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Author for correspondence:
Robert M. Larkin
e-mail: larkinr@msu.edu

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Influence of plastids on light signalling and development

Robert M. Larkin[1,2]

[1]Michigan State University-Department of Energy Plant Research Laboratory, and [2]Department of Biochemistry and Molecular Biology, Michigan State University, Room 106 Plant Biology Building, East Lansing, MI 48824, USA

In addition to their contribution to metabolism, chloroplasts emit signals that influence the expression of nuclear genes that contribute to numerous plastidic and extraplastidic processes. Plastid-to-nucleus signalling optimizes chloroplast function, regulates growth and development, and affects responses to environmental cues. An incomplete list of plastid signals is available and particular plastid-to-nucleus signalling mechanisms are partially understood. The plastid-to-nucleus signalling that depends on the *GENOMES UNCOUPLED* (*GUN*) genes couples the expression of nuclear genes to the functional state of the chloroplast. Analyses of *gun* mutants provided insight into the mechanisms and biological functions of plastid-to-nucleus signalling. *GUN* genes contribute to chloroplast biogenesis, the circadian rhythm, stress tolerance, light signalling and development. Some have criticized the *gun* mutant screen for employing inhibitors of chloroplast biogenesis and suggested that *gun* alleles do not disrupt significant plastid-to-nucleus signalling mechanisms. Here, I briefly review *GUN*-dependent plastid-to-nucleus signalling, explain the flaws in the major criticisms of the *gun* mutant screen and review the influence of plastids on light signalling and development.

1. Introduction

Most photosynthesis-related proteins that reside in chloroplasts are encoded by nuclear genes. Extraplastidic signalling mechanisms, plastid-to-nucleus signalling mechanisms and the integration of these signalling mechanisms are major regulators of photosynthesis-associated nuclear genes (PhANGs) [1–3]. Plastid-to-nucleus signalling affects numerous plastidic and extraplastidic processes, such as the biogenesis of chloroplasts and amyloplasts [3–6], the circadian rhythm [7,8], DNA replication [3], the transcription of genes that encode ribosomal RNA by RNA polymerase I [9], development [10] and the optimization of photosynthesis to various qualities of light [3]. Plastid-to-nucleus signalling also contributes to the response to wounding, biotic stress, abiotic stress and sugar [2,3,9,11–14]. Thus, plastid-to-nucleus signalling broadly affects plant cells by optimizing chloroplast function and helping to coordinate extrachloroplastic processes with chloroplast function. Known plastid signals include hydrogen peroxide, 3′-phosphoadenosine 5′-phosphate, β-cyclocitral, methylerythritol cyclodiphosphate, thiols and particular proteins [2,3,9,13,15]. Nonetheless, our knowledge of plastid signals and plastid-to-nucleus signalling mechanisms is incomplete. Here, I review *GENOMES UNCOUPLED* (*GUN*)-dependent plastid-to-nucleus signalling and the influence of plastids on light signalling and development. I also explain the flaws in the major criticisms of the *gun* mutant screen.

2. The *gun* mutant screen

Chloroplasts are derived from proplastids during germination and leaf development. When chloroplast biogenesis is blocked with mutant alleles or inhibitors, the transcription of most PhANGs is severely downregulated. Thus, dysfunctional chloroplasts were proposed to emit signals that negatively regulate the transcription of PhANGs [16,17]. Attenuating the activity of well-functioning chloroplasts was found to also activate this signalling [18,19].

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The *gun* mutant screen was the first screen for mutant alleles that disrupt plastid-to-nucleus signalling [17,20]. *gun* mutant screens use reporter genes to screen for *Arabidopsis* mutants that transcribe elevated levels of PhANGs when chloroplast biogenesis is blocked with norflurazon treatments [5,18,20–22]. Norflurazon specifically inhibits phytoene desaturase, which is required for carotenoid biosynthesis and chloroplast biogenesis [17]. *gun* alleles either attenuate negative regulators or promote positive regulators of PhANG expression [5,18,20–25].

Voigt *et al.* [26] suggest that the *gun* mutant screen is problematic because blocking chloroplast biogenesis with norflurazon causes 'a plethora of secondary effects and induces artificial and complex metabolic situations that are unlikely to reflect natural stimuli relevant for plastid signalling' (p. 504). Others appear to support this interpretation [27–29]. Whether a mutant screen uses natural, stressful or unnatural growth conditions does not matter. For example, mutant screens with seedlings that grew and developed abnormally provided major advances to our understanding of hormone signalling [30,31]. Additionally, the unfolded protein response [32] and mitochondria-to-nucleus signalling [33] were discovered using mutant screens that, similar to the *gun* mutant screen, employed inhibitors or mutant alleles that cause severe organellar dysfunction [34–37].

