

Vacuole Biogenesis in Germinating Seeds



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Abstract

In germinating seeds, vacuole provides the beginning of cell elongation in embryo organs. Three patterns of vacuole biogenesis are demonstrated during seed imbibition, namely preservation of vacuoles formed earlier at seed maturation; restoration from protein-storage vacuole; de novo formation from provacuoles in endoplasmic reticulum.

Keywords: Seed; Embryo germination; Cell elongation; Vacuole; Provacuole; Protein-storage; vacuole and de novo

Abbreviations: PSV: Protein-Storage Vacuole; TIP: Tonoplast vacuolar aquaporins

Introduction

Vacuole is a cell organelle presenting only in plants, being a structural component distinguishing a plant cell from an animal cell. Vacuole represents a cavity inside the cytoplasm surrounded by a membrane (tonoplast) and filled with water and some dissolved substances. Their existence in plant cells is directly due to the immobile way of plant living and the necessity to compensate immobility by increasing cell length and surface in order to enlarge their contacts with soil water and mineral elements (roots) and light (leaves). With plant age, the contents of vacuole changes, in young cells they contain mainly osmotic substances and enzymes, whereas in old cells they are transformed to storage vacuoles containing reserve proteins or to the reservoirs containing various secondary compounds.

Discussion

In germinating seeds, vacuoles appear in imbibing embryo cells prior to growth beginning [1,2]. Their osmotically active contents drives the inflow of water into vacuole. In its turn, vacuole exerts turgor pressure through cytoplasm on loosened cell walls and induces the extension of cell wall resulting in cell volume increase (elongation). This is the way how plant cell growth in embryos (germination) starts. In various germinating seeds, various organs start to elongate but provide a common result – the beginning of seedling development. Most seeds, especially in non-tropic regions, belong to orthodox type. They desiccate after maturation, are shed, then stored in dry state (6-10% water content in embryos) and wait for spring optimum

conditions of water supply and temperature to germinate. In the orthodox seeds, germination starts by cell elongation either in hypocotyls or in basal radical cells [1]. In hypocotyl, the growth period of which is short, vacuoles of dry seeds are represented by protein storage vacuoles (previously called protein bodies) filled during seed maturation with reserve proteins. In imbibing seeds, protein degradation and mobilization are the starting point of their restoration to vacuole [2]. Water enters dry seeds by diffusion like in any capillary-porous body containing much hydrophilic constituents. For example, in broad bean seeds, proteolysis of legumin, main reserve protein, begins at 45-55 % water content by proteinases [3] present inside protein-storage vacuoles (PSV). Further proteolysis is exerted by endopeptidases located in cytoplasm [3]. At water content of 68%, restoration of vacuole from PSV is evident [2]. These vacuoles then fuse and enlarge. At initiation of cell elongation, large vacuoles occupy the central position in embryo cells pushing cytoplasm to cell periphery that permits the plant cell to maintain a rather large cell volume on the case of relatively small cytoplasm contents.

The same pattern of vacuole biogenesis from PSV takes place in the basal radical cells [2]. However, meristematic root cells lack PSV. For them, another pathway of vacuole biogenesis is characteristic [2]. In the meristematic cells and their derivatives small provacuoles are present. Presumably, they are the predecessors of vacuoles [4] formed from the lagunae of endoplasmic reticulum and Golgi vesicles. Now provacuole origination from the subdomains of endoplasmic reticulum [5]

is the predominant hypothesis. In *Arabidopsis* root tip cells they look like brightly fluorescent small cytoplasmic structures containing tonoplast aquaporin TIP1:1 [6] or three aquaporins [7].

There are also so-called recalcitrant seeds grown mainly in tropical and subtropical regions, in which the end of maturation is not accompanied by desiccation [8]. The embryo cells remain highly hydrated (62-65%) and preserve this hydration level after shedding. These cells lack PSV that apparently corresponds to the finding that their main reserve substance is sucrose, a water-soluble compound. In imbibing seeds [8], there is no de novo vacuole formation in embryo cells, they only preserve the vacuoles formed earlier at seed maturation. These vacuoles were evident under electron microscope and after vital staining with Neutral red [9]. Such vacuoles were characteristic for both hypocotyl and root. Their preservation permits embryo cells in imbibing seeds to immediately commence the cell elongation and germination [8].

Therefore, three patterns of vacuole biogenesis take place in germinating seeds. In recalcitrant seeds, vacuoles are preserved in shedded seeds and are not formed de novo. In orthodox seeds, they are restored from PSV in hypocotyls, i.e. the organs with limited growth period. However, in roots, the organs growing for a long time, initial vacuole formation from PSV in basal radicle cells is not sufficient, and cell proliferation in meristematic cells is accompanied by de novo vacuole formation from provacuoles. Independent of vacuole biogenesis pattern, in all germinating seeds these vacuoles then fuse and enlarge, thus producing a large central vacuole and favoring the cell elongation. It must be also mentioned that in mature seed embryos of all seeds [2,10-12], the vacuolar membranes contain aquaporins, proteins forming water channels. The presence of TIP 3 (previous name α -TIP) and TIP 1 (γ TIP) indicates the readiness of vacuole to intensify the water transport if water channels are opened by phosphorylation. In growing embryo cells the amount of aquaporins per cell manifold increases due to active expression of TIP genes [2,11]. In the course of vacuole restoration from PSV, the TIP composition changes, namely the PSV markers of tonoplast (TIP 3 aquaporins) [12] are substituted for TIP 1 aquaporins, the markers of vacuoles in elongating cells [13,14]. These water channels are usually opened in elongating cells [2] and provide rapid water transport functioning in germinated seeds.

Conclusion

Vacuoles are the obligatory participants of events preparing the initiation and maintenance of cell growth in embryo cells of

germinating seeds. Various patterns of their biogenesis during seed imbibition are due to seed biology closely related to plant habitat.

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