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Figure legends

Figure 1. Irregular and regular $[Ca^{2+}]_{cyt}$ oscillations observed in elongated *Arabidopsis* pollen tubes 5 h after dissemination under the *in vitro* condition.

- A. ECFP, Venus, and Ratio (Venus/ECFP) images of an elongated pollen tube 5 h after dissemination. A tip-focused $[Ca^{2+}]_{cyt}$ gradient was observed. The ratio (Venus/ECFP) in a tip area with a diameter of 6 μm was measured. Bar represents 10 μm .
- B. The ratio change in the tip region and the elongation of a normally growing pollen tube. Primarily irregular oscillations were observed, although relatively regular oscillations also were identified in the range marked with a blue line.
- C. The ratio change in the tip region of a relatively slowly growing pollen tube (blue line) and the distance moved during monitoring. Regular $[Ca^{2+}]_{cyt}$ oscillations with a large amplitude were observed.
- D. Regular oscillations followed by irregular oscillations. Regular oscillations were detected in a stopped or slowly growing pollen tube, whereas irregular oscillations were observed in a growing pollen tube.

Figure 2. Titration curves for YC3.60 and YC4.60.

Figure 3. Irregular oscillations observed in *Arabidopsis* pollen tubes growing through a papilla cell wall (*in vivo* condition) and in pollen tubes growing through a style and then on germination medium (*semi-in vivo* condition).

- A. A ratio image of a pollen tube growing through a papilla cell obtained approximately 7.5 min after penetration. A tip-focused $[Ca^{2+}]$ gradient was observed. The papilla cell is marked with a white line.
- B. The ratio change in the tip of the pollen tube shown in (A) and the elongation of a pollen tube 5 min after penetration. The amplitude and period of the oscillations were

irregular.

C. Ratio images of a pollen tube that has just passed through the style.

D. The ratio change in the tip of the pollen tube shown in (C) and elongation during monitoring. The amplitude and periodicity of the oscillations were irregular.

Figure 4. The YC3.60 spectrum in the presence of ionophore (A) or in the presence of ionophore and EGTA (B).

Figure 5. The effects of inhibitors on *Arabidopsis* pollen tube elongation.

Figure 6. The effects of inhibitors on the changes in the $[Ca^{2+}]_{cyt}$ in the tip region of *Arabidopsis* pollen tubes growing in the semi-*in vivo* condition.

A. Ratio images of a pollen tube. The numbers in each figure correspond to each point in the graph shown in B. After the addition of CPA, the $[Ca^{2+}]_{cyt}$ increased in a large area containing the tip region (white line).

B. The ratio change in the tip region, an area from the pollen tube shown in (A) with a diameter of 6 μm . Blue line indicates distance from origin. Before the addition of CPA, irregular fluctuations were observed. Two minutes after the addition of CPA (shown with a green arrow), regular oscillations were induced. These oscillations were inhibited by the addition of Gd^{3+} (shown with a green arrow). Red arrows (1) - (5) correlate to the ratio images in Figure 6A.

Figure 7. The effects of Gd^{3+} on the changes in the $[Ca^{2+}]_{cyt}$ in the tip region of *Arabidopsis* pollen tubes growing in the semi-*in vivo* condition.

A. Ratio images of a pollen tube before and after the addition of Gd^{3+} . Numbers in each figure correspond to each point in the graph shown in B. After the addition of Gd^{3+} , the

- tip-focused $[Ca^{2+}]_{cyt}$ gradient disappeared and the tip region burst (a white arrow in 3).
- B. The ratio change in the tip region before and after the addition of Gd^{3+} (a green arrow) and the elongation of the pollen tube. Red arrows (1) - (3) correlate to the ratio images in Figure 7A. Blue line indicates distance from origin.

Figure 8. Imaging of the $[Ca^{2+}]_{ER}$ in *Arabidopsis* pollen tubes growing in the semi-*in vivo* condition. The YFP image shows the ER, which was localized throughout the pollen tube. In the ratio (Venus/ECFP) images of the ER, high concentration areas were scattered throughout the pollen tube.

Figure 9. The effect of CPA on the $[Ca^{2+}]_{ER}$ in *Arabidopsis* pollen tubes growing in the semi-*in vivo* condition.

