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Review

Large-scale genetic analysis of chloroplast biogenesis in maize ☆

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ABSTRACT

Background: Chloroplast biogenesis involves a collaboration between several thousand nuclear genes and ~100 genes in the chloroplast. Many of the nuclear genes are of cyanobacterial ancestry and continue to perform their ancestral function. However, many others evolved subsequently and comprise a diverse set of proteins found specifically in photosynthetic eucaryotes. Genetic approaches have been key to the discovery of nuclear genes that participate in chloroplast biogenesis, especially those lacking close homologs outside the plant kingdom. *Scope of Review:* This article summarizes contributions from a genetic resource in maize, the Photosynthetic Mutant Library (PML). The PML collection consists of ~2000 non-photosynthetic mutants induced by *Mu* transposons. We include a summary of mutant phenotypes for 20 previously unstudied maize genes, including genes encoding chloroplast ribosomal proteins, a PPR protein, tRNA synthetases, proteins involved in plastid transcription, a putative ribosome assembly factor, a chaperonin 60 isoform, and a NifU-domain protein required for Photosystem I biogenesis. *Major Conclusions:* Insertions in 94 maize genes have been linked thus far to visible and molecular phenotypes with the PML collection. The spectrum of chloroplast biogenesis genes that have been genetically characterized in maize is discussed in the context of related efforts in other organisms. This comparison shows how distinct organismal attributes facilitate the discovery of different gene classes, and reveals examples of functional divergence between monocot and dicot plants. *General Significance:* These findings elucidate the biology of an organelle whose activities are fundamental to agriculture and the biosphere. This article is part of a Special Issue entitled: Chloroplast Biogenesis.

1. Introduction

The chloroplast is a dynamic organelle whose ultrastructural and proteome complexity are comparable to those of free-living bacteria. Chloroplasts evolved from an endosymbiont of cyanobacterial ancestry, and were likely retained by their primordial host due to their photosynthetic capacity [reviewed in [1]]. Subsequently, massive gene transfer from the endosymbiont to the nucleus, integration of the organelle into host signaling and metabolic pathways, and coevolution of the nuclear and chloroplast genomes produced an organelle whose bacterial ancestry remains apparent but is embellished by numerous physiological and molecular novelties [reviewed in [2,3]].

The biogenesis of the photosynthetic apparatus in extant chloroplasts requires the coordinated expression of several thousand nuclear genes and ~100 chloroplast genes, followed by a complex series of protein targeting and assembly steps that lead to the elaboration of the thylakoid membrane system and the biogenesis of the multisubunit complexes that perform the core reactions of photosynthesis. Furthermore, the composition and activities of the photosynthetic apparatus adapt readily to environmental influences such as light and temperature. Mechanisms underlying the biogenesis and adaptation of the photosynthetic apparatus are, in general, poorly understood [reviewed in [4–6]]. An added layer of complexity arises in multicellular plants, in which chloroplasts belong to an organelle family, the plastids, that adopt different forms in different cell types [reviewed in [7]]. The differentiation of non-photosynthetic proplastids into chloroplasts occurs in conjunction with the differentiation of leaf cells from meristematic progenitors. Furthermore, two distinct photosynthetic cell types in C4 plants - mesophyll and bundle sheath - harbor chloroplasts with distinct morphologies, intracellular distribution, and enzymatic profiles [reviewed in [8]].

Chloroplast biogenesis and photosynthesis *per se* are nicely amenable to analysis by classical genetic approaches ("forward genetics") because defects in photosynthesis can be detected with simple screens and photosynthesis is dispensable when an alternative source of reduced carbon is provided. Despite the increasing ease of reverse-genetic approaches, forward genetics remains a powerful method for dissecting complex biological processes. For example, a screen based on chlorophyll fluorescence parameters yielded a rich harvest of nuclear

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genes functioning in the synthesis, assembly, and regulation of the thylakoid NADH dehydrogenase-like complex (NDH) [reviewed in [9]]. Screens for mutants with defects in plastid gene expression revealed functions of several "domains of unknown function", screens for the loss of specific photosynthetic complexes identified novel photosystem assembly factors, and screens for mutants with defects in non-photochemical quenching and state transitions screens provided important insights into mechanisms of photosynthetic acclimation [reviewed in [3,10–12]].

