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Tubular extensions of plant organelles and their implications on retrograde signaling

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Abstract

Tubular extensions emerging from plastids, termed stromules, have received renewed attention due to advancements in imaging techniques. Stromules are widespread in plant and algal species; however, their role in organelle communication and physiology is yet to be elucidated. Initially, stromules were thought to facilitate interplastid communication; however, this proposition is still debated. Stromules with diameters of 0.3-0.8 µm enable protein movement via diffusion and Adenosine Triphosphate (ATP)-dependent transport. Stromule formation is more evident in non-photosynthetic plastids and is induced by various biotic and abiotic stresses, suggesting the involvement of stress-triggered signal transduction via phytohormones and redox changes. Recent studies have emphasized the significance of stromules in plant immunity, especially in response to viral and bacterial effectors, where they serve as conduits for the transport of retrograde signaling molecules from the plastids to the nucleus. Peroxules and matrixules, extending from peroxisomes and mitochondria, respectively, are parallel tubular extensions that were originally found in plant cells, while similar structures also exist in mammalian cells. The response of these extensions to stress may contribute to the management of Reactive Oxygen Species (ROS) and organelle proliferation. This short review discusses the potential roles of the organelle extensions in retrograde signaling pathways.

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Introduction

Intricate inter-organelle communication is crucial to cellular functions, enabling cells to respond to environmental stimuli and orchestrate physiological responses. Among the diverse mechanisms that facilitate this communication, tubular extensions from plastids, peroxisomes, and mitochondria have attracted significant scientific interest. Stromules are slender plastid extensions that were initially proposed to mediate inter-plastid communication but remain a topic of debate. Additionally, peroxules from peroxisomes and matrixules from mitochondria exhibit similar dynamic behaviors, potentially signifying shared functions. It is quite interesting that the three different organelles share the property of extending tubules, implying physiological advantages of assuming this form. As discussed below, there is also a known commonality in that these extensions from each organelle are often induced by some kind of stress. This raises the possibility that these extensions may be a manifestation of the cell's response to stress, and furthermore, that the extensions may function as "passageways" carrying some stress signals. This review summarizes the current understanding of these tubular extensions, shedding light on their implications in organelle dynamics, stress responses, and retrograde signaling pathways.

Stromules: potential conduits for the transport of retrograde signaling molecules from plastids

Thin tubular extensions that extend from plastids in plant and algal cells were described in the literature as early as the 1880s[1]

but were neglected until their re-discovery using stroma-targeted Green Fluorescent Protein (GFP).[2] These plastid extensions were shown to contain stroma and were bound by double membranes (inner and outer envelopes of plastids). Thus, they were termed "stromules" (*i.e.,* stroma-filled tubules).[3] Currently, it is widely accepted that stromule formation is a general and ubiquitous feature of plastids, having been reported in various species of plants and green algae.[4]

The initial study on stromule re-discovery[2] argued, as the title states, that stromules could allow the exchange of molecules (*e.g.,* proteins) between plastids, thereby establishing an "inter-plastid communication system." In fact, images showing connections between two (or more) plastids, mediated by stromules, have been recorded.[5] If stromules play a role in relaying information from the plastid body to other compartments, understanding the types and sizes of molecules that can flow through them is critical. Stromules, which are usually 0.3-0.8 µm in diameter, allow the movement of protein complexes of at least 550 kDa,[6] whereas much larger structures such as thylakoids, organellar DNA (as the form of nucleoids), and ribosomes are excluded from stromules.[7] The motion of GFP in stromules was analyzed using fluorescence correlation spectroscopy.[8] This revealed that GFP moved in stromules via two distinct modes: diffusion- and Adenosine Triphosphate (ATP)-dependent active transport (at approximately 0.12 µm/s). The diffusion coefficient in stromules was found to be 50-100 times lower than that in the cytosol and aqueous solution, indicating a high viscosity of the stroma within stromules. Therefore, active transport is likely the principal mechanism for long-distance protein movement in stromules. Although the underlying mechanism of this active transport remains to be elucidated, live imaging has demonstrated the formation of blebs that move along the stromules.[9] Thus, the observed blebs might serve as "packets" for protein cargos during their long-distance active transport via stromules.

