er in the same lineage because both are mechanisms to avoid inbreeding. The obvious corollary, that gender dimorphism is more likely to evolve in groups that are self-compatible, has often been discussed (9, 10, 20). Yet, if scenarios like that proposed here are common, gender dimorphism may frequently evolve in lineages with self-incompatibility without negating inbreeding avoidance as a selective mechanism. The pathway presented here reinforces the importance of inbreeding avoidance in the evolution of gender dimorphism and could explain why a negative association between gender dimorphism and self-incompatibility has been difficult to find (10). Although gender dimorphism has been widely studied, many aspects are not fully understood, and new scenarios, such as the one presented here, surely await discovery.

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Alteration of Stimulus-Specific Guard Cell Calcium Oscillations and Stomatal Closing in *Arabidopsis det3* Mutant

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Cytosolic calcium oscillations control signaling in animal cells, whereas in plants their importance remains largely unknown. In wild-type *Arabidopsis* guard cells abscisic acid, oxidative stress, cold, and external calcium elicited cytosolic calcium oscillations of differing amplitudes and frequencies and induced stomatal closure. In guard cells of the V-ATPase mutant *det3*, external calcium and oxidative stress elicited prolonged calcium increases, which did not oscillate, and stomatal closure was abolished. Conversely, cold and abscisic acid elicited calcium oscillations in *det3*, and stomatal closure occurred normally. Moreover, in *det3* guard cells, experimentally imposing external calcium-induced oscillations rescued stomatal closure. These data provide genetic evidence that stimulus-specific calcium oscillations are necessary for stomatal closure.

Cytosolic calcium ($[Ca^{2+}]_{cyt}$) oscillations are an integral component of cell signaling, and the frequency, amplitude, and spatial localization of oscillations control the efficiency and specificity of cellular responses in animals (1–3). In plant cells $[Ca^{2+}]_{cyt}$ oscillations are induced by multiple stimuli (4–9); however, it remains unknown whether oscillations are required to elicit physiological responses in plants. Here we show that the *Arabidopsis det3* mutant abolishes guard cell $[Ca^{2+}]_{cyt}$ oscillations and stomatal closure in response to oxidative stress and extracellular calcium ($[Ca^{2+}]_{ext}$), but not to abscisic acid (ABA) and cold. Restoring $[Ca^{2+}]_{ext}$-induced $[Ca^{2+}]_{cyt}$ oscillations in *det3* guard cells rescued stomatal closure, suggesting that $[Ca^{2+}]_{cyt}$ oscillations are essential for stomatal closure.

Stomatal closure follows increases in guard cell $[Ca^{2+}]_{cyt}$ (10), and endomembrane calcium transport contributes to the $[Ca^{2+}]_{cyt}$ signal (7, 11–13). Genetic impairment of endomembrane calcium transport could therefore provide a direct approach for dissecting $[Ca^{2+}]_{cyt}$ signals. The *de-etiolated 3* (*det3*) *Arabidopsis* mutant has reduced endomembrane energization owing to a 60% reduction

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in expression of the C-subunit of the V-type H^+–adenosine triphosphatase (V-ATPase) (14). det3 was identified by its failure to repress light-specific development in the dark; it has a dwarf phenotype, but normal stomatal development (14). Figure 1 shows DET3 promoter–driven DET3–green fluorescent protein (GFP) fluorescence in Arabidopsis guard cells (Fig. 1A), protoplasts (Fig. 1C), and isolated vacuoles (Fig. 1D) and indicates that DET3 is expressed in guard cells and is localized on multiple membranes including both endoplasmic reticulum (ER) and vacuolar membranes. We hypothesized that endomembrane de-energization in det3 guard cells could affect $[Ca^{2+}]_{cyt}$ signaling because Ca^{2+} sequestration into intracellular stores is H^+ gradient–dependent in many organisms (15–19).

