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Review

# Calcium - a central regulator of pollen germination and tube growth<sup>☆</sup>

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## ABSTRACT

Pollen tubes grow rapidly by very fast rates and reach extended lengths to bring about fertilization during plant reproduction. The pollen tube grows exclusively at its tip. Fundamental for such local, tip-focused growth are the presence of internal gradients and transmembrane fluxes of ions. Consequently, vegetative pollen tube cells are an excellent single cell model system to investigate cell biological processes of vesicle transport, cytoskeleton reorganization and regulation of ion transport. The second messenger  $\text{Ca}^{2+}$  has emerged as a central and crucial modulator that not only regulates but also integrates the coordination each of these processes. In this review we reflect on recent advances in our understanding of the mechanisms of  $\text{Ca}^{2+}$  function in pollen tube growth, focusing on its role in basic cellular processes such as control of cell growth, vesicular transport and intracellular signaling by localized gradients of second messengers. In particular we discuss new insights into the identity and role of  $\text{Ca}^{2+}$  conductive ion channels and present experimental addressable hypotheses about their regulation. This article is part of a Special Issue entitled: 12th European Symposium on Calcium.

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## 1. Introduction

During sexual reproduction of plants, germination of the male gametophyte (the pollen grain) and proper elongation of the growing tube are essential processes. A plethora of different activities and signals are involved in guiding the male sperm cells to their target, the haploid egg cell (the female gametophyte). In angiosperms after landing on the stigma the desiccated pollen grain rapidly rehydrates and starts to germinate. Subsequently, a pollen tube is growing out of the grain, penetrating the stigma tissue and starts to grow obliviously towards the ovule [1]. To find the right way to the ovule pollen tubes have to perceive and integrate guiding signals from the female tissue. However, even without such external guiding signals pollen grains are able to germinate and establish a polar growing tube *in vitro* due to a “self-organizing signaling network” [2]. This is one feature that renders this tip-growing cell an excellent model system and tool for investigation of various polar growth processes by quantification of germination rates and tube elongation. Remarkably, pollen tubes can elongate with a tremendous speed. Such pollen tubes growth rates have been reported to range from 1000  $\mu\text{m}/\text{h}$  for lily pollen [2–5] and can reach up to 14400  $\mu\text{m}/\text{h}$  in fast growing species like *Tradescantia* or *Hemerocallis* [6]. This is considerably faster than

described growth rates of neuronal axons, which grow at a speed comparable to that of root hairs ( $\sim 20\text{--}50 \mu\text{m}/\text{h}$ ) [7,8]. Another remarkable feature of growing pollen tubes is the distance they cover. To deliver the sperm cells to the ovule, pollen tubes have to transcend distances like a few hundred micrometers in *Arabidopsis* and up to 50 cm in maize [6,9]. Expansion of the growing pollen tube exclusively occurs at the extreme apex. This exclusive apical growth results from coordinated changes in cell wall properties, endo- and exocytosis, cytoskeletal regulation, changes in ion concentrations and possibly subsequent changes in turgor pressure. Hypotheses that consider the prevalence of either a “cell wall model” or a “hydrodynamic model” as driving forces for pollen tube growth have recently been controversially discussed [10,11]. It still appears to represent an open question whether adjustments of turgor pressure take place in order to promote growth and so far no changes in turgor pressure in growing pollen tubes have been observed [11,12]. However, a contribution of the existing turgor pressure in the cell to its elongation appears conceivable. Complex interwoven networks of signaling events regulating the above mentioned components have evolved and are currently intensely investigated. Several signaling molecules function in these signaling networks during pollen germination and tube growth.  $\text{Ca}^{2+}$  appears to be involved in most if not in all of these processes. In the 1960s Brewbaker and Kwack were the first to describe the necessity of  $\text{Ca}^{2+}$  for pollen germination and pollen tube growth [13]. In the 1990s significant advancements have been made in characterizing and measuring the  $\text{Ca}^{2+}$  gradient within the tube and ion influxes from the extracellular space by  $\text{Ca}^{2+}$  sensitive dyes and ion selective vibrating electrodes [14–17]. Nowadays the importance of the tip-focused  $\text{Ca}^{2+}$  gradient and apical