- A. Ratio images of the ER. The numbers in each figure correspond to each point in the graph shown in B.
- B. The ratio change in the tip region. After the addition of CPA, the ratio gradually decreased. After the addition of more CPA, the ratio decreased further.

Figure 10. Regular and irregular $[Ca^{2+}]_{cyt}$ oscillations observed in elongated *N. tabacum* pollen tubes 5 h after dissemination under the *in vitro* condition.

- A. ECFP, Venus, and Ratio (Venus/ECFP) images of an elongated pollen tube 5 h after dissemination. A tip-focused $[Ca^{2+}]_{cyt}$ gradient was observed. The ratio was measured in a tip area with a diameter of 8 μm . Bar represents 10 μm .
- B. The ratio change in the tip region and elongation during monitoring of a normally growing pollen tube. Regular oscillations were observed but the amplitude was small.
- C. The ratio change in the tip region of a normally growing pollen tube and the distance moved during monitoring. The amplitude and periodicity of the oscillations were

irregular.

D. Regular oscillations in a pollen tube that was not growing. The amplitude was larger than that in (A).

Figure 11. Irregular oscillations observed in *N. tabacum* pollen tubes growing on the stigma (*in vivo* condition) and in pollen tubes growing through a style and then on germination medium (*semi-in vivo* condition).

A. The ratio change in the tip and elongation of a pollen tube growing on a stigma. The amplitude and periodicity of the oscillations were irregular, whereas the growth rate was relatively low.

B. The ratio change in growing pollen tubes under the *semi-in vivo* condition. The amplitude and periodicity were irregular.

Supplementary videos

Supplementary Video 1. Irregular $[Ca^{2+}]_{cyt}$ oscillations in a normally growing *Arabidopsis* pollen tube under the *in vitro* condition.

Supplementary video 2. Regular $[Ca^{2+}]_{cyt}$ oscillations in a slowly growing *Arabidopsis* pollen tube under the *in vitro* condition.

Supplementary video 3. Regular oscillations followed by irregular oscillations in an elongated *Arabidopsis* pollen tube under the *in vitro* condition.

Supplementary video 4. Irregular oscillations observed in an *Arabidopsis* pollen tube growing through a papilla cell wall (*in vivo* condition).

Supplementary video 5. Irregular oscillations observed in an *Arabidopsis* pollen tube growing through a style and then on germination medium (*semi-in vivo* condition).

Supplementary video 6. The effects of CPA and Gd^{3+} on the changes in the $[Ca^{2+}]_{cyt}$ in the tip region of *Arabidopsis* pollen tubes growing in the *semi-in vivo* condition.

Supplementary video 7. The effects of Gd^{3+} on the changes in the $[Ca^{2+}]_{cyt}$ in the tip region of an *Arabidopsis* pollen tube growing in the semi-*in vivo* condition.

Supplementary video 8. Imaging of the $[Ca^{2+}]_{ER}$ in an *Arabidopsis* pollen tube growing in the semi-*in vivo* condition.

Supplementary video 9. Regular $[Ca^{2+}]_{cyt}$ oscillations observed in an elongated *N. tabacum* pollen tube.

Supplementary video10. Irregular $[Ca^{2+}]_{cyt}$ oscillations observed in an elongated *N. tabacum* pollen tube.

Supplementary video11. Regular $[Ca^{2+}]_{cyt}$ oscillations observed in a stopping *N. tabacum* pollen tube.

Supplementary video12. Irregular oscillations observed in a *N. tabacum* pollen tube growing on the stigma (*in vivo* condition).

Supplementary video13. Irregular oscillations observed in a *N. tabacum* pollen tube growing through a style and then on germination medium (semi-*in vivo* condition).

