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Nuclear dynamics and programmed cell death in *Arabidopsis* root hairs

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ABSTRACT

In this paper we demonstrate the coupling of nuclear migration to the base of *Arabidopsis* root hairs with programmed cell death (PCD). Nuclear migration and positioning are fundamental processes of eukaryotic cells. To date, no evidence for a direct connection between nucleus migration and PCD has been described in the literature. Based on the findings of our previous study, we hereby further establish the regulatory role of caspase-3-like/DEVDase in root hair death and demonstrate nuclear migration to a position close to the root hair basement during PCD. In addition, continuous observation and statistical analysis have revealed that the nucleus disengages from the root hair tip and moves back to the root after the root hair grows to a certain length. Finally, pharmacological studies have shown that the meshwork of actin filaments surrounding the nucleus plays a pivotal role in nuclear movement during root hair PCD, and the basipetal movement of the nucleus is markedly inhibited by the caspase-3 inhibitor, Ac-DEVD-CHO.

1. Introduction

Tubular-shaped root hairs are essential for nutrient and water uptake, as well as anchorage of a plant to the soil [1]. As a single-cell model, root hairs are easily visualized and accessible to a variety of experimental manipulations. Therefore, hair cells in the root epidermis have been used for more than a century as a simple model for studies on cell specification, differentiation, and polarity [1–3,12].

The development of *Arabidopsis* root hairs can be divided into four phases: i) cell fate specification, ii) initiation, iii) subsequent tip growth, and iv) maturation. It has been well demonstrated that these four phases involve different cellular and genetic processes [2,4]. During the cell fate specification phase, the epidermal cells that are in contact with two underlying cortical cells (in the H position) form root hairs, whereas epidermal cells overlying a single cortical cell (in the N position) develop into non-hair cells [5–7]. This position-dependent differentiation results in a striped pattern of hair cell files along the long axis of the root [7]. At the subsequent initiation phase, Rho-related GTPases (or Rop) move along the cortex of trichoblasts in the root epidermis to the site of root hair formation, initiating a protrusion or bulge [8], and secretory vesicles containing new membranes and cell walls are transported to the tip of the bulge. Then, the root hair enters the third developmental phase: tip growth, where the apical cytoplasmic area of the root hair is densely packed with endoplasmic reticula (ER), Golgi cisternae, and mitochondria, whereas at the extreme apex, a high density of vesicles emerge [9–11]. After the root hair grows to a certain length, it enters the final maturation phase, in which a large vacuole invades the extreme apex of the root hair [3,12].

The position of the nucleus in the hair cell constantly changes according to the developmental phase of the root hair. Before the initiation stage, the nucleus is in the trichoblasts of the root epidermis, and thereafter, during bulge formation and sustained tip growth, the nucleus migrates to the tubular-shaped hair cell [13]. The nucleus moves forward during root hair elongation and is located at a distinct distance from the apex during tip growth [14–16]. Moreover, through an optical trap technique, Ketelaar et al. demonstrated that nuclear movement is a component of the growth machinery that is involved in root hair elongation [16]. After growth arrest, the nucleus moves away from the tip and eventually obtains a random position in the hair [16].

Root hair cells survive for only 2 to 3 weeks and then die off, however, so far, studies on root hairs have only focused on the four developmental phases, leaving the mechanisms underlying root hair death elusive. Shishkova and Dubrovsky [17] found that nuclear DNA undergoes fragmentation (a hallmark feature of PCD in plants)in dead root hairs of Sonoran Desert Cactaceae, indicating that after growth arrest, root hairs die via an intracellular program. PCD is an active process controlled by certain genes to

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kill cells. Several important processes relating to plant development (e.g., vessel element promotion, disassembly of suspensor cells, detachment of root cap cells, stamen dehiscence, petal senescence, and morphological differentiation of leaves) result from PCD [18–27]. Our preliminary investigations on the cellular characteristics of *Arabidopsis* root hair death have shown the involvement of protoplast retraction and nuclear DNA fragmentation, both are considered hallmark features of apoptotic-like programmed cell death in plants. Therefore, we propose that the death of *Arabidopsis* root hairs belongs to PCD, which is mediated by initiation of PCD-related genes [28].

Based on the results of our previous research, we hereby further determined that caspase-3-like/DEVDase regulated *Arabidopsis* root hair PCD. In addition, we studied the coupling of nuclear migration to the base of root hairs with the PCD process. Finally, the meshwork of actin filaments surrounding the nucleus was investigated in terms of its pivotal role in nucleus movement during root hair PCD.

2. Materials and methods

2.1. Plant growth

Seeds of *Arabidopsis thaliana* were surface sterilized and grown vertically on semi-solid half-strength Murashige and Skoog (or 1/2 MS) agar plates supplemented with 3.0%

sucrose in a controlled growth room with 16 h light: 8 h dark cycles at 22 °C ± 2 °C.