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influx of this ion, as well as its contribution to polar growth of the tube are firmly established [4,18–21]. Functions that the second messenger ion  $\text{Ca}^{2+}$  fulfills in pollen range from a key role in the prevention of self-fertilization [22,23], regulation of the cytoskeleton via actin binding proteins [24–26], influence on vesicle dynamics and membrane trafficking [27,28] to its role as a constituent of the cell wall [29]. In this review we discuss the various functions of  $\text{Ca}^{2+}$  in the initiation and regulation of pollen germination as well as during the polar growth of the tube.

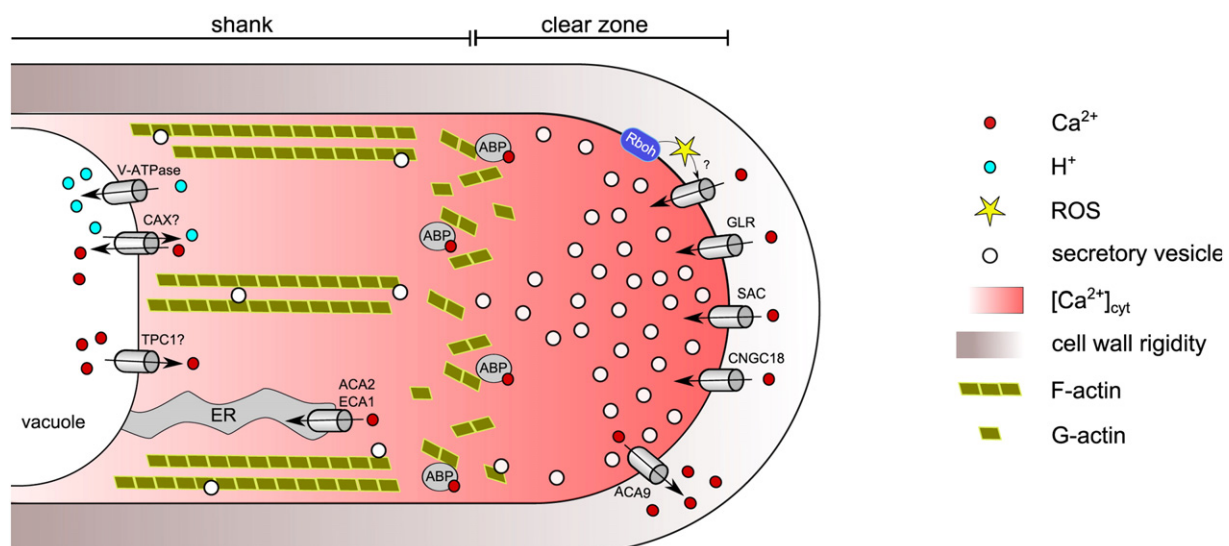
## 2. A tip-focused calcium gradient is essential for pollen germination and tube growth

In 1975 Jaffe et al. described patterns of  $\text{Ca}^{2+}$  accumulations and fluxes in growing pollen tubes of *L. longiflorum* which formed a tip-focused gradient and required an influx at the tip [30]. Today it is well established that in all species that have been studied the cytosolic concentration of free  $\text{Ca}^{2+}$  is highest in close proximity to the plasma membrane at the tip of the growing pollen tube [6,31–35]. The cytoplasmic  $\text{Ca}^{2+}$  concentration ranges from 2–10  $\mu\text{M}$  in the apical 20  $\mu\text{m}$  of the tip region to 20–200 nm in the shank of the tube. In contrast extracellular  $\text{Ca}^{2+}$  concentrations vary in a range of 10–10000  $\mu\text{M}$ . Apical influx of  $\text{Ca}^{2+}$  ions from the extracellular space represents the predominant source establishing this gradient in combination with subapical secretion of this ion to internal stores and/or across the plasma membrane (Fig. 1) [14,16–18,36–40]. The necessity of this steep  $\text{Ca}^{2+}$  gradient for growth has been shown in the 1990s by application of  $\text{Ca}^{2+}$  channel blockers (e.g.  $\text{La}^{3+}$ ) or injection of 1,2-bis(o-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid (BAPTA) buffer which inhibits elongation and at the same time abrogates the  $\text{Ca}^{2+}$  gradient [14,16–18]. Interestingly, the described cytosolic  $\text{Ca}^{2+}$  gradient is not only necessary for elongation of the tube. Additionally, it impacts on growth direction and alteration of its focal point leads to reorientation of the growth axis toward the site of the higher  $\text{Ca}^{2+}$  concentration [19]. The establishment of the growth directing  $\text{Ca}^{2+}$  gradient already begins in the rehydrated pollen grain where it defines the site where the tube will eventually protrude [33]. Treatment with the  $\text{Ca}^{2+}$  channel blocker nifedipine prevents establishment of the gradient, as well as germination [33,41]. Experiments with latrunculin B (Lat-B), which prevents actin polymerization, furthermore revealed a connection between actin dynamics and the  $\text{Ca}^{2+}$  gradient in lily pollen [42]. Application of Lat-B to growing