Wild-type (WT) Arabidopsis stably expressing yellow cameleon 2.1 (YC2.1) under the control of the constitutive 35S promoter was used to measure $[Ca^{2+}]_{cyt}$ signals in stomatal guard cells (20–22). Oscillations in $[Ca^{2+}]_{cyt}$ were rapidly induced by 1 or 10 mM extracellular calcium in all 84 WT guard cells tested (Fig. 2, A and B). $[Ca^{2+}]_{cyt}$ oscillations were smaller and of higher frequency for 1 mM $[Ca^{2+}]_{ext}$ (average $[Ca^{2+}]_{cyt}$ at peak $\approx$ 160 nM; period = 161 $\pm$ 20 s) than for 10 mM $[Ca^{2+}]_{ext}$ ($[Ca^{2+}]_{cyt} \approx$ 1020 nM; period = 396 $\pm$ 23 s) [see also (4, 8)]. Oscillations ceased after 45 to 60 min (Fig. 2B) and preceded maximal stomatal closure, which was measured after 3 hours. In det3 guard cells, increasing $[Ca^{2+}]_{ext}$ from 0 to 1 or 10 mM caused immediate $[Ca^{2+}]_{cyt}$ increases which, unlike in the WT, subsequently failed to oscillate but consisted of small, rapid spikes superimposed on prolonged $[Ca^{2+}]_{cyt}$ increases (n = 55 of 56 cells) (Fig. 2, C and D) ($[Ca^{2+}]_{cyt} \approx$ 350 nM for 1 mM $[Ca^{2+}]_{ext}$ and $\approx$ 1250 nM for 10 mM $[Ca^{2+}]_{ext}$). Additionally, the total integrated $[Ca^{2+}]_{cyt}$ increase over an $\approx$30-min period was higher in det3 [5910 $\pm$ 785 nM·min (n = 6)] than in WT [3010 $\pm$ 263 nM·min (n = 6)] at 10 mM $[Ca^{2+}]_{ext}$.

The absence of $[Ca^{2+}]_{ext}$-induced $[Ca^{2+}]_{cyt}$ oscillations in det3 guard cells was consistent with a marked effect on $[Ca^{2+}]_{ext}$-induced stomatal closure. Addition of $[Ca^{2+}]_{ext}$ to preopened WT stomata caused closure, whereas in det3 stomatal closure was abolished at all $[Ca^{2+}]_{ext}$ concentrations tested (Fig. 2E).

To further investigate the role of $[Ca^{2+}]_{cyt}$ oscillations and det3 in guard cell signaling, we measured $[Ca^{2+}]_{cyt}$ and stomatal movements in response to other stimuli. In WT guard cells ABA caused repetitive $[Ca^{2+}]_{cyt}$ transients (Fig. 3A), or more prolonged oscillations (Fig. 3B), with a magnitude of $\sim$500 nM and a period of 468 $\pm$ 41 s (n = 40) for transients and 333 $\pm$ 35 s (n = 6) for oscillations (Fig. 3, A and B). In det3, ABA caused $[Ca^{2+}]_{cyt}$ transients or oscillations with near-identical magnitudes (Fig. 3, C and D) and periods (476 $\pm$ 32 s, n = 26 for transients and 328 $\pm$ 36 s, n = 4 for oscillations; P > 0.15 for WT versus det3 periods for both transients and oscillations). ABA