Table 1. $[Ca^{2+}]$ oscillation and growth rate of the pollen tube in *Arabidopsis* and *Nicotiana tabacum*

| Condition | Growth rate ($\mu\text{m}/\text{min}$) | Max/Min ratio | Amplitude range (μM) | $[Ca^{2+}]_{\text{cyt}}$ | Exp. No. |
|--------------------------|---|-----------------------------|--------------------------------------|--------------------------|----------|
| <i>Arabidopsis</i> | | | | | |
| <i>in vitro</i> | 2.0 ± 1.8 | $4.5 \pm 0.2 / 3.9 \pm 0.3$ | 0.3 ± 0.2 | $0.3 - 0.4$ | 45 |
| <i>in vitro</i> | < 1.0 | $7.2 \pm 0.5 / 3.5 \pm 0.2$ | 1.8 ± 0.3 | $0.3 - 1.0$ | 5 |
| <i>in vivo</i> | 3.6 ± 0.5 | $4.0 \pm 0.4 / 3.4 \pm 0.3$ | 0.4 ± 0.3 | $0.3 - 0.4$ | 10 |
| semi- <i>in vivo</i> | 3.1 ± 0.8 | $5.1 \pm 0.5 / 4.5 \pm 0.5$ | 0.3 ± 0.2 | $0.4 - 0.5$ | 48 |
| <i>Nicotiana tabacum</i> | | | | | |
| <i>in vitro</i> | 2.8 ± 1.6 | $3.9 \pm 0.2 / 3.5 \pm 0.2$ | 0.2 ± 0.1 | $0.3 - 0.4$ | 17 |
| <i>in vitro</i> | < 1.0 | $4.8 \pm 0.4 / 2.8 \pm 0.2$ | 1.0 ± 0.1 | $0.1 - 0.5$ | 5 |
| <i>in vivo</i> | 0.2 ± 0.1 | $3.5 \pm 0.2 / 3.3 \pm 0.1$ | 0.1 ± 0.1 | 0.3 | 15 |
| semi- <i>in vivo</i> | 1.2 ± 0.2 | $3.9 \pm 0.4 / 3.4 \pm 0.4$ | 0.3 ± 0.2 | $0.3 - 0.4$ | 30 |

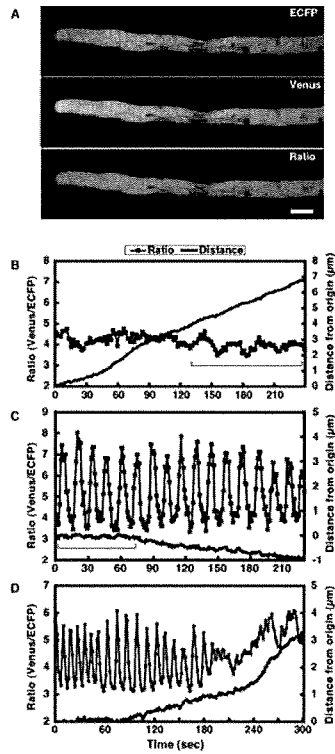


Figure 1. Irregular and regular $[Ca^{2+}]_i$ oscillations in the elongated *Arabidopsis* pollen tubes 5 hr after dissemination under the *in vitro* condition.

A. ECFP, Venus and Ratio (Venus/ECFP) images. A tip-focused $[Ca^{2+}]_i$ gradient was observed. The ratio in the tip area with a diameter of $6 \mu m$ was measured. Bar represents $10 \mu m$.

B. The ratio change and the elongation of a normally growing pollen tube. Primarily irregular oscillation were observed, although relatively regular oscillations also were in the range marked with a blue line.

C. The ratio change in a stopped pollen tube (blue line) and the distance during monitoring. Regular $[Ca^{2+}]_i$ oscillation with a large amplitude were observed.

D. Regular oscillations followed by an irregular oscillation. Regular oscillations were detected in a stopped or slowly growing pollen tube, whereas irregular oscillations were observed in a growing pollen tube.