tubes leads to growth retardation, followed by growth arrest and simultaneously the  $\text{Ca}^{2+}$  gradient dissipates. Besides this essential  $\text{Ca}^{2+}$  gradient there are also other ionic gradients existing in a growing pollen tube such as  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{H}^+$ . The latter gradient creates a constitutive alkaline band at the base of the clear zone, which contains mainly vesicles but no large organelles, and an acidic tip [43]. Although three studies suggest that pH gradients are not required or associated with tip growth in *Lilium longiflorum* or *Agapanthus umbellatus* pollen tubes [44–46], subsequent studies with *Lilium longiflorum* and tobacco pollen tubes led to the assumption that proton gradients exist and that they may be associated with polarized pollen tube growth [32,43,47]. Also tightly regulated fluxes of anions like most notably  $\text{Cl}^-$  appear to be crucial for pollen germination and pollen tube growth. This not very well understood aspect of pollen biology has been comprehensively reviewed and discussed in an excellent recent review [48]. Interestingly, the chloride anion has a reversed direction of flow compared to cations as it was observed to leak out at the tip and enter the tube at the shank [49]. The essential requirement of efficient  $\text{K}^+$  transport into the growing pollen tube has been corroborated by analyses of the plasma membrane inwardly rectifying  $\text{K}^+$  channel SPIK of the Shaker family. The respective *spik* mutant revealed an impairment of pollen tube growth [9]. Simultaneous modeling of diverse ion fluxes in growing pollen tubes based on all currently available experimental evidence supported a model for pollen electric polarization establishment and a role of transport system polarization in ion flux regulation (discussed in detail in [6]).

## 3. Calcium oscillations and tube growth

The above mentioned growth rates of pollen tubes represent peak values. In fact growth rates of elongating pollen tubes oscillate in many species. And so does the cytosolic  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_{\text{cyt}}$ ) as virtually all growth related processes in the growing pollen tube oscillate [4,36].  $[\text{Ca}^{2+}]_{\text{cyt}}$  and the growth rate synchronously oscillate with amplitudes of 3–4 fold changes. Both oscillate with the same period (15–60 s) and in phase. Oscillations of  $[\text{Ca}^{2+}]_{\text{cyt}}$  lag 10–40° behind growth rate, whereas influx of  $\text{Ca}^{2+}$  lags 11 s behind the growth peak in *Lilium longiflorum*. This corresponds to a phase shift of  $\sim 140^\circ$  relating to a period of  $\sim 30$  s [50]. Measurements that confirmed the latter finding were performed with an ion-selective vibrating electrode, which does not directly measure ion fluxes across the plasma membrane but fluxes from the extracellular space into the tube. These observations led to the



**Fig. 1.**  $\text{Ca}^{2+}$  regulated processes and changes in  $\text{Ca}^{2+}$  concentration in a growing pollen tube. This schema illustrates the diverse  $\text{Ca}^{2+}$  fluxes and concentration as well as their impact on actin organization in the pollen tube. See main text for further details.

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