Cytokinins

Chemistry, Activity, and Function

Edited by

David W. S. Mok, Ph.D.

and

Machteld C. Mok, Ph.D.

Department of Horticulture

and

Center for Gene Research and Biotechnology

Oregon State University

Corvallis, Oregon

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Chapter 14

Plastid Genes and Chloroplast Biogenesis

Ralf Reski

TABLE OF CONTENTS

I. PLASTIDS ARE A HETEROGENOUS ORGANELLE CLASS

Plastids are the DNA-containing organelles with the widest range of different forms and functions. Interconversion between different plastid types implies marked changes in their architecture and physiology. Furthermore, these changes go hand in hand with differentiation processes of cells and organs during plant development (reviewed by Herrmann et al.).[1] Energy, organic substances, and oxygen are provided by photosynthesis, and most of the photosynthetic products are formed in chloroplasts. They also catalyze key reactions in the reductive nitrogen and sulfur pathway, in lipid synthesis, and they synthesize important secondary metabolites. Specialized modifications are leukoplasts, amyloplasts, elaioplasts, and chromoplasts, which are characteristic for different tissues, exhibit marked structural differences, and lack chlorophyll. These organelle forms are involved in various fundamental biological processes, such as carbohydrate or lipid storage and plant propagation. During leaf senescence in angiosperms, chloroplast structures are degraded, temporally forming the last plastid type, gerontoplasts.[2] In vascular plants chloroplast biogenesis from less-differentiated organelle forms occurs: in vegetation tips the differentiation process starts from colorless proplastids. Angiosperms exhibit an additional pathway for chloroplast biogenesis: grown in darkness, proplastids differentiate to pale-yellow etioplasts with characteristic paracrystalline internal structures. Upon illumination, etioplasts develop into chloroplasts. In addition to light, in dicotyledonous angiosperms the biogenesis of chloroplasts is also regulated by intrinsic developmental signals that control leaf differentiation.[3] However, in monocots the processes of primary leaf development and chloroplast development are uncoupled.[4] The common view is that in vascular plants only mesophyll cells house mature chloroplasts, indicating that cell-specific signals are also important for chloroplast biogenesis.[5] Nevertheless, recent findings demonstrate active, but not fully differentiated, chloroplasts even in pith cells.[6] Finally, the developmental stage of the chloroplast itself appears to regulate the expression of nuclear genes coding for plastid proteins.[3]

There are several established experimental systems to study chloroplast biogenesis and correlated cell and organ morphogenesis at the molecular level.

The light-controlled differentiation is studied during greening of etiolated seedlings. Morphogenetic changes occur within a few hours[7] and are thus amenable to molecular analysis. Much work on chloroplast biogenesis in higher plants, particularly on the photocontrol of gene expression, has therefore been carried out with this etioplast/chloroplast transition.[1] However, this system is restricted to angiosperms, and plastid gene regulation during etioplast/chloroplast transition appears to be different from the one during regular proplastid/chloroplast transition.[8]

The *Arabidopsis thaliana det1* mutant develops as a light-grown plant even in darkness. The recessive nuclear mutation affects a variety of light-regulated traits, including leaf development, plastid morphology, and gene expression. Most probably, the wild-type *det1* gene product is a negative regulator in a signal transduction pathway that couples leaf and chloroplast development to light perception. This implies a constitutive expression of light-regulated genes in cell types where these genes are normally silent. In line with this model is the observation that cell cultures of this particular mutant become green even in the absence of cytokinins, conditions where normally no chloroplast biogenesis takes place.[9]

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Leaves of monocots are widely used to study plastid differentiation processes. Cell division occurs only in a basal meristem, resulting in a gradient of cells, with increasing age, from the base to the tip.[10] In a diurnal light regime, a continuous transition of proplastids to chloroplasts occurs in parallel to the basipetal increase in cell age.

In cereals the formation of plastid ribosomes is prevented by growth temperatures above 32°C.[11] The ribosome-deficient plastids resemble proplastids in size and structure. They do not differentiate into chloroplasts, although carotinoids are synthesized, and enzymes for chlorophyll biosynthesis are functioning, within these plastids.[12-14] In contrast to treating plants with inhibitors of protein synthesis, the heat-induced deficiency of plastid ribosomes provides a system for studying interactions between plastids and the cytosol, with minimal risk of toxic side effects or damage by photodestruction (reviewed by Feierabend and Berberich[15]). Ribosome-deficient plastids can also be obtained from the barley nuclear mutant *albostrians,* with the advantage of ruling out side effects of elevated temperatures.[16]

II. PLASTIDS ARE SEMIAUTONOMOUS ORGANELLES

Plant cells are characterized by three different genomes that have their own machinery for gene expression: the prokaryotic ancestors of plastids and mitochondria lost their autonomy by losing most of their genes during the establishment of endosymbiosis.[17] Some of these prokaryotic genes were transferred into the eukaryotic background of the nucleus. The problems arising for a coordinated expression of these genes, and the subsequent transport of their products into the ancestral organelle, are obvious and require the coevolution of three different genomes in one single cell.[1,17] The eukaryon achieved its capability of photosynthesis by a process that started with the engulfment of autonomous prokaryotic cells, continued with the establishment of endosymbiosis between eukaryon and chloroplast, and culminated in the domestication of virile remnants (chloroplasts) to nongreen derivatives (proplastids, amyloplasts, gerontoplasts, chromoplasts, etc.) with specialized functions in higher plants. Thus, the phenomenon of plastid differentiation demonstrates the domestication of the prokaryon by the eukaryon and, finally, the evolutionary aspect of plant development.

The activity of genes involved in this differentiation of plastids has to be under temporal and spatial control. Furthermore, these genes have to respond to multiple extrinsic signals notifying changes in the environment of the plant. Therefore, the role and importance of a specific signal, whether extrinsic or intrinsic, in the complex regulatory network around chloroplast biogenesis and accompanying cell and organ morphogenesis is likely to differ between the major plant groups.

Based on results from the *aurea* mutant[18,19] and the *ghost* mutant[20] of tomato, the *albostrians* mutant[21] and the *albina f17* mutant[22] from barley, and carotinoid-deficient mutants in maize,[23] and from norflurazon treated plants,[24,25] the *consensus* is now that the expression of some light-inducible nuclear genes is modulated by a factor generated by differentiated chloroplasts. This plastidic factor seems to be responsible for the expression of a certain set of nuclear genes: genes coding for proteins that are plastid constituents or that are, at least indirectly, involved in chloroplast biogenesis.[26-31] These observations reveal a common *trans*-acting factor modulating the expression of those nuclear genes that originally were coded by the prokaryotic DNA of the ancestors of chloroplasts. Nevertheless, the nature of the postulated plastidic factor is unknown.[30,32]

A common view about gene regulation during light-induced chloroplast biogenesis is that it is transcriptional for nuclear genes, but translational or posttranslational for plastid genes.[33] Nevertheless, the situation seems to be more complex, as several plastid differentiation processes have been shown to be accompanied not only by changes in general transcriptional activity,[34] but also by changes in the relative transcription rates of individual genes, indicating the existence of gene-specific mechanisms for transcriptional regulation.[1] Examples are the development of proplastids to chloroplasts in *Hordeum* and *Sorghum,*[8,35] light-dependent transformation of mature barley etioplasts to chloroplasts,[36] amyloplast formation in cultured tobacco cells,[37] and chromoplast formation in tomatoes.[38]

Several mechanisms for changes in the transcriptional activity of plastid genes during plastid differentiation have been suggested; changes in the ptDNA copy number per cell can only confer to the nonspecific regulation of plastid genes and do not seem to play a vital role in chloroplast biogenesis.[39] Developmentally related changes in DNA methylation were observed in some studies[38] but not in others.[8,40] Likewise, variations in the conformation of the plastid DNA template, and differences in the three-dimensional structure of plastid nucleoids,[41] have not been unequivocally proven yet. Differences in promoter strength and competition, changes in RNA polymerase to template ratios,[35] and different half-lives of mRNAs may influence transcription rates and cause accumulation rates that differ relative to each other as well.[42,43] However, such

models are based on the hypothesis that all DNA circles in a given plastid are, to the same extent, physiologically active and that the *in vivo* situation is reflected by our *in vitro* data. In addition to these theories are two just emerging models for differential gene regulation.

The first model is that changes in the molecular composition of the transcriptional apparatus itself can cause differences in gene expression and the subsequent plastid differentiation processes. There are two possibilities: either these changes in gene expression can be caused by changes in the populations of several putative RNA-polymerases, or a varying specificity can be conferred, as in prokaryotes, by sigma factors associated with a core RNA-polymerase.

Plastid DNA encodes the *rpo* genes, with all polypeptides resembling the bacterial RNA-polymerase, and these genes are transcribed and translated.[44] Additionally, first evidence is available about sigma-like factors in the plastids of higher plants.[45-47] Nevertheless, plastid extracts contain two fractions with RNA-polymerase activity, in all plants examined so far, whether alga or weed. These fractions differ from each other in biochemical terms (soluble vs. bound, molecular weight, composition) and were reported to differ in their preference to transcribe certain plastid genes, although these reports have given conflicting results.[33,44,48-53] Whether the two fractions indeed contain different enzymes or just different states of one common core enzyme with various auxiliary proteins is still unknown.[54] However, in the last decade there have been hints for a nuclear-encoded plastidic RNA-polymerase.[55-57] Utilizing barley with ribosome-deficient plastids, evidence was solidified for a plastidic RNA-polymerase translated on extraplastidic ribosomes.[15] This nuclear-encoded enzyme seems to be constitutively active in proplastids, chloroplasts, and senescing plastids, whereas the soluble fraction, possibly encoded by the prokaryotic *rpo* genes, develops activity solely in differentiated chloroplasts,[58,59] indicating a differential function of two different RNA-polymerases, at least in barley. Nevertheless, transcript levels of plastid genes coding proteins of the photosynthetic apparatus only accumulate during chloroplast biogenesis, pointing to the importance of posttranscriptional regulation.