Mutant screens are judged by their specificity, not by whether they use natural or unnatural growth conditions. *gun* mutant screens repeatedly yielded mutant alleles of genes that contribute to a small number of processes [5,18,21,23–25]. These data indicate that *gun* mutant screens specifically disrupted a few distinct mechanisms that downregulate the expression of PhANGs when chloroplast biogenesis is blocked, not complex metabolic situations. The findings that these mechanisms appear conserved in all plants tested [17] and contribute to stress tolerance [4,5,11,14], the circadian rhythm [7,8] and development [38–41] provide evidence that they contribute plastid-to-nucleus signalling in natural environments.

Mutant alleles yielded by the *gun* mutant screens affect an extraplastidic blue-light photoreceptor, a chloroplastic pentatricopeptide repeat protein named GUN1, that may affect the expression of the plastid genome and enzymes that contribute to tetrapyrrole metabolism, which occurs within the plastid (see electronic supplementary material, figure S1). Tetrapyrroles include chlorophylls, sirohaem, haem and phytochromobilin (see electronic supplementary material, figure S2) [3]. The *gun* alleles that attenuate tetrapyrrole metabolism were suggested to cause resistance to norflurazon [42,43] because norflurazon was thought to promote collisions between ground-state triplet oxygen and triplet chlorophyll, which yield singlet oxygen ($^1O_2$), a toxic reactive oxygen species (ROS). This $^1O_2$ was thought to block chloroplast biogenesis [16,17]. Recent data provide compelling evidence that norflurazon blocks chloroplast biogenesis not by affecting the levels of $^1O_2$ but perhaps by causing the misfolding of the chlorophyll- and carotenoid-binding proteins of the thylakoid membranes [44]. Nonetheless, Kim & Apel [44] suggest that $^1O_2$ may transiently accumulate when chloroplast biogenesis is blocked with a norflurazon treatment because the expression of one $^1O_2$-inducible gene was higher at 3 days than at 5 days. Alternatively, signals unrelated to chloroplastic $^1O_2$ may cause these differences in expression.

The finding that a mutant allele of the Mg-chelatase subunit gene *ChlI* named *cs* that accumulated 40% of the chlorophyll found in wild-type was not a *gun* mutant and that a mutant allele of the Mg-chelatase subunit gene *ChlH* named *gun5* that accumulated 70% of the chlorophyll found in wild-type was a *gun* mutant indicates that the upregulation of PhANG expression in *gun5* was not caused by attenuated chlorophyll biosynthesis [23]. In other words, *gun5* did not facilitate chloroplast biogenesis in norflurazon-treated seedlings by attenuating chlorophyll biosynthesis, which reduced the levels of $^1O_2$. The finding that etiolated *sigma factor2* (*sig2*) *gun5* double mutants expressed higher levels of PhANGs than the etiolated *sig2* (i.e. in the dark and without norflurazon) [19] also indicates that *gun5* regulated PhANG expression without increasing the levels of $^1O_2$, regardless of whether this increase in ROS is transient, localized or sustained. Additionally, mutant alleles that were obtained from *gun* mutant screens were not obtained from screens for norflurazon-resistant mutants, and in contrast to norflurazon-resistant mutants, *gun* mutants were not resistant to low concentrations of norflurazon [45]. Other attempts to show that *gun* mutants are resistant to norflurazon were not successful [26,44,45]. Indeed, ROS and blocking chloroplast biogenesis with norflurazon appeared to activate distinct plastid-to-nucleus signalling mechanisms [44,46].

The preponderance of the data is consistent with tetrapyrroles regulating nuclear gene expression in plants, as they do in *Chlamydomonas reinhardtii*, without inducing an increase in the levels of $^1O_2$ [3], and with particular *gun* alleles disrupting this signalling by disrupting tetrapyrrole metabolism. Popular models suggest that haem or the chlorophyll precursor Mg-protoporphyrin IX serve as plastid signals [3,22,29]. The finding that the moderate *cs* allele of *ChlI* does not cause *gun* phenotypes [23] and that severe mutant alleles of *ChlI* that cause albinism do cause *gun* phenotypes [47] combined with the finding that mild alleles of *ChlH* (i.e. *gun5*) cause *gun* phenotypes [23] is consistent with mild defects in the tetrapyrrole-binding subunit of Mg-chelatase (i.e. ChlH) and severe defects in non-tetrapyrrole-binding subunits of Mg-chelatase (e.g. ChlI) causing *gun* phenotypes by diverting protoporphyrin IX to ferrochelatase, the enzyme that converts protoporphyrin IX to haem (see electronic supplementary material, figure S2). An increase in haem biosynthesis appears to induce PhANG expression [22]. Perhaps the protoporphyrin IX-binding activity of ChlH or other protoporphyrin IX-binding proteins, for example GUN4 (electronic supplementary material, figure S2), sequester excess tetrapyrroles in leaky mutants such as *cs*.