2.2. Pharmacological studies

All chemicals were purchased from Sigma (St. Louis, MO, USA) unless otherwise indicated. Casepase-3 inhibitor Ac-DEVD-CHO and latrunculin B (LatB) were dissolved at final concentrations of 10 and 1 mg/mL, respectively, in anhydrous dimethyl sulfoxide (DMSO), with the amount of DMSO not exceeding 0.1% of the total volume of the solution. The appropriate amounts of stock solutions were added.

2.3. NaCl treatment

According to Hogg et al. [29], six-day-old *A. thaliana* seedlings were incubated in 6-cm Petri dishes containing 5 mL of 100 mM NaCl solution for 5 min, followed by two washes with sterile 1/2 MS solution. Afterwards, seedlings were kept in constant light at room temperature for 24 h until scoring.

2.4. Dye loading

The root hairs were stained with 1 μg/mL solution of FDA and 2 μg/mL DAPI by direct addition of diluted solutions to the culture medium. After 5-min incubation, the medium containing the dye was washed three times with fresh liquid medium containing one-half strength Murashige and Skoog salts and immediately examined under fluorescent light.

2.5. Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) assay

A TUNEL assay was performed with a TUNEL apoptosis detection kit (DeadEnd Fluorometric TUNEL System; Promega, USA). Briefly, 8 days after germination, seedlings were fixed in 4% (w/v) paraformaldehyde overnight. Then, the fixed seedlings were treated with a disassociation solution containing 1% (w/v) cellulose R-10 and 0.1% (w/v) pectinase Y-23 for 30 min, followed by rinsing in PBS for three times. Then, the seedlings were treated with 0.2% Triton X-100 for 30 min. The seedlings were then manipulated according to the manufacturer's instructions.

3. Results

3.1. Effects of caspase-3 inhibitor Ac-DEVD-CHO on root hair development

According to our preliminary study, dead hair cells show typical features of PCD, including protoplast shrinking and DNA fragmentation [28]. After death, tubular-shaped root hairs finally twist (Fig. 1a). To determine whether *Arabidopsis* root hairs undergo PCD, the effect of a caspase-3 inhibitor (Ac-DEVD-CHO) on root hair development was examined in seedlings, which had been previously demonstrated to block PCD in various eukaryote species [30–32]. A culture medium with different concentrations of Ac-DEVD-CHO (10 nM, 20 nM, and 100 nM) was used, and the percentage of viable root hairs was counted after 6 days of culture. The results showed that root hairs in a medium with 100 nM Ac-DEVD-CHO were abnormal (data not shown), whereas those with 10 nM or 20 nM Ac-DEVD-CHO showed no side effects, thereby indicating that a high concentration of Ac-DEVD-CHO is toxic to cells. Therefore, culture media with 10 nM or 20 nM Ac-DEVD-CHO were utilized in subsequent experiments. Fig. 1b shows that in all three replicates, 20 nM Ac-DEVD-CHO significantly increased the survival rate of root hairs ($P < 0.05$), and 10 nM Ac-DEVD-CHO significantly increased the survival rate of root hairs in two repeat experiments. This effect might be attributable to the lower concentration of the inhibitor. In summary, the results showed that caspase-3 inhibitor, Ac-DEVD-CHO, decreased the death rate of *Arabidopsis* root hairs, thus indicating that full-grown root hairs initiate a PCD process that could be reverted by a caspase-3 inhibitor.

3.2. TUNEL-positive nuclei in natural death root hairs are located at the base

The TUNEL assay was performed to detect cleavage of nuclear DNA in root hairs. The majority of TUNEL-positive nuclei in natural death root hairs were located close to the base of the hairs (Fig. 2d–f). To quantify the above phenomenon, a total of 23 TUNEL-positive root hairs were examined. We measured the length of the total root hair (FL) and the distance between the nuclear center and the base of the root hairs (NL), separately, and the ratio of NL/FL was calculated to determine the position of the nucleus. Statistical analysis showed that the ratio of more than 78% TUNEL-positive root hairs was <0.5 (Fig. 3), indicating that most of the nuclei in natural death root hairs were located near the base.

3.3. Position of nuclei in growing, full-grown, and dead Arabidopsis root hairs

To further explore the movement of nuclei during the PCD process in root hairs, a ProAAC1:H2B-tdTomato transgenic line whose nucleus can be conveniently observed via fluorescence microscopy was used [33]. Then, fluorescein diacetate (FDA) staining was performed to determine cell viability, and the position of the nucleus in growing, full-grown, and dead root hairs was measured, respectively. The tips of growing root hairs (FDA-live cells, green) contained secretory vesicles, whereas the full-grown root hairs (FDA-live cells, green) showed a large vacuole occupying the entire extreme apex area. Moreover, the nuclei were fusiform in shape in the growing root hairs, whereas in full-grown root hairs, the nuclei were shorter. In dead root hairs, some aggregates emerged, and the nuclei were round in shape (Fig. 4). FL and NL were measured separately, and the ratio of NL/FL was applied