type RNA polymerases in Arabidopsis. Science 277:809-811

Hess WR, Börner T (1999) Organellar RNA polymerases of higher plants. Inter Rev Cytol 190:1-59

Hess WR, Prombona A, Fieder B, Subramanian AR, Börner T (1993) Chloroplast rps15 and the rpoB/C1/C2 gene cluster are strongly transcribed in ribosome-deficient plastids: evidence for a functioning non-chloroplast-encoded RNA polymerase. EMBO J 12:563-571

Hess WR, Hoch B, Zeltz P, Hübschmann T, Kössel H, Börner T (1994) Inefficient rpl2 splicing in barley mutants with ribosome-deficient plastids. Plant Cell 6:1455-1465

Hippler M, Bock R (2004) Chloroplast proteomics. Prog Bot 65:90-105

Hiratsuka J, Shimada H, Whittier R, Ishibashi T, Sakamoto M, Mori M, Kondo C, Honji Y, Sun C-R, Meng B-Y, Li Y-Q, Kanno A, Nishizawa Y, Hirai A, Shinozaki K, Sugiura M (1989) The complete sequence of the rice (Oryza sativa) Chloroplast genome: Intermolecular recombination between distinct tRNA genes accounts for a major plastid DNA inversion during the evolution of cereals. Mol Gen Genet 217:185-194

Horlow C, Goujaud J, Lépingle A, Missonier C, Bourgin J-P (1990) Transmission of paternal chloroplasts in tobacco (Nicotiana tabacum). Plant Cell Rep 9:249-252

Ikehara T, Uchida H, Suzuki L, Nakamura S (1996) Chloroplast nucleoids in large number and large DNA amount with regard to maternal inheritance in Chlamydomonas reinhardtii. Protoplasma 194:11-17

Isono K, Niwa Y, Satoh K, Kobayashi H (1997) Evidence for transcriptional regulation of plastid photosynthesis genes in Arabidopsis thaliana roots. Plant Physiol 114:623-630

Jansen RK, Raubeson LA, Boore JL, de Pamphilis CW, Chumley TW, Haberle RC, Wyman SK, Alverson AJ, Peery R, Herman SJ, Fourcade HM, Kuehl JV, McNeal JR, Leebens-Mack J, Cui L (2005) Methods for obtaining and analyzing whole chloroplast genome sequences. Methods Enzymol 395:348-384

Jenkins BD, Kulhanek DJ, Barkan A (1997) Nuclear mutations that block group II RNA splicing in maize chloroplasts reveal several intron classes with distinct requirements for splicing factors. Plant Cell 9:283-296

Jeong SY, Rose A, Meier I (2003) MFP1 is a thylakoid-associated, nucleoid-binding protein with a coiled-coil structure. Nucleic Acids Res 31:5175-5185

Joet T, Cournac L, Horvath EM, Medgyesy P, Peltier G (2001) Increased sensitivity of photosynthesis to antimycin A induced by inactivation of the chloroplast ndhB gene. Evidence for a participation of the NADH-dehydrogenase complex to cyclic electron flow around photosystem I. Plant Physiol 125:1919-1929

Jukes TH, Osawa S (1990) The genetic code in mitochondria and chloroplasts. Experientia 46:1117-1126

Kahlau S, Aspinall S, Gray JC, Bock R (2006) Sequence of the tomato chloroplast DNA and evolutionary comparison of solanaceous plastid genomes. J Mol Evol 63:194-207

Kaneko T, Tabata S (1997) Complete genome structure of the unicellular cyanobacterium Synechocystis sp. PCC6803. Plant Cell Physiol 38:1171-1176

Kaneko T, Sato S, Kotani H, Tanaka A, Asamizu E, Nakamura Y, Miyajima N, Hirosawa M, Sugiura M, Sasamoto S, Kimura T, Hosouchi T, Matsuno A, Muraki A, Nakazaki N, Naruo K, Okumura S, Shimpo S, Takeuchi C, Wada T, Watanabe A, Yamada M, Yasuda M, Tabata S (1996) Sequence analysis of the genome of the unicellular cyanobacterium Synechocystis sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. DNA Res 3:109-136

Khakhlova O, Bock R (2006) Elimination of deleterious mutations in plastid genomes by gene conversion. Plant J 46:85-94

Kobayashi T, Takahara M, Miyagishima S-y, Kuroiwa H, Sasaki N, Ohta N, Matsuzaki M, Kuroiwa T (2002) Detection and localization of a chloroplast-encoded HU-like protein that organizes chloroplast nucleoids. Plant Cell 14:1579-1589

Kowallik KV, Stoebe B, Schaffran I, Kroth-Pancic P, Freier U (1995) The chloroplast genome of a chlorophyll a+c-containing alga, Odontella sinensis. Plant Mol Biol Rep 13:336-342

Krause K, Krupinska K (2000) Molecular and functional properties of highly purified transcriptionally active chromosomes from spinach chloroplasts. Physiol Plant 109:188-195

Kumar D, Mukherjee S, Reddy MK, Tewari KK (1995) A novel single-stranded DNA-specific endonuclease from pea chloroplasts. J Exp Bot 46:767-776

Kuroiwa T (1989) The nuclei of cellular organelles and the formation of daughter organelles by the "plastid-dividing ring". Bot Mag Tokyo 102:291-329

Kuroiwa T (1991) The replication, differentiation, and inheritance of plastids with emphasis on the concept of organelle nuclei. Int Rev Cytol 128:1-62

Lee D, Natesan E (2006) Evaluating genetic containment strategies for transgenic plants. Trends Biotechnol 24:109-114

Li W, Ruf S, Bock R (2006) Constancy of organellar genome copy numbers during leaf development and senescence in higher plants. Mol Gen Genomics 275:185-192