3. Integration of light and plastid-to-nucleus signalling

Plastid-responsive promoter elements were identified in transgenic plants with reporter genes in which expression was driven by PhANG-promoter mutants after blocking chloroplast biogenesis with norflurazon treatments. Light and plastid signals typically depend on the same composite promoter element to regulate transcription. These composite elements are composed of at least two common promoter elements [1,16,18]. Light and plastid signals appear distinct because plastid signals can regulate PhANG expression in the dark [5,19,48]. Plastid-to-nucleus signalling that is activated by chloroplastic ROS uses distinct promoter elements from those activated by blocking chloroplast biogenesis with norflurazon treatments [46].

A *gun* mutant screen specifically disrupted *CRYPTOCHROME1* (*CRY1*) [5], which encodes an extraplastidic blue-light receptor [49]. Null alleles of *CRY1* did not cause resistance to inhibitors of chloroplast biogenesis and did not acclimatize chloroplasts to stress. When chloroplast biogenesis was blocked, CRY1 became a negative regulator of genes that encode type I proteins of the major light-harvesting complex of photosystem II (Lhcb1) because LONG HYPOCOTYL5 (HY5) was converted from a positive to a negative regulator of *Lhcb1* expression [5]. HY5 is a bZIP-type transcription factor that acts downstream of cry1 and other photoreceptors. HY5 binds ACGT-containing promoter elements, for example the G box, and contributes to numerous light-regulated processes [50]. The idea that these findings are explained by *cry1* attenuating $^1O_2$-dependent plastid-to-nucleus signalling [29] conflicts with the findings that *cry1* and *hy5* were *gun* mutants when chloroplast biogenesis was blocked with lincomycin treatments [5], which do not require light to block plastid development [48], and that *cry1* and *hy5* mutants are more sensitive to excess light than wild-type [5]. Chloroplasts also appear to affect light signalling mediated by cry2 and phytochrome A [51].

cry1 appeared to have no effect, upregulate or downregulate the expression of *Lhcb* [52–55] depending on experimental conditions and was required to drive a $^1O_2$-independent reaction that induces programmed cell death after $^1O_2$ accumulated in chloroplasts [56]. Thus, the nature of cry1 signalling is influenced by cellular context. Although cry1 was a positive regulator of other PhANGs besides *Lhcb1* regardless of whether chloroplast biogenesis was blocked [5], blocking chloroplast biogenesis severely attenuated the light-induced expression of other PhANGs [57].

gun1 cry1 and *gun1 hy5* synergistically attenuated the plastid regulation of *Lhcb* genes and chloroplast biogenesis, consistent with the integration of light and plastid-to-nucleus signalling [5]. I speculate that a novel plastid signal ‘rewires’ light signalling by affecting the activity of transcription factors that act downstream of photoreceptors. ABSCISIC ACID INSENSITIVE4 (ABI4) acts downstream of GUN1 and helps to downregulate the expression of *Lhcb1.2* by binding CCAC, which overlaps the G box in the *Lhcb1.2* promoter [18]. The plastid regulation of PhANGs appears to require additional transcription factors and promoter elements because ABI4 did not contribute to the plastid regulation of *Lhcb1.1* or *RbcS* [3,58] and CCAC did not contribute to the plastid regulation of an *Lhcb* gene from *Nicotiana plumbaginifolia* when chloroplast biogenesis was blocked with a norflurazon treatment [46]. GOLDEN2-LIKE 1 (GLK1) and PHD-type transcription factor with transmembrane domains (PTM) also contribute to this signalling [3] (see electronic supplementary material, figure S1). The integration of light and plastid-to-nucleus signalling appears to promote chloroplast biogenesis [5], development [39], the accumulation of anthocyanins [39,40,59], photoperiodic responses [59] and programmed cell death [56].