Fig. 1. Localization in Arabidopsis guard cells of a COOH-terminal DET3-GFP fusion expressed under the control of the DET3 promoter in plasmid pPZP221 (14). (**A**) GFP fluorescence is excluded from the vacuoles (vc), chloroplasts (ch), and nucleus (nu) and is concentrated in the ER. Bar, 5 μm. (**B**) Autofluorescence in nontransformed cell. Bar, 5 μm. (**C**) In guard cell protoplasts, GFP fluorescence is excluded from vacuoles (vc) and the nucleus (nu) and is concentrated in the ER. Bar, 4 μm. (**D**) In ruptured guard cell protoplasts fluorescence was associated with released vacuolar membranes (vc) and the collapsed nucleus (nu), but not the plasma membrane (pm). Bar, 4 μm.
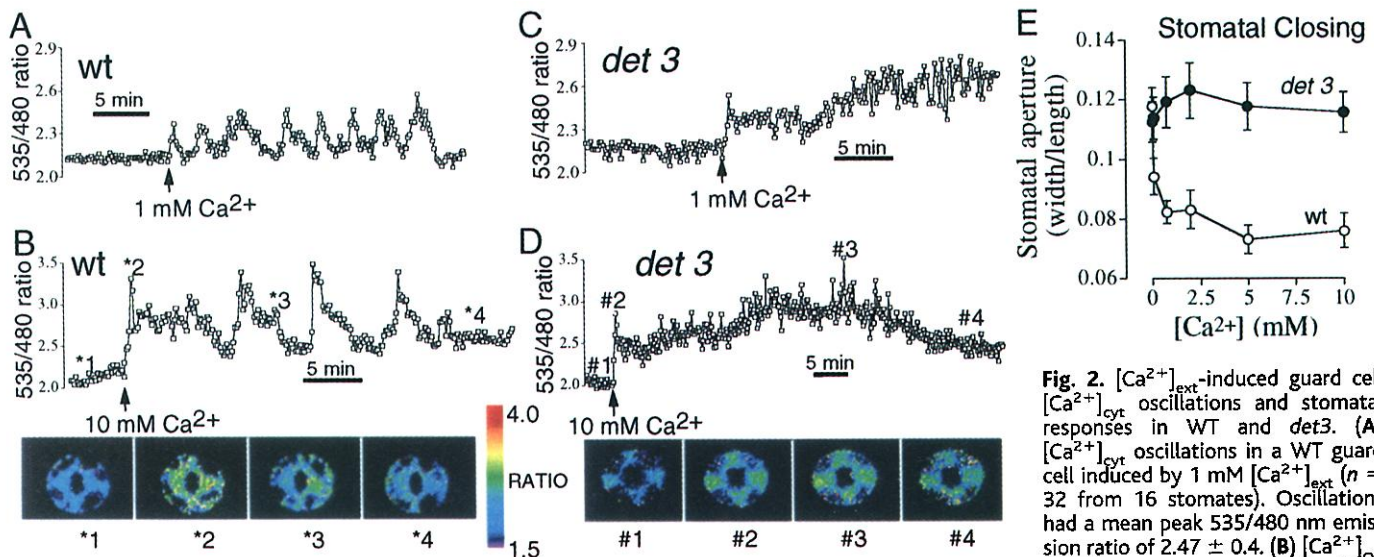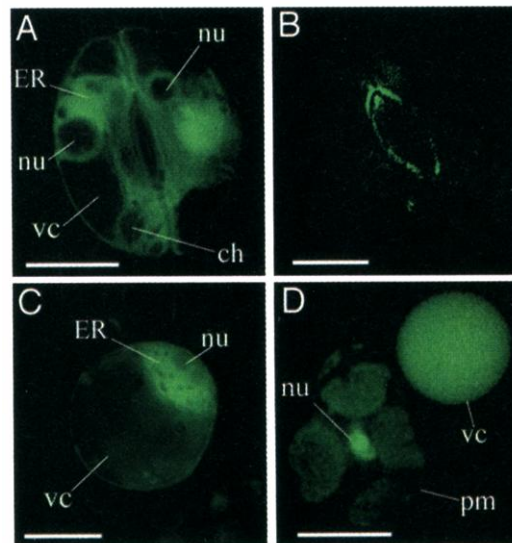

Fig. 2. $[Ca^{2+}]_{ext}$-induced guard cell $[Ca^{2+}]_{cyt}$ oscillations and stomatal responses in WT and det3. (**A**) $[Ca^{2+}]_{cyt}$ oscillations in a WT guard cell induced by 1 mM $[Ca^{2+}]_{ext}$ (n = 32 from 16 stomates). Oscillations had a mean peak 535/480 nm emission ratio of 2.47 $\pm$ 0.4. (**B**) $[Ca^{2+}]_{cyt}$ oscillations in a WT guard cell induced by 10 mM $[Ca^{2+}]_{ext}$ (n = 52 from 26 stomates). Oscillations had a mean peak 535/480 nm ratio of 3.21 $\pm$ 0.16. The trace is from the right-hand cell of the stomate in the lower panel. Images indicate the 535/480 nm ratio at points *1 to *4. (**C**) Increase of det3 guard cell $[Ca^{2+}]_{cyt}$ induced by 1 mM $[Ca^{2+}]_{ext}$ (n = 21 cells from 11 stomates). Increases had a mean peak 535/480 nm ratio of 2.83 $\pm$ 0.31. (**D**) Increase of det3 guard cell $[Ca^{2+}]_{cyt}$ induced by 10 mM extracellular calcium (n = 34 from 17 stomates). Increases had a mean peak 535/480 nm ratio of 3.26 $\pm$ 0.39. The trace is from the right-hand cell of the stomate in the lower panel. Images indicate the 535/480 nm ratio at points #1 to #4. (**E**) Increasing $[Ca^{2+}]_{ext}$ caused stomatal closure in WT (○) but not det3 (●). Data are the mean $\pm$ SEM of 120 stomata from four replicates for each $[Ca^{2+}]_{ext}$.