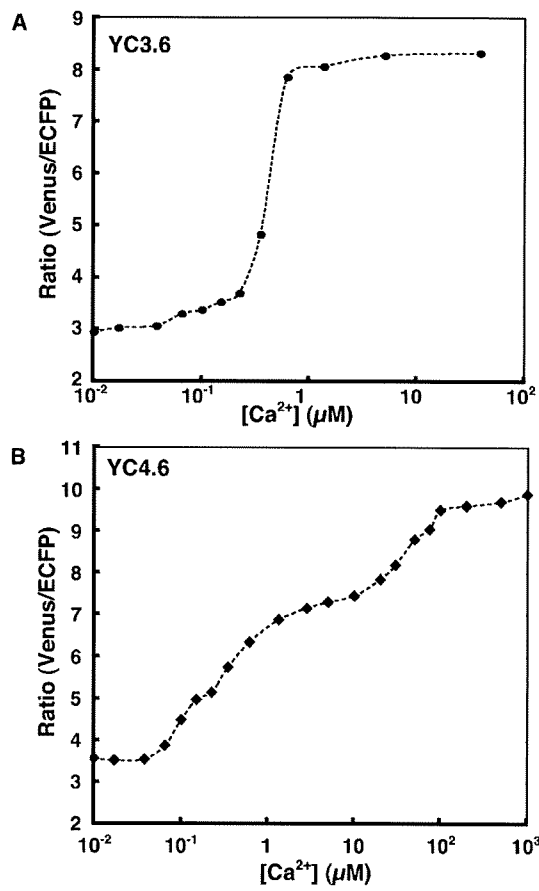


Figure 2. Titration curves for YC3.60 (A) and YC4.60 (B).

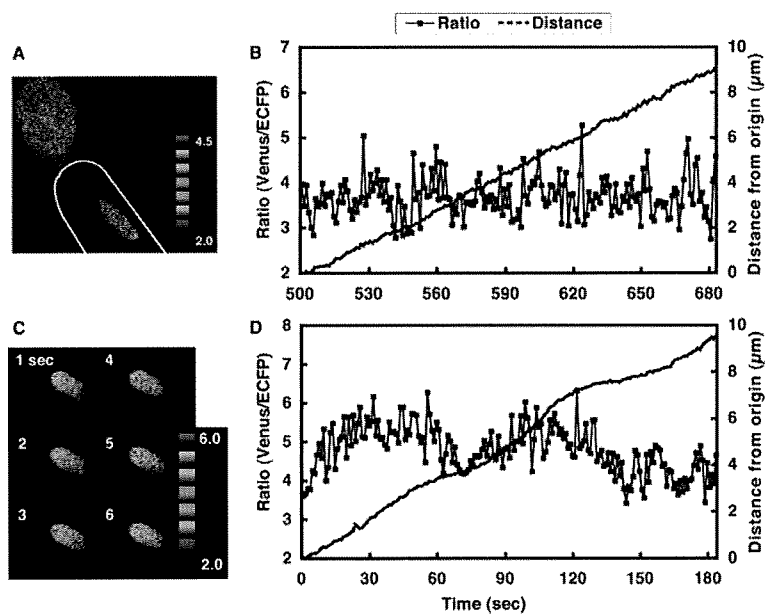


Figure 3. Irregular oscillations observed in *Arabidopsis* pollen tubes growing through a papilla cell wall (the *in vivo* condition) and in pollen tubes growing through a style and then on germination medium (the semi-*in vivo* condition).
 A. A ratio image of a pollen tube growing through a papilla cell obtained approximately 7.5 min after penetration. A tip focused $[Ca^{2+}]$ gradient was observed. The papilla cell is marked with a white line.
 B. The ratio change in the tip of the pollen tube shown in (A) and the elongation of a pollen tube 5 min after penetration. The amplitude and periodicity of the oscillations were irregular.
 C. Ratio images of a pollen tube that has just passed through the style.
 D. The ratio change in the tip of the pollen tube shown in (C) and elongation during monitoring. The amplitude and period of the oscillations were irregular.