The second model is that RNA-editing may be a mechanism involved in the differential regulation of gene expession. Editing was observed with kinetoplast RNA, mitochondrial, and nuclear genes.[60-62] All groups of plastid genes can be affected as well.[63-65] The main function of RNA editing is the creation of an mRNA that can be translated into a biologically active protein either by creating an initiator codon or by restoring codons for conserved amino acids. However, at the moment there are only first hints that differential mRNA editing really regulates gene expression. If so, quantitative Northern blots as well as *in vitro* transcription assays would not necessarily reflect the respective *in vivo* situation. According to our present knowledge, all editing processes in higher plants seem to bring down the peptide sequences to a *consensus* that is represented by the liverwort *Marchantia polymorpha*. This bryophyte is the first plant in which the total nucleotide sequence of ptDNA, as well as mtDNA, has been determined.[66,67] Our analyses of mitochondrial and plastid genes of the moss *Phys-comitrella patens* reveal that moss and liverwort share significant gene homology different from higher plants.[68-72] Thus, RNA-editing of plastid genes might be a regulatory mechanism evolved during the "domestication of chloroplasts."

Analyzing plastid differentiation we find a complex informational network between at least two different genomes in a single cell across several membranes.[1,73] Therefore, we have to dismiss the sedative simplification that each plant species reacts the same way as any other, neglecting the ongoing coevolution of these genomes.[17] For example, the plastid genes of monocots and dicots diverged 100 to 200 million years ago.[74] Why should they use the same dialect in their communication with nuclear genes?

III. PLASTIDS ARE TARGETS FOR CYTOKININ ACTION

Since the pioneering work of Mothes,[75] plastids have seemingly been a primary target of cytokinin action; numerous papers reported on the hormone-stimulated synthesis of chloroplast components during etioplast/chloroplast transition in greening seedlings, cotyledons, or tissue cultures. In detached leaves and in cell culture, both depleted in endogenous cytokinin, exogenously applied hormone stimulates this transition in light and in darkness. In contrast, cytokinins do not influence structural or physiological plastid parameters in intact plants. Likewise, cytokinins retard senescence in detached leaves, including maintenance, or even promote the synthesis of plastid components, but have no effect on senescence retardation of albino barley leaves, or in N-deficient, chlorophyll-free detached leaves of tobacco (reviewed by Parthier[76]).

Cytokinin-induced, as well as light-induced, chloroplast biogenesis is at the molecular level characterized by the accumulation of transcripts from plastid genes. A number of polyadenylated

transcripts exhibit enhanced steady-state levels in plants treated with naturally occurring cytokinins or with their nonphysiological substitutes kinetin or benzyladenine, as well. Interestingly, the hormone seems to act only on a certain set of nuclear genes: genes coding for proteins that are plastid constituents or that are at least indirectly involved in chloroplast biogenesis.[31,77-80] These are the same eukaryotic remnants of prokaryotic ancestors that were suspected to be regulated by a yet-unknown plastidic factor. Nevertheless, this factor is triggering the expression of the *rbc*S and *cab* genes to a much higher extent than the expression of the gene coding chlorophyll synthetase.[81] Furthermore, this factor seems not to interfere with light responsiveness of nuclear genes, at least in monocots.[82]

It is still a controversy whether the enhancement of steady-state transcript levels of cytokinin-responsive genes is due to enhanced transcription rates or posttranscriptional events, although post-transcriptional regulation[83] is favored. Translational control by cytokinins has been described as polyribosome formation,[84] modification of the secondary structure of poly(A$^+$)RNA, which leads to "unmasking" of the message,[85] phosphorylation of ribosomal proteins,[86] or regulation of tRNA activity.[87] Posttranslational control may be conferred by retarded protein degradation.[88] Stimulation of correct RNA editing might be another posttranscriptional mechanism modulated by cytokinin.

Already the early reports of Stetler and Laetsch[89] and Seyer et al.[90] describe a correlation between the two cellular processes promoted by cytokinin: the cell cycle and chloroplast biogenesis. In one case an inverse relationship between chloroplast differentiation and tissue growth was observed (either chloroplasts differentiated or cells divided); in the latter a restriction of cytokinin action on chloroplast biogenesis, to the cell growth phase, was found: cytokinin added during the stationary phase was inactive. A similar correlation was described by Suzuki et al.,[91] who found preferential synthesis of plastid DNA prior to that of nuclear DNA, suggesting that ptDNA is synthesized and stored in large amounts to prepare for the following multiple cell divisions without organelle DNA synthesis. Thus, a communication between genome and plastome does exist, and cytokinin presumably interferes with it.

Repeatedly, a coaction of cytokinin and light, in plastid differentiation and multiplication, has been observed.[76] Even in molecular terms, a coaction of light and cytokinin is detectable.[92-94] Molecular genetic analysis of the regulation of cytokinin-responsive nuclear genes is scarce, since, up to now, no gene expression has been known to be triggered specifically by cytokinin. Thus, the vast amount of data on light-regulated genes[95] has to serve as a guideline for further characterizing the cytokinin stimulus on gene expression. In dicots, the expression of several "photogenes" (e.g., *rbc*S, *rbc*L) is seen only after exposure of the plants to light, leading to the idea that light is required for the expression of these genes. Leaf and chloroplast development also require exposure to light, again suggesting a control mechanism.[4] In monocots the situation is quite different, as primary leaf development and chloroplast biogenesis are uncoupled. Nevertheless, leaf expansion and the expression of several "photogenes" still seem to be coupled; in contrast to dicots, they occur at near-light-grown levels, even in dark-grown plants.[34]

The most extensively studied light-responsive genes are *rbc*S and *cab*. Expression of these genes coding for the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbc*S) and the chlorophyll a/b-binding proteins (*cab*), respectively, is regulated by light at the transcriptional level, by sets of *cis*-acting elements and *trans*-acting factors. Moreover, their expression is dependent on genome–plastome interaction as well as on cytokinin. The light signal is mediated via phytochrome, although the expression of many light-responsive genes is modulated by more than one wavelength, e.g., blue light. In fact, there is increasing evidence that blue light plays an important role in modulating plastid differentiation and the accompanying expression of plastome- as well as genome-coded genes for plastid polypeptides.[96-100] A plastid promoter has been characterized that specifically responds to blue light: the light-responsive promoter (LRP) of the *psb*D-*psb*C operon.[101] While genes such as *rbc*L, *atp*B, and 16 S rRNA are fully active in dark-grown barley, the LRP has evolved a light-regulated specificity unlike other chloroplast promoters. Interestingly, LRP is highly conserved in monocots, less in dicots, and was not found in liverwort chloroplast DNA, indicating differences in light-induced gene expression between the major plant groups. The same indication was given by analysis of the nuclear phytochrome gene of the moss *Ceratodon purpureus*. In contrast to all known phytochromes, the 3′ terminus of *phyCer* codes for a putative polypeptide with striking homology to protein kinases.[102] These results suggest that phytochrome in moss is a light-regulated protein kinase, revealing a simple transduction pathway for the light stimulus. Protein kinases are prime candidates for members of the signal transduction pathway for several stimuli in all kinds of plants; e.g., Kulaeva et al. proposed that cytokinin-binding proteins (CBPs) in barley may have protein kinase activity.[103]

Both light-responsive genes *rbc*S and *cab* are not single-copy genes, but represent multigene families with members possessing individual responsiveness to light in an organ- and developmental-specific manner.[104-108] An interaction between light responsiveness and an endogenous developmental program has also been proven for the expression of plastome-encoded genes.[109,110] Furthermore, Eckes et al. described the organ-specific expression of three cDNA clones from potato, that are regulated by light and are correlated with chloroplast development.

Regulatory circuits are even more complex; in addition to being cytokinin modulated, developmentally programmed, and light responsive, an endogenous oscillator also controls the expression of the multigene families of *cab* and *rbc*S. Thus, mRNA levels of these genes exhibit distinct circadian rhythms,[112,113] as do other light-responsive genes such as those coding for nitrate reductase and phosphoenolpyruvate carboxylase.[114,115] Further extrinsic and intrinsic signals interfering with cytokinin action on chloroplast biogenesis and accompanying gene expression are temperature[116] and auxin.[117]

Bearing these complex parameters in mind, it is small wonder that reports on the molecular genetic analysis of cytokinin action are scarce. However, cytokinins seem to interfere with, or modulate, the cooperation between the two genomes, but the molecular mechanisms of this regulatory circuit are far from being understood. Nevertheless, the use of mutants with altered response to external cytokinin as well as *ipt*-transformants[118,119] should facilitate the molecular analysis of cytokinin action.

IV. THE MOLECULAR ANALYSIS OF CHLOROPLAST DIVISION

Since Schimper, 110 years ago, suggested the continuity of plastids,[120] studies on the number, orientation, and division of chloroplasts have been undertaken to trace the mechanisms that provide for their nearly equal distribution into daughter cells during cytokinesis.[121] It is now generally assumed that the different plastid types can multiply by constriction division.[122-124] Additionally, species- as well as organ-specific correlations between nuclear DNA content and plastid number per cell are well documented.[125] However, genes involved in plastid division have not been identified yet, although they have been known from prokaryotes for nearly 30 years.[126,127] From inhibitor studies it was postulated that these genes are encoded by nuclear DNA in higher plants,[128] an indication supported by a nuclear mutant of *Arabidopsis thaliana*, hampered in chloroplast division.[129] Obviously, these yet-unknown genes are members of the regulatory network around plastid differentiation, woven by nuclear and plastid genes.[29,130,131]

The genome–plastome interaction in chloroplast multiplication can be seen in electron micrographs, as division appears to be a two-step process: (1) it is preceded by events within the plastid, such as the disintegration of starch grains and thylakoid membranes at the prospective constriction site, and is then followed by (2) the occurence of a plastid dividing ring (PD ring) outside the plastid around this constriction. This cytoplasmic PD ring is composed of a bundle of actin-like filaments and can be found around dividing plastids of red, brown, and green algae, mosses and ferns (archegoniates), and gymnosperms. In angiosperms, the plastid dividing ring is a doublet that is composed of an outer ring and an inner ring. In any case plastid division occurs by contraction of the filament bundle (reviewed by Kuroiwa[132]).