Fig. 3. ABA-induced guard cell $[Ca^{2+}]_{cyt}$ oscillations and stomatal responses in WT and det3. **(A)** Repetitive, transient $[Ca^{2+}]_{cyt}$ increases in a WT guard cell induced by 10 μM ABA (n = 40 from 28 stomates). Transients had a mean peak 535/480 nm ratio of 3.07 ± 0.29. **(B)** Oscillations of WT guard cell $[Ca^{2+}]_{cyt}$ induced by 10 μM ABA

(n = 6 from three stomates). Oscillations had a mean peak 535/480 nm ratio of 2.91 ± 0.31. **(C)** Repetitive, transient increases in det3 guard cell $[Ca^{2+}]_{cyt}$ induced by 10 μM ABA (n = 26 from 15 stomates). Transients had a mean peak 535/480 nm ratio of 3.09 ± 0.22. **(D)** Oscillations of det3 guard cell $[Ca^{2+}]_{cyt}$ induced by 10 μM ABA (n = 4 from two stomates). Oscilla-

tions had a mean peak 535/480 nm ratio of 2.98 ± 0.31. Transients occurred in both guard cells of all responsive stomata (ABA-induced $[Ca^{2+}]_{cyt}$ increases were observed in 81% of cells, n = 76 from 96 stomates). **(E)** ABA induced stomatal closure in WT (○) and det3 (●). Data are the mean ± SEM of 120 stomata from four replicates.
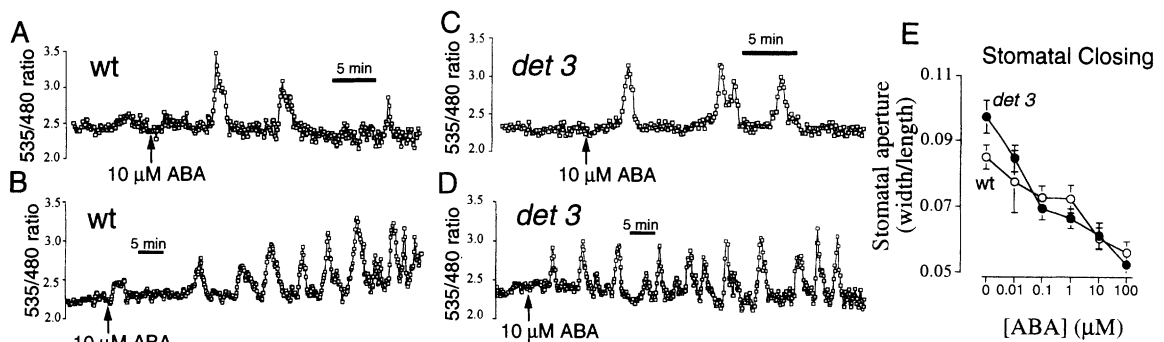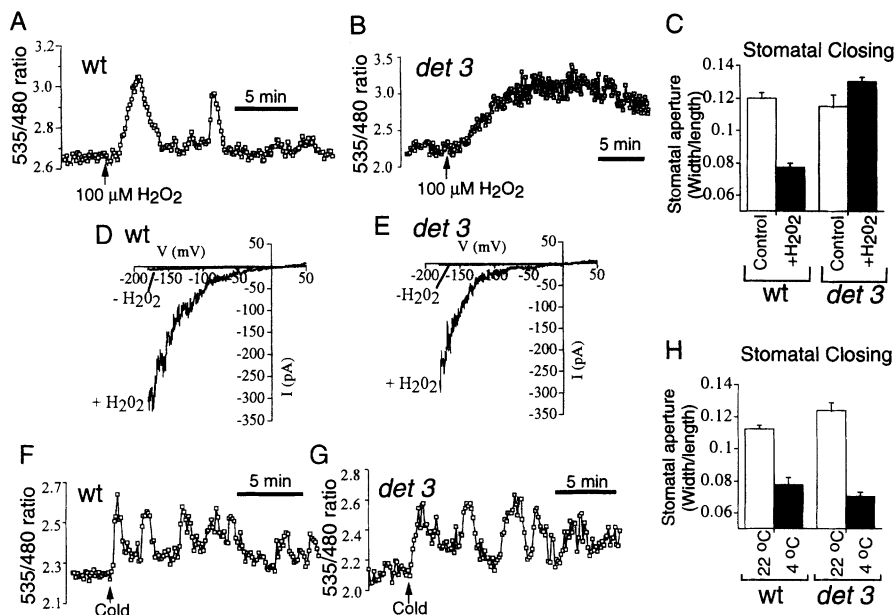