2. Maize as a model organism for the genetic analysis of chloroplast biogenesis

Phenotype-driven genetic analyses of chloroplast processes have concentrated on four organisms: the green alga *Chlamydomonas reinhardtii*, the C3 dicot *Arabidopsis thaliana*, the C3 monocot *Oryza sativa*, and the C4 monocot *Zea mays*. These organisms span a considerable phylogenetic distance and embed their chloroplasts in diverse developmental and physiological contexts. Each offers a distinct set of attributes that impacts which experimental approaches are most easily employed and the types of mutants that are recovered. *Chlamydomonas* is the only organism that allows the ready manipulation of both the nuclear and chloroplast genomes, but it cannot serve as a model for the many aspects of chloroplast biology that are specific to land plants. The expansive genomic tools available for *Arabidopsis* are unrivaled, and the ease of growing large numbers of mutants in a small space make *Chlamydomonas* and *Arabidopsis* well suited to high throughput metabolite and fluorescence-based screens [13]. On the other hand, the large seed reserves of maize and rice support rapid heterotrophic growth of non-photosynthetic mutants for several weeks without the need for specialized growth media. At eight days post germination, a non-photosynthetic maize seedling is typically 10 cm tall with a fresh weight of approximately 0.5 gm (see photographs in Figs. 1 through 5). This provides ready access to non-photosynthetic mutant tissue for biochemical analysis, and fosters comprehensive analyses of molecular phenotypes using methods that can be onerous in *Arabidopsis*. Maize and rice have proven to be particularly useful for studying mutations that either directly or indirectly cause the loss of plastid ribosomes: this condition causes embryo lethality in *Arabidopsis*, but typically yields albino seedlings in cereals whose molecular defects can be informative [reviewed in [14]]. This feature likely accounts for the fact that the nuclear gene complement involved in chloroplast RNA splicing in land plants has been elucidated primarily through genetic and biochemical approaches in maize (see below).

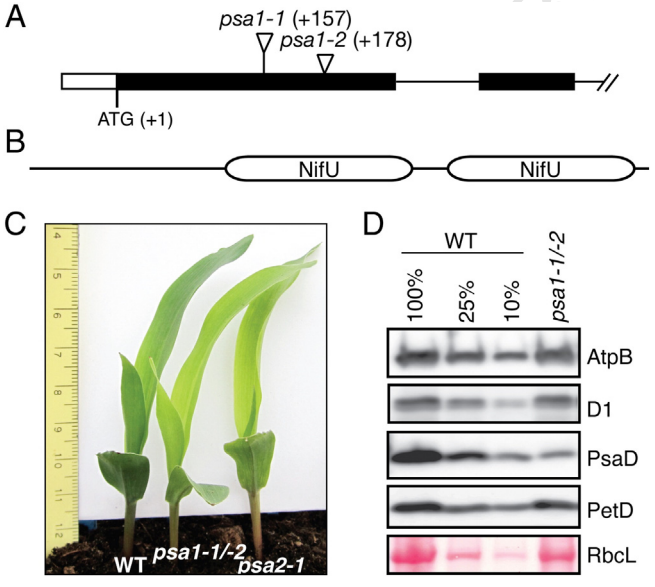

Fig. 1. Overview of *psa1* mutants. (A) Positions of the *Mu* insertions in the *psa1* gene. The nucleotide positions relative to the start codon are indicated. The insertion site sequences are provided in Supplementary Fig. 1. (B) Domain architecture of PSA1. (C) Phenotype of *psa1* mutants. Plants were grown for nine days in soil. (D) Immunoblot profile of core subunits of photosynthetic enzyme complexes. A single blot was probed sequentially with antibodies to the indicated proteins. The Ponceau S stained blot below illustrates the abundance of RbcL, the large subunit of Rubisco.