In general, stromule formation in plants is more pronounced in non-green (non-photosynthetic) plastids than that in chloroplasts.[10] Moreover, stromules are induced by various biotic and abiotic stresses, implying their role in stress-associated signal transduction from the plastid body. Stromule-inducing stresses include the exogenous application of hydrogen peroxide (H_2O_2) and salicylic acid (a defense-related phytohormone); high temperatures; excess sucrose and glucose; low phosphate; drought; high salinity; and infections by viruses, bacteria, fungi, and oomycetes.[11-14] It is likely that these stresses do not necessarily affect stromule induction directly and individually; however, they may act through the integration of information by phytohormones, such as abscisic acid[15] and salicylic acid,[12,16] as well as the change of redox status.[17,18]

Among the studies on the relationship between stress and stromules, the role of stromules in plant immunity has received particular attention in recent years, following the publication of a landmark paper by Caplan *et al*.[12] This study is the first to show that both the tobacco mosaic virus effector protein p50 and *Xanthomonas campestris* (a plant-disease-causing bacterium) effector protein AvrBS2 along with its receptor BS2, known to activate Hypersensitive Response Programmed Cell Death (HR-PCD) in plants, induces excessive formation of stromules. Moreover, the frequent and close associations of stromules with the nuclear envelopes in *Nicotiana benthamiana* plants were also revealed. This suggests that the induction of stromules is a general response during the beginning phase of HR PCD caused by viral and bacterial effector proteins. It also implies a possible role of stromules as conduits for retrograde-signaling-molecule transport from plastids to the nucleus during the plant immune response. Caplan *et al*.[12] further demonstrated the plastid-to-nucleus movement of H_2O_2 and N Receptor-Interacting Protein 1 (NRIP1, the plastid-localized defense protein)[19] in *N. benthamiana* during a p50-induced defense. The level of H_2O_2 was monitored in the plastid stroma- and nucleus-targeted versions of the fluorescent sensor protein HyPer.[20] The stroma-targeted HyPer signal was abundant at the interface between the plastids/stromules and the nucleus. In addition, when H_2O_2 bursts were induced in the perinuclear plastids by laser scanning of the region of interest, the nuclear-targeted HyPer signal increased. This study also showed evidence for the possible translocation of NRIP1 from the plastid stroma to the nucleus by employing an elaborate experimental strategy. NRIP1 was fused with the Nuclear Export Sequence (NES) and Cyan Fluorescent Protein (CFP) N- and C-terminally, respectively. The NES was supposed to be cleaved off after the import of NES-NRIP1-CFP into the plastid stroma, where the N-terminal transit peptide of imported proteins is excised. This system ensures that CFP fluorescence from the nucleus is derived from the stroma-localized NRIP1-CFP because NES-NRIP1-CFP cannot enter the nucleus due to the presence of NES. In this experimental system, an increased fluorescence signal from NRIP1-CFP was observed in the nucleus during the p50-induced defense response. Stress-triggered plastid-to-nucleus protein migration was also observed in a redox-sensitive transcriptional coactivator, Non-expressor of Pathogenesis-Related genes 1 (NPR1), which is considered to be involved in the expression of retrograde-signaling-related nuclear genes under salt stress and exogenous H_2O_2 application.[21] Intriguingly, NPR1 has been suggested to be loaded from the plastid stroma into cytoplasmic vesicles, possibly arising from the tip shedding of stromules.[9] Together, these results indicate the possible role of stromules as conduits for retrograde signaling.

Currently, there is no evidence for the fusion of plastid envelope membranes with the nucleus.[22] Therefore, how signaling molecules inside double-membrane-bound plastids/stromules move into or at least convey the signal to the nucleus (which is also surrounded by double membranes) remains an open question. At the ultrastructural level, the tips of stromules and main bodies of plastids were in close contact, but separated by a thin layer of cytosol from the nuclear envelope during p50-triggered HR-PCD.[12] At the sites of those connections, numerous perforations were observed on the nuclear envelope; however, it was unclear whether these perforations were nuclear pores. In addition to the means of protein-mediated retrograde signaling, a concept of "Reactive Oxygen Species (ROS) wave" or "ROS-induced ROS production" was recently proposed for ROS-mediated inter-organellar signaling.[23] ROS molecules such as H_2O_2 cannot move long distances because they are rapidly scavenged by cells. According to the concept of the ROS wave, it is not the ROS molecules themselves that are propagated between organelles but the change in the status of ROS (*e.g.*, rapid overaccumulation of ROS known as "ROS burst"). In this scenario, it is assumed that a small portion of ROS generated excessively inside plastids is first exported to the cytosol via aquaporins (or other kinds of transmembrane pore proteins). Then, it diffuses over a short distance of the cytosol at the organelle-organelle connection sites and enters the neighboring organelles (including the nucleus) via aquaporins or nuclear pores,[24] triggering the next round of ROS burst therein.[23] However, in relation to this concept, the identity of the perforations on the nuclear envelope at the junction of plastids/stromules,[12] needs further clarification.