Fig. 4. Guard cell $[Ca^{2+}]_{cyt}$ oscillations and stomatal responses to H_2O_2 and cold in WT and det3. **(A)** Repetitive, transient increases in WT guard cell $[Ca^{2+}]_{cyt}$ induced by 100 μM H_2O_2 (n = 24 from 13 stomates). Transients had a mean peak 535/480 nm ratio of 3.11 ± 0.31 and a period, when two increases occurred, of 366 ± 31 s. **(B)** Increase of det3 guard cell $[Ca^{2+}]_{cyt}$ induced by 100 μM H_2O_2 (n = 22 from 13 stomates). Increases had a mean peak 535/480 nm ratio of 3.31 ± 0.46. **(C)** One hundred micromolar H_2O_2 induced stomatal closure in WT (left bars) but not det3 (right bars). Data are the mean ± SEM of 180 stomata from three replicates. Hyperpolarization activated calcium-permeable currents in **(D)** WT (n = 9) and **(E)** det3 (n = 6) guard cell protoplasts induced by H_2O_2. **(F)** Oscillations of WT guard cell $[Ca^{2+}]_{cyt}$ induced by cold (n = 20 from 10 stomates). Oscillations had a mean peak 535/480 nm ratio of 2.66 ± 0.31. **(G)** Oscillations of det3 guard cell $[Ca^{2+}]_{cyt}$ induced by cold (n = 18 from nine stomates). Oscillations had a mean peak 535/480 nm ratio of 2.61 ± 0.29. **(H)** Cold induced stomatal closure in WT (left) and det3 (right). Data are the mean ± SEM of 180 stomata from three replicates.

also induced stomatal closure to the same extent in WT and det3 (Fig. 3E).

Hydrogen peroxide (H_2O_2) also induced $[Ca^{2+}]_{cyt}$ increases in WT guard cells (23, 24), consisting of one or two separate transients (Fig. 4A) with peak magnitudes of ~700 nM (n = 24). In det3 guard cells H_2O_2 caused larger (≈1450 nM), more prolonged $[Ca^{2+}]_{cyt}$ increases (Fig. 4B, n = 22 cells), and H_2O_2-induced stomatal closure

was abolished (Fig. 4C). H_2O_2-induced $[Ca^{2+}]_{cyt}$ signaling in guard cells requires activation of a plasma membrane calcium influx current (I_{Ca}) that shows enhanced activity at negative membrane potentials (24). Electrophysiological measurement (22) of I_{Ca} in guard cell protoplasts showed identical I_{Ca} activation by H_2O_2 in WT and det3 (Fig. 4, D and E).

Cold increases plant cell $[Ca^{2+}]_{cyt}$ (25). In

WT guard cells cold elicited small, repetitive $[Ca^{2+}]_{cyt}$ transients (amplitude ≈ 125 nM $[Ca^{2+}]_{cyt}$; frequency, 154 ± 11 s; n = 20 cells) (Fig. 4F). Cold-induced transients of similar amplitude and frequency (amplitude ≈ 125 nM $[Ca^{2+}]_{cyt}$; frequency, 162 ± 21 s; n = 18 cells) were measured in det3 guard cells (P < 0.01) (Fig. 4G). Cold elicited stomatal closure to the same extent in det3 and WT (P < 0.05) (Fig. 4H).

Calcium contents (predominantly vacuolar and ER) measured by elemental x-ray microanalysis (26) in intact guard cells of open stomates were indistinguishable between WT and det3 (calcium as weight percent of total K^+, Na^+, and Ca^{2+} = 34.3 ± 1.2% for WT and 34.7 ± 0.7% for det3, n = 90 each). These data suggest that the residual 40% V-ATPase activity is sufficient to allow long-term intracellular calcium accumulation in det3. Therefore, disruption of $[Ca^{2+}]_{ext}$- and oxidative stress–induced $[Ca^{2+}]_{cyt}$ oscillations in det3 guard cells (Figs. 2, C and D, and 4B) does not appear to be due to depletion of intracellular calcium stores.