Liere K, Link G (1995) RNA-binding activity of the matK protein encoded by the chloroplast trnK intron from mustard (Sinapis alba L.). Nucleic Acids Res 23:917-921

Lilly JW, Havey MJ, Jackson SA, Jiang J (2001) Cytogenomic analyses reveal the structural plasticity of the chloroplast genome in higher plants. Plant Cell 13:245-254

Liu J-W, Rose RJ (1992) The spinach chloroplast chromosome is bound to the thylakoid membrane in the region of the inverted repeat. Biochem Biophys Res Commun 184:993-1000

Lung B, Zemann A, Madej MJ, Schuelke M, Techritz S, Ruf S, Bock R, Hüttenhofer A (2006) Identification of small non-coding RNAs from mitochondria and chloroplasts. Nucleic Acids Res 34:3842-3852

Maier RM, Neckermann K, Igloi GL, Kössel H (1995) Complete sequence of the maize chloroplast genome: gene content, hotspots of divergence and fine tuning of genetic information by transcript editing. J Mol Biol 251:614-628

Majeran W, Wollman F-A, Vallon O (2000) Evidence for a role of ClpP in the degradation of the chloroplast cytochrome b6f complex. Plant Cell 12:137-149

Maliga P (2004) Plastid transformation in higher plants. Annu Rev Plant Biol 55:289-313

Marano MR, Carrillo N (1991) Chromoplast formation during tomato fruit ripening. No evidence for plastid methylation. Plant Mol Biol 16:11-19

Martin W, Rujan T, Richly E, Hansen A, Cornelsen S, Lins T, Leister D, Stoebe B, Hasegawa M, Penny D (2002) Evolutionary analysis of Arabidopsis, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. Proc Natl Acad Sci USA 99:12246-12251

Maul JE, Lilly JW, Cui L, dePamphilis CW, Miller W, Harris EH, Stern DB (2002) The Chlamydomonas reinhardtii plastid chromosome: islands of genes in a sea of repeats. Plant Cell 14:2659-2679

Medgyesy P, Fejes E, Maliga P (1985) Interspecific chloroplast recombination in a Nicotiana somatic hybrid. Proc Natl Acad Sci USA 82:6960-6964

Medgyesy P, Páy A, Márton L (1986) Transmission of paternal chloroplasts in Nicotiana. Mol Gen Genet 204:195-198

Millen RS, Olmstead RG, Adams KL, Palmer JD, Lao NT, Heggie L, Kavanagh TA, Hibberd JM, Gray JC, Morden CW, Calie PJ, Jermiin LS, Wolfe KH (2001) Many parallel losses of infA from chloroplast DNA during angiosperm evolution with multiple independent transfers to the nucleus. Plant Cell 13:645-658

Misumi O, Suzuki L, Nishimura Y, Sakai A, Kawano S, Kuroiwa H, Kuroiwa T (1999) Isolation and phenotypic characterization of Chlamydomonas reinhardtii mutants defective in chloroplast DNA segregation. Protoplasma 209:273-282

Mogensen HL (1996) The hows and whys of cytoplasmic inheritance in seed plants. Am J Bot 83:383-404

Mohr G, Perlman PS, Lambowitz AM (1993) Evolutionary relationships among group II intron-encoded proteins and identification of a conserved domain that may be related to maturase function. Nucleic Acids Res 21:4991-4997

Monod C, Takahashi Y, Goldschmidt-Clermont M, Rochaix J-D (1994) The chloroplast ycf8 open reading frame encodes a photosystem II polypeptide which maintains photosynthetic activity under adverse growth conditions. EMBO J 13:2747-2754

Morden CW, Wolfe KH, dePamphilis CW, Palmer JD (1991) Plastid translation and transcription genes in a non-photosynthetic plant: intact, missing and pseudo genes. EMBO J 10:3281-3288

Mukherjee SK, Reddy MK, Kumar D, Tewari KK (1994) Purification and characterization of a eukaryotic type 1 topoisomerase from pea chloroplasts. J Biol Chem 269:3793-3801

Muller HJ (1964) The relation of recombination to mutational advance. Mutat Res 1:2-9

Munekage Y, Hashimoto M, Miyake C, Tomizawa K-i, Endo T, Tasaka M, Shikanai T (2004) Cyclic electron flow around photosystem I is essential for photosynthesis. Nature 429:579-582

Nakano T, Murakami S, Shoji T, Yoshida S, Yamada Y, Sato F (1997) A novel protein with DNA binding activity from tobacco chloroplast nucleoids. Plant Cell 9:1673-1682

Neale DB, Marshall KA, Sederoff RR (1989) Chloroplast and mitochondrial DNA are paternally inherited in Sequoia sempervirens D. Don Endl. Proc Natl Acad Sci USA 86:9347-9349

Ngernprasirtsiri J, Kobayashi H, Akazawa T (1988a) DNA methylation occurred around lowly expressed genes of plastid DNA during tomato fruit development. Plant Physiol 88:16-20

Ngernprasirtsiri J, Kobayashi H, Akazawa T (1988b) DNA methylation as a mechanism of transcriptional regulation in nonphotosynthetic plastids in plant cells. Proc Natl Acad Sci USA 85:4750-4754

Nishimura Y, Misumi O, Matsunaga S, Higashiyama T, Yokota A, Kuroiwa T (1999) The active digestion of uniparental chloroplast DNA in a single zygote of Chlamydomonas reinhardtii is revealed by using the optical tweezer. Proc Natl Acad Sci USA 96:12577-12582

Nishimura Y, Misumi O, Kato K, Inada N, Higashiyma T, Momoyama Y, Kuroiwa T (2002) An mt+ gamete-specific nuclease that targets mt- chloroplasts during sexual reproduction in C. reinhardtii. Genes & Dev 16:1116-1128