Peroxules, matrixules, and mitochondrial nanotunnels: tubular extensions of peroxisomes and mitochondria

Peroxisomes and mitochondria also have the ability to intermittently extend thin-membrane tubules, similar to stromules, which were named peroxules and matrixules, respectively.[25] Though the dynamic nature of these organelle extensions was initially studied extensively in plant cells,[26] similar structures were later reported to exist in mammalian cells.[27] This implied that the formation of thin tubules from peroxisomes and mitochondria may be a common feature shared among multicellular eukaryotes. Similar to stromules, the formation of peroxules in plant cells is induced by exogenous H_2O_2 application.[28] Additionally, high light exposure[29] and treatment with Cd,[30] which lead to ROS generation, could also induce peroxule formation. In the plant response to Cd, nitric oxide is necessary for peroxule induction.[31] Moreover, the peroxisomal protein Peroxin 11a (PEX11a) is also essential for Cd-triggered peroxule formation in *Arabidopsis thaliana*.[30] One of the proposed functions of peroxules is the rapid scavenging of ROS, facilitated by an increase in the surface area to volume ratio via tubulation.[27] Peroxules are also involved in protein transport such as the transfer of the Sugar-Dependent1 (SDP1) lipase, which hydrolyzes triacylglycerols into fatty acids and glycerol, from the peroxisomal membrane to the lipid body in plant cells.[32] Alternatively, the formation of peroxules might be a preliminary phase of peroxisome proliferation by fission. This is validated by the observation in plant cells that prolonged ROS stress, such as exposure to exogenous H_2O_2 application or intense light, causes a change in the peroxisome morphology from spherical bodies (emanating peroxules) to vermiform peroxisomes, which eventually break up into smaller peroxisomes.[28] Likewise, exposure to H_2O_2 and ultraviolet light induced complete tubulation of peroxisomes, possibly followed by fission, in cultured human hepatoblastoma cells,[33] whereas no peroxule-like structures were observed by Schrader *et al*. To the best of the author's knowledge, peroxules have been observed neither in control (non-stressed) nor in oxidatively stressed mammalian cells to date, while a peroxule-like structure was reported to appear in Peroxin 5 (PEX5)-deficient fibroblasts overexpressing the mitochondrial Rho GTPase MIRO1.[34] The author refers the reader to recent excellent reviews by Sandalio *et al.*[35,36] for further detailed information on the stress-dependent formation of peroxules in plants.

Mitochondria are dynamic organelles that move along the cytoskeleton, interact with other organelles, and change their morphology via elongation, fission, and fusion.[37] Dynamic changes in mitochondrial shape have been linked to various physiological and stress conditions, including cell type, nutrient supply, cell death, disease, impaired oxidative phosphorylation, damaged mitochondrial DNA, aging, heat stress, oxidative stress by ROS, and bacterial and viral infections.[38,39] Additionally, mitochondria transiently form tubular membrane extensions in plant cells, termed matrixules[25,40]. Similar mitochondrial extensions in mammalian cells, referred to as "mitochondrial nanotunnels",[41,42] were reportedly induced by various stress conditions, such as dysregulation of calcium homeostasis,[43] exposure to Mn,[44] and inhibition of respiratory complex III.[45] The functions of such stress-induced extensions remain unelucidated, although it has been speculated that the increased interconnectivity of mitochondria by "nanotunneling" might exert a protective effect.[27] In plant cells, in addition to the double-membrane-bound, matrix-containing matrixules, outer mitochondrial membrane-derived, matrix-free protrusions have also been observed.[46] This structure, designated as Mitochondrial Outer-membrane Protrusions (MOPs), were more prominent in senescent leaves and after dark treatment, suggesting a senescence-associated function of MOPs.

As with plastids, retrograde signaling from the mitochondria to the nucleus has been well studied, mainly in yeast and mammalian cells.[47] Additionally, a peroxisome-derived retrograde signaling pathway, potentially utilizing signaling molecules such as phytohormones, ROS, and reactive nitrogen species, has been presumed in plant cells.[48] Currently, it is unknown whether the mitochondrial and peroxisomal membrane extensions play a role in retrograde signaling. In mitochondrial nanotunnels of mammalian cells, the interconnection and content exchange between non-adjacent mitochondria are considered to play a principal role, rather than the signaling conduits toward the nucleus.[41] Moreover, light-irradiation-induced peroxules interact physically with spherical mitochondria in plant cells.[29] This observation led to a working model in which peroxules increase the proximity between mitochondria and peroxisomes, thereby facilitating the peroxisomal recruitment of division proteins commonly employed in mitochondria and peroxisomes (*e.g.*, dynamin-related protein, DRP3). This eventually results in an increased peroxisomal population, which is advantageous for tackling high light-triggered ROS stress.

Conclusions

The exploration of tubular extensions from various plant organelles, such as stromules, peroxules, and matrixules, has provided intriguing insights into organelle dynamics and their responses to environmental stimuli. Although the functions of these extensions require further elucidation, their presence underscores the complexity of inter-organelle communication and their importance in plant biology. Investigations into their roles during stress responses and their potential involvement in retrograde signaling pathways hold promise for uncovering novel aspects of cellular communication. As the research advances, new avenues for further exploration will emerge. Exploring the molecular machinery underlying the transport of signaling molecules within these extensions and deciphering their regulatory mechanisms are paramount. Additionally, understanding how these extensions interface with other cellular structures such as the nucleus and other organelles will shed light on the broader implications of their roles in cellular communication.

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