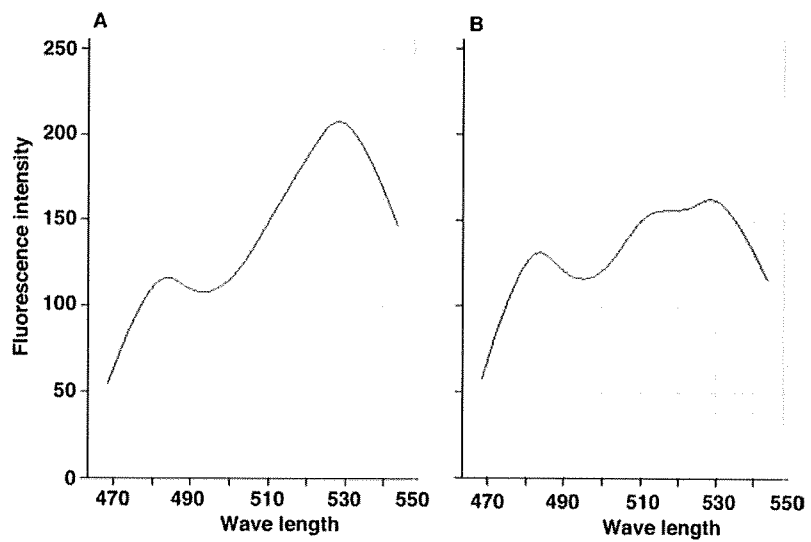


Figure 4. The YC3.60 spectrum in the presence of ionophore (A) or in the presence of ionophore and EGTA (B).

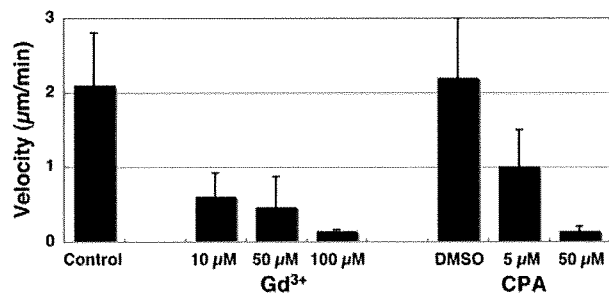


Figure 5. The effects of inhibitors on *Arabidopsis* pollen tube elongation.

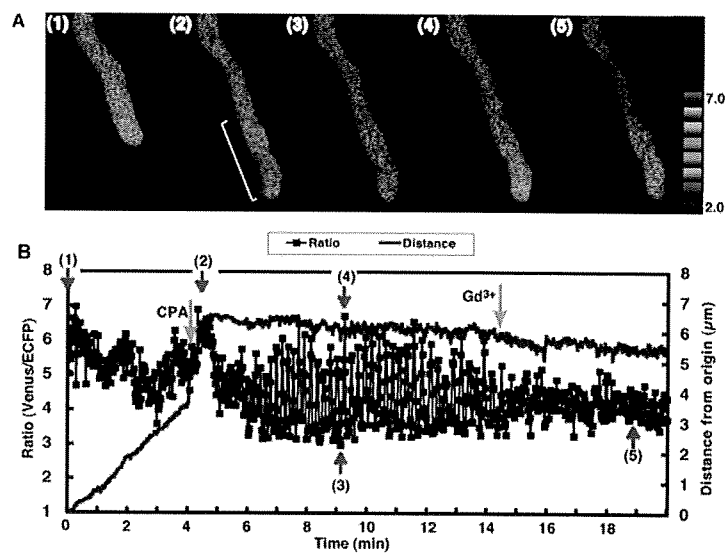


Figure 6. The effects of inhibitors on the changes in the $[Ca^{2+}]_{55T}$ in the tip region of *Arabidopsis* pollen tubes growing in the semi-*in vivo* condition.

A. Ratio images of a pollen tube. The numbers in each figure correspond to each point in the graph shown in B. After the addition of CPA, the $[Ca^{2+}]_{55T}$ increased in a large area containing the tip region (a white line).

B. The ratio change in the tip region, an area from the pollen tube shown in (A) with a diameter of 6 μm and the elongation of the pollen tube. Blue line indicates the distance to the origin. Before the addition of CPA, irregular oscillations were observed. Two minutes after the addition of CPA (shown with a green arrow), regular oscillations were induced. These oscillations were inhibited by the addition of Gd^{3+} (shown with a green arrow). Red arrows (1) - (3) correlate to the ratio images in Figure 6A.

