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Endocytosis and Membrane Recycling in Pollen Tubes

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Abstract In plants, tip-growing cells are an ideal system to investigate signal transduction mechanisms and, among these, pollen tubes are one of the favourite models. Many signalling pathways have been identified during germination and tip growth and, not surprisingly, the apical secretory machinery, essential for tip growth, seems to be an intersection point for all these pathways. Here we review previous data on the pollen tube endocytic machinery and its coupling to the exocytic delivery of new cell wall material. Additionally, we discuss new methodologies and how these are shaping our current working hypothesis to explain endocytosis in pollen tubes.

1 Introduction

Pollen tubes, the active male gametophytes of seed plants, are the vectors carrying the male sperm cells to the egg cell of the female gametophyte in the ovules of seed plants. Unlike most plant cells in which growth occurs by modification of the existing wall and the insertion of new material throughout its surface, pollen tubes extend strictly at their apex, undergoing a specialized type of growth called tip growth. Pollen tubes are thought to derive from the haustoria by which the primitive microgametophytes fed on the host sporophyte. They may grow extremely rapidly, with rates up to 1 cm/h like in lily, and their growth is often oscillatory (de Graaf et al. 2001; Holdaway-Clarke and Hepler 2003). Most studies on pollen tubes are performed with in vitro cultures of bicellular pollen (e.g. lily and tobacco) that supposedly grow autonomously. But even in optimized media, pollen tubes growing in vitro never achieve the high speed and length of those growing *in planta* as they lack the biochemical, physiological and physical environment of the pistil.

Pollen tubes bear similarity to other tip-growing cells like root hairs and moss and fern protonemal cells, with which they share a general cytoplasmic organization. Perhaps one of the most striking features of these cells is their growth mode that depends on polarized exocytosis at the growing tip

and incorporation of new wall material. Quantitative data in pollen tubes has revealed, however, that the quantity of membrane delivered by exocytosis is clearly in excess for the cell growth rate (Steer and Steer 1989), indicating that, coupled to secretion, an underlying recycling process must take place. With the significant advances in molecular analysis and fluorescent probes, the mechanisms that drive this vesicle trafficking start to be unveiled. Here we review recent data and technological advances in the study of endocytosis/exocytosis in pollen tubes and discuss how they help us to dissect old hypotheses and raise new questions.

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Exocytosis and Membrane Retrieval in Pollen Tubes

Until live imaging of secretory vesicles (SVs) became possible, mapping and quantification of endocytosis/exocytosis relied on the analysis of static electron microscopic images. Two main approaches were used and, together, they established the core information we now discuss and test.

The first approach was that of Morré and van der Woude (1974), who calculated the number of SVs needed for growth from the vesicle volume and the corresponding increase in wall volume for *Lilium* pollen tubes. This approach assumes a high similarity in the SVs, similar densities of SV content and the secreted wall, and negligible effects of processing for electron microscopy. Their estimation suggested identical vesicle requirements for both wall and membrane production. This, however, can be true only if the vesicle diameter is very high in comparison with the wall diameter. SVs with diameters smaller than or near the wall thickness necessarily will carry much more membrane material than required for tip expansion. A similar approach was used for tobacco by Derksen et al. (1995) but using rapid freeze fixing and substitution, which minimizes ultrastructural artefacts. They showed that the SV content and the wall at the tip exhibited similar electron densities (Fig. 1), although behind the tip the primary wall became thinner and appeared to lose wall material. The SV diameter was clearly smaller than the width of the wall at the tip.

The other approach was that by Picton and Steer (1981). They calculated the production rate of SV from the increase in SV numbers at the Golgi stacks by inhibiting their export from the Golgi with cytochalasin D. This approach omits possible effects of processing for electron microscopy, as only SV numbers need to be counted and is limited by the time exposure to the drug (prolonged cytochalasin D treatment perturbs SV production). Another drawback is that secretion depends not only on production rates but also on transport and fusion rates with the complete exclusion of other secretion sites, i.e. for the formation of the secondary wall. The procedure was used for *Tradescantia* pollen tubes after different treatments and was confirmed by