The correlation in det3 between stimuli for which guard cell $[Ca^{2+}]_{cyt}$ oscillations are disrupted and stomatal closure is abolished strongly suggests that $[Ca^{2+}]_{cyt}$ oscillations are necessary for stomatal closure. To critically test this hypothesis, we experimentally imposed $[Ca^{2+}]_{cyt}$ oscillations in guard cells using hyperpolarization-mediated calcium influx (9, 11). Exchanging the bathing solution every 300 s between high-KCl (depolarizing) and low-KCl (hyperpolarizing) buffers (22), and adding $[Ca^{2+}]_{ext}$ concomitantly with the hyperpolarizing buffer, imposed repetitive $[Ca^{2+}]_{cyt}$ increases in WT guard cells (Fig. 5A, n = 20 cells) and resulted in stomatal closure (Fig. 5B, left panel, "Post Osc"). Removing external calcium with 10 mM EGTA prevented oscillations (n = 8) and inhibited stomatal closure when external K^+

Fig. 5. Repetitive hyperpolarizations impose $[Ca^{2+}]_{cyt}$ oscillations in guard cells. (A) $[Ca^{2+}]_{cyt}$ oscillations in a WT guard cell resulting from exchanges between depolarizing (100 mM KCl, zero added Ca^{2+}) (■) and hyperpolarizing buffers (0.1 mM KCl, 10 mM Ca^{2+}) (□) (n = 20 from 10 stomates). (B) (Left) Inducing $[Ca^{2+}]_{cyt}$ oscillations close preopened [Control (Pre-osc)] stomata in WT (Post Osc). Oscillation-induced closure is elicited by continuous hyperpolarization (Hyp) but inhibited by prolonged $[Ca^{2+}]_{cyt}$ increase induced by stepwise hyperpolarization (Step). (Right) Oscillation-induced closure in det3. Data are the mean ± SEM of 160 stomata from four replicates. (C) One exchange from depolarizing to hyperpolarizing buffer (n = 12 from six stomates). (D) Continuous transfer to hyperpolarization buffer (n = 32 from 16 stomates). (E) A stepwise hyperpolarization induces prolonged $[Ca^{2+}]_{cyt}$ increases (n = 20 from 10 stomates). (F) Depolarizing to hyperpolarizing buffer exchanges as in (A) in det3 guard cells (n = 18 from 11 stomates).
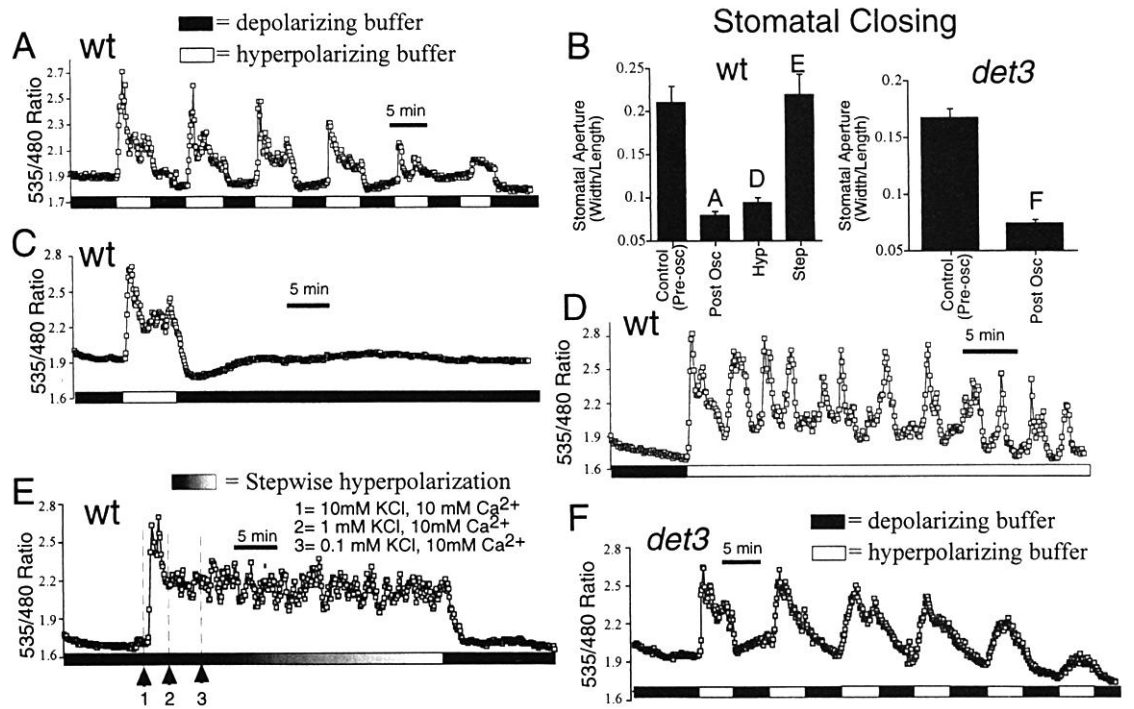
was repetitively exchanged (27). One 300-s hyperpolarization resulted in a single calcium transient, indicating that spontaneous oscillations were not initiated (Fig. 5C, n = 12 cells). Continuous transfer into the hyperpolarizing buffer (0.1 mM K⁺) (Fig. 5D) induced oscillations with a higher frequency than oscillations induced by 10 mM $[Ca^{2+}]_{ext}$ at 5 mM K⁺ [period = 178 ± 31 s, n = 32 compared with 396 ± 23 (Fig. 2B)] and resulted in stomatal closure (Fig. 5B, left panel, "Hyp"). However, imposing hyperpolarizations by decreasing external K⁺ stepwise from 100 mM to 10, 1, and 0.1 mM, concomitant with the addition of 10 mM $[Ca^{2+}]_{ext}$, produced prolonged $[Ca^{2+}]_{cyt}$ increases in WT guard cells (Fig. 5E) similar to $[Ca^{2+}]_{ext}$-induced $[Ca^{2+}]_{cyt}$ increases in det3 (compare Figs. 2, C and D, and 5E). Notably, prolonged $[Ca^{2+}]_{cyt}$ increases in WT also failed to elicit stomatal closure (Fig. 5B, left panel, "Step").

