MINI-REVIEW

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Enslaved bacteria as new hope for plant biotechnologists

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Abstract The most distinguishing feature of the plant cell is a DNA-containing organelle that sets plants apart from all other organisms: the chloroplast. Compelling evidence supports an endosymbiotic origin for chloroplasts. According to this theory, chloroplasts are descendants of formerly free-living cyanobacterial ancestors which entered an endosymbiotic relationship with a pre-eukaryotic cell and were ultimately integrated into the metabolism of the host cell. Chloroplasts retain many prokaryotic features and their gene expression system still closely resembles that of their eubacterial ancestors. During the past decade, our knowledge about chloroplast biology has benefited immensely from a most remarkable methodological breakthrough: the development of transformation technologies for chloroplast genomes. Moreover, recent advances in the manipulation of higher plant chloroplast genomes have created unprecedented opportunities for the genetic engineering of plants and promise to overcome many of the problems associated with conventional transgenic technologies. This review describes the state of the art in genetic engineering of higher plant chloroplast genomes and highlights the tremendous potential of these technologies for the biotechnology of the future.

Introduction

Plastids and mitochondria as prokaryotic systems of endosymbiotic origin

The making of the eukaryotic cell is one of evolution’s most remarkable feats, and scientific understanding of this process has itself been evolving at a rapid pace (Martin and Müller 1998; Brock et al. 1999). The rise of eukaryotes, possibly as early as 2,700 million years ago, is closely connected to the acquisition of two cell organelles: the mitochondrion and the plastid (best known in its green differentiation form, the chloroplast). The structural complexity of eukaryotic cells and the fact that prokaryotes had existed at least one billion years before the first eukaryotes appeared led to the idea that eukaryotic cell organelles could have originated from formerly free-living prokaryotes. An attractive mechanistic explanation for such an evolutionary conversion of prokaryotes into mitochondria or plastids has been provided by the endosymbiosis theory (for review, see e.g. Gray 1993; Gray et al. 1999): a bacterium was engulfed by a pre-eukaryotic host cell and, instead of being digested, became domesticated. This process involved the gradual integration of the endosymbiont into the metabolism of the host cell by establishing a division of labor and inventing sophisticated regulatory networks to coordinate the host’s gene expression with that of the endosymbiont. Genetically, the evolutionary optimization of the endosymbiosis was accompanied by the loss of dispensable or redundant genetic information and the massive translocation of genetic information, particularly from the endosymbiont to the host genome (Martin and Herrmann 1998). Contemporary organellar genomes are greatly reduced and contain only a small proportion of the genes that their free-living ancestors had possessed.

Using molecular methods, the origins of organelles have been traced back to specific taxa of Eubacteria: whereas Cyanobacteria were identified as the presumptive
ancestors of plastids, z-Proteobacteria are most closely related to mitochondria (Gray 1993). The present-day organelles are believed to be of monophyletic origin, in that all extant lineages of eukaryotes harbor mitochondria originating from one and the same endosymbiosis event (Lang et al. 1997; Andersson et al. 1998). Likewise, plastids in all lineages of plant evolution have a common cyanobacterial ancestor (Ozeki et al. 1989; Bhattacharya and Medlin 1998; Tomitani et al. 1999).

**Gene expression in chloroplasts**

The chloroplast genome of higher plants is a circular molecule of double-stranded DNA, typically in the size range 120–160 kb (Fig. 1). Whereas the genome of the cyanobacterium *Synechocystis* contains more than 3,000 genes (Kaneko et al. 1996; Kaneko and Tabata 1997), chloroplast genomes of higher plants harbor only approximately 120 genes (Sugiura 1992), illustrating the dramatic reduction that the endosymbiotic's genome has suffered during evolution.

The picture that has emerged from the complete sequencing of several plastid genomes over the past decade is that the chloroplast has retained a largely prokaryotic system of gene organization and expression. Most plastid-encoded genes are organized in operons and hence produce monocistronic mRNAs by co-transcription. This is in striking contrast to gene expression in the plant nuclear genome, where almost all genes are transcribed as monocistronic mRNAs. The gene order in several chloroplast operons is remarkably conserved and still closely resembles that of Cyanobacteria and other Eubacteria (Stoebel and Kowalkik 1999). For example, the genes for the ribosomal proteins L23, L2, S19, L22, S3 and L16 are part of an operon in *Escherichia coli* (S10 operon) and are found in exactly the same order within the *rpoA* operon of chloroplasts (Fig. 1).

The prokaryotic origin of chloroplasts is also mirrored by the molecular mechanisms of practically all steps in plastid gene expression. Plastid transcription is carried out by a eubacterial-type RNA polymerase, the subunits of which are encoded in the chloroplast DNA (*rpoA, rpoB, rpoC1 and rpoC2*; Fig. 1). As in Eubacteria, a set of sigma-like factors interact with this plastid-encoded RNA polymerase, conferring promoter-specific binding and mediating transcriptional regulation in response to environmental cues. Many, but not all, promoters of plastid genes resemble bacterial promoters in that their core sequence consists of the typical −10 (TATA box) and −35 elements (Igloi and Kössel 1992). Recently, a second, nuclear-encoded, RNA polymerase activity could be identified which utilizes non-consensus promoters. The enzyme turned out to be closely related to single-subunit bacteriophage RNA polymerases (Hedtke et al. 1997; Hess and Börner 1999). Interestingly, the phage-like polymerase is predominantly active in undifferentiated plastids and preferentially transcribes plastid genetic system genes (e.g. rRNA genes, ribosomal protein genes) whereas the *E. coli*-like enzyme

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**Fig. 1** Structure of a typical chloroplast genome of a higher plant. The physical map of the plastid DNA from maize (*Zea mays*) is shown. The circular genome contains two large inverted repeat regions (*IR_A* and *IR_B*) which separate the molecule into a large single copy region (*LSC*) and a small single copy region (*SSC*). Major gene classes are: *rrn* (*rRNA* genes), *trn* (*tRNA* genes), *rpl* (ribosomal proteins of the large subunit), *rps* (ribosomal proteins of the small subosomal subunit), *rpo* (RNA polymerase subunits), *psa* (subunits of photosystem I), *psb* (subunits of photosystem II), *pet* (subunits of the cytochrome b6f complex), *atp* (subunits of the ATP synthase), *ndh* (subunits of an NAD(P)H dehydrogenase) and *ycf* (conserved open reading frames). Genes located on the outside of the circle are transcribed clockwise, genes located on the inside are transcribed counterclockwise.