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SHORT COMMUNICATION



Dynamic coordination of plastid morphological change by cytoskeleton for chloroplast-nucleus communication during plant immune responses

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ABSTRACT

Considering their sessile life, plants must efficiently coordinate their resources and energy for maintaining their presence in normal living conditions and for defending themselves against environmental threats. Collaboration between multiple subcellular compartments is a common strategy in several biological processes to modify cells' architecture for their growth and development and to respond to acute changes in the environment. When plants defend themselves against microbial pathogens, chloroplasts generate tubular structures – so-called stromules- to facilitate chloroplast movement towards nuclei during innate immunity. Morphological changes and movements of stromules are directed by interactions with microtubule and actin cytoskeleton. Microtubules provide a direction for the stromule extension, while actin filaments restrict stromule retraction which provides a driving force for repositioning of chloroplast near nucleus during plant immune responses. These findings implicated a critical role for stromules in signal transduction from chloroplast to the nucleus in plant defense.

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Text

Eukaryotic cells contain sophisticated subcellular compartmental organization that includes the nucleus, the endomembrane system and small and large organelles encompassing endosymbiotic compartments such as chloroplasts and mitochondria in plants.¹ This compartmentation efficiently coordinates complex intracellular metabolic processes by imposing a physical barrier to sequester metabolites and macromolecules, for controlling their flux between organelles.² Several recent studies proposed that organelles can dynamically interact, and in doing so, contribute to the plant's physiological responses to environmental changes.^{3–5} Under stress conditions, various organelles undergo a change in their morphology and inter-organelle communication, possibly to exchange metabolites, proteins, or lipids to coordinate various signal transduction cascades.^{6–8} Stroma-filled tubular protrusions of chloroplast called stromules provide proximity between the chloroplast and the nucleus for transferring molecules such as reactive oxygen species (ROS) and defense proteins during immune responses.³

Stromules protrude from all types of plastids and observed in several plant tissues, including leaf, shoot, roots, and fruits (reviewed in⁹). Stromules have been observed for more than 80 years, but in-depth studies of their dynamics began after the advent of green fluorescent protein (GFP) to visualize protein location in plant cells by confocal microscopy.¹⁰ The biological function of stromules remains mostly speculative, with initially proposed function in plant stress responsive signaling by

observations that stromule induction is correlated with stress responses.^{11–15} We demonstrated in 2015 a potential function of stromules in plant immunity for the first time.³ In *Nicotiana benthamiana*, stromules are induced when N immune receptor together with a chloroplastic N Receptor Interacting Protein 1 (NRIP1) recognizes p50 effector from *Tobacco Mosaic Virus* (TMV).³ Interestingly, stromules provide a conduit to transfer defense protein, such as NRIP1, and pro-defense signaling molecule, such as hydrogen peroxide (H₂O₂) into the nucleus.³ Remarkably, stromule induction is a common immune response to various pathogens.³ In addition, stromules can be induced by exogenous treatment of pro-defense molecules, such as salicylic acid (SA) and H₂O₂,³ implicating that stromules might be involved in a general defense mechanism. Stromules rarely allow exchange of stromal components between plastids^{16,17} and frequently associate with the plasma membrane, endoplasmic reticulum, nuclei, and mitochondria,^{4,18,19} suggesting the role of stromule in inter-organelle communication.

Recently, using extensive time-lapse imaging we showed that stromules are very dynamic and they maintain their lengths by constant repetition of the extension and the retraction through coordinated function of microtubules (MT) and actin cytoskeleton.¹⁹ In *N. benthamiana* leaf epidermis, 5–20% of chloroplasts have stromules under normal growth conditions.^{3,19} However, this frequency fluctuates significantly since the time-lapsed image sequences revealed that even stromules from individual chloroplast under normal growth conditions in a cell repeatedly extend and retract.¹⁹ Stromules in normal growth

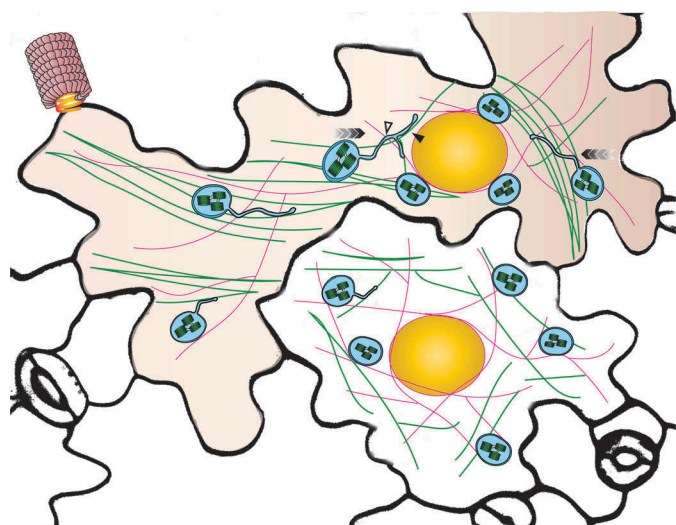


Figure 1. Schematics of stromule-directed perinuclear clustering of chloroplast controlled by microtubules and actin filaments.