In det3 guard cells, repetitive hyperpolarizations and the concomitant addition of 10 mM $[Ca^{2+}]_{ext}$ also induced $[Ca^{2+}]_{cyt}$ oscillations (Fig. 5F, n = 18 of 22 cells), which contrasted with the prolonged $[Ca^{2+}]_{cyt}$ increases induced by $[Ca^{2+}]_{ext}$ at constant external K⁺ (Fig. 2, C and D). Remarkably, imposing $[Ca^{2+}]_{cyt}$ oscillations in det3 guard cells elicited $[Ca^{2+}]_{ext}$-induced stomatal closure (Fig. 5B, right panel). Therefore, by restoring oscillations in det3 guard cells, the impairment of $[Ca^{2+}]_{ext}$-induced stomatal closure was rescued.

The $[Ca^{2+}]_{cyt}$ oscillations elicited by ABA, cold, $[Ca^{2+}]_{ext}$, and oxidative stress had different amplitudes and frequencies (Figs. 2, A and B; 3, A and B; and 4, A and F), and all induced stomatal closure. Oscillations in $[Ca^{2+}]_{cyt}$ result from the interaction of three processes: extracellular calcium influx, intracellular calcium release, and sequestration into intracellular stores or across the plasma membrane (28, 29). In guard cells the stimuli $[Ca^{2+}]_{ext}$ (4) and oxidative stress (24) activate calcium influx from the extracellular space. In det3 guard cells these stimuli caused an initial $[Ca^{2+}]_{cyt}$ increase (Figs. 2, C and D, and 4B), suggesting that this initial calcium influx is unaffected, as also indicated by identical I_{Ca} activation in WT and det3 (Fig. 4, D and E). However, in det3 these stimuli caused prolonged $[Ca^{2+}]_{cyt}$ increases, suggesting that disruption of $[Ca_{2+}]_{cyt}$ oscillations is due to impaired endomembrane Ca^{2+} uptake. Disruption of endomembrane Ca^{2+} uptake in det3 could occur by a number of possible mechanisms. Ca^{2+}/H^+ antiporter activity, which is involved in Ca^{2+} homeostasis in plant cells (16), may be reduced. Alternatively, a proposed direct facilitation of Ca^{2+}-ATPase activity by V-ATPases (17, 19) might be disrupted in det3. Additionally, endomembrane lumen or cytosolic pH changes in det3 may have effects on Ca^{2+} transporters.

Disruption of $[Ca^{2+}]_{cyt}$ oscillations in response to $[Ca^{2+}]_{ext}$ and oxidative stress but not ABA and cold suggests that different mechanisms generate oscillations for these stimuli. These mechanisms may involve different Ca^{2+} transporters located at separate intracellular calcium stores, as is found in animal cells (18, 19), although certain components can be shared among stimuli (9, 24, 25).