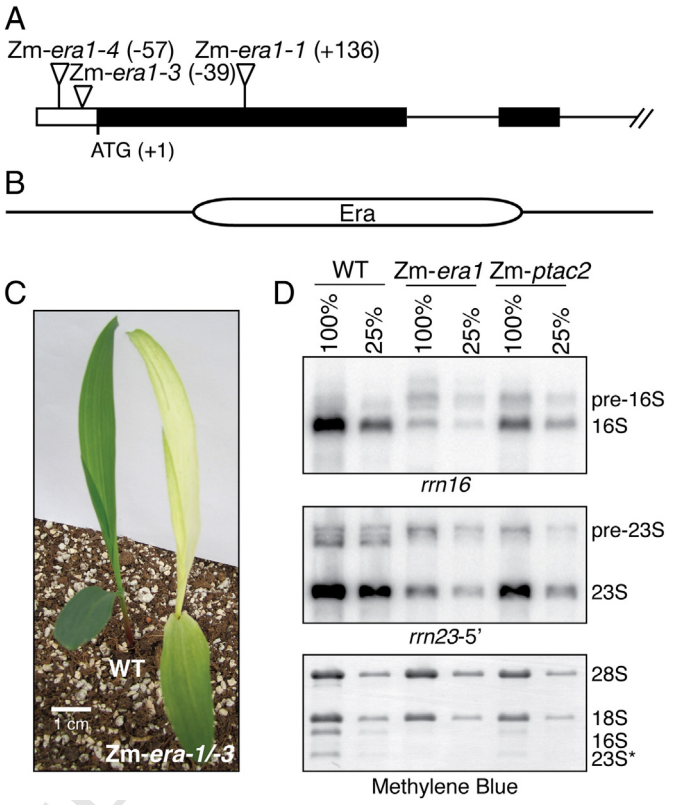

Fig. 2. Overview of Zm-*era1* mutants. (A) Positions of the *Mu* insertions in the Zm-*era1* gene. The nucleotide positions relative to the start codon are indicated. The insertion site sequences are provided in Supplementary Fig. 1. (B) Domain architecture of Zm-Era1. (C) Phenotype of Zm-*era1* mutants. The pictured individual is the heteroallelic progeny of a cross between a strong and weak allele. (D) RNA gel blot hybridizations showing defects in the processing and accumulation of chloroplast rRNAs. The upper and middle blots were probed with *rrn16* and *rrn23*, respectively. The methylene blue-stained membrane is shown below to illustrate relative loading.

The use of maize for the genetic dissection of chloroplast processes was pioneered by Don Miles, who was the first to use "high chlorophyll fluorescence" (*hcf*) to screen for non-photosynthetic mutants in plants [15,16]. Miles initially screened EMS-mutagenized maize, but chemical mutagens were soon supplanted by the *Mutator* (*Mu*) transposon system as the mutagen of choice [17,18]. However, the high copy number of *Mu* transposons (~100 insertions per genome) hindered the assignment of causal insertions in *Mu* lines, and only one of the causal mutations in the Miles collection has been reported [17,19]. This challenge of the *Mu* system was overcome with the recent development of high-throughput methods for sequencing *Mu* insertion sites and linking them to specific phenotypes [20,21].

3. Overview of the PML mutant collection

The PML collection was assembled as a tool to deeply sample the complement of nuclear genes required for the biogenesis of photosynthetically competent chloroplasts in plants. The collection consists of ~2000 independently arising mutants that were selected from *Mu*-active maize lines based on seedling chlorophyll deficiency (pale green, albino, yellow, virescent, etc.) or an *hcf* phenotype. The latter screen was abandoned early in the project because the vast majority

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