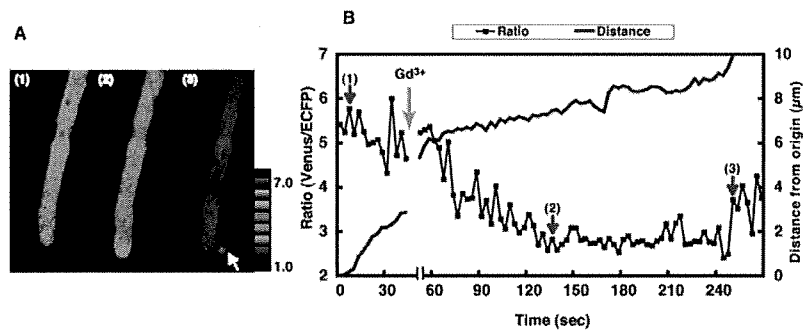


Figure 7. The effects of Gd^{3+} on the changes in the $[Ca^{2+}]_{cyt}$ in the tip region of *Arabidopsis* pollen tubes growing in the semi-*in vivo* condition.

A. Ratio images of a pollen tube before and after the addition of Gd^{3+} . Numbers in each figure correspond to each point in the graph shown in B. After the addition of Gd^{3+} , the tip-focused $[Ca^{2+}]_{cyt}$ gradient disappeared and the tip region burst (a white arrow in 3).

B. The ratio change in the tip region before and after the addition of Gd^{3+} (a green arrow) and the elongation of the pollen tube. Red arrows (1) - (3) correlate to the ratio images in Figure 7A. Blue line indicates the distance to the origin.

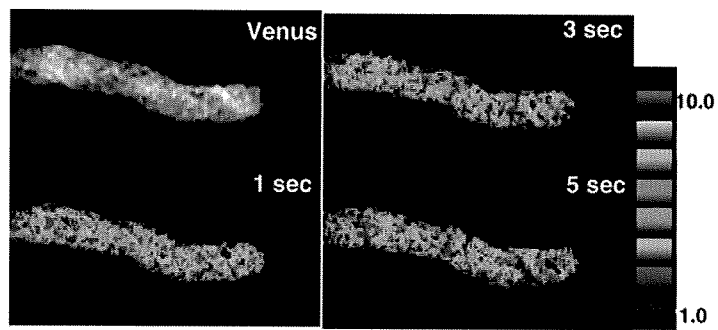


Figure 8. Imaging of the $[Ca^{2+}]_{ER}$ in *Arabidopsis* pollen tubes growing in the semi-*in vivo* condition.

The Venus image shows the ER, which was localized throughout the pollen tube. In the ratio images of the ER, high concentration areas were scattered throughout the pollen tube.

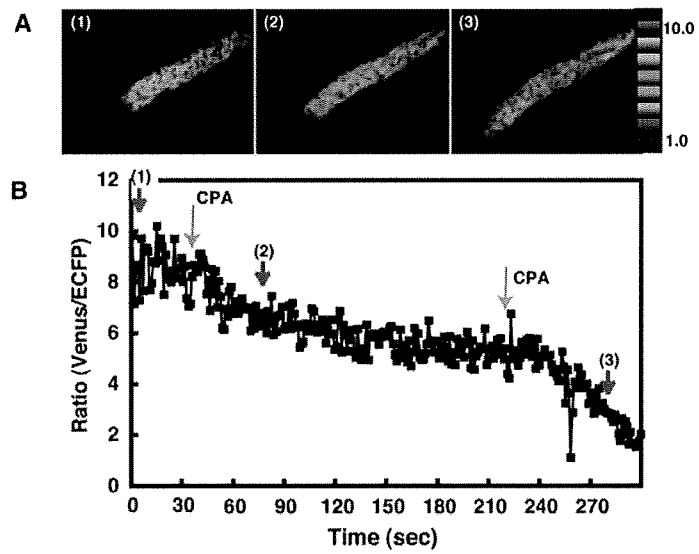


Figure 9. The effect of CPA on the $[Ca^{2+}]_{ER}$ in *Arabidopsis* pollen tubes growing in the semi-*in vivo* condition.

A. Ratio images of the ER. The numbers in each figure correspond to each point in the graph shown in B.

B. The ratio change in the tip region. After the addition of CPA (shown with a green arrow), ratio gradually decreased. After the addition of more CPA (shown with a green arrow) the ratio decreased further. (1), (2) and (3) pointed with red arrows correlate to the ratio images in Figure 9A.