We have concentrated our studies on the mode of action of the physiological cytokinin N^6-(Δ^2-isopentenyl)adenine on differentiation of the haploid protonema of the moss *Physcomitrella patens*.[133] Special interest is directed at the mutant PC22, which is defective in chloroplast division, and thus the majority of cells possesses only one chloroplast per cell (Figures 1 and 2). Nevertheless, these macrochloroplasts have a normal internal fine structure (Figure 3). Under white-light conditions, they are severed during cell division by the enlarging cell plate, this being a, so far, unknown mechanism of plastid division. The defect of this particular mutant can be partially compensated for by exogenous cytokinin[134] and by blue-light treatment.[31] Cytokinin-induced chloroplast division is accompanied by a transient accumulation of several plastid polypeptides and a dramatic increase in *rbc*L mRNA levels. Such molecular changes cannot be detected under blue-light conditions with already divided chloroplasts, indicating a coaction of cytokinin and light quality in chloroplast division at the molecular level.[135]

Restriction Fragment Length Polymorphism (RFLP) analyses revealed methylation of a putative regulatory gene (*zfp*A gene[136]) near the *rbc*L in chloroplast mutant PC22,[135] as a possible form of transcriptional control in plastid differentiation. We sequenced the 3′ region of the wild-type *rbc*L and found a tRNA gene that was, so far, only known from the ptDNA of the liverwort *Marchantia*,[68] and an open reading frame (ORF) of 948 bp with homology to the *zfp*A from *Pisum*. The deduced polypeptide of this ORF contains a sequence (–Cys–X2–Cys–X15–Cys–X2–Cys–) that

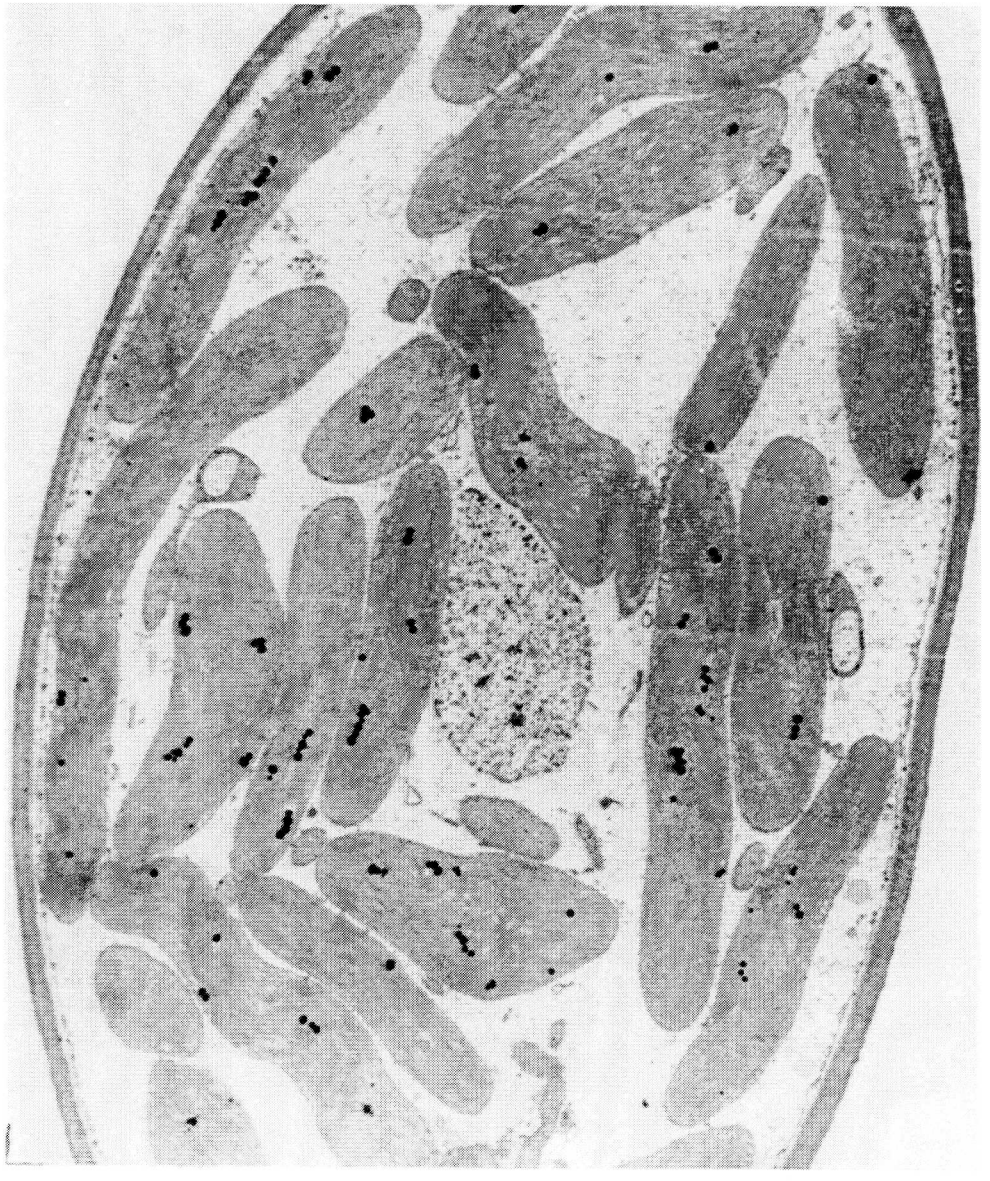

Figure 14-1 Electron micrographs of a cross-section through one protonema cell of (a) the wild type of *Physcomitrella patens* and (b) the chloroplast mutant PC22 derived from it. Note the cup-shaped giant chloroplast in the mutant.

may fold to form a zinc-finger element.[69] These elements can interact with nucleic acids, thereby revealing a regulatory function of the respective polypeptide. However, the function of this protein is still unknown and is a subject of debate.[137,138] Nevertheless, sequence comparison revealed that this protein contains two distinct domains: the hydrophilic zinc-finger element and a hydrophobic C-terminal region.[69] Moreover, *zfp*A may be a marker gene for plastid evolution, as it is highly homologous within the archegoniates and dicots, respectively, but differs significantly between the two groups.[139] Furthermore, it is mutilated in the plastid DNA of monocots. In the moss we found

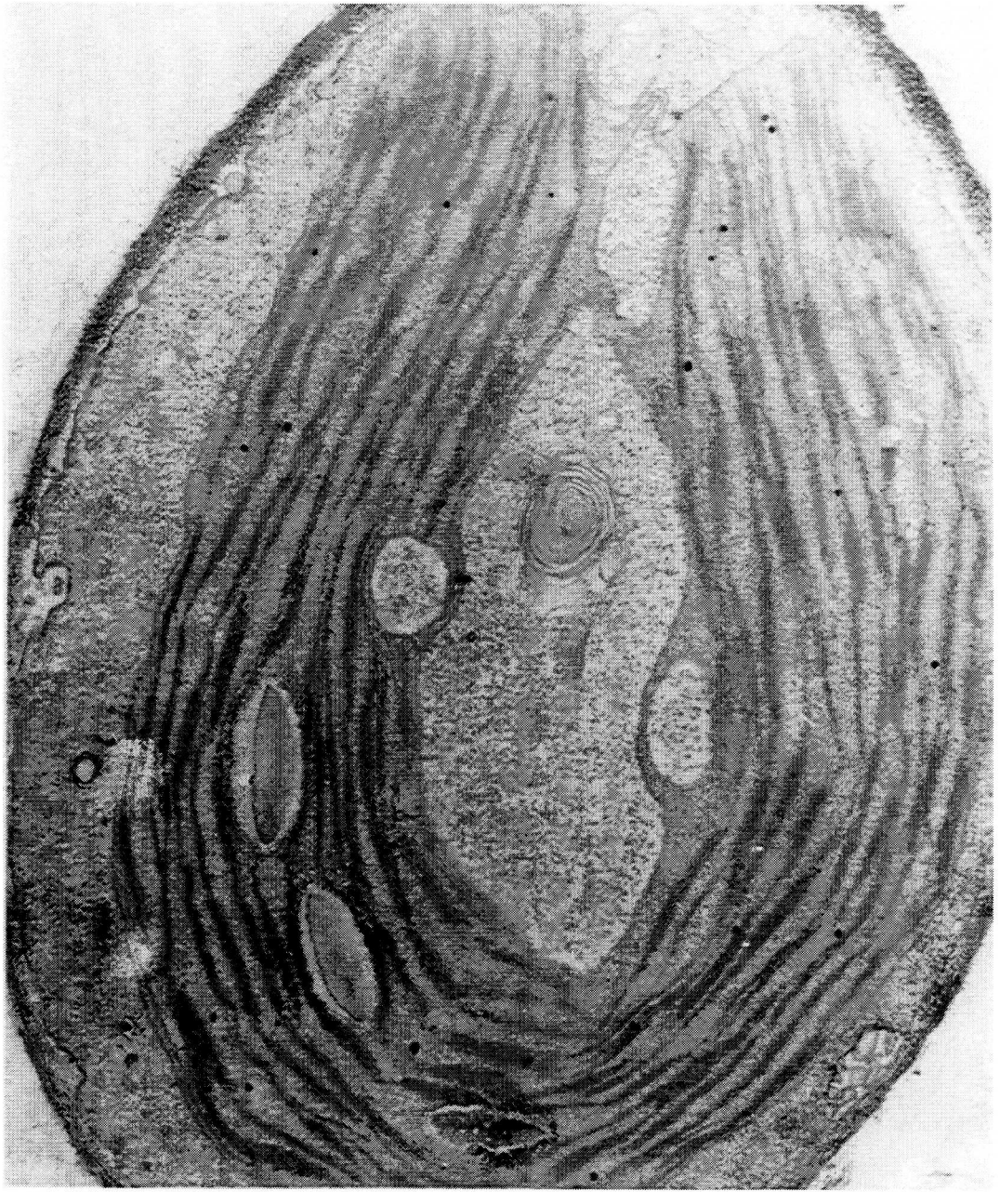

Figure 14-1 (continued).