In Tobacco Mosaic virus (TMV) infected cell (shaded), chloroplasts generate long stromules by constant extension and retraction events. These stromules extend along microtubules (green lines) and anchored (open arrowhead) to actin filaments (magenta lines). Microtubules become longer, less curved, and more parallel to each other in infected cells compared to uninfected cells (white). The anchor point (black arrowhead) restricts the stromule retraction providing a driving force for chloroplast body movement (motion arrows for direction). Actin filaments around the nucleus anchor the tip of stromule at the proximity of the nucleus and then stromules retract along the microtubule, resulting in chloroplasts' bodies cluster around nucleus. This clustering might provide the close location between chloroplasts and nucleus, allowing the efficient inter-organellar communication during plant immune responses.

condition are morphologically diverse, and range from short to long and thin to wide. Recent evidence indicates that MTs and actin filaments (AFs) differentially organize stromule dynamics in plant cells.¹⁹ Previously, AF inhibitor treatments in non-green plant tissues resulted in the reduction of stromule frequency.²⁰ Additionally, myosin inhibitor 2,3 butanedion 2-monoxime (BDM) treatment affected stromule movement and length, implying that AFs might have a regulatory role for stromules.²¹ However, treatment with MT inhibitor amiprophosmethyl (AMP) also reduced stromules²⁰ suggesting that both MTs and AFs might regulate stromule morphology and motility. However, detailed mechanisms of movement were not studied. In Kumar et al. (2018), contribution of MTs and AFs was revisited with comprehensive live cell image analyses using *N. benthamiana* leaves during normal growth conditions as well as during plant immune responses.¹⁹ Surprisingly, co-visualization of the stromule marker and cytoskeleton marker over time revealed that MTs but not AFs provide tracks for stromule extension.¹⁹ Non-invasive MT inhibition treatment with minimal effect to the AF organization reduced stromule length dramatically by increasing stromule retraction rate. Furthermore, stabilization of MTs through silencing of γ -tubulin complex protein 4 (GCP4) homolog in *N. benthamiana* resulted in increased stromule frequency and stromule length by reduced extension and retraction velocities.¹⁹ On the other hand, AFs provide anchor points to stromules and the body of chloroplasts. Remarkably, some of the chloroplast movement occurs in a stromule-dependent manner. Time-lapsed image analysis revealed that stromules anchored to

the AF directs chloroplast movement to the anchor points by retraction events.¹⁹

During plant immune responses, chloroplasts cluster around the nucleus.^{3,19} In *N. benthamiana* leaf epidermal cells, more than half of observed cells contain nuclei which are surrounded by more than 4 chloroplasts during immune response.¹⁹ Interestingly, this perinuclear clustering of chloroplasts during plant immunity is dramatically affected by the disruption of AFs by cytochalasin D drug treatment but not by MT inhibitor Oryzalin treatment.¹⁹ These findings suggested that stromule-directed movement of chloroplast bodies was predominantly regulated by AF during plant immune responses, providing the proximity between chloroplast and nucleus for efficient communication during plant defense. It is possible that previously a role for MTs was not uncovered due to the timing of the inhibitor treatment and future research will need to be conducted to determine if MTs play an initial role in guiding stromules to nuclei.

Organelle-organelle interactions have been observed in plant cells under different stress conditions (reviewed in¹). Extremely high light irradiation generates extensions from peroxisomes, called peroxules, which might connect to other organelles, although the roles of these connections remain unknown.²² Biochemical studies have shown that mitochondria and chloroplasts exchanged lipid contents under phosphate starvation conditions, with no visual evidence of real interaction between mitochondria and chloroplast.²³ Interestingly, ROS translocation from the chloroplast body to the nucleus without stromule induction has been observed in cells exposed to high light.⁵ These reports indicate that plant organelles might follow well-orchestrated mechanisms to change their morphology and motility in response to environmental perturbations. Identification and characterization of regulatory components that mediate induction of change in organelle morphology is required for better understanding of dynamic contribution of organelles in plant responses to the environmental stresses.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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