Nishiyama R, Ito M, Yamaguchi Y, Koizumi N, Sano H (2002) A chloroplast-resident DNA methyltransferase is responsible for hypermethylation of chloroplast genes in Chlamydomonas maternal gametes. Proc Natl Acad Sci USA 99:5925-5930

Nishiyama R, Wada Y, Mibu M, Yamaguchi Y, Shimogawara K, Sano H (2004) Role of a nonselective de novo DNA methyltransferase in maternal inheritance of chloroplast genes in the green alga, Chlamydomonas reinhardtii. Genetics 168:809-816

Ohta N, Matsuzaki M, Misumi O, Miyagishima S-y, Nozaki H, Tanaka K, Shin-I T, Kohara Y, Kuroiwa T (2003) Complete sequence and analysis of the plastid genome of the unicellular red alga Cyanidioschyzon merolae. DNA Res 10:67-77

Ohyama K, Fukuzawa H, Kohchi T, Shirai H, Sano T, Sano S, Umesono K, Shiki Y, Takeuchi M, Chang Z, Aota S-i, Inokuchi H, Ozeki H (1986) Chloroplast gene organization deduced from complete sequence of liverwort Marchantia polymorpha chloroplast DNA. Nature 322:572-574

Ohyama K, Fukuzawa H, Kohchi T, Sano T, Sano S, Shirai H, Umesono K, Shiki T, Takeuchi M, Chang Z, Aota S-i, Inokuchi H, Ozeki H (1988) Structure and organization of Marchantia polymorpha chloroplast genome. I. Cloning and gene identification. J Mol Biol 203:281-298

Oldenburg DJ, Bendich AJ (2004) Most chloroplast DNA of maize seedlings in linear molecules with defined ends and branched forms. J Mol Biol 335:953-970

Orsat B, Monfort A, Chatellard P, Stutz E (1992) Mapping and sequencing of an actively transcribed Euglena gracilis chloroplast gene (ccsA) homologous to the Arabidopsis thaliana nuclear gene cs (ch-42). FEBS Lett 303:181-184

Osawa S, Jukes TH, Watanabe K, Muto A (1992) Recent evidence for evolution of the genetic code. Microbiol Rev 56:229-264

Palmer JD (1983) Chloroplast DNA exists in two orientations. Nature 301:92-93

Palmer JD, Thompson WF (1982) Chloroplast DNA rearrangements are more frequent when a large inverted repeat sequence is lost. Cell 29:537-550

Palmer JD, Nugent JM, Hebron LA (1987) Unusual structure of geranium chloroplast DNA: A triple-sized inverted repeat, extensive gene duplications, multiple inversions, and two repeat families. Proc Natl Acad Sci USA 84:769-773

Pfalz J, Liere K, Kandlbinder A, Dietz K-J, Oelmüller R (2006) pTAC2, -6, and -12 are components of the transcriptionally active plastid chromosome that are required for plastid gene expression. Plant Cell 18:176-197

Reith M (1995) Molecular biology of rhodophyte and chromophyte plastids. Annu Rev Plant Physiol Plant Mol Biol 46:549-575

Reith M, Munholland J (1993) A high-resolution gene map of the chloroplast genome of the red alga Porphyra purpurea. Plant Cell 5:465-475

Reith M, Munholland J (1995) Complete nucleotide sequence of the Porphyra purpurea chloroplast genome. Plant Mol Biol Rep 13:333-335

Revill MJW, Stanley S, Hibberd JM (2005) Plastid genome structure and loss of photosynthetic ability in the parasitic genus Cuscuta. J Exp Bot 56:2477-2486

Rochaix J-D (1997) Chloroplast reverse genetics: new insights into the function of plastid genes. Trends Plant Sci 2:419-425

Rogalski M, Ruf S, Bock R (2006) Tobacco plastid ribosomal protein S18 is essential for cell survival. Nucleic Acids Res 34:4537-4545

Rolland N, Dorne A-J, Amoroso G, Sültemeyer DF, Joyard J, Rochaix J-D (1997) Disruption of the plastid ycf10 open reading frame affects uptake of inorganic carbon in the chloroplast of Chlamydomonas. EMBO J 16:6713-6726

Ruf S, Kössel H, Bock R (1997) Targeted inactivation of a tobacco intron-containing open reading frame reveals a novel chloroplast-encoded photosystem I-related gene. J Cell Biol 139:95-102

Rujan T, Martin W (2001) How many genes in Arabidopsis come from cyanobacteria ? An estimate from 386 protein phylogenies. Trends Genet 17:113-121

Sager R, Ishida MR (1963) Chloroplast DNA in Chlamydomonas. Proc Natl Acad Sci USA 50:725-730

Sager R, Ramanis Z (1976) Chloroplast genetics of Chlamydomonas. II. Mapping by cosegregation frequency analysis. Genetics 83:323-340

Sakai A, Yamashita H, Nemoto Y, Kawano S, Kuroiwa T (1991) Transcriptional activity of morphologically intact proplastid-nuclei (nucleoids) isolated from tobacco cultured cells. Plant Cell Physiol 32:835-843

Salvador ML, Klein U, Bogorad L (1998) Endogenous fluctuations of DNA topology in the chloroplast of Chlamydomonas reinhardtii. Mol Cell Biol 18:7235-7242

Sasaki Y, Sekiguchi K, Nagano Y, Matsuno R (1993a) Chloroplast envelope protein encoded by chloroplast genome. FEBS Lett 316:93-98

Sasaki Y, Hakamada K, Suama Y, Nagano Y, Furusawa I, Matsuno R (1993b) Chloroplast-encoded protein as a subunit of acetyl-CoA carboxylase in pea plant. J Biol Chem 268:25118-25123