prokaryotic promoter consensus sequences (-10, -35, Shine Dalgarno) as well as three TATA boxes that are known to be typical eukaryotic promoter sequences. Additionally, these elements are involved in the differential control of the *Sinapis alba* plastid *psb*A gene, depending on the state of plastid differentiation.[140] However, a multiplication of TATA boxes has not previously been reported for plastid promoters and reveals a complex mechanism of transcriptional control. A gene-specific hybridization probe detected three transcripts of low abundance with apparent molecular weights larger than the gene. RNA accumulation patterns differed between wild-type and chloroplast mutants. Additionally, under the influence of cytokinin, the high-molecular-weight transcript of the mutant decreased in amount (Figure 4). Using riboprobes we ensured that these transcripts were

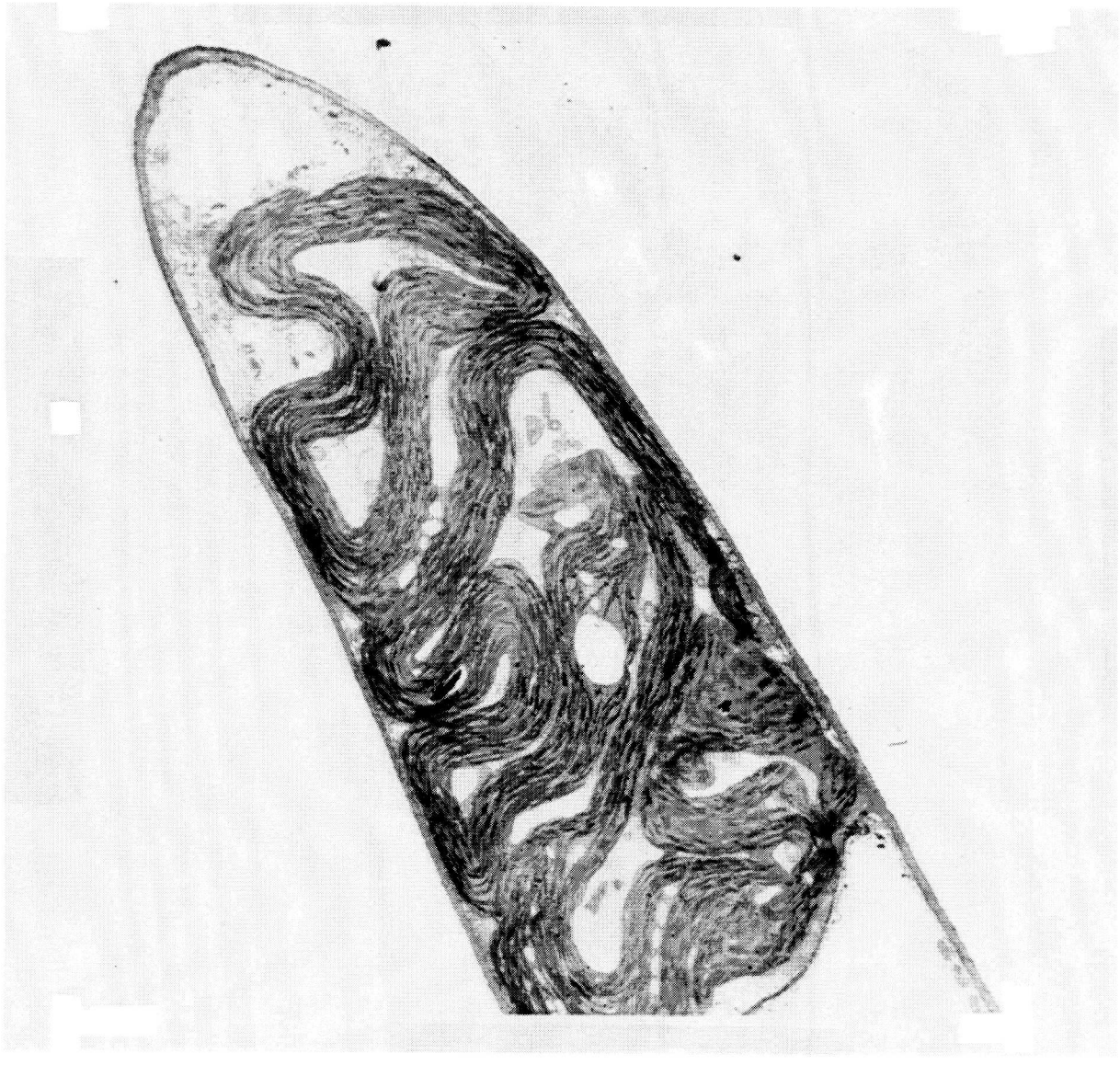

Figure 14-2 Electron micrograph of a longitudinal section through a PC22 protonema tip cell with one giant chloroplast.

encoded by the same ptDNA strand, thus being immature transcripts including *zfp*A sequences.

We performed Northern blots for several plastid genes with the *Physcomitrella* wild type, and two cytokinin-sensitive mutants derived from it (PC22 and P24), P24 not obviously being a chloroplast mutant. In every case we found an accumulation of immature plastid transcripts in both mutants (e.g., Figure 5). The amounts of high-molecular-weight transcripts were less in the wild type, as well as in mutant protonemata treated with cytokinin.[141] Reports on qualitative changes in the RNA processing patterns of plastid genes are infrequent, although most of them are organized in polycistronic transcription units, as in the ancestral bacterial operons.[142-146] Evidence came from light-induced chloroplast development in mustard,[109] maize,[147] and barley.[148] Likewise, in an *Oenothera* plastome, mutant processing of plastid proteins, whether encoded by ptDNA or by nucDNA, was affected.[149] Thus, processing of mRNAs and proteins might be one way for cytokinins to modulate plastid gene expression posttranscriptionally.

Obviously, Western analyses will need to be employed to prove changes in zfp A-protein amounts upon cytokinin treatment of the moss mutants, in

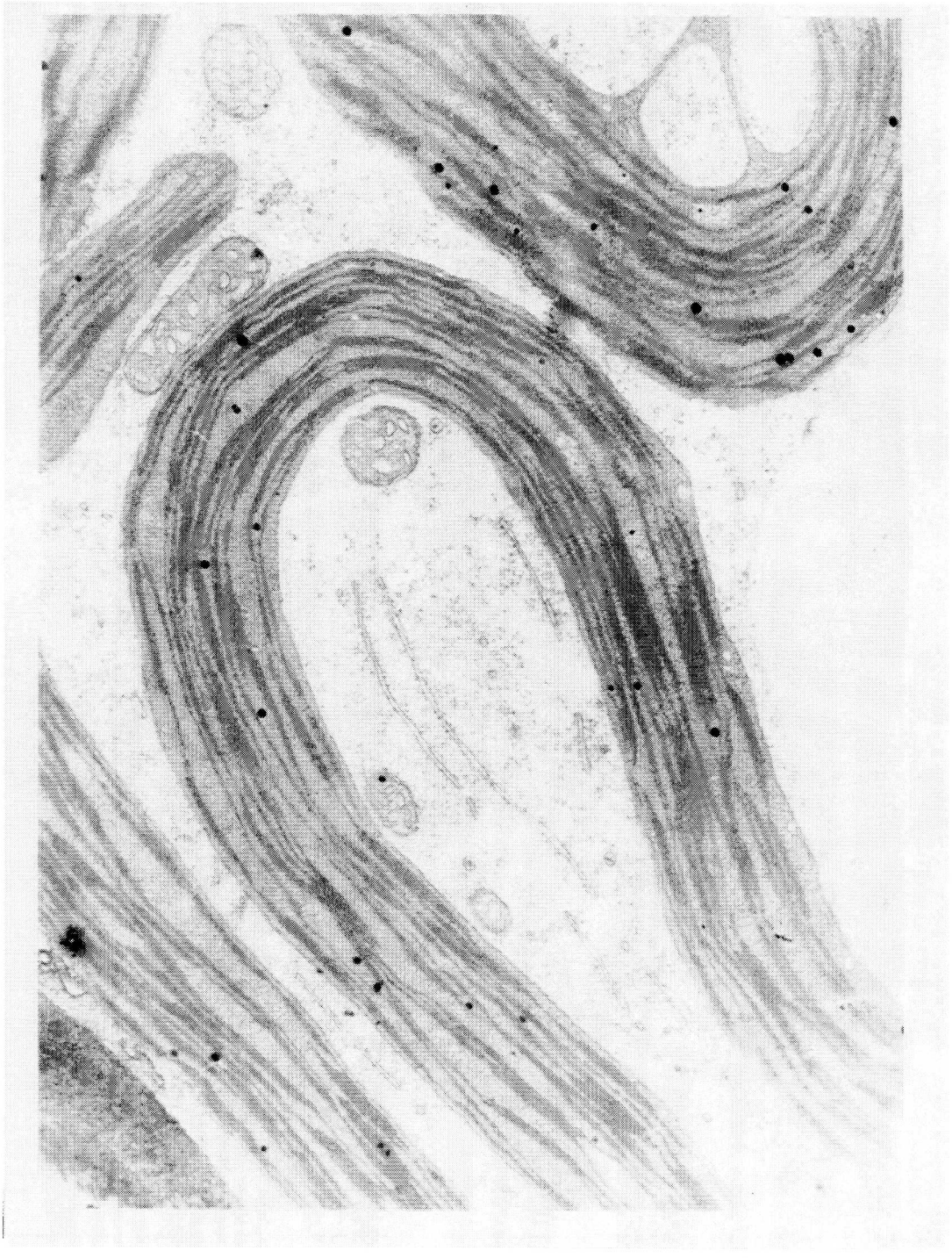

Figure 14-3 Electron micrograph of a PC22 protonema cell showing a normal internal fine structure of the mutant chloroplast.