The idea that tetrapyrroles affect the integration of light and plastid-to-nucleus signalling by affecting HY5 [28] conflicts with the finding that *hy5* was a *gun* mutant when chloroplast biogenesis was blocked with lincomycin treatments [5]. Such lincomycin treatments do not activate plastid-to-nucleus signalling that depends on tetrapyrroles [16,18]. However, the finding that feeding the chlorophyll precursor Mg-protoporphyrin IX to mature plants downregulated PhANG expression in wild-type but not in *gun1*, *abi4* and *hy5* [28,60] provides evidence that tetrapyrroles are negative regulators of PhANG expression. These findings are also consistent with tetrapyrroles acting upstream of GUN1 and with tetrapyrroles affecting the integration of light and plastid-to-nucleus signalling within the plastid. By contrast, Woodson *et al*. [22] provided compelling evidence that haem is a positive regulator of PhANG expression. Additionally, the finding that *gun5* upregulates PhANG expression in etiolated *gun5 sig2* seedlings relative to etiolated *sig2* seedlings and that this effect is not observed in light-grown seedlings [19] is consistent with light-regulated development affecting tetrapyrrole signalling.

4. Plastid signals contribute to development

The abnormal leaf development of variegated mutants and reticulate mutants provided early evidence that plastid signals contribute to development. The leaves of reticulate mutants resemble webs or nets because they accumulate significantly different levels of chlorophyll in the vasculature and lamina. Leaves of variegated mutants have green and yellow/white sectors. Variegated and reticulate phenotypes are caused by deficiencies in proteins that affect chloroplast function and are often linked to abnormal leaf development. Thus, these mutant alleles were suggested to disrupt plastid-to-nucleus signalling mechanisms that contribute to leaf development [10,61]. For example, the yellow and white sectors of variegated mutants had abnormal palisade cells [10,61,62] and an attenuated cell division and cell expansion response of palisade cells to excess light [63]. The green sectors of variegated leaves resembled wild-type leaves grown in optimal conditions [62] or resembled wild-type leaves exposed to excess light [64]. Additionally, *immutans* (*im*) had variegated leaves, attenuated biogenesis of non-photosynthetic plastids and short roots. Thus, signals from non-photosynthetic plastids appear to affect development [64]. The distinct PhANG expression phenotypes of variegated mutants are consistent with multiple plastid-to-nucleus signalling mechanisms affecting development [61,65]. The depletion of essential metabolites during their transport from the bundle sheath cells to the lamina was suggested to cause reticulate phenotypes [66] because many of the mutant alleles that cause reticulate phenotypes disrupt amino acid or purine metabolism [10,65–67]. The finding that *scabra3* (*sca3*) exhibited reticulate phenotypes without directly affecting the biosynthesis of essential metabolites [10] provides evidence that metabolic deficiencies may not underpin all reticulate phenotypes.

Dysfunctional plastids caused other developmental abnormalities that are consistent with plastid signals contributing to development, such as pale and elongated leaves, reduced apical dominance [68] and lack of aerial organ development [69]. Blocking chloroplast biogenesis with a norflurazon treatment appeared to affect leaf size in part by inhibiting the transition from cell-proliferation-based morphogenesis to cell-expansion-based morphogenesis [38]. Deficiencies in mitochondrial and chloroplastic RNA helicases affected nuclear gene expression and promoted the formation and function of plasmodesmata [70].

Particular mutant alleles that disrupt plastid-to-nucleus signalling affect development. Mutant alleles that disrupt *GUN1* and the integration of light and *GUN1*-dependent signalling affected the unfolding and expansion of the cotyledons [39,40], the development of epidermal pavement cells and stomata of cotyledons and the elongation of the hypocotyl [39].

Other connections between plastid function and hypocotyl elongation were reported [71,72]. *GUN1* also linked plastid function to shifts in the adaxial–abaxial gene expression that promote the expansion of the leaf lamina [41]. Additionally, *GUN1* contributed to the inhibition of germination by abscisic acid [40]. Similarly, the inhibition of germination by abscisic acid depended on the chloroplast-localized Whirly1 protein [73], which translocates from within the chloroplast to the nucleus [15]. Mutant alleles that activate 3′-phosphoadenosine 5′-phosphate-dependent plastid-to-nucleus signalling [3] also caused developmental abnormalities in leaves, such as short and round leaves with short petioles, undulating leaves, abnormal leaf vasculature and mesophyll cells [74].

In summary, plastid-to-nucleus signalling is integrated with extraplastidic signalling and contributes to a number of processes. The *gun* mutant screen specifically disrupts plastid-to-nucleus signalling mechanisms that are broadly significant. Light signalling and development are modulated by a number of different plastid signals. The influence of plastid signals on development and environmental responses provides optimism that future research on plastid-to-nucleus signalling will help agriculture to adapt to our changing environment.

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