Sasaki Y, Konishi T, Nagano Y (1995) The compartmentation of acetyl-coenzyme A carboxylase in plants. Plant Physiol 108:445-449

Sato N, Albrieux C, Joyard J, Douce R, Kuroiwa T (1993) Detection and characterization of a plastid envelope DNA-binding protein which may anchor plastid nucleoids. EMBO J 12:555-561

Scharff LB, Koop H-U (2006) Linear molecules of tobacco ptDNA end at known replication origins and additional loci. Plant Mol Biol 62:611-621

Sekine K, Hase T, Sato N (2002) Reversible DNA compaction by sulfite reductase regulates transcriptional activity of chloroplast nucleoids. J Biol Chem 277:24399-24404

Shanklin J, DeWitt ND, Flanagan JM (1995) The stroma of higher plant plastids contain ClpP and ClpC, functional homologs of Escherichia coli ClpP and ClpA: an archetypal two-component ATP-dependent protease. Plant Cell 7:1713-1722

Shi L, Zhu T, Mogensen HL, Smith SE (1991) Paternal plastid inheritance in alfalfa: plastid nucleoid number within generative cells correlates poorly with plastid number and male plastid transmission strength. Curr Genet 19:399-401

Shikanai T, Endo T, Hashimoto T, Yamada Y, Asada K, Yokota A (1998) Directed disruption of the tobacco ndhB gene impairs cyclic electron flow around photosystem I. Proc Natl Acad Sci USA 95:9705-9709

Shimada H, Sugiura M (1991) Fine structural features of the chloroplast genome: comparison of the sequenced chloroplast genomes. Nucleic Acids Res 19:983-995

Shinozaki K, Ohme M, Tanaka M, Wakasugi T, Hayashida N, Matsubayashi T, Zaita N, Chunwongse J, Obokata J, Yamaguchi-Shinozaki K, Ohto C, Torazawa K, Meng BY, Sugita M, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Kato A, Tohdoh N, Shimada H, Sugiura M (1986) The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J 5:2043-2049

Sijben-Müller G, Hallick RB, Alt J, Westhoff P, Herrmann RG (1986) Spinach plastid gene coding for initiation factor IF-1, ribosomal protein S11 and RNA polymerase α-subunit. Nucleic Acids Res 14:1029-1042

Simpson CL, Stern DB (2002) The treasure trove of algal chloroplast genomes. Surprises in architecture and gene content, and their functional implications. Plant Physiol 129:957-966

Stein DB, Palmer JD, Thompson WF (1986) Structural evolution and flip-flop recombination of chloroplast DNA in the fern genus Osmunda. Curr Genet 10:835-841

Sugiura M (1989) The chloroplast chromosomes in land plants. Annu Rev Cell Biol 5:51-70

Sugiura M (1992) The chloroplast genome. Plant Mol Biol 19:149-168

Szmidt AE, Alden T, Hällgren J-E (1987) Paternal inheritance of chloroplast DNA in Larix. Plant Mol Biol 9:59-64

Testolin R, Cipriani G (1997) Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in the genus Actinidia. Theor Appl Genet 94:897-903

Tewari KK, Wildman SG (1966) Chloroplast DNA from tobacco leaves. Science 153:1269-1271

Thanh ND, Medgyesy P (1989) Limited chloroplast gene transfer via recombination overcomes plastome-genome incompatibility between Nicotiana tabacum and Solanum tuberosum. Plant Mol Biol 12:87-93

Tilney-Bassett RAE (1984) The genetic evidence for nuclear control of chloroplast biogenesis in higher plants. In: Ellis, R J (ed) Chloroplast Biogenesis. Cambridge Univ Press, Cambridge, pp13-50

Tilney-Bassett RAE (1994) Nuclear control of chloroplast inheritance in higher plants. J Heredity 85:347-354

Umen JG, Goodenough UW (2001) Chloroplast DNA methylation and inheritance in Chlamydomonas. Genes & Dev 15:2585-2597

Wakasugi T, Tsudzuki J, Ito S, Nakashima K, Tsudzuki T, Sugiura M (1994) Loss of all ndh genes as determined by sequencing the entire chloroplast genome of the black pine Pinus thunbergii. Proc Natl Acad Sci USA 91:9794-9798

Wakasugi T, Nagai T, Kapoor M, Sugita M, Ito M, Ito S, Tsudzuki J, Nakashima K, Tsudzuki T, Suzuki Y, Hamada A, Ohta T, Inamura A, Yoshinaga K, Sugiura M (1997) Complete nucleotide sequence of the chloroplast genome from the green alga Chlorella vulgaris: The existence of genes possibly involved in chloroplast division. Proc Natl Acad Sci USA 94:5967-5972

Wakasugi T, Tsudzuki T, Sugiura M (2001) The genomics of land plant chloroplasts: gene content and alteration of genomic information by RNA editing. Photosynthesis Res 70:107-118

Wang T, Li Y, Shi Y, Reboud X, Darmency H, Gressel J (2004) Low frequency transmission of a plastid-encoded trait in Setaria italica. Theor Appl Genet 108:315-320

Wimpee CF, Wrobel RL, Garvin DK (1991) A divergent plastid genome in Conopholis americana, an achlorophyllous parasitic plant. Plant Mol Biol 17:161-166

Wolfe KH, Li W-H, Sharp PM (1987) Rates of nucleotide substitutions vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. Proc Natl Acad Sci USA 84:9054-9058

Wolfe KH, Morden CW, Ems SC, Palmer JD (1992) Rapid evolution of the plastid translational apparatus in a nonphotosynthetic plant: Loss or accelerated sequence evolution of tRNA and ribosomal protein genes. J Mol Evol 35:304-317

Wolfe KH, Morden CW, Palmer JD (1992) Function and evolution of a minimal plastid genome from a nonphotosynthetic parasitic plant. Proc Natl Acad Sci USA 89:10648-10652

