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Review article

Features of cues and processes during chloroplast-mediated retrograde signaling in the alga *Chlamydomonas*

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ABSTRACT

Retrograde signaling is an intracellular communication process defined by cues generated in chloroplast and mitochondria which traverse membranes to their destination in the nucleus in order to regulate nuclear gene expression and protein synthesis. The coding and decoding of such organellar message(s) involve gene medleys and metabolic components about which more is known in higher plants than the unicellular organisms such as algae. *Chlamydomonas reinhardtii* is an oxygenic microalgal model for genetic and physiological studies. It harbors a single chloroplast and is amenable for generating mutants. The focus of this review is on studies that delineate retrograde signaling in *Chlamydomonas* vis a vis higher plants. Thus, communication networks between chloroplast and nucleus involving photosynthesis- and ROS-generated signals, functional tetrapyrrole biosynthesis intermediates, and Ca^{2+}-signaling that modulate nuclear gene expression in this alga are discussed. Conceptually, different signaling components converge to regulate either the same or functionally-overlapping gene products.

1. Introduction

Cellular organization in plants has evolved to maximize sensing machineries and multi-tiered transduction systems that enable their adaptation to and survival in variable habitats. Thus, plants being sessile have developed intricate mechanisms for perceiving a variety of external stimuli generated by a constantly changing environment around them and integrating them to modulate and sustain growth and development, including defensive responses against biotic and abiotic stresses. Ancestral prokaryotes were equipped with full genomic potential to live as independent life forms and survived with autotrophic growth. It is estimated that primitive plastids evolved over a billion years ago via endosymbiosis between cyanobacteria and eukaryotic hosts [1]. Endosymbiosis led to the ingression of chloroplast and chloroplast-function in higher plants, which led to selective reduction in total coding capacity of the organelle via deletion of genes that were no more needed because of endosymbiosis. This led to movement of many genes and their integration within the host's nuclear genome [2–4]. However, the chromist algae followed a different route whose description is beyond the scope of this review.

Subcellular structures in a plant cell include the plasma membrane-cell wall complex, endoplasmic reticulum (ER)-golgi complex, vacuolar compartment, nucleus-nuclear complex, mitochondria, chloroplast(s), and peroxisomes [5]. Plant nucleus encodes a majority of chloroplast-housed proteins – 3500–4000 in number, which are post translationally transported and imported into the chloroplast [6,7]. The chloroplast and mitochondria-housed genomes are responsible for only about 100 open reading frames (ORFs) [8]. The segregation of intracellular genomes demanded that a regulated network of signaling and coordination of bi-directional communication between the nucleus and each organelle be in place for the fool-proof programming of cellular metabolism and survival of the organism. Alongwith such a reassembly of gene networks, selective and specific mechanisms evolved for 'error-prone' targeting of nuclear-encoded proteins to various organelles and vice versa [9,10], to streamline specific gene targeting.

That chloroplasts and nucleus within a cell communicated became apparent when nuclear-encoded cytosolic protein synthesis was found perturbed following an induced repression of plastid protein synthesis [11]. Such an intracellular communication became known as retrograde signaling while the cues from the nucleus that perturb/regulate organellar function(s) are termed anterograde signaling (Fig. 1) [11–14]. In essence, the coding and decoding of organellar message(s) that alter nuclear gene expression and/or cellular metabolism is central to retrograde signaling. Several functional components that are

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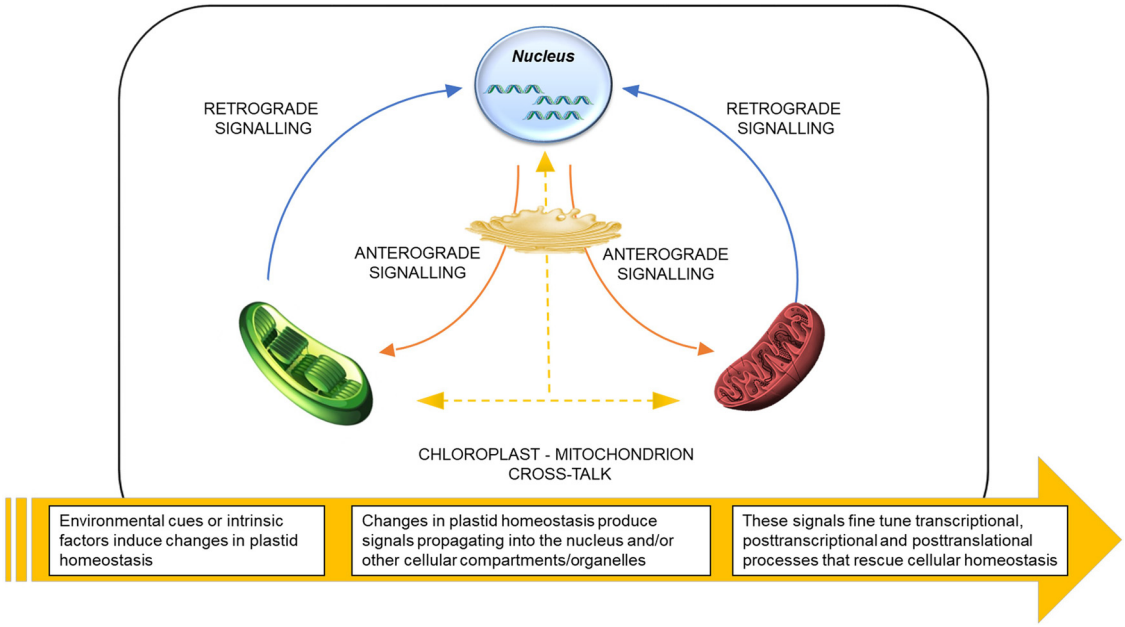