In det3 guard cells, the perfect correlation between those stimuli for which $[Ca^{2+}]_{cyt}$ oscillations were disrupted and stomatal closure was abolished strongly suggests that calcium oscillations are required for stomatal closure. Imposing oscillations rescued $[Ca^{2+}]_{ext}$-induced stomatal closure in det3, supporting previous hypotheses that calcium oscillations are required for physiological responses in guard cells (30). Prolonged $[Ca^{2+}]_{cyt}$ increases in det3 (Fig. 2, C and D) or WT (Fig. 5, B and E) failed to elicit stomatal closure, suggesting, as in animal cells (1–3), that disruption of oscillations has a negative effect on some physiological responses. Therefore, regulation of the mechanism(s) in guard cells necessary to mediate stomatal closure is probably encoded by a pattern of periodic $[Ca^{2+}]_{cyt}$ increases. Overall, these findings strongly suggest that in Arabidopsis guard cells, $[Ca^{2+}]_{cyt}$ oscillations are essential to elicit stomatal closure.

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High Direct Estimate of the Mutation Rate in the Mitochondrial Genome of *Caenorhabditis elegans*

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Mutations in the mitochondrial genome have been implicated in numerous human genetic disorders and offer important data for phylogenetic, forensic, and population genetic studies. Using a long-term series of *Caenorhabditis elegans* mutation accumulation lines, we performed a wide-scale screen for mutations in the mitochondrial genome that revealed a mutation rate that is two orders of magnitude higher than previous indirect estimates, a highly biased mutational spectrum, multiple mutations affecting coding function, as well as mutational hotspots at homopolymeric nucleotide stretches.

Understanding the onset of mitochondrial disease and effective evolutionary analysis require accurate estimates of the rate and pattern of mitochondrial mutation, both of which have been the focus of recent controversy. Phylogenetic estimates of the substitution rate in the control region and protein-coding sequences of the human mitochondrial genome range from 0.02 to 0.26 per site per 10^6 years (My) (*1*, *2*). By contrast, pedigree analyses of the human control region and protein-coding sequences suggest that the substitution rate is much higher: ~2.5 per site per My (*3*, *4*). This discrepancy may be a consequence of mutational hotspots in the control region and/or the mitochondrial disease state of the individuals included in the pedigree analyses (*5*, *6*). Because the rate and pattern of mitochondrial substitution observed over phylogenetic time are a function of both the baseline mutational spectrum and its subsequent modification by natural selection, they likely provide a highly biased view of the rate and pattern of mutation. Unfortunately, almost all of our current estimates are based on indirect arguments and observations that may be biased by the consequences of selection (*7*, *8*).

A direct estimate of the mitochondrial mutation rate and pattern was accomplished by sequencing 10,428 base pairs (bp) of the mitochondrial genomes of 74 *Caenorhabditis elegans* mutation accumulation (MA) lines maintained for an average of 214 generations by single-progeny descent (*9–12*). Each MA line was propagated in a benign environment across generations by a single, random worm. This resulted in an effective population size of each MA line equal to one; hence, the efficiency of natural selection was reduced to a minimum, ensuring that all mutations, except those with extreme effects, accumulated over time in a neutral manner. These lines are known to have undergone a substantial decline in productivity, survival to maturity, generation time, and fitness as a consequence of deleterious-mutation accumulation (*12*).

Among the 74 MA lines, we analyzed 771,672 bp and observed 26 mutations for a total mutation rate equal to 1.6×10^{-7} per site per generation ($\pm 3.1 \times 10^{-8}$), or based on an average generation time of 4 days, 14.3 per site per My (± 2.8). Sixteen of these mutations were base substitutions (Table 1), yielding a direct estimate of the mitochondrial mutation rate for base substitutions equal to 9.7×10^{-8} per site per generation ($\pm 2.4 \times 10^{-8}$), or 8.9 per site per My (± 2.2). This observed rate is two orders of magnitude higher than the phylogenetic estimates discussed above and exceeds rates derived from pedigree analyses (*1–4*). The 16 base substitutions occurred across unique sites in 15 different MA lines, suggesting that the observed rate of base substitution is a product neither of hotspots nor of lines predisposed to mitochondrial mutation. Furthermore, the observed numbers of lines with 0, 1, and 2 mutations are nearly identical to those expected on the basis of a Poisson distribution (*13*).

Animal mitochondrial DNA (mtDNA) evolution is characterized by a strong bias toward transition (G↔A or T↔C) substitutions, but it has been unclear whether this phenomenon is a consequence of selection or of the baseline mutational spectrum (*14*, *15*). Comparison of two natural isolates of *C. elegans*, N2 (England) and RC301 (Germany), revealed 27 transitions and 2 transversions in mtDNA (*16*). Similarly,

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