Xie Z, Merchant S (1996) The plastid-encoded ccsA gene is required for heme attachment to chloroplast c-type cytochromes. J Biol Chem 271:4632-4639

Xie Z, Culler D, Dreyfuss BW, Kuras R, Wollman F-A, Girard-Bascou J, Merchant S (1998) Genetic analysis of chloroplast c-type cytochrome assembly in Chlamydomonas reinhardtii: one chloroplast locus and at least four nuclear loci are required for heme attachment. Genetics 148:681-692

Zhang Q, Liu Y, Sodmergen (2003) Examination of the cytoplasmic DNA in male reproductive cells to determine the potential for cytoplasmic inheritance in 295 angiosperm species. Plant Cell Physiol 44:941-951

Zhang Z, Green BR, Cavalier-Smith T (1999) Single gene circles in dinoflagellate chloroplast genomes. Nature 400:155-159

Zoschke R, Liere K, Börner T (2007) From seedling to mature plant: Arabidopsis plastidial genome copy number, RNA accumulation and transcription are differentially regulated during leaf development. Plant J, in press

Zubko MK, Day A (1998) Stable albinism induced without mutagenesis: a model for ribosome-free plastid inheritance. Plant J 15:265-271

Zubko MK, Day A (2002) Differential regulation of genes transcribed by nucleus-encoded plastid RNA polymerase, and DNA amplification, within ribosome-deficient plastids in stable phenocopies of cereal albino mutants. Mol Genet Genomics 267:27-37

List of abbreviations

CF: coupling factor, cyt b_6f: cytochrome b_6f complex, DAPI: 4',6-diamidino-2-phenylindole, GM: genetically modified, IR: inverted repeat, kb: kilobase pairs, LSC: large single copy region, mt: mating type, NEP: nuclear-encoded RNA polymerase, ORF: open reading frame, PEP: plastid-encoded RNA polymerase, PSI: photosystem I, PSII: photosystem II, ptDNA: plastid DNA, RFLP: restriction fragment length polymorphism, Rubisco: ribulose 1,5-bisphosphate carboxylase/oxygenase, SSC: small single copy region; *ycf*: hypothetical chloroplast reading frame

Figure legends

Figure 1 Physical map of the tomato (*Solanum lycopersicum*, formerly *Lycopersicon esculentum*) plastid genome as a typical example of a plastid genome in higher plants (modified from Kahlau et al. 2006). Genes inside the circle are transcribed clockwise, genes outside the circle are transcribed counterclockwise. The two large inverted repeat regions IR_A and IR_B are shown as fat lines. Asterisks indicate intron-containing genes, introns are depicted as open boxes. For gene products and their functions, compare Table 1.

Figure 2 Cytological mechanisms leading to maternal plastid inheritance in higher plants. See text for details and Table 2 for example species.

Tables

Table 1. Plastid-encoded genes and conserved open reading frames (*ycf* = hypothetical chloroplast reading frame) in higher plants.

Gene	Gene product	Functions and remarks
psaA	A subunit of PSI	reaction center subunit, essential for PSI function
psaB	B subunit of PSI	reaction center subunit, essential for PSI function
psaC	C subunit of PSI	essential cofactor-binding subunit
psaI	I subunit of PSI	small subunit, not essential for PSI function
psaJ	J subunit of PSI	small subunit, not essential for PSI function
ycf3	Ycf3 protein	essential PSI assembly factor, contains three tetratricopeptide (TPR) repeats
ycf4	Ycf4 protein	essential PSI assembly factor
psbA	D1 protein of PSII	reaction center, also termed 'herbicide-binding protein', essential for PSII function
psbB	CP47 subunit of PSII	inner antenna protein, essential for PSII function
psbC	CP43 subunit of PSII	inner antenna protein, essential for PSII function
psbD	D2 protein of PSII	reaction center, essential for PSII function
psbE	α-subunit of cytochrome b_{559}	essential for PSII assembly/stability/function, protection of PSII against photoinhibition, dark reduction of plastoquinone, cyclic electron transfer?
psbF	β-subunit of cytochrome b_{559}	essential for PSII assembly/stability/function, protection of PSII against photoinhibition, dark oxidation of plastoquinol
psbH	H subunit of PSII	small subunit associated with CP47, involved in PSII assembly, stabilization and photoprotection
psbI	I subunit of PSII	small subunit, involved in stabilization of PSII dimers and PSII-LHCII supercomplexes

psbJ	J subunit of PSII	small subunit, involved in assembly of the water-splitting complex and intra-complex electron transfer
psbK	K subunit of PSII	small subunit associated with CP43, presumably involved in PSII assembly/stability
psbL	L subunit of PSII	small subunit, involved in PSII dimerization and PSII-LHCII supercomplex formation, required for assembly of the water-splitting complex
psbM	M subunit of PSII	small subunit, function unknown
psbN	N subunit of PSII	function unknown, assignment as PSII subunit uncertain
psbT	T subunit of PSII	small subunit, involved in repair of photodamaged PSII reaction centers
psbZ	Z subunit of PSII	small subunit, couples the light-harvesting complex protein CP26 to PSII
petA	cytochrome f	core subunit of cyt b_6f complex, essential for cyt b_6f function
petB	cytochrome b_6	core subunit of cyt b_6f complex, essential for cyt b_6f function
petD	subunit IV of cyt b_6f	essential for cyt b_6f function
petG	G subunit of cyt b_6f	small subunit, essential for cyt b_6f assembly/stability in *Chlamydomonas*
petL	L subunit of cyt b_6f	small subunit, not essential for cyt b_6f function, involved in complex stabilization
petN	N subunit of cyt b_6f	small subunit, essential for cyt b_6f assembly/stability
atpA	ATP synthase α-subunit	CF_1, nucleotide-binding site
atpB	ATP synthase β-subunit	CF_1, catalytic site
atpE	ATP synthase ε-subunit	CF_1, regulation of CF_1CF_0 activation, required for proton gating
atpF	ATP synthase b-subunit	CF_0, binding of CF_1
atpH	ATP synthase c-subunit	CF_0, proton translocation

