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SHORT COMMUNICATION

Cyclic nucleotide-gated channel 18 functions as an essential Ca^{2+} channel for pollen germination and pollen tube growth in *Arabidopsis*

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ABSTRACT

We recently revealed that cyclic nucleotide-gated channel 18 (CNGC18) functioned as the main Ca^{2+} channel in pollen tube tips for pollen tube guidance to ovules by regulating external Ca^{2+} influx in *Arabidopsis*. In this study, we found that the reduction of external Ca^{2+} concentration ($[Ca^{2+}]_{ext}$) from 10 mM to 5 mM, and further to 2 mM, led to the decreases of pollen germination percentages, but led to the increases of the percentages of ruptured pollen grains and tubes, and branched pollen tubes *in vitro* in *cngc18-17* compared with wild type. The second point mutant allele *cngc18-22* showed similar phenotypes, including reduced pollen germination percentages, increased percentages of ruptured pollen tubes, but did not show obvious different percentages of ruptured pollen grains and branched pollen tubes compared with wild type. These data demonstrate that CNGC18 plays essential roles in pollen germination and tube growth as a Ca^{2+} channel in *Arabidopsis*.

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Sperm cells of flowering plant have lost their motility, and need to be transported by the tip growth of pollen tubes to ovules for double fertilization,[1] and pollen tube growth is guided by multiple attractants from female reproductive tissues.[1,2] A cytosolic Ca^{2+} gradient in pollen tube tips plays central roles in both pollen tube growth and orientation,[3–6] and plasma membrane Ca^{2+} channels in pollen tube tips have been proposed as the main regulators for the Ca^{2+} gradient and pollen tube growth by mediating and regulating external Ca^{2+} influx. The diverse attractants from female gametophytes may guide the pollen tube growth by regulating the activity of the Ca^{2+} channels, and by consequently regulating the Ca^{2+} gradient. The expression of 8 channel genes has been detected in *Arabidopsis* pollen grains and tubes, including 2 *glutamate receptor-like* (*GLR*) channels (1.2 and 3.7) and 6 *cyclic nucleotide-gated channels* (*CNGCs*) (*7, 8, 9, 10, 16*, and *18*),[7–10] and all the 8 channels have been characterized as inward Ca^{2+} channels.[10–12] We recently found that CNGC18 is the main Ca^{2+} channel in pollen tube tips for pollen tube guidance to ovules, whereas the other 7 Ca^{2+} channels play no role, or only a negligible role, if any, in pollen tube guidance in *Arabidopsis*.[12] Considering that the forming and polar growth of normal pollen tubes are the prerequisite for pollen tube guidance, we have proposed that CNGC18 has 2 functions in pollen tube tips.[12] First, CNGC18 mediates external Ca^{2+} influx to establish and maintain the Ca^{2+} gradient in pollen tube tips as an inward Ca^{2+} channel. This function of CNGC18 could be essential for pollen germination and the elongating polar growth of pollen tubes. The pollen grains of T-DNA knockout mutant *cngc18-1* cannot germinate to normal pollen tubes,[7] probably because of the absence of this function of CNGC18.[12] Second, CNGC18 is a key player for pollen tube guidance, and this function of CNGC18 has been confirmed and reported recently.[12]

We have performed *in vitro* pollen germination assay using 10 mM Ca^{2+} condition,[12] because this $[Ca^{2+}]_{ext}$ is the most suitable for a high *in vitro* pollen germination percentage in *Arabidopsis*.[13] The pollen germination percentages were approximate 70% for all the 2 point mutants *cngc18-17* and *cngc18-22*, the 2 rescued lines COM1 and COM2, and Columbia wild type in the $[Ca^{2+}]_{ext}$ condition.[12] To test the function of CNGC18 as a Ca^{2+} channel in pollen germination and pollen tube growth, we performed *in vitro* pollen germination and tube culturing experiments using different external Ca^{2+} concentrations, especially low external Ca^{2+}. The experimental results showed that the pollen germination percentages were significantly reduced in *cngc18-17* and *cngc18-22*, were slightly reduced in COM1, but were not significantly altered in COM2 and wild type, upon the reduction of $[Ca^{2+}]_{ext}$ from 10 mM to 5 mM, and further to 2 mM (Fig. 1A),[12] demonstrating that CNGC18 plays an essential role in pollen germination as a Ca^{2+} channel. We also observed ruptured pollen grains and pollen tubes, and branched pollen tubes (Fig. 1B). We then analyzed the $[Ca^{2+}]_{ext}$ dependence of pollen grain and pollen tube rupturing, as well as pollen tube branching. The experimental results showed that the percentage of ruptured pollen grains was increased significantly from 18% to 37.6% for *cngc18-17* upon the reduction of $[Ca^{2+}]_{ext}$ from 10 mM to 2 mM, whereas the percentages of the ruptured pollen grains