Fig. 1. *Schematic illustration of plant intracellular communication networks*. Imbalance of plastid homeostasis induced by either environmental or intrinsic cues determines which signaling molecules will be synthesized or called upon to transmit information to the nucleus. As a consequence, remodeling of gene expression patterns, and activation of posttranscriptional and posttranslational processes takes place. Anterograde signaling (nucleus to organelle) is represented by orange arrows; retrograde signaling (organelle to nucleus) by blue arrows, and intra-organellar communication by yellow dashed arrows.

associated with a number of retrograde signaling pathways in plants have been identified and the list continues to grow (see for comprehensive reviews [13,15–18]). Some of these have been mentioned here. Alternative splicing frequency was found to enhance in response to changing environmental condition, resulting in the synthesis of different transcripts from a unique gene, thereby affecting nuclear gene expression [19,20]. Such a process was found to positively impact plant development and its response to stress [21]. The redox state of chloroplast-localized photosynthetic-component plastoquinone (PQ) is known to change in response to environmental perturbations and is considered as one of the signals involved in splicing events [22,23]. However, the mechanism of PQ-related input to retrograde signaling remains to be fully elucidated [24,25]. Another suggested component of plant retrograde signaling is the plastid nucleotide binding GUN1 protein, which contains a pentatricopeptide repeat, and may coordinate nuclear photosynthetic gene expression [26] (Fig. 2). Other signals that have been linked to redox regulation and retrograde signaling include state-transitions, photosystem II (PSII) protein turnover and repair, and thioredoxin redox state.

Retrograde signal transduction can emanate or end via components that traverse nucleus, organelles (chloroplasts/mitochondria), and other intracellular complexes (Figs. 1 and 2). Chloroplast is an important site providing light harvesting, photochemistry, and photosynthesis which are among the essential processes necessary for sustenance of life. It has also become apparent that these processes and their fine tuning are quickly adjusted in response to many environmental cues. Such developments have led to highlighting chloroplasts(s) as an environment-sensing organelle. The unicellular oxygenic green alga *Chlamydomonas reinhardtii* contains a single chloroplast and is a recognized experimental model system for experimental biology. Several distinct features of central metabolism and chloroplast-mediated retrograde signaling have been revealed in this organism. Here, we review these features and compare them to other biological models. In particular, we examine gene expression markers (e.g., light harvesting proteins) and molecular transducers (e.g., tetrapyrrole intermediates, reactive oxygen species and calcium ions) involved in cellular homeostasis.

2. *Chlamydomonas reinhardtii* as a model

C. reinhardtii has been used as a model organism for contemporary plant biology research, including regulation and acclimation of the photosynthetic reactions, chloroplast function, and chloroplast biogenesis, a distinctive feature being that its photosynthetic machinery, photochemical reactions and carbon assimilation metabolism are highly similar to that of the higher plants. *Chlamydomonas* offers a short life cycle, well-characterized genetics and fully sequenced genomes. Moreover, molecular tools for easy genetic transformation of its nucleus, mitochondria and chloroplast are available [27–29]. Basic genetic studies are simplified by its vegetative haploid growth and controlled sexual cycle enabling tetrad analysis and identification of induced-mutant phenotypes. Also, there is no need of crosses for obtaining homozygous lines as in *Arabidopsis*. Moreover, unlike higher plants that contain hundreds of chloroplasts, *Chlamydomonas* cell houses a single cup-shaped chloroplast, which occupies almost half of the cell volume. This bodes well for studies on chloroplast retrograde signaling preventing any complexity due to asynchronous messages that could emanate from multiple chloroplasts within a cell. Metabolically, the chloroplast is also the site for the biosynthesis of amino acids, fatty acids, carotenoids and chlorophylls, and assimilation of sulphates and nitrites. A salient kit of protein transporters regulates fluxes and homeostasis of ions and metabolites. These metabolites and transporters are critical in maintaining retrograde signaling and communication between the chloroplast and the rest of the cell.

The internal architecture of the *Chlamydomonas* chloroplast is built by interconnected, functionally specialized compartments where photosynthetic biochemistry is orchestrated [30]. The lobes of the cup-shaped chloroplast contain mature thylakoids that catalyze the light reactions of photosynthesis, while the large base of the chloroplast hosts a specialized pyrenoid enriched in the carbon fixing enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) complexes. Pyrenoid tubules are cylindrical microstructures physically bridging thylakoid stacks to pyrenoids. The spatial separation of light-dependent from light-independent reactions of photosynthesis in the algal cell is an essential adaptation step imposed by the need to extract CO_2 from the water phase. Since photosynthesis is not mandatory for *Chlamydomonas*