atpI	ATP synthase a-subunit	CF_0, proton translocation
ndhA	A subunit of NAD(P)H dehydrogenase	chlororespiration, cyclic electron transfer, absent from gymnosperm plastomes
ndhB	B subunit of NAD(P)H dehydrogenase	chlororespiration, cyclic electron transfer, absent from gymnosperm plastomes
ndhC	C subunit of NAD(P)H dehydrogenase	chlororespiration, cyclic electron transfer, absent from gymnosperm plastomes
ndhD	D subunit of NAD(P)H dehydrogenase	chlororespiration, cyclic electron transfer, absent from gymnosperm plastomes
ndhE	E subunit of NAD(P)H dehydrogenase	chlororespiration, cyclic electron transfer, absent from gymnosperm plastomes
ndhF	F subunit of NAD(P)H dehydrogenase	chlororespiration, cyclic electron transfer, absent from gymnosperm plastomes
ndhG	G subunit of NAD(P)H dehydrogenase	chlororespiration, cyclic electron transfer, absent from gymnosperm plastomes
ndhH	H subunit of NAD(P)H dehydrogenase	chlororespiration, cyclic electron transfer, absent from gymnosperm plastomes
ndhI	I subunit of NAD(P)H dehydrogenase	chlororespiration, cyclic electron transfer, absent from gymnosperm plastomes
ndhJ	J subunit of NAD(P)H dehydrogenase	chlororespiration, cyclic electron transfer, absent from gymnosperm plastomes
ndhK	K subunit of NAD(P)H dehydrogenase	chlororespiration, cyclic electron transfer, absent from gymnosperm plastomes
rbcL	Rubisco large subunit	CO_2 fixation
rpoA	RNA polymerase α-subunit	transcription, *E.coli*-like plastid RNA polymerase (PEP)

rpoB	RNA polymerase β-subunit	transcription, *E.coli*-like plastid RNA polymerase (PEP)
rpoC1	RNA polymerase β'-subunit	transcription, *E.coli*-like plastid RNA polymerase (PEP)
rpoC2	RNA polymerase β''-subunit	transcription, *E.coli*-like plastid RNA polymerase (PEP)
matK	intron maturase	splicing factor for group II introns
rrn16	16S ribosomal RNA	translation, small ribosomal subunit
rrn23	23S ribosomal RNA	translation, large ribosomal subunit
rrn5	23S ribosomal RNA	translation, large ribosomal subunit
rrn4.5	23S ribosomal RNA	translation, large ribosomal subunit
trnA-UGC	tRNA-Alanine(UGC)	translation
trnC-GCA	tRNA-Cysteine(GCA)	translation
trnD-GUC	tRNA-Aspartate(GUC)	translation
trnE-UUC	tRNA-Glutamate(UUC)	translation, tetrapyrrole biosynthesis
trnF-GAA	tRNA-Phenylalanine(GAA)	translation
trnG-GCC	tRNA-Glycine(GCC)	translation
trnG-UCC	tRNA-Glycine(UCC)	translation
trnH-GUG	tRNA-Histidine(GUG)	translation
trnI-CAU	tRNA-Isoleucine(CAU)	translation
trnI-GAU	tRNA-Isoleucine(GAU)	translation
trnK-UUU	tRNA-Lysine(UUU)	translation
trnL-CAA	tRNA-Leucine(CAA)	translation
trnL-UAA	tRNA-Leucine(UAA)	translation
trnL-UAG	tRNA-Leucine(UAG)	translation

trnM-CAU	tRNA-Methionine(CAU)	translation
trnfM-CAU	tRNA-N-Formyl-methionine(CAU)	translation initiation
trnN-GUU	tRNA-Asparagine(GUU)	translation
trnP-UGG	tRNA-Proline(UGG)	translation
trnQ-UUG	tRNA-Glutamine(UUG)	translation
trnR-ACG	tRNA-Arginine(ACG)	translation
trnR-UCU	tRNA-Arginine(UCU)	translation
trnS-GCU	tRNA-Serine(GCU)	translation
trnS-GGA	tRNA-Serine(GGA)	translation
trnS-UGA	tRNA-Serine(UGA)	translation
trnT-GGU	tRNA-Threonine(GGU)	translation
trnT-UGU	tRNA-Threonine(UGU)	translation
trnV-GAC	tRNA-Valine(GAC)	translation
trnV-UAC	tRNA-Valine(UAC)	translation
trnW-CCA	tRNA-Tryptophan(CCA)	translation
trnY-GUA	tRNA-Tyrosine(GUA)	translation
rps2	ribosomal protein S2	translation, small ribosomal subunit
rps3	ribosomal protein S3	translation, small ribosomal subunit
rps4	ribosomal protein S4	translation, small ribosomal subunit
rps7	ribosomal protein S7	translation, small ribosomal subunit