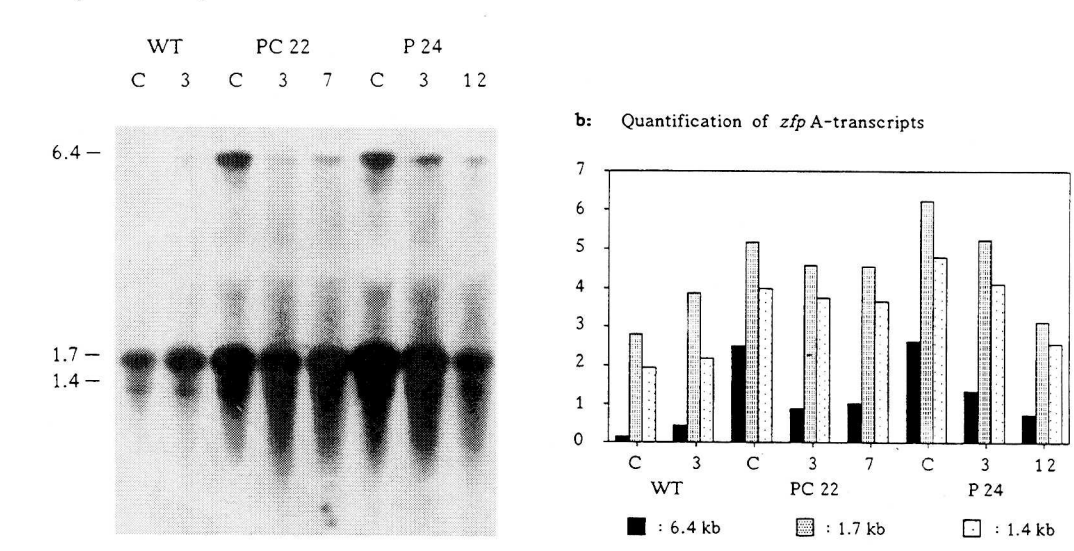

Figure 14-4 Northern analysis of *Physcomitrella zfp*A transcripts. [WT: wild-type; PC22: cytokinin-sensitive chloroplast mutant; P24: cytokinin-sensitive mutant; C: protonemata grown 12 days without cytokinin; 3: protonemata after 9 days of culture with additional 3 days in cytokinin; 7: protonemata after 5 days of culture with additional 7 days in cytokinin; 12: protonemata after 12 days in cytokinin (compare to References 135 and 154).] (a:) Northern blot using a radioactive *zfp*A-specific gene probe. (b:) Densitometric quantification of the autoradiogram shown in (a). (Unit: pixel × 1000. Transcript sizes are given in kilobases.)

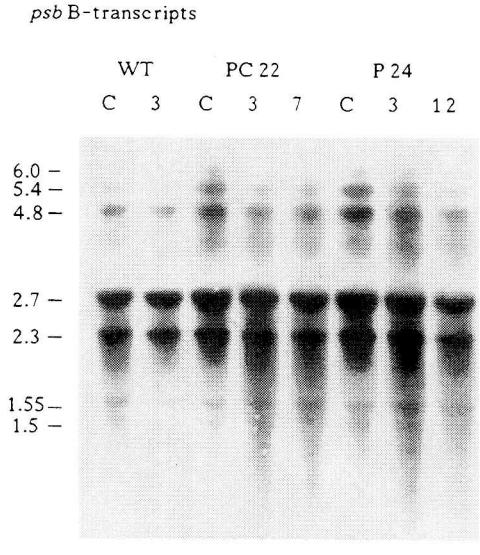

Figure 14-5 Northern analysis of *Physcomitrella psb*B transcripts. [WT: wild-type; PC22: cytokinin-sensitive chloroplast mutant; P24: cytokinin-sensitive mutant; C: protonemata grown 12 days without cytokinin; 3: protonemata after 9 days of culture with additional 3 days in cytokinin; 7: protonemata after 5 days of culture with additional 7 days in cytokinin; 12: protonemata after 12 days in cytokinin (compare to References 135 and 154).] (Transcript sizes are given in kilobases.)

order to correlate them to the proposed enhancing effect on transcript processing. Furthermore, the postulated interaction of zfpA protein with nucleic acids has to be proven. However, we do not yet know if the mutation affecting plastid division in this particular moss mutant is located on nucDNA or on ptDNA.[150] Furthermore, most of the data on plastid gene regulation during chloroplast development result from *in vitro* studies, and there is a need for plastid transformation systems to study gene regulation *in vivo*. First reports on the successful transformation of the giant chloroplast of *Chlamydomonas,*[151] as well as of higher-plant chloroplasts,[152,153] demonstrate the feasibility of such an approach. The macrochloroplast of PC22 seems to be a prime candidate for chloroplast transformation

of multicellular plants, as it is amenable for microinjection.[134] Thus, we will try to unravel the informational network around cytokinin-induced chloroplast division, with mutant curing experiments. Furthermore, we will attempt to transform moss mutants with *ipt* constructs, in order to manipulate endogenous cytokinin concentrations.

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REFERENCES

1. **Herrmann, R. G., Westhoff, P., and Link, G.,** Biogenesis of plastids in higher plants, in *Plant Gene Research, Cell Organelles*, Herrmann, R. G., Ed., Springer-Verlag, New York, 1992, 275.
2. **Becker, W. and Apel, K.,** Differences in gene expression between natural and artificially induced leaf senescence, *Planta*, 189, 74, 1993.
3. **Mullet, J. E.,** Chloroplast development and gene expression, *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 39, 475, 1988.
4. **Dale, J. E.,** The control of leaf expansion, *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 39, 276, 1988.
5. **Dean, C. and Leech, R. M.,** Genome expression during normal leaf development, *Plant Physiol.*, 69, 904, 1982.
6. **Van Cleve, B., Forreiter, C., Sauter, J. J., and Apel, K.,** Pith cells of poplar contain photosynthetically active chloroplasts, *Planta*, 189, 70, 1993.
7. **Tobin, E. M. and Silverthorne, J.,** Light regulation of gene expression in higher plants, *Ann. Rev. Plant Physiol.*, 36, 569, 1985.
8. **Krupinska, K.,** Transcriptional control of plastid gene expression during development of primary foliage leaves of barley grown under a daily light-dark regime, *Planta*, 186, 294, 1992.
9. **Chory, J., Peto, C., Feinbaum, R., Pratt, L., and Ausubel, F.,** *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light, *Cell*, 58, 991, 1989.
10. **Boffey, S. A. and Leech, R. M.,** Chloroplast DNA levels and the control of chloroplast division in light-grown wheat leaves, *Plant Physiol.*, 69, 1387, 1982.
11. **Feierabend, J. and Mikus, M.,** Occurrence of a high temperature sensitivity of chloroplast ribosome formation in several higher plants, *Plant Physiol.*, 59, 863, 1977.
12. **Feierabend, J. and Schrader-Reichhardt, U.,** Biochemical differentiation of plastids and other organelles in rye leaves with a high-temperature-induced deficiency of plastid ribosomes, *Planta*, 129, 133, 1976.
13. **Rademacher, E. and Feierabend, J.,** Formation of chloroplast pigments and sterols in rye leaves deficient in plastid ribosomes, *Planta*, 129, 147, 1976.
14. **Hess, W. R., Schendel, R., Rüdiger, W., Fieder, B., and Börner, T.,** Components of chlorophyll biosynthesis in a barley *albina* mutant unable to synthesize δ-aminolevulinic acid by utilizing the transfer RNA for glutamic acid, *Planta*, 188, 19, 1992.
15. **Feierabend, J. and Berberich, T.,** Heat-induced ribosome-deficiency of plastids — mechanisms and applications, in *The Translational Apparatus of Photosynthetic Organelles*, Mache, R. and Subramanian, A. R., Eds., NATO ASI Series Vol. H55, Berlin, 1991, 215.
16. **Börner, T., Manteuffel, R., and Wellburn, A. R.,** Enzymes of plastid ribosome-deficient mutants. Chloroplast ATPase (CF1), *Protoplasma*, 98, 153, 1979.
17. **Gray, M. W.,** The endosymbiont hypothesis revisited, *Int. Rev. Cytol.*, 141, 233, 1992.
18. **Parks, B. M., Jones, A. M., Adamse, P., Korneef, M., Kendrick, R. E., and Quail, P. H.,** The *aurea* mutant of tomato is deficient in spectrophotometrically and immunochemically detectable phytochrome, *Plant Mol. Biol.*, 9, 97, 1987.
19. **Sharrock, R. A., Parks, B. M., Korneef, M., and Quail, P. H.,** Molecular analysis of the phytochrome deficiency in an *aurea* mutant of tomato, *Mol. Gen. Genet.*, 213, 9, 1988.
20. **Giuliano, G. and Scolnik, P. A.,** Transcription of two photosynthesis-associated nuclear gene families correlates with the presence of chloroplasts in leaves of the variegated tomato *ghost* mutant, *Plant Physiol.*, 86, 7, 1988.
21. **Hess, W. R., Schendel, R., Börner, T., and Rüdiger, W.,** Reduction of mRNA level for two nuclear encoded light regulated genes in the barley mutant *albostrians* is not correlated with phytochrome content and activity, *J. Plant Physiol.*, 138, 292, 1991.

