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Commentary

Role of ArF-BAR in Fungal Pathogenesis

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ABSTRACT

The establishment of invaginations and protrusions in the plasma membrane and the shaping of intracellular organelles require equilibrium between generation and stabilization of membrane curvature. The intrinsic shape of BAR-(Bin–Amphiphysin–Rvs) superfamily proteins helps them to bind and generate curvature in the plasma membrane. Thus, evolutionarily conserved BAR domain-containing proteins act as the central regulator of membrane dynamics. Loss-of-function mutation of the F-BAR domain-containing gene in *Ascochyta rabiei* (ArF-BAR) results in compromised virulence. ArF-BAR participates in various cellular functions including endocytosis, actin polymerization, oxidative stress tolerance, penetration of the fungal hyphae in the host tissue, formation of septa, and secretion of a fungal effector. ArF-BAR functions downstream to stress-responsive transcription factor ArCRZ1. Overall, understanding the role ArF-BAR unravels the multifaceted interplay underlying endocytosis and fungal virulence.

Keywords: Ascochyta rabiei; Planta; Menadione; Fungal effector

DESCRIPTION

For any living cell, dynamics of micro- and macro-molecules are required to control vital cellular activities such as sensing environmental cues, cellular uptakes, signal transduction, transportation, and allocation of nutrients at different cytosolic compartments. Thus, collectively, membrane trafficking controls the fate of cells. The important events of vesicular dynamics, viz. endocytosis, and exocytosis help in sensing environmental cues and help the invading pathogen counter host-generated stress. Endocytosis is a cellular process by which cells internalize macromolecules, surface proteins, and associated membrane receptors and initiates downstream signaling. Through endocytosis, cells communicate with one another and their surroundings. Endocytosis is a vital process that allows cells to maintain the dynamics of membranes and proteins embedded in membranes and helps facilitates invagination. Various components are extensively involved in endocytosis, such as phosphatidylinositol phosphate, clathrin, actin, and most importantly, BAR-domain protein recruited at the membrane to facilitate invagination. BAR-domain Superfamily proteins are categorized into four distinct subfamilies: a classical BAR or N-terminal-BAR (N-BAR), extended-FCH-BAR (F-BAR), IMD/Inverse BAR (I-BAR), and Pinkbar [1]. In yeast, animals, and plants, the mechanism of endocytosis is extensively studied; however, understanding the intricate mechanism of endocytosis and endosomal trafficking in the growth and virulence of phytopathogenic filamentous fungi is emerging.

In the natural environment, amidst shifting ecological conditions such as temperature, drought, and salinity, plants are accompanied by several diverse microorganisms. Among various biotic threats, fungal pathogens with a share of 70-80% of an annual loss are the most devastating and cause huge yield loss [2]. Initially, during host-pathogen interaction, the fungal pathogens activate the defense response of the host. On the other hand, to subvert host-immune response, fungal pathogen secretes an arsenal of specialized molecules called effector proteins. However, the elongated hyphal architecture comprising the increased distance between the sub-apical nucleuses and invading tip poses a challenge for the secretion of fungal effectors. Fungi overcome the intracellular challenge through long-range signaling mediated by vesicular dynamics. The

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ancient process of vesicular dynamics serves as the regulatory mechanism for various cellular processes.

Ascochyta rabiei is a necrotrophic fungal pathogen that infects the foliar parts of the plant. It is a causal organism of the Ascochyta blight (AB) disease of chickpea. AB disease has unique symptoms of concentric rings in the lesions on leaves, stems, and pods [3]. Recent studies from Sinha et al., revealed the the F-BAR domain-containing gene from *A rabiei* (ArF-BAR) is indispensable for the virulence of *A. rabiei* [4]. ArF-BAR is involved in various cellular functions. ArF-BAR is a potential candidate for fungal virulence, as the mutant (Δarf-bar) showed severely compromised AB symptoms during in *planta* infections. ArF-BAR functions during the early phase of infection, as the ArF-BAR transcript is upregulated in the initial hours of in *planta* infection. In *A. rabiei*, the nature of the F-BAR-domain in ArF-BAR is evolutionarily conserved, as it is extensively involved in endocytosis. The *Δarf-bar* mutant showed impairment in internalization of styryl type dye FM4-64 widely used for membrane internalization studies. Also, the number of actin patches that participate in endocytosis is reduced in the mutants. A Calcineurine responsive zinc-finger transcription factor, ArCRZ1, binds to the Cis-regulatory element of ArF-BAR and regulates the expression of ArF-BAR. Loss of functional copy of ArCRZ1 (Δarcrz1) exhibits a reduction in disease symptoms during in *planta* infection. Δarcrz1 phenocopies Δarf-bar in virulence. In the complementation strains, where Δarf-bar is complemented with ArF-BAR; and Δarcrz1 is complemented with ArCRZ1 the virulence of the mutants are restored similar to that of the wild-type *A. rabiei*.

ArF-BAR is specifically localized at the growing hyphal tip and septa. Proper arrangement of septa is required for the maintenance of normal hyphal architecture to promote pathogenicity. The first cell formed from the growing end of the hyphal tip remains elongated in the *Δarf-bar* mutant compared to the WT. Loss of ArF-BAR results in delaying of septa formation, as the *Δarf-bar* mutant lacked regularly spaced septa. During host-pathogen interaction, there is an outburst of reactive oxygen species [5]. ArF-BAR promotes oxidative stress tolerance in *A. rabiei* during infection. The *Δarf-bar* mutants are extremely sensitive toward oxidative stress inducers such as menadione and H_2O_2. Thus, ArF-BAR has an imperative role in oxidative stress tolerance of A. rabiei. In response to oxidative stress, pathogens deliver an array of effector proteins. Here, in *A. rabiei*, ArF-BAR mediates the secretion of a candidate effector protein Ar93. Unlike *Δarf-bar* mutants, the presence of Ar93 has been exclusively observed in the culture filtrate of wild-type (WT) fungus. However, the expression of Ar93 in tissue lysate of *Δarf-bar* mutant is similar to that of WT. Overall; the study further supports the potential role of ArF-BAR protein in the secretion of fungal effectors.

These shreds of evidence suggest that the ArCRZ1 regulated F-BAR domain-containing protein is involved in vesicular trafficking and has a potential role in oxidative stress tolerance, hyphal architecture maintenance, and regulates the secretion of fungal effectors that collectively help in countering the host defense systems and contribute to fungal virulence. Further, the study can explore targets of such virulence factors in the host, and a pathway of regulation of virulence can be discovered in the host.

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