rps8	ribosomal protein S8	translation, small ribosomal subunit
rps11	ribosomal protein S11	translation, small ribosomal subunit
rps12	ribosomal protein S12	translation, small ribosomal subunit
rps14	ribosomal protein S14	translation, small ribosomal subunit
rps15	ribosomal protein S15	translation, small ribosomal subunit
rps16	ribosomal protein S16	translation, small ribosomal subunit
rps18	ribosomal protein S18	translation, small ribosomal subunit
rps19	ribosomal protein S19	translation, small ribosomal subunit
rpl2	ribosomal protein L2	translation, large ribosomal subunit
rpl14	ribosomal protein L14	translation, large ribosomal subunit
rpl16	ribosomal protein L16	translation, large ribosomal subunit
rpl20	ribosomal protein L20	translation, large ribosomal subunit
rpl22	ribosomal protein L22	translation, large ribosomal subunit
rpl23	ribosomal protein L23	translation, large ribosomal subunit, inactive pseudogene in Caryophyllidae
rpl32	ribosomal protein L32	translation, large ribosomal subunit
rpl33	ribosomal protein L33	translation, large ribosomal subunit
rpl36	ribosomal protein L36	translation, large ribosomal subunit
infA	translation initiation factor 1	translation, inactive pseudogene or gene lost (and transferred to the nucleus) in several lineages
clpP	catalytic subunit of the protease Clp	ATP-dependent protein degradation, essential for cell survival

accD	acetyl-CoA carboxylase subunit	fatty acid biosynthesis, essential for cell survival
ycf5 / *ccsA*	subunit A of the system II complex for c-type cytochrome biogenesis	required for heme attachment to chloroplast c-type cytochromes
ycf10	inner envelope protein	presumably involved in the uptake of inorganic carbon
ycf1	putative Ycf1 protein	essential gene, function unknown
ycf2	putative Ycf2 protein	essential gene, function unknown, contains a putative nucleotide-binding domain
ycf15	unknown	ORF with unclear functional significance
sprA	small RNA	function unknown

Table 2. Modes and mechanisms of plastid inheritance.

Mode of inheritance	**Cytological mechanism**	**Examples**
Maternal (Lycopersicon type)	Plastid exclusion from the generative cell during the first haploid pollen mitosis	*Beta vulgaris, Brassica campestris, Gossypium hirsutum, Solanum lycopersicum, Nicotiana alata, Petunia hybrida, Prunus avium, Spinacia oleracea*
Maternal (Solanum type)	Plastid degradation in the generative cell	*Convallaria majalis, Epilobium spec., Fritillaria imperialis, Hosta japonica, Solanum tuberosum*
Maternal (Triticum type)	Plastid exclusion during fertilization	*Hordeum vulgare, Pisum sativum, Triticum aestivum, Zea mays*
Maternal	Selective degradation	*Chlamydomonas reinhardtii*

(Chlamydomonas type)	of the paternal ptDNA after syngamy, fusion of maternal and paternal plastids	
Biparental (Pelargonium type)	Presence of plastids in sperm cells, transmission into the zygote	*Medicago sativa, Oenothera spec., Pelargonium spec., Rhododendron spec., Hypericum spec.*
Paternal	Presence of plastids in sperm cells and transmission into the zygote, disintegration of maternal plastids in the egg cell	*Abies alba, Actinidia deliciosa, Larix decidua, Pinus taeda, Pseudotsuga menziesii, Sequoia sempervirens*

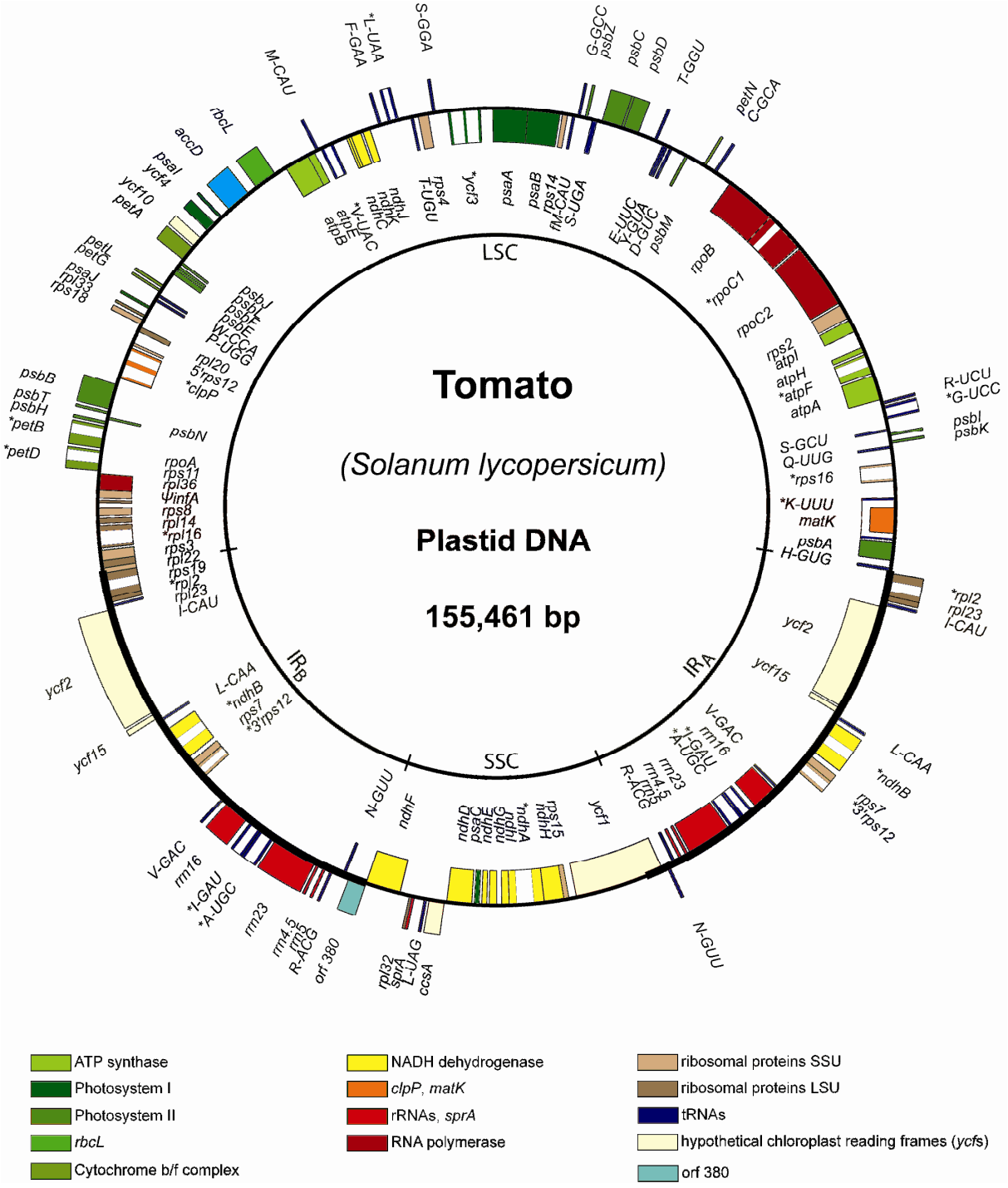
Tomato
(Solanum lycopersicum)
Plastid DNA
155,461 bp
LSC
IRB
SSC
IRA
ATP synthase
Photosystem I
Photosystem II
rbcL
Cytochrome b/f complex
NADH dehydrogenase
clpP, matK
rRNAs, sprA
RNA polymerase
ribosomal proteins SSU
ribosomal proteins LSU
tRNAs
hypothetical chloroplast reading frames (ycfs)
orf 380

Figure 1

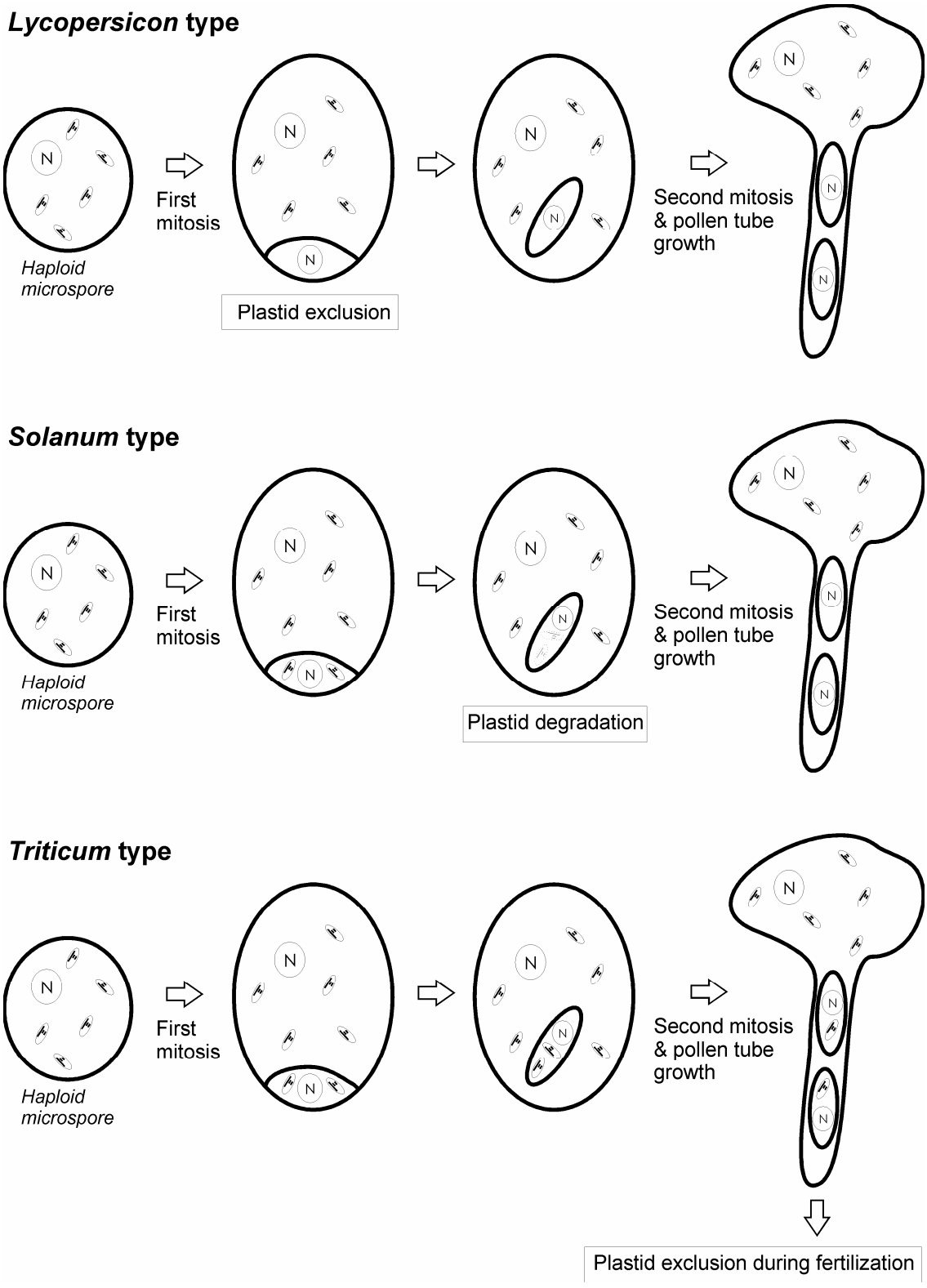
Lycopersicon type
N
Haploid microspore
First mitosis
N
N
Plastid exclusion
N
N
Second mitosis & pollen tube growth
N
N
Solanum type
N
Haploid microspore
First mitosis
N
N
N
N
Plastid degradation
Second mitosis & pollen tube growth
N
N
Triticum type
N
Haploid microspore
First mitosis
N
N
N
N
Second mitosis & pollen tube growth
N
N
Plastid exclusion during fertilization

Figure 2