22. **Batschauer, A., Mösinger, E., Krenz, K., Dörr, I., and Apel, K.,** The implication of a plastid-derived factor in the transcriptional control of nuclear genes encoding the light-harvesting chlorophyll a/b binding protein, *Eur. J. Biochem.*, 154, 625, 1986.
23. **Burgess, D. G. and Taylor, W. C.,** The chloroplast affects the transcription of a nuclear gene family, *Mol. Gen. Genet.*, 214, 89, 1988.
24. **Burgess, D. G. and Taylor, W. C.,** Chloroplast photooxidation affects the accumulation of cytosolic messenger RNA encoding chloroplast proteins in maize, *Planta*, 170, 520, 1987.
25. **Oelmüller, R., Schuster, C., and Mohr, H.,** Physiological characterization of a plastidic signal required for nitrate-induced appearance of nitrate and nitrite reductases, *Planta*, 174, 75, 1988.
26. **Stockhaus, J., Schell, J., and Willmitzer, L.,** Correlation of the expression of the nuclear photosynthetic gene ST-LS1 with the presence of chloroplasts, *EMBO J.*, 8, 2445, 1989.
27. **McHale, N. A., Kawata, E. E., and Cheung, A. Y.,** Plastid disruption in a thiamine requiring mutant of *Nicotiana sylvestris* blocks accumulation of specific nuclear and plastid mRNAs, *Mol. Gen. Genet.*, 221, 203, 1990.
28. **Börner, T.,** Chloroplast control of nuclear gene function, *Endocytobios, Cell Res.*, 3, 265, 1986.
29. **Taylor, W. C.,** Regulatory interactions between nuclear and plastid genomes, *Ann. Rev. Plant Physiol.*, 40, 211, 1989.
30. **Oelmüller, R.,** Photooxidative destruction of chloroplasts and its effect on nuclear gene expression and extraplastidic enzyme levels, *Photochem. Photobiol.*, 49, 229, 1989.
31. **Reski, R. and Abel, W. O.,** Analyzing cytokinins and plastid differentiation at the molecular level: interference with genome/plastome interaction, light quality, developmental state and endogenous oscillators (mini-review), in *Physiology and Biochemistry of Cytokinins in Plants*, Kamínek, M., Mok, D. W. S., and Zazímalová, E., Eds., SPB Academic Publishing, The Hague, 1992, 255.
32. **Susek, R. E. and Chory, J.,** A tale of two genomes: role of a chloroplast signal in coordinating nuclear and plastid genome expression, *Aust. J. Plant Physiol.*, 19, 387, 1992.
33. **Gruissem, W.,** Chloroplast gene expression: how plants turn their plastids on, *Cell*, 56, 161, 1989.
34. **Baumgartner, B. J., Rapp, J. C., and Mullet, J. E.,** Plastid transcription activity and DNA copy number increase early in barley chloroplast development, *Plant Physiol.*, 89, 1011, 1989.
35. **Schrubar, H., Wanner, G., and Westhoff, P.,** Transcriptional control of plastid gene expression in greening *Sorghum* seedlings, *Planta*, 183, 101, 1990.
36. **Klein, R. R. and Mullet, J. E.,** Light-induced transcription of chloroplast genes. *psb*A transcription is differentially enhanced in illuminated barley, *J. Biol. Chem.*, 265, 1895, 1990.
37. **Sakai, A., Kawano, S., and Kuriowa, T.,** Conversion of proplastids to amyloplasts in tobacco cultured cells is accompanied by changes in the transcriptional activities of plastid genes, *Plant Physiol.*, 100, 1062, 1992.
38. **Ngernprasirtsiri, J., Kobayashi, H., and Akazawa, T.,** DNA methylation as a mechanism of transcriptional regulation in nonphotosynthetic plastids in plant cells, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 4750, 1988.
39. **Deng, X. W. and Gruissem, W.,** Control of plastid gene expression during development: the limited role of transcriptional regulation, *Cell*, 49, 379, 1987.
40. **Marano, M. R. and Carillo, N.,** Chromoplast formation during tomato fruit ripening. No evidence for plastid DNA methylation, *Plant Mol. Biol.*, 16, 11, 1991.
41. **Zaitlin, D., Hu, J., and Bogorad, L.,** Binding and transcription of relaxed DNA templates by fractions of maize chloroplast extracts, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 876, 1989.
42. **Harpster, M. and Apel, K.,** The light-dependent regulation of gene expression during plastid development in higher plants, *Physiol. Plant.*, 64, 147, 1985.
43. **Deng, X. W., Tonkyn, J. C., Peter, G. F., Thornber, J. P., and Gruissem, W.,** Post-transcriptional control of plastid mRNA accumulation during adaptation of chloroplasts to different light quality environments, *Plant Cell*, 1, 645, 1989.
44. **Hu, J. and Bogorad, L.,** The chloroplast RNA polymerase: the 180-, 120-, and 38-kilodalton polypeptides are encoded in chloroplast genes, *Proc. Natl. Acad. Sci. U.S.A.*, 87, 1531, 1990.
45. **Jolly, S. O. and Bogorad, L.,** Preferential transcription of cloned maize chloroplast DNA sequences by maize chloroplast RNA polymerase, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 822, 1980.

46. **Lerbs, S., Bräutigam, E., and Mache, R.,** DNA-dependent RNA polymerase of spinach chloroplasts: characterization of α-like and τ-like polypeptides, *Mol. Gen. Genet.*, 211, 459, 1988.
47. **Tiller, K., Eisermann, A., and Link, G.,** The chloroplast transcription apparatus from mustard (*Sinapis alba* L.). Evidence for three different transcription factors which resemble bacterial sigma factors, *Eur. J. Biochem.*, 198, 93, 1991.
48. **Hallick, R. B., Lipper, C., Richards, O. C., and Rutter, W. J.,** Isolation of transcriptionally active chromosome from chloroplasts of *Euglena gracilis*, *Biochemistry*, 15, 3039, 1976.
49. **Greenberg, B. M., Narita, J. O., DeLuca-Flaherty, C., Gruissem, W., Rushlow, K. A., and Hallick, R. B.,** Evidence for two RNA polymerase activities in *Euglena gracilis* chloroplasts, *J. Biol. Chem.*, 259, 14880, 1984.
50. **Narita, J. O., Rushlow, K. E., and Hallick, R. B.,** Characterization of a *Euglena gracilis* chloroplast RNA polymerase specific for ribosomal genes, *J. Biol. Chem.*, 260, 11194, 1985.
51. **Reiss, T. and Link, G.,** Characterization of transcriptionally active DNA-protein complexes from chloroplasts and etioplasts of mustard (*Sinapis alba* L.), *Eur. J. Biochem.*, 148, 207, 1985.
52. **Gruissem, W., Greenberg, B. M., Zurawski, G., and Hallick, R. B.,** Chloroplast gene expression and promoter identification in chloroplast extracts, *Methods Enzymol.*, 118, 253, 1986.
53. **Richter, G. and Ottersbach, N.,** Blue-light-dependent chloroplast differentiation in cultured plant cells: evidence for transcriptional control of plastid genes, *Bot. Acta*, 103, 168, 1990.
54. **Igloi, G. L. and Kössel, H.,** The transcription apparatus of chloroplasts, *Crit. Rev. Plant Sci.*, 10, 525, 1992.
55. **Bünger, W. and Feierabend, J.,** Capacity for RNA synthesis in 70 S ribosome deficient plastids of heat-bleached rye leaves, *Planta*, 149, 163, 1980.
56. **Siemenroth, A., Wollgiehn, R., Neumann, D., and Börner, T.,** Synthesis of ribosomal RNA in ribosome-deficient plastids of the mutant "albostrians" of *Hordeum vulgare* L., *Planta*, 153, 547, 1981.
57. **Lerbs, S., Bräutigam, E., and Parthier, B.,** Polypeptides of DNA-dependent RNA polymerase of spinach chloroplasts: characterization by antibody-linked polymerase assay and determination of sites of synthesis, *EMBO J.*, 4, 1661, 1985.
58. **Falk, J., Schmidt, A., and Krupinska, K.,** Characterization of plastid DNA transcription in ribosome deficient plastids of heat-bleached barley leaves, *J. Plant Physiol.*, 141, 176, 1993.
59. **Hess, W. R., Prombona, A., Fieder, B., Subramanian, A. R., and Börner, T.,** Chloroplast *rps*15 and the *rpo*B/C1/C2 gene cluster are strongly transcribed in ribosome-deficient plastids: evidence for a functioning non-chloroplast-encoded RNA polymerase, *EMBO J.*, 12, 563, 1993.
60. **Benne, R.,** RNA editing in trypanosomes: is there a message? *Trends Genet.*, 6, 177, 1990.
61. **Hiesel, R., Wissinger, B., Schuster, W., and Brennicke, A.,** RNA editing in plant mitochondria, *Science*, 246, 1632, 1989.
62. **Weissmann, C., Cattaneo, R., and Billeter, M. A.,** Sometimes an editor makes sense, *Nature*, 343, 697, 1990.
63. **Hoch, B., Maier, R. M., Appel, K., Igloi, G. L., and Kössel, H.,** Editing of a chloroplast mRNA by creation of an initiation codon, *Nature*, 353, 178, 1991.
64. **Kudla, J., Igloi, G. L., Metzlaff, M., Hagemann, R., and Kössel, H.,** RNA editing in tobacco chloroplasts leads to the formation of a translatable *psb*L mRNA by a C to U substitution within the initiation codon, *EMBO J.*, 11, 1099, 1992.
65. **Maier, R. M., Hoch, B., Zeltz, P., and Kössel, H.,** Internal editing of the maize chloroplast *ndhA* transcript restores codons for conserved amino acids, *Plant Cell*, 4, 609, 1992.
66. **Ohyama, K., Fukuzawa, H., Kohchi, T., Shrai, H., Sano, T., Sano, S., Shiri, H., Umesono, K., Shiki, Y., Takeuchi, M., Chang, Z., Aota, S., Inokuchi, H., and Ozeki, H.,** Chloroplast gene organization deduced from complete sequence of liverwort *Marchantia polymorpha* chloroplast DNA, *Nature*, 322, 572, 1986.
67. **Oda, K., Yamato, K., Ohta, E., Nakamura, Y., Takemura, M., Nozato, N., Akashi, K., Kanegae, T., Ogura, Y., Kohchi, T., and Ohyama, K.,** Gene organization deduced from the complete sequence of liverwort *Marchantia polymorpha* mitochondrial DNA. Primitive form of plant mitochondrial genome, *J. Mol. Biol.*, 223, 1, 1992.
68. **Kasten, B., Wehe, M., Reski, R., and Abel, W. O.,** *trnR-CCG* is not unique to the plastid DNA of the liverwort *Marchantia*: gene identification from the moss *Physcomitrella patens*, *Nucleic Acids Res.*, 19, 5074, 1991.