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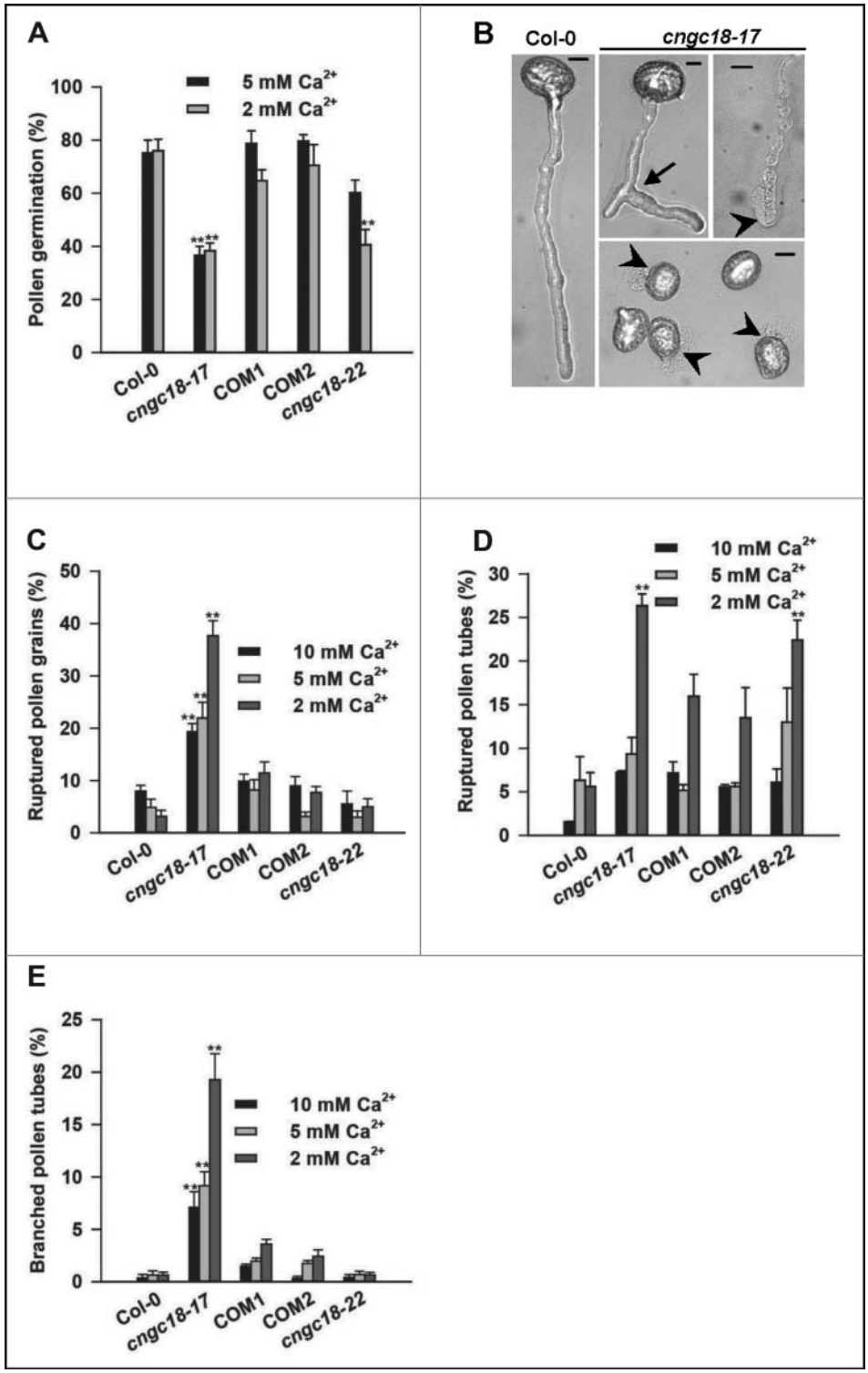

Figure 1. CNGC18 plays essential roles in pollen germination and pollen tube growth in *Arabidopsis*. (A) The analysis of $[Ca^{2+}]_{ext}$ dependence of pollen germination. (B) Pictures showing typical normal pollen tube of Col-0, a branched pollen tube, ruptured pollen grains and a ruptured pollen tube of *cngc18-17*. The branched pollen tube is indicated by an arrow, and the ruptured pollen grains and pollen tube are indicated by arrowheads. Scale bars equal to 10 μm. (C-E) The analysis of $[Ca^{2+}]_{ext}$ dependence of pollen grain rupturing (C), pollen tube rupturing (D), and pollen tube branching (E). Three replicates were performed. Error bars represent means ± SEM. **Significant differences from Col-0 ($P < 0.01$).

were not altered significantly in *cngc18-22*, COM1, COM2 and wild type upon the similar $[Ca^{2+}]_{ext}$ reduction from 10 mM to 2 mM (Fig. 1C). The percentages of ruptured pollen tubes was increased from 7% to 26% for *cngc18-17*, from 6% to 22% for *cngc18-22*, from 7% to 15% for COM1, and from 5% to 13% for COM2, upon the $[Ca^{2+}]_{ext}$ reduction from 10 mM to 2 mM (Fig. 1D). The percentages of branched pollen tubes was increased significantly from 7% to 17% for *cngc18-17*, but not altered in *cngc18-22*, COM1, COM2 and wild type upon the similar $[Ca^{2+}]_{ext}$ reduction from 10 mM to 2 mM (Fig. 1E). These phenotypes of *cngc18-17* and *cngc18-22* could be caused by insufficient and/or abnormal external Ca^{2+}

influx for pollen grains and pollen tubes. These data demonstrate that CNGC18 plays essential roles as an inward Ca^{2+} channel in both pollen germination and the elongating polar growth of pollen tubes in *Arabidopsis*.

Disclosure of potential conflicts of interest

The authors declare no conflict of interest.

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