69. **Kasten, B., Wehe, M., Kruse, S., Reutter, K., Abel, W. O., and Reski, R.,** The plastome-encoded *zfp*A gene of a moss contains prokaryotic as well as eukaryotic promoter consensus sequences and its RNA abundance is modulated by cytokinin, *Curr. Genet.*, 22, 327, 1992.
70. **Marienfeld, J. R., Reski, R., and Abel, W. O.,** The first analysed archegoniate mitochondrial gene (*cox*3) exhibits extraordinary features, *Curr. Genet.*, 20, 319, 1991.
71. **Marienfeld, J. R., Reski, R., and Abel, W. O.,** Chondriom analysis of the moss *Physcomitrella patens*, *Crypt. Bot.*, 3, 23, 1992.
72. **Reutter, K., Wehe, M., Kasten, B., Abel, W. O., and Reski, R.,** Cloning plastid DNA of the moss *Physcomitrella patens, Mitt. Inst. Allg. Bot. Hamburg*, 24, 35, 1992.
73. **Keegstra, K. and von Heijne, G.,** Transport of proteins into chloroplasts, in *Plant Gene Research. Cell Organelles*, Herrmann, R. G., Ed., Springer-Verlag, New York, 1992, 353.
74. **Wolfe, K. H., Gouy, M., Yang, Y.-W., Sharp, P. M., and Li, W.-H.,** Data of the monocot-dicot divergence estimated from chloroplast DNA sequence data, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 6201, 1989.
75. **Mothes, K.,** Über das Altern der Blätter und die Möglichkeit ihrer Wiederverjüngung, *Naturwissenschaften*, 47, 337, 1960.
76. **Parthier, B.,** The role of phytohormones (cytokinins) in chloroplast development, *Biochem. Physiol. Pflanzen*, 174, 173, 1979.
77. **Seyer, P. and Lescure, A.-M.,** Evidence for changes in plastid mRNA populations during cytokinin-induced chloroplast differentiation in tobacco cell suspensions, *Plant Sci. Lett.*, 36, 59, 1984.
78. **Lerbs, S., Lerbs, W., Klyachko, N. L., Romanko, E. G., Kulaeva, O. N., Wollgiehn, R., and Parthier, B.,** Gene expression in cytokinin- and light-mediated plastogenesis of *Cucurbita* cotelydons: ribulose-1,5-bisphosphate carboxylase/oxygenase, *Planta*, 162, 289, 1984.
79. **Teyssendier de la Serve, B., Axelos, M., and Péaud-Lenoël, C.,** Cytokinins modulate the expression of genes encoding the protein of the light harvesting chlorophyll a/b complex, *Plant Mol. Biol.*, 5, 155, 1985.
80. **Parthier, B.,** Hormone-induced alterations in plant gene expression, *Biochem Physiol. Pflanzen*, 185, 289, 1989.
81. **Hess, W. R., Blank-Huber, M., Fieder, B., Börner, T., and Rüdiger, W.,** Chlorophyll synthetase and chloroplast $tRNA^{glu}$ are present in heat-bleached, ribosome-deficient plastids, *J. Plant Physiol.*, 139, 427, 1992.
82. **Börner, T. and Hess, W. R.,** Altered nuclear, mitochondrial and plastid gene expression in white barley cells containing ribosome-deficient plastids, in *Plant Mitochondria*, Brennicke, A. and Kück, U., Eds., VCI, Mannheim, 1993, in press.
83. **Flores, S. and Tobin, E.M.,** Cytokinin modulation of LHCP mRNA levels: the involvement of post-transcriptional regulation, *Plant Mol. Biol.*, 11, 409, 1988.
84. **Ohya, T. and Suzuki, H.,** Cytokinin-promoted polyribosome formation in excised cucumber cotyledons, *J. Plant Physiol.*, 133, 295, 1988.
85. **Jackowski, G., Jarmolowski, A., and Szweykowska, A.,** Kinetin modifies the secondary structure of poly(A)RNA in cucumber cotyledons, *Plant Sci.*, 52, 67, 1987.
86. **Yakovleva, L. A., Klueva, N. Y., and Kulaeva, O. N.,** Phosphorylation of ribosome proteins as a mechanism of phytohormone regulation of protein synthesis in plants, in *Physiology and Biochemistry of Cytokinins in Plants*, Kamínek, M., Mok, D. W. S., and Zazímalová, E., Eds., SPB Academic Publishing, The Hague, 1992, 169.
87. **Romanov, G. A.,** Cytokinins and tRNAs: a new insight into the old problem, *Sov. Plant Physiol.*, 37, 1196, 1990.
88. **Lamattina, L., Anchoverri, V., Conde, R. D., and Lezica, R. P.,** Quantification of the kinetin effect on protein synthesis and degradation in senescing wheat leaves, *Plant Physiol.*, 83, 497, 1987.
89. **Stetler, D. and Laetsch, W. M.,** Kinetin-induced chloroplast maturation in cultures of tobacco tissue, *Science*, 149, 1387, 1965.
90. **Seyer, P., Marty, D., Lescure, A. M., and Péaud-Lenoël, C.,** Effect of cytokinin on chloroplast cyclic differentiation in cultured tobacco cells, *Cell Differentiation*, 4, 187, 1975.
91. **Suzuki, T., Kawano, S., Sakai, A., Fujie, M., Kuroiwa, H., Nakamura, H., and Kuroiwa, T.,** Preferential mitochondrial and plastid DNA synthesis before multiple cell divisions in *Nicotiana tabacum, J. Cell Sci.*, 103, 831, 1992.
92. **Cotton, J. L. S., Ross, C. W., Byrne, D. H., and Colbert, J. T.,** Down-regulation of phytochrome mRNA abundance by red light and benzyladenine in etiolated cucumber cotyledons, *Plant Mol. Biol.* 14, 707, 1990.

93. **Jayabaskaran, C., Kuntz, M., Guillemaut, P., and Weil, J.-H.,** Variations in the levels of chloroplast tRNAs and aminoacyl-tRNA synthetase in senescing leaves of *Phaseolus vulgaris, Plant Physiol.*, 92, 136, 1990.
94. **Lu, J. L., Ertl, J. R., and Chen, C.-M.,** Cytokinin enhancement of the light induction of nitrate reductase transcript levels in etiolated barley leaves, *Plant Mol. Biol.*, 14, 585, 1990.
95. **Gilmartin, P. M., Sarokin, L., Memelink, J., and Chua, N.-H.,** Molecular light switches for plant genes, *Plant Cell*, 2, 369, 1990.
96. **Fluhr, R. and Chua, N.-H.,** Developmental regulation of two genes encoding ribulose-bisphosphate carboxylase small subunit in pea and transgenic tobacco plants: phytochrome response and blue-light induction, *Proc. Natl. Acad. Sci. U.S.A.*, 83, 2358, 1986.
97. **Richter, G., Dudel, A., Einspanier, R., Dannhauer, I., and Hüsemann, W.,** Blue-light control of mRNA level and transcription during chloroplast differentiation in photomixotrophic and photoautotrophic cell cultures (*Chenopodium rubrum* L.), *Planta*, 172, 79, 1987.
98. **Gamble, P. E. and Mullet, J. E.,** Blue light regulates the accumulation of two *psb*D-*psb*C transcripts in barley chloroplasts, *EMBO J.*, 8, 2785, 1989.
99. **Marrs, K. A. and Kaufman, L. S.,** Blue-light regulation of transcription in nuclear-encoded genes in pea, *Proc. Natl. Acad. Sci. U.S.A.*, 86, 4492, 1989.
100. **Oelmüller, R., Kendrick, R. E., and Briggs, W. R.,** Blue-light mediated accumulation of nuclear-encoded transcripts coding for proteins of the thylakoid membrane is absent in the labile phytochrome deficient *aurea* mutant of tomato, *Plant Mol. Biol.*, 13, 223, 1989.
101. **Christopher, D. A., Kim, M., and Mullet, J. E.,** A novel light-regulated promoter is conserved in cereal and dicot chloroplasts, *Plant Cell*, 4, 785, 1992.
102. **Algarra, P., Lindner, S., and Thümmler, F.,** Biochemical evidence that phytochrome of the moss *Ceratodon purpureus* is a light-regulated protein kinase, *FEBS Lett.*, 315, 69, 1993.
103. **Kulaeva, O. N., Novikova, G. V., Selivankina, Y., Karavaiko, N. N., and Moshkov, I. E.,** Protein kinases and cytokinin-binding proteins in plant cell response to cytokinin, in *Physiology and Biochemistry of Cytokinins in Plants*, Kamínek, M., Mok, D. W. S., and Zazímalová, E., Eds., SPB Academic Publishing, The Hague, 1992, 121.
104. **Kaufman, L. S., Thompson, W. F., and Briggs, W. R.,** Different red light requirements for phytochrome-induced accumulation of *cab* RNA and *rbc* RNA, *Science*, 226, 1447, 1984.
105. **Sugita, M. and Gruissem, W.,** Developmental, organ-specific, and light dependent expression of the tomato ribulose-1,5-bisphosphate carboxylase small subunit gene family, *Proc. Natl. Acad. Sci. U.S.A.*, 84, 7104, 1987.
106. **Silverthorne, J., Wimpee, C. F., Yamada, T., Rolfe, S. A., and Tobin, E. M.,** Differential expression of individual genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase in *Lemna gibba, Plant Mol. Biol.*, 15, 49, 1990.
107. **Hobbs, S. L. A., Pelcher, L. E., DeLong, C. M. O., Anderson, M., and Mahon, J. D.,** Genetic variability in the amount of ribulose-1,5-bisphosphate carboxylase/oxygenase and its small subunit mRNA in pea, *Planta*, 180, 510, 1990.
108. **Brusslan, J. A. and Tobin, E. M.,** Light-independent developmental regulation of *cab* gene expression in *Arabidopsis thaliana* seedlings, *Proc. Natl. Acad. Sci. U.S.A.*, 89, 7791, 1992.
109. **Dietrich, G., Detschey, S., Neuhaus, H., and Link, G.,** Temporal and light control of plastid transcript levels for proteins involved in photosynthesis during mustard (*Sinapis alba* L.) seedling development, *Planta*, 172, 393, 1987.
110. **Fiebig, C., Neuhaus, H., Teichert, J., Röcher, W., Degenhardt, J., and Link, G.,** Temporal and spatial pattern of plastid gene expression during crucifer seedling development and embryogenesis, *Planta*, 181, 191, 1990.
111. **Eckes, P., Schell, J., and Willmitzer, L.,** Organ-specific expression of three leaf/stem specific cDNAs from potato is regulated by light and correlated with chloroplast development, *Mol. Gen. Genet.*, 199, 216, 1985.
112. **Ernst, D., Apfelböck, A., Bergmann, A., and Weyrauch, C.,** Rhythmic regulation of the light-harvesting chlorophyll a/b protein and the small subunit of ribulose-1,5-bisphosphate carboxylase mRNA in rye seedlings, *Photochem. Photobiol.*, 52, 29, 1990.
113. **Riesselmann, S. and Piechulla, B.,** Effect of dark phases and temperature on the chlorophyll a/b binding protein mRNA level oscillations in tomato seedlings, *Plant Mol. Biol.*, 14, 605, 1990.
114. **Deng, M. D., Moureaux, T., Leydecker, M.-T., and Caboche, M.,** Nitrate-reductase expression is under the control of a circadian rhythm and is light inducible in *Nicotiana tabacum* leaves, *Planta*, 180, 257, 1990.

115. **Thomas, M., Cretin, C., Vidal, J., Keryer, E., Gadal, P., and Monsinger, E.,** Light-regulation of phosphoenolpyruvate carboxylase mRNA in leaves of C4 plants: evidence for phytochrome control on transcription during greening and for rhythmicity, *Plant Sci.*, 69, 65, 1990.
116. **Humbeck, K., Melis, A., and Krupinska, K.,** Chilling affects chloroplast development in barley primary foliage leaves, *J. Plant Physiol.*, in press, 1994.
117. **Dominov, J. A., Stenzler, L., Lee, S., Schwarz, J. J., Leisner, S., and Howell, H. H.,** Cytokinins and auxins control the expression of a gene in *Nicotiana plumbaginifolia* cells by feedback regulation, *Plant Cell*, 4, 451, 1992.
118. **Beinsberger, S. E. I., Valck, R. L. M., Clijsters, H. M. M., de Greef, J. A., and van Onckelen, H. A.,** Effects of enhanced cytokinin levels in *ipt*-transgenic tobacco, in *Physiology and Biochemistry of Cytokinins in Plants*, Kamínek, M., Mok, D. W. S., and Zazímalová, E., Eds., SPB Academic Publishing, The Hague, 1992, 77.
119. **Schmülling, T., Schell, J., and Spena, A.,** Construction of cytokinin overproducing mutants in higher plants, in *Physiology and Biochemistry of Cytokinins in Plants*, Kamínek, M., Mok, D. W. S., and Zazímalová, E., Eds., SPB Academic Publishing, The Hague, 1992, 87.
120. **Schimper, A. F.,** Über die Verteilung der Chlorophyllkörner und Farbkörper, *Bot. Ztg.*, 41, 105, 1883.
121. **Reski, R., Wehe, M., Kasten, B., Reutter, K., Marienfeld, J. R., and Abel, W. O.,** The molecular analysis of chloroplast division (mini-review), *Crypt. Bot.*, 3, 18, 1992.
122. **Chaley, N., Possingham, J. V., and Thompson, W. W.,** Chloroplast division in spinach leaves examined by scanning electron microscopy and freeze-etching, *J. Cell Sci.*, 46, 87, 1980.
123. **Whatley, J. M.,** The ultrastructure of plastids in roots, *Int. Rev. Cytol.*, 85, 175, 1983.
124. **Lawrence, M. E. and Possingham, J. V.,** Observations of microtubule-like structures within spinach plastids, *Biol. Cellulaire*, 52, 77, 1984.
125. **Butterfaß, T.,** Patterns of chloroplast reproduction. A developmental approach to protoplasmic plant anatomy, *Cell Biol. Monogr.*, 6, 1, 1979.
126. **Van de Putte, P. J., van Dillewijn, J., and Rörsch, A.,** The selection of mutants of *Escherichia coli* with impaired cell division at elevated temperature, *Mutat. Res.*, 1, 121, 1964.
127. **Lutkenhaus, J. F., Wolf-Watz, H., and Donachie, W. D.,** Organisation of genes in the *fts*A-*env*A region of the *Escherichia coli* genetic map and identification of a new *fts* locus: *fts*2, *J. Bacteriol*, 142, 615, 1980.
128. **Leonard, J. M. and Rose, R. J.,** Sensitivity of chloroplast division cycle to chloramphenicol and cycloheximide in cultured spinach leaves, *Plant Sci. Lett.*, 14, 159, 1979.
129. **Pyke, K. A. and Leech, R. M.,** Chloroplast division and expansion is radically altered by nuclear mutations in *Arabidopsis thaliana*, *Plant Physiol.*, 99, 1005, 1992.
130. **Parthier, B.,** The cooperation of nuclear and plastid genomes in plastid biogenesis and differentiation, *Biochem. Physiol. Pflanzen*, 177, 283, 1982.
131. **Hagemann, R.,** Plastid genetics in higher plants, in *Plant Gene Research. Cell Organelles*, Herrmann, R. G., Ed., Springer-Verlag, New York, 1992, 66.
132. **Kuroiwa, T.,** The replication, differentiation, and inheritance of plastids with emphasis on the concept of organelle nuclei, *Int. Rev. Cytol.*, 128, 1, 1991.
133. **Reski, R. and Abel, W. O.,** Induction of budding on chloronemata and caulonemata of the moss, *Physcomitrella patens*, using isopentenyladenine, *Planta*, 165, 354, 1985.
134. **Abel, W. O., Knebel, W., Koop, H.-U., Marienfeld, J. R., Quader, H., Reski, R., Schnepf, E., and Spörlein, B.,** A cytokinin-sensitive mutant of the moss, *Physcomitrella patens*, defective in chloroplast division, *Protoplasma*, 152, 1, 1989.
135. **Reski, R., Wehe, M., Hadeler, B., Marienfeld, J. R., and Abel, W. O.,** Cytokinin and light quality interact at the molecular level in the mutant PC22 of the moss *Physcomitrella, J. Plant Physiol.*, 138, 236, 1991.
136. **Sasaki, Y., Nagano, Y., Morioka, S., Ishikawa, H., and Matsuno, R.,** A chloroplast gene encoding a protein with one zinc finger, *Nucleic Acids Res.*, 17, 6217, 1989.
137. **Nagano, Y., Matsuno, R., and Sasaki, Y.,** Sequence and transcriptional analysis of the gene cluster *trn*Q-*zfp*A-*psa*I-ORF231-*pet*A in pea chloroplasts, *Curr. Genet.*, 20, 431, 1991.
138. **Smith, A. G., Wilson, R. M., Kaethner, T. M., Willey, D. L., and Gray, J. C.,** Pea chloroplast genes encoding a 4 kDa polypeptide of photosystem I and a putative enzyme of C1 metabolism, *Curr. Genet.*, 19, 403, 1991.

139. **Reski, R. and Kruse, S.,** Does a finger point to plastid evolution? Submitted.
140. **Eisermann, A., Tiller, K., and Link, G.,** In vitro transcription and DNA binding characteristics of chloroplast and etioplast extracts from mustard (*Sinapis alba*) indicate differential usage of the *psb*A promoter, *EMBO J.*, 9, 3981, 1990.
141. **Kruse, S., Quast, S., Abel, W. O., and Reski, R.,** Cytokinin-promoted processing of plastid transcripts in cytokinin-sensitive moss mutants, submitted.
142. **Westhoff, P. and Herrmann, R. G.,** Complex RNA maturation in chloroplasts: the *psb*B operon from spinach, *Eur. J. Biochem.*, 171, 551, 1988.
143. **Ohyama, K.,** Organization and expression of genes of plastid chromosomes from nonangiospermous land plants and green algae, in *Plant Gene Research. Cell Organelles*, Herrmann, R. G., Ed., Springer-Verlag, New York, 1992, 137.
144. **Palmer, J. D.,** Comparison of chloroplast and mitochondrial genome evolution in plants, in *Plant Gene Research. Cell Organelles*, Herrmann, R. G., Ed., Springer-Verlag, New York, 1992, 99.
145. **Whittier, R. F. and Sugiura, M.,** Plastid chromosomes from vascular plants — genes, in *Plant Gene Research. Cell Organelles*, Herrmann, R. G., Ed., Springer-Verlag, New York, 1992, 164.
146. **Kanno, A. and Hirai, A.,** A transcription map of the chloroplast genome from rice (*Oryza sativa*), *Curr. Genet.*, 23, 166, 1993.
147. **Crossland, L. D., Rodermel, S. R., and Bogorad, L.,** Single gene for the large subunit of ribulosebisphosphate carboxylase in maize yields two differentially regulated mRNAs, *Proc. Natl. Acad. Sci. U.S.A.*, 81, 4060, 1984.
148. **Gamble, P. E., Berends, T., Sexton, T., and Mullet, J. E.,** Light-dependent changes in *psb*D and *psb*C transcripts of barley chloroplasts: accumulation of two transcripts maintains *psb*D and *psb*C translation capability in mature chloroplasts, *EMBO J.*, 7, 1289, 1988.
149. **Johnson, E. M., Schnabelrauch, L. S., and Sears, B. B.,** A plastome mutation affects processing of both chloroplast and nuclear DNA-encoded plastid proteins, *Mol. Gen. Genet.*, 225, 106, 1991.
150. **Rother, S., Hadeler, B., Orsini, J. M., Abel, W. O., and Reski, R.,** Fate of a mutant macrochloroplast in somatic hybrids, *J. Plant Physiol.*, 143, 72, 1994.
151. **Boynton, J. E., Gillham, N. W., Newman, S. M., and Harris, E. H.,** Organelle genetics and transformation of *Chlamydomonas*, in *Plant Gene Research. Cell Organelles*, Herrmann, R. G., Ed., Springer-Verlag, New York, 1992, 4.
152. **Svab, Z., Hajdukiewitz, P., and Maliga, P.,** Stable transformation of plastids in higher plants, *Proc. Natl. Acad. Sci. U.S.A.*, 87, 8526, 1990.
153. **Spörlein, B., Streubel, M., Dahlfeld, G., Westhoff, P., and Koop, H.-U.,** PEG-mediated plastid transformation: a new system for transient gene expression assays in chloroplasts, *Theor. Appl. Genet.*, 82, 717, 1991.
154. **Ye, F., Gierlich, J., Reski, R., Marienfeld, J. R., and Abel, W. O.,** Isoenzyme analysis of cytokinin sensitive mutants of the moss *Physcomitrella patens*, *Plant Sci.*